

Received: 2005.01.31
Accepted: 2005.03.24
Published: 2005.10.15

Signal transduction in human pancreatic cancer: roles of transforming growth factor β , somatostatin receptors, and other signal intermediates

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Source of support: by the Methodist Hospital Foundation grant 39935, and the St Luke's Episcopal Hospital Roderick D. MacDonald Research Fund 05RDM005 (M. Li), NIH K08 CA85822 (W. E. Fisher), and NIH RO1 DE15543 (Q. Yao).

Summary

Pancreatic cancer is a devastating disease because of the lack of early detection markers and effective treatments. It is the fourth leading cause of cancer-related death in western countries, including the United States. The mechanisms of pancreatic cancer progression remain unknown. Transforming growth factor β (TGF- β), a multifunctional cytokine, regulates cell growth and differentiation in healthy tissues, yet fails to do so in pancreatic cancer. Alterations of the TGF- β and TGF- β receptor/Smad signal transduction pathway have been implicated in pancreatic cancer. Furthermore, both the TGF- β receptor and Smad proteins interact with a variety of cellular signal pathways, such as the somatostatin receptors (SSTRs), ERK1/2, and Wnt signal transduction cascades. This suggests that pancreatic cancer is a multi-gene-controlled malignancy and that effective treatments for pancreatic cancer should be aimed at multiple targets. In this review, we summarized the major signal intermediates involved in pancreatic cancer signal transduction pathways and specifically discussed how alterations in the regulatory functions of TGF- β and Smad proteins allow for pancreatic carcinogenesis.

Key words: pancreatic cancer • TGF- β • SSTR

Full-text PDF: http://www.aite-online/pdf/vol_53/no_5/8146.pdf

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Pancreatic cancer is a devastating disease because of the lack of early detection markers and effective treatments. It is the fourth leading cause of cancer-related death in western countries, including the United States. Despite some recent progress in chemotherapy, radiation therapy, and surgical resections, the overall survival rate of pancreatic cancer is still less than 5%^{20, 42}. Numerous efforts have been made in an attempt to elucidate the mechanism of this malignant cancer development, and several pathways and putative factors have been shown to play important roles in regulating pancreatic cancer growth. One of these important factors is the transforming growth factor β (TGF- β) and its associated signal transduction pathways^{13, 15, 65}.

TGF- β SIGNAL TRANSDUCTION PATHWAY

TGF- β s are pluripotent cytokines that regulate cell differentiation, growth, and migration^{3, 49, 55, 57, 58, 62}. TGF- β , activin, bone morphogenetic protein (BMP), and other related proteins are the known members of the TGF- β superfamily^{9, 15}. Three isoforms (TGF- β 1, TGF- β 2, and TGF- β 3) have been identified that are encoded by different genes but bind to and function through the same receptor pathway^{13, 48, 57}. Pancreatic cancer cells over-express all three TGF- β isoforms, and the over-expression is associated with angiogenesis, fibrosis, and macrophage infiltration. How a cytokine that normally restricts cell division and growth triggers events that may lead to conditions in

which tumors can form remains unclear. Early in tumorigenesis, TGF- β inhibits cancer growth by signaling through an intact intracellular signal transduction pathway that may suppress epithelial cell growth⁵⁰. In later stages of pancreatic cancer, however, proper responses to TGF- β are ablated, possibly due to signal transduction pathway defects. The production of TGF- β isoforms is unregulated, leading to angiogenesis and decreased immune surveillance⁵⁰. TGF- β causes grossly divergent effects on the same cell in varying stages of pancreatic cancer.

