Distinct Roles of Smad2-, Smad3-, and ERK-dependent Pathways in Transforming Growth Factor-β1 Regulation of Pancreatic Stellate Cellular Functions*

Hirohide Ohnishi‡‡, Tomohiko Miyata‡, Hiroshi Yasuda‡, Yukihiro Satoh‡, Kazunobu Hanatsuka‡, Hiroki Kitaz, Akira Ohashi‡, Kiichi Tamada‡, Noriko Makita‡, Taroh Iiri‡, Namiki Ueda‡‡, Hirosato Mashima‡‡, and Kentaro Sugano‡

From the ‡Department of Gastroenterology, Jichi Medical School, Tochigi 329-0498, Japan, the ‡Division of Gastroenterology, Showa University Fujigaoka Hospital, Kanagawa 227-8501, Japan, the Department of Endocrinology and Nephrology, University of Tokyo School of Medicine, Tokyo 113-8655, Japan, and the **Department of Gastroenterology, University of Tokyo School of Medicine, Tokyo 113-8655, Japan

Pancreatic stellate cells (PSCs) play a major role in promoting pancreatic fibrosis. Transforming growth factor-β1 (TGF-β1) regulates PSC activation and proliferation in an autocrine manner. The intracellular signaling pathways of the regulation were examined in this study. Immunoprecipitation and immunocytochemistry revealed that Smad2, Smad3, and Smad4 were functionally expressed in PSCs. Adenovirus-mediated expression of Smad2, Smad3, or dominant-negative Smad2/3 did not alter TGF-β1 mRNA expression level or the amount of autocrine TGF-β1 peptide. However, expression of dominant-negative Smad2/3 inhibited PSC activation and enhanced their proliferation. Co-expression of Smad2 with dominant-negative Smad2/3 restored PSC activation inhibited by dominant-negative Smad2/3 expression without changing their proliferation. By contrast, co-expression of Smad3 with dominant-negative Smad2/3 attenuated PSC proliferation enhanced by dominant-negative Smad2/3 expression without altering their activation. Exogenous TGF-β1 increased TGF-β1 mRNA expression in PSCs. However, PD98059, a specific inhibitor of mitogen-activated protein kinase kinase (MEK1), inhibited ERK activation by TGF-β1, and consequently attenuated TGF-β1 enhancement of its own mRNA expression in PSCs. We propose that TGF-β1 differentially regulates PSC activation, proliferation, and TGF-β1 mRNA expression through Smad2-, Smad3-, and ERK-dependent pathways, respectively.

Pancreatic stellate cells (PSCs)† were recently identified, isolated, and characterized (1, 2). In the normal pancreas, PSCs possess fat droplets containing vitamin A and are quiescent. In the quiescent state they are characterized by desmin-positive but α-smooth muscle actin (α-SMA)-negative staining (1). When cultured in vitro, PSCs are autostimulated (autotransformed), changing their morphological and functional features (2). PSCs commence losing vitamin A containing lipid droplets, highly proliferating, increasing expression of α-SMA, and producing and secreting extracellular matrix components such as collagen and fibronectin. Namely, PSCs are autotransformed to myofibroblast-like cells. In vivo, PSCs are also activated during both human and experimental pancreatic fibrosis (3). Therefore, PSCs are believed to play an important role in pancreatic fibrogenesis.

TGF-β1 is one of the major profibrogenic cytokines in various tissues. Recently, evidence for TGF-β participation in pancreatic fibrogenesis has been mounting. In this regard, it has been noted that transgenic mice overexpressing TGF-β1, in islet cells develop fibrosis of exocrine pancreas (4). Moreover, inhibition of TGF-β1 by anti-TGF-β1 antibody reduced extracellular matrix production in rat cerulein pancreatitis (5). TGF-β1 has also been shown to promote PSC activation and collagen production and to inhibit proliferation of PSCs in an autocrine manner (6, 7). In human chronic pancreatitis tissue, TGF-β1 expression was observed in acinar cells adjacent to areas of fibrosis and in spindle cells in fibrotic bands (3). Thus, TGF-β1 is thought to promote pancreatic fibrosis, in part by modulating PSC functions. However, the intracellular signaling pathway(s) through which TGF-β1 regulates PSC functions is still uncertain.

