行政院國家科學委員會專題研究計畫　成果報告

Pentoxifylline 治療腎小管間質纖維化

計畫類別：個別型計畫
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執行單位：國立臺灣大學醫學院內科

計畫主持人：林水龍
共同主持人：蔡敦仁
計畫參與人員：呂育璇

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行政院國家科學委員會補助專題研究計畫

■成果報告

□期中進度報告

（計畫名稱）

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成果報告類型(依經費核定清單規定繳交)：■精簡報告　□完整報告

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執行單位：國立台灣大學醫學院內科

中　華　民　國　　94　　年　　12　　月　　14　　日

中文摘要

關鍵詞: Pentoxifylline，結締組織生長因子，上皮間質轉分化，Smad，轉化生長因子β1

Pentoxifylline(PTX)為結締組織生長因子(CTGF)的強利抑制劑，但抑制機轉並不清楚。本研究不僅證實PTX可以抑制轉化生長因子β1(TGF- β1)誘發CTGF的表現，也證實PTX可以抑制NRK-49F纖維母細胞表現第一型膠元蛋白與NRK-52E近端腎小管上皮細胞表現α-smooth muscle actin(α-SMA)。進一步的研究發現Smad3/4的活化為TGF- β1誘發CTGF基因轉錄所必須，雖然PTX無法干擾TGF- β1活化Smad2/3及其與Smad4的結合及後續的移位至細胞核內，但PTX可以抑制TGF-β1刺激Smad3/4所活化的reporter，也可以抑制CTGF的promoter的活化。因此吾人推測PTX可能經由干擾與Smad3/4協同刺激CTGF轉錄的分子以達到抑制CTGF的表現。吾人進一步證實PTX經由增加細胞內cyclic adenine monophosphate (cAMP)以活化蛋白質磷酸化脢A(PKA)，而PKA的抑制劑H89可以逆轉PTX抑制CTGF轉錄的作用，但 dibutyryl cAMP及forskolin可以造成與PTX相同的作用。因此吾人結論: PTX透過活化PKA以抑制TGF-β1刺激Smad3/4所活化的CTGF轉錄並進一步抑制CTGF促進腎臟細胞產生纖維化的作用。

英文摘要

Key words: Pentoxifylline, connective tissue growth factor, epithelial-mesenchymal transdifferentiation, Smad, transforming growth factor-β1

Pentoxifylline (PTX) is a potent inhibitor of connective tissue growth factor (CTGF), but its underlying mechanism is poorly understood. Here, we demonstrated that PTX inhibited not only transforming growth factor-β1 (TGF- β1)-induced CTGF expression, but also CTGF-induced collagen I (α1) [Col I (α1)] expression in NRK-49F and α-smooth muscle actin expression in NRK-52E cells. The mechanism by which PTX reduced CTGF in NRK-49F and NRK-52E was investigated. Activation of Smad3/4 was essential for TGF-β1-induced CTGF transcription, but PTX did not interfere with TGF-β1 signaling to Smad2/3 activation, association with Smad4, and their nuclear translocation. However, PTX was capable of blocking activation of TGF-β1-induced Smad3/4-dependent reporter as well as CTGF promoter, suggesting that PTX affects a factor that acts cooperatively with Smad3/4 to execute transcriptional activation. We found that PTX increased intracellular cyclic adenine monophosphate (cAMP) and caused cAMP response element binding protein phosphorylated. The protein kinase A (PKA) antagonist H89 abolished the inhibitory effect of PTX on Smad3/4-dependent CTGF transcription, whereas dibutyryl cAMP and forskolin recapitulated the inhibitory effect. In conclusion, our results indicate that PTX inhibits CTGF expression by interfering with Smad3/4-dependent CTGF transcription through PKA and blocks the profibrogenic effects of CTGF on renal cells.

報告內容

前言

Almost all forms of kidney diseases progressing to end-stage renal failure are characterized by diffuse fibrosis, in which tubulointerstitial fibrosis is considered the most important determinant of progressive renal injury. Tubulointerstitial fibrosis is characterized by the accumulation of myofibroblasts and extracellular matrix (ECM). Increasing evidence indicates that transforming growth factor-β1 (TGF-β1) is a key mediator in renal fibrosis. Therefore, it would be of great importance to elucidate downstream mediators of TGF-β1's profibrogenic effects and to develop new antifibrotic strategies. Connective tissue growth factor (CTGF) has been known to act downstream of TGF-β1 to regulate ECM synthesis. CTGF expression is increased in mesangial cells, proximal tubular epithelial cells, and interstitial fibroblasts of fibrotic kidneys. Neutralization of CTGF by antisense oligodeoxynucleotide (ODN) significantly attenuates tubulointerstitial fibrosis despite the sustained TGF-β1 level. These data suggest that CTGF may be a potential target to prevent progressive tubulointerstitial fibrosis.

