The Notch pathway mediates the angiotensin II-induced synthesis of extracellular matrix components in podocytes

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Abstract. The Notch pathway is known to contribute to the development of glomerular disease. Angiotensin II (Ang II), an important member of the renin-angiotensin system, stimulates the accumulation of extracellular matrix components in glomerular disease; however, the exact mechanisms involved remain to be elucidated. In the present study, we aimed to investigate the effects of the Notch pathway on the synthesis of extracellular matrix components in Ang II-stimulated podocytes. Mouse podocytes were stimulated with Ang II (10^{-6} mol/l). The activation of the Notch pathway was inhibited by a vector carrying short hairpin RNA (shRNA) targeting Notch1 (sh-Notch1) or by γ-secretase inhibitor (GSI). The protein levels of Notch1, Notch intracellular domain 1 (NICD1), hairy and enhancer of split-1 (Hes1), matrix metalloproteinase (MMP)-2, MMP-9, transforming growth factor-β1 (TGF-β1), type IV collagen and laminin were determined by western blot analysis. The Notch1, Hes1, MMP-2, MMP-9, TGF-β1, type IV collagen and laminin mRNA levels were detected by RT-PCR. The MMP-2 and MMP-9 activity was measured using a cell active fluorescence assay kit. The levels of TGF-β1, type IV collagen and laminin were determined in the culture medium of the podocytes by enzyme-linked immunosorbent assay (ELISA). Our results revealed that Ang II upregulated Notch1, NICD1, Hes1, TGF-β1, type IV collagen and laminin expression and downregulated MMP-2 and MMP-9 expression in the cultured podocytes. The inhibition of the Notch pathway by sh-Notch1 or GSI increased MMP-2 and MMP-9 expression, decreased the TGF-β1 level and suppressed type IV collagen and laminin expression. The inhibition of the Notch pathway by sh-Notch1 or GSI also increased MMP-2 and MMP-9 activity, and decreased TGF-β1 levels, type IV collagen levels and laminin secretion. These findings indicate that the Notch pathway potentially mediates the Ang II-induced synthesis of extracellular matrix components in podocytes through the regulation of MMPs and TGF-β1.

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Introduction

It is considered that the dysfunction of podocytes, which are a type of glomerular epithelial cell, plays an important role in progressive renal diseases, including focal segmental glomerulosclerosis (FSGS) and diabetic nephropathy (DN) (1,2). Podocyte injury is also associated with a higher degree of albuminuria and leads to the development of glomerulosclerosis. It has recently been demonstrated that the Notch pathway plays an important role in the onset and development of glomerular disease (3). Niranjan *et al* (4) reported that the Notch pathway was activated in podocytes in patients with FSGS and DN and in mouse models of these diseases. The Notch pathway is an evolutionarily conserved and widely used intercellular signaling pathway that influences cellular proliferation and differentiation (5,6). The binding of a ligand induces a conformational change in the Notch receptor. This allows an extracellular metalloprotease to cleave the receptor, which then allows the γ-secretase-mediated protease to release the Notch intracellular domain (NICD). NICD travels into the nucleus where it activates the transcription of downstream genes, such as the hairy and enhancer of split (Hes) gene.

It is known that alterations in hemodynamics contribute to the development of glomerular disease. Alterations in hemodynamics in glomerular disease activate angiotensin II (Ang II), an important member of the renin-angiotensin system, which damages podocytes and results in the accumulation of extracellular matrix (ECM) components and basement membrane thickening in the glomeruli (7,8). It is well recognized that in glomerular disease, Ang II increases the synthesis of ECM components, including collagens and laminin (9,10). Koshizaka *et al* (11) found that Ang II induced podocyte apoptosis through the activation of the Notch pathway in cultured podocytes. However, it is unclear as to whether the Notch pathway mediates the Ang II-induced ECM synthesis in podocytes.

Matrix metalloproteinases (MMPs) constitute a family of extracellular soluble or membrane-bound proteases that

collectively degrade or proteolytically modify essentially all the main components of the ECM, including collagens, laminin and proteoglycans. Gelatinases (e.g., MMP-2 and MMP-9), which degrade type IV collagen and laminin, are important constitutional elements of the basement membrane of the glomeruli (12,13). It is well established that transforming growth factor-β1 (TGF-β1) is a potent stimulator of ECM production in glomerular injury and may be the most important growth factor in determining the extent of renal fibrosis following injury. The exposure of cultured podocytes and mesangial cells to TGF-β1 has been shown to increase the production of type IV collagen and laminin (14-16). Previous studies have demonstrated that Ang II decreases matrix degradation due to a reduction in MMP activity and promotes the synthesis of ECM components through TGF-β1 (17,18).

In the present study, we aimed to investigate the hypothesis that Ang II may induce the activation of the Notch pathway in podocytes. The activation of the Notch pathway may alter the production of ECM through MMPs and TGF-β1 in Ang II-stimulated podocytes. The inhibition of the activation of the Notch pathway can prevent ECM accumulation and glomerulosclerosis.