Sma- and Mad-related proteins (Smads) are a group of recently identified molecules that function as intracellular signaling mediators and modulators of TGF-β family members (8, 9). Smads can be classified into three groups: receptor-regulated Smads (R-Smads), common mediator Smads (Co-Smads), and inhibitory Smads (I-Smads). In TGF-β signaling pathway, Smad2 and Smad3 function as R-Smads, Smad4 functions as a Co-Smad, and Smad7 functions as an I-Smad. Upon TGF-β binding to TGF-β type II receptor, the type II receptor kinase phosphorylates the GS domain of TGF-β type I receptor, leading to activation of the type I receptor. The activated type I receptor kinase phosphorylates Smad2 and Smad3 (R-Smads) at two serine residues in the SSX motif at their extreme C termini (10, 11). Phosphorylated Smad2 and Smad3 form oligomeric complexes with Smad4 (Co-Smad); the complexes then translocate into the nucleus. These complexes then activate the transcription of target genes. Thus, TGF-β intracellular signaling involves dual Smad-dependent pathways, namely, Smad2-
and Smad3-dependent pathways. In addition to Smad-mediated signaling pathways, other signaling pathways have also been shown to mediate TGF-β signaling. For example, TGF-β activates Rho-GTPase, mitogen-activated protein kinases, and protein kinase B (12). However, a direct link between these mediators and TGF-β receptors has not been demonstrated unequivocally (12). Because TGF-β stimulus is transduced through the multiple intracellular pathways described above, the elucidation of the signaling pathways through which TGF-β regulates PSC functions is likely to provide new insights related to the molecular pathogenesis of pancreatic fibrosis. We therefore conducted the present study to examine these pathways by applying adenovirus-mediated overexpression of Smad2 and Smad3. Moreover, because Smad2 and Smad3 compete with each other at the receptor and for Smad4-binding steps for their activation, overexpression of Smad2 and Smad3 blocks endogenous Smad2 and Smad3 functions by competing at the binding steps to TGF-β receptor and Smad4. Thus, the possibility remains that the observed effects of Smad2 and Smad3 overexpression on PSC functions may result from the blockade of endogenous Smad2 and Smad3 function but may not result from the enhancement of Smad2 and Smad3 activity by their overexpression. To exclude this possibility, we investigated Smad2- and Smad3-specific roles in TGF-β1 regulation of PSC functions by co-expression of dominant-negative Smad2/3 with Smad2 or Smad3. Although the dominant-negative Smad2/3 mutant was generated by substituting Gln for Asp-407 of Smad3, which is defective in TGF-β-receptor-dependent phosphorylation, this mutant possesses a dominant-negative effect on both Smad2 and Smad3 (13). Thus, we designated the mutant as dominant-negative Smad2/3. In this way, expression of dominant-negative Smad2/3 blocks both endogenous Smad2 and Smad3 functions at TGF-β-receptor-dependent phosphorylation step. Therefore, the co-expression of Smad2 or Smad3 with dominant-negative Smad2/3 rescues only Smad2- or Smad3-dependent pathways, respectively. Thus, we can examine Smad2- and Smad3-specific signaling pathways. Using this method, we demonstrated that TGF-β1 activates PSCs through a Smad2-dependent pathway and inhibits their proliferation through a Smad3-dependent pathway. Moreover, TGF-β1 enhanced its own mRNA expression and peptide secretion of PSCs through an ERK-dependent pathway.