Pentoxifylline (PTX), a non-selective phosphodiesterase inhibitor, exerts potent inhibitory effects against cell proliferation, inflammation, and ECM accumulation. There is increasing evidence that PTX markedly reduces proteinuria in patients with membranous nephropathy or diabetes. PTX can downregulate the gene expression of CTGF and collagen in remnant nephropathy or acute Thy 1 glomerulonephritis. PTX downregulates CTGF despite sustained TGF-β1 in angiotensin II-stimulated interstitial fibroblasts and mesangial cells. Although PTX inhibits TGF-β1-induced collagen expression in human peritoneal mesothelial cells through modulating mitogen-activated protein kinase (MAPK), the detailed mechanism by which PTX downregulates CTGF in renal cells is unknown. Moreover, it remains unclear whether PTX reduces profibrogenic effects of CTGF on interstitial fibroblasts and proximal tubular epithelial cells, which concert in the progressive tubulointerstitial fibrosis.

研究目的

In this study, we investigated the mechanism by which PTX inhibited TGF-β1-induced CTGF in interstitial fibroblasts and proximal tubular epithelial cells. Our results indicate that PTX inhibits not only CTGF expression by interfering with Smad3/4-dependent CTGF transcription through protein kinase A (PKA), but also profibrogenic effects of CTGF on renal cells.

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研究方法

Cell Culture and Transfection

Normal rat kidney fibroblasts (NRK-49F) and proximal tubular epithelial cells (NRK-52E) were cultured as stated previously. Subconfluent cells were placed in medium with 0.5% fetal bovine serum (Gibco BRL, Rockville, MD) for 24 h before TGF-β1 or PTX treatment. Lipofectamine® 2000 reagent (Invitrogen, Carlsbad, CA) was used for transfection of plasmids. G418 was used to select cell lines after transfection with CTGF/wt, AS-CTGF, and pCDNA3, and hygromycin B to select NRK-52E with pTRE2 or pTRE2-CTGF. NRK-52E with pTRE2-CTGF was maintained in medium containing doxycycline (2 μg/ml) to inhibit CTGF expression.

RNA and Protein Extraction, Northern and Western Blot Analyses

Total RNA isolated for Northern blot analysis was performed as previously. Human type I collagen (α1) [Col I (α1)], rat TGF-β1, CTGF, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) RNA probes were synthesized as previously.

Total cellular proteins were extracted by RIPA buffer, and nuclear proteins were obtained using

methods as described. For detection of CTGF secreted into by renal cells, media were concentrated with Centricon-10® (Millipore, Bedford, MA) as described. Equal amount of proteins was subjected to Western blot analysis.

Luciferase Assay

pRL-TK vectors were cotransfected to normalize the experimental firefly reporter activities. Firefly and renilla luciferase activities were quantified with the Dual-Luciferase® Reporter Assay System (Promega, Madison, WI).

Immunoprecipitation

Immunoprecipitation was performed as described previously. Briefly, 250 μg of total cellular extracts were immunoprecipitated overnight with 1 μg of anti-p-Smad2/3 followed by precipitation with 20 μl of protein A/G Plus-Agarose (Santa Cruz Biotechnology). The resulting precipitated complexes were separated by SDS-polyacrylamide gel electrophoresis and analyzed by Western blot analyses.

Enzyme Immunoassay

cAMP and cyclic guanine monophosphate (cGMP) concentrations were measured using enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI) as described previously.

Statistical Analyses

Statistical analyses were carried out using GraphPad Prizm® (GraphPad Software, Inc., San Diego, CA). The statistical significance was evaluated by one-way analysis of variance using the Bonferroni correction application.

結果

Figure 1. Pentoxifylline (PTX) inhibited profibrogenic gene expression in renal interstitial fibroblasts stimulated by transforming growth factor-β1. (A) PTX downregulated connective tissue growth factor (CTGF) and type I collagen (α1) [Col I (α1)] mRNA expression in transforming growth factor-β1 (TGF-β1)-stimulated interstitial fibroblasts. Interstitial fibroblasts (NRK-49F) were stimulated with 5 ng/ml TGF-β1 in the presence or absence of 1 mM PTX for various times as indicated. The levels of CTGF, Col I (α1), and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) mRNAs were determined by Northern blot analyses. Normalized data from three independent experiments were expressed in bar charts as mean + S.D. and analyzed by one-way analysis of variance using the Bonferroni correction application. *, $P < 0.05$ versus non-TGF-β1-stimulated cells and **, $P < 0.05$ versus TGF-β1-stimulated cells in the absence of PTX, respectively. (B) PTX reduced CTGF protein expression in TGF-β1-stimulated NRK-49F. Total cellular extracts and concentrated conditioned media were prepared and then subjected to Western blot analyses with antibodies to CTGF and β-actin as indicated. The signals of cellular CTGF (cCTGF) and soluble CTGF (sCTGF) were normalized against those of β-actin and albumin respectively. Normalized data from three independent experiments were expressed and analyzed as described in (A). (C) PTX downregulated α-smooth muscle actin (α-SMA) expression in TGF-β1-stimulated NRK-49F. Cells stimulated with TGF-β1 were treated with PTX of varying concentrations for 48 h. Total cellular extracts were subjected to Western blot analyses with antibodies to α-SMA and β-actin as indicated. Normalized data were expressed and analyzed as described in (A). (D) PTX suppressed CTGF transcription in TGF-β1-stimulated NRK-49F. pGL3-CTGF-transfected cells were stimulated with TGF-β1 in the presence or absence of PTX for

4 h. Luciferase assays were performed with cellular extracts. The results of luciferase assays were expressed as the relative activity of pGL3-CTGF over pGL3-basic and normalized to the renilla luciferase activity from cotransfected pRL-TK vectors. The raw luciferase activities for pGL3-basic were 134.8 ± 18.3 from four independent experiments. The results were analyzed as described in (A).

