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Review

Transforming Growth Factor-β: Biology and Clinical Relevance

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Transforming growth factor-β is a pleiotropic growth factor that has enthralled many investigators for approximately two decades. In addition to many reports that have clarified the basic mechanism of transforming growth factor-β signal transduction, numerous laboratories have published on the clinical implication/application of transforming growth factor-β. To name a few, dysregulation of transforming growth factor-β signaling plays a role in carcinogenesis, autoimmunity, angiogenesis, and wound healing. In this report, we will review these clinical implications of transforming growth factor-β.

Keywords: TGF-β, Smad, cell cycle, tumor suppressor, signal, wound healing

Introduction

TGF-β signaling pathway's elucidation has shown it to be very complex with multiple levels of regulation, involved in many processes, including development, wound healing, fibrosis, carcinogenesis, angiogenesis, and immunity to name a few. Herein, we outline some of the clinically relevant aspects of this intriguing, and rapidly progressing field.

Signaling

In mammalian cells, there are three subtypes of TGF-β ligands, β1, β2, and β3. They are encoded by separate genes, but signal through the same signaling cascade. TGF-β binds to a transmembrane, heteromeric complex of serine/threonine kinases, which are comprised of "type I" and "type II" receptors. Once the TGF-β ligand binds to the receptor complex, TGF-β type II receptor (TβRII) phosphorylates the GS domain of the type I receptor (TβRI). This in turn activates TβRI, which then autophosphorylates itself and phosphorylates downstream target proteins (Fig. 1) (Roberts and Sporn, 1990; Derynck *et al*., 2001).

Smads, a family of proteins, are important mediators in the TGF-β signaling cascade (Shi and Massague, 2003). Smad2 and Smad3 are bound to SARA (SMAD anchor for receptor activation) in the cytoplasm (Tsukazaki *et al*., 1998) which presents Smad2 and Smad3 to the activated TGF-β receptor complex. TβRI then directly phosphorylates the carboxy terminal of Smad2 and Smad3, resulting in decreased affinity to SARA and heterotrimerization of Smad2 and Smad3 with Smad4 (Derynck and Zhang, 2003). This entire complex then translocates into the nucleus via the nucleoporins within the nuclear pore complex (Xu *et al*., 2002), and transcriptionally regulates multiple effector genes. The Smad2/3/4 complex's stay within the nucleus is transient, as it becomes dephosphorylated, and shuttled back out to the cytoplasm, where it becomes rephosphorylated to repeat its trip once again (Inman *et al*., 2002). Smad6 and Smad7 bind the type I receptor, thereby competitively inhibiting Smad2 and Smad3 phosphorylation (Itoh *et al*., 2000). TGF-β induces the expression of Smad7. Therefore, Smad7 acts in a negative feedback loop to regulate the intensity or duration of the TGF-β signal. However, the aberrant expression and the continued presence of Smad7 may cause TGF-β resistance. Smurf1 and Smurf2 are an E3 ubiquitin ligase, which recognize TGF-β activated receptors, and target them for ubiquintylation and degradation (Kavsak *et al*., 2000); (Ebisawa *et al*., 2001). Jab1/CSN5, which is a component of the COP9 signalosome complex, associates constitutively with Smad7 and that overexpression of Jab1/CSN5 causes the translocation of Smad7 from the nucleus to the cytoplasm, promoting its degradation and thus releasing Smad7-mediated suppression of the TGF-β signaling (Kim *et al*., 2004).

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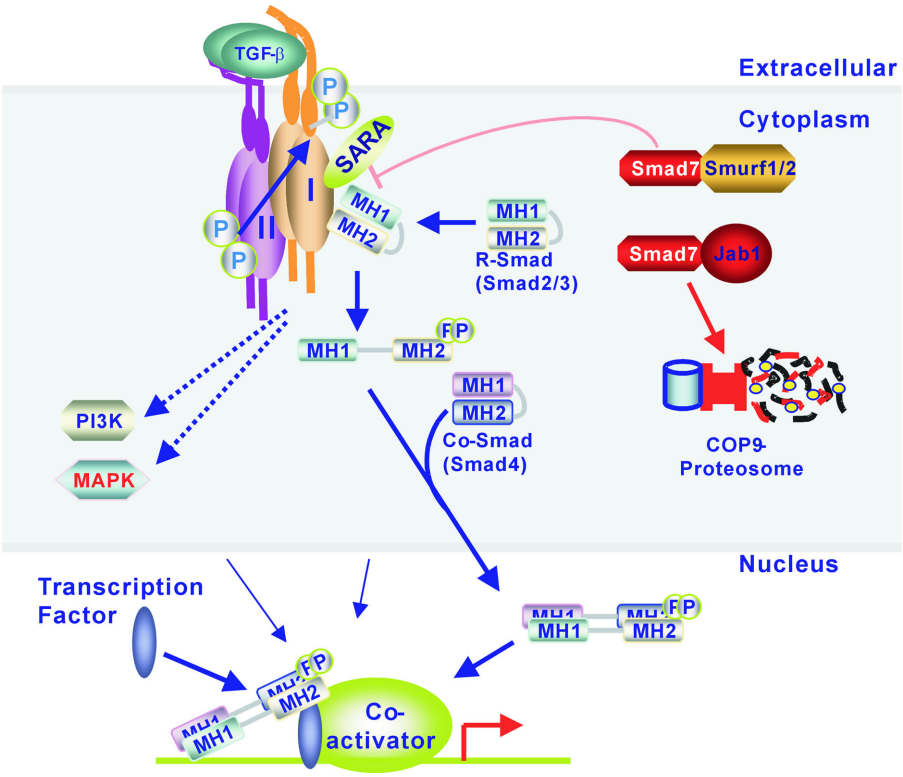