EXPERIMENTAL PROCEDURES

Materials—Nycodenz, Pronase, and anti-α-SMA antibody were purchased from Sigma. DNase I and collagenase P were from Roche Applied Science. Anti-Smad2, anti-Smad3, anti-Smad4, and anti-ERK antibodies were purchased from Cell Signaling (Beverly, MA). Horseradish peroxidase (HRP)-conjugated donkey anti-goat IgG, HRP-conjugated donkey anti-mouse IgG, HRP-conjugated donkey anti-rabbit IgG, and Cy3-conjugated donkey anti-goat IgG antibodies were from Jackson ImmunoResearch (West Grove, PA). PD98059 was from Calbiochem (San Diego, CA).

Isolation and Culture of Rat Pancreatic Stellate Cells—Rat pancreatic stellate cells were prepared as described (1). Briefly, rat pancreas was digested in Gey’s balanced salt solution supplemented with 0.05% collagenase P, 0.02% Pronase, and 0.1% DNase. After filtration through nylon mesh, the cells were centrifuged at 13,200 × g for 20 min. The cells were collected from the band just above the interface of the Nycodenz solution and the aqueous layer, washed, and resuspended in Iscove’s modified Dulbecco’s medium containing 10% fetal calf serum, 100 units/ml penicillin, and 100 mg/ml streptomycin. PSCs were cultured in a 5% CO2 atmosphere at 37 °C. All of the experiments were carried out using PSCs from passages 2 and 3.

Immunoprecipitation and Western Blotting—Immunoprecipitation was performed as described previously (14). Western blotting was carried out as described before (15), using enhanced chemiluminescence reagent to visualize the secondary antibody.

Adenovirus Infection—Recombinant adenoviruses containing recombinant Smad DNAs were kindly provided by Dr. Miyazono (University of Tokyo, Tokyo, Japan). For a single adenovirus infection, the cells were infected with a recombinant adenovirus at a dose of 10 plaque-forming units (pfu)/cell in the culture media described above. In the experiments using double adenovirus infection, the cells were infected with dominant-negative Smad2/3 adenovirus (AdDN-Smad2/3) at a dose of 10 pfu/cell, simultaneously with Smad2 (AdSmad2) or Smad3 (AdSmad3) adenovirus at doses of 1, 5, or 10 pfu/cell. Subsequent experiments were performed 48 h after infection. An adenovirus expressing β-galactosidase (AdLacZ) was used as an infection control.

Immunofluorescence Microscopy—Immunofluorescence microscopy was performed as described previously (15, 16). The samples were examined by epifluorescence microscopy (see Fig. 2) and confocal fluorescence microscopy (see Fig. 3) (Fluoview FV300; Olympus, Tokyo, Japan) using an Olympus BX51 microscope. The images were digitized and then processed using Photoshop 5.0 software (Adobe Systems Inc., Mountain View, CA).

Measurement of DNA Synthesis—DNA synthesis was determined by measuring [3H]thymidine incorporation into cells. [3H]Thymidine was added to the culture medium and incubated for 2 h, and the incorporation of radioactivity was measured as described previously (17).

Measurement of TGF-β1 Peptide Secretion—TGF-β1 peptide secretion was examined by determining the concentration of TGF-β1 peptide in a culture medium of PSCs using a commercial kit from DRG International (Mountainide, NJ), according to the manufacturer’s instructions.

Competitive Reverse Transcription-PCR of TGF-β1 mRNA—Total RNA was obtained from PCS cells by using ISOGEN (Wako, Tokyo, Japan), followed with synthesis of double-stranded DNA as described.
previously (18). Competitive PCR of TGF-/H92521 mRNA was performed using the competitive PCR kit for rat TGF-/H92521 (Maxim Biotech Inc., San Francisco, CA) according to the manufacturer’s instructions. In this method, 189- and 250-bp PCR fragments are generated by amplifying a DNA competitor and rat TGF-/H92521 cDNA, respectively.