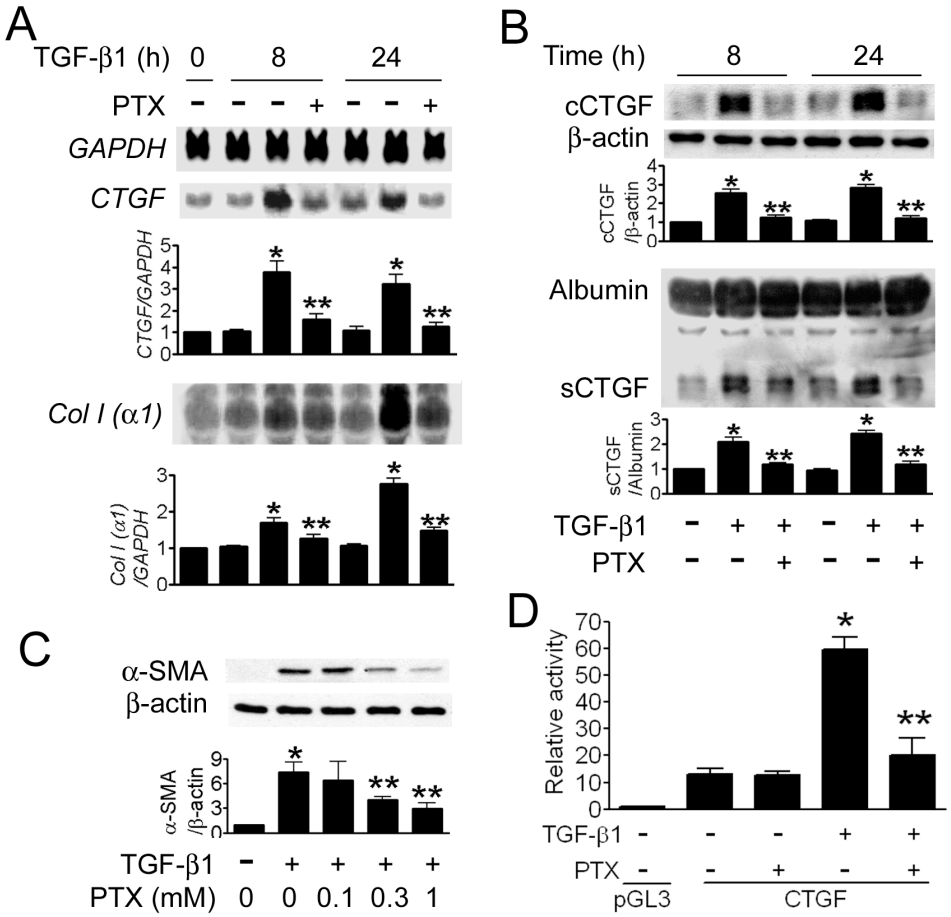

Figure 2. PTX inhibited profibrogenic gene expression in proximal tubular epithelial cells stimulated by angiotensin II or TGF-β1. (A) PTX downregulated the gene expression of CTGF, but not TGF-β1 in angiotensin II-stimulated proximal tubular epithelial cells. Proximal tubular epithelial cells (NRK-52E) were stimulated with 1 μM angiotensin II in the presence or absence of PTX for 8 h. The levels of TGF-β1, CTGF, and GAPDH mRNAs were determined by Northern blot analyses. (B) PTX downregulated CTGF and Col I (α1) in TGF-β1-stimulated NRK-52E. Cells stimulated with 5 ng/ml TGF-β1 were treated with PTX of varying concentrations for 24 h. The signals of CTGF and Col I (α1) form three independent experiments were normalized and analyzed as described in Figure 1A. (C) PTX reduced CTGF protein expression in TGF-β1-stimulated NRK-52E. Normalized data from Western blot analyses performed for total cellular extracts and concentrated conditioned media were expressed in bar charts and analyzed as described in Figure 1B. (D) PTX downregulated the expression of α-SMA in TGF-β1-stimulated NRK-52E. Cells were stimulated with TGF-β1 in the presence or absence of 1 mM PTX for various times. Total cellular extracts were subjected to Western blot analyses with antibodies to α-SMA and β-actin as indicated. Normalized data from three independent experiments were expressed and analyzed as described in Figure 1A. (E) PTX suppressed CTGF transcription in TGF-β1-stimulated NRK-52E. Luciferase assays were performed and the normalized relative activity of pGL3-CTGF over pGL3-basic were analyzed as described in Figure 1D. The raw luciferase activities for pGL3-basic were 271.3 ± 37.2

from four independent experiments.