TGF- β receptors

TGF- β ligands signal through two heterotetrameric serine/threonine kinase receptors known as TGF- β receptor I (T β RI) and TGF- β receptor II (T β RII)^{45, 53}. T β RII becomes constitutively active and heterodimerizes with T β RI once it binds to TGF- β ligand⁶⁷. The heterodimerization of the two receptors allows T β RII to trans-phosphorylate the GS domain of T β RI and activate the kinase domain of T β RI^{19, 68}. Activated T β RI phosphorylates a class of molecules known as receptor-regulated Smads (R-Smads) at an SSXS motif near the carboxy terminus, as shown in Fig. 1^{1, 60}. Alternatively, phosphorylated T β RI may interact directly with cell-cycle proteins, such as cyclin B, as an anti-proliferative control measure⁴⁶. In this case, up-regulation of TGF- β receptors may allow cells an additional level of control over growth and proliferation³⁸. After prolonged signaling, the

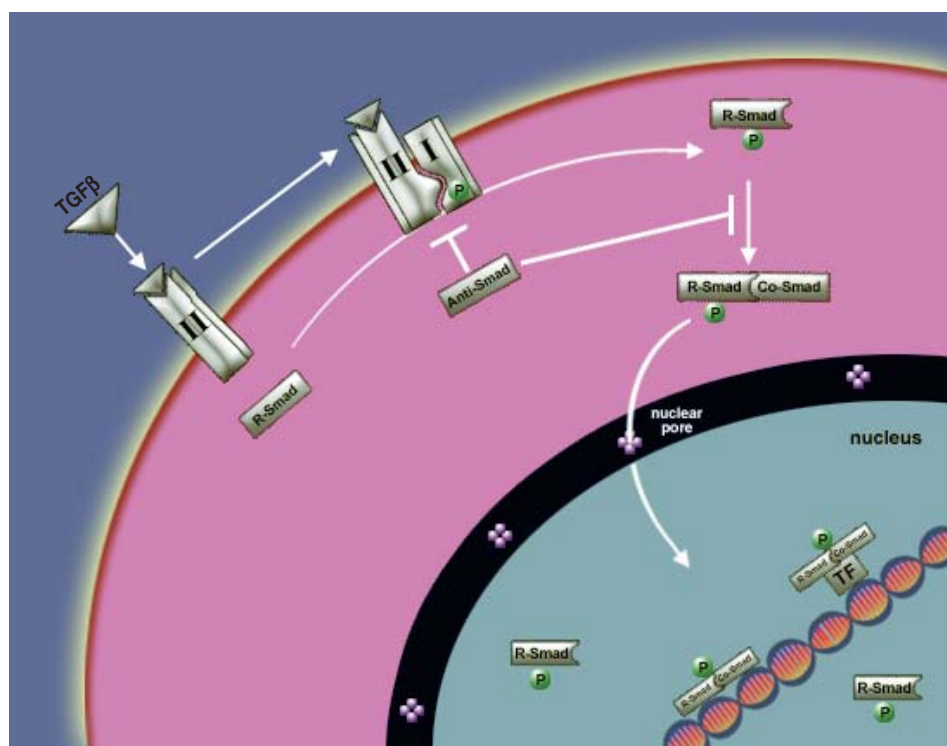


Figure 1. Diagram of the TGF- β signal transduction pathway. TGF- β receptor II (T β RII) binds to TGF- β ligands, forms a dimer with TGF- β receptor I (T β RI), and transphosphorylates T β RI. R-Smads are phosphorylated by the activated T β RI. Phosphorylated R-Smads complex with Co-Smad and translocate to the nucleus, where they mediate transcription directly or indirectly. Anti-Smads may block T β RII/R-Smad interactions, phosphorylation of R-Smads, or R-Smad/Co-Smad dimerization.

receptors are degraded in a ubiquitin-mediated, proteasome-dependent fashion⁵³. Alterations of T β R expression may produce decreased anti-proliferative responses in TGF- β -targeted cells by disrupting appropriate interactions with R-Smads or cell-cycle proteins.

