Role of microRNA-29b in angiotensin II-induced epithelial-mesenchymal transition in renal tubular epithelial cells

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Abstract. Angiotensin II (Ang II) has been proven to induce epithelial-mesenchymal transition (EMT). The aim of the present study was to determine the role of microRNA-29b (miR-29b) during Ang II-induced EMT. For this purpose, we used spontaneously hypertensive rats (SHRs) and age-matched Wistar-Kyoto (WKY) rats. The levels of Ang II and its receptor in the kidneys of the SHRs are significantly higher than those in the age-matched WKY rats. As shown by RT-qPCR, the expression of miR-29b in the renal cortex was lower in the SHRs than in the WKY rats. For *in vitro* experiments, NRK-52E renal tubular epithelial cells were treated with 10^{-7} M Ang II; we found that the expression of miR-29b was decreased in the cells treated with Ang II. In addition, transfection of the NRK-52E cells with miR-29b inhibitor led to the downregulation of miR-29b in these cells, and increased the expression of transforming growth factor (TGF)-β, α-smooth muscle actin (α-SMA) and collagen I (Col I). Similar results were observed with the induction of Ang II expression in the NRK-52E cells. By contrast, the upregulation of miR-29b by transfection with miR-29b mimics inhibited the overexpression of these genes induced by Ang II. These results suggest that miR-29b plays an important role in Ang II-induced EMT.

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Introduction

MicroRNAs (miRNAs or miRs) are small endogenous RNAs that modulate gene expression by targeting the 3' untranslated regions (3' UTRs) of mRNAs to inhibit translation or promote mRNA degradation (1,2). In recent years, the miR-29 family has been implicated in the fibrosis of multiple organs (3). Spontaneously hypertensive rats (SHRs) are an ideal animal model of hypertension. The kidneys of adult SHRs express high levels of extracellular matrix (ECM) proteins, α-smooth muscle actin (α-SMA) and matrix metalloproteinases (MMPs) than age-matched Wistar-Kyoto (WKY) rats (4). This indicates that the kidneys of adult SHRs have already developed fibrosis.

Angiotensin II (Ang II), the main peptide of the renin-angiotensin-aldosterone system (RAAS), plays an important role in the occurrence and development of hypertension, as well as in epithelial-mesenchymal transition (EMT), which is a crucial step in the development of renal fibrosis. In the kidneys of young SHRs, the levels of Ang II, as well as those of its receptor are significantly higher than those in WKY rats (5,6). Ang II increases the expression and the synthesis of ECM proteins, including multiple collagens and fibrillins (7). In the present study, we aimed to determine whether miR-29b is the downstream gene of Ang II and to elucidate its role in Ang II-induced EMT.

Materials and methods

Rats and measurement of blood pressure. Male (15 weeks old) SHRs and age-matched WKY rats were acquired from the Chinese Academy of Sciences, Shanghai, China. They were housed under standardized conditions with controlled temperature (20-26˚C) and humidity (40-70%) and exposed to a 12-h light/dark cycle. Systolic blood pressure (SBP) was measured by the tail-cuff method using a PowerLab data acquisition and analysis system (ADInstruments Inc., Sydney, Australia) under slightly restrained and quiet conditions for 5 days prior to the experiments. The average of 3 pressure readings was recorded. The animal study was approved by the Animal

Welfare Committee of Wenzhou Medical University following state and institutional regulations.

