Article

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LncRNA Meg3 Aggravates Renal Fibrosis Caused by Unilateral Ureteral Obstruction in Rats by Activating the Hedgehog Pathway

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Background: The hedgehog signaling pathway exerts vital functions in regulating epithelial-to-mesenchymal transition (EMT) in renal interstitial fibrosis (RIF). It was reported that lncRNA-maternally expressed gene 3 (lncRNA Meg3) can regulate hepatic fibrosis by regulating the expression of smoothened (Smo) in the hedgehog signaling pathway. However, the specific role of lncRNA Meg3 in renal fibrosis resulting from unilateral ureteral obstruction (UUO) by regulating the hedgehog signaling pathway has not been reported. Hence, this research aimed to expound the effects of lncRNA Meg3 on renal fibrosis induced by UUO in rats via the hedgehog pathway.

Methods: Peripheral blood was collected from patients with chronic kidney disease (CKD, CKD group) and healthy volunteers (Normal group) at the same period. In addition, 6-week-old male Sprague-Dawley (SD) rats were divided to Sham, UUO, UUO+shRNA Negative control (shNC), and UUO+sh-Meg3 groups, and their kidney tissues and serum were gathered. Next, quantitative real-time polymerase chain reaction (qRT-PCR) was employed for detecting the lncRNA Meg3 expression level in the serum of patients and renal tissue of rats; kits for testing levels of blood urea nitrogen (BUN), creatinine (Cr), hydroxyproline (HYP), and 24-hour urine protein (24-up) in rats of each group; hematoxylin and eosin (HE) staining and Masson staining for observing kidney tissue and renal fibrosis level in rats; western blot for measuring levels of collagen type III (Col III), α-Smooth muscle actin (α-SMA), fibronectin, E-cadherin, sonic hedgehog (Shh), patched (Ptch) protein, smoothened (Smo) protein and glioma-associated oncogene homolog 1 (Gli1) protein expression.

Results: LncRNA Meg3 was highly expressed in CKD patients and UUO rats ($p < 0.01$). In contrast to the UUO+shNC group, knocking down lncRNA Meg3 improved renal injury, relieved pathological renal lesions, and reduced kidney fibrosis and related protein levels. It inhibited the hedgehog pathway in kidney tissues of UUO rats ($p < 0.05$ and $p < 0.01$).

Conclusions: LncRNA Meg3 can aggravate UUO-induced rat renal fibrosis by activating the hedgehog pathway.

Keywords: lncRNA-maternally expressed gene 3; hedgehog pathway; unilateral ureteral obstruction; renal fibrosis; rat

Introduction

Chronic kidney disease (CKD) is a condition characterized by a high incidence (about 12%) and prevalence rate worldwide [1], and its mortality is second only to acquired immune deficiency syndrome (AIDS) and diabetes [2]. Currently, CKD has been regarded as a worldwide public health problem owing to its properties of high incidence, high mortality, high medical costs, easily concealed condition, and easily combined with a variety of complications [3].

Renal interstitial fibrosis (RIF) is an unavoidable pathological process for most CKD developing to the final stage, accompanied by main pathological manifestations of epithelial-to-mesenchymal transition (EMT) in renal tubules. EMT is a tubular epithelial cell migration to the renal interstitium and transdifferentiation into myofibroblasts to secrete many cell-extracellular matrix (ECM) and deposit in renal interstitium [4]. Such a process is dominated by various factors and affected by complex signaling pathways. Therefore, the development of CKD can be effectively alleviated by blocking EMT in RIF. Many studies have confirmed that activating the hedgehog signaling pathway is significantly associated with the occurrence of EMT in cells [5–7].

