Soluble Type II Transforming Growth Factor-β Receptor Attenuates Expression of Metastasis-associated Genes and Suppresses Pancreatic Cancer Cell Metastasis[1]

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a deadly malignancy that frequently metastasizes and that overexpresses transforming growth factor-βs (TGF-βs). To determine whether TGF-βs can act to enhance the metastatic potential of PDAC, PANC-1 human pancreatic cancer cells were transfected with an expression construct encoding a soluble type II TGF-β receptor (sTβRII) that blocks cellular responsiveness to TGF-β1. When injected s.c. in athymic mice, PANC-1 clones expressing sTβRII exhibited decreased tumor growth in comparison with sham-transfected cells and attenuated expression of plasminogen activator inhibitor 1 (*PAI-1*), a gene associated with tumor growth. When tested in an orthotopic mouse model, these clones formed small intrapancreatic tumors that exhibited a suppressed metastatic capacity and decreased expression of plasminogen activator inhibitor 1 and the metastasis-associated urokinase plasminogen activator. These results indicate that TGF-βs act *in vivo* to enhance the expression of genes that promote the growth and metastasis of pancreatic cancer cells and suggest that sTβRII may ultimately have a therapeutic benefit in PDAC.

Introduction

TGF-βs[3] are structurally related polypeptide growth factors that regulate many cellular processes, including cell proliferation and differentiation, migration, deposition of the extracellular matrix, immunosuppression, motility, and cell death (1, 2). TGF-βs enhance the synthesis of matrix proteins, such as proteoglycans, fibronectin, laminin, collagens, tenascin, and vitronectin; increase the synthesis of protease inhibitors while decreasing the synthesis of matrix-degrading proteases; and enhance the expression of cell adhesion molecules, such as integrins (3, 4). TGF-βs are synthesized as precursors that undergo proteolytic cleavage, leading to the generation of biologically active 25-kDa dimers (1, 2). The mature forms of TGF-β1 and -β2 share 70% amino acid sequence homology, and the mature form of TGF-β3 shares 80% homology with the other two TGF-βs (1, 2).

TGF-βs enhance the proliferation of cells of mesenchymal origin and inhibit the proliferation of many types of epithelial cells (1, 2). TGF-βs act by binding to TβRII, which is constitutively active as a serine/threonine kinase (5, 6). After ligand binding, TβRII heterodimerizes with and activates TβRI. Activation of TβRI leads to the phosphorylation of Smad2 and Smad3 and induces their heterodimerization with Smad4 (7, 8). These Smad complexes then translocate to the nucleus where they regulate gene transcription (9).

PDAC is a deadly disease in which nonsurgical therapy is ineffective and in which the majority of patients harbor metastatic lesions at presentation, precluding the possibility for curative surgical intervention (10). These cancers frequently overexpress all three mammalian TGF-β isoforms (11). This aberrant overexpression occurs in the cancer cells within the tumor mass despite the fact that TGF-βs are most often expressed at high levels by mesenchyme-derived rather than epithelium-derived cells and is associated with decreased patient survival (11). Several alterations that interfere with the ability of TGF-βs to inhibit pancreatic cancer cell growth have been reported, including a high frequency of Smad4 mutations (12), overexpression of inhibitory Smad6 (13) and Smad7 (14), and underexpression of TβRI (15). Consequently, pancreatic cancer cell-derived TGF-βs cannot act to suppress the growth of the cancer cells. Instead, they may promote pancreatic tumor growth *in vivo* by acting on the pericancerous cellular elements, such as endothelial cells and fibroblasts (4), because these cells do not harbor Smad4 mutations. Furthermore, in pancreatic cells that express high levels of Smad7, TGF-βs may act directly on the cancer cells to enhance the expression of growth-promoting genes (14).

We recently reported that COLO-357 pancreatic cancer cells expressing sTβRII exhibit attenuated growth in a s.c., nonmetastatic nude mouse model (16). It is not known, however, whether this attenuated growth could suppress the metastatic potential of pancreatic cancer cells because the s.c. mouse model is nonmetastatic. Therefore, in the present study, the growth of PANC-1 human pancreatic cancer cells was tested in a metastatic mouse model. PANC-1 cells were used because they express all three TGF-β isoforms (17) and exhibit increased *in vitro* invasiveness in response to TGF-β

Received 10/12/2001; revised 11/29/01; accepted 12/3/01.
[1] This work was supported in part by United States Public Health Service Grant CA-75059, awarded by the National Cancer Institute to M. K. M. A. R-G. was the recipient of a postdoctoral fellowship award from the George E. Hewitt Foundation for Medical Research. UCI Undergraduate Research Opportunities Grants supported M. R. and S. R.
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[3] Abbreviations used: TGF-β, transforming growth factor-β; sTβRII, soluble type II TGF-β receptor; TβRI, type I TGF-β receptor; PDAC, pancreatic ductal adenocarcinoma; HA, hemagglutinin; uPA, urokinase plasminogen activator; PAI-1, plasminogen activator inhibitor 1.