RESULTS

Expression of Smad2, Smad3, and Smad4 Proteins in PSCs—We first examined the expression of R-Smads (Smad2 and Smad3) and Co-Smad (Smad4) in rat PSCs. To this end, we performed immunoprecipitation of Smad2, Smad3, and Smad4 from crude extract of rat PSCs, using antibodies specific to each Smad. As shown in Fig. 1, all of the three Smads were immunoprecipitated from rat PSCs, suggesting that essential components of both Smad2- and Smad3-dependent TGF-/H92521 signaling pathways are present in PSCs.

TGF-/H92521 Induced the Nuclear Accumulation of both Smad2 and Smad3 in PSCs—We next examined whether Smad2- and Smad3-dependent TGF-/H92521 signaling pathways are functioning in PSCs. Because the levels of endogenously expressed Smad2 and Smad3 are not sufficient to detect by immunochemistry using specific antibodies, we used adenovirus-mediated overexpression of Smad2 and Smad3 in PSCs. We first determined the infection efficiency by using AdLacZ infection and in situ staining with X-gal. As shown in Fig. 2, more than 98% of

Table I

TGF-/H92521 peptide concentration (ng/ml) in the culture medium of PSCs infected with AdSmad2, AdSmad3, or AdDNSmad2/3

|                | LacZ | Smad2 | Smad3 | DN-Smad2/3 |
|----------------|------|-------|-------|-------------|
| TGF-/H92521 peptide concentration (ng/ml) | 2.65 ± 0.45 | 2.23 ± 0.22 | 2.55 ± 0.28 | 2.26 ± 0.36 |

FIG. 3. Characterization of dominant-negative Smad2/3 expressed in PSCs. PSCs were infected with 10 pfu/cell AdDNSmad2/3 (A and B) or combinations of 10 pfu/cell AdDNSmad2/3 + 1 pfu/cell AdSmad2 (C and D), 10 pfu/cell AdDNSmad2/3 + 1 pfu/cell AdSmad2 (E and F), or 10 pfu/cell AdDNSmad2/3 + 10 pfu/cell AdSmad2 (G and H). The cells were stained with anti-Smad2 (C-H) or anti-Smad3 (A and B) antibodies before (A, C, E, and G) and after (B, D, F, and H) 2 h of stimulation with 10 pM TGF-/H92521. Bars, 40 μm.

FIG. 4. Overexpression of Smad2, Smad3, or dominant-negative Smad2/3 did not alter TGF-/H92521 mRNA expression level in PSCs. PSCs were infected with AdLacZ, AdSmad2, AdSmad3, or AdDNSmad2/3. TGF-/H92521 mRNA expression in PSCs was determined with competitive reverse transcription-PCR 48 h after the infection.
AdLacZ-infected PSCs expressed β-galactosidase (Fig. 2, A and B). In PSCs infected with AdSmad2 and AdSmad3, sufficient expression of Smad2 and Smad3 was observed by immunocytochemistry (Fig. 2, C and E). When treated with 10 pM TGF-β1, both Smad2 and Smad3 accumulated in the nucleus (Fig. 2, D and F). These data indicate that functional Smad2- and Smad3-dependent signaling pathways are present in PSCs.

**Fig. 5. Effects of Smad2, Smad3, and dominant-negative Smad2/3 overexpression on α-SMA expression in PSCs.** PSCs were infected with AdLacZ, AdSmad2, AdSmad3, or AdDNSmad2/3. For double adenovirus infection experiments, PSCs were infected with the combination of AdSmad2 and AdDNSmad2/3 or that of AdSmad3 and AdDNSmad2/3. After 48 h of incubation, total homogenates were prepared from PSCs, and aliquots of 10 μg of protein of the homogenates were subjected to electrophoresis on 10% sodium dodecyl sulfate-polyacrylamide gels and transferred to nitrocellulose membranes. Western blotting was carried out using anti-α-SMA monoclonal antibodies. The results shown are representative of three independent experiments.

