Screening and Identification of the Up-Regulated Genes in Human Mesangial Cells Exposed to Angiotensin II

Xiubin LIANG, Hong ZHANG, Anyu ZHOU, Ping HOU, and Haiyan WANG

Accumulation of extracellular matrix (ECM) in the glomerular mesangium is a common feature of many progressive renal diseases. Angiotensin II (Ang II) plays a major role in the progression of chronic kidney diseases in part by induction of ECM. However, the precise molecular signals responsible for this effect are unknown. To explore possible molecular mechanisms of ECM production related to Ang II, we screened and identified genes up-regulated by Ang II in cultured human mesangial cells (MC). Detection of up-regulated genes was determined by mRNA populations from human MC with and without Ang II stimulation (10^{-6} mol/l, 24 h) by suppression subtractive hybridization. Reverse Northern blot analysis was performed to screen for differentially expressed genes. Full-length cDNAs of three novel genes were isolated by rapid amplification of cDNA ends (RACE)-polymerase chain reaction (PCR). One of these novel genes, AngRem104, was further investigated by Northern blot, Western blot and reverse transcription (RT)-PCR. The bioinformatics analysis implied that AngRem104 coded for a nuclear protein that was widely expressed in various normal human tissues. Moreover, up-regulation of AngRem104 induced by Ang II was time-dependent and was dose-dependently blocked by the Ang II type 1 receptor antagonist, Losartan. Interestingly, we also demonstrated that AngRem104 was associated with increased fibronectin expression. We conclude that AngRem104 is a novel human gene that is related to the expression of fibronectin and that is up-regulated by Ang II in human MC. These findings may lead to new insights into the mechanisms of glomerular sclerosis associated with Ang II. (Hypertens Res 2003; 26: 225–235)

Key Words: angiotensin II, gene cloning, mesangial cells, extracellular matrix

Introduction

Angiotensin II (Ang II), the principle effector molecule of the renin-angiotensin system (RAS), has long been known to regulate blood pressure, salt and fluid homeostasis. Recent findings have indicated that Ang II also plays a critical role in the progress of glomerular sclerosis (1). Ang II has been shown to stimulate glomerular mesangial cells (MC) to produce extracellular matrix (ECM) components such as fibronectin (FN), whose accumulation is a hallmark of progressive glomerular disease. It has also been well established that human MC are a critical factor in glomerular sclerosis. After stimulation by Ang II, human MC have been shown to over-express a number of genes, such as transforming growth factor-β (TGF-β) and plasminogen-activator inhibitor type-1 (PAI-1) (2–4). In addition, angiotensin converting enzyme inhibitors (ACEIs) and Ang II type 1 receptor (AT1R) antagonists have been shown to improve the renal pathologic changes of renal disease and retard the progression of chronic glomerular diseases (5–7). However, the molecular events occurring in human MC stimulated by Ang II are not yet clearly understood. In our previous study, we used human MC as a model to identify genes that were involved in Ang II-induced ECM accumulation. Consistent with the other published reports (8, 9), we found that Ang II
stimulation (10−6 mol/l) of human MC resulted in a time-dependent increase in the expression of FN at 6, 12, 18 and 24 h after treatment, as detected by reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA). Moreover, FN expression was significantly increased at 24 h in the Ang-II-stimulated human MC. Therefore, in the present study, to investigate the molecular mechanism of ECM accumulation induced by Ang II, we performed suppression subtractive hybridization (SSH) to screen and identify the over-expressed genes in human MC stimulated by Ang II.

Methods

Cell Lines and Cell Culture

Primary human MC that were obtained from Clonetics Corp. (Clonetics, San Diego, USA) were cultured in RPMI 1640 medium (Life Technologies, Baltimore, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco BRL, Grand Island, USA), 100 unit/ml penicillin, 100 µg/ml streptomycin, 12.5 mmol/l HEPES buffer, 2 mmol/l l-alanyl-l-glutamine and insulin (5 µg/ml)-transferrin (5 µg/ml)-sodium selenit (5 ng/ml) media supplement (Sigma, St. Louis, USA), and cells were maintained in 5% CO2 at 37 ºC.