(18). When these cells were stably transfected with a cDNA construct encoding a human sTβRII (pMHsTβRII), there was a marked decrease in their metastatic potential *in vivo* compared with sham-transfected cells.

Materials and Methods

Animals. Four- to six-week-old female *nu/nu* (nude) mice (Harlan; Indianapolis, IN) were used for tumor implantation for both the s.c. and orthotopic models. Mice were housed in our animal facility within a sterile environment.

Construction of a Mammalian Expression Vector. The cDNA of human TβRII served as the template for PCR amplification of the sequence encoding the extracellular domain of TβRII (nucleotides 1–477, including the signal sequence), as reported previously (16). The cDNA was ligated into the *Hin*dIII/*Eco*721-digested pMH expression vector (Boehringer-Mannheim, Indianapolis, IN), which is driven by a highly efficient immediate early human cytomegalovirus promoter sequence and is tagged with the HA epitope at its COOH terminus. Authenticity was confirmed by sequencing. The pMH plasmid containing the *G418* resistance gene (neomycin) was used for generation of control clones (sham) expressing the vehicle vector alone.

Cell Culture. PANC-1 cells (American Type Culture Collection, Rockville, MD) were grown in DMEM supplemented with 8% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 5% fungazone (complete medium) and maintained in monolayer culture at 37°C in humidified air with 5% CO_2. To select for cells containing the neomycin resistance gene, the medium was supplemented with 1.25 mg/ml G418. For TGF-β1 (a gift from Genentech, Inc., South San Francisco, CA) experiments, cells were incubated in serum-free medium (DMEM containing 0.1% BSA, 5 μg/ml transferrin, 5 ng/ml sodium selenite, antibiotics, and fungazone). PANC-1 cells were transfected in a stable manner with the pMHsTβRII plasmid (10 μg), using the Lipofectamine method (Life Technologies, Inc., Gaithersburg, MD) as reported previously (19). After expansion of each individual clone, cells were screened for expression of pMHsTβRII by Northern blotting.

RNA Extraction and Northern Blot Analysis. Total RNA was extracted by the single-step acid guanidine thiocyanate-phenol-chloroform method. Northern blot analysis was performed as reported previously (20). The cDNA probes included a 500-bp *Hin*dIII/*Eco*RI fragment of the human pMHsTβRII cDNA, a 1.5-kb *Pst*1 fragment of the human *uPA* gene (American Type Culture Collection, Manassas, VA), a 500-bp *Sac*II/*Pst*1 fragment of the human *PAI-1* gene, and a 190-bp *Bam*HI fragment of mouse 7S cytoplasmic cDNA, which cross-hybridizes with human 7S RNA. The 7S probe was used to confirm equal RNA loading (20). Blots were exposed to Kodak Biomax MS films at −80°C.

Immunohistochemistry. To assess TβRII and HA immunoreactivity, tumors from s.c. lesions were removed and immediately divided. Tissues were fixed in 4% formaldehyde and embedded in paraffin wax. Paraffin-embedded sections (4 μm) from tumor tissue derived from sham-transfected or pMHsTβRII-transfected cells were cut and mounted on poly-L-lysine-coated glass slides and air-dried overnight at room temperature. Representative sections from each case were examined by the streptavidin-peroxidase technique, using appropriate positive and negative controls. Endogenous peroxidase activity was blocked by incubation for 30 min with 0.3% hydrogen peroxide in methanol. Tissue sections were incubated for 15 min (room temperature) with 10% normal goat serum and then incubated for 16 h at 4°C with either anti-HA antibody (0.4 μg/ml) or anti-TβRII antibody (0.2 μg/ml), which recognizes the epitope corresponding to the full-length TβRII, in PBS containing 1% BSA. In addition, tumor samples from the orthotopic model were embedded in OCT compound, frozen in liquid nitrogen, and stored at −80°C. Cryostat sections were then prepared and stained with an anti-HA antibody (0.25 μg/ml).