Long non-coding RNAs (lncRNAs) are RNA sequences exceeding 200 nucleotides in length. Many recent studies have stated that lncRNAs significantly affect epigenetic and cellular physiological regulation [8]. LncRNA-maternally expressed gene 3 (lncRNA Meg3) is a novel lncRNA molecule encoded by Meg3 at chromosome 14q32.3 in humans [9]. The role of lncRNA Meg3 in EMT

has been reported by several studies [8,10]. Moreover, lncRNA Meg3 expression is down-regulated in the process of hepatic fibrosis; the hedgehog pathway-mediated EMT may participate in hepatic stellate cell activation by the interaction of lncRNA Meg3 with smoothened (Smo) protein [10–12]. However, there are no reports on whether inhibiting lncRNA Meg3 in RIF can protect the kidney by regulating Smo, inhibiting the hedgehog signaling pathway activity the occurrence of and EMT in CKD. Unilateral ureteral obstruction (UUO), mainly characterized by renal tubule injury caused by obstructed urine flow, is a standard model for exploring the mechanism of renal fibrosis [13]. Therefore, taking the experimental rat model of UUO as the research object, this paper investigated the regulatory role of lncRNA Meg3 in UUO-induced renal fibrosis through the hedgehog pathway. Through a series of discussions, this paper was expected to offer a reference basis for exploring new biomarkers applied to appraise UUO-induced renal fibrosis.

Materials and Methods

Clinical Samples

CKD patients offered the peripheral blood samples (CKD group) admitted to the Second People's Hospital of Three Gorges University and healthy volunteers (Normal group) at the same period. The inclusion criteria of patients were shown as follows: ① all patients met the following diagnostic criteria of CKD: duration of illness >3 months, glomerular filtration rate (GFR) <60 mL/(min·1.73 m^2); ② age >18 years old; ③ patients had complete medical records and received treatment actively. However, patients were excluded if they ① were accompanied by malignant tumor and acute kidney injury; ② suffered from blood, immune, and infectious diseases; ③ were pregnant or lactating. All included subjects and their families signed informed consent. Besides, this study was approved via the ethics committee of the Second People's Hospital of Three Gorges University (approval number: 202310). Also, this research was conducted in accordance with the Declaration of Helsinki.

Experimental Animals and Grouping

Forty male Sprague-Dawley (SD) rats, aged 6 weeks weighing 200–250 g were purchased from the Guangdong Medical Experimental Center. They were selected and randomly assigned to four groups (Sham, UUO, UUO+shRNA Negative control (shNC), and UUO+sh-Meg3 groups). Next, rats in each group were intraperitoneally injected with 1% pentobarbital sodium for anesthesia. Subsequently, the Sham group rats only received laparotomy but did not undergo ligation, and after surgery, they were intraperitoneally injected with normal saline 0.1 mL/day. In the UUO group, the left kidney and left ureter of rats were exposed layer by layer through laparotomy; the left ureter near the renal pelvis was ligated with 3–0 silk thread, then normal saline was injected intraperitoneally 0.1 mL/day after surgery. The rats in UUO+shNC and UUO+sh-Meg3 groups were made into the ureter adjacent to the renal pelvis of shRNA-adenovirus vectors (0.1 mL/day, 1×10^{14} virus genome copies per milliliter) and shRNA-adenovirus lncRNA Meg3 (0.1 mL/day, 1×10^{14} virus genome copies per milliliter), respectively, after UUO [14,15]. Both shRNA and lncRNA Meg3 shRNA were synthesized by Thermo Fisher Scientific. All intraperitoneal injections in each group were continued for 21 days. The rats were sacrificed under anesthesia on the 21st day after surgery. Notably, 1 day before sacrificing rats, they were put into metabolism cages to collect 24-hour urine for measuring 24-hour urine protein (24-up). Additionally, before the euthanasia of rats, blood was collected from their abdominal aorta, and the supernatant was acquired upon centrifugation at 3000 r/min for 15 minutes for the determination of blood urea nitrogen (BUN), creatinine (Cr), and hydroxyproline (HYP) levels. After blood collection, an intraperitoneal injection of 150 mg/kg pentobarbital sodium was performed to euthanize the rats; then, the renal tissues were collected quickly. Some tissues were fixed with 4% paraformaldehyde to observe pathological morphology, and others were stored at –80 °C to detect gene and protein expression levels. Each animal protocol was approved through the Experimental Animal Ethics Committee of Guangdong Medical Experimental Center (approval number: C202304-3) and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Renal Function Tests

According to the instructions of Cr (C011-2-1), HYP (A030-2-1), and BUN (C013-2-1) assay kits, the content of Cr, HYP, and BUN was measured in the collected serum. All three kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). In addition, the content of 24-up was tested using an automatic biochemistry analyzer.