SSH

SSH was performed with a Clontech PCR-select™ cDNA Subtraction Kit (Clontech, Palo Alto, USA) following the manufacturer’s protocol. In brief, RNA prepared from human MC that were stimulated with Ang II (Sigma) (10−6 mol/l, 24 h) was used as a tester, whereas RNA prepared from the human MC without Ang II stimulation was used as a driver. Double-stranded cDNAs were synthesized from 2 µg of mRNA as the tester or driver, respectively, and digested with Rsal. Two types of adapter, provided by the manufacturer, were independently ligated to the tester cDNA. First and second hybridizations were performed with the excess of driver cDNA and the subtractive products were amplified by PCR.

Reverse Northern Blot Analysis

Reverse Northern blots were performed to screen the over-expressed clones. The SSH final subtracted products were subcloned into pGEM-T easy vector (Promega, Madison, USA). After transformation, bacterial clones were randomly picked and plasmid clones with inserts were purified using a plasmid purification system (Qiagen, Hilden, Germany). The cDNA inserts were released by EcoRI and subjected to 2% agarose gel electrophoresis, denatured with 1.5 mol/l NaCl + 0.5 Eq/l NaOH, neutralized with 0.5 mol/l Tris-HCl (pH 7.4) + 1.5 mol/l NaCl, and transferred to nylon membranes. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as an internal control. The transferred DNAs were hybridized with [α-32P]dCTP-radiolabeled tester and driver cDNAs independently, which reflected the mRNA expression with or without Ang II stimulation, respectively. The over-expressed clones were sequenced by an automated DNA sequence analyzer (ABI PRISM 310; Genetic Analyzer, Perkin-Elmer, Foster City, USA) and a GenBank homology search was performed.

Northern Blot Analysis

Northern blots were performed as described previously (10). Total RNA was isolated from human MC with or without Ang II-stimulation using Trizol reagent (Life Technologies). Thirty micrograms of total RNA was subjected to 2.2 mol/l formaldehyde 1% agarose electrophoresis and capillary-transferred to the Hybond N+ nylon membranes (Amersham, Arlington Heights, USA). cDNA inserts of differentially expressed genes were digested with EcoRI, purified using a QiAEX II Kit (Qiagen), and radiolabeled with [α-32P]dCTP using the random primer labeling system (Promega). The membranes were hybridized with α-32P-radiolabeled cDNA probes (1 × 106 cpm/ml) at 42 ºC in 50% formamide, 5 µl saline sodium citrate (SSC), 1 µl Denhardt’s solution, 50 mmol/l sodium phosphate (pH 7.0) and 200 µg/ml salmon sperm DNA for 24 h. Filters were washed at the high stringency condition (four times at room temperature for 15 min in 1 × SSC / 0.1% sodium dodecyl sulfate (SDS), followed by two times at 50°C in 0.1 × SSC/0.1% SDS). The membranes were also hybridized with a glyceraldehyde-3-phosphate as an internal control. A commercial human Multiple Tissue Expression (MTE™) Northern Blot panel (Clontech) was used to detect the distribution of novel cDNAs in normal tissues according to the above description.

Isolation of the Full-Length cDNA of Novel Genes

The 5’ and 3’ rapid amplification of cDNA ends (RACE) reactions were performed using a SMART RACE cDNA Amplification kit following the manufacturer’s instructions (Clontech). Double-stranded cDNAs were synthesized from 1.0 µg of mRNA and subjected to the 5’ and 3’ RACE. The primers are listed in Table 1. The 5’ and 3’ RACE products were subcloned into pGEM-T Easy vector (Promega) and sequenced by automated DNA sequencing (ABI PRISM 310 Analyzer: Perkin-Elmer). The full-length of every novel gene was amplified by end-to-end PCR using the upstream and downstream primers. At least four different clones were sequenced to ensure the fidelity of Taq polymerase.

Homology and Nucleotide Sequencing Analysis

Analysis of the nucleotide sequencing of novel genes was performed using the program GENETYX-WIN (Software Development, Tokyo, Japan). Proteomics tools of the Expert
Protein Analysis System (ExPASy) server of the SWISS Institute of Bioinformatics (http://cn.expasy.org/tools/) were used to analyze the primary sequence and motifs of the AngRem104 protein.

Generation of AngRem104 Eukaryotic Expression Plasmid Constructs

The sense and antisense full-length coding sequence of AngRem104, which was amplified by PCR using the sense primer (5’-ATGTCAGATTATAATCCTGATG-3’) and antisense primer (5’-ACTGTAGAGGAGTCCTAGG-3’), was ligated into pcDNA3.1/V5-His-TOPO vector (Invitrogen, San Diego, USA) following the manufacturer’s protocol to construct the mammalian expression vector. All constructs were sequenced to ensure proper in-frame ligation and Taq polymerase fidelity. Constructs were subcloned and the plasmid cDNAs were purified by means of a Midi plasmid preparation kit (Qiagen).

