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MiR-30c-5p inhibits high glucose-induced EMT and renal fibrogenesis by down-regulation of JAK1 in diabetic nephropathy

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Abstract. – OBJECTIVE: Diabetic nephropathy (DN) is one of the most serious complications of diabetes mellitus (DM) and has become the major cause of end-stage renal failure. MicroRNAs (miRs) play key roles in many pathologic processes for initiating and progressing, including DN. Epithelial-mesenchymal transition (EMT) and renal fibrogenesis are important features of DN. However, the role of miR-30c-5p in high glucose (HG)-induced EMT and renal fibrogenesis is not clear. This study was aimed at determining the regulatory network of miR-30c-5p and JAK1 in DN.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) and Western blot assays were performed to detect expressions of miR-30c-5p, JAK1, vimentin, α-SMA, and E-cadherin. The possible binding sites between miR-30c-5p and JAK1 were predicted by TargetScan online database and verified by Luciferase report assay. The secretion of fibronectin (FN) and Collagen IV (Col IV) in the supernatant was detected by Enzyme-linked immunosorbent (ELISA) assay.

RESULTS: MiR-30c-5p was downregulated and JAK1 was upregulated in renal fibrosis tissue and HG stimulated HK2 cells. Transfection of miR-30c-5p inhibited HG-induced EMT and renal fibrogenesis in HK2 cells, which was reversed by miR-30c-5p inhibitor. Moreover, JAK1 was confirmed as a direct target of miR-30c-5 and knockdown of JAK1 markedly inhibited HG-induced renal fibrogenesis and EMT in HK2 cells. Furthermore, overexpression of JAK1 attenuated the inhibitory effect of miR-30c-5p on HG-induced EMT and renal fibrogenesis in HK2 cells.

CONCLUSIONS: MiR-30c-5p evidently inhibited HG-induced EMT and renal fibrogenesis by down-regulation JAK1 in DN, providing a promising therapeutic strategy for the treatment of DN.

Key Words:
Diabetic nephropathy, MiR-30c-5p, JAK1, High glucose, Epithelial-mesenchymal transition, Renal fibrogenesis.

Abbreviations

DN = Diabetic nephropathy; DM = diabetes mellitus; EMT = Epithelial-mesenchymal transition; HG = high glucose; ECM = extracellular matrix; miRNA = MicroRNA; DMEM = Dulbecco's Modified Eagle's Medium; ELISA = Enzyme-linked immunosorbent assay; OD = Optical density; SD = standard deviation.

Introduction

Diabetic nephropathy (DN) is one of the most serious complications of diabetes mellitus (DM) and has become the major cause of end-stage renal failure[1]. The worldwide prevalence of diabetic nephropathy is increasing, and DN has become a medical catastrophe for public health around the world[2]. Pathological features of DN include thickening of the basement membrane, tubular epithelial-mesenchymal transition (EMT), extracellular matrix (ECM) accumulation, glomerular sclerosis, and tubulointerstitial fibrosis (TIF), which ultimately lead to irreversible kidney damage[3,4]. Researchers[5,6] have demonstrated that high glucose (HG) stimulated EMT in renal tubular cells is an important mechanism of DN tubulointerstitial fibrosis. Thus, it is imperative to search for an effective therapeutic target for diagnosis and therapeutics of DN. MicroRNA (miRNA) is a family of small non-coding RNA (which contains about 22 nucleotides) and plays a key role in the regulation of gene expression at the posttranscriptional level[7]. Moreover, miRNAs play critical roles in a variety of physiological and pathological processes, such as growth and differentiation of organisms, cell proliferation and signal transduction, inflammation, and endocrine diseases[8,9]. Bullock et al[10] have shown that miRNAs have essential roles in EMT. The miR-30 family consists of five members, miR-

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30a, b, c, d, and e. Among these, miR-30c could be protective against diabetic nephropathy by suppressing EMT[11]. In addition, previous studies[12,13] demonstrated that EMT in cancer cells could be promoted via knockdown of miR-30c-5p. However, the role of miR-30c-5p in HG-induced EMT and renal fibrogenesis is not clear. The JAK-STAT pathway induces varieties of cellular responses by transmitting signals from extracellular ligands which induce many cytokines and chemokines, growth factors, and hormones to the nucleus directly[14]. In the past few years, the role of the JAK/STAT pathway in DN and other chronic kidney diseases has attracted much attention[15]. It has been reported that the expression levels of JAK1, 2, and 3 in diabetic kidneys were significantly upregulated compared with normal subjects[16]. However, reports on JAK1 in DN remain poorly understood. In the present study, the expression levels of miR-30c-5p and JAK1 in renal tissues and HK2 cells were first detected. Furthermore, we investigated the effect of miR-30c-5p and JAK1 on DN and explored the regulatory network of miR-30c-5p and JAK1, providing a promising therapeutic strategy for the treatment of DN.

Patients and Methods

Clinical Samples and Cell Culture

A total of 10 pairs of renal tissues and adjacent normal tissues were collected from the Zhongshan Hospital Affiliated to Dalian University between January 2017 to January 2018. The written informed consents were obtained from all patients and our investigation was approved by the Ethics Committee of the Zhongshan Hospital Affiliated to Dalian University. All samples were immediately frozen in liquid nitrogen and stored in a freezer at −80°C until use.

Human kidney 2 cell lines were purchased from American Tissue Culture Collection (ATCC; Manassas, VA, USA). The cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (HyClone, South Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) and maintained in a humidified atmosphere containing 5% CO_2 at 37°C.

Reagent and Cell Transfection

MiR-30c-5p mimic (miR-30c-5p) and its matched negative controls (miR-NC), miR-30c-5p inhibitor and its matched negative controls (inhibitor-NC), Lentivirus-mediated shRNA interference targeting JAK1 (sh-JAK1) and empty lentiviral vector (sh-NC), JAK1 expression plasmid (pcDNA-JAK1) and its matched negative controls (pcDNA-control) were obtained from GenePharma Co. ltd. (Shanghai, China). HK-2 cells were transfected with these oligonucleotides or plasmids by Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) according to the instructions of the manufacturer.