Renal Pathology Testing

Hematoxylin and eosin (HE) staining (Sigma-Aldrich, St. Louis, MO, USA) was performed on tissue samples. Specifically, the tissues were paraffin-embedded, sectioned, and fixed with 4% paraformaldehyde. Next, they were dewaxed, dehydrated, and then soaked with xylene. After that, the sections were washed and immersed in 70%, 80%, 90%, 95%, and 100% ethanol successively. Later, 1×PBS solution (Sigma-Aldrich, USA) was employed for rinsing, hematoxylin for nuclear staining, hydrochloric acid of alcohol for color separation, double distilled water for soaking, and eosin for staining. After that, the samples were immersed in the ethanol solution with a concentration from high to low in a jar in turn. Lastly, the sections were sealed with neutral gum, observed using a microscope, and scanned.

As for Masson staining, the samples were sectioned, dewaxed, and dehydrated with the same procedures as HE staining. Masson compound staining solution was dripped for kidney tissue section staining. Next, the sections were rinsed with distilled water, dried, and covered with phosphomolybdic acid and aniline blue for staining. Washed and dried again, the sections were differentiated using a differentiation solution and immersed with ethanol in a jar. Subsequently, the sections were rinsed and immersed using xylene. Afterward, they were sealed, observed under a microscope, scanned, and assessed with Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA). Ultimately, the image analysis software was employed to calculate the percentage of fibrosis area of the pathological sections of rat renal tissues.

qRT-PCR

Total RNA extraction of clinical blood samples and rat renal tissues was performed utilizing the TRIzol kit (Life Technologies Corporation, Gaithersburg, MD, USA). Later, the total RNA obtained was reversely transcribed into cDNA by corresponding kits (TaKaRa, Osaka, Japan). Next, the SuperScript III Reverse Transcriptase kit (Thermo Fisher Scientific, Waltham, MA, USA) was applied to test the expression of lncRNA. Referring to the manufacturers' handbook, the SYBR® Premix Ex TaqTM II kit (TaKaRa Japan) was adopted for quantitative real-time polymerase chain reaction (qRT-PCR). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control of lncRNA expression. The relative transcription level of target genes was calculated through the relative quantitative method ($2^{-\Delta\Delta Ct}$ method) [14]. Besides, all samples were provided with 3 wells, and each test was repeated 3 times. The primer sequences of Human LncRNA Meg3 were shown as follows: Forward: 5′-GGGTCCCCTGATTCTCTCCT-3′; Reverse: 5′-TCAGAATCTCCTGGGAGGGG-3′. The primer sequences of Human GAPDH were established as follows: Forward: 5′-GTGGCTGGCTCAGAAAAAGG-3′; Reverse: 5′-GGGGAGATTCAGTGTGGTGG-3′. The primer sequences of Rat LncRNA Meg3 were shown as follows: Forward: 5′-TGGCCTTGGCTGAAGCTCT-3′; Reverse: 5′-GAAGACTGGTGTGAGCCGATGA-3′. The primer sequences of Rat GAPDH were displayed as follows: Forward: 5′-GGCACAGTCAAGGCTGAGAATG-3′; Reverse: 5′-ATGGTGGTGAAGACGCCAGTA-3′. All the above primers were synthesized through Sangon Biotech (Shanghai) Co., Ltd.

