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Prostaglandin E2 receptor subtypes 1 and 2 play a role in TGF-β1-induced renal fibrosis by regulating endoplasmic reticulum stress

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Abstract. – OBJECTIVE: This study aimed to investigate the effects of prostaglandin E2 receptor subtypes 1 (EP1) and 2 (EP2) on endoplasmic reticulum (ER) stress induced by TGF-β1 in mouse mesangial cells (MCs) and to explore its potential mechanisms.

MATERIALS AND METHODS: Mouse mesangial cells were isolated and cultured. EP-siRNAs were transfected into mesangial cells for silencing EP1 and EP2. Mesangial cell proliferation was assessed by the CCK-8 method. Expression of PGE2 was measured by enzyme-linked immunosorbent assay (ELISA). GRP78, TRPC1, ERK1/2, and phospho-ERK1/2 levels were examined by Western blot.

RESULTS: TGF-β1 induced mesangial cell proliferation and increased PGE2 secretion. Besides, TGF-β1 significantly upregulated GRP78 and TRPC1 expression at the protein level. Phospho-ERK1/2 protein amounts were also increased (*p*<0.05). Compared with the TGF-β1 group, cell proliferation in the EP1-siRNA+TGF-β1 group was reduced, while GRP78, TRPC1, and ERK1/2 protein amounts were downregulated (*p*<0.05). EP1 agonist significantly enhanced above changes and their activities (*p*<0.05). EP1 antagonist significantly attenuated the above changes (*p*<0.05). Compared with TGF-β1 group, cell proliferation in EP2-siRNA+TGF-β1 group was increased, while GRP78, TRPC1, and ERK1/2 protein amounts were increased (*p*<0.05). EP2 agonist significantly attenuated the above changes (*p*<0.05).

CONCLUSIONS: EP1 receptor may increase TGF-β1-induced cell damage by increasing the activities of GRP78, TRPC1, and ERK1/2 *via* ER stress. Meanwhile, the EP2 receptor may reduce TGF-β1-induced cell damage by suppressing GRP78, TRPC1, and ERK1/2 activities, also *via* ER stress. EP1 inhibition and EP2 stimulation may be a therapeutic option for delaying renal fibrosis.

Key Words:
Prostaglandin E2 receptor, Mesangial cell, Transforming growth factor-β1, Endoplasmic reticulum stress.

Introduction

Chronic kidney disease (CKD) has high incidence and poor prognosis. In addition, CKD treatment requires high medical expenses, making this disease a global public health burden[1]. Renal fibrosis is the common pathway for the progression of all types of CKD to end-stage renal disease (ESRD), and pathologically initiates many CKDs. It negatively affects renal interstitial cell proliferation and leads to excessive extracellular matrix (ECM) deposition, ultimately causing kidney failure. Transforming growth factor-β1 (TGF-β1) is considered the main and potent fibrogenic growth factor[2], as well as an important regulator of ECM deposition and renal fibrosis progression.

The endoplasmic reticulum (ER) represents the main site of cell protein processing and calcium accumulation, and is involved in protein folding and cell apoptosis. Breaking the steady state of the ER would lead to altered protein transport and Ca^{2+} release, which subsequently causes ER stress (ERS). In glomerular MCs, the calcium-operated Ca^{2+} channel (SOC) is associated with calcium signaling[3]. Meanwhile, TRPC1 and TRPC4 expression is evident in transient receptor potential cation channel protein (TRPC)[4]. High glucose regulatory protein (GRP78) is one of the most important endoplasmic reticulum stress markers, and specifically reflects ERS response.

Mitogen-activated protein kinase (MAPK) signaling is a critical signal transduction network in the cell, which is involved in transduction of extracellular signals from the cell surface to the interior, and represents a common pathway for intracellular information transmission. Multicellular organisms have a variety of MAPKs, of which ERK1/2, JNK, and p38 are widely studied[5].

Prostaglandin E2 (PGE2) represents a major arachidonic acid metabolite in the kidney, and exerts

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its effects through interactions with four 7-transmembrane G-protein-coupled prostaglandin receptors (EP1, EP2, EP3, and EP4). Regulating multiple physiological and pathophysiological processes in the kidney, all EP receptors are localized in the kidney and are responsible for the various PGE2 effects. Upon activation, EP1 is involved in regulating intracellular calcium levels and increasing intracellular Ca^{2+} amounts. A significant *in situ* hybridization EP1 signal has been reported in the mesangial area, and high glucose-induced mesangial cell proliferation can be almost completely inhibited by EP1 antagonists[6]. EP2 is a Gs protein-coupled receptor. Ganesh et al[7] have shown that EP2 receptor expression is significantly higher in rats after 5/6 nephrectomy compared with control group. However, the effects and mechanisms of EP1 and EP2 in kidney disease remains unclear. To assess the functions of EP1 and EP2 receptors in renal fibrosis and CKD, we cultured primary wild-type MCs, inhibited EP1 and EP2 expression by RNA interference, and observed changes in ERS and renal fibrosis markers upon addition of EP1 and EP2 agonists and antagonists. This study revealed roles for EP1 and EP2 in the pathogenesis of renal fibrosis, as well as the function played by ERS in this process, thereby providing new methods for slowing down and treating CKD.