Materials and methods

Cell culture. Conditionally immortalized mouse podocytes were purchased from the Cell Resource Center (Peking Union Medical College, Beijing, China). In this cell line, a temperature-sensitive mutant of the SV40 virus large T-cell antigen (tsA58 Tag) is controlled by a γ-interferon-inducible H-2K^b promoter. The podocytes were firstly cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) (both from Gibco-BRL, Gaithersburg, MD, USA) and 10 U/ml γ-interferon (PeproTech, Rochy Hill, NJ, USA) in a 33˚C 5% CO_2 atmosphere to induce proliferation, and were then incubated in RPMI-1640 medium supplemented with 10% FBS and deprived of γ-interferon in a 37˚C 5% CO_2 atmosphere for 14 days to induce quiescence and the differentiated phenotype, as previously described (19). The podocytes were grown to 75-85% confluence under growth restrictive conditions and growth-arrested in serum-free RPMI-1640 for 24 h to synchronize cell growth. After this time period, the medium was changed to fresh serum-free medium containing Ang II (10^{-6} mol/l; Sigma, St. Louis, MO, USA) at the indicated time points, as previously described (11). The transient transfection of the podocytes with a vector carrying short hairpin RNA (shRNA) targeting Notch1 (sh-Notch1) or a negative control vector (sh-Scramble) (Jingsai Corp., Wuhan, China) was carried out using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. For inhibition experiments, the cells were treated with γ-secretase inhibitor (GSI; Sigma) at 1 μmol/l for 30 min prior to stimulation with Ang II.

Western blot analysis. The cells were lysed in lysis buffer (20 mmol/l Tris·HCl, 2.5 mmol/l EDTA, 10% glycerol, 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, 10 mmol/l sodium pyrophosphate, 50 mmol/l NaF, 1 mmol/l sodium vanadate and 1 mmol/l PMSF) and the protein concentrations were measured by Coomassie brilliant blue assay. Protein (40 μg) was loaded and separated on a SDS-polyacrylamide gel and then transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore Corp., Billerica, MA, USA). The membrane was blocked with 5% dry milk and incubated overnight at 4˚C with rabbit anti-Notch1 (ab65297; 1:200 dilution), anti-NICD1 (ab52301; 1:200 dilution), anti-Hes1 (ab71599; 1:2,000 dilution) (all from Abcam, Cambridge, MA, USA), anti-MMP-2 (10737-2-AP; 1:500 dilution; Proteintech, Chicago, IL, USA), anti-MMP-9 (sc-10737; 1:400 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-TGF-β1 (18978-1-AP; 1:1,000 dilution), anti-type IV collagen (19797-1-AP; 1:1,000 dilution), anti-laminin (19698-1-AP; 1:1,000 dilution) (all from Proteintech) and anti-β-actin (sc-130656; 1:1,000 dilution; Santa Cruz Biotechnology, Inc.) polyclonal antibodies. After washing, the membrane was incubated with goat anti-rabbit IgG horseradish peroxidase conjugate (SA00001-2; 1:5,000 dilution; Proteintech). Proteins in western blot analysis were quantified following acquisition and analysis of the image using the software of a UVP Image Station Lab Works version 4.5. Proteins expression was quantified by comparison with the internal control, β-actin.

Reverse transcription PCR (RT-PCR). Total RNA was extracted from the podocytes using TRIzol reagent (Invitrogen) according to the instructions of the manufacturer and reverse transcribed using oligo(dT) primers in the presence of avian myeloblastosis virus reverse transcriptase to yield cDNA. The cDNA was amplified on a 7900HT Sequence Detection system (Applied Biosystems, Foster City, CA, USA) at default thermal cycling conditions: 2 min at 50˚C, 10 min at 95˚C for enzyme activation and then 40 cycles of 15 sec at 95˚C for denaturation and 1 min at 60˚C for annealing and extension. The results were analyzed using the relative standard curve method of analysis/ΔC_t method of analysis. The primers used for PCR were as follows: 18S forward, 5'-CGC CGC TAG AGG TGA AAT TC-3' and reverse, 5'-CCA GTC GGC ATC GTT TAT GG-3' (149 bp); Notch1 forward, 5'-GTG GAT GAC CTA GGC AAG TCG-3' and reverse, 5'-GTC TCC TCC TTG TTG TTC TGC A-3' (118 bp); Hes1 forward, 5'-CAC GAC ACC GGA CAA ACC A-3' and reverse, 5'-GCC GGG AGC TAT CTT TCT TAA GTG-3' (148 bp); MMP-2 forward, 5'-CAG GGA ATG AGT ACT GGG TCT ATT-3' and reverse, 5'-ACT CCA GTT AAA GGC AGC ATC TAC-3' (118 bp); MMP-9 forward, 5'-CAA TCC TTG CAA TGT GGA TG-3' and reverse, 5'-TAA GGA AGG GGC CCT GTA AT-3' (128 bp); TGF-β1 forward, 5'-ACC GCA ACA ACG CAA TCT ATG-3' and reverse, 5'-ATT CCG TCT CCT TGG TTC AG-3' (196 bp); type IV collagen forward, 5'-GTC AAA CTA CTG CTA TCC CTC CGT GTC-3' and reverse, 5'-CAT TCT ATA AAT GGA CTG GCT CGG AAT-3' (162 bp); laminin forward, 5'-CCT GCC AAA TTC CTC GGT AAC-3' and reverse, 5'-ACA TCG TAG GCA GAC GGC TG-3' (101 bp).