Smad proteins

Smads, the transcription factors that regulate cellular responses to TGF- β , are distributed throughout the cytoplasm and nucleus and may be classified by function as follows: receptor-regulated Smads (R-Smads 1, 2, 3, 5, and 8), common Smad (Co-Smad 4), and antagonist Smads (Anti-Smads 6 and 7)^{14, 18, 69}. All Smad proteins have two conserved mad homology (MH) domains, an N-terminal MH1 and a C-terminal MH2, that are joined by a proline-rich linker sequence^{16, 61}. Duration of Smad-mediated signaling may play a critical role in TGF- β response specificity⁵⁰.

R-Smads

The five R-Smads may be categorized according to the protein interactions and pathways involved. Smad2 and Smad3 mediate activin and TGF- β signal transduction, while Smad1, Smad5, and Smad8 mediate BMP signals^{9, 58}. R-Smads directly interact with activated T β RI in the receptor complex and, as a result, are phosphorylated at three serine residues in a C-terminal SSXS motif^{41, 47, 61}. Active, phosphorylated Smad2 or Smad3 can heterodimerize with Smad4, the common Smad protein, or heterotrimerize as a Smad2-Smad3-Smad4 complex⁵⁰. Both the heterodimers and heterotrimers translocate to the nucleus and regulate gene expression (Fig. 1). Studies with Smad4-null cell lines have shown that Smad2 and Smad3 are able to translocate to the nucleus on their own, but these R-Smads alone are unable to activate reporter genes¹⁹. This suggests that an R-Smad/Co-Smad complex is required for transcriptional regulation.

A missense mutation in an arginine residue present in the MH1 domain of Smad2 and Smad3 has been identified in pancreatic tissue samples from multiple patients. Xu et al.⁶⁹ observed that, although this alteration did not interfere with the majority of the R-Smad's cellular functions, the mutation directed the R-Smad protein for degradation via the UbC^{H5} family of E2 ubiquitin ligases. Similar results were also found in mutations in the MH2 domain; therefore, disruption of the steady-state kinetics of R-Smads due to amino-acid substitutions represents another pathway through which cells may become resistant to the suppressive effects of TGF- β .

Co-Smad

Smad4, also called DPC4 (deleted in pancreatic carcinomas), is recognized as a key tumor suppressor, as it is defective in more than 50% of pancreatic cancers^{17, 27}. Because it interacts with Smads 1, 2, 3, and 5, Smad4 participates in the intracellular signaling pathways of all three classes of TGF- β ⁹. This indicates that wild-type Smad4 in the TGF- β signal pathway of pancreatic cells may be critical in maintaining an environment that inhibits tumorigenesis. Upon heterodimerization or heterotrimerization with activin/TGF- β R-Smads, the complexes translocate into the nucleus where they can directly interact with DNA or the DNA binding proteins of TGF- β -regulated genes^{32, 49}. Zawel et al.⁷² showed that Smad3 and Smad4 might transcriptionally activate some portions of the genome by interacting with an 8-base-pair palindromic sequence (5'-GTCTAGAC-3') present in Smad binding elements (SBE). SBE-like sequences and three concatamerized CAGA repeats have been found in the plasminogen activator inhibitor (PAI-1) promoter^{8, 12}. Smad4 activates PAI-1 transcription by direct binding to the SBE-like sequence, while Smad3 and Smad4 are able to regulate transcription at the CAGA sites. The MH1 domain may be responsible for the DNA binding abilities of Smad3 and Smad4, but the MH2 domain mediates interaction with other transcription factors, such as AP-1, sp1, μ E3, and the vitamin D receptor^{33, 51, 70, 74}.

A nonsense mutation producing a C-terminal 38-amino-acid truncation in Smad4, rendering it unable to heterodimerize with R-Smads, has been identified in TGF- β unresponsive cells^{27, 50}. Maurice et al.⁵⁰ discovered that the proteasome degrades this mutant Smad4 molecule and that degradation of truncated Smad4 occurs in many cells that harbor defective Co-Smad. Transfection with wild-type Smad4 restores TGF- β responsiveness in Smad4 deficient cells, demonstrating the importance of Smad4^{50, 59}.