RT-qPCR analysis of miR-29b expression in the renal cortex. Total RNA was extracted from the renal cortex of SHRs and WKY rats using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The purity of the total RNA was determined using a spectrophotometer (DU800; Beckman Coulter, Miami, FL, USA) and its quality was determined by formaldehyde denaturation gel electrophoresis. The miRNAs were isolated and purified using the miRNA isolation kit (Ambion). The reverse transcription of the miRNAs was performed using the miScription Reverse Transcription kit (Qiagen, Valencia, CA, USA) according to the manufacturer's recommendations. The miRNA specific real-time quantitative polymerase chain reaction (RT-qPCR) primers were used in RT-qPCR together with the miScript Universal Primer which was included in the kit. The sequence of the rno-miR-29b specific primer was 5'-TAGCACCATTTGAAATCAGTGTT-3', and that of the U6 primer was 5'-CAAGGATGACACGCAAATTCG-3'. The PCR reaction was performed using the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Bedford, MA, USA). The amplification program was 95˚C for 15 min and then 40 cycles consisting of 95˚C for 10 sec and 60˚C for 35 sec. The relative amount of miR-29b was normalized to that of the U6 RNA. The ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) was used to analyze the data, and the ΔΔCT method was used to calculate the relative expression of the sample gene. The relative quantification (RQ) of gene expression was analyzed using the $2^{-\Delta\Delta CT}$ method, as previously described (8): $RQ = 2^{-\Delta\Delta CT}$ (CT indicates the cycles required by the fluorescence signal intensity to reach the threshold value in the PCR amplification process, $\Delta CT_{sample} = CT_{sample}$ - CT $U6_{sample}$, $\Delta CT_{control} = CT_{control}$ - CT $U6_{control}$, $\Delta\Delta CT = \Delta CT_{sample} - \Delta CT_{control}$). The experiment was repeated 3 times.

Cell line. The renal tubular epithelial cells line (NRK-52E) was obtained from the Cell Bank of the Chinese Academy of Sciences. The NRK-52E cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (low glucose; Gibco-BRL, Gaithersburg, MD, USA) containing 5% fetal bovine serum (FBS; Gibco-BRL) and incubated in a humidified atmosphere of 95% O_2, 5% CO_2 at 37˚C in a CO_2 incubator. When the cells grew to 90% confluency, they were then harvested by a brief exposure to 0.05% trypsin-EDTA (Gibco-BRL) and passaged every 3 days.

Treatment with Ang II. The NRK-52E cells were cultured into 6-well plates at the density of $1x10^5$ cells/well. They were then divided into 2 groups: the Ang II group (treatment with 10^{-7} M Ang II) and the blank control (BC) group (without extra treatment). The following experiments were carried out after the cells were cultured at 37˚C in an incubator containing 5% CO_2 for 24 or 48 h.

RT-qPCR analysis of miR-29b expression in the Ang II and blank control group. Total RNA was extracted using TRIzol reagent (Invitrogen). The purity and quality of the total RNA were determined as described above. The microRNA isolation kit (Ambion) was used to detach small molecular RNA less than 100 nt, and the miScript Reverse Transcription kit (Qiagen) was used to synthesize the cDNA through reverse transcription. The PCR conditions and the method of data analysis were as described above. The experiment was repeated 3 times.

Synthesis of miR-29b mimics and miR-29b inhibitor. The positive-sense strand of miR-29b mimics (Sigma-Aldrich, St. Louis, MO, USA) was as follows: 5'-UAGCACCAUUUGA AAUCAGUGUU-3' and its antisense strand was 5'-CACUG AUUUCAAAUGGUGCUAUU-3', having no homology with the rat genome. The positive-sense strand of the miR-29b inhibitor (Sigma-Aldrich) was as follows: 5'-AACACUGA UUUCAAAUGGUGCUA-3' and its antisense strand was 5'-CAGUACUUUUGUGUAGUACAA-3', having no homology with the rat genome.

Flow cytometry for the assessment of the transfection efficiency. A total of 5 μl FAM-NC miRNA/5 μl Lipofectamine™ 2000 (Invitrogen) was transfected into the NRK-52E cells. The transfection efficiency was assessed using a flow cytometer (Becton-Dickinson, San Jose, CA, USA) 24 h following transfection.

Transfection with miR-29b inhibitor and miR-29b mimics. miR-29b was either downregulated or upregulated in the NRK-52E cells by transfection with miR-29b inhibitor or miR-29b mimics, respectively. A single-cell suspension was prepared and the cells were cultured in 6-well plates at the density of $1x10^5$ cells/well 24 h prior to transfection. The NRK-52E cells were transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. For the downregulation, the cells were divided into 3 groups: downregulation (DR) group (transfection with miR-29b inhibitor), negative control (NC) group (transfection with miRNA synthesized randomly) and the blank control (BC) group (no transfection). For the upregulation, the cells were divided into 4 groups: the upregulation (UR) group (transfection with miR-29b and treatment with 10^{-7} M Ang II 24 h following transfection), the negative control-$_{Ang\ II}$ ($NC_{Ang\ II}$) group (transfection with miRNA synthesized randomly and treatment with 10^{-7} M Ang II 24 h following transfection), the Ang II group (treatment with 10^{-7} M Ang II) and the blank control (BC) group. The following experiments were carried out after the cells were cultured at 37˚C in an incubator containing 5% CO_2 for 24 or 48 h.