Enzyme activity assay. The activity of MMP-2 and MMP-9 in the serum-free conditioned medium was assayed using a cell active MMP-2 and MMP-9 fluorescence assay kit (GenMed Scientifics, Arlington, MA, USA), according to the manufacturer's instructions. The relative fluorescence units were determined with an excitation wavelength of 330 nm and an emission wavelength of 400 nm. The consistency of the fluorescent polypeptide segments was calculated on the basis of the

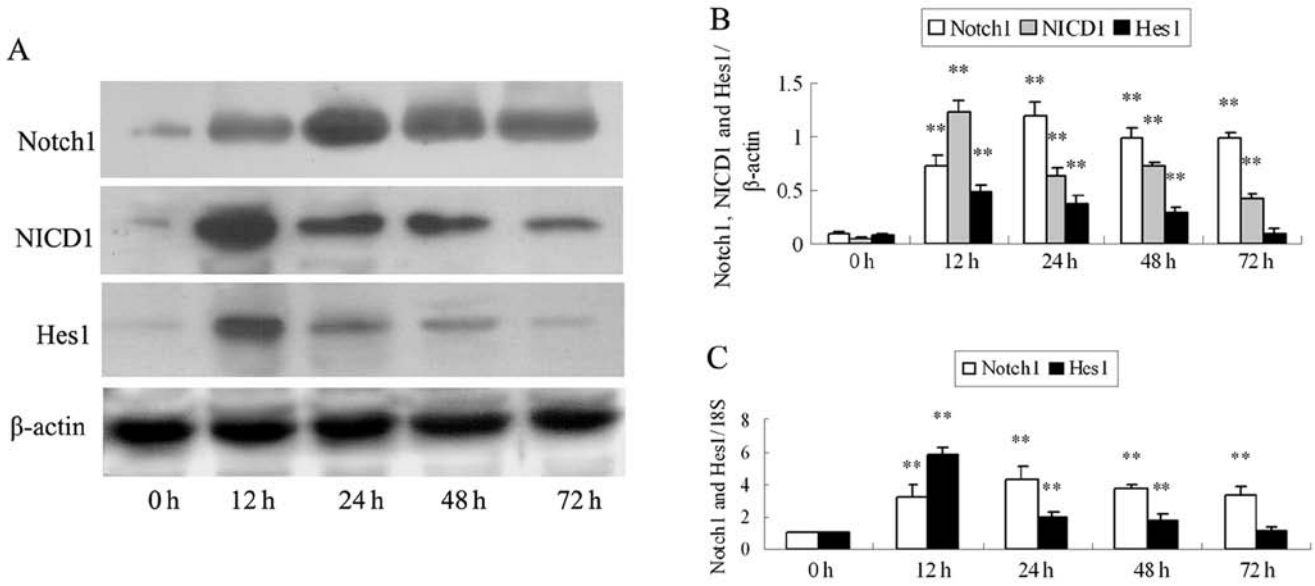

Figure 1. Time course of the activation of the Notch pathway by angiotensin II (Ang II) in podocytes. Podocytes were incubated with Ang II (10^{-6} mol/l) for 0-72 h. (A) The protein expression of Notch1, Notch intracellular domain 1 (NICD1) and hairy and enhancer of split-1 (Hes1) was measured by western blot analysis. (B) The protein levels of Notch1, NICD1 and Hes1 were quantified by densitometry. Protein expression was normalized to β-actin. (C) The mRNA expression of Notch1 and Hes1 was detected by RT-PCR. The mRNA expression was normalized to 18S. Data are presented as the means ± standard deviation (SD), n=6. **P<0.01 vs. Ang II (0 h).

relative fluorescence units. MMP-2 and MMP-9 activity was expressed as nmol/mg/min.

Enzyme-linked immunosorbent assay (ELISA). After the cells were cultured in 6-well plates under the different experimental conditions, the supernatants were collected. The TGF-β1, type IV collagen and laminin protein levels were quantified using a commercial quantikine enzyme-linked immunosorbent assay kit (ELISA; R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Statistical analysis. Data presented as bar graphs are the means ± standard deviation (SD) of at least 3 independent experiments. Statistical analysis was performed using the Student's t-test with SPSS version 13.0 software (SPSS Inc., Chicago, IL, USA). The results were considered statistically significant at P<0.05.