Anti-Smads

Smad6 and Smad7 act as negative regulators of the TGF- β signal pathway and serve as inhibitors of TGF- β -induced, anti-proliferative cellular responses. Anti-Smads may inhibit propagation of response by blocking R-Smads' interaction with T β RI, phosphorylation by T β RI, and heterodimerization with Smad4^{30, 34, 52}. Increased expression of Smad7, which is observed in some pancreatic cancers, down-regulates TGF- β anti-proliferative responses in COLO-357 pancreatic cancer cells, while having no effect on the cytokine's induction of PAI-1³⁷. TGF- β can cause over-expression of Smad7, and this may represent yet

another mechanism through which a cell may become resistant to cell-cycle inhibition by TGF- β through this negative feedback loop². Similar phenotypic patterns of resistance to TGF- β also might exist in cells that over-express Smad6³⁹.

ALTERED SMADS PATHWAY IN PANCREATIC CANCER

At least one factor of the TGF- β transduction pathway is mutated in nearly all pancreatic cancer cells. Modifications at any step in the TGF- β pathway can increase the chance of cancer development. Nicolas et al.⁵³ found that an attenuated TGF- β pathway in Panc-1 and PT45 cells leads to a decrease in T β RI, reduces the time for activated R-Smad/Co-Smad complexes remaining in the nucleus, and causes an inability of p21 expression maintenance, resulting in accelerated growth of the cells. The attenuation signal is known to occur through defects in either MH1 or MH2 domains of Smads 2, 3, and 4 that cause an increase in Smad degradation. In addition to Co-Smad and R-Smad defects, Anti-Smads are over-expressed in human pancreatic cancer. This leads to a loss of anti-proliferative responses without interfering with TGF- β -mediated induction of PAI-1, thus allowing for decreased anchorage dependence and other hallmarks of transformed cells³⁷.

Though instances of mutations in R-Smads, Anti-Smads, and T β Rs do occur in pancreatic cancer, the overwhelming majority of genetic alterations are found in the Smad4 protein^{13, 73}. The Smad family of proteins are encoded in two gene clusters located at 15q21-22 (Smad3 and Smad6) and 18q21 (Smad2, Smad4, and Smad7), and losses of these regions are frequently found in pancreatic cancer³⁶. In a genetic analysis of 12 pancreatic cancer patients, functional loss of R-Smads and increases in Anti-Smads occurred at negligible levels, while Smad4 defects were discovered in 5 of the 12 patients³⁶. The DPC4/Smad4 gene is rearranged in 42% of a series of pancreatic cancer cell lines³⁵. Clinically, 50% of pancreatic cancer patients have Smad4 alterations that would ablate or decrease the cell-cycle control of TGF- β , again suggesting a critical role of Smad4 protein in pancreatic cancer cells.

GENES REGULATED BY TGF- β /SMAD SIGNAL TRANSDUCTION PATHWAY

As sequence-specific transcription activators, some Smads interact with SBEs in promoters and regulatory regions of many genes, such as p15 and p21. As an inhibitor of cyclin-dependent kinases, p21 causes cell-cycle arrest at the G1 to S phase transition. In response to TGF- β 1 stimulation, Smad4 induces p21

expression, the key anti-proliferative effector response^{29, 43}. In response to TGF- β , cells may fortify their extracellular matrix (ECM), as activated Smad complexes target genes encoding aggrecan, biglycan (BGN), collagens, and other ECM proteins⁷. The proteoglycan BGN arrests the cell-cycle in G1 phase in a Smad-independent fashion and serves as a marker for TGF- β 1 pathway activation⁶⁶.