Quantitative Real-Time Polymerase Chain Reaction Assay

Total RNA was obtained from tissues and cells using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) referring to the manufacturer's instructions. Next, complementary DNA (cDNA) was synthesized using Reverse Transcription Kit (TaKaRa, Dalian, China) or TaqMan microRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Subsequently, the cDNA was diluted and used for qRT-PCR by SYBR green detection kit (Toyobo, Tokyo, Japan) on a CFX96 Real-time PCR system (Bio-Rad; Hercules, CA, USA) according to the amplification instructions. All primers were purchased from Sangon Biotech (Shanghai, China) and listed below: miR-30c-5p (Forward, 5'-GCCGCTGTAAACATCCTACACT-3'; Reverse, 5'-GTGCAGGGTCCGAGGT-3'); JAK1 (Forward, 5'-TGCTCCTGAGTGTGTTGAGG–3'; Reverse, 5'-AGGTCAGCCAGCTCCTTACA-3'); FN (Forward, 5'-CGGTGGCTGTCAGTCAAAG-3'; Reverse, 5'-AAACCTCGGCTTCCTCCATAA-3'); Col IV (Forward, 5'-GGACTACCTGGAACAAAAGGG-3'; Reverse, 5'-GCCAAGTATCTCACCTGGATCA-3'); E-cadherin (Forward, 5'-CTACCAGCCCAAAGTGTGTG-3'; Reverse, 5'-GTGTTATCGTGATTATCCGTGA-3'); α-SMA (Forward, 5′-AAAAGACAGCTACGTGGGTGA-3'; Reverse, 5'-GCCATGTTCTATCGGGTACTTC-3'); Vimentin (Forward, 5'-CGCCAACTACATCGACAAGG-3'; Reverse, 5'-GGCTTTGTCGTTGGTTAGCT-3'); U6 snRNA (Forward, 5'-CTCGCTTCGGCAGCACA-3'; Reverse, 5'-AACGCTTCACGAATTTGCGT-3'); GAPDH (Forward, 5'-CGTGGGCCGCCCTAGGCACCA-3'; Reverse 5'-TTGGCTTAGGGTTCAGGGGGG-3'). The relative expression of miRNA and mRNA was calculated by the $2^{-\Delta\Delta Ct}$ method using U6 snRNA or glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as internal control.

Western Blot Assay

Tissues or HK2 cells were harvested and lysed in radioimmunoprecipitation assay (RIPA) ly-

sis buffer (Sigma-Aldrich, St. Louis, MO, USA) containing protease inhibitor (Sigma-Aldrich, St. Louis, MO, USA) for 30 min at 4°C. Next, the supernatant was collected by centrifuging at high speed of 12,000 x g for 10 min. Total protein concentration was detected by bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Rockford, IL, USA). An equal amount of proteins was separated by 8-10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, the gels were transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Next, membranes were blocked in 5% (w/v) non-fat milk in Tris-buffered saline and 0.1% Tween-20 (TBST) buffer for 2 h at room temperature and then incubated with specific primary antibodies against E-cadherin (#3195), vimentin (#5741), α-SMA (#19245), and GAPDH (#5174; Cell Signaling Technology, Danvers, MA, USA) at 1:1000 dilutions in 5% non-fat milk overnight at 4°C shaker, and then washed 3 times with TBST. After that, the membranes were incubated by horseradish peroxidase (HRP)-conjugated secondary antibodies (Sangon Biotech, Shanghai, China) at 1:4000 dilutions for 2 h at room temperature. The membranes again were washed 3 times in TBST. Finally, the protein bands were detected by enhanced chemiluminescence (ECL) chromogenic substrate (Thermo Fisher Scientific, Waltham, MA, USA) and imaged using a chemiluminescent gel imaging system (Bio-Rad, Hercules, CA, USA).

Enzyme-Linked Immunosorbent Assay (ELISA)

HK2 cells were transfected with miR-30c-5p mimic, miR-30c-5p inhibitor, sh-JAK1, miR-30c-5p+pcDNA-JAK1 or their matched controls and then stimulated with HG for 24 h. The protein expression of FN and Col IV in the supernatant was detected by ELISA kit (NeoScientific, Cambridge, MA, USA) according to the ELISA kit instructions. The Optical density (OD) was detected by microplate reader (Bio-Teck, Winooski, VT, USA) at 450 nm.

Luciferase Reporter Assay

The binding sites of miR-30c-5p and JAK1 were predicted by TargetScan online software. The 3' untranslated regions (3'-UTR) of JAK1 containing wild-type or mutant binding sites of miR-30c-5p were amplified and cloned into pGL3 plasmids (Promega, Madison, WI, USA), namely, pGL3-JAK1-3′UTR-WT and pGL3-JAK1-3′UTR-MUT. The miR-30c-5p mimic or miR-30c-5p inhibitor was co-transfected with WT or MUT luciferase reporter plasmids into HK2 cells according to the protocols of the manufacturer. After transfection for 24 h in HG, luciferase activities were examined using Dual-Luciferase Assay Kit (Promega, Madison, WI, USA).

Statistical Analysis

Data of our research were shown as the mean ± standard deviation (SD) of at least three independent experiments. The significance differences between groups were analyzed by Student's *t*-test using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) Prism. $p<0.05$ was considered as statistically significant difference.

Results

MiR-30c-5p Was Downregulated and JAK1 Was Upregulated In Renal Fibrosis Tissues and HG-Induced HK2 Cells

To confirm the potential role of miR-30c-5p and JAK1 in the processes of renal fibrosis, qRT-PCR and Western blot analysis were performed to explore the expression of miR-30c-5p and JAK1 in both tissues and HK2 cells. The results demonstrated that the expression of miR-30c-5p was significantly downregulated in renal fibrosis tissues compared with the corresponding normal tissues (Figure 1A). Next, we investigated the expression of miR-30c-5p in HG-induced HK2 cells. The cells were cultured in normal glucose (NG, 5.5 mM) or high glucose (HG, 30 mM) for 6, 12, 24, and 48 h, respectively. We found that the expression of miR-30c-5p was significantly downregulated in HG-induced cells, and it reached the lowest at 24 h with a time-dependent manner (Figure 1B). Moreover, the qRT-PCR and Western blot analysis revealed that the mRNA and protein levels of JAK1 were markedly upregulated in contrast to normal tissues (Figure 1C and 1D). Subsequently, the mRNA and protein levels of JAK1 were detected in HG and NG, and it was found drastically upregulated in HG-induced HK2 cells (Figure 1E and 1F). From these data, we predicted that miR-30c-5p may negatively regulate JAK1.