Results

Ang II activates the Notch pathway in podocytes. To determine the effects of Ang II on the activation of the Notch pathway, we measured the Notch1, NICD1 and Hes1 levels in the podocytes stimulated with Ang II for 0, 12, 24, 48 and 72 h (Fig. 1). Notch1 protein and mRNA expression increased within 12 h of Ang II stimulation, peaked at 24 h, and then slightly decreased at 48 and 72 h (all P<0.01); the maximum protein expression levels of NICD1 were detected as early as 12 h and then the expression levels declined, but did not return to the basal levels (Ang II stimulation at 0 h) (all P<0.01; Fig. 1A and B); Hes1 protein and mRNA expression also peaked at 12 h and then decreased at 24 and 48 h (P<0.01; Fig. 1A and C). No changes in Hes1 protein and mRNA expression were detected in the cultured podocytes at 0 and 72 h (P>0.05; Fig. 1).

Transfection with sh-Notch1 or pre-treatment with GSI increases MMP and decreases TGF-β1 expression in the Ang II-stimulated podocytes. Compared with the cells stimulated with Ang II at 0 h, the protein levels of Notch1, NICD1 and Hes1 significantly increased at 12 h (Fig. 2A and B; all P<0.01). Transfection with sh-Notch1 decreased the Ang II-induced the protein overexpression of Notch1, NICD1 and Hes1 in the podocytes (all P<0.01; Fig. 2A and B). RT-PCR revealed similar changes in Notch1 and Hes1 mRNA expression following transfection (Fig. 2C). GSI decreased the NICD1 and Hes1 protein and the Hes1 mRNA expression in the podocytes stimulated with Ang II at 12 h (all P<0.01; Fig. 2). Western blot analysis and RT-PCR revealed that GSI did not inhibit Notch1 overexpression induced by Ang II at 12 h (all P>0.05). Ang II stimulation decreased MMP-2 and MMP-9 protein and mRNA expression at 12 h (all P<0.01; Fig. 3). Compared with the cells stimulated with Ang II, the MMP-2 and MMP-9 protein and mRNA levels significantly increased in the podocytes transfected with sh-Notch1 or pre-treated with GSI (all P<0.01). Incubation with Ang II for 12 h resulted in a significant upregulation in TGF-β1 protein and mRNA expression compared with the cells stimulated with Ang II at 0 h (all P<0.01). However, the changes observed in the TGF-β1 expression level in the Ang II-stimulated podocytes were reversed by transfection with sh-Notch1 or by the addition of GSI to the Ang II culture medium (all P<0.01).

Transfection with sh-Notch1 or pre-treatment with GSI increases MMP activity in the Ang II-stimulated podocytes. When the podocytes were incubated with Ang II for 12 h, Ang II markedly decreased MMP-2 and MMP-9 activity. However, transfection with sh-Notch1 or pre-treatment with GSI enhanced the Ang II-induced inhibition of MMP-2 and MMP-9 activity (all P<0.01; Fig. 4).