Intact TGF- β signaling also regulates expression of somatostatin receptor subtype-2 (SSTR-2), a G protein-coupled receptor with antitumorigenic properties⁵⁶. Most pancreatic cancers, however, fail to express functional somatostatin receptors (SSTRs), making them non-responsive to somatostatin and somatostatin analog treatment^{4, 44, 64}. Reintroduction of SSTR-2 gene in pancreatic cancer cells inhibits cell proliferation both *in vitro* and *in vivo*^{6, 21}. Restoration of wild-type Smad4 in SSTR-2-negative pancreatic cancer cells also induces the expression of SSTR-2 and inhibition of cell growth (Fig. 2). This indicates that functional interaction between the TGF- β and SSTR-2 pathways exists and highlights the importance of the pathways in regulating pancreatic cancer growth⁵⁶.

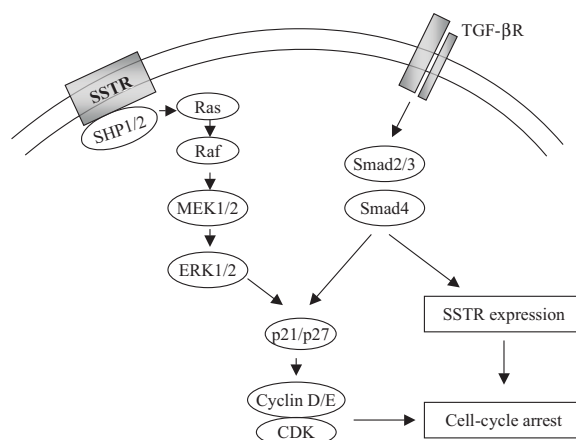


Figure 2. Interaction between TGF- β and SSTR signal transduction pathways. SSTR-2 inhibits cell proliferation by stimulating extracellular regulated kinases (ERK1/2) signaling via a SHP-1-SHP-2-PI3K/Ras-Rap1/B-Raf/MEK pathway. Activated ERK1/2 stimulates production of cyclin D1 and p21, a cyclin kinase inhibitor that is responsible, in part, for the arrest of cell growth. The SSTR-ERK1/2 pathway also interacts with the TGF- β pathway, both the SSTR-ERK1/2 and TGF- β pathways up-regulate the expression of p27, an inhibitor of cyclinE-cdk2 kinase activity, leading to cell-cycle arrest. The expression of SSTR can be up-regulated by wild-type Smad4 as well.

TGF- β -RELATED PATHWAYS AND INTERMEDIATES IN PANCREATIC CANCER

Several other signal transduction pathways play important roles in pancreatic cancer, signifying that pancreat-

ic cancer is a multi-gene-related malignancy. The cross-talk between different pathways might be essential in regulating the proliferation of pancreatic cancer cells.

SSTR signal pathway

SSTRs mediate pancreatic cancer cell growth inhibition^{4, 6, 21, 64}. Five known subtypes of SSTRs exist in humans, rats, and mice. All five receptor subtypes are coupled to the inhibition of adenylyl cyclase via a pertussis-toxin-sensitive GRP binding protein⁵⁴. Some of the subtypes are linked to tyrosine phosphatase (SSTR-1 and SSTR-2), calcium channels (SSTR-2), a Na⁺/H⁺ exchanger (SSTR-2), MAP kinase/*c-fos* (SSTR-3 and SSTR-4), and PLA-2 (SSTR-5). Recently, a new mechanism was proposed in which SSTR-2 inhibits cell proliferation by stimulating extracellular regulated kinases (ERK1/2) signaling via a SHP-1-SHP-2-PI3K/Ras-Rap1/B-Raf/MEK pathway⁵⁶. The ERK1/2 pathway, a major regulator of cellular proliferation, interacts with the TGF- β pathway. When constitutively activated, the ERK1/2 pathway is involved in pancreatic cancer development. Activated ERK1/2 stimulates the production of cyclin D1 (a cell proliferation enhancer), and p21 (a cyclin kinase inhibitor) that is responsible, in part, for the arrest of cell growth. A delicate balance between signal intensity and longevity is required to maintain cells in homeostasis. Normally, Ras activates Raf-1 by phosphorylation so that Raf-1 can phosphorylate MEK1. Activated MEK1 then phosphorylates ERK1 and ERK2. Both ERK proteins then phosphorylate factors that control proliferation and differentiation responses. The presence of oncogenic, constitutively active Ras correlates to a down-regulation of T β RRII expression²². Smad2 and Smad3 can be phosphorylated by MAP kinase intermediates^{11, 40, 71}. TGF- β activates Ras and recruits its assistance in growth arrest and other negative regulatory functions^{23, 28}. Activation of a serine/threonine phosphatase by TGF- β 1 inhibits ERK2 activation²⁴. Also, both the TGF- β and SSTR-ERK1/2 pathways up-regulate the expression of p27 (an inhibitor of cyclinE-cdk2 kinase activity) leading to cell-cycle arrest^{21, 56} (Fig. 2). These findings are indicative of the cross-talk that occurs in these two signal transduction pathways in pancreatic cancer.