**Fig. 6. Effects of Smad2, Smad3, and dominant-negative Smad2/3 overexpression on DNA synthesis in PSCs.** PSCs were infected with AdLacZ, AdSmad2, AdSmad3, or AdDNSmad2/3. For double adenovirus infection experiments, PSCs were infected with the combination of AdSmad2 and AdDNSmad2/3 or that of AdSmad3 and AdDNSmad2/3. After 48 h of incubation, DNA synthesis was determined with a [3H]thymidine uptake assay. The values are expressed as the means ± S.E. for three independent experiments. *, p < 0.05; **, p < 0.01, by analysis of variance.
these adenoviruses infections on diverse PSC functions modu-
lated by autocrine TGF-β₁, regardless of the effect of the in-
fection on the amount of autocrine TGF-β₁.

**Effects of Smad2, Smad3, and Dominant-negative Smad2/3**

**Overexpression on PSC Activation**—We examined involvement of Smad2 and Smad3 in PSC activation using adenovirus-
mediated overexpression of the proteins. PSC activation was
examined by determining the amount of α-SMA protein in
PSCs with Western blotting. Overexpression of dominant-neg-
ative Smad2/3 inhibited PSC activation (Fig. 5), indicating that
TGF-β₁ activates PSCs through a Smad-dependent pathway.
Moreover, overexpression of Smad2 but not Smad3 enhanced
PSC activation, suggesting that TGF-β₁ activates PSCs
through a Smad2-dependent but not a Smad3-dependent path-
way. However, because both Smad2 and Smad3 compete with
each other for receptor and for Smad4 binding steps for the
activation of their pathways as described above, the possibility
remained that the positive effect of Smad2 overexpression on
PSC activation may have resulted from inhibition of endoge-
nous Smad3 function and not the involvement of Smad2-de-
pendent pathway in TGF-β₁ induced PSC activation. To ex-
clude this possibility, we investigated Smad2- and Smad3-
specific roles in TGF-β₁-induced PSC activation by co-infection
of AdDNaSmad2/3 with AdSmad2 or AdSmad3. As shown in Fig.
5, AdSmad2 co-infection with AdDNaSmad2/3 rescued PSC ac-
tivation inhibited by AdDNaSmad2/3. On the other hand, AdS-
mad3 co-infection with AdDNaSmad2/3 did not alter PSC ac-
tivation inhibited by AdDNaSmad2/3. These data suggest that
TGF-β₁ activates PSCs through a Smad2-dependent pathway.

**Effects of Smad2, Smad3, and Dominant-negative Smad2/3**

**Overexpression on PSC Proliferation**—We next examined the
pathway through which TGF-β₁ inhibits PSC proliferation.
PSC proliferation was examined by determining DNA synthe-
sis by means of [³H]thymidine incorporation. AdDNaSmad2/3
infection enhanced PSC proliferation, whereas AdSmad3 infec-
tion inhibited it (Fig. 6), suggesting that TGF-β₁ inhibits PSC
proliferation through a Smad3-dependent pathway. Moreover,
results of the co-infection method showed that AdSmad3 co-
infection with AdDNaSmad2/3 inhibited PSC proliferation en-
hanced by AdDNaSmad2/3. On the other hand, AdSmad2 co-
infection with AdDNaSmad2/3 did not alter PSC proliferation augmented by AdDNaSmad2/3 infection. These data suggest that
TGF-β₁ inhibits PSC proliferation through a Smad3-de-
pendent pathway.