Fig. 1. Mechanism of signaling by TGF-β. TGF-β's bind and activate TGF-β receptor complex. Activated type I receptor propagates the signal downstream by directly phosphorylating receptor-regulated Smads (Smad2 and Smad3) at a conserved SSXS motif at the carboxyl terminus of the protein, which assemble into heteromeric complexes with the common mediator Smad4. Smad2 and Smad3 can be presented to TβRI by scaffolding proteins such as SARA. Smad complexes translocate to the nucleus where they control transcription of target genes cooperatively with other transcription factors, co-activators and co-repressors. Complexes of Smad7 and Smurf1 or Smurf2 (dark blue) mediate the termination of signaling by promoting the poly-ubiquitination and degradation of activated receptors. However, Jab1 enhances TGF-β signaling by binding to Smad7 and inducing degradation of Smad7 through the COP9-proteosome pathway. Target gene expression and cellular response are also modulated by TGF-β via activation of PI3K and various MAPK pathways.

TGF-β mediated growth inhibition

TGF-β is a potent mediator of growth inhibition in a variety of cell types, including epithelial cells. Downstream targets of TGF-β regulated transcription include $p15^{INK4B}$ (Hannon & Beach, 1994) and $p21^{CIP1}$ (Datto *et al.*, 1995) both of which are important "brakes" in the cell cycle. $p21^{CIP1}$, a cyclin dependent kinase inhibitor, interacts with CDK2/cyclin A and CDK2/cyclin E complexes, preventing progression through the cell cycle (Reynisdottir *et al.*, 1995). In TGF-β mediated cell cycle arrest of human mammary epithelial cells, $p15^{INK4B}$ interacts with both CDK4 and CDK6, with loss of cyclin D1, $p21^{CIP1}$, and $p27^{Kip1}$ from these complexes. The displaced $p27^{Kip1}$ then interacts with CDK2/cyclin E complexes, causing inhibition of this complex (Sandhu *et al.*, 1997).

In addition, TGF-β inhibits the expression of c-Myc, CDK4, and CDC25A, all of which are involved in driving cellular growth (Derynck *et al.*, 2001). c-Myc overexpression causes cells to become resistant to TGF-β induced growth suppression (Alexandrow *et al.*, 1995). Thus, decreasing the levels of c-Myc would repress its inhibitory effects on TGF-β's growth suppression (Warner *et al.*, 1999). TGF-β also decreases the levels of CDK4 (Ewen *et al.*, 1993), and CDC25A (Iavarone and Massague, 1997), a tyrosine phosphatase involved in the activation of CDK4.

TGF-β signaling is also involved in the induction of apoptosis, the mechanism of which is not completely understood at this time. One component identified in rat prostate cancer cells is ARTS (apoptosis-like protein the TGF-β signaling pathway). This molecule sensitizes cells normally resistant to TGF-β induced apoptosis. It is normally found in the mitochondria, but localizes to the nucleus in apoptosis. Expression of a mutant form of ARTS abrogates the activation of caspase 3 and apoptosis (Larisch *et al.*, 2000). Other proteins that have been implicated in TGF-β induced apoptosis is TIEG1 (TGF-β inducible early response gene) (Tachibana *et al.*, 1997) and DAPK (death-associated protein kinase) (Jang *et al.*, 2002).

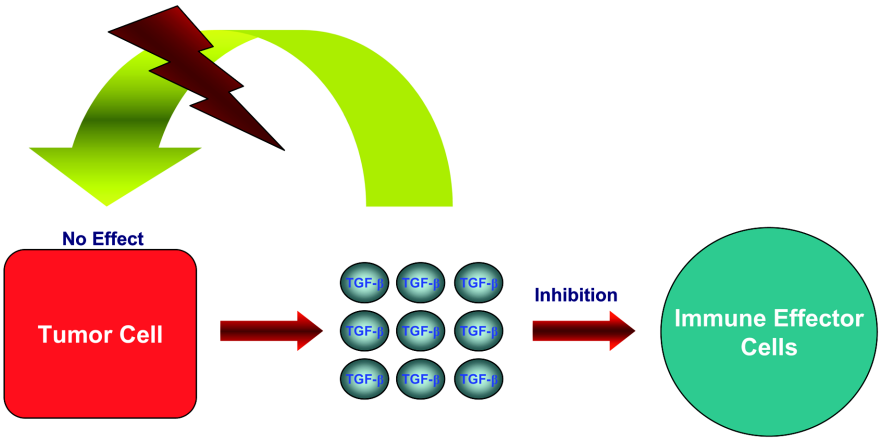

Fig. 2. Schema for potential effect of tumor-derived TGF-β.