Up-Regulation of MiR-30c-5p Inhibited HG-Induced EMT and Renal Fibrogenesis In HK2 Cells

To explore the effects of miR-30c-5p on HG-induced EMT and renal fibrogenesis, HK2 cells

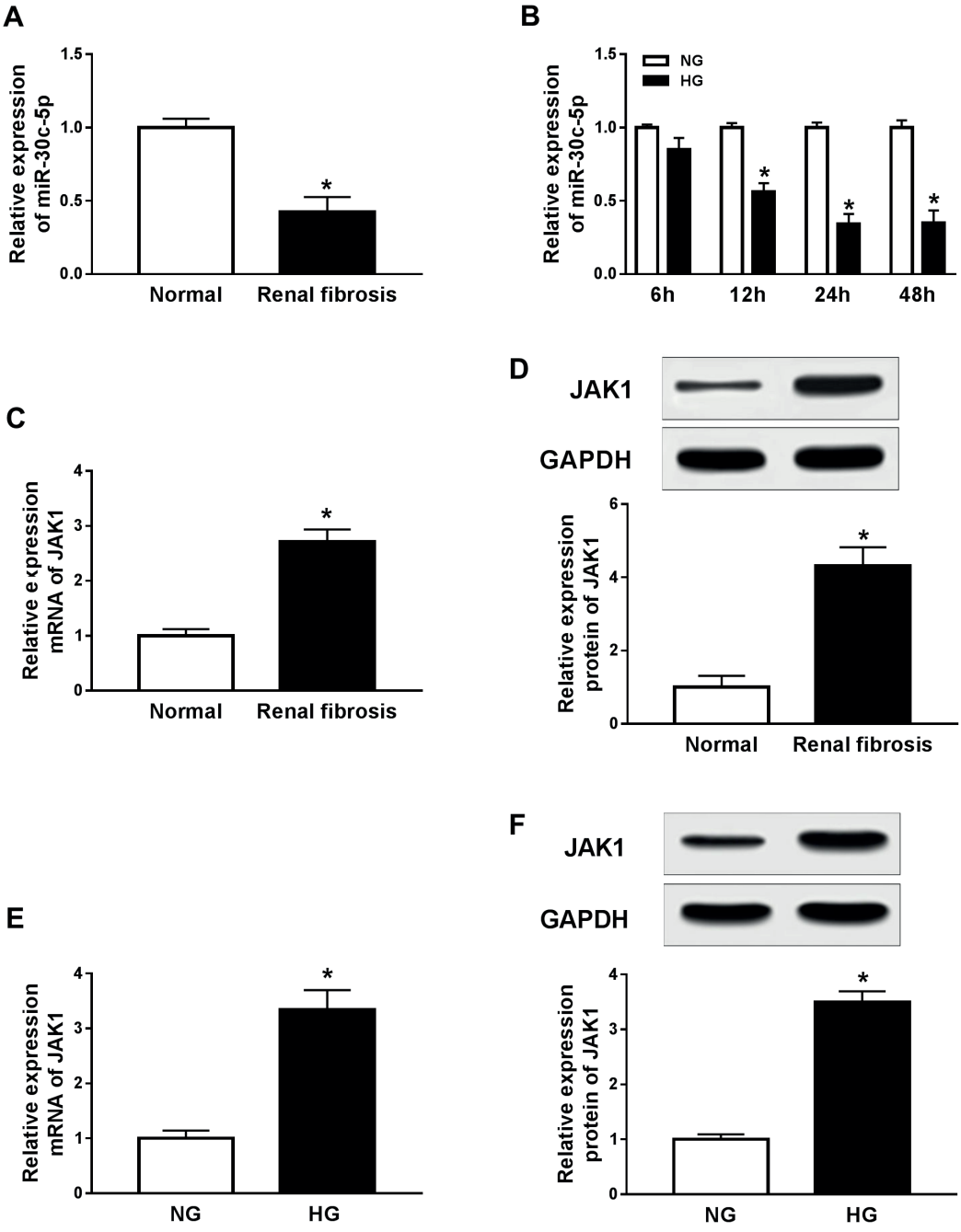

Figure 1. MiR-30c-5p was downregulated and JAK1 was upregulated in renal fibrosis tissues and HG-induced HK2 cells. **A**, Level of miR-30c-5p in normal (n = 10) and renal fibrosis tissues (n = 10) was detected by qRT-PCR. **B**, HK2 cells were cultured with NG (5 mM) or HG (30 mM) for 6, 12, 24 and 48 h, and the expression of miR-30c-5p was measured by qRT-PCR. **C** and **D**, The mRNA and protein expression levels of miR-30c-5p in renal fibrosis tissues were detected by qRT-PCR and Western blot assays. **E** and **F**, Expression of JAK1 in HG and NG was measured by qRT-PCR and Western blot assays. *$p<0.05$.

were transfected with miR-30c-5p mimic and stimulated in HG for 24 h. First, qRT-PCR assay revealed that overexpression of miR-30c-5p abolished the effect of HG on the expression of miR-30c-5p (Figure 2A). Additionally, increase expression of the mesenchymal marker a-SMA and vimentin, as well as a decrease expression of the epithelial marker E-cadherin, are essen-

tial features of EMT. qRT-PCR and Western blot analysis proved that HG stimulation evidently decreased the expression of E-cadherin and markedly promoted expression of vimentin and α-SMA, whereas transfection of miR-30c-5p mimic eliminated these effects (Figure 2B and 2C). Subsequently, qRT-PCR and ELISA analysis showed that the expression and secretion of FN and Col IV were significantly increased under HG stimulation, whereas the expression and secretion were markedly reduced following transfection of miR-30c-5p mimic (Figure 2D and 2E). Our data indicated that transfection of miR-30c-5p mimic inhibited EMT and renal fibrogenesis induced by HG in HK2 cells.