**TGF-β₁ Enhanced TGF-β₁ mRNA Expression of PSCs**

**through an ERK-dependent Pathway**—As described above,
TGF-β₁ mRNA expression of PSCs was not affected by the
infection of AdSmad2, AdSmad3, or AdDNaSmad2/3, suggesting that the regulation of TGF-β₁ mRNA expression in PSCs is
independent of Smad-dependent signaling pathways. Thus, we
next attempted to elucidate the regulatory mechanism of
TGF-β₁ mRNA expression in PSCs. To this end, we first exam-
ined whether TGF-β₁ modulates its own mRNA expression in
PSCs. As shown in Fig. 7A, the addition of exogenous TGF-β₁
into the culture medium of PSCs enhanced TGF-β₁ mRNA ex-
pression in a dose-dependent manner, indicating TGF-β₁ autoinduction independent of Smad-mediated signaling. Be-
cause mitogen-activated protein kinases including ERKs are
also TGF-β signaling mediators (12), we examined the partic-
ipation of ERK-dependent pathway in the autoinduction of
TGF-β₁ mRNA in PSCs. For this purpose, we blocked ERK ac-
tivation by using the MEK1 inhibitor PD98059. PD98059
pretreatment decreased TGF-β₁ mRNA expression in PSCs
(Fig. 7B, first and second lanes). Moreover, the addition of
exogenous TGF-β₁ into the culture medium could not enhance
TGF-β₁ mRNA expression in PSCs pretreated with PD98059
(Fig. 7B, first and second lanes). Consistent with these data,
PD98059 pretreatment decreased TGF-β₁ peptide secretion
from PSCs (Fig. 7C). Finally, we confirmed that TGF-β₁ ac-
tivates ERK in PSCs (Fig. 7D, first and second lanes), and
PD98059 pretreatment blocked ERK activation (Fig. 7D, third
and fourth lanes). These data indicate that TGF-β₁ autoinduc-
tion in PSCs is regulated through an ERK-dependent pathway.

**DISCUSSION**

In this study, we demonstrated that TGF-β₁ regulates vari-
ous PSC functions through distinct intracellular signaling
pathways. Adenovirus-mediated dominant-negative Smad2/3
expression inhibited PSC activation and enhanced their prolif-
eration but did not alter TGF-β₁ mRNA expression. Co-expres-
sion of Smad2 with dominant-negative Smad2/3 restored the
PSC activation inhibited by dominant-negative Smad2/3 ex-
pression. In contrast, co-expression of Smad3 with dominant-
negative Smad2/3 attenuated the proliferation enhanced by
dominant-negative Smad2/3 expression. Moreover, exogenous
TGF-β₁ increased TGF-β₁ mRNA expression in PSCs, and
MEK1 inhibitor PD98059 blocked it. Accordingly, TGF-β₁ en-
hances PSC activation, inhibits their proliferation, and in-
increases their TGF-β1 mRNA expression through Smad2-, Smad3-, and ERK-dependent pathways, respectively. Although TGF-β1 signaling is mediated by both Smad2- and Smad3-dependent pathways, their functional difference has been uncertain. However, studies using targeted homozygous deletion of Smad2 and Smad3 genes in mice revealed their distinct functions in embryo development. Smad2 knockout mice are embryonic lethal because of the defects of left-right patterning and mesoderm induction (19, 20). In contrast, Smad3 knockout mice are viable but are smaller than wild-type littermates and show forelimb malformation and die because of immune function defects (21, 22). In addition, using hepatic cell lines (H9252) derived from the Smad3 knockout mice, Schnabl et al. (23) recently elucidated the Smad3 specific role in cellular function. They reported that Smad3 is necessary for TGF-β-mediated inhibition of HSC proliferation but not for HSC activation, which is consistent with our data on Smad3 function in patients. However, the specific role of Smad2 in HSC function has not yet been demonstrated. Recently, using fibroblasts derived from both embryos of Smad2 and Smad3 knockout mice, Piek et al. (24) reported that Smad2 and Smad3 mediated the transcription of distinct genes in fibroblasts stimulated by TGF-β1. Indeed, these cell systems are useful for comparing the functions of Smad2 and Smad3 in cells derived from embryos of knockout mice. However, because Smad2 knockout is lethal to embryonic mice because of embryonic development defects, it had been difficult to examine specific roles of Smad2 and Smad3 concurrently in fully differentiated cells derived from mature organs. In the present study, however, we have demonstrated specific roles of Smad2 and Smad3 in TGF-β1 regulation of PSC functions isolated from the mature pancreas by employing adenovirus-mediated co-expression of Smad2 or Smad3 with dominant-negative Smad2/3. It is noteworthy that this method can be widely applied for the study on TGF-β1 intracellular signaling pathway in a variety of mature organs because adenovirus-mediated gene transfer is highly effective in various cell types.