TGF-β, a tumor suppressor and a tumor promoter

The TGF-β's are a family of ligands that potently inhibit growth and induce apoptosis in nontransformed human colon epithelium. Numerous studies have now demonstrated that the TGF-β receptor complex and its downstream signaling intermediates constitute a tumor suppressor pathway. Inactivation of either of the two transmembrane serine/threonine kinases called the TβRI and TβRII is now known to underlie a wide variety of human pathologies including carcinogenesis (de Caestecker *et al*., 2000; Kim *et al*., 2000). Human gastric and colon cancers are in general functionally resistant to TGF-β growth inhibition. In human gastric and colon cancers, this resistance is due to the genetic alteration including the mutation of the TβRII resulting from microsatellite instability (Park *et al*., 1994; Markowitz *et al*., 1995; Myeroff *et al*., 1995). By contrast, the most common mechanism of loss of expression of the TβRII involves transcriptional repression. The observation that TβRII inactivation coincides with acquisition of invasiveness and frank malignancy raises the question of whether TβRII inactivation may directly promote tumor invasiveness as well as release cells from TGF-β mediated growth inhibition. Moreover, TGF-β resistant gastric and colon cancers often produce significant amounts of TGF-β. Such secreted TGF-β could contribute to tumor pathogenesis via paracrine effects on neighboring stromal cells, via inducing the growth of new blood vessels, and via local immune suppression.

Inhibition of TGF-β encourages proliferation of early tumor cells, but paradoxically, in late tumor cells, activation of TGF-β seems to enhance tumor growth and invasion. For instance, in hereditary non-polyposis colorectal cancer (HNPCC), a repeat stretch of adenines in TβRII gene are prone to mutations due to microsatellite instabilities, which give rise to nonfunctional truncated proteins (Markowitz *et al*., 1995; Lu *et al*., 1996). And mutations in the TβRII have been found sporadically in gliomas, and as well as in cancers of the colon, stomach, endometrium, pancreas, liver, and breast (Siegel and Massague, 2003). Overall, 20-25% of all colon cancers have an inactivating mutation in TβRII (Grady *et al*., 1999). Mutations of TβRI have been found in ovarian, breast, pancreatic cancers, as well as in T-cell lymphoma (Siegel and Massague, 2003). And homozygotes of an attenuated form of TβRI allele have a higher risk of developing colon cancer (Pasche *et al*., 1999).

Experimentally, expression of wild type TβRII in gastric, colon, or breast cancer cell lines which lack functional TβRII results in growth inhibition and reduced anchorage independence (Wang *et al*., 1995; Sun & Chen, 1997; Chang *et al*., 1997). And expression of active TGF-β1 in mouse keratinocytes rendered them more resistant to the formation of benign skin lesions in a skin chemical carcinogenesis model (Cui *et al*., 1996). These studies suggest that TGF-β is indeed a "tumor suppressor."

However, tumors have increased production of TGF-β (Derynck *et al*., 1987), not less, which suggests a pro-growth role of this ligand in tumorigenesis (Fig. 2). In the experiment described above with mouse keratinocytes, the rate of progression from benign tumors to invasive spindle carcinomas was increased. And transgenic mice expressing TGF-β1 in the liver spontaneously developed hepatocellular tumors (Factor *et al*., 1997). Sequestration of TGF-β ligand with soluble TGF-β receptors inhibited metastasis of mammary tumors to the lung (Muraoka *et al*., 2002). These studies point to a growth promoting role for TGF-β in late tumorigenesis. Thus, in early tumor development, escape from TGF-β's growth inhibition allows proliferation. However, in late tumorigenesis, TGF-β's effects on the tumor microenvironment, including fibroblasts, endothelial cells, and immune cells, promote angiogenesis and suppress immune response to lead to tumor invasion and metastasis.

Angiogenesis

Growth of new blood vessels from pre-existing ones is

essential in supplying the growing tumor's metabolic needs. TGF-β1 is a potent inducer of angiogenesis. It can induce capillary formation of endothelial cells cultured on collagen matrix (Madri *et al*., 1988). Transgenic mice lacking functional TGF-β1 or the TβRII are embryonically lethal, in part, due to defects in vasculogenesis and angiogenesis (Martin *et al*., 1995). And mice lacking the TβRI have defective angiogenesis. TGF-β1 overexpression in prostate carcinoma enhanced angiogenesis whereas neutralizing antibodies decreased it in immunocompromised mice (Ueki *et al*., 1992). TGF-β can induce angiogenesis by increasing the expression of VEGF (vascular endothelial growth factor) (Pertovaara *et al*., 1994). TGF-β can also create favorable environmental condition for the growth and maintenance of new blood vessels. It upregulates MMP-2 and MMP-9, which degrade extracellular matrix, and allow for subsequent invasion and migration of endothelial cells (Derynck *et al*., 2001). Thus, TGF-β is pro-angiogenic in two ways, first by directly stimulating cytokines involved in endothelial growth, and second by inducing a micro-environment favorable for endothelial migration, invasion, and survival.