Western Blot

A total of 1 mL protein lysis buffer (Beyotime Biotechnology, Shanghai, China) was mixed well with rat renal tissues. After homogenization, ultrasonic treatment, and centrifugation of the mixtures, the supernatant was collected and placed in an RNase-free centrifuge tube for subsequent trials. Next, BSA protein standards (Beyotime Biotechnology, China) were diluted, and the protein standard solution (20 uL) was supplemented. The samples were diluted and dissolved to 20 uL with 1×PBS solution. Next, the BCA working solution was added, the absorbance value was measured, and the concentration of protein samples was calculated. Subsequently, the separating gel and concentrate gel were placed in a glass gel mold, respectively. After several treatments, protein samples were added to the mold, and the voltage was set to 80 V. After the protein marker was fully separated and decreased to the separating gel interface, the voltage was adjusted to 120 V. The electrotransfer was performed in an electric transfer tank with a constant current of 200 mA for 2 hours. Next, the cellulose membrane was blocked, and one hour later, it was incubated with the primary antibody (Abcam, Cambridge, UK) on a shaker at 4 °C overnight. On the next day, the membrane was supplemented with the secondary antibody Goat Anti-Mouse IgG H&L (HRP) (1:5000, ab205719, Abcam) and Goat Anti-Rabbit IgG H&L (HRP) (1:5000, ab205718, Abcam) for 1 hour of incubation. Subsequently, the cellulose membrane was incubated with a chemiluminescent solution (Sigma-Aldrich, USA), and the gel imaging system was utilized to analyze the relative expression of the target protein. The primary antibody consisted of collagen type III (Col III, 1:1000, ab184993, Abcam), α-Smooth muscle actin (α-SMA, 1:1000, ab108424, Abcam), fibronectin (1:1000, ab268021, Abcam), E-cadherin (1:1000, ab212059, Abcam), sonic hedgehog (Shh, 1:1000, PA5-96777, Thermo Fisher Scientific), patched (Ptch, 1:1000, PA1-46222, Thermo Fisher Scientific), smoothened (1:1000, ab236465, Abcam) and glioma-associated oncogene homolog 1 (Gli1, 1:1000, ab273018, Abcam), β-actin (1:1000, ab8226, Abcam).

Statistical Analysis

SPSS 21.0 statistical software (IBM Corp., Armonk, NY, USA) was responsible for the data analysis in this paper: *t*-test for comparison between two groups and one-factor analysis of variance for comparison among multiple groups. The experimental results were expressed as the mean ± standard deviation, and $p < 0.05$ indicated significant differences.

Results

High LncRNA Meg3 Expression in Chronic Kidney Disease Patients and Unilateral Ureteral Obstruction Rats

As displayed in Fig. 1A,B, the CKD group patients exhibited significantly increased lncRNA Meg3 expression in the serum relative to the normal patients ($p < 0.01$). The expression level of lncRNA Meg3 was not significantly different in the renal tissues of rats in the Sham group at 0, 3, 7, 14, and 21 days ($p > 0.05$). LncRNA Meg3 expression in

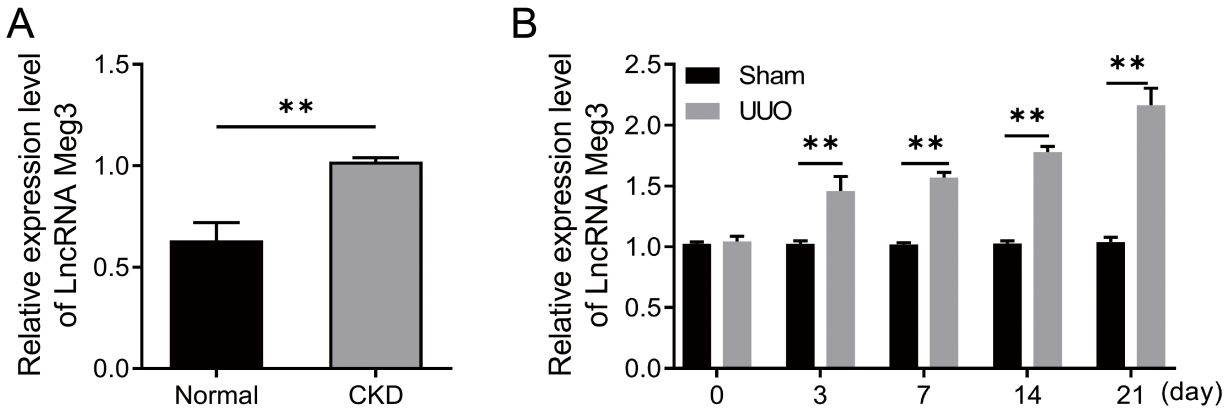

Fig. 1. LncRNA Meg3 is highly expressed in chronic kidney disease patients and unilateral ureteral obstruction rats. (A) qRT-PCR was employed to assess the lncRNA Meg3 expression level in the serum of patients in the CKD and Normal groups (n = 20). (B) qRT-PCR was utilized to test the expression levels of lncRNA Meg3 in renal tissues of the Sham and UUO groups at 0, 3, 7, 14 and 21 days after surgery (n = 6). $^{**}p < 0.01$. LncRNA Meg3, lncRNA-maternally expressed gene 3; qRT-PCR, quantitative real-time polymerase chain reaction; CKD, chronic kidney disease; UUO, unilateral ureteral obstruction.