SEL1L pathway

The human ortholog of *C. elegans*' suppressor-enhancer-lin gene (*sel1*), known as SEL1L, encodes a negative regulator of Notch, a protein involved in cell differentiation^{25, 26}. Notch may be regulated in a competitive fashion by the fibronectin type II domain of SEL1L, as it is homologous to a fibronectin domain found in Notch. In healthy pancreatic tissue, SEL1L is expressed at high levels in cell membranes

of acinar and endocrine cells, but 36% of pancreatic cancer cells are void of both SEL1L and DPC4 expression⁵. The same group discovered that when SEL1L is over-expressed, Smad4 and activin A levels are up-regulated. Although it is well known that pancreatic cancer tissues often have lost their SEL1L expression, genetic analysis reveals no gross mutations in the gene, located at 14q31, suggesting that perhaps some post-transcriptional alteration of the SEL1L product is responsible for the loss of SEL1L.

Apc pathway

Adenomatous polyposis coli (*Apc*) tumor suppressor gene, a member of the Wnt signaling pathway, serves to mediate apoptosis and to decrease the amount of the transcription factor β -catenin, thereby controlling cellular division. The Apc protein controls the β -catenin level by forming a complex with β -catenin and marking the transcription factor for degradation. Defects in expression allow an increase in β -catenin that can mediate inappropriate activation of genes such as *c-myc* and *cyclin D1*, and this improper activation leads to a cascade of signaling events that stimulates cell growth and division^{10, 31, 63}. Mice heterozygous for a mutation in the *Smad4* gene locus are predisposed to pancreatic cancer only in the presence of a concurrent *Apc* mutation, suggesting the cooperation between the TGF- β and Wnt signaling pathways¹⁰.

CONCLUSIONS

The precise mechanisms through which the TGF- β signal transduction pathway shifts from eliciting an anti-proliferative response to a state favorable for tumorigenesis are uncertain. A growing body of evidence supports the idea that defects in TGF- β intermediates and other related molecules, including SSTR and ERK1/2, augment the transcription of genes that promote cell proliferation and pancreatic cancer development. Altered or inactive tumor suppressor proteins, most notably Smad4, may release cells from TGF- β -mediated negative regulation. Currently available treatment regimens offer little hope of survival for pancreatic cancer patients. Therefore, the development of novel methods of diagnosis, treatment, and prevention of pancreatic cancer depends on continuing efforts to understand the complex, interwoven pathways that lead to pancreatic tumorigenesis.

ACKNOWLEDGMENT

We thank Dr. Xin-Hua Feng (Baylor College of Medicine) for helpful discussions and valuable suggestions, and thank Chris Boyd and Xiaochi (Lily) Wang for excellent assistance in preparing the figures.

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