Tumor invasion

Invasion through the basement membrane is a critical step in tumor progression and requires transdifferentiation of epithelial cells to mesenchymal type. This involves loss of cell-cell contact, with acquisition of increased fibroblastic characteristics. This transition is commonly referred to as the epithelial-mesenchymal transition (EMT) and is not only found in tumors, but also in development (Siegel and Massague, 2003). TGF-β plays a critical role in the transdifferentiation of epithelium to mesenchyme of embryonic cardiac, palatal, and hair follicle development (Derynck *et al*., 2001). In keratinocytes, the predominant cytoskeleton switches from intermediate filament to vimentin during transdifferentiation (Oft *et al*., 1996) while there is a decreased expression of E-cadherin, the calcium dependent cell-cell adhesion molecule. Tumors commonly downregulate E-cadherin, and its overexpression can suppress tumor invasion (Thiery, 2002). Because TGF-β induces expression of SNAIL and SIP1, zinc-finger transcription factors that repress expression of E-cadherin (Comijn *et al*., 2001); (Peinado *et al*., 2003), it has been suggested that TGF-β cause differentiation of epithelial cells to those of mesenchymal phenotype, thereby making these cells more invasive, with greater metastatic potential. This hypothesis is supported by the observation that the expression of dominant negative TβRII reversed colon cancer cells to an epithelial phenotype *in vitro* (Oft *et al*., 1998) and in squamous cell carcinomas *in vivo* (Portella *et al*., 1998).

Immune suppression

TGF-β: negative regulator of immune system

Since its initial discovery by Roberts *et al*. (1990), TGF-β has been shown to be a critical negative regulator of immune system. In TGF-β1 knock-out mice, animals die of diffuse autoimmune response 3-4 weeks after birth (Kulkarni *et al*., 1993). Further studies have demonstrated that TGF-β inhibits interleukin-2 (IL-2) production, leading to inhibition of T-cell proliferation. In addition, TGF-β inhibits the ability of naïve CD4 and CD8 T cells to develop into mature effector T cells, TH1 or TH2, or cytotoxic lymphocytes, respectively. In macrophages and monocytes, TGF-β is both stimulatory and inhibitory. TGF-β is a potent chemoattaractant for human blood monocytes and activates phagocytic activity in macrophages. However, TGF-β has also been shown to deactivate macrophages by suppressing nitric oxide and reactive oxygen intermediates.

Aside from a direct inhibitory effect of TGF-β on immune cells, TGF-β can act on non-immune cells and can contribute to the immunosuppressive effect in the host. Interactions between thymic stromal cells and immune cells are the basis for T cell selection and have an impact on final T cell repertoire (Sebzda *et al*., 1999). For example, TGF-β expressed from thymic stromal cells regulates the differentiation of CD4+/CD8+ double positive stages (Plum *et al*., 1995). Another example is the inhibitory effect of TGF-β on the production of IL-7 by non-lymphoid stromal cells, which are important for the development of B cells (Tang *et al*., 1997).

Multiple investigators have suggested that the principal immune-suppressive factor secreted by tumor cells is TGF-β (Chouaib *et al*., 1997; Wojtowicz-Praga, 1997). Indeed, it has been reported that the overexpression of TGF-β in the highly immunogenic C3H tumors led to tumor growth and escape from immune-surveillance without affecting the levels of expression of MHC I or tumor-specific antigen (Torre-Amione *et al*., 1990) while TGF-β neutralization resulted in MCF-7 tumor rejection (Arteaga *et al*., 1993). In addition, transfection of antisense TGF-β in a rat glioma cell line and the Duning rat prostate tumor MatLyLu led to a complete eradication of tumor xenografts *in vivo* (Fakhrai *et al*., 1996; Matthews *et al*., 2000). Lastly, it has been shown that the overexpression of soluble TβRII resulted in tumor suppression in a mouse thymoma model (Won *et al*., 1999). These observations, taken together, support the hypothesis that malignant cells produce TGF-β to escape immune surveillance and suggest that the elimination of TGF-β from tumor cells enhances host anti-tumor immune response.

TGF-β insensitive immune cells and tumor rejection

An alternative to eliminating the production of TGF-β from

tumor cells is to render immune cells insensitive to TGF-β. In this regard, Gorelik and Flavell have demonstrated that T-cells transfected with dominant negative form of TβRII (TβRIIDN) were able to reject tumor (Gorelik and Flavell, 2001). The same authors also reported that the eradication of tumors did not occur if tumors were established 7 days prior to infusion with the TβRIIDN-expressing T-cells. These results suggest that the immune-mediated rejection of established tumors requires additional cell types, in addition to T-cells.

In this regard, the transplantation of TGF-β-insensitive hematopoietic stem cells has been reported to eliminate cancer cells in mice (Shah *et al*., 2002b). Unfortunately, the animals eventually died of diffuse inflammation of peripheral organs that is characteristic of graft-versus-host disease (GVHD) at seven months after initiation of treatment. Analysis of these animals demonstrated a dramatic expansion of macrophages (Shah *et al*., 2002a). A similar activation of immune cells was also observed in TGF-β knockout animals which showed an excess inflammation in peripheral organs (Kulkarni *et al*., 1993).

Wound healing

Wound healing after injury requires a complex coordination of cells, beginning with the influx of inflammatory cells, transdifferentiation of epithelial to mesenchymal cells, mobilization of fibroblasts, then finally laying down of extracellular matrix. Degranulation of platelets at sites of injury releases a bolus of TGF-β1, thereby increasing the production of ECM by inducing various collagen gene promoters (Flanders, 2004) while decreasing ECM degradation by inhibiting matrix degrading enzymes and increasing expression of MMP inhibitors (Yuan and Varga, 2001).