Down-Regulation of MiR-30c-5p Promotes HG-Induced Renal Fibrogenesis and EMT In HK2 Cells

To further investigate the effects of miR-30c-5p on HG-induced renal fibrogenesis and EMT, HK2 cells were transfected with miR-30c-5p inhibitor and then stimulated with HG for 24 h. As displayed in Figure 3A, transfection of miR-30c-5p inhibitor dramatically inhibited the expression of miR-30c-5p *vs.* HG control group. Simultaneously, qRT-PCR and Western blot analysis detected the mRNA and protein levels of E-cadherin, α-SMA, and vimentin in HK2 cells. Results showed that after transfection of miR-30c-5p inhibitor with HG stimulation, the expression of vimentin and α-SMA was evidently promoted and E-cadherin was significantly decreased (Figure 3B and 3C). Moreover, we further demonstrated that the expression and secretion of FN and Col IV were significantly increased in HK2 cells after transfected with miR-30c-5p inhibitor (Figure 3D and 3E). These findings revealed that transfection of miR-30c-5p inhibitor promoted HG-induced EMT and renal fibrogenesis in HK2 cells.

JAK1 Acts As a Target Gene of MiR-30c-5p In HK2 Cells

The potential binding sites of miR-30c-5p and JAK1 were predicted by TargetScan online website, suggesting the potential binding sites between miR-30c-5p and JAK1 (Figure 4A). To further confirm the prediction, the effect of miR-30c-5p on the relative luciferase activity of the JAK1 3'UTR-WT or JAK1 3'UTR-MUT reporter was detected by the luciferase reporter assay. Our data showed that overexpression of miR-30c-5p notably reduced the luciferase activity and transfection of miR-30c-5p inhibitor markedly elevated the luciferase activity in HK2 cells transfected with JAK1 3'UTR-WT, but no evident effect was found on luciferase activity of JAK1 3'UTR-MUT reporter (Figure 4B and 4C). Additionally, RT-qPCR and Western blot analysis verified that the transfection of miR-30c-5p mimic resulted in the evident down-regulation of JAK1 level and knockdown miR-30c-5p showed an opposite effect in HK2 cells (Figure 4D and 4E). Our findings indicated that JAK1was a target of miR-30c-5p.

Knockdown of JAK1 Inhibited HG-Induced Renal Fibrogenesis and EMT In HK2 Cells

To further explore the effect of JAK1 on HK2 cells, sh-JAK1 was transfected in HK2 cells with HG stimulation for 24 h. qRT-PCR and Western blot analysis demonstrated that HG-induced HK2 cells (Figure 5A and 5B). Furthermore, qRT-PCR and Western blot analysis were performed to determine the mRNA and proteins levels of E-cadherin, vimentin, and α-SMA in HK2 cells. As shown in Figure 5C and 5D, the cells transfected with sh-JAK1 led to a significant increment of E-cadherin expression and an evident reduction of vimentin and α-SMA expressions. Simultaneously, qRT-PCR and ELISA analysis showed that the expression and secretion of FN and Col IV were significantly increased under HG stimulation, which was abolished following transfection of sh-JAK1 in HK2 cells (Figure 5E and 5F). These data revealed that transfection of sh-JAK1 suppressed HG-induced EMT and renal fibrogenesis in HK2 cells.

MiR-30c-5p Suppressed HG-Induced EMT and Renal Fibrogenesis By Targeting JAK1

To confirm that miR-30c-5p directly targeted JAK1 to suppress HG-induced EMT and renal fibrogenesis in HK2 cells, miR-30c-5p mimic and pcDNA-JAK1 were co-transfected into HK2 cells with HG stimulation for 24 h. The qRT-PCR and Western blot analysis indicated that the mRNA and protein levels of vimentin and α-SMA were markedly reduced and E-cadherin wad drastically increased after transfection with miR-30c-5p mimic, whereas overexpression of JAK1 attenuated these effects (Figure 6A and 6B). Additionally, we demonstrated that transfection of miR-30c-5p mimic dramatically reduced the expression and secretion of FN and Col IV in HK2 cells, while transfection of pcDNA-JAK1 significantly reversed miR-30c-5p-mediated reduction of these effects (Figure 6C and 6D). We detected that miR-