For immunohistochemistry, bound HA and TβRII antibodies were detected with biotinylated goat antirabbit IgG secondary antibodies and streptavidin-peroxidase complexes, using diaminobenzidine tetrahydrochloride as the substrate. Sections were counterstained with Mayer's hematoxylin. Sections incubated with nonimmune rabbit IgG or with secondary antibodies alone did not yield positive immunoreactivity.

Cell Growth Assays. PANC-1 sham-transfected or pMHsTβRII-transfected cells were seeded in complete DMEM at a density of 1.0×10^5 cells/well in 12-well plates. Cells were incubated for 24 h prior to incubation for 48 h in serum-free medium in the absence or presence of TGF-β1. Cell growth was then determined by cell counting using a hemocytometer, and data were expressed as a percentage of control cell growth.

Growth Characteristics in s.c. and Orthotopic Models. Initially, cells expressing the empty vector alone (sham) or pMHsTβRII were injected s.c. into female athymic (nude) mice, as reported previously (16). Tumors were measured externally on the indicated days, and tumor volume was determined by the equation: volume $= (l \times h \times w) \times \pi/4$, where l is length, h is height, and w is width of the tumor. The mice were sacrificed 49 days after injection, when tumor burden in control mice approached the allowable limit.

To generate intrapancreatic tumors, the s.c. tumors were aseptically resected and immediately placed into complete DMEM. Three separate tumors from each group (sham, clone 18, or clone 19) were pooled and minced together into pieces of ~2 mm^3. For each group (sham, clone 18, or clone 19), nude mice were implanted with three tumor fragments that were introduced into the pancreas via a surgical flap. The mice were anesthetized with a cocktail of xyla-ject and keta-ject (Phoenix Pharm., St. Joseph, MD), a median incision was made, and the portion of the pancreas near the spleen was exposed (21). Tumor pieces were implanted under a pancreatic flap that was sutured with a 6-0 absorbable suture (ETHICON, Somerville, NJ). The abdominal wall and skin were then closed with 3-0 silk sutures (ETHICON).

After implantation, mice were inspected weekly for tumor formation by palpation. All mice were sacrificed 2 months after implantation. At autopsy, the pancreas and other organs harboring metastatic lesions were resected. All studies with mice were approved by the University of California Irvine

Institutional Animal Care and Use Committee (protocol 98-1298).

Statistics. Statistical analysis was performed with SigmaStat software (Jandel Scientific, San Raphael, CA) and Prism software (Graphpad Software, Inc., San Diego, CA). Student's two-sided *t* test was used when indicated. $P < 0.05$ was taken as the level of significance. Image Quant software (Molecular Dynamics, Sunnyvale, CA) was used to quantitate the intensity of bands from Northern blots.

Results

Effects of pMHsTβRII on TGF-β1 Actions and s.c. Tumor Growth. Sham-transfected PANC-1 cells and clones C18 and C19 stably transfected with pMHsTβRII, which encodes the extracellular domain (amino acids 1–159) of human TβRII, exhibited the endogenous (~5.2 kb) TβRII mRNA transcript (Fig. 1*A*). Clones C18 and C19 expressed, in addition, the (~0.8 kb) sTβRII mRNA transcript (Fig. 1*A*). In contrast, sham-transfected PANC-1 cells that were transfected with the pMH empty vector for use as controls did not express sTβRII mRNA (Fig. 1*A*). The sham-transfected cells exhibited doubling times of ~23 h and were growth-inhibited by 10 and 30 pM TGF-β (Fig. 1*B*). Although clones C18 and C19 exhibited similar doubling times that ranged from 24 to 29 h, they were not growth-inhibited by either concentration of TGF-β1 (Fig. 1*B*).

The tumorigenicity of pMHsTβRII-expressing PANC-1 cells and sham-transfected cells was compared after s.c. injection in athymic nude mice. Clones transfected with pMHsTβRII consistently formed smaller tumors compared with tumors arising from sham-transfected cells. There was significant inhibition (72%) of tumor growth in the pMHsTβRII-transfected clones beginning at 7 days postinjection (Fig. 1*C*), which was most pronounced at 49 days postinjection (83%). At this time point, endogenous TβRII immunoreactivity was present in the tumors derived from sham-transfected PANC-1 cells (Fig. 2*A*). As expected, these tumors did not exhibit any HA immunoreactivity (Fig. 2*C*). In contrast, strong TβRII (Fig. 2*B*) and moderate HA (Fig. 2*D*) immunoreactivity were evident in the PANC-1 cells expressing the pMHsTβRII construct. In general, TβRII immunoreactivity exhibited a heterogeneous pattern of distribution within the tumors (Fig. 2*B*), indicating that there was variable but persistent expression of sTβRII *in vivo*.