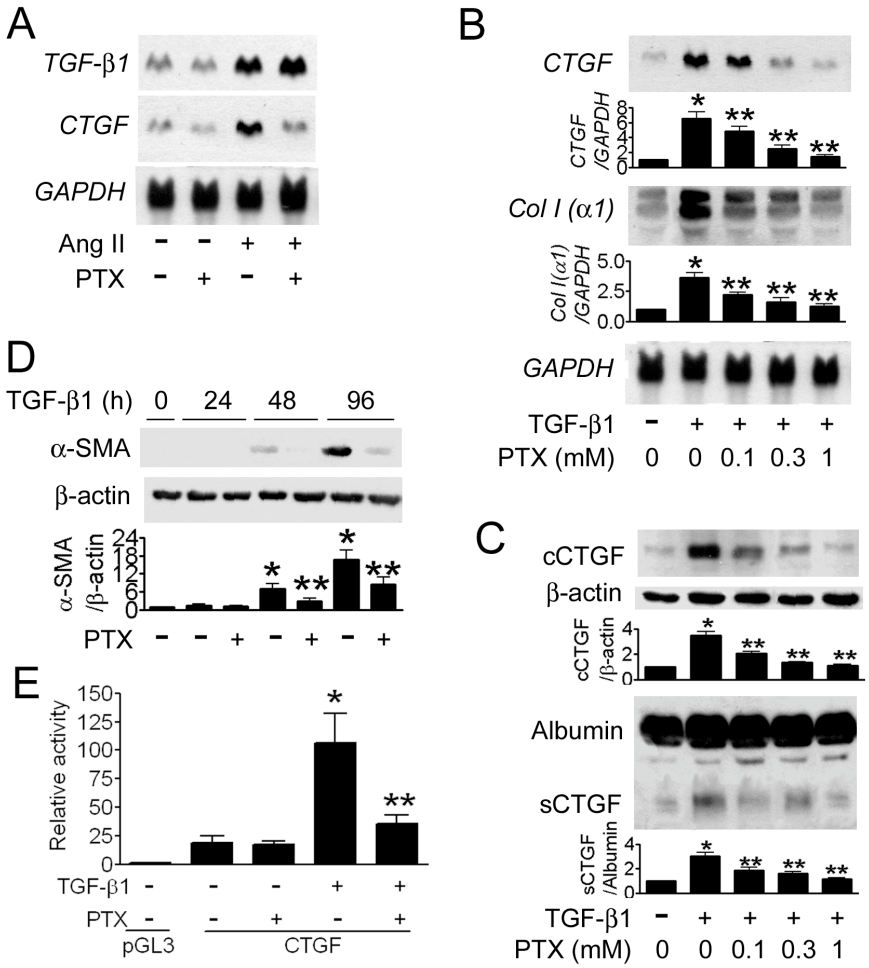

Figure 3. PTX blocked CTGF-mediated induction of collagen in renal interstitial fibroblasts. (A) Antisense CTGF gene transfection blocked the Col I (α1) expression in TGF-β1-stimulated NRK-49F. Antisense CTGF (AS-CTGF)- or pCDNA3-tranfected cells were stimulated with or without 5 ng/ml TGF-β1 for 24 h. Total cellular extracts and concentrated conditioned media were subjected to Western blot analyses with antibodies to CTGF and β-actin as indicated. Total RNA was subjected to Northern blot analyses for Col I (α1) and GAPDH. (B) PTX blocked CTGF-mediated induction of collagen in NRK-49F. Cells with constitutive expression of CTGF (CTGF/wt) or control vector (pCDNA3) were treated with or without 1 mM PTX for 24 h. Western and Northern blot analyses were performed as described in (A). Representatives of Northern and Western blot analyses in (A and B) are one of three independent experiments with similar results.