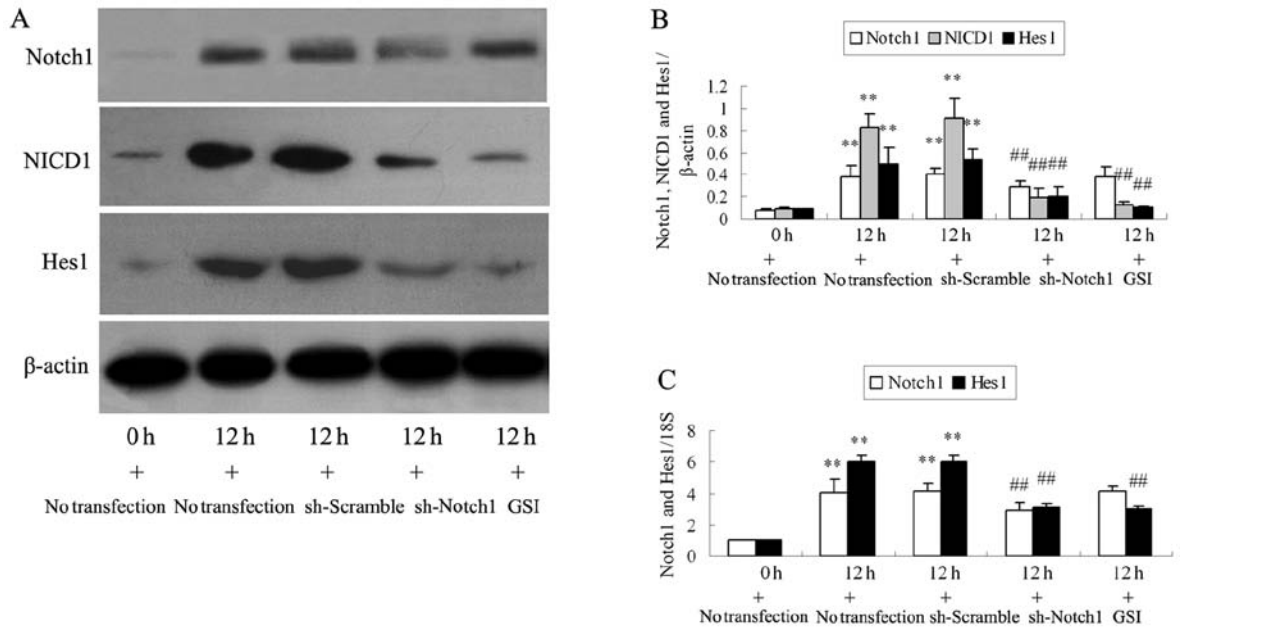

Figure 2. Effect of transfection with short hairpin RNA (shRNA) targeting Notch1 (sh-Notch1) or pre-treatment with γ-secretase inhibitor (GSI; 1 μmol/l) on the Notch pathway in angiotensin II (Ang II)-stimulated podocytes. (A) The protein expression of Notch1, Notch intracellular domain 1 (NICD1) and hairy and enhancer of split-1 (Hes1) was measured by western blot analysis. (B) The protein levels of Notch1, NICD1 and Hes1 were quantified by densitometry. Protein expression was normalized to β-actin. (C) The mRNA levels of Notch1 and Hes1 were detected by RT-PCR. The mRNA expression was normalized to 18S. Data are presented as the means ± standard deviation (SD), n=6. **P<0.01 vs. Ang II (0 h); $^{\#\#}$P<0.01 vs. Ang II (12 h) + sh-Scramble.

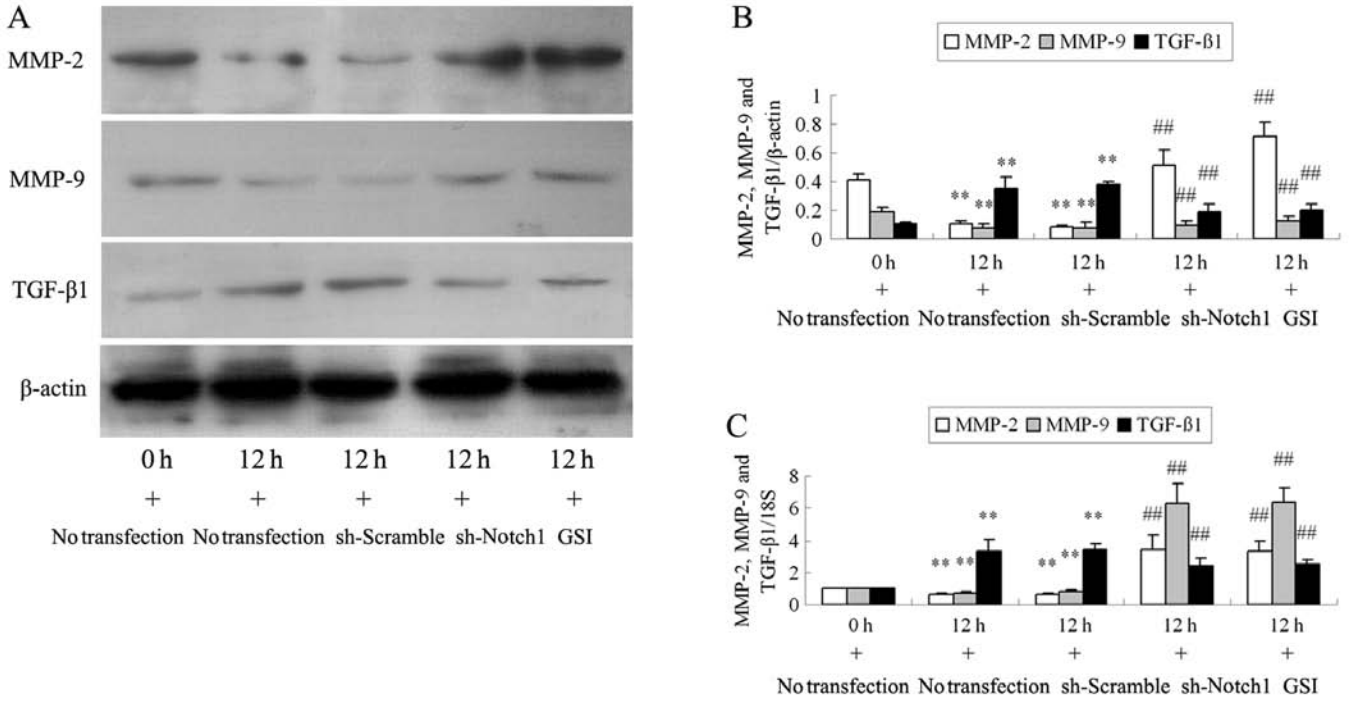

Figure 3. Effect of transfection with short hairpin RNA (shRNA) targeting Notch1 (sh-Notch1) or pre-treatment with γ-secretase inhibitor (GSI; 1 μmol/l) on the expression of matrix metalloproteinase (MMP)-2, MMP-9 and transforming growth factor-β1 (TGF-β1) in angiotensin II (Ang II)-stimulated podocytes. (A) The protein expression of MMP-2, MMP-9 and TGF-β1 was measured by western blot analysis. (B) The protein levels of MMP-2, MMP-9 and TGF-β1 were quantified by densitometry. Protein expression was normalized to β-actin. (C) The mRNA levels of MMP-2, MMP-9 and TGF-β1 were detected by RT-PCR. The mRNA expression was normalized to 18S. Data are presented as the means ± standard deviation (SD), n=6. **P<0.01 vs. Ang II (0 h); $^{\#\#}$P<0.01 vs. Ang II (12 h) + sh-Scramble.