Our present observations that TGF-β1 activates PSCs through a Smad2-dependent pathway and inhibits PSCs proliferation through a Smad3-dependent pathway provide a novel therapeutic strategy for pancreatic fibrosis. Because TGF-β1 is a key activator of PSCs (7) and a major inducer of pancreatic fibrosis (4), the therapeutic effect of TGF-β1 stimulius inhibition on pancreatic fibrosis has been extensively studied. For example, Menke et al. (5) reported that inhibition of TGF-β1 by injection of neutralizing TGF-β1 antibody reduced extracellular matrix formation in pancreatitis in vivo. However, TGF-β1 is also an autocrine inhibitor of PSC proliferation (6). Thus, blockade of TGF-β1 activity promotes PSC proliferation. If TGF-β1 stimuli on PSC activation could be selectively blocked without diminishing the TGF-β1 inhibitory effect on PSC proliferation, it could be a more potent therapeutic method for pancreatic fibrosis. In this respect, our present data indicate that selective blockade of the Smad2-dependent pathway without affecting the Smad3-dependent pathway can be a novel strategy for the treatment of pancreatic fibrosis.

Our data on TGF-β1, mRNA expression and peptide secretion of PSCs are important. Because TGF-β1, mRNA expression and TGF-β1 peptide secretion of PSCs were not affected by overexpression of Smad2, Smad3, or dominant-negative Smad2/3, we could apply their overexpression and co-expression to observe Smad2- and Smad3-specific roles in the regulation of PSC function by autocrine TGF-β1. We also demonstrated that TGF-β1 enhanced its own mRNA expression through a Smad-independent but ERK-dependent pathway. To date, TGF-β1 has been shown to augment the expression of its own mRNA (25) in both normal and transformed cells, and the promoter sequences of TGF-β1 gene responsive to the autoinduction have been identified (26). In addition, Yue and Mulder (27) reported that MEK-ERK pathway activation is required for TGF-β1 expression induced by TGF-β1, a TGF-β1 isoform derived from a gene distinct from that of TGF-β1. However, the intracellular signaling pathway of TGF-β1 autoinduction has never been demonstrated. Thus, to our knowledge, this is the first report that has elucidated the intracellular signaling pathway of TGF-β1 autoinduction.

In conclusion, we showed that TGF-β1 regulates PSC activation, proliferation, and TGF-β1 mRNA expression through Smad2-, Smad3-, and ERK-dependent pathways, respectively. These observations provide new insights for understanding the mechanism of pancreatic fibrosis and developing a novel therapeutic strategy for its treatment.

Acknowledgments—We are grateful to Mie Sikata and Takako Inshijima for technical and secretarial assistance and Dr. Kohei Miyazono of University of Tokyo for Smad2, Smad3, and dominant-negative Smad2/3 adenoviral vectors.