Materials and Methods

Materials and Reagents

17-phenyl PGE2 (EP1 agonist), SC19220 (EP1 antagonist), butaprost (EP2 agonist), AH6809 (EP2 antagonist) and EP1 and EP2 antibodies were all provided by Cayman (Ann Arbor, MI, USA). Recombinant human TGF-β1 was provided by PeproTech (Rocky Hill, NJ, USA). Cholecystokinin-octapeptide (CCK-8) and TRIzol reagent were manufactured by Invitrogen (Carlsbad, CA, USA), while mouse PGE2 enzyme-linked immunosorbent assay (ELISA) kit was obtained from R&D Systems (Minneapolis, MN, USA). The Real Time quantitative Polymerase Chain Reaction (PCR) kit was manufactured by Roche (Basel, Switzerland). Rabbit anti-mouse extracellular signal-regulated kinases 1 and 2 (ERK1/2) and phosphorylated ERK1/2 monoclonal antibodies (Cell Signaling, Danvers, MA, USA), anti-mouse β-actin, GRP78, and TRPC1 monoclonal antibodies (Abcam, CA, MA, USA), as well as horseradish peroxidase(HRP)-labeled goat anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were employed.

Animals and Ethics Committee Approval

C57BL/6 mice were obtained from Nantong University and singly housed under a 12 h - 12 h light/dark cycle at 22±3°C, with freely available rodent chow and water. All efforts were made for minimizing suffering and reducing the amounts of mice utilized. All the experiments on animals strictly complied with the ethical standards of the Animal Care and Use Committee of Nantong University. This investigation was approved by the Animal Ethics Committee of Nantong University Animal Center.

Isolation and Culture of Primary Mesangial Cells (MCs)

The mice were sacrificed under anesthesia, and the kidneys were extracted (with capsules). After the kidney capsule was removed, the cortex was minced and submitted to collagenase (1 mL/kidney) digestion at 37°C with shaking for 40 min. Then, the digested products were filtered with mesh, and the glomeruli were harvested and cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA). Purified primary mesangial cells (MCs) could be obtained in 5-8 generations.

Transfection of Small Interfering RNAs (siRNAs)

Specific siRNAs for EP1 or EP2 were designed and synthesized by GenePharma (Shanghai, China). The density of mouse MCs in primary culture was adjusted to 1×10^5/mL, and MCs were seeded in 6-well plates with culture medium containing serum but no antibiotics in a 37°C incubator with 5% CO_2. After overnight culture to 70-80% confluency, the above medium was substituted by serum free medium. EP1-siRNA, EP2-siRNA or negative control (NC)-siRNA were transfected into wild type mouse MCs following the protocol of Lipofectamine™ 2000 transfection reagent (Invitrogen, Carlsbad, CA, USA). After 12 h of culture, 10 μg/L of TGF-β1 was added for 24 h, and EP1 and EP2 mRNA levels in each group were assessed by quantitative real-time PCR. The siRNAs with the highest interference efficiencies were selected.

Quantitation of MC Proliferation by CCK-8

Cells seeded at 5×10^4/well in sterile 96-well plates were cultured in 100 μL complete medium. When cells reached 70-80% confluency, siRNA

transfection was performed. After 4-6 h, the culture medium was substituted by complete medium with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 10 μg/L TGF-β1 for 24 h. CCK-8 reagent was supplemented, and then cells were incubated at 37°C in the dark for 2-4 h. Absorbance was measured by a dual-wavelength method (570 nm with background wavelength at 630 nm).

Detection of PGE2 Content in MCs by Enzyme-Linked Immunosorbent Assay (ELISA)

Mouse MCs in logarithmic growth phase seeded in 6-well plates (1×10^5/well) were cultured in a 37°C incubator with 5% CO_2. After synchronous quiescence, siRNA transfection was performed separately. Upon incubation for 4-6 h, 10 μg/L TGF-β1 was added. An equal volume of 0.1% bovine serum albumin (BSA) was added to control group. After 24 h, supernatants were collected for PGE2 content assessment following the instructions of the specific ELISA kit.

Quantitative Real Time PCR

Total RNA extraction was carried out with TRIzol reagent (Invitrogen, Carlsbad, CA, USA), respectively, followed by measurement of RNA concentration using an ultraviolet spectrophotometer (Hitachi, Tokyo, Japan). The complementary Deoxyribose Nucleic Acid (cDNA) was synthesized according to the instructions of the PrimeScript™ RT MasterMix kit (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed according to RT-PCR kit instructions (Roche, Basel, Switzerland). The sequences of the PCR primers are shown in Table I. The PCR reaction system was 20 μL, and the amplification conditions were as follows: (1) predenaturation at 95°C for 10 min; (2) 40 cycles of 95°C for 15 s and 60°C for 60 s; and (3) dissociation curve at 95°C for 15 s, 60°C for 1 h, 95°C for 15 s, and 60°C for 15 s. The cycle threshold (CT) value was recorded after the reaction was complete, and the experiment was repeated three times. The CT value of the housekeeping gene was subtracted from the CT value of each group of genes to obtain ΔCT, and the ΔCT of normal group was subtracted from that of each group to obtain ΔΔCT. The difference in mRNA expression between experimental group and control group was analyzed by the $2^{-\Delta\Delta CT}$ method.