Growth Properties of sTβRII-expressing Clones in an Orthotopic Model. s.c. tumors arising from pancreatic cancer cells do not metastasize. Therefore, tissue minces from these tumors were next implanted into the pancreas of nude mice because this orthotopic model is known to yield metastases (21). Tissue fragments from the s.c. tumors from three mice previously injected with sham-transfected cells were implanted directly into the pancreas of three nude mice as described in "Materials and Methods." The resulting pancreatic tumors were large (0.8–1.1 cm) and formed multiple metastatic lesions, including lesions in the liver, spleen, local lymph nodes, and distal lymph nodes. In contrast, when three mice were implanted with sTβRII-derived tumor tissue minces, only one mouse grew a large primary tumor (0.8 cm), and it formed only a few metastatic lesions (liver, peripancreatic lymph nodes). One mouse formed a small (0.3 cm) primary tumor, and the other mouse did not form a tumor; neither mouse had any metastatic lesions.

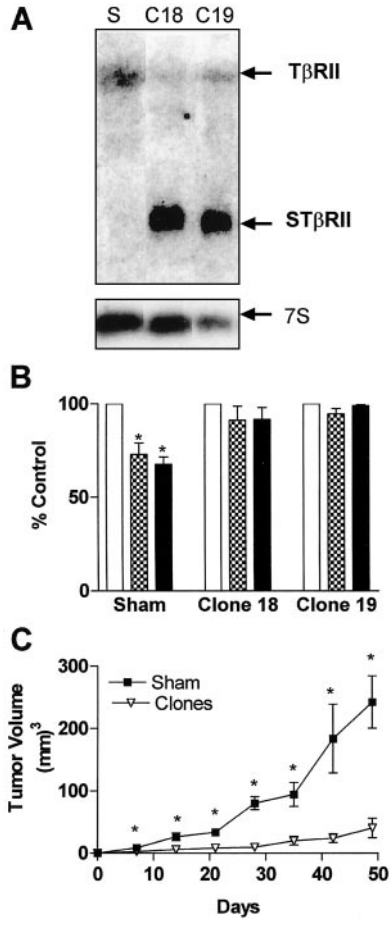

Fig. 1. Transfection of pMHsTβRII in PANC-1 cells. *A*, expression of sTβRII mRNA. Northern blot analysis of total RNA (20 μg/lane) from sham-transfected PANC-1 cells (*S*) or from clones that were stably transfected with pMHsTβRII (*C18* and *C19*) was carried out with a human soluble TβRII cDNA probe (5×10^5 cpm/ml; 1-day exposure). 7S cDNA was used as a loading control (5×10^4 cpm/ml; 2-h exposure). *B*, effects of TGF-β1 on cell growth. Sham-transfected cells (*Sham*) and *clones 18* and *19* were seeded in 12-well plates (100,000 cells/well) and incubated for 24 h in complete DMEM. Cells were then placed in serum-free medium in the absence (□) or presence of 10 pM (▩) or 30 pM (■) TGF-β1 for 48 h. Results are expressed as percentage of control and are the means ± SE (*bars*) of three determinations per experiment from three experiments; *bars* (SE) for the control groups were exceedingly small. *, $P < 0.01$ when compared with respective untreated controls. *C*, tumor growth in s.c. model. Athymic nude mice received s.c. injections (2×10^6 cells/site; two sites per mouse) of sham-transfected cells (five mice) and clones C18 (four mice) and C19 (four mice). Data are the means ± SE (*bars*). *, $P < 0.001$ when compared with the sham control.