the UUO group was increased on days 3, 7, 14, and 21, and markedly higher than in the Sham group ($p < 0.01$). The difference on day 0 between the Sham and UUO groups was not statistically significant ($p > 0.05$) (Fig. 1B). Consequently, abnormal lncRNA Meg3 expression may significantly influence CKD and act as a potential candidate target.

Knocking down LncRNA Meg3 Improves Renal Injury in Unilateral Ureteral Obstruction Rats

According to the experimental results, compared with the Sham group, the UUO group rats presented notably raised levels of the lncRNA Meg3 expression, BUN, Cr, HYP, and 24-up in renal tissues ($p < 0.05$ and $p < 0.01$). However, knocking down lncRNA Meg3 decreased the levels of the lncRNA Meg3 expression, BUN, Cr, HYP, and 24-up compared with the UUO+shNC group ($p < 0.05$ and $p < 0.01$). The differences between the UUO group and the UUO+shNC group were not statistically significant ($p > 0.05$) (Fig. 2A–E). In conclusion, downregulating lncRNA Meg3 expression can alleviate kidney injury in UUO rats.

Knockdown of LncRNA Meg3 Improves Pathological Renal Lesions in Rats Receiving Unilateral Ureteral Obstruction

The HE staining outcomes displayed that the morphological structure of renal tissues in the Sham group was normal. No epithelial cell necrosis, inflammatory cell infiltration, or proliferation of connective tissue of renal tubules were observed. The renal tissues in the UUO and UUO+shNC groups presented expanded renal tubules, epithelial cell necrosis and shedding, obvious inflammatory cell infiltration, and connective tissue proliferation of renal tubules. After the knockdown of lncRNA Meg3, the rats exhibited improved pathological renal lesions, slightly denatured renal tubules, reduced epithelial cell necrosis, mild inflammatory cell infiltration, and decreased connective tissue proliferation in renal tissues (Fig. 3). The results showed that lncRNA Meg3 reduced renal pathological injury in UUO rats.

Knocking down LncRNA Meg3 Alleviates Renal Fibrosis and Reduces Related Protein Levels in Rats Receiving Unilateral Ureteral Obstruction

The observation results of Masson staining are displayed in Fig. 4A. The Sham group showed normal renal morphology; the UUO and UUO+shNC groups exhibited severe renal tubular fibrosis; knocking lncRNA Meg3 down could significantly reduce renal fibrosis in rats. In addition, the statistical results for the percent of fibrosis area revealed that the percentage of fibrosis area was remarkably raised in the UUO group compared to the Sham group ($p < 0.01$); compared to the UUO+shNC group, the percentage of renal fibrosis area was significantly reduced after knocking lncRNA Meg3 down ($p < 0.01$); there was no statistical significance between the UUO+shNC group and the UUO group ($p > 0.05$) (Fig. 4B). Furthermore, western blot outcomes showed that compared to the Sham group, the Col III, α-SMA, and fibronectin protein levels in the renal tissue of rats in the UUO group were markedly up-regulated ($p < 0.01$). In contrast, the E-cadherin protein level decreased ($p < 0.01$). In comparison with the UUO+shNC group, the levels of Col III, α-SMA and fibronectin proteins in rat renal tissue notably decreased upon knockdown of lncRNA Meg3 ($p < 0.01$), while the E-cadherin protein level increased ($p < 0.01$); besides, the differences between the UUO group and UUO+shNC group were not statistically significant ($p > 0.05$) (Fig. 4C,D). It was suggested that the knockdown of lncRNA Meg3 in UUO rats delayed the progression of the disease by reducing renal fibrosis.