Western Blot

After 24 h of culture, cells were harvested using cell lysis buffer, and protein amounts were determined. Equal amounts of protein were resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), electro-transferred onto polyvinylidene difluoride (PVDF) membranes (Roche, Basel, Switzerland), and blocked with 5% BSA for 2 h. Then, GRP78, TRPC1, ERK1/2, and phosphorylated ERK1/2 monoclonal antibodies (1:1000) were added to the membranes and incubated overnight at 4°C, respectively. This was followed by incubation with horseradish peroxidase (HRP)-labeled secondary antibodies (1:1000) for 2 h in ambient conditions and development with the Bio-Rad (Hercules, CA, USA) developer.

Immunohistochemistry

Conventional sectioning of paraffin embedded specimens and dehydration were performed. Sections were placed in antigen retrieval solution and heated in a pressure cooker to boiling. The pressure valve was closed for 1-4 min after complete pressure release. After cooling to room temperature, the specimens underwent phosphate-buffered saline (PBS) washes and treatment with 3% hydrogen peroxide (H_2O_2, 10 min). This was followed by incubation with goat serum (working solution) in ambient conditions for 20 min. Then, the specimens were successively incubated with primary (4°C, 24 h) and secondary (37°C, 30 min) antibodies, followed by diaminobenzidine (DAB) color development (Solarbio, Beijing, China).

Table I. PCR primer sequences.

Gene	Primer (5'-3')	Fragment size (bp)
EP1	Sense strand TAACGATGGTCACGCGATGG Antisense strand ATGCAGTAGTGGGCTTAGGG	291
EP2	Sense strand ATACTTAGGCCACCGGTCCT Antisense strand TGAAGCGCATCCTCACAACT	153
GAPDH	Sense strand AGAAGGAAATGGCTGCAGAA Antisense strand GCTCGGCTTCCAGTATTGAG	238

Statistical Analysis

Measurement data $\bar{x}\pm s$ was assessed by one-way analysis of variance (ANOVA) for multiple groups, followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ indicated statistical significance. Statistical Product and Service Solutions (SPSS) 16.0 (SPSS Inc., Chicago, IL, USA) was employed for all statistical analyses.

Results

MC Proliferation after TGF-β1 Stimulation and Expression of PGE2 in MC Culture Supernatants

After siRNA transfection, MCs were administered 10 ng/mL TGF-β1. Immunoblot analysis revealed that EP1 protein levels were lower in EP1-siRNA group compared with the normal control and NC-siRNA groups ($p<0.05$, Figures 1A and 1B). Similarly, EP2 protein expression in EP2-siRNA group was significantly reduced ($p<0.05$, Figures 1C and 1D).

In CCK-8 assay, after treatment with 10 ng/mL TGF-β1 for 24 h, the cell proliferation ability was significantly enhanced in comparison with normal control cells ($p<0.05$). In comparison with TGF-β1 group, EP1-siRNA+TGF-β1 group showed significantly less cells, while EP2-siRNA+TGF-β1 group showed significantly increased cell number ($p<0.05$). The differences were statistically significant, as shown in Figures 1E and 1F.

ELISA results revealed that after treatment with 10 ng/mL TGF-β1 for 24 h, PGE2 amounts in cell culture supernatants were significantly increased compared with those of normal control cells ($p<0.05$). PGE2 levels in cell culture supernatants of the EP1-siRNA+TGF-β1 group were reduced in comparison with those of the TGF-β1 and NC-siRNA+TGF-β1 groups. Similarly, PGE2 amounts in the EP2-siRNA+TGF-β1 group were also significantly decreased ($p<0.05$), as shown in Figures 1G and 1H.

Effects of SiRNA Transfection on the Expression Levels of TGF-β1-Induced GRP78, TRPC1, and ERK1/2 in Mouse MCs

Immunoblot revealed that after administration of 10 ng/mL TGF-β1 to MCs, GRP78 and TRPC1 protein amounts in the TGF-β1, NC-siRNA+TGF-β1, and EP1-siRNA+TGF-β1 groups were significantly increased in comparison with those of normal control cells (Figures 2A-2C) ($p<0.05$). Compared with TGF-β1 and NC-siRNA+TGF-β1 groups, GRP78 and TRPC1 protein amounts in EP1-siRNA+TGF-β1 group were significantly decreased (Figures 2A-2C), while those of EP2-siRNA+TGF-β1 group were further upregulated with statistical significance (Figures 2D-2F all $p<0.05$).