REFERENCES

1. Apte, M. V., Haber, P. S., Applegate, T. L., Norton, I. D., McCaughan, G. W., Korsten, M. A., Pirolo, R. C., and Wilson, J. S. (1998) Gastroenterology 115, 421–432
2. Bachem, M. G., Schneider, E., Gross, H., Weidenbach, H., Schmid, R. M., Menke, A., Siech, M., Beger, H., Grunert, A., and Adler, G. (1998) Gastroenterology 115, 413–420
3. Haber, P. S., Keogh, G. W., Apte, M. V., Moran, C. S., Stewart, N. L., Crawford, D. H., Pirolo, R. C., McCaughan, G. W., Ramm, G. A., and Wilson, J. S. (1999) Am. J. Pathol. 155, 1081–1095
4. Lee, M. S., Gu, D., Peng, L., Curnide, S., Arnoux, M., Kralh, T., Gurushan-thaihaai, D., Wilson, C., Loskutoff, D. L., and Fox, H. (1995) Am. J. Pathol. 147, 42–52
5. Menke, A., Yamaguchi, H., Gross, T. M., and Adler, G. (1997) Gastroenterology 113, 295–303
6. Kruse, M. L., Hildebrand, B. P., Timke, C., Fulsch, U. R., and Schmidt, W. E. (2000) Regul. Pept. 90, 47–52
7. Apte, M. V., Haber, P. S., Darby, S. J., Rodgers, S. C., McCaughan, G. W., Korsten, M. A., Pirolo, R. C., and Wilson, J. S. (1999) Gut 44, 534–541
8. Heldin, C. H., Miyazono, K., and ten Dijke, P. (1997) Nature 390, 465–471
9. Massague, J. (1998) Annu. Rev. Biochem. 67, 753–791
10. Macias-Silva, M., Abdollah, S., Pirolo, R. C., and Wilson, J. S. (1999) Nature 390, 791–792
11. Liu, X., Sun, Y., Constantinou, S., N. K., Karam, E., Weinberg, R. A., and Lodish, H. F. (1997) Proc. Natl. Acad. Sci. U. S. A. 91, 10689–10674
12. Attisano, L., and Wrana, J. L. (1996) Cell 87, 1215–1229
13. Ohnishi, H., Mine, T., Shibata, H., Ueda, N., Tsuichida, T., and Fujita, T. (1999) J. Biol. Chem. 274, 30773–30779
14. Ohnishi, H., Mine, T., Mine, T., Shibata, H., Ueda, N., Tsuichida, T., and Fujita, T. (1999) J. Clin. Invest. 105, 9378–9383
15. Suzuki, J., Ohnishi, H., Shibata, H., Wada, A., Hirayama, T., Iiri, T., Ueda, N., Kanamaru, C., Tsuichida, T., Mashima, H., Yasuda, H., and Fujita, T. (2001) J. Clin. Invest. 108, 363–370
16. Kanamaru, C., Yasuda, H., Takeda, M., Ueda, N., Suzuki, J., Tsuichida, T., Mashima, H., Ohnishi, H., and Fujita, T. (2001) J. Biol. Chem. 276, 45636–45641
17. Ohnishi, H., Ogusho, N., Tanaka, S., Mogami, H., Nobeusawa, R., Mashima, H., Furukawa, M., Mine, T., Shimada, O., Ishikawa, H., and Kojima, I. (1995) J. Clin. Invest. 95, 2304–2314
18. Nomura, M., and Li, E. (1998) Nature 393, 786–790
19. Weinstein, M., Yang, X., Li, C., Xu, X., Getay, J., and Deng, C. X. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8178–8183
20. Datto, M. B., Frederick, J. P., Pan, L., Borton, A. J., Zhuang, Y., and Wang, X. F. (1999) Mol. Cell. Biol. 19, 2495–2504
21. Yang, X., Letterio, J. J., Lechleider, R. J., Chen, L., Hayman, R., Gu, H., Roberts, A. B., and Deng, C. (1999) EMBO J. 18, 1290–1291
22. Schnabl, B., Kweon, Y. O., Frederick, J. P., Wang, X. F., Ripple, R. A., and Brenner, D. A. (2001) Hepatology 34, 89–100
23. Piek, E., Ju, W. J., Heyer, J., Escalante-Alcalde, D., Stewart, C. L., Weinstein, M., Deng, C., Kercherlapati, R., Bottinger, E. P., and Roberts, A. B. (2001) J. Biol. Chem. 276, 19396–19402
24. Van Obberghen-Schilling, E., Roche, N. S., Flanders, K. C., Sporn, M. B., and Roberts, A. B. (1988) J. Biol. Chem. 263, 7741–7746
25. Kim, M. J., Jeang K. T., Glick, A. B., Sporn, M. B., and Roberts, A. B. (1989) J. Biol. Chem. 264, 7041–7045
26. Yue, J., and Mulder, K. M. (2000) J. Biol. Chem. 275, 30765–30773