Topical application of TGF-β improves healing, even in radiation-impaired wounds (Bernstein *et al*., 1991). Wound healing is believed to signal mainly through Smad3, but not Smad2. The Smad2 knockout mice are embryonically lethal, and fail to gastrulate, form mesoderm, and have left-right patterning defects (Nomura & Li, 1998). The Smad3 KO mice have skeletal abnormalities and defects in T-cell immunity (Yang *et al*., 1999; Yang *et al*., 2001). In addition, Smad3 null mice have faster cutaneous wound healing, due to the absence of TGF-β mediated growth inhibition of keratinocytes (Ashcroft *et al*., 1999). The Smad3 KO mice also have been reported to be resistant to TGF-β-induced pulmonary fibrosis, and to have decreased cutaneous damage and fibrosis following ionizing irradiation (Flanders *et al*., 2002).

Aberrant TGF-β signaling has been implicated in pathologic fibrotic conditions involving skin, liver, kidney, eye, lung, and heart (Flanders, 2004). For instance, increased levels of TGF-β were found in hepatic fibrosis induced by murine schistosomiasis or CCl4-treatment (Czaja *et al*., 1989). It is thought that the release of TGF-β by necrotic hepatocytes trigger the transdifferentiation of hepatic stellate cells into fibroblast like cells (Furukawa *et al*., 2003). And in the lung, TGF-β seems to be important in the regulation of ECM. Inhalation of adenovirus expressing TGF-β1 can induce pulmonary fibrosis in WT mice, but not in Smad3 null mice (Bonniaud *et al*., 2004). However, the Smad3 KO mice develop enlarged airspace, similar to emphysema, due to decreased amounts of ECM. In the kidney, abnormal TGF-β signaling has been suggested to play a role in diabetic nephropathy by increasing glomerulosclerosis and fibrosis. Because TGF-β is intimately involved in wound healing and fibrosis, mediated mostly by its actions on the transdifferentiation of epithelial cells to mesenchymal ones and regulation of ECM, TGF-β may be used to improve wound healing. On the other hand, the inhibition of Smad3 may prevent fibrotic diseases of various organs.

Conclusion

TGF-β is a multi-functional growth factor that has numerous clinical implications. Because TGF-β's effects depend on the context of the experiments, it has been an arduous process to understand the role of TGF-β in clinical pathology. Nevertheless, significant progress has been made in the last few years by many investigators. Further clarification of the precise role of TGF-β in different biological context will likely lead to new and novel therapeutic strategies for various diseases such as cancer, autoimmunity, and pathologic fibrosis.

References

Alexandrow, M. G., Kawabata, M., Aakre, M. and Moses, H. L. (1995) Overexpression of the c-Myc oncoprotein blocks the growth-inhibitory response but is required for the mitogenic effects of transforming growth factor β1. *Proc. Natl. Acad. Sci. USA* **92**, 3239-3243.

Arteaga, C. L., Hurd, S. D., Winnier, A. R., Johnson, M. D., Fendly, B. M. and Forbes, J. T. (1993) Anti-transforming growth factor (TGF)-beta antibodies inhibit breast cancer cell tumorigenicity and increase mouse spleen natural killer cell activity. Implications for a possible role of tumor cell/host TGF-beta interactions in human breast cancer progression. *J. Clin. Invest.* **92**, 2569-2576.

Ashcroft, G. S., Yang, X., Glick, A. B., Weinstein, M., Letterio, J. L., Mizel, D. E., Anzano, M., Greenwell-Wild, T., Wahl, S. M., Deng, C. and Roberts, A. B. (1999) Mice lacking Smad3 show accelerated wound healing and an impaired local inflammatory response. *Nat. Cell Biol.* **1**, 260-266.

Bernstein, E. F., Harisiadis, L., Salomon, G., Norton, J., Sollberg, S., Uitto, J., Glatstein, E., Glass, J., Talbot, T. and Russo, A. (1991) Transforming growth factor-beta improves healing of radiation-impaired wounds. *J. Invest. Dermatol.* **97**, 430-434.

Bonniaud, P., Kolb, M., Galt, T., Robertson, J., Robbins, C., Stampfli, M., Lavery, C., Margetts, P. J., Roberts, A. B. and

Gauldie, J. (2004) Smad3 null mice develop airspace enlargement and are resistant to TGF-β-mediated pulmonary fibrosis. *J. Immunol.* **173**, 2099-2108.

Chang, J., Park, K., Bang, Y.-J., Kim, W. S., Kim, D. and Kim, S.-J. (1997) Expression of transforming growth factor beta type II receptor reduces tumorigenicity in human gastric cancer cells. *Cancer Res.* **57**, 2856-2859.

Chouaib, S., Asselin-Paturel, C., Mami-Chouaib, F., Caignard, A. and Blay, J. Y. (1997) The host-tumor immune conflict: from immunosuppression to resistance and destruction. *Immunol. Today* **18**, 493-497.

Comijn, J., Berx, G., Vermassen, P., Verschueren, K., van Grunsven, L., Bruyneel, E., Mareel, M., Huylebroeck, D. and van Roy, F. (2001) The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. *Mol. Cell* **7**, 1267-1278.

Cui, W., Fowlis, D. J., Bryson, S., Duffie, E., Ireland, H., Balmain, A. and Akhurst, R. J. (1996) TGFβ1 inhibits the formation of benign skin tumors, but enhances progression to invasive spindle carcinomas in transgenic mice. *Cell* **86**, 531-542.

Czaja, M. J., Weiner, F. R., Flanders, K. C., Giambrone, M. A., Wind, R., Biempica, L. and Zern, M. A. (1989) In vitro and in vivo association of transforming growth factor-beta 1 with hepatic fibrosis. *J. Cell Biol.*, **108**, 2477-2482.