RT-qPCR analysis of miR-29b, transforming growth factor (TGF)-β, α-SMA and collagen (Col) I expression following transfection. Total RNA was extracted through the one-step method with TRIzol reagent (Invitrogen) 24 h after transfection and the cells were cultured until they reached 70-80% confluence. The purity and quality of the total RNA were determined as described above. The methods and steps of reverse transcription, PCR amplification and data analysis for miR-29b were as described above. The RevertAid™ First Strand cDNA Synthesis kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) was adopted to synthesize cDNA through reverse transcription. The RT-qPCR primers (synthesized by Invitrogen) are presented in Table I. The PCR reaction was performed using the ABI 7500 Fast Real-Time PCR System.

Table I. Primers used for RT-qPCR.

mRNA	Primer sequences
α-SMA	Forward: CTTCTATAACGAGCTTCGC
	Reverse: TCCAGAGTCCAGCACAAT
TGF-β	Forward: AGGCGGTGCTCGCTTTGT
	Reverse: GATTGCGTTGTTGCGGTCC
Col I	Forward: ACTCAGCCCTCTGTGCCT
	Reverse: CCTTCGCTTCCATACTCG
MMP-2	Forward: AGCTCCCGGAAAAGATTGAT
	Reverse: TCCAGTTAAAGGCAGCGTCT
β-actin	Forward: GAGGGAAATCGTGCGTGAC
	Reverse: AGGAAGGAAGGCTGGAAG

α-SMA, α-smooth muscle actin; TGF-β, transforming growth factor-β; Col I, collagen I; MMP-2, matrix metalloproteinase 2.

The relative amount of each mRNA was normalized to that of β-actin using the $2^{-\Delta CT}$ method. The amplification program was 95˚C for 10 min and then 40 cycles consisting of 95˚C for 15 sec and 60˚C for 60 sec. The relative amount of each mRNA was normalized to that of β-actin. The ABI Prism 7900HT Sequence Detection System (SDS 2.2.2 software) was used to analyze the data, and ΔΔCT method was used to calculate the relative expression of the sample gene. The relative quantification (RQ) of gene expression was analyzed by the $2^{-\Delta\Delta CT}$ method as follows: $RQ = 2^{-\Delta\Delta CT}$ ($\Delta CT_{sample} = CT_{sample} - CT_{\beta\text{-actin sample}}$, $\Delta CT_{control} = CT_{control} - CT_{\beta\text{-actin control}}$, $\Delta\Delta CT = \Delta CT_{sample} - \Delta CT_{control}$). The experiment was repeated 3 times.

Western blot analysis of the protein expression of TGF-β, α-SMA and Col I following transfection. Forty-eight hours after transfection, the cells were washed twice with ice-cold PBS and then lysed in 200 μl of ice-cold lysis buffer (RIPA:PMSF, 99:1; Sigma). The cells lysates were lysed further by ultrasound. The lysates were then centrifuged at 15,000 x g for 15 min at 4˚C, and the supernatants were collected and stored at -80˚C. The protein concentration was determined by BCA protein assay (Beyotime, Jiansu, China). The lysates (30 μg of protein) were separated on 10% polyacrylamide gels using SDS-PAGE and transferred onto nitrocellulose (NC) membranes. After transferring, the membranes were blocked for 2 h at 4˚C with 5% skimmed milk, incubated overnight at 4˚C with each primary antibody (rabbit anti-α-SMA antibody 1:500 dilution; rabbit anti-TGF-β antibody 1:300 and rabbit anti-Col I antibody 1:500 dilution; Abcam, Bristol, UK), washed 3 times in TBST buffer, incubated with secondary antibody [at 1:4,000 dilution, goat anti-rabbit IgG (H+L) IRDye® 800CW; LI-COR Biosciences, Lincoln, NE, USA] for 2 h at room temperature and washed 3 times in TBST buffer. The blots were analyzed using the Odyssey Infrared Imaging System (Li-COR Biosciences). The relative amount of protein on each blot was normalized to that of β-actin, and the semi-quantitative analysis of the blots was carried out using the AlphaEaseFC Imaging System (Alpha Innotch,San Leandro,CA, USA). The experiment was repeated at least 3 times.