Immunoblot demonstrated that upon administration of 10 ng/mL TGF-β1 to MCs, the protein expression levels of ERK1/2 in the TGF-β1, NC-siRNA+TGF-β1, and EP1-siRNA+TGF-β1 groups were significantly increased in comparison with those of normal control cells ($p<0.05$). Compared with TGF-β1 and NC-siRNA+TGF-β1 groups, ERK1/2 protein amounts in EP1-siRNA+TGF-β1 group were decreased, while those of EP2-siRNA+TGF-β1 group were further increased, with statistical significance ($p<0.05$), as shown in Figures 2H-2K.

Effects of 17-Phenyl PGE2 (EP1 Agonist), SC19220 (EP1 Antagonist), Butaprost (EP2 agonist), and AH6809 (EP2 Antagonist) on TGF-β1-Induced GRP78 and TRPC1 Expression in Mouse MCs

Immunoblot indicated that GRP78 and TRPC1 protein amounts in TGF-β1 group were significantly elevated in comparison with control values ($p<0.05$). In comparison with TGF-β1 group, GRP78 and TRPC1 protein amounts in 17-phenyl PGE2 group were markedly elevated, while those of SC19220 group were remarkably reduced, with statistical significance ($p<0.05$), as shown in Figures 3A-3F.

In addition, GRP78 and TRPC1 protein amounts in TGF-β1 group were significantly higher than control values ($p<0.05$). In comparison with TGF-β1 group, GRP78 and TRPC1 protein amounts in the butaprost group showed a significant reduction, while those of AH6809 group were markedly elevated ($p<0.05$), as shown in Figures 3G-3L.

Immunohistochemical Changes in the EP-siRNA Mice Model with 5/6 Nephrectomy

The renal tissue was fixed with 4% formalin perfusion and paraffin embedded for sectioning. The immunohistochemical changes of the renal glomeruli were observed. In comparison with the amounts in WT CON group, connective tissue growth factor and GRP78 levels in some renal