Datto, M. B., Li, Y., Panus, J. F., Howe, D. J., Xiong, Y. and Wang, X. F. (1995) Transforming growth factor β induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism. *Proc. Natl. Acad. Sci. USA* **92**, 5545-5549.

de Caestecker, M. P., Piek, E. and Roberts, A. B. (2000) Role of transforming growth factor-β signaling in cancer. *J. Natl. Cancer Inst.* **92**, 1388-1402.

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

Derynck, R., Goeddel, D. V., Ullrich, A., Gutterman, J. U., Williams, R. D., Bringman, T. S. and Berger, W. H. (1987) Synthesis of messenger RNAs for transforming growth factors alpha and beta and the epidermal growth factor receptor by human tumors. *Cancer Res.* **47**, 707-712.

Derynck, R. and Zhang, Y. E. (2003) Smad-dependent and Smad-independent pathways in TGF-β family signalling. *Nature*, **425**, 577-584.

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

Ewen, M. E., Sluss, H. K., Whitehouse, L. L. and Livingston, D. M. (1993) TGF β inhibition of Cdk4 synthesis is linked to cell cycle arrest. *Cell* **74**, 1009-1020.

Factor, V. M., Kao, C. Y., Santoni-Rugiu, E., Woitach, J. T., Jensen, M. R. and Thorgeirsson, S. S. (1997) Constitutive expression of mature transforming growth factor beta1 in the liver accelerates hepatocarcinogenesis in transgenic mice. *Cancer Res.* **57**, 2089-2095.

Fakhrai, H., Dorigo, O., Shawler, D. L., Lin, H., Mercola, D., Black, K. L., Royston, I. and Sobol, R. E. (1996) Eradication of established intracranial rat gliomas by transforming growth factor β antisense gene therapy. *Proc. Natl. Acad. Sci. USA* **93**, 2909-2914.

Flanders, K. C. (2004) Smad3 as a mediator of the fibrotic response. *Int. J. Exp. Pathol.* **85**, 47-64.

Flanders, K. C., Sullivan, C. D., Fujii, M., Sowers, A., Anzano, M. A., Arabshahi, A., Major, C., Deng, C., Russo, A., Mitchell, J. B. and Roberts, A. B. (2002) Mice lacking Smad3 are protected against cutaneous injury induced by ionizing radiation. *Am J. Pathol.* **160**, 1057-1068.

Furukawa, F., Matsuzaki, K., Mori, S., Tahashi, Y., Yoshida, K., Sugano, Y., Yamagata, H., Matsushita, M., Seki, T., Inagaki, Y., Nishizawa, M., Fujisawa, J. and Inoue, K. (2003) p38 MAPK mediates fibrogenic signal through Smad3 phosphorylation in rat myofibroblasts. *Hepatology* **38**, 879-889.

Gorelik, L. and Flavell, R. A. (2000) Abrogation of TGF â signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. *Immunity.* **12**, 171-181.

Gorelik, L. and Flavell, R. A. (2001) Immune-mediated eradication of tumors through the blockade of transforming growth factor-β signaling in T cells. *Nat. Med.* **7**, 1118-1122.

Grady, W. M., Myeroff, L. L., Swinler, S. E., Rajput, A., Thiagalingam, S., Lutterbaugh, J. D., Neumann, A., Brattain, M. G., Chang, J., Kim, S. J., Kinzler, K. W., Vogelstein, B., Willson, J. K. and Markowitz, S. (1999) Mutational inactivation of transforming growth factor β receptor type II in microsatellite stable colon cancers. *Cancer Res*, **59**, 320-324.

Hannon, G. J. and Beach, D. (1994) $pl5^{INK4B}$ is a potential effector of TGF-β-induced cell cycle arrest. *Nature* **371**, 257-261.

Iavarone, A. and Massague, J. (1997) Repression of the CDK activator Cdc25A and cell-cycle arrest by cytokine TGF-β in cells lacking the CDK inhibitor p15. *Nature* **387**, 417-422.

Inman, G. J., Nicolas, F. J. and Hill, C. S. (2002) Nucleocytoplasmic shuttling of Smads 2, 3, and 4 permits sensing of TGF-β receptor activity. *Mol. Cell.* **10**, 283-294.

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

Jang, C. W., Chen, C. H., Chen, C. C., Chen, J. Y., Su, Y. H. and Chen, R. H. (2002) TGF-β induces apoptosis through Smad-mediated expression of DAP-kinase. *Nat. Cell Biol.* **4**, 51-58.

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

Kim, B.-C., Lee, H.-J., Park, S. H., Lee, S., Karpova, T. S., McNally, J. G., Felici, A., Lee, D. K. and Kim. S.-J. (2004) Jab1/CSN5, a component of the COP9 signalosome, regulates transforming growth factor β signaling by binding to Smad7 and promoting its degradation. *Mol. Cell. Biol.* **24**, 2251-2262.

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

Kulkarni, A. B., Huh, C. G., Becker, D., Geiser, A., Lyght, M., Flanders, K. C., Roberts, A. B., Sporn, M. B., Ward, J. M. and Karlsson, S. (1993) Transforming growth factor β_1 null mutation in mice causes excessive inflammatory response and early death. *Proc. Natl. Acad. Sci. USA* **90**, 770-774.