Analysis of SMA and Col I expression by immunofluorescence staining. The NRK-52E cells were fixed in 4% paraformaldehyde solution, permeabilized with 0.5% Triton X-100 for 10 min, blocked for 45 min with 5% serum at room temperature, incubated overnight at 4˚C with primary antibodies (rabbit anti-α-SMA antibody 1:200 dilution; rabbit anti-Col I antibody 1:100 dilution; Abcam), washed 3 times in PBS, incubated with DyLight 549 conjugated donkey anti-rabbit antibodies (1:500; Jackson ImmunoResearch Laboratories West Grove, PA, USA) at 37˚C for 1 h and washed 4 times in PBS. The cells were then stained with 4',6-diamidino-2-phenylindole to visualize the nuclear content. Fluorescence images were acquired using a laser scanning confocal microscope (Carl Zeiss, Jena, Germany).

Statistical analysis. The data were analyzed using SPSS 18.0 software. All experiments were repeated 3 times and the results are presented as the means ± SE. Differneces between multiple groups were analyzed by one-way ANOVA followed by Tukey's post hoc comparisons. Differences were considered statistically significant at $P<0.05$.

Results

Blood pressure measurement. The average SBP of the SHRs and WKY rats was 191.3±16.6 and 121.8±14.7 mmHg, respectively, and the difference between them was statistically significant ($P<0.05$).

Differential expression of miR-29b in the renal cortex between the SHRs and WKY rats. RT-qPCR revealed that the expression of miR-29b in the renal cortex of the SHRs (0.76±0.01) was significantly lower than that of the WKY rats (1.00±0.00) ($P<0.05$).

Ang II downregulates miR-29b expression. The expression of miR-29b in the NRK-52E cells was confirmed by RT-qPCR following treatment with 10^{-7} M Ang II. The expression of miR-29b in the Ang II group (0.56±0.06) was significantly downregulated compared with that of the blank control (BC) group (1.00±0.00).

Transfection with miR-29b inhibitor upregulates the expression levels of TGF-β, α-SMA and Col I. To further examine the function of miR-29b, the NRK-52E cells were transfected with miR-29b inhibitor. The transfection efficiency was determined by flow cytometry 24 h after 5 μl FAM-NC miRNA/5 μl Lipofectamine 2000 were transfected into the NRK-52E cells. The results of flow cytometry revealed that the transfection efficiency of the NRK-52E cells was 95.14% (Fig. 1).

The results of miR-29b RT-qPCR revealed that the expression level of miR-29b was significantly lower in the downregulation (DR) group than in the negative control (NC) and blank control (BC) group ($P<0.05$), while there was no statistically significant difference between the latter 2 groups ($P>0.05$) (Fig. 2A).

The results of RT-qPCR also revaled that the mRNA expression levels TGF-β, α-SMA and Col I in the DR group were significantly higher than those of the BC and NC group

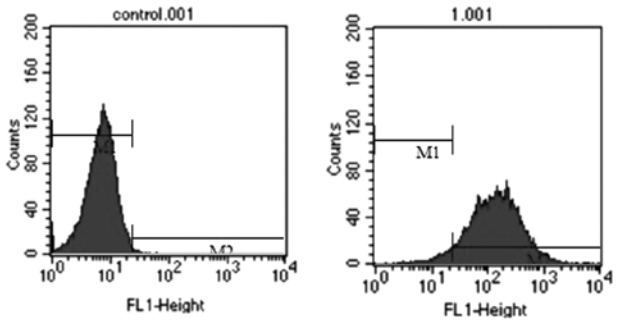

Figure 1. Transfection efficiency of NRK-52E cells following transfection with miR-29b inhibitor detected by flow cytometry. (Left panel) Blank control group of NRK-52E cells (transfection efficiency, 0.25%); (right panel) NRK-52E cells transfected with inhibitor (transfection efficiency, 95.14%).

(P<0.05), while there was no statistically significant difference between the latter 2 groups (P>0.05) (Fig. 2B).

The results of western blot analysis indicated that the protein expression levels of TGF-β, α-SMA and Col I in the DR group were significantly higher than those of the BC and NC group (P<0.05), while there was no statistically significant difference between the latter 2 groups (P>0.05) (Fig. 2C).