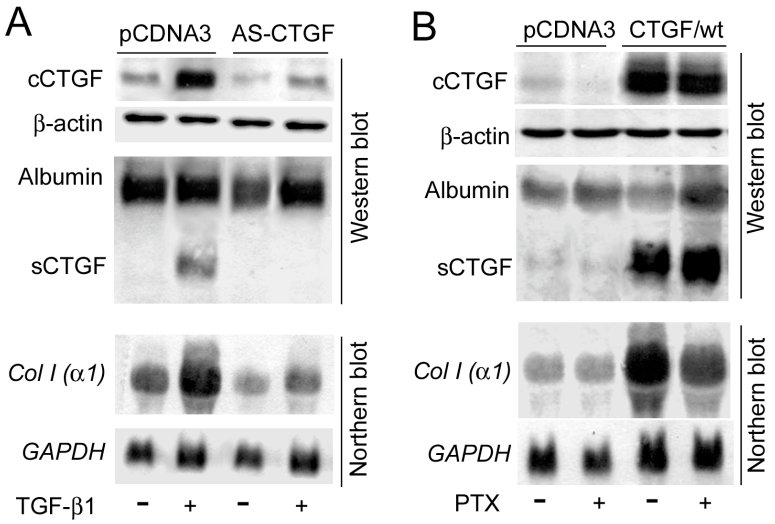

Figure 4. PTX blocked CTGF-mediated induction of α-SMA in proximal tubular epithelial cells. (A) AS-CTGF gene transfection blocked α-SMA expression in TGF-β1-stimulated NRK-52E. AS-CTGF- or pCDNA3-tranfected cells were stimulated with or without 5 ng/ml TGF-β1 for 96 h. Total cellular extracts were subjected to Western blot analyses with antibodies to CTGF, α-SMA, and β-actin as indicated. (B) PTX blocked CTGF-mediated induction of α-SMA in NRK-52E. After withdrawing doxycycline, cells with inducible expression of CTGF (pTRE2-CTGF) or control vector (pTRE2) were treated with or without 1 mM PTX for 96 h. Western blot analyses were performed as described in (A) and Northern blot analyses were performed for Col I (α1) and GAPDH. Representatives of Northern and Western blot analyses in (A and D) are one of three independent experiments with similar results.

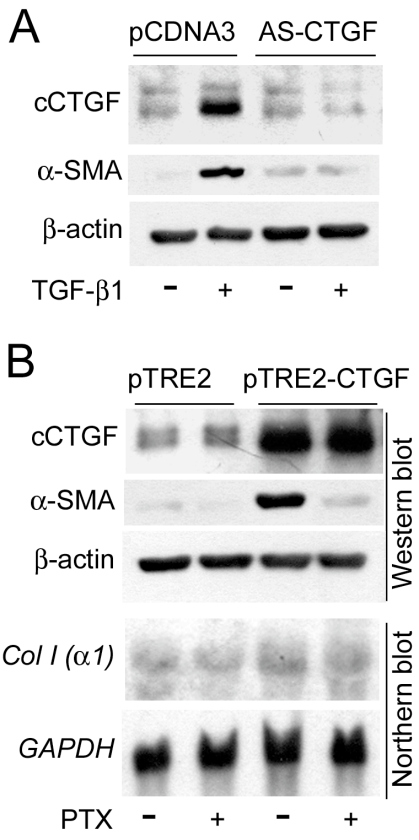

Figure 5. Smad mediated TGF-β1 signaling to the activation of CTGF transcription. (A) TGF-β1 activated Smad2/3. NRK-49F were stimulated with or without 5 ng/ml TGF-β1 for various times as indicated. Total cellular extracts were subjected to Western blot analysis with antibodies to phosphorylated Smad2/3 (p-Smad2/3) and Smad2/3. Representatives of Western blot analyses are one of three independent experiments with similar results. (B) Smad binding element in the CTGF promoter region was essential for TGF-β1-induced gene transcription. pGL3-CTGF- or pGL3-CTGF-ΔSmad-transfected NRK-49F were stimulated with or without TGF-β1 for 4 h. Luciferase assays were performed and the results were expressed as the relative activity of pGL3-CTGF or pGL3-CTGF-ΔSmad over pGL3-basic after normalization to the renilla luciferase activity from cotransfected pRL-TK vectors from four independent experiments. (C) Dominant negative Smads inhibited TGF-β1-induced CTGF transcription. pGL3-CTGF-transfected NRK-49F were cotransfected with or without C-terminally truncated forms of Smad3 (Smad3DN) and Smad4 (Smad4DN) as indicated. Transfected cells with or without TGF-β1 stimulation for 4 h were harvested for luciferase assay. The results were expressed as the relative activity over those of non-TGF-β1-stimulated cells after normalization to the renilla luciferase activity as described previously. Data are expressed as mean + S.D. in (B and C). *, $P < 0.05$ versus non-TGF-β1-stimulated cells and **, $P < 0.05$ versus TGF-β1-stimulated pGL3-CTGF-transfected cells in (B) or cells without cotransfected Smad3DN or Smad4DN in (C).