Larisch, S., Yi, Y., Lotan, R., Kerner, H., Eimerl, S., Tony Parks, W., Gottfried, Y., Birkey Reffey, S., de Caestecker, M. P.,

Danielpour, D., Book-Melamed, N., Timberg, R., Duckett, C. S., Lechleider, R. J., Steller, H., Orly, J., Kim, S. J. and Roberts, A. B. (2000) A novel mitochondrial septin-like protein, ARTS, mediates apoptosis dependent on its P-loop motif. *Nat. Cell Biol.* **2**, 915-921.

Lu, S. L., Zhang, W. C., Akiyama, Y., Nomizu, T. and Yuasa, Y. (1996) Genomic structure of the transforming growth factor beta type II receptor gene and its mutations in hereditary nonpolyposis colorectal cancers. *Cancer Res.* **56**, 4595-4598.

Madri, J. A., Pratt, B. M. and Tucker, A. M. (1988) Phenotypic modulation of endothelial cells by transforming growth factor-beta depends upon the composition and organization of the extracellular matrix. *J. Cell Biol.* **106**, 1375-1384.

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

Martin, J. S., Dickson, M. C., Cousins, F. M., Kulkarni, A. B., Karlsson, S. and Akhurst, R. J. (1995) Analysis of homozygous TGF beta 1 null mouse embryos demonstrates defects in yolk sac vasculogenesis and hematopoiesis. *Ann. N. Y. Acad. Sci.* **752**, 300-308.

Matthews, E., Yang, T., Janulis, L., Goodwin, S., Kundu, S. D., Karpus, W. J. and Lee, C. (2000) Down-regulation of TGF- β1 production restores immunogenicity in prostate cancer cells. *Br. J. Cancer* **83**, 519-525.

Muraoka, R. S., Dumont, N., Ritter, C. A., Dugger, T. C., Brantley, D. M., Chen, J., Easterly, E., Roebuck, L. R., Ryan, S., Gotwals, P. J., Koteliansky, V. and Arteaga, C. L. (2002) Blockade of TGF-β inhibits mammary tumor cell viability, migration, and metastases. *J. Clin. Invest.* **109**, 1551-1559.

Myeroff, L. L., Parsons, R., Kim, S.-J., Hedrick, L., Cho, K. R., Orth, K., Mathis, M., Kinzler, K. W., Lutterbaugh, J., Park, K., Bang, Y.-J., Lee, H. Y., Park, J.-G., Lynch. H. T., Roberts, A. B., Vogelstein, B. and Markowitz, S. D. (1995) A transforming growth factor beta receptor type II gene mutation common in colon and gastric but rare in endometrial cancers with microsatellite instability. *Cancer Res.* **55**, 5545-5547.

Nomura, M. and Li, E. (1998) Smad2 role in mesoderm formation, leftright patterning and craniofacial development. *Nature* **393**, 786-790.

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

Oft, M., Peli, J., Rudaz, C., Schwarz, H., Beug, H. and Reichmann, E. (1996) TGF-beta1 and Ha-Ras collaborate in modulating the phenotypic plasticity and invasiveness of epithelial tumor cells. *Genes Dev.* **10**, 2462-2477.

Park, K., Kim, S.-J., Bang, Y.-J., Park, J.-G., Kim, N. K., Roberts, A. B. and Sporn, M. B. (1994) Genetic changes in the transforming growth factor β (TGF-β) type II receptor gene in human gastric cancer cells: correlation with sensitivity to growth inhibition by TGF-β. *Proc. Natl. Acad. Sci. USA* **91**, 8772-8776.

Pasche, B., Kolachana, P., Nafa, K., Satagopan, J., Chen, Y. G., Lo, R. S., Brener, D., Yang, D., Kirstein, L., Oddoux, C., Ostrer, H., Vineis, P., Varesco, L., Jhanwar, S., Luzzatto, L., Massague, J. and Offit, K. (1999). *TβR-I(6A)* is a candidate tumor susceptibility allele. *Cancer Res.* **59**, 5678-5682.

Peinado, H., Quintanilla, M. and Cano, A. (2003) Transforming growth factor β-1 induces snail transcription factor in epithelial cell lines: mechanisms for epithelial mesenchymal transitions. *J. Biol. Chem.* 278, 21113-21123.

Pertovaara, L., Kaipainen, A., Mustonen, T., Orpana, A., Ferrara, N., Saksela, O. and Alitalo, K. (1994) Vascular endothelial growth factor is induced in response to transforming growth factor-beta in fibroblastic and epithelial cells. *J. Biol. Chem.* **269**, 6271-6274.

Plum, J., De Smedt, M., Leclercq, G. and Vandekerckhove, B. (1995) Influence of TGF-beta on murine thymocyte development in fetal thymus organ culture. *J. Immunol.* **154**, 5789-5798.

Portella, G., Cumming, S. A., Liddell, J., Cui, W., Ireland, H., Akhurst, R. J. and Balmain, A. (1998) Transforming growth factor beta is essential for spindle cell conversion of mouse skin carcinoma *in vivo*: implications for tumor invasion. *Cell Growth Differ.* **9**, 393-404.

Reynisdottir, I., Polyak, K., Iavarone, A. and Massague, J. (1995) Kip/Cip and Ink4 Cdk inhibitors cooperate to induce cell cycle arrest in response to TGF-beta. *Genes Dev.*, **9**, 1831-1845.