The results of immunofluorescence staining also revealed that the expression levels of α-SMA and Col I in the DR group were significantly higher than those of the BC and NC group (Fig. 2D and E).

Transfection with miR-29b mimics downregulates the expression levels of TGF-β, α-SMA and Col I. The NRK-52E cells were transfected with miR-29b mimics, and treated with Ang II (10^{-7} M) following transfection. The results of RT-qPCR demonstrated that the expression level of miR-29b was markedly higher in upregulation (UR) group than in the $NC_{Ang\ II}$ and Ang II group (P<0.05), while there was no statistically significant difference between the latter 2 groups (P>0.05) (Fig. 3A).

The results of real-time PCR revealed that the mRNA expression levels of TGF-β, α-SMA and Col I were significantly lower in the UR group than in the Ang II and $NC_{Ang\ II}$ group, while there was no statistically significant difference between the latter 2 groups (P>0.05); the expression levels of both these latter groups were significantly higher than the levels of the BC group (Fig. 3B).

The results of western blot analysis indicated that the protein expression levels of TGF-β, α-SMA and Col I were significantly lower in the UR group than in the Ang II and $NC_{Ang\ II}$ group (P<0.05), while there was no statistically significant difference between the latter 2 groups (P>0.05); the expression levels of both these latter groups were significantly higher than the levels of the BC group (P<0.05) (Fig. 3C).

The results of immunofluorescence staining revealed that the expression levels of α-SMA and Col I in the UR group were significantly lower than those of the Ang II and $NC_{Ang\ II}$ group (Fig. 3D and E).

Discussion

The present study demonstrated that the expression level of miR-29b in the renal cortex of SHRs was decreased compared with age-matched WKY rats. Ang II induced the downregulation of miR-29b in the NRK-52E cells *in vitro*, and we hypothesized that low level of miR-29b expression in the renal cortex of SHRs may be related to the high level of Ang II. We came to this hypothesis on the basis of the study of Matsushima *et al* (6), who demonstrated that the levels of Ang II and the density of Ang II receptor in the kidneys of young SHRs were significantly higher than those of age-matched WKY rats. We also thus hypothesized that the downregulation of miR-29b expression may have a similar effect on NRK-52E cells as that observed following treatment with Ang II, while the upregulation of miR-29b expression may protect NRK-52E cells from EMT induced by Ang II.

The expression level of miR-29b in the renal cortex between adult SHRs and WKY rats was analyzed by RT-qPCR. We found that the expression level of miR-29b in the SHRs was decreased compared with that of the WKY rats. The study of Matsushima *et al* mentioned above testified high levels of Ang II and its receptor in the kidneys of SHRs. In our study, we treated NRK-52E cells with Ang II *in vitro*, and found (by RT-qPCR) that this induced the downregulation of miR-29b. Ang II promotes renal fibrosis by increasing the expression of fibrosis-associated genes, such as TGF-β, α-SMA and Col I (9-12). In recent years, a number of studies have demonstratd the effects of miR-29b on fibrosis (13-15). However, to the best of our knowledge, it has rarely been reported that Ang II regulates miR-29b expression. Liu *et al* (16) reported that Ang II induced renal functional injury and fibrosis through the upregulation of SP1 and the downregulation of miR-29b expression, while Smad7 may play a protective role in Ang II-induced hypertensive kidney injury. These data and the data presented in our study suggest that Ang II decreases the expression level of miR-29b. Possibly, the low levels of miR-29b expression in the renal cortex of SHRs may be related to the high levels of Ang II. However, the specific mechanisms involved remain unclear. We hypothesized that at least two distinct intracellular signaling pathways are involved. One pathway involves nuclear factor-κB (NF-κB) activated by Ang II by binding to type I angiotensin II receptor (AT1R) (17,18). NF-κB activates another transcription factor, YY1, which directly combines with the promoter of miR-29b2/c to suppress its expression (19). Another pathway involves mitogen-activated protein kinase (MAPK) activated by the Ang II-phospholipase C (PLC)-protein kinase C (PKC) signaling pathway; MAPK activates proto-oncogene c-myc, which directly binds to the promoter of miR-29b2/c to suppress its expression (20-22).