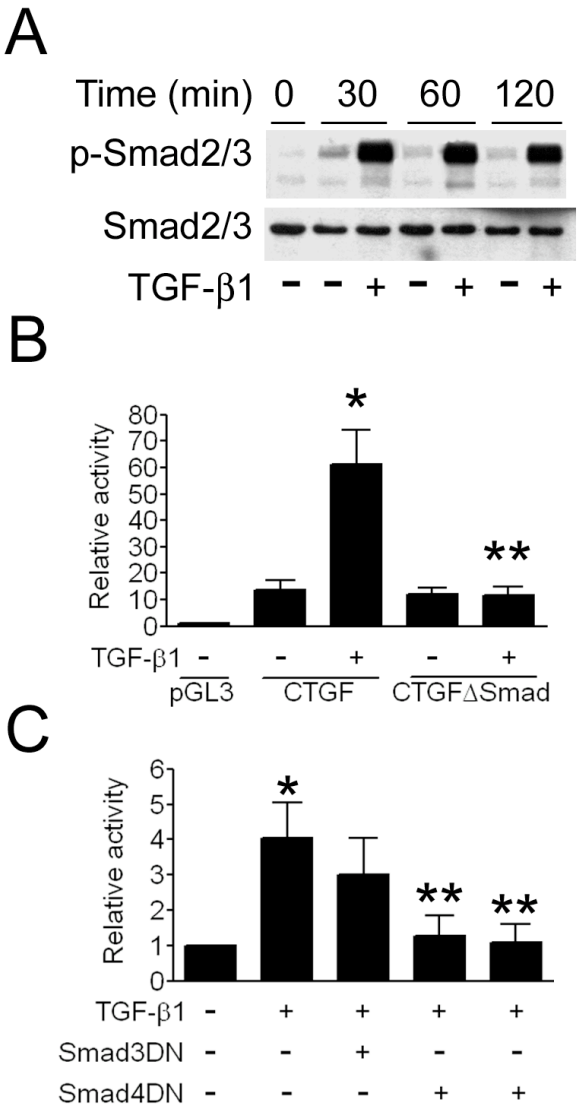

Figure 6. PTX did not interfere with activation and nuclear translocation of Smad proteins induced

by TGF-β1. (A) PTX did not suppress the activation of Smad2/3 in TGF-β1-stimulated NRK-49F. Cells were stimulated with 5 ng/ml TGF-β1 in the presence or absence of 1 mM PTX for various times as indicated. Total cellular extracts were subjected to Western blot analyses with antibodies against p-Smad2/3 and Smad2/3. (B) PTX did not affect the association of activated Smad2/3 with Smad4. NRK-49F were treated as described in (B) and then harvested for total cellular extracts preparation. Total cellular extracts were immunoprecipitated with antibodies against p-Smad2/3. The precipitated complexes were subjected to Western blot analyses with antibodies against p-Smad2/3 and Smad4. (C) PTX did not block the nuclear translocation of activated Smad in TGF-β1-stimulated NRK-49F. Cells were treated with or without TGF-β1and PTX for 30min. Nuclear proteins were prepared and subjected to Western blot analyses with antibodies against p-Smad2/3, Smad4, and Sp1. Representatives of Western blot analyses in (A through C) are one of three independent experiments with similar results. (D) PTX did not inhibit the activation and nuclear accumulation of smad2/3 induced by TGF-β1. NRK-49F and NRK-52E were stimulated with TGF-β1 for 30min in the presence or absence of PTX. Cells were fixed by 4% paraformaldehyde and then immunostained with antibodies against p-Smad2/3. The reactions on sections were detected with diaminobenzidine. Shown are the representatives of three independent experiments.

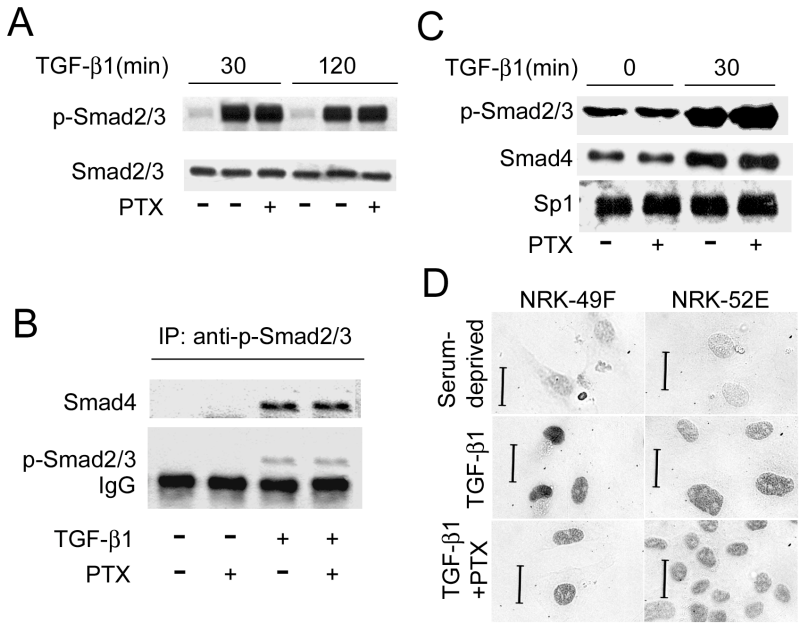

Figure 7. PTX inhibits TGF-β1-induced CTGF expression in renal interstitial fibroblasts by blocking Smad3/4-dependent transcription through activating protein kinase A. (A) PTX blocked TGF-β1-induced activation of Smad3/4-dependent reporter through PKA in NRK-49F. (CAGA)9-MLP-Luc -transfected cells were stimulated with 5 ng/ml TGF-β1 for 4 h in the presence of various agents including PTX (1 mM), N-[2-(4-bromocinnamylamino)ethyl]-5-isoquinoline (H89, 5 μM), dibutyryl cAMP (db-cAMP, 2 mM) or forskolin/3-isobutyl-1-methylxanthine (FK/IBMX, 20 μM/200 μM). Results of luciferase assays were expressed as the relative activity of (CAGA)9-MLP-Luc over MLP-Luc and normalized to the renilla luciferase activity from cotransfected pRL-TK vectors. The raw luciferase activities for MLP-Luc were 71.5 $\pm$ 25.1 from four independent experiments. Data are expressed as mean + S.D. *, $P < 0.05$ versus non-TGF-β1-stimulated cells and **, $P < 0.05$ versus TGF-β1-stimulated cells in the absence of various agents