Roberts, A. B. and Sporn, M. B. (1990). *Peptide growth factors and their receptors: Handbook of Experimental Pharmacology* pp. 421-472. Springer-Verlag, Heidelberg, Germany.

Sandhu, C., Garbe, J., Bhattacharya, N., Daksis, J., Pan, C. H., Yaswen, P., Koh, J., Slingerland, J. M. and Stampfer, M. R. (1997) Transforming growth factor beta stabilizes p15INK4B protein, increases p15INK4B-cdk4 complexes, and inhibits cyclin D1-cdk4 association in human mammary epithelial cells. *Mol. Cell Biol.* **17**, 2458-2467.

Sebzda, E., Mariathasan, S., Ohteki, T., Jones, R., Bachmann, M. F. and Ohashi, P. S. (1999) Selection of the T cell repertoire. *Annu. Rev. Immunol.* **17**, 829-874.

Shah, A. H., Tabayoyong, W. B., Kim, S. Y., Kim, S. J., Van Parijs, L. and Lee, C. (2002a) Reconstitution of lethally irradiated adult mice with dominant negative TGF-β type II receptor-transduced bone marrow leads to myeloid expansion and inflammatory disease. *J. Immunol.* **169**, 3485-3491.

Shah, A. H., Tabayoyong, W. B., Kundu, S. D., Kim, S. J., Van Parijs, L., Liu, V. C., Kwon, E., Greenberg, N. M. and Lee, C. (2002b) Suppression of tumor metastasis by blockade of transforming growth factor β signaling in bone marrow cells through a retroviral-mediated gene therapy in mice. *Cancer Res*, **62**, 7135-7138.

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

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

Sun, L. and Chen, C. (1997) Expression of transforming growth Factor β type III receptor suppresses tumorigenicity of human breast cancer MDA-MB-231 cells. *J. Biol. Chem.* **272**, 25367-25372.

Surh, C. D. and Sprent, J. (1994) T-cell apoptosis detected *in situ* during positive and negative selection in the thymus. *Nature* **372**, 100-103.

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

2374.

Tang, J., Nuccie, B. L., Ritterman, I., Liesveld, J. L., Abboud, C. N. and Ryan, D. H. (1997) TGF-beta down-regulates stromal IL-7 secretion and inhibits proliferation of human B cell precursors. *J. Immunol.* **159**, 117-125.

Thiery, J. P. (2002) Epithelialmesenchymal transitions in tumour progression. *Nat. Rev. Cancer* **2**, 442-454.

Torre-Amione, G., Beauchamp, R. D., Koeppen, H., Park, B. H., Schreiber, H., Moses, H. L. and Rowley, D. A. (1990) A highly immunogenic tumor transfected with a murine transforming growth factor type β_1 cDNA escapes immune surveillance. *Proc. Natl. Acad. Sci. USA* **87**, 1486-1490.

Tsukazaki, T., Chiang, T. A., Davison, A. F., Attisano, L. and Wrana, J. L. (1998) SARA, a FYVE domain protein that recruits Smad2 to the TGFβ receptor. *Cell* **95**, 779-791.

Ueki, N., Nakazato, M., Ohkawa, T., Ikeda, T., Amuro, Y., Hada, T. and Higashino, K. (1992) Excessive production of transforming growth-factor β_1 can play an important role in the development of tumorigenesis by its action for angiogenesis: validity of neutralizing antibodies to block tumor growth. *Biochim. Biophys. Acta* **1137**, 189-196.

Wang, J., Sun, L., Myeroff, L., Wang, X., Gentry, L. E., Yang, J., Liang, J., Zborowska, E., Markowitz, S., Willson, J. K. and Brattain, M. G. (1995) Demonstration that mutation of the type II transforming growth factor β receptor inactivates its tumor suppressor activity in replication error-positive colon carcinoma cells. *J. Biol. Chem.* **270**, 22044-22049.

Warner, B. J., Blain, S. W., Seoane, J. and Massague, J. (1999) Myc downregulation by transforming growth factor β required for activation of the $p15^{Ink4b}$ G_1 arrest pathway. *Mol. Cell. Biol.* **19**, 5913-5922.

Wojtowicz-Praga, S. (1997) Reversal of tumor-induced immunosuppression: a new approach to cancer therapy. *J. Immunother.* **20**, 165-177.

Won, J., Kim, H., Park, E. J., Hong, Y., Kim, S. J. and Yun, Y. (1999) Tumorigenicity of mouse thymoma is suppressed by soluble type II transforming growth factor β receptor therapy. *Cancer Res.* **59**, 1273-1277.

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

Yang, X., Chen, L., Xu, X., Li, C., Huang, C. and Deng, C. X. (2001) TGF-β/Smad3 signals repress chondrocyte hypertrophic differentiation and are required for maintaining articular cartilage. *J. Cell Biol.* **153**, 35-46.

Yang, X., Letterio, J. J., Lechleider, R. J., Chen, L., Hayman, R., Gu, H., Roberts, A. B. and Deng, C. (1999) Targeted disruption of SMAD3 results in impaired mucosal immunity and diminished T cell responsiveness to TGF-β. *Embo J.* **18**, 1280-1291.

Yuan, W. and Varga, J. (2001) Transforming growth factor-repression of matrix metalloproteinase-1 in dermal fibroblasts involves Smad3. *J. Biol. Chem.* **276**, 38502-38510.