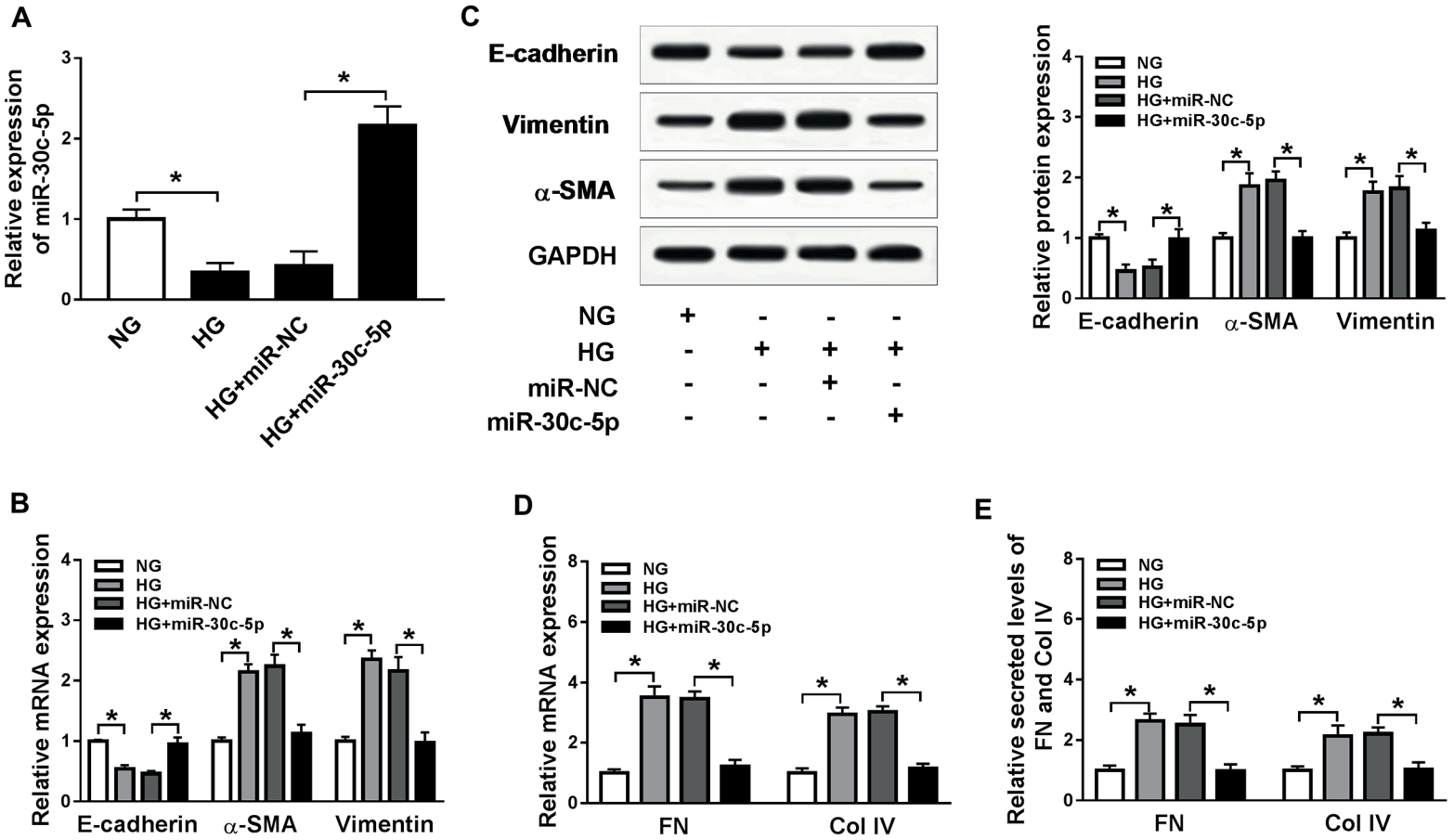

Figure 2. Effect of miR-30c-5p mimic on HG-induced EMT and renal fibrogenesis of HK2 cells. HK2 cells were transfected with miR-30c-5p mimic and stimulated in HG for 24 h. **A**, Level of miR-30c-5p in HK2 cells were determined by qRT-PCR. **B** and **C**, The mRNA and protein expressions of E-cadherin, a-SMA and Vimentin were detected by qRT-PCR and Western blot assays, respectively. **D**, The mRNA expression of FN and Col IV were measured by qRT-PCR. **E**, Secretion of FN and Col IV were detected by ELISA assay. *$p<0.05$.

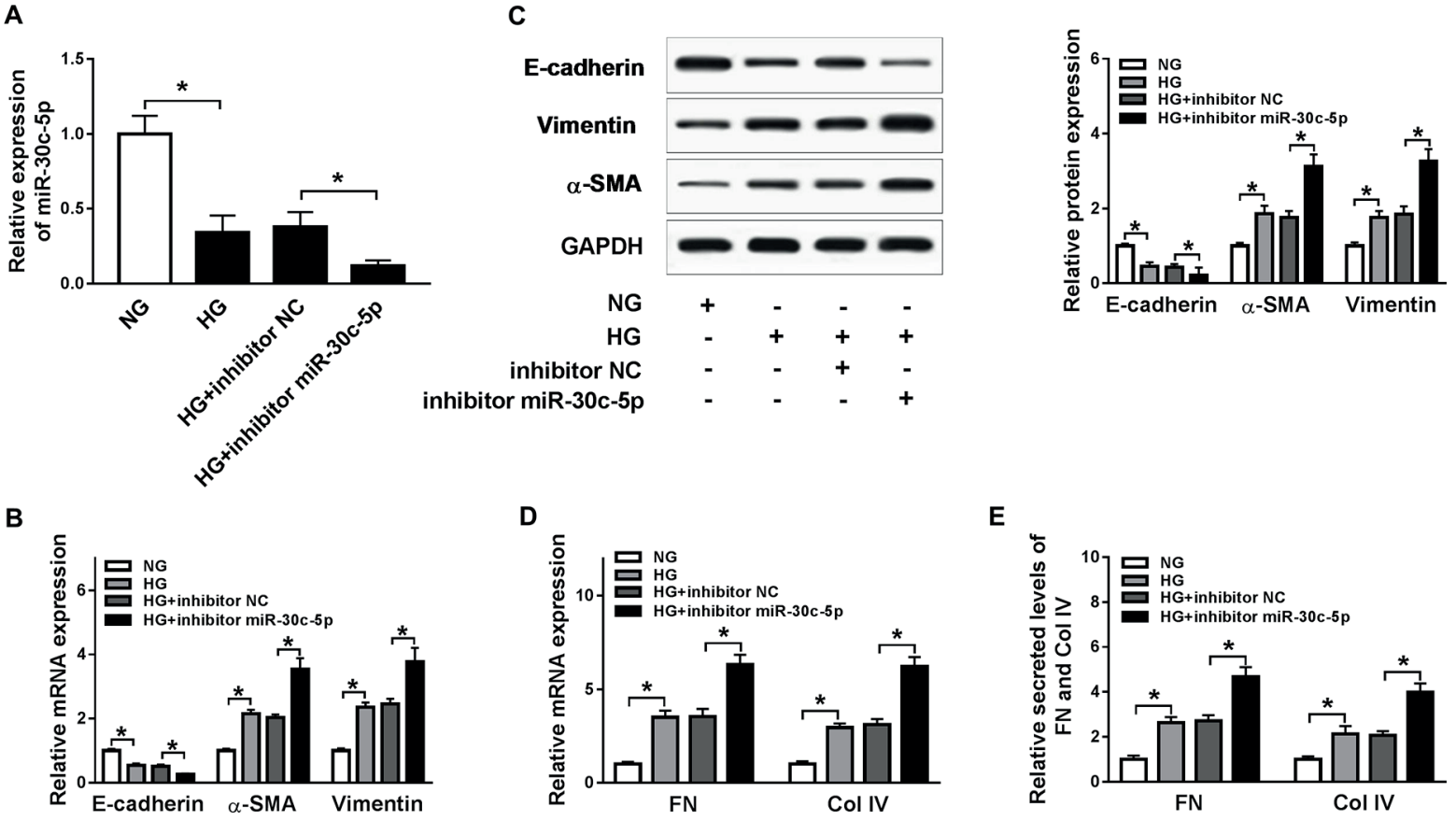

Figure 3. Effect of miR-30c-5p inhibitor on HG-induced EMT and renal fibrogenesis of HK2 cells. HK2 cells were transfected with miR-30c-5p inhibitor and stimulated in HG for 24 h. **A**, Level of miR-30c-5p in HK2 cells was determined by qRT-PCR. **B** and **C**, mRNA and protein expressions of E-cadherin, a-SMA and Vimentin were detected by qRT-PCR and Western blot assays, respectively. **D**, The mRNA expression of FN and Col IV was measured by qRT-PCR. **E**, Secretion of FN and Col IV was detected by ELISA assay. *$p<0.05$.