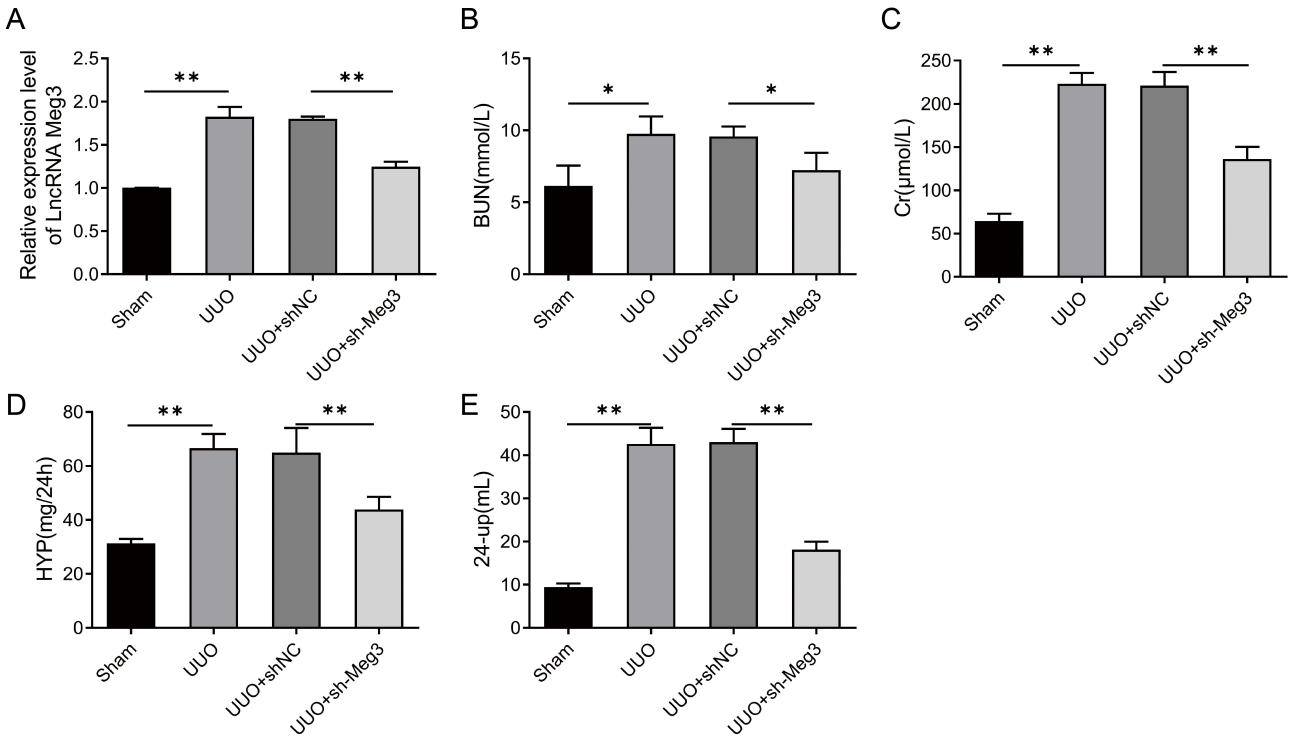

Fig. 2. Knocking down lncRNA Meg3 improves the renal injury of unilateral ureteral obstruction rats. (A) LncRNA Meg3 expression in renal tissues of the Sham, UUO, UUO+shNC and UUO+sh-Meg3 groups by qRT-PCR (n = 6). (B–E) The levels of BUN (B), Cr (C), HYP (D) and 24-up (E) in serum of rats in the Sham group, UUO, UUO+shNC and UUO+sh-Meg3 groups were determined by kits (n = 6). *$p < 0.05$, **$p < 0.01$. shNC, shRNA Negative control; BUN, blood urea nitrogen; Cr, creatinine; HYP, hydroxyproline; 24-up, 24-hour urine protein.