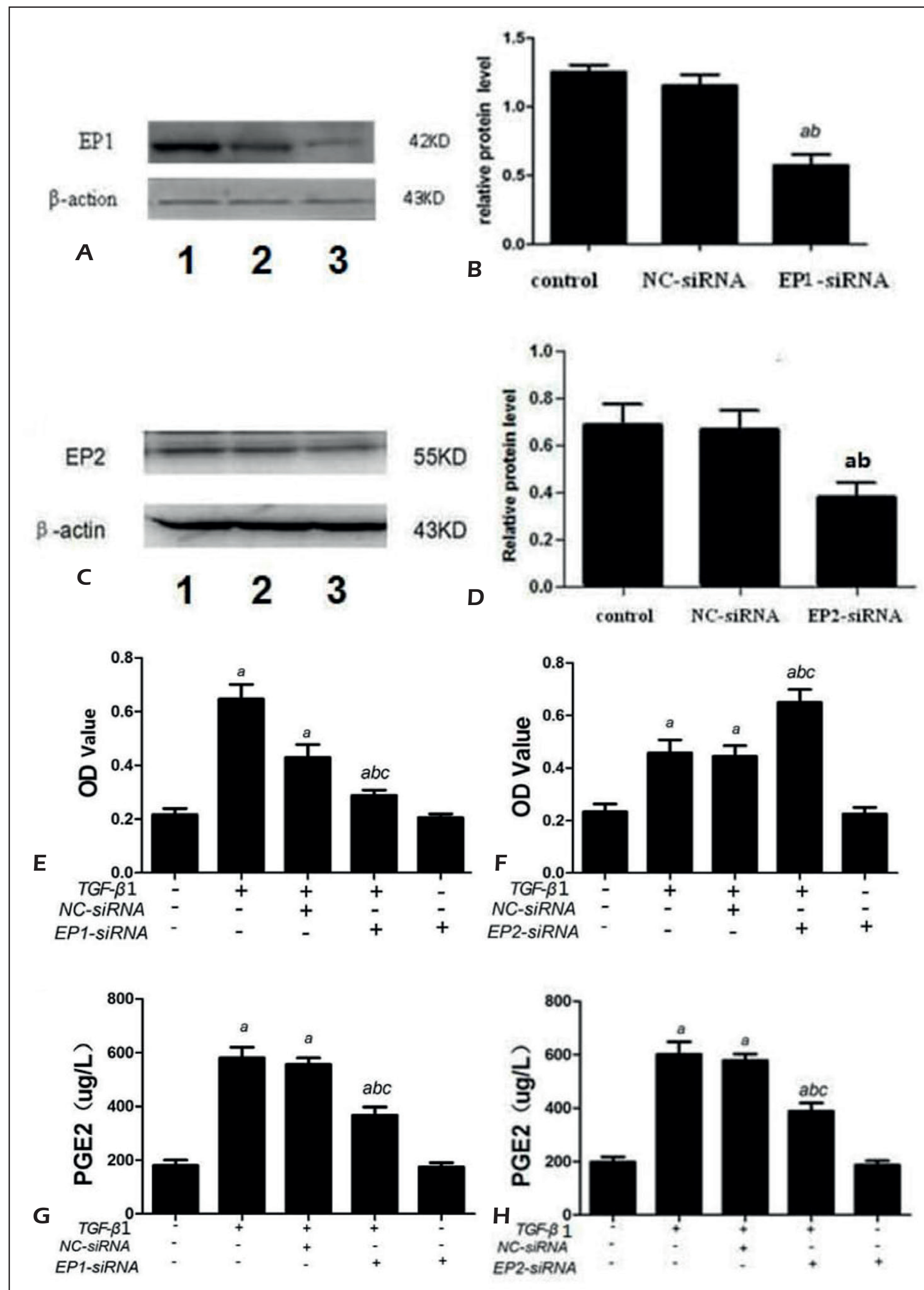

Figure 1. MCs proliferation after TGF-β1 stimulation and PGE2 levels in MC culture supernatants. **A**, and **B**, EP1 protein expression in glomerular MCs of each group after siRNA transfection. **C**, and **D**, EP2 protein expression in glomerular MCs of each group after siRNA transfection. **E**, and **F**, MCs proliferation in each group after siRNA transfection. **G**, and **H**, PGE2 content in MCs of each group after siRNA transfection. 1, Normal control group; 2, NC-siRNA group; 3, EP1/EP2-siRNA group. $^{a}p<0.05$, *vs.* normal control group; $^{b}p<0.05$, *vs.* NC-siRNA group; $^{c}p<0.05$, *vs.* NC-siRNA+TGF-β1 group.

glomeruli in renal tissue samples from the 5/6 nephrectomy group were markedly elevated. In comparison with EP1-siRNA CON group, EP1-siRNA group showed reduced GRP78 and TRPC1 densities in the renal glomeruli. In comparison with EP2-siRNA CON group, EP2-siRNA group had higher GRP78 and TRPC1 densities in the renal glomeruli, suggesting that EP1 receptor stimulation and EP2 receptor inhibition promoted renal fibrosis, as shown in Figures 4A-4D.