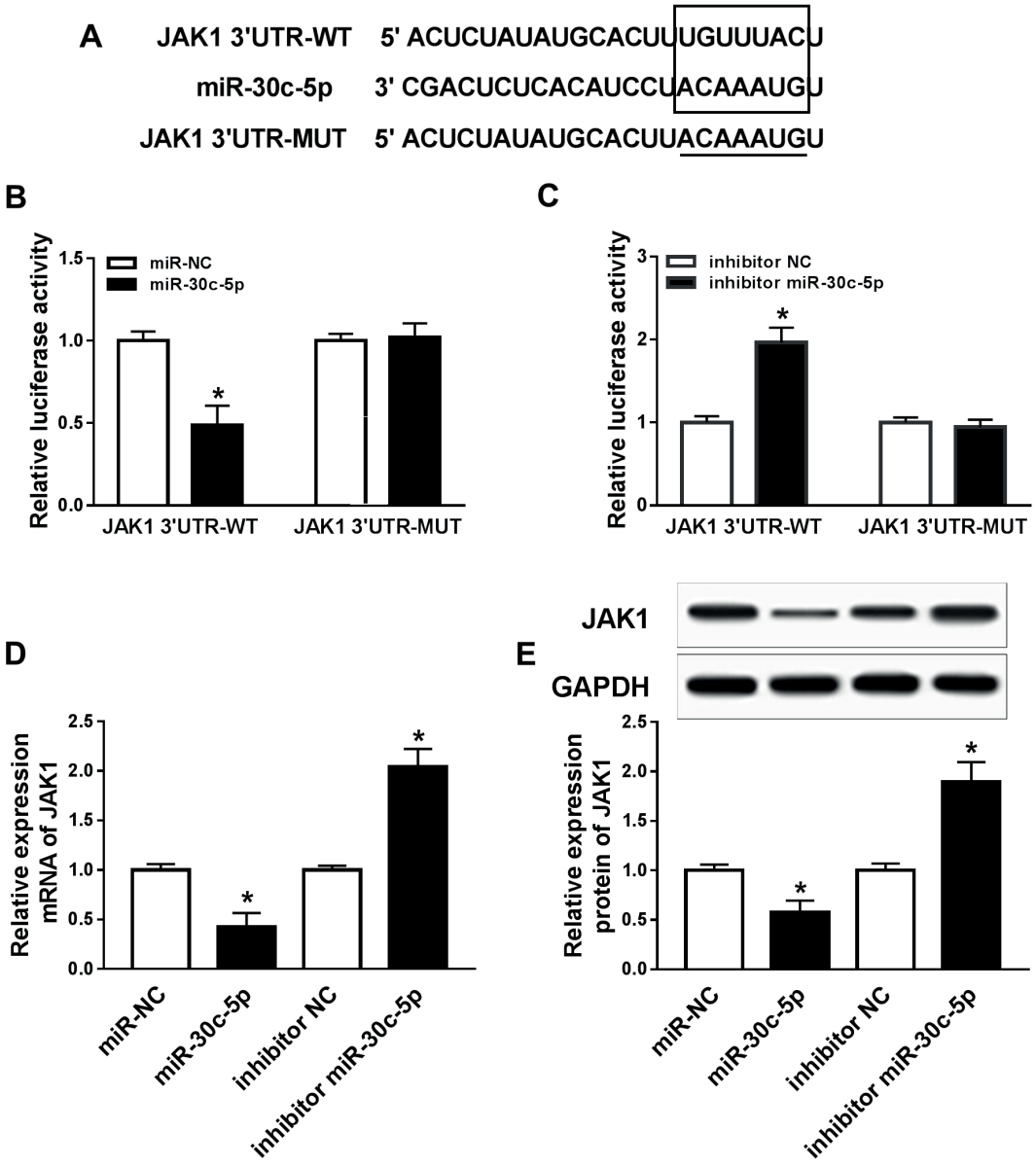

Figure 4. JAK1 was a direct target of miR-30c-5p. MiR-30c-5p mimic or inhibitor was transfected and then stimulated with HG in HK2 cells for 24 h. **A**, Potential binding sites of miR-30c-5p and JAK1 were predicted by TargetScan online software. **B** and **C**, HK2 cells were co-transfected with JAK1-3′UTR-WT or JAK1-3′UTR-MUT reporter and miR-30c-5p mimic or inhibitor, followed by the detection of relative luciferase activity. **D** and **E**, The mRNA and protein expressions of JAK1 detected by qRT-PCR and Western blot assays, respectively. *$p<0.05$.

30c-5p inhibited HG-induced EMT and renal fibrogenesis by targeting JAK1.

Discussion

DN is usually considered to be the leading cause of chronic kidney disease worldwide[17]. Some studies[18,19] have revealed that miRNAs are frequently dysregulated in many serious diseases, including DN. In this research, we focused on the role and mechanism of miR-30c-5p in EMT and renal fibrogenesis by regulation of JAK1.

MicroRNA-30 family has been confirmed to be an important player in the development of tissues and organs and the pathogenesis of many diseas-

es[20]. The previous finding has demonstrated that miR-30c-5p would suppress migration, invasion, and EMT of gastric cancer by targeting MTA1[21]. It was reported that overexpression of miR-30c reduced renal fibrosis in DN to improve kidney structure and function[22]. In addition, miR-30c was downregulated in DN and renal dysfunction of DN in db/db mice was attenuated by overexpression of miR-30c[11]. In our results, we demonstrated that expression of miR-30c-5p was significantly inhibited in renal fibrosis tissues and HG-stimulated cells, which was in line with our previous findings.