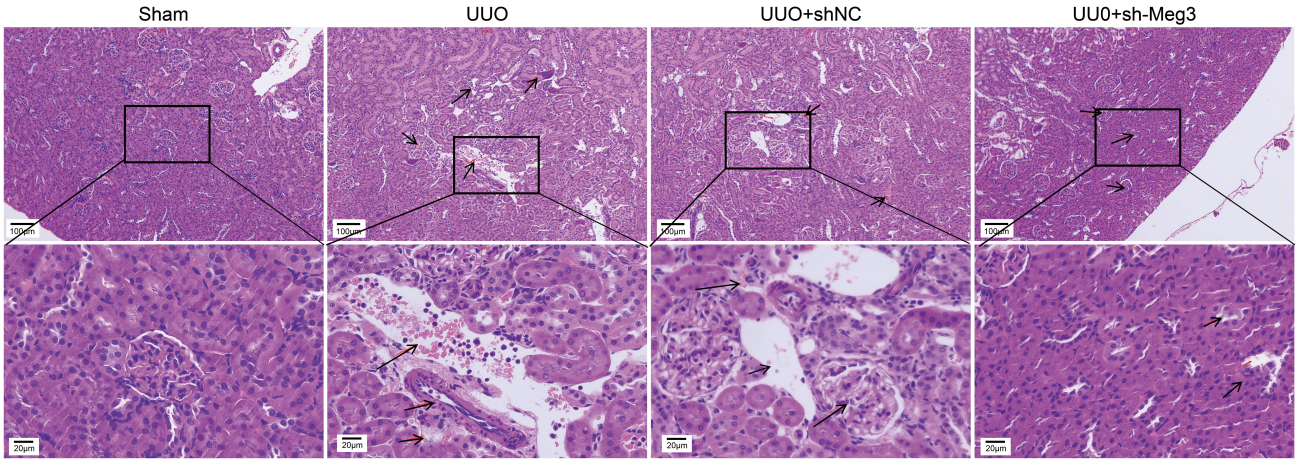

Fig. 3. Knocking down lncRNA Meg3 improves pathological renal lesions in rats undergoing. The pathological changes of renal tissue in the Sham group, UUO group, UUO+shNC group, and UUO+sh-Meg3 group were observed by HE staining (The arrows indicate the infiltration of inflammatory cells in the tissue). HE, hematoxylin and eosin.

Knockdown of LncRNA Meg3 Inhibits the Hedgehog Pathway in the Renal Tissue of Rats Receiving Unilateral Ureteral Obstruction

Briefly speaking, the UUO group rats presented much higher levels of Shh, Smo, and Gli1 protein expression ($p < 0.01$) while notably lower Ptch level ($p < 0.01$) than the Sham group. In comparison with the UUO+shNC group, the expression levels of Shh, Smo, and Gli1 proteins in renal tissues of rats markedly decreased ($p < 0.01$), while the protein level of Ptch notably increased ($p < 0.01$) after knockdown of lncRNA Meg3. The difference between the UUO and UUO+shNC groups had no statistical signif-