A larger experiment was then carried out in which four mice were implanted with sham-derived tissue minces and eight mice were implanted with pMHsTβRII-expressing clones (Table 1). All four mice implanted with sham-derived tissue minces mice grew large pancreatic tumors (0.8–1.2 cm), and three of the mice exhibited tumor spread to multiple sites, including liver, spleen, adrenals, perirectum, and kidneys (Table 1). The lymph nodes adjacent to the aorta, omen-

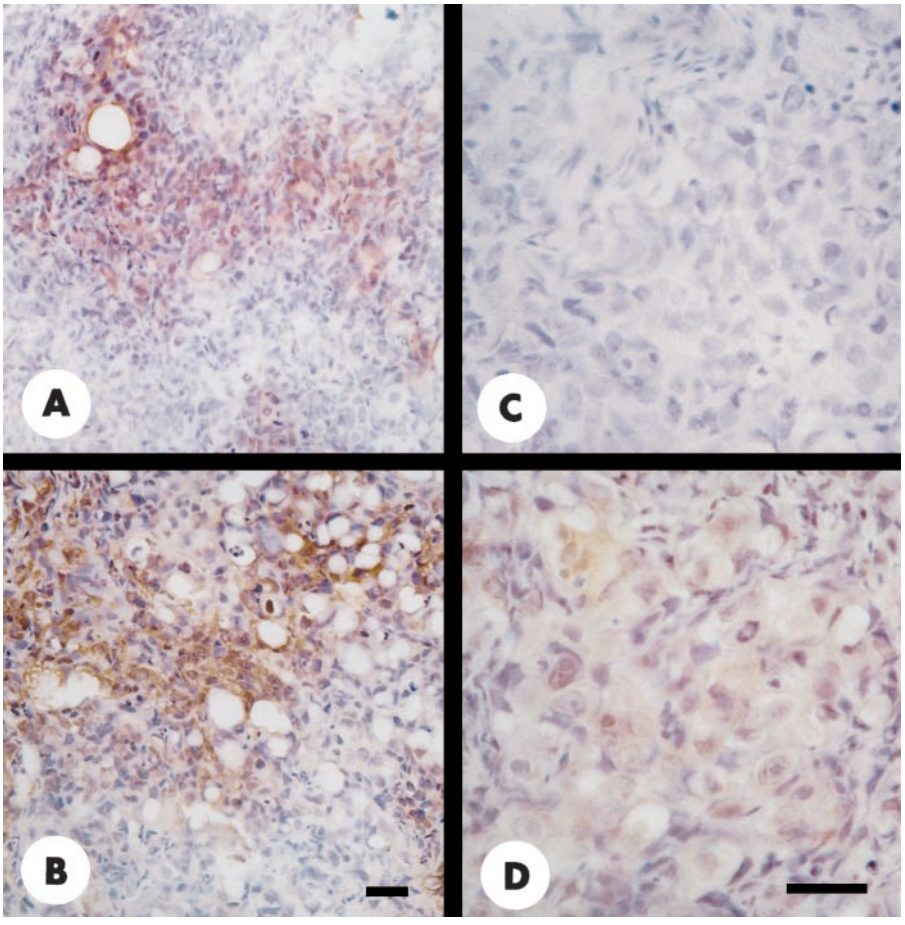

Fig. 2. TβRII immunoreactivity in tumors derived from PANC-1 cells. An anti-TβRII antibody recognizing the full-length TβRII (*A* and *B*) and an anti-HA antibody recognizing the HA epitope of the pMHsTβRII construct (*C* and *D*) were used. In the tumor tissue derived from the sham-transfected cells, there was weak to moderate immunostaining for endogenous TβRII (*A*) but undetectable HA immunoreactivity (*C*). Strong TβRII (*B*) and moderate HA (*D*) immunoreactivity was present in the tumor tissue derived from cancer cells expressing the pMHsTβRII construct. *Bar*, 25 μm.

Table 1 Primary and metastatic lesions

	Sham-transfected				sTβRII clones							
	A	B	C	D	18A	18B	18C	18D	19A	19B	19C	19D
Primary tumor	+++[a]	+++	+++	+++	+	++	+++	+	−	+	−	−
Liver		M										
Spleen		M										
Adrenal gland				M								
Perirectum		M										
Kidneys				M/M								
Peritoneal seeding	No	No	Yes	No	No	No	Yes	No	No	No	No	No
Lymph nodes												
Periaortic			M	M								
Omental			M									
Gastric				M								
Mesenteric		M		M			M					

[a] +++, large tumor; ++, medium tumor; +, small tumor; M, metastatic site; M/M, bilateral metastasis.

tum, mesentery, and stomach also contained metastatic foci (Table 1). An example of a pancreatic tumor exhibiting metastases to the mesenteric lymph nodes and spleen is shown in Fig. 3. In contrast, only one of the eight mice implanted with pMHsTβRII-expressing clones developed a large primary tumor (1.2 cm), and this mouse developed peritoneal seeding and mesenteric lymph involvement (Table 1). In addition, one mouse implanted with pMHsTβRII-expressing cells developed a medium-sized primary tumor (~0.8 cm in diameter), three mice developed very small (~.3 cm in diameter) primary tumors, and three mice did not form any tumors (Table 1). None of these 7 mice developed any metastases. Thus, altogether only 2 of 11 (18%) mice implanted with pMHsTβRII-expressing clones exhibited metastatic lesions.