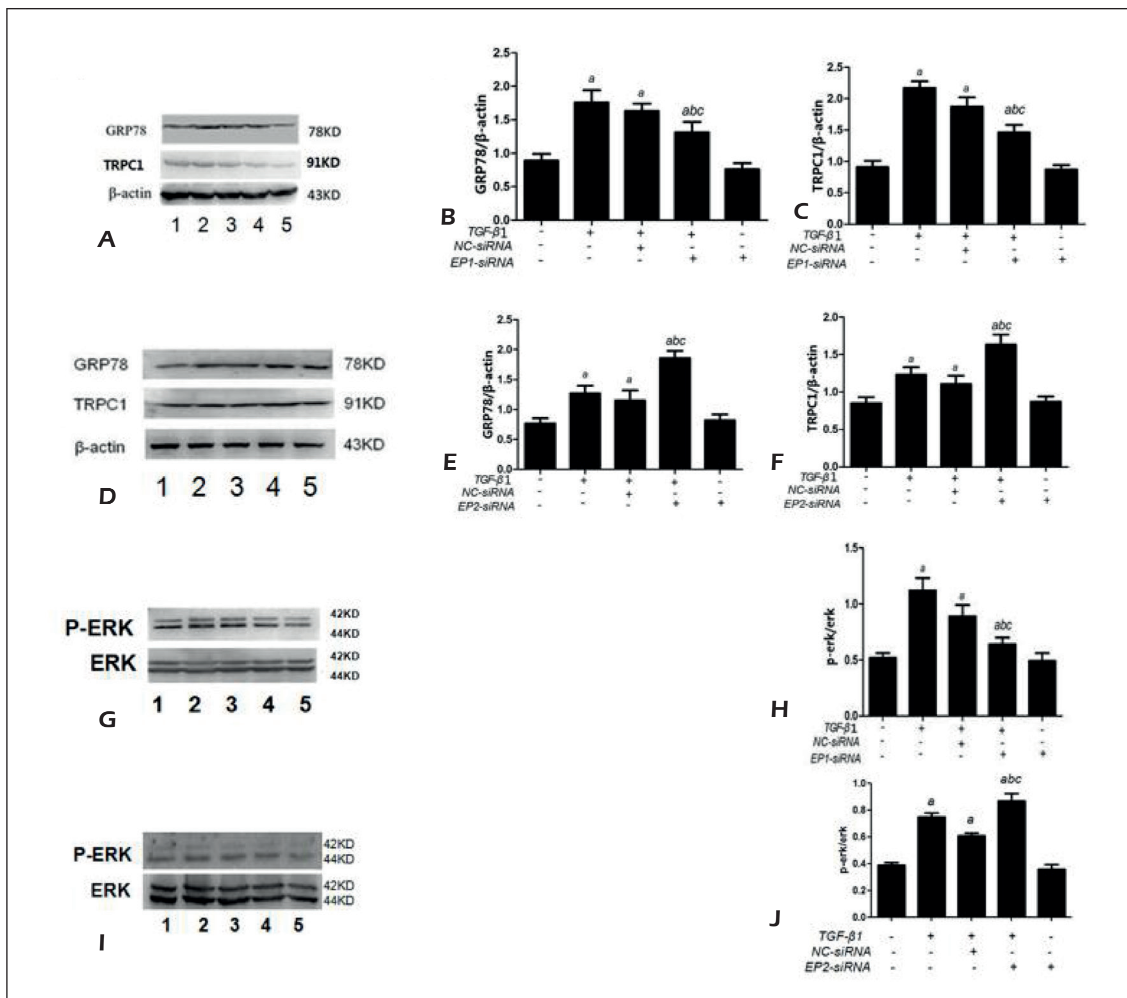

Figure 2. Effects of siRNA transfection on TGF-β1-induced GRP78, TRPC1, and ERK1/2 protein levels in mouse MCs. **A**-**C**, GRP78 and TRPC1 protein amounts in MCs after transfection with EP1-siRNA. **D**-**F**, GRP78 and TRPC1 protein amounts in MCs after transfection with EP2-siRNA. **G**, and **H**, ERK1/2 protein amounts in MCs after transfection with EP1-siRNA. **I**, and **J**, ERK1/2 protein amounts in MCs after transfection with EP2-siRNA. 1, Normal control group; 2, TGF-β1 (10 μg/L) group; 3, NC-siRNA+TGF-β1 (10 μg/L) group; 4, EP1/EP2-siRNA+TGF-β1 (10 μg/L) group; 5, EP1/EP2-siRNA group. $^{a}p<0.05$, *vs.* normal control group; $^{b}p<0.05$, *vs.* TGF-β1 group; $^{c}p<0.05$, *vs.* NC-siRNA+TGF-β1 group.

Discussion

The main pathological characteristic of renal fibrosis is the activation of renal ECM-producing cells, among which MCs constitute one of the main groups. When injured, the proliferation and phenotype of MCs may change to produce the major ECM constituents, including fibronectin (FN) and connective tissue growth factor (CTGF), which may directly lead to and exacerbate ECM deposition in glomeruli, thereby impairing the renal tissue structure and ultimately leading to renal fibrosis. TGF-β1 represents a well-known fibrogenic growth factor and an important regulator of ECM deposition and renal fibrosis progression. TGF-β1 has certain functions in the pathological changes of MCs hypertrophy in diabetes mellitus and other glomerular diseases[8]. TGF-β1 upregulates α1(I) collagen mRNA expression by activating the P38 pathway and promotes the synthesis of collagen and FN accumulation through the ERK1/2 pathway[9,10].

Endoplasmic reticulum stress (ERS) is a pathological state in which the endoplasmic reticulum is dysfunctional; Ca^{2+} homeostasis is lost, and misfolded or unfolded proteins accumulate in the lumen of the ER. Appropriate ERS maintains intracellular homeostasis and protects cell functions, while excessive ERS disrupts ER homeostasis and induces apoptosis[11]. It is known that ERS has a critical function in high glucose-associated phenotypic transformation of human