EMT is an important step in the process of renal fibrosis. TGF-β has been acknowledged to play an important role in EMT by inducing the expression of α-SMA, which is an important marker of EMT and stimulates the production of ECM, including Col I and Col III (15,23,24). To further examine the function of miR-29b, NRK-52E cells were transfected with miR-29b inhibitor. The results of RT-qPCR, western blot analysis and immunofluorescence staining revealed that miR-29b inhibitor induced a marked upregulation in the expression of TGF-β, α-SMA and Col I in the NRK-52E cells. Our data suggest that the downregulation of miR-29b expression promotes EMT. Members of the miR-29 family are negatively regulated by TGF-β/Smad3 and play a protective role in renal fibrosis by inhibiting the deposition of ECM and preventing EMT (25,26). At present, only approxi-

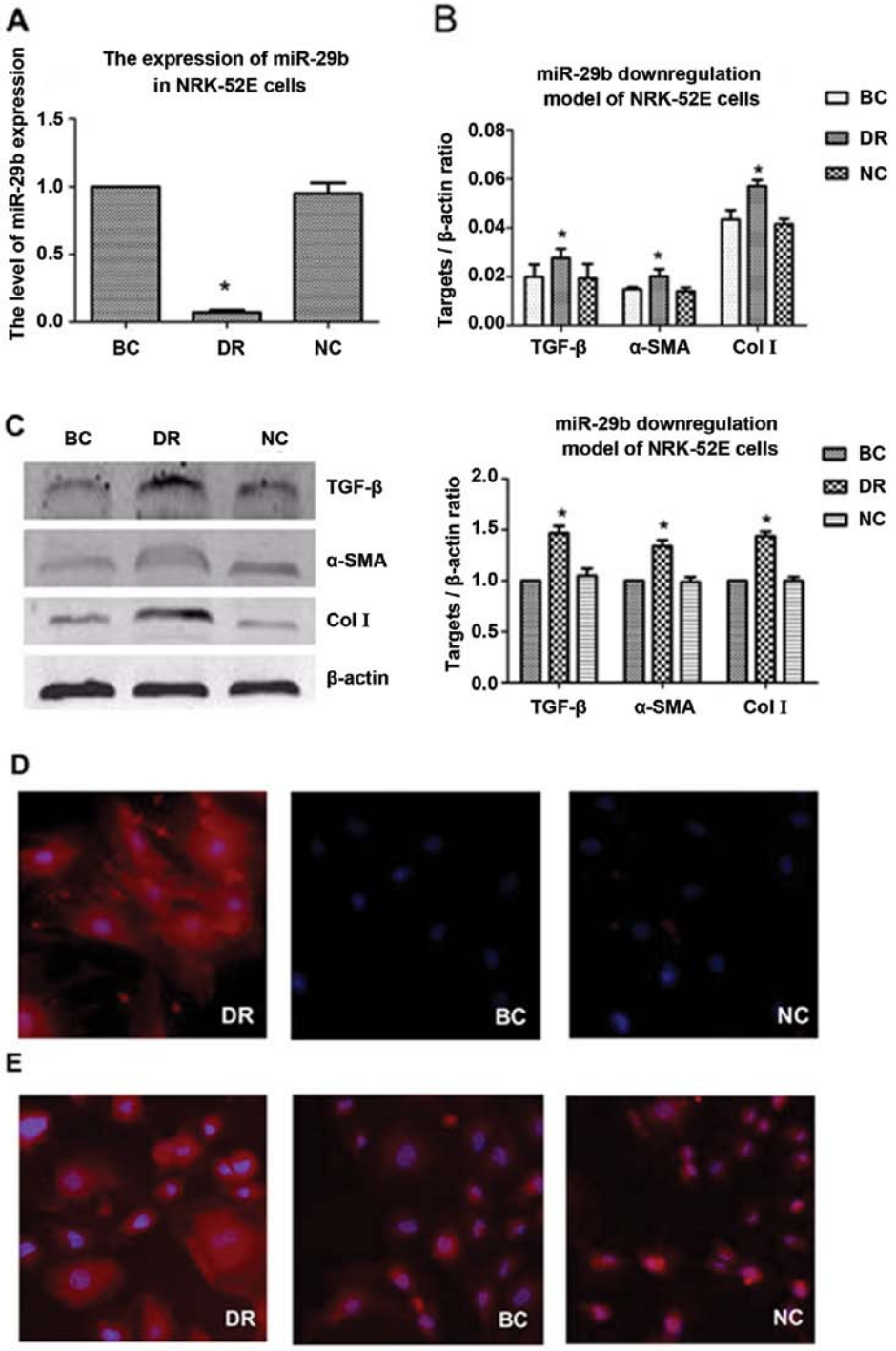

Figure 2. Expression levels of miR-29b, transforming growth factor (TGF)-β, α-smooth muscle actin (α-SMA) and collagen I (Col I) analyzed by RT-qPCR, western blot analysis and immunofluorescence staining following downregulation of miR-29b. (A) RT-qPCR analysis of miR-29b expression; *P<0.05 compared with the BC group and NC group. (B) RT-qPCR analysis of TGF-β, α-SMA and Col I expression; *P<0.05 compared with the BC group and NC group. (C) Western blot analysis of TGF-β, α-SMA and Col I expression; representative blots show TGF-β, α-SMA and Col I; *P<0.05 compared with the BC group and NC group. (D) Immunofluorescence staining of α-SMA; representative images demonstrating the fluorescence of α-SMA protein (red) and nuclear (blue) expression in NRK-52E cells in the different groups. (E) Immunofluorescence staining of Col I; representative images demonstrating the fluorescence of Col I protein (red) and nuclear (blue) expression in NRK-52E cells in the different groups. BC, blank control group; DR, downregulation group (transfection with miR-29b inhibitor); NC, negative control group.

mately 40 target genes of 7,000 candidates which were predicted by miRNA target prediction software (PicTar, TargetScan and miRBase) have been confirmed, chief among these were ECM genes, such as various collagens, fibrillin, elastin and protocadherin (12,15,19,24,27,28).

Furthermore, in our study, NRK-52E cells were transfected with miR-29b mimics and treated with Ang II 24 h following transfection. The results of RT-qPCR, western blot analysis and immunofluorescence staining demonstrated that the overexpression of miR-29b markedly suppressed the Ang II-induced