As expected, tumors forming after orthotopic implantation of sham-transfected cells did not exhibit HA immunoreactiv-

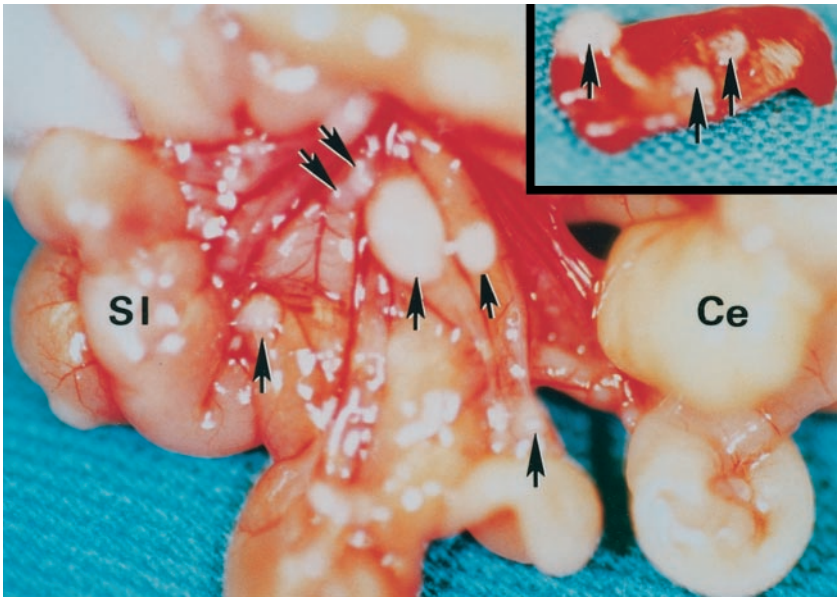

Fig. 3. Tumor metastases in the orthotopic model. The pancreas of a nude mouse was implanted with pancreatic tumor fragments derived from sham-transfected cells, as described in "Materials and Methods." The *arrows* indicate metastatic lesions in the mesenteric lymph nodes. *SI*, small intestine; *Ce*, cecum. *Inset*, metastatic lesions in the spleen (indicated by *solid arrows*).

ity (Fig. 4*A*). Furthermore, the tumor from the mouse implanted with pMHsTβRII-expressing clones that developed metastases exhibited weak HA immunoreactivity (Fig. 4*B*). In contrast, in the other mice implanted with pMHsTβRII-expressing clones (which were not metastatic), all of the tumors exhibited strong HA immunoreactivity (Fig. 4*C*).

PAI-1 and *uPA* are growth- and metastasis-associated genes that are overexpressed in PDAC (22–24). Therefore, their expression in both the s.c. and orthotopic models was analyzed next (Fig. 5). Both mRNA moieties were expressed at high levels in tumors from sham-transfected cells, irrespective of the model (Fig. 5). In contrast, their expression was below the level of detection in the normal pancreas (Fig. 5*B*). uPA mRNA levels ($\sim$2.4 kb) were especially elevated in the orthotopic model because a brief exposure time (6 h) and less RNA (20 μg/lane) was required in this model compared with the s.c. model (Fig. 5). In both models, there was a 55–60% decrease in the PAI-1 mRNA transcript levels ($\sim$2.2 and 3 kb) in the tumors derived from pMHsTβRII-expressing clones when compared with the corresponding sham tumors (Fig. 5). In contrast, only the intrapancreatic model exhibited decreased uPA mRNA levels (53%) with the pMHsTβRII-expressing tumors (Fig. 5).