Analysis of the Expression of Eukaryotic Constructs

The human MC (Clonetics) were transfected transiently with the sense AngRem104-pcDNA3.1/V5-His-TOPO, the antisense AngRem104-pcDNA3.1/V5-His-TOPO, and the pcDNA3.1/V5-His-TOPO plasmid DNA by LipofectAMINT2000 reagent (Invitrogen). For each transfection, 10 µl lipofectin reagent and 1 µg plasmid DNA were used. A β-gal staining kit (Invitrogen) was used to evaluate the efficiency of each transfection.

Western Blot Analysis

The sense-, antisense-AngRem104-pcDNA3.1/V5-His-TOPO and pcDNA3.1/V5-His-TOPO plasmid DNA were transfected into human MC, and human MC were collected at 24 h after transfection. Protein preparation was performed according to the manufacturer’s instructions (Santa Cruz Biotechnology, Santa Cruz, USA). The extracted proteins (5 µg) were subjected to 10% polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE), and transferred electrophoretically onto a nitrocellulose membrane (Bio-Rad, Hercules, USA). The transfected protein from human MC was probed with anti-V5-HRP-antibody (Invitrogen). The reaction bands were detected by enhanced chemiluminescence (Pierce Chemical, Rockford, USA).

RT-PCR

To detect the expressions of FN, RT-PCR was performed and a 500-base pair (bp) segment for FN was amplified. The upstream primer of FN was 5’-TGGAACTTCTACCAGTGCGAC-3’ and the downstream primer was 5’-TGTCTTCACCCCGTGCTGGAACGTAC-3’.

Table 1. PCR Primer Sequences for the Three Novel Genes

<table>
<thead>
<tr>
<th>Primer sequences for full-length PCR</th>
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<tbody>
<tr>
<td>5’-ACGCCGGGAGAACAAGGGTTCCT-3’</td>
</tr>
<tr>
<td>5’-GGCAATTAGTAGATATTAAATCCAAAGCC-3’</td>
</tr>
</tbody>
</table>

Results

Screening of Up-Regulated Genes in Human MC Exposed to Ang II

To screen for up-regulated genes that were involved in the Ang II-induced accumulation of ECM in human MC, SSH was performed. The cDNAs differentially expressed between human MC with and without Ang II-stimulation (10⁻⁶ mol/l, 24 h) were subtracted. One hundred and eighteen subtracted clones were randomly selected for reverse Northern blot analysis. The expressions of 55 of the 118 clones were significantly up-regulated (Fig. 1).

Identification of Up-Regulated Clones

To confirm the up-regulated expression of the isolated genes, the expression of 18 clones that were selected from the 55
clones screened by reverse Northern blot analysis was further confirmed by Northern blot analysis. The expression of each of the 18 clones was significantly (1–3 times) higher than that of the control (Fig. 2).

Classification of Up-Regulated Genes

To classify up-regulated genes, a GenBank homology search was performed. The search revealed that 15 clones were completely matched with known genes (Table 2). In addition, three clones were novel and uncharacterized genes (clones 46, 52 and 104). Clones 52 and 104 did not show any significant homology with known genes deposited in a non-redundant GenBank + EMBL + DDBJ + PDB database, and even failed to show significant homology to known genes in an EST database. One clone (clone 46) also did not show any significant homology with the known genes, but it matched with Human EST clones (gb|AC016775.5| and gb|AC020781.4|) that were located at chromosome 11. The three novel genes were named AngRem104, AngRem52, and

Fig. 1. Reverse Northern blot analysis of 118 clones. The differentially expressed cDNAs were subcloned into pGEM-T easy vector, and 118 clones were randomly picked. Reverse Northern blotting was performed for selected up-regulated genes. The cDNA insert of each clone and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; internal control) were transferred to nylon membranes and hybridized with [α-32P]dCTP-radiolabeled tester and driver cDNA populations independently. The expressions of 55 clones (arrows) were significantly increased in the T (Tester) compared with the expression in the D (Driver). An asterisk (*) denotes GAPDH.
AngRem46, respectively, where the prefix AngRem means Ang II-related gene in MC.