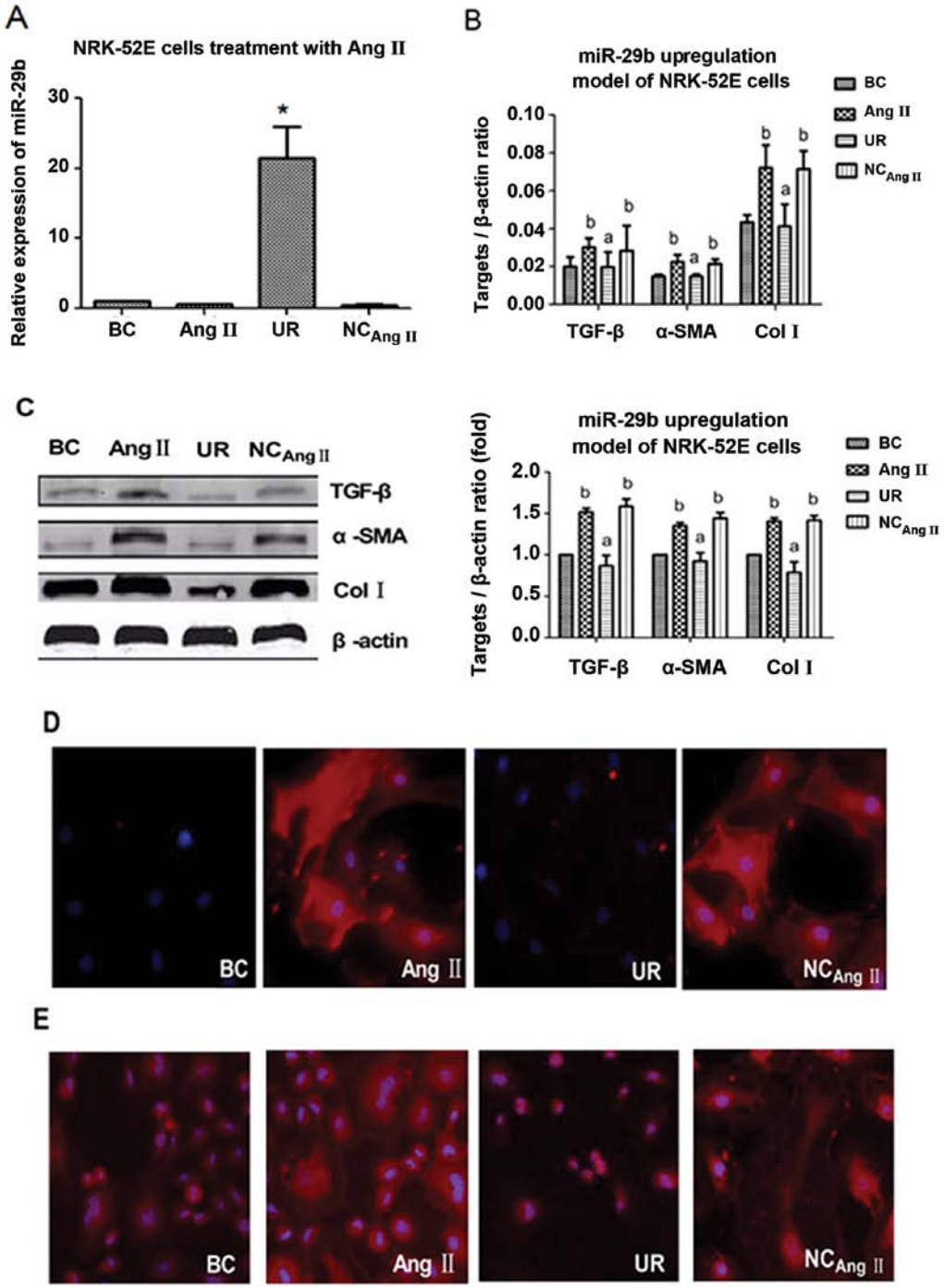

Figure 3. Expression levels of miR-29b, transforming growth factor (TGF)-β, α-smooth muscle actin (α-SMA) and collagen I (Col I) analyzed by RT-qPCR, western blot analysis and immunofluorescence staining following upregulation of miR-29b 24 h before treatment with 10^{-7} M angiotensin II (Ang II). (A) RT-qPCR analysis of miR-29b expression; *P<0.05 compared with the Ang II group and $NC_{Ang\ II}$ group. (B) RT-qPCR analysis of TGF-β, α-SMA and Col I expression; ^{a}P<0.05 compared with the Ang II group and $NC_{Ang\ II}$ group; ^{b}P<0.05 compared with the BC group. (C) Western blot analysis of TGF-β, α-SMA and Col I expression; representative blots show TGF-β, α-SMA and Col I; ^{a}P<0.05 compared with the Ang II group and $NC_{Ang\ II}$ group; ^{b}P<0.05 compared with the BC group. (D) Immunofluorescence staining of α-SMA; representative images demonstrating the fluorescence of α-SMA protein (red) and nuclear (blue) expression in NRK-52E cells in the different groups. (E) Immunofluorescence staining of Col I; representative images demonstrating the fluorescence of Col I protein (red) and nucleus (blue) in NRK-52E cells in different groups. BC, blank control group; Ang II, Ang II group (treatment with 10^{-7} M Ang II); UR, upregulation group (transfection with miR-29b mimics 24 h before treatment with 10^{-7} M Ang II); $NC_{Ang\ II}$, negative control-Ang II group (without transfection 24 h before treatment with 10^{-7} M Ang II).

upregulation of TGF-β, α-SMA and Col I. These results suggest that the overexpression of miR-29b negatively modulates EMT. miR-29 regulates multiple signal pathways, such as the TGF-β/Smad (15), NF-κB (19,22), Wnt/β-catenin (29) and MAPK (30) pathways, by suppresing the expression of target genes. The TGF-β/Smad pathway is considered the most important. Luna *et al* (31) found that the overexpression of miR-29b inhibited fibrosis by suppressing TGF-β1 expression at the mRNA and protein level. miR-29b directly suppresses the expression of ECM proteins, such as various collagens, fibrillin, elastin and

protocadherin (27,28). These data, as well as the data presented in our study illustrate that miR-29b negatively modulates EMT. Moreover, Ang II induces the downregulation of miR-29b expression and promotes NRK-52E cells to undergo EMT. The downregulation of miR-29b has a similar effect on NRK-52E cells as that observed following treatment with Ang II, while the upregulation of miR-29b protects NRK-52E cells from Ang II-induced EMT.

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