Discussion

TGF-βs generally inhibit the proliferation of epithelial cells (1, 2). Nonetheless, several observations have implicated increased TGF-β expression with epithelial cell tumorigenesis (4). Thus, TGF-β1 mRNA levels are increased in many human cancers, including carcinomas of the pancreas, kidney, liver, and breast (4). Rat prostate, human E1A-transformed 293 tumor cells, and mouse Meth A sarcoma cells transfected to overexpress TGF-β1 exhibit increased tumorigenicity when compared with control cells (4, 25). *In vivo*-produced TGF-β1 induces estrogen-independent tumorigenicity of human breast cancer cells in athymic mice (26). These earlier studies are supported by more recent observations documenting that suppression of TGF-β actions with a soluble or dominant-negative receptor approach leads to attenuated breast cancer metastasis (27, 28), decreased mouse thymoma tumorigenicity (29), and attenuated malignant transformation of keratinocytes (30).

In the present study, expression of sTβRII in transfected clones was confirmed by Northern blotting and by demonstration that transfected cells were not growth-inhibited by TGF-β1 *in vitro*. These observations indicate that expression of sTβRII interfered with the ability of exogenous TGF-β1 to activate the endogenous TβRII. Compared with sham-transfected cells, PANC-1 clones expressing pMHsTβRII, as confirmed by immunostaining, yielded small tumors in the s.c. model. PANC-1 clones expressing pMHsTβRII also formed smaller primary tumors and exhibited decreased metastatic potential in the orthotopic model when compared with the sham-transfected cells. The only pMHsTβRII-expressing clone that yielded metastases exhibited relatively weak HA immunoreactivity, indicating that strong and persistent expression of pMHsTβRII, rather than weak and heterogeneous expression, may be required to attenuate the metastatic potential in the orthotopic model. These observations indicate that sTβRII has the potential to be a potent suppressor of PANC-1-derived tumor growth and metastasis.

There was a marked up-regulation of uPA and PAI-1 in the s.c. and intrapancreatic tumors that formed with sham-transfected PANC-1 cells, compared with the levels observed in the normal pancreas. Although the exact site of expression within the tumor mass was not determined, in the case of uPA this up-regulation was especially marked in the orthotopic model. Furthermore, expression of pMHsTβRII was associated with decreased PAI-1 expression in both models, as well as with decreased levels of uPA mRNA in the orthotopic model. Inasmuch as targeted deletion of either PAI-1 or uPA results in attenuated tumor formation and growth in

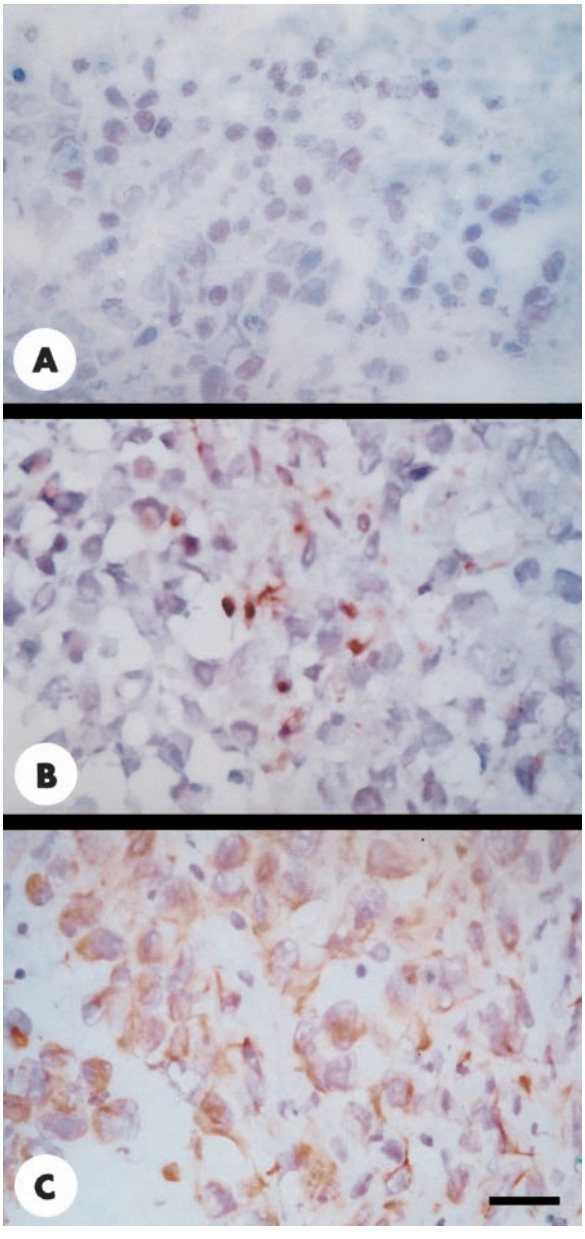

Fig. 4. HA immunoreactivity in intrapancreatic tumors. An anti-HA antibody was used to detect sTβRII expression in the intrapancreatic tumors. *A*, tumor from sham-transfected cells was devoid of HA immunoreactivity. *B*, tumor from clone 18C exhibited weak HA immunoreactivity. *C*, Tumor from clone 19B exhibited strong HA immunoreactivity. *Bar*, 25 μm.