Isolation and Sequencing Analysis of Full-Length cDNAs of Novel Genes

To obtain the full-length cDNAs of three novel genes, 3’ and 5’ RACE-PCR were performed. AngRem104 (GenBank accession number: AF367870) contained 1,690-bp nucleotides and encoded 347-amino acid (aa) peptides. The nucleotide sequence (CTGATGT) surrounding the initiation codon at nt 90–92 reasonably fits a Kozak consensus site, suggesting that it may be a site of translation initiation (11). A poly(A) tail with a potential polyadenylation signal

Table 2. Classification of Up-Regulated Known Genes

<table>
<thead>
<tr>
<th>Classification</th>
<th>Clone No.</th>
<th>Gene</th>
<th>GenBank accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular matrix</td>
<td>54</td>
<td>thrombospondin 1</td>
<td>gb ⊕ M25631.1 ⊕</td>
</tr>
<tr>
<td></td>
<td>97</td>
<td>type I (α2) collagen</td>
<td>gb ⊕ J03464.1 ⊕</td>
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<tr>
<td>Cytoskeleton components</td>
<td>19</td>
<td>α-smooth muscle actin</td>
<td>gb ⊕ J05192.1 ⊕</td>
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<tr>
<td></td>
<td>75</td>
<td>Caldesmon 1</td>
<td>gb ⊕ M64110.1 ⊕</td>
</tr>
<tr>
<td></td>
<td>87</td>
<td>cytoplasmic-γ isoform of actin</td>
<td>gb ⊕ M1624.7 ⊕</td>
</tr>
<tr>
<td>Synthesis and metabolism related molecules</td>
<td>28</td>
<td>aldolase A</td>
<td>gb ⊕ BC004333.1 ⊕</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>elongation factor-1γ</td>
<td>gb ⊕ BC004215.1 ⊕</td>
</tr>
<tr>
<td></td>
<td>38</td>
<td>S28 (ribosomal protein S28)</td>
<td>gb ⊕ BC000354.1 ⊕</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>farnesyl pyrophosphate synthetase</td>
<td>gb ⊕ J05262.1 ⊕</td>
</tr>
<tr>
<td>Catabolism related molecules</td>
<td>101</td>
<td>ubiquitin protein ligase</td>
<td>gb ⊕ AF251046.1 ⊕</td>
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<tr>
<td></td>
<td>105</td>
<td>cathepsin U</td>
<td>gb ⊕ AF070448.1 ⊕</td>
</tr>
<tr>
<td>Others</td>
<td>11</td>
<td>ion transport regulator 5</td>
<td>ref ⊕ NM-021909 ⊕</td>
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<td></td>
<td>59</td>
<td>PAI-1</td>
<td>gb ⊕ M14083.1 ⊕</td>
</tr>
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<td></td>
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<tr>
<td></td>
<td>100</td>
<td>nucleolar protein B23</td>
<td>gb ⊕ M26697.1 ⊕</td>
</tr>
</tbody>
</table>
(AATAAA) at the 3'-untranslated region was present (Fig. 3). AngRem52 (GenBank accession number: AY040225) contained 3,170-bp nucleotides and encoded 340-aa peptides. The nucleotide sequence (ACAATGG) surrounding the initiation codon at nt 16–18 reasonably fits a Kozak consensus site. A poly(A) tail with a potential polyadenylation signal (AATAAA) at the 3'-non-coding region was present (Fig. 4). AngRem46 (GenBank accession number:AY040224) contained 416-bp nucleotides and encoded 60-aa peptides. The nucleotide sequence (GAGATGC) surrounding the initiation codon at nt 70–72 reasonably fits a Kozak consensus site. A poly(A) tail with a potential polyadenylation signal (AATAAA) at the 3'-coding region was present (Fig. 5).

**Fig. 3.** Nucleotide and predicted amino acid sequences of the AngRem104 cDNA. The full-length AngRem104 cDNA was 1,690 bp. It contained a 1,041-bp open reading frame flanked by 5'- and 3'-untranslated regions. The Kozak sequences is boxed, and poly(A) signals are underlined.