Transfection with sh-Notch1 or pre-treatment with GSI decreases type IV collagen and laminin expression in the Ang II-stimulated podocytes. As shown by western blot analysis (Fig. 5A and B), type IV collagen and laminin protein

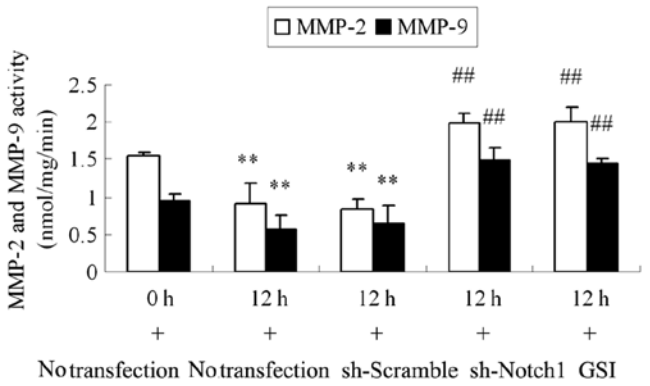

Figure 4. Effect of transfection with short hairpin RNA (shRNA) targeting Notch1 (sh-Notch1) or pre-treatment with γ-secretase inhibitor (GSI; 1 μmol/l) on the activity of matrix metalloproteinase (MMP)-2 and MMP-9 in angiotensin II (Ang II)-stimulated podocytes. Data are presented as the means ± standard deviation (SD), n=6. **P<0.01 vs. Ang II (0 h); ##P<0.01 vs. Ang II (12 h) + sh-Scramble.

expression was induced by Ang II at 12 h compared with the podocytes treated with Ang II at 0 h; these expression levels were efficiently inhibited by transfection with sh-Notch1 or pre-treatment with GSI (P<0.05 or P<0.01). Compared with the podocytes stimulated with Ang II at 0 h, the mRNA levels of type IV collagen and laminin were increased in the Ang II-stimulated podocytes at 12 h. Their mRNA expression was inhibited following transfection with sh-Notch1 or pre-treatment with GSI (all P<0.01; Fig. 5C).

Transfection with sh-Notch1 or pre-treatment with GSI decreases Ang II-induced TGF-β1, type IV collagen and laminin secretion. We also examined the concentrations of TGF-β1, type IV collagen and laminin in the culture medium of the podocytes using ELISA (Fig. 6). We found that the podocytes stimulated with Ang II for 12 h showed higher levels of TGF-β1, type IV collagen and laminin in the supernatants than those cultured with Ang II at 0 h (P<0.05 or P<0.01). Compared to the podocytes stimulated with Ang II, which induced the overexpression of TGF-β1, type IV collagen and laminin in the supernatants, transfection with sh-Notch1 or pre-treatment with GSI significantly decreased the expression of TGF-β1, type IV collagen and laminin (P<0.05 or P<0.01; Fig. 6).

Discussion

The Notch pathway is an evolutionarily conserved local cell-signaling mechanism that participates in a variety of cellular processes and is important in glomerular development (20,21). It has been found that the activation of the Notch pathway in podocytes plays a role in the development of glomerular disease (4). In our previous study, it was revealed that the activation of the Notch pathway in high glucose-stimulated podocytes altered the apoptotic pathway and induced apoptosis (22). Alterations in hemodynamics in glomerular disease induce higher levels of Ang II, which also interacts with the Notch pathway in podocytes (11). In this study, we also observed the activation of the Notch pathway in Ang II-stimulated podocytes. Notch1 seems to be a modulatory target of Ang II in podocytes. Ang II induces Notch1 cleavage in podocytes and the release of