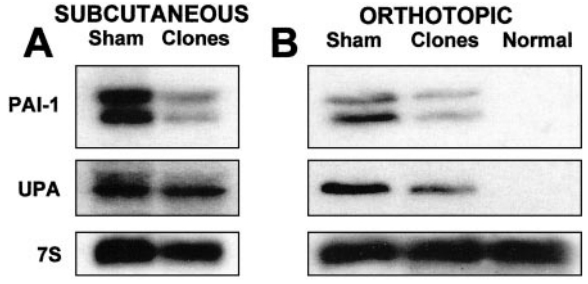

Fig. 5. Relative expression of PAI-1 and uPA. *A*, s.c. model. Total RNA was prepared and pooled from s.c. tumors (33 μg/lane) from three mice receiving injections of sham-transfected cells (*Sham*) and three mice receiving injections of pMHsTβRII-transfected cells (*Clones*). Northern blot analysis was carried out using the PAI-1 cDNA probe (5×10^5 cpm/ml; 1-day exposure). The membrane was reprobed with the uPA cDNA probe (5×10^5 cpm/ml; 3-day exposure). A 7S cDNA probe (*7S*) was used as a loading control (5×10^4 cpm/ml; 1-day exposure). *B*, intrapancreatic orthotopic model. Total RNA (20 μg/lane) was prepared and pooled from intrapancreatic tumors derived from four mice implanted with sham-transfected cells (*Sham*) and four mice implanted with pMHsTβRII-transfected cells (*Clones*). Total RNA (20 μg/lane) was also prepared from normal pancreatic tissues from two mice implanted with pMHsTβRII-transfected cells that failed to yield tumors (*Normal*). Northern blot analysis was carried out as in *panel A*, but with a reduced exposure time after hybridization with the uPA cDNA probe (6 h).

mice (31), our findings suggest that sTβRII may suppress tumor growth and invasion by attenuating the expression of PAI-1 and uPA and that this phenomenon may be especially important in the orthotopic model.

TGF-βs are initially released as latent molecules that form complexes with latent binding protein, and their biological effectiveness is dependent on their activation by such proteins as plasmin, uPA and its receptor, the insulin-like growth factor II receptor, and tissue transglutaminase (32, 33). Furthermore, PAI-1, uPA and its receptor, TGF-β, and tissue transglutaminase have all been implicated in promoting angiogenesis (34, 35). The relevance of these observations to the results in the present study is underscored by several facts. Thus, TGF-βs, uPA and its receptor, PAI-1, and the insulin-like growth factor-II receptor are overexpressed in PDAC, and pancreatic cancer cell lines express tissue transglutaminase (11, 22, 36, 37). In addition, PAI-1 is up-regulated by TGF-β in pancreatic cancer cells (38), and increased TGF-β expression correlates with increased PAI-1 levels in PDAC (24). uPA indirectly activates TGF-β by activating plasmin (39), and reduced expression of uPA and PAI-1 correlates with attenuated tumorigenicity in Smad4 reconstituted cancer cells (40). Taken together, these observations suggest that sTβRII may act by interfering with important regulatory loops between TGF-βs, uPA, and PAI-1 in an orthotopic model that recapitulates the alterations observed in PDAC.

In summary, the present results suggest that there are several potential beneficial consequences to blocking TGF-β actions *in vivo* by the sTβRII approach. These advantages include suppression of intrapancreatic tumor growth and local as well as distant metastases, suppression of angiogenesis (16), inhibition of PAI-1 and uPA overexpression, and potentially, suppression of uPA-mediated TGF-β activation. Together these findings suggest that sTβRII targets many of the deleterious aspects that occur as a consequence of TGF-β overexpression in PDAC. Therefore, sTβRII may ultimately have a distinct therapeutic benefit in the treatment of this malignancy.

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