respectively. (B) PTX caused a dose-dependent increase in intracellular cyclic adenosine monophosphate (cAMP) levels of NRK-49F. Cells were treated with vehicle (VEH), PTX or FK/IBMX. cAMP concentrations per mg cellular protein were measured using enzyme immunoassay kits and expressed as mean ± S.D. from three independent experiments performed in quadruplicate. #, $P < 0.05$ versus VEH-treated. (C) H89 blocked the phosphorylation of CREB induced by PTX in NRK-49F. Cells were treated with 1 mM PTX for 15min in the presence or absence of 5 μM H89. Parallel study was performed with the treatment of db-cAMP or FK/IBMX. Nuclear extracts were prepared for Western blot analysis with antibody against phosphorylated cAMP response element binding protein (p-CREB). Shown blot is the representative of three independent experiments with similar results. (D) PTX reduced CTGF mRNA levels in TGF-β1-stimulated NRK-49F through PKA. Cells were stimulated with TGF-β1 in the presence of various agents as indicated. Total RNA was subjected to Northern blot analyses for CTGF and GAPDH. (E) PTX blocked TGF-β1-induced CTGF transcription through PKA in NRK-49F. pGL3-CTGF -transfected cells were stimulated with TGF-β1 for 4 h in the presence of various agents as indicated. The results of luciferase assays were expressed as described in Figure 8C. Data are expressed as mean ± S.D. *, $P < 0.05$ versus non-TGF-β1-stimulated cells and **, $P < 0.05$ versus TGF-β1-stimulated cells in the absence of various agents respectively.

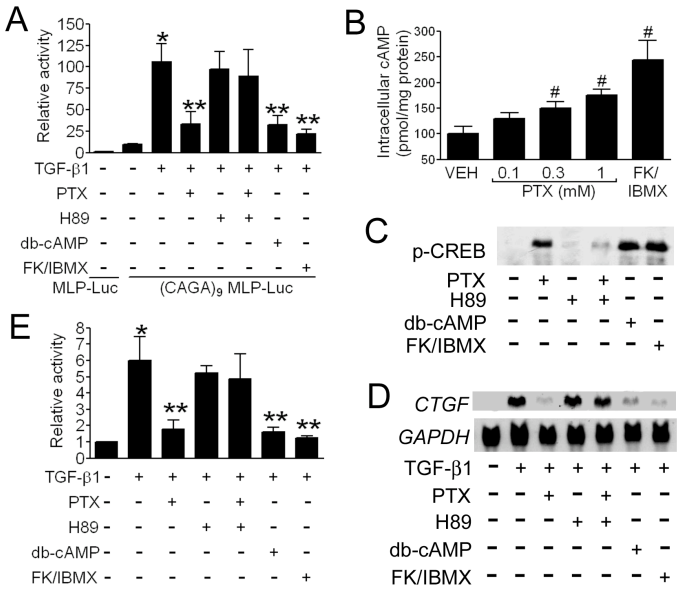

Figure 8. PTX inhibits TGF-β1-induced CTGF expression in proximal tubular epithelial cells by blocking Smad3/4-dependent transcription through activating protein kinase A. (A) PTX blocked TGF-β1-induced activation of Smad3/4-dependent reporter through PKA in NRK-52E. (CAGA)9-MLP-Luc -transfected cells were stimulated with 5 ng/ml TGF-β1 for 4 h in the presence of various agents as indicated. The result of luciferase assays were expressed and analyzed as described in Figure 7A. The raw luciferase activities for MLP-Luc were 83.7 ± 30.6 from four independent experiments. (B) PTX reduced CTGF mRNA levels in TGF-β1-stimulated NRK-52E through PKA. Cells were stimulated with TGF-β1 in the presence of various agents as indicated. Total RNA was subjected to Northern blot analyses for CTGF and GAPDH. (C) PTX blocked TGF-β1-induced CTGF transcription through PKA in NRK-52E. pGL3-CTGF -transfected cells were stimulated with TGF-β1 for 4 h in the presence of various agents as indicated. Luciferase assay and data analysis were performed as described in Figure 7E.

討論

Considerable evidence indicates that CTGF plays a pivotal role in TGF-β1-dependent

tubulointerstitial fibrosis. In this study, we represent the first demonstration that PTX blocked TGF-β1-induced CTGF and CTGF-induced profibrogenic effect on renal cells.