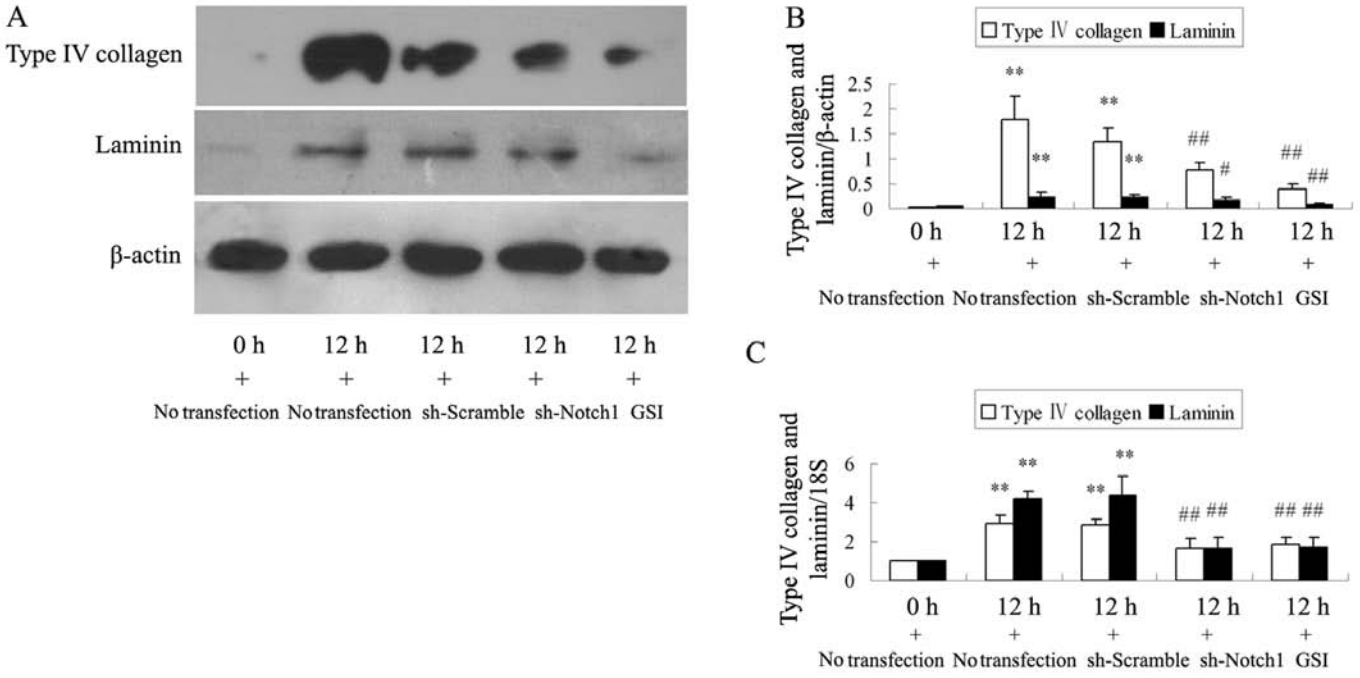

Figure 5. Effect of transfection with short hairpin RNA (shRNA) targeting Notch1 (sh-Notch1) or pre-treatment with γ-secretase inhibitor (GSI; 1 μmol/l) on the expression of type IV collagen and laminin in angiotensin II (Ang II)-stimulated podocytes. (A) The protein expression of type IV collagen and laminin was measured by western blot analysis. (B) The protein levels of type IV collagen and laminin were quantified by densitometry. Protein expression was normalized to β-actin. (C) The mRNA levels of type IV collagen and laminin were detected by RT-PCR. The mRNA expression was normalized to 18S. Data are presented as the means ± standard deviation (SD), n=6. **P<0.01 vs. Ang II (0 h); #P<0.05 and ##P<0.01 vs. Ang II (12 h) + sh-Scramble.

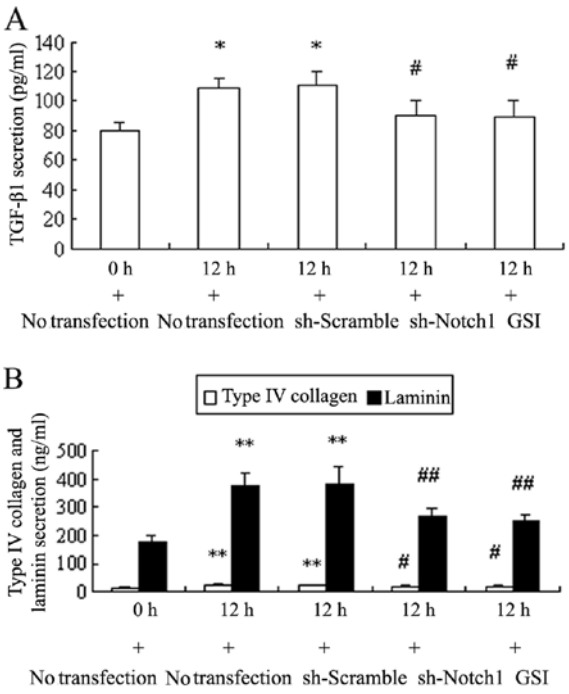

Figure 6. Effect of transfection with short hairpin RNA (shRNA) targeting Notch1 (sh-Notch1) or pre-treatment with γ-secretase inhibitor (GSI; 1 μmol/l) on the concentrations of the transforming growth factor-β1 (TGF-β1), type IV collagen and laminin in the culture medium of angiotensin II (Ang II)-stimulated podocytes. (A) The protein expression of TGF-β1 was analyzed by enzyme-linked immunosorbent assay (ELISA). (B) The protein expression of type IV collagen and laminin was analyzed by ELISA. Data are presented as the means ± standard deviation (SD), n=6. *P<0.05, **P<0.01 vs. Ang II (0 h); $^{\#}$P<0.05 and $^{\#\#}$P<0.01vs. Ang II (12 h) + sh-Scramble.