**Distribution of Three Novel Genes in Human Normal Tissues**

To demonstrate the tissue distribution of novel genes, human MTE™ Northern blots were analyzed, including normal human tissues of the heart, lung, placenta, brain, liver, muscle, kidney and pancreas. AngRem104 was widely expressed in the human heart, placenta, liver, muscle, kidney and pancreas. There was no obvious expression in the human lung or brain. The size of AngRem104 transcription was 1.7 kb, which consisted of the full-length of AngRem104 by sequence analysis. Notably, AngRem104 showed two other transcripts (4.5 kb and 5 kb, respectively) in the placenta. These two transcripts were considered alternative splicing.
Fig. 4. Nucleotide and predicted amino acid sequences of the AngRem52 cDNA. The full-length AngRem52 cDNA was 3,170 bp. It contained a 1,020-bp open reading frame flanked by 5' and 3' untranslated regions. The Kozak sequence is boxed, and poly(A) signals are underlined.
isoforms or homologous genes. AngRem52 showed a single transcript of \( \sim 3.2 \) kb, that was widely expressed in all of the tissues on the Northern blot panel. AngRem46 was also widely expressed in various tissues, although only weak signals were observed in the pancreas, and its transcript was \( \sim 400 \) bp in length (Fig. 6).

AngRem46 mRNA is Localized in the Cellular Nucleus

To analyze the primary sequence and motif of AngRem46 protein, a bioinformatics tool-based on-line server was used. The predicted protein had a calculated isoelectric point of 4.95. No amino terminal (N-terminal) signal peptide, transmembrane domain, peroxisomal targeting signal (C-terminal), or membrane retention motifs were found; however, use of the Reinhardt’s method predicted that the protein AngRem104 was subcellularly localized at the cellular nucleus.
AngRem104 mRNA Is Up-Regulated in Ang II-Stimulated Human MC and Blocked by an AT1R Antagonist (Losartan)

To examine the effect of Ang II on AngRem104 expression, the expressions of AngRem104 were then detected by Northern blot analysis at 6, 12, 24 and 48 h after treatment with Ang II (10⁻⁶ mol/l). The expression of AngRem104 was significantly increased at 6 h, and this increased expression was maintained up to 48 h (Fig. 7A). To determine whether the regulatory effect of Ang II on AngRem104 expression was specific to human MC, a blocking experiment was performed using Losartan (Merck & Co Inc., Whitehouse Station, USA) in serial concentrations (10⁻⁴, 10⁻⁵, 10⁻⁶ mol/l). The results showed that the up-regulated expression of AngRem104 induced by Ang II was dose-dependently blocked by Losartan (Fig. 7B).

Fibronectin Is the Up-Regulated Gene Related to AngRem104 Over-Expression

To investigate the function of AngRem104, we constructed sense- and antisense-AngRem104 mammalian expression vectors (sense- and antisense-AngRem104-pcDNA3.1/V5-His-TOPO constructs). The results of β-gal staining showed that the transfection efficiency reached a peak of 45% at 24 h after the Eukaryotic constructs had been transfected into human MC (data not shown). The alternative expressions of sense- and antisense-AngRem104 in transfected human MC were detected by Western blot analysis. The results revealed that the expression of AngRem104 fusion protein was present when human MC was transfected with sense-AngRem104, whereas the expression was suppressed when human MC was transfected with antisense-AngRem104 (Fig. 8). In addition, RT-PCR was performed to detect the expression of fibronectin in human MC transfected with the sense- and antisense-AngRem104 constructs. Figure 9 shows that FN was more highly expressed in human MC that were transfected with the sense-AngRem104 construct than in those that were transfected with the antisense-AngRem104 or those that were not transfected at all (controls). Moreover, the over-expression of FN induced by Ang II was significantly suppressed by the transfection of antisense-AngRem104.

Discussion

In addition to exerting vasoactive effects, Ang II has been shown to act as a growth factor that modulates the cell growth, synthesis and degradation of ECM in the kidney. Ang II induced the synthesis and secretion of FN, and of type I and III collagen from MC (7, 8, 12). In our previous study, Ang II was dramatically increased in the local renal tissue of puromycin nephropathy rats, and this increase was related with the progression of puromycin nephropathy. Enalapril, an ACEI, and Irbesartan, an AT1R antagonist, have been shown to decrease the Ang II activities in local renal tissue, to retard the progression of puromycin nephropathy.
nephropathy, and to ameliorate the pathologic lesions, such as glomerular sclerosis and tubular interstitial fibrosis (2, 13). These investigations suggested that Ang II was a critical factor for the progression of chronic renal diseases and played an important role in glomerular sclerosis and tubular interstitial fibrosis. However, the molecular mechanism of these effects of Ang II remains poorly understood.