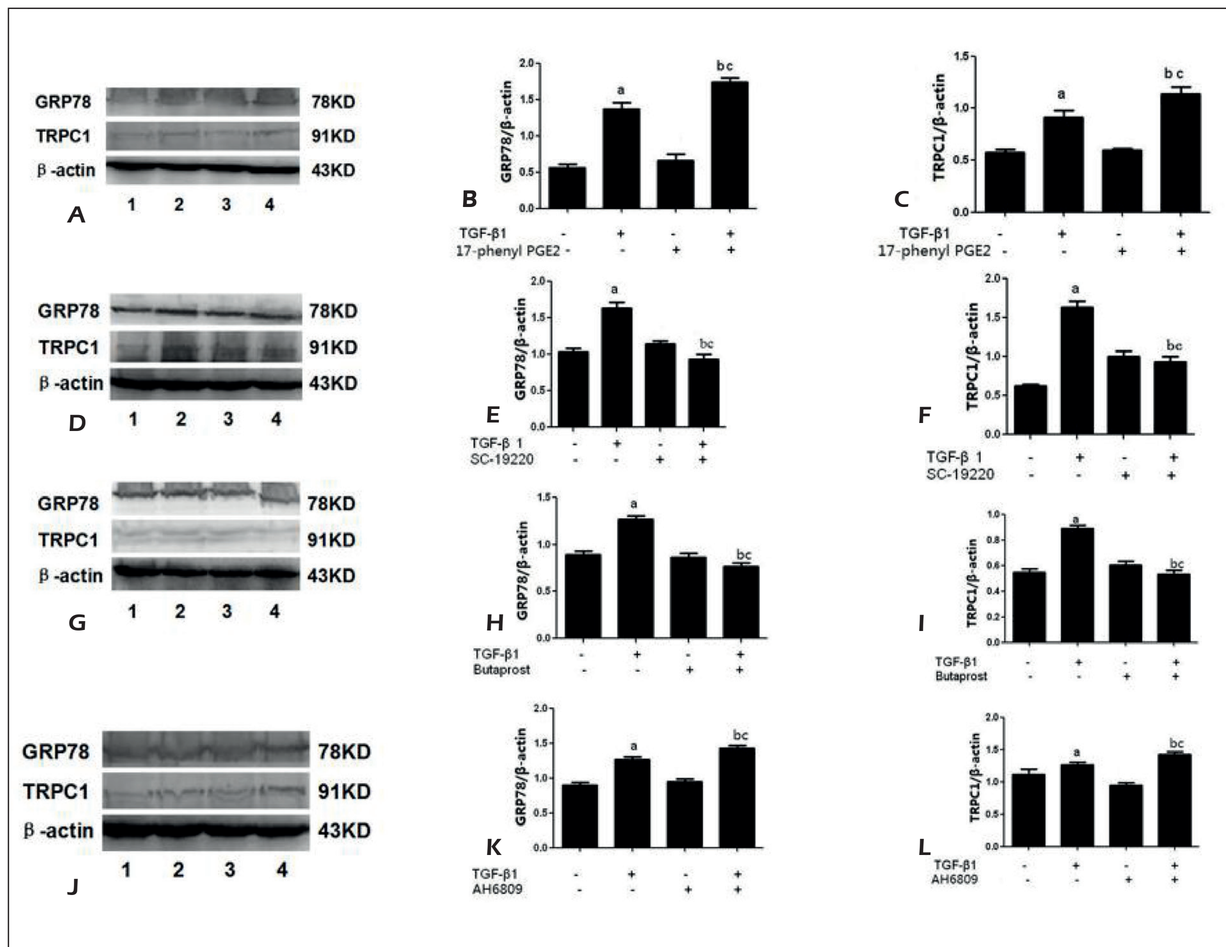

Figure 3. Effects of 17-phenyl PGE2, SC19220, butaprost, and AH6809 on TGF-β1-induced GRP78 and TRPC1 protein expression in mouse MCs. **A-C**, Effects of 17-phenyl PGE2. **D-F**, Effects of SC19220. G-I, Effects of butaprost. **J-L**, Effects of AH6809. 1, Normal control group; 2, TGF-β1 group; 3, agonist/antagonist group; 4, TGF-β1+ agonist/antagonist group. [a]$p<0.05$, *vs.* normal control group; [b]$p<0.05$, *vs.* agonist/antagonist group; [c]$p<0.05$, *vs.* TGF-β1 group.

MCs. GRP78 is an important molecular marker of ERS. GRP78 amounts in pancreatic islet β cells are increased under high glucose stimulation in diabetic nephropathy[12], confirming that high glucose induces the ERS response. Fonseca et al[13] demonstrated GRP78 is upregulated in the MCs and podocytes of rats with anti-Thy1 nephritis, while phospho-ERK (P-ERK) and eukaryotic initiation factor 2 (eIF2) amounts are significantly increased. These results indicate that the ERK pathway may be associated with ERS and might participate in its development. Studies have shown that ERS activates apoptotic pathways, including the c-Jun NH 2-terminal kinase (JNK) and P38-CHOP pathways, to promote macrophage apoptosis in advanced atherosclerotic lesions[14,15]. PGE2 is the most widely distributed and diverse active substance in tissues, and represents an important metabolite of arachidonic acid. To date, PGE2 has four known subtypes, namely EP1, EP2, EP3, and EP4, which display different distribution patterns and physiological functions in the renal tissue. EP1 is mainly arranged on the collecting duct to regulate calcium ion levels. EP3 is distributed in the collecting duct and the medullary loop, and is coupled with the suppressive G protein (Gi) to negatively regulate adenylate cyclase (cAMP). EP2 is expressed in blood vessels, while EP4 is found in glomeruli and collecting ducts. The function of EP2 and EP4 is opposite to that of EP3; they are both non-suppressive Gs-associated receptors which enhance cAMP levels[16]. As shown above, GRP78 and TRPC1 amounts were elevated in MCs after TGF-β1 stimulation. Moreover, GRP78 and TRPC1 protein amounts were remarkably decreased after EP1 receptor silencing or administration of a specific EP1 antagonist. After treatment with a specific EP1 agonist,