NICD1, which travels to the nucleus where it activates the Hes1 gene. The activation of the Notch pathway induced by Ang II was inhibited by Notch1 shRNA or GSI.

ECM accumulation in glomeruli is considered the most common destructive pathway associated with chronic glomerular disease, which is characterized by the remodeling of the interstitial ECM, resulting in the excessive deposition of ECM components, including type IV collagen and laminin (23). MMPs are zinc-containing endopeptidases that are involved in the remodeling of the ECM and are crucial for tissue development and homeostasis. MMP-2 and MMP-9 cleave the denatured collagens and laminin, as well as some chemokines. In view of their matrix-degrading capacity, MMP-2 and MMP-9 were originally considered to be beneficial to chronic renal fibrogenesis due to their perceived potential to lessen interstitial matrix accumulation and deposition (12,13,24). Studies on renal fibrosis have demonstrated that a decrease in MMP levels promotes the deposition of ECM in the context of renal fibrosis (11,25). Ang II has been shown to inhibit the angiotensin converting enzyme II (ACE2)-induced MMP-2 activity through the Ang II type-1 receptor (AT1R) and extracellular signal-regulated kinase 1 and 2 (ERK1/2) signaling pathway in human cardiofibroblasts (26). Under normal glucose conditions, Ang II induces a dose-dependent downregulation in MMP-2; on the contrary, MMP-9 is upregulated by Ang II (27). However, we found that Ang II inhibited the protein and mRNA expression, as well as the activity of MMP-2 and MMP-9 in podocytes. An enhanced MMP activity has been shown to be responsible for the development of disease due to their ability to degrade type IV collagen and laminin (28,29). Zhou *et al* (30) found that the downregulation of Notch1 decreased the migration and invasion capacities of hepatocellular carcinoma cells by regulating CD44v6, E-cadherin, MMP-2, MMP-9 and urokinase-type plasminogen activator (uPA). In this study, after the activation of the Notch pathway was inhibited by Notch1 shRNA or GSI in Ang II-stimulated podocytes, MMP-2 and MMP-9 expression and activity increased. These results indicated that Ang II inhibited MMP-2 and MMP-9 through the Notch pathway in podocytes.

It is well established that TGF-β1 is a potent stimulator of ECM production in glomerular injury and may be the most important growth factor in determining the extent of renal fibrosis following injury. It has been demonstrated that TGF-β1 mediates the production of type IV collagen and laminin in mesangial cells under conditions of high glucose *in vitro* (31). The exposure of cultured conditionally immortalized human podocytes to TGF-β1 has also been shown to increase the production of the basement membrane components (32). In the present study, we found that the stimulation of podocytes with Ang II induced type IV collagen and laminin accumulation by increasing TGF-β1 mRNA and protein synthesis. Ang II has been shown to induce the expression and production of TGF-β1 through a mechanism dependent on reactive oxygen species production in mouse skeletal muscle cells (33). In another study, Ang II-induced cardiac hypertrophy and fibrosis were significantly enhanced by the increase in TGF-β1 expression in fibroblast growth factor 16 (Fgf16) knockout mice (34). Furthermore, our finding that the inhibition of the Notch pathway by Notch1 shRNA or GSI markedly prevented the Ang II-induced upregulation of TGF-β1 suggests that Ang II induces TGF-β1 expression through the Notch pathway. Aoyagi-Ikeda *et al* (35) found that Notch induced myofibroblast differentiation through a TGF-β-Smad3 pathway that activated SMA gene transcription in alveolar epithelial cells, and increased migratory behavior in pulmonary fibrosis. In a previous study, blocking Notch signaling by the γ-secretase inhibitor, DAPT, was shown to significantly attenuate liver fibrosis, decrease the expression of TGF-β1 and suppress the endothelial-to-mesenchymal transition (EMT) process in a rat hepatic stellate cell line (36). In this study, we found that the activity of MMP-2 and MMP-9 was reduced, and TGF-β1 expression was increased in Ang II-stimulated podocytes. Imbalances between the synthesis and degradation of ECM proteins are considered to play important roles in the progression of glomerular sclerosis in glomerular disease (37).

In conclusion, our data demonstrate that the Ang II-induced activation of the Notch pathway, which inhibited MMP activity, increased TGF-β1 expression and increased the synthesis of ECM components in podocytes. Thus, the activation of the Notch pathway plays a role in Ang II-induced glomerular injury. The blockade of the Notch pathway may thus be an effective method for the treatment of glomerular disease.

Acknowledgements

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