JAK-STAT pathways play important activating roles in varieties of disease processes[23]. It was reported that JAK1 is involved in many cytokine signaling *in vivo,* and its overexpression and gene mutation are related to tumor development[24-26]. Zhang et al[27] indicated that the major phenotypic changes of diabetic kidney disease were reversed after treating with JAK1/2 inhibitor. Similarly, we found that the expression level of JAK1 was increased notably in renal fibrosis tissues and HG-stimulated cells. These results demonstrated that miR-30c-5p and JAK1 were important regulatory factors in DN.

EMT is an indispensable process in the development and progression of DN[28]. Increasing reports[29,30] have found that EMT, which is in renal tubular epithelial cells, plays a key role during the

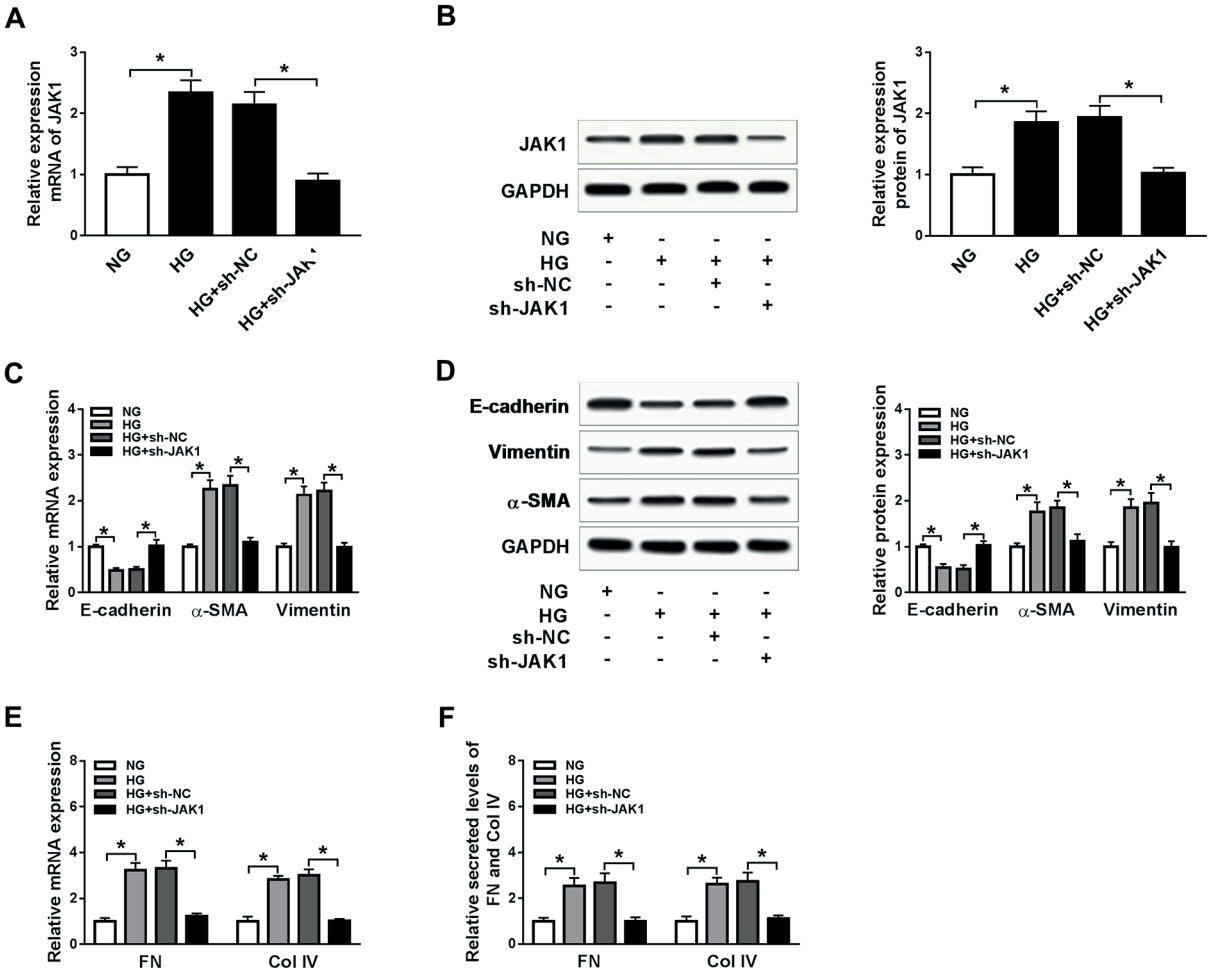

Figure 5. Knockdown of JAK1 inhibited HG-induced renal fibrogenesis and EMT in HK2 cells. The sh-JAK1 was transfected in cells and then stimulated with HG for 24 h. **A** and **B**, The mRNA and protein levels of JAK1 were detected by qRT-PCR and Western blot assays. **C** and **D**, The mRNA and protein expressions of E-cadherin, a-SMA and Vimentin were detected using qRT-PCR and Western blot assays, respectively. **E**, The mRNA expression of FN and Col IV was measured by qRT-PCR. **F**, The secretion of FN and Col IV was detected by ELISA assay. *$p<0.05$.