Although CTGF is considered to be the downstream mediator of TGF-β1 in profibrogenesis, it is found to have distinct fibrogenic effects on different renal cells. CTGF mediates the upregulatory effect of TGF-β1 on collagen I in mesangial cells and fibroblasts, however, it mainly transduces EMT signal to tubular epithelial cells. In fact, remnant kidney and coculture system have shown that CTGF secreted by tubular epithelial cells has paracrine effect on ECM synthesis in fibroblasts. Because antisense ODN therapy reducing CTGF attenuates kidney disease, agents targeting CTGF should be promising to ameliorate tubulointerstitial fibrosis. In agreement with previous findings on mesangial cells and NRK-49F, we demonstrated that PTX significantly inhibited TGF-β1-induced CTGF and Col I (α1) in NRK-52E; in addition, PTX also blocked TGF-β1-induced α-SMA. Thus, the inhibition of PTX on CTGF expression is a common effect in many renal cell types. Furthermore, CTGF overexpression was capable of recapitulating TGF-β1's effect in NRK-49F and NRK-52E. However, overexpression of CTGF could not overcome the downregulatory effect of PTX on Col I (α1) in NRK-49F and rescue the downregulation of α-SMA in NRK-52E, either. These findings indicate that PTX can block profibrogenic effects of TGF-β1 through inhibiting both CTGF expression and profibrogenic effects of CTGF on renal cells. Because of the dual-blockade, we found that the inhibitory effects of PTX on CTGF could be translated into the attenuation of in vivo tubulointerstitial fibrosis system.

In this study, we demonstrated that PTX downregulated TGF-β1-induced CTGF through blocking Smad3/4-dependent transcription via PKA. Agents that elevate intracellular cAMP have been reported to inhibit CTGF expression and cell proliferation in TGF-β1-stimulated renal fibroblasts and mesangial cells. No evidence has shown that increased cAMP in tubular epithelial cells can recapitulate such an inhibition, although it is demonstrated in ovarian granulose cells. Although increased cAMP increases CTGF protein by inhibiting its breakdown without affecting mRNA level in bovine endothelial cells, it has been reported to downregulate the expression of several additional TGF-β1 inducible genes in most sensitive cells. Thus, sensitivity to cAMP appears to be a common feature for many TGF-β1-regulated genes and one common TGF-β1-induced signaling pathway may be the target of cAMP-activated molecules. Smad proteins transduce signals from the TGF-β1 receptors to regulate the target genes, including collagen and α-SMA. Cross-talks with other signaling pathways may contribute to the regulation of TGF-β1's effects. In this study, we demonstrated that Smad, not MAPK and PI3K/Akt pathways, mediated TGF-β1's signaling into CTGF transcription in NRK-49F and NRK-52E. We further demonstrated that PTX inhibited TGF-β1-induced Smad3/4-dependent CTGF transcription without affecting Smad activation and nuclear translocation, which is in agreement with findings in other cell types. Schiller et al. demonstrate that transcription coactivators CREB-binding protein (CBP) and p300 are essential for Smad3/4-dependent gene transcription. In the absence of effect on nuclear translocation and DNA binding of Smad3/4 complexes, cAMP-elevating agents can interfere with Smad3/4-dependent gene transcription in nucleus via reduction of Smad3/4-CBP/p300 interactions and possible sequestration of CBP and p300 by phosphorylated CREB in a PKA-dependent manner.

Smad mediates TGF-β1 signaling to the activation of α-SMA transcription in mouse AKR-2B fibroblasts and normal skin fibroblasts. However, the activation of Smad is not enough to induce α-SMA expression in NRK-52E because such expression was blocked by antisense CTGF gene transfection, a finding in agreement with that in human proximal tubular epithelial cells. Whereas, interference with TGF-β1-induced Smad signaling by peroxisome proliferator-activated receptor gamma activation or hepatocyte growth factor treatment has been shown to downregulate α-SMA expression in normal skin fibroblasts and human kidney epithelial cells. Thus, we speculate that

PTX inhibits TGF-β1-induced expression of α-SMA in NRK-52E by inhibiting CTGF expression through blocking Smad-dependent gene transcription.

The nature of signaling pathways distal to the CTGF stimulation that are regulated by PTX remains to be defined. Recent evidence shows that CTGF can activate Smad pathway in human proximal tubule cells and cortical fibroblasts, a potential target of PTX in disruption of CTGF signaling. In addition to the Smad pathway, small G protein Rho has been shown to be indispensable for α-SMA activation in porcine proximal tubular epithelial cells. Interestingly, PKA can phosphorylate Rho and regulate its activity negatively. We are now undergoing studies to examine the signaling pathways distal to recombinant CTGF stimulation in NRK-49F and NRK-52E that will be crucial for Col I (α1) and α-SMA expression and the possible functional position of PTX.

In conclusion, our results demonstrated that PTX not only inhibited CTGF expression by interfering with Smad3/4-dependent CTGF transcription through PKA, but also blocked profibrogenic effects of CTGF on renal cells.