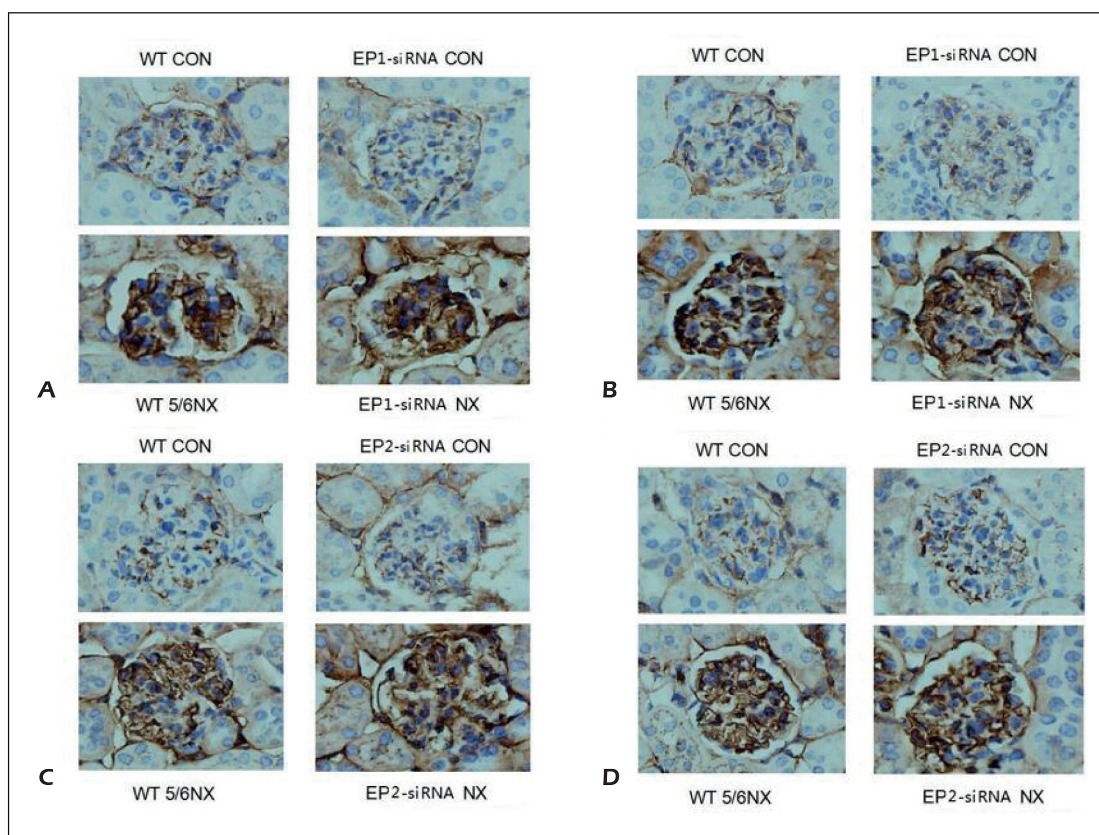

Figure 4. Immunohistochemical changes in the EP-siRNA mouse model with 5/6 nephrectomy. **A**, Immunohistochemical observations of GRP78 expression in the renal tissue. Typical expression of GRP78 in the glomeruli and renal tubules of the WT and EP1-siRNA groups (×400). **B**, Immunohistochemical observations of TRPC1 expression in the renal tissue. Typical expression of TRPC1 in the glomeruli and renal tubules of the WT and EP1-siRNA groups (×400). **C**, Immunohistochemical observations of GRP78 expression in the renal tissue. Typical expression of GRP78 in the glomeruli and renal tubules of the WT and EP2-siRNA groups (×400). **D**, Immunohistochemical observations of TRPC1 expression in the renal tissue. Typical expression of TRPC1 in the glomeruli and renal tubules of the WT and EP2-siRNA groups (×400).

GRP78 and TRPC1 protein amounts were markedly elevated, suggesting that GRP78 and TRPC1 are involved in ERS. However, inhibition of EP1 receptor expression had an inhibitory effect on ERS. After EP2 receptor silencing or administration of a specific EP2 antagonist, GRP78 and TRPC1 amounts were markedly elevated than those of TGF-β1 group. After treatment with an EP2 agonist, GRP78 and TRPC1 protein levels were remarkably lower compared with those of TGF-β1 group. These findings suggest EP1 receptor inhibition and EP2 receptor simulation downregulate GRP78 and TRPC1, which may in turn inhibit ERS-induced apoptosis, thereby reducing MCs damage.

MAPKs constitute a family of intracellular serine/threonine protein kinases, including ERK, p38MAPK, and JNK, which regulate multiple physiological processes, including cell proliferation, cell death, and functional synchronization between cells, etc[17]. The ERK pathway is essential in renal fibrosis. ERK and p38 are activated in renal tubular epithelial cells of diabetic rats, which might control glucose-associated cell hypertrophy and TGF-β1 biosynthesis[18]. Chi et al[19] have also shown that EP1 receptor-mediated cell proliferation is regulated by the ERK/MARK pathway in TGF-β1-treated MCs in rats. As shown above, TGF-β1 increased ERK phosphorylation levels in MCs of all groups, and ERK phosphorylation levels were decreased after EP1 receptor silencing or administration of a specific EP1 antagonist. ERK phosphorylation levels were markedly increased after administration of an EP1 agonist. After knockdown of EP2 receptor or administration of a specific EP2 antagonist, ERK phosphorylation levels were remarkably elevated in comparison with control values. After treatment with an EP2

agonist, ERK phosphorylation levels were markedly decreased. These findings suggested that TGF-β1-induced cell proliferation may be partly associated with EP1-mediated phosphorylation of ERK. Thus, inhibiting the EP1 receptor and antagonizing the EP2 receptor may decrease ERK expression, which may reduce MC damage.

Conclusions

We showed that EP1 receptor inhibition and EP2 receptor stimulation could reduce TGF-β1-associated cell proliferation and downregulate ERS-related indicators (GRP78 and TRPC1), reducing TGF-β1-induced injury in mouse MCs, which might be associated with the inhibition of excessive ERK signaling activation. These results indicate that EP1 receptor inhibition and EP2 receptor stimulation may be promising treatments for delaying renal fibrosis.

Acknowledgments

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Conflict of Interests

The Authors declare that they have no conflict of interests.

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