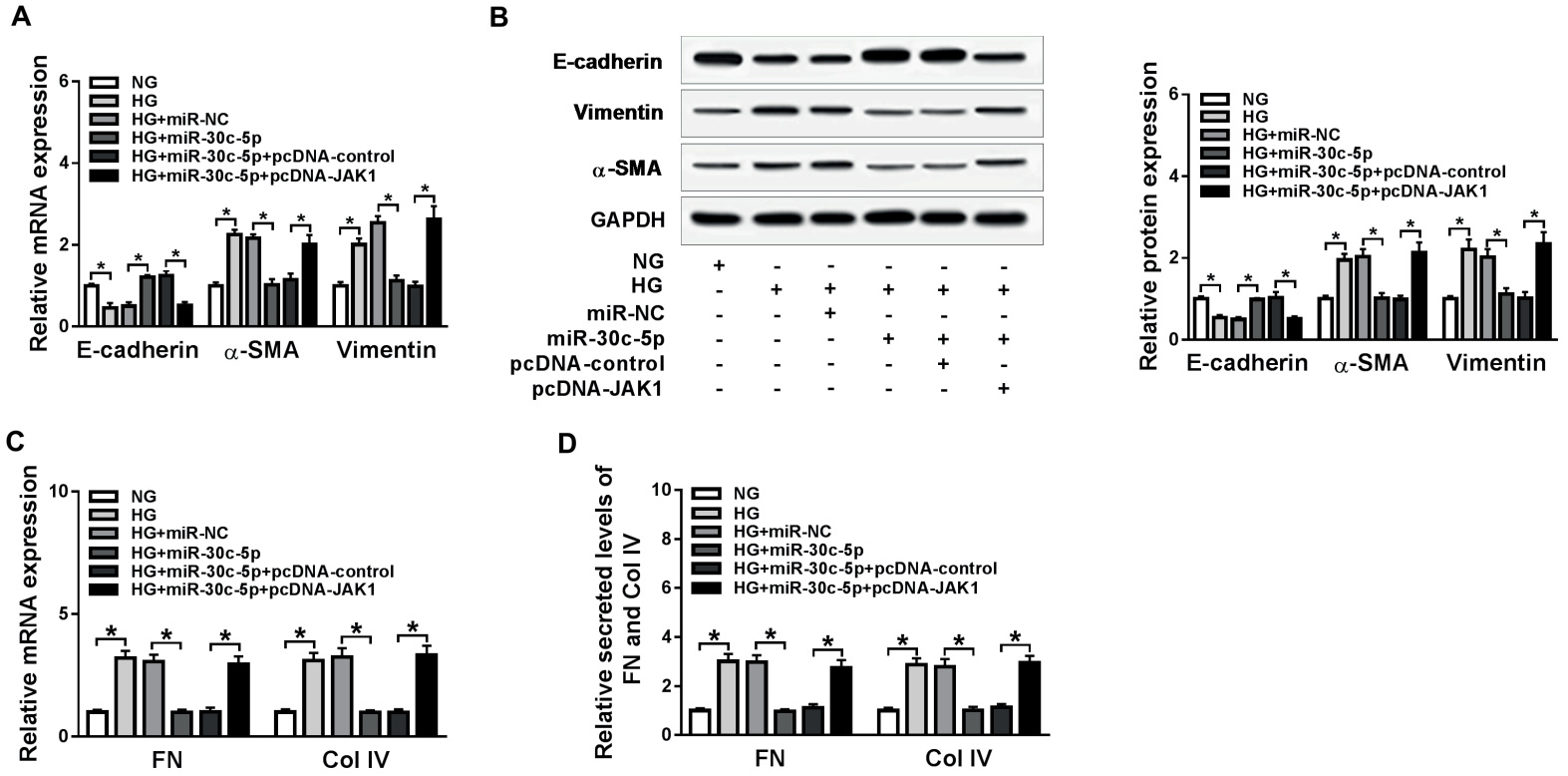

Figure 6. MiR-30c-5p suppressed HG-induced EMT and renal fibrogenesis by targeting JAK1. HK2 cells were transfected miR-30c-5p mimic or miR-30c-5p+ pcDNA-JAK1 and then stimulated with HG for 24 h. **A** and **B**, The mRNA and protein expressions of E-cadherin, a-SMA and Vimentin were detected by qRT-PCR and Western blot assays, respectively. **C**, The mRNA expression of FN and Col IV was measured by qRT-PCR. **D**, Secretion of FN and Col IV was detected by ELISA assay. *p<0.05.

development and progression of DN and organ fibrosis. If miR-30c-5p plays an important role in EMT, it would be vital to determine whether its overexpression improves EMT induced by HG. To determine this, miR-30c-5p mimic was transfected into HK2 cells under HG condition to assess the expression levels of EMT-related genes and protein. The results indicated that transfection of miR-30c-5p mimic inhibited EMT induced by HG in HK2 cells.

EMT accelerates the early development of diabetic renal interstitial fibrosis[31]. Renal fibrosis is a common feature of chronic kidney diseases and diabetic nephropathy[32]. FN and Col IV are considered markers of renal fibrogenesis and can cause renal fibrosis when accumulated in DN[33]. It has proved that the expression and secretion of FN and Col IV in the culture medium were conspicuously reduced after transfection of miR-23a inhibitor in HK2 cells[34]. In our research, it was found that the expression and secretion of FN and Col IV were markedly reduced following transfection of miR-30c-5p mimic in HK2 cells, and the transfection of sh-JAK1 also presents the same phenomenon. In our data, it was showed that transfection of miR-30c-5p mimic and sh-JAK1 inhibited HG-induced EMT and renal fibrogenesis in HK2 cells.

Wang et al[22] demonstrated that renal fibrosis is promoted by down-regulation of miR-30c through target CTGF in diabetic nephropathy. However, more targets on miR-30c have not been reported yet. In this report, JAK1 was predicted as a target of miR-30c-5p through Targetscan online website. Moreover, our data revealed that overexpression of miR-30c-5p could prevent the expression of HG-induced JAK1 and down-regulation of miR-30c-5p led to an evident increment of JAK1 expression in HK2 cells. These results demonstrated the existence of inverse regulatory relationship between miR-30c-5p and JAK1. Additionally, overexpression of JAK1 inhibited the role of miR-30c-5p in HG-induced EMT and fibrosis. This indicated that JAK1 was directly targeted by miR-30c-5p.

Conclusions

We showed that miR-30c-5p and JAK1 were downregulated and upregulated, respectively, in renal fibrotic tissue and HG stimulated HK2 cells. Transfection of miR-30c-5p suppressed HG-induced EMT and renal fibrogenesis in HK2 cells, which was reversed by miR-30c-5p inhibitor. Moreover, JAK1 was indicated as a target of miR-30c-5p and its overexpression attenuated the inhibitory effect of miR-30c-5p on HG-induced EMT and fibrosis. Collectively, overexpression of miR-30c-5p prevented HG-induced EMT and renal fibrogenesis by targeting expression of JAK1. In addition, potential therapeutic avenues for preventing renal fibrosis in DN would be provided by further research of the function and significance of these anti-fibrotic miRNAs and their specific targets.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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