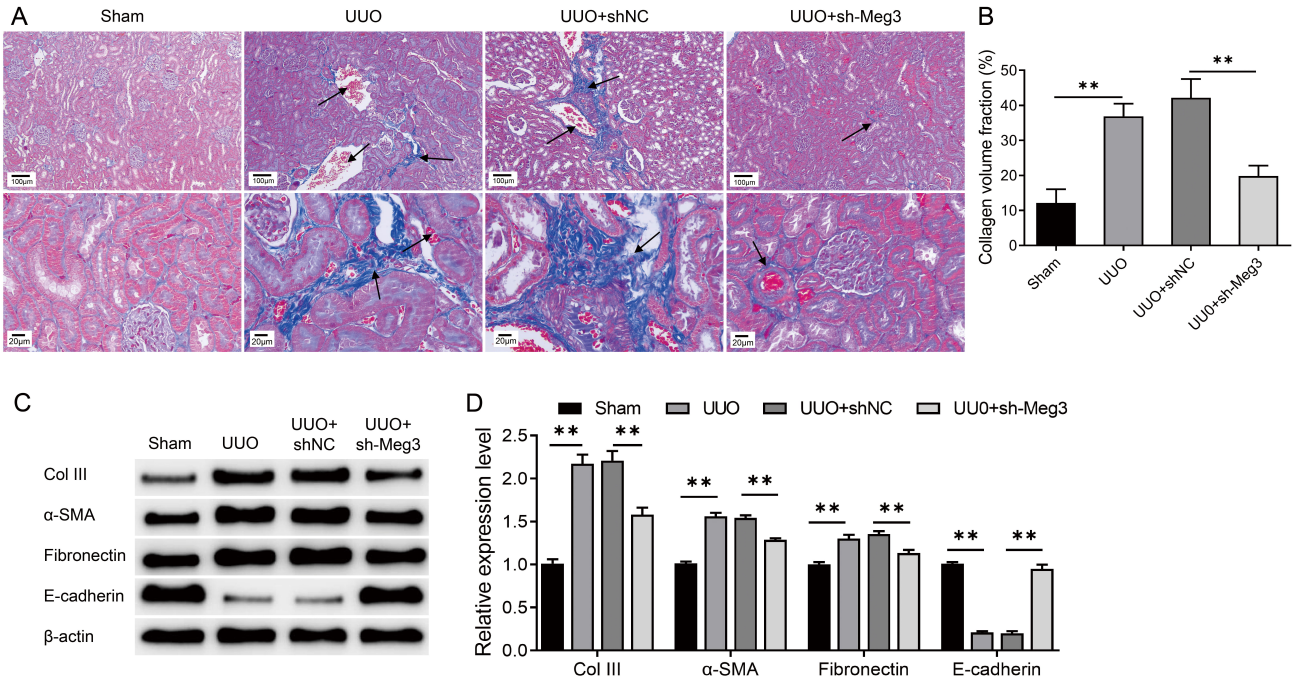

Fig. 4. Knocking down lncRNA Meg3 reduces renal fibrosis and related protein levels in rats treated by unilateral ureteral obstruction. (A) The observation of Masson staining for renal fibrosis of rats in the Sham, UUO, UUO+shNC, and UUO+sh-Meg3 groups (The arrow indicates that the collagen fibers are dyed blue). (B) Analysis of fibrosis area percentage in each group. (C) The Col III, α-SMA, fibronectin and E-cadherin protein levels in the renal tissues of the Sham, UUO, UUO+shNC, and UUO+sh-Meg3 group rats were tested by western blot (n = 6). (D) Statistical analysis of protein levels of Col III, α-SMA, fibronectin, and E-cadherin in each group (n = 6). $^{**}p < 0.01$. Col III, collagen type III; α-SMA, α-Smooth muscle actin.

icance ($p > 0.05$) (Fig. 5A,B). Therefore, lncRNA Meg3 may promote renal injury in UUO rats through activating the Hedgehog pathway.

Discussion

Renal fibrosis, a prevalent pathological process of CKD leading to end-stage renal failure [16], is mainly caused by a variety of factors, including inflammation, external injury, hypoxia, ischemia, matrix deposition and remodeling, and myofibroblast activation and migration [17]. Many diseases, including obstructive nephropathy, chronic pyelonephritis, chronic glomerulonephritis, hereditary renal disease, and systemic lupus erythematosus nephropathy, are associated with renal fibrosis [18,19]. Most scholars believe renal diseases can be alleviated by preventing the EMT process in renal fibrosis. However, finding effective biomarkers of renal fibrosis and clarifying its exact mechanism are still significant challenges in treating kidney disease.

In recent years, lncRNAs have attracted a lot of attention. There is increasing evidence that lncRNAs affect multiple biological processes of the body, such as EMT, autophagy, proliferation, protein synthesis, and apoptosis [20,21]. Also, lncRNAs have momentous functions in the pathological process of renal fibrosis. Zhou *et al.* [22] discovered that lncRNA HOTAIR expression was notably up-regulated in the UUO model, and silencing lncRNA HOTAIR could alleviate EMT and renal fibrosis by suppressing the Notch1 pathway. Moreover, lncRNA TCONS_00088786 expression was up-regulated in renal tissues of UUO animal models, and silencing lncRNA TCONS_00088786 expression alleviated renal fibrosis by reducing Col I and Col III expression levels [23]. LncRNA Meg3 is a new lncRNA molecule. Studies have pointed out that lncRNA Meg3 expression is down-regulated in the epithelial cells of human renal cortical