SSH is a highly efficient cDNA subtractive method, and has been used to selectively amplify differentially expressed cDNA fragments and suppress non target DNA amplification at the same time (14). The important advantage of this method is that the mRNA abundance could be equalized and the rarely expressed mRNAs could be effectively amplified and cloned (15). In the present study, SSH was adopted to screen the up-regulated genes in human MC stimulated by Ang II. Among the 18 up-regulated genes, 15 known genes and 3 novel genes were identified.

Among the 15 known genes, several are well known to be up-regulated in Ang II-stimulated mesangial cells, such as type I (α2) collagen (6, 16), which is an ECM component, PAI-1 (17), which is an inhibitor of ECM degradation, and smooth muscle actin α (18). These results suggest that Ang II exerts dual effects on ECM, i.e., that Ang II up-regulates the synthesis of ECM components of cultured MC and also inhibits the degradation of ECM by up-regulating the ECM degradation inhibitors. Several genes, such as thrombospondin 1 (TSP-1), caldesmon 1 and farnesyl pyrophosphate synthetase, have been shown to be over-expressed in Ang II-stimulated non-MC, which are also known to be related to glomerular disease (19–22). Prior to the present report, however, there has been no account of the changes in the expression of these genes in Ang II-stimulated MC.

Three novel genes were found in addition to the known genes described above. These were AngRem104, AngRem52 and AngRem46 (GenBank accession Nos. AF367870, AY040225 and AY040224, respectively). Their full-length cDNAs were 1,690 bp, 3,170 bp and 416 bp, respectively, and their predicted open reading frames encoded 347-aa, 340-aa and 60-aa polypeptides, respectively. Moreover, we found that all three of these novel genes were widely expressed in various human normal tissues, indicating that they might play important roles in regulating the function of various tissues.

In the functional study of AngRem104, we found that AngRem104 was up-regulated by Ang II stimulation in cultured human MC, and that this over-expression could be dose-dependently blocked by the AT1R antagonist, Losartan. In addition, our study revealed that the expression of one of the major ECM components, fibronectin, was dramatically up-regulated by AngRem104. This finding suggested that AngRem104 was a new putative molecule that was up-regulated by Ang II and might partially participate in the regulation of the over-expression of fibronectin induced by Ang II in human MC. FN is a high-molecular-mass adhesive glycoprotein implicated in a wide variety of cellular properties, including cell adhesion, differentiation, proliferation, migration and apoptosis (23). It has been reported that the expression of FN is regulated by many molecules, such as TGF-β (24, 25), cAMP (25), epidermal growth factor (EGF), platelet-derived growth factor (PDGF) (26), and γ-interferon (27). Transfection of cells with any of various oncogenes (src, ras, mos, and EIA) can lead to decreased expression of FN (28). To date, however, there have been few investigations into the mechanism of FN regulation.

How did AngRem104 regulate the expression of FN? According to the predicted result of the bioinformatics trials, AngRem104 coded for a nuclear protein, indicating that AngRem104 might play a role in the regulation of gene transcription in the nucleus. Therefore, possible mechanisms by which AngRem104 may have induced the up-regulation of FN could include binding with response elements of the FN promoter as trans-acting factors; interaction with a known binding protein on the FN promoter during transcriptional regulation; alteration of nuclear RNA splicing or polyadenylation; or alteration of nuclear stability during post-transcriptional regulation. Each of these hypotheses of the functions of AngRem104 merits further investigation.

In summary, in the present study we used SSH to screen for genes that were over-expressed in human MC in response to stimulation with Ang II. Ang II-stimulated human MC showed over-expressions of TSP-1, caldesmon 1 and other known genes that are thought to play a role in ECM accumulation probably played a role in the observed ECM accumulation. Interestingly, AngRem104, one of the three novel genes, was found to increase the fibronectin expression and could be specifically up-regulated by Ang II in human MC. Conceivably, AngRem104 might be another key molecule involved in the renal pathologic process. Further characterization of the function of AngRem104 and identification of the particular cross-reactions among Ang II, AngRem104 and fibronectin, would be expected to provide new insights into the pathogenesis of various renal diseases. In general, functional studies of these known and novel genes might provide new clues for exploring the mechanisms of glomerular sclerosis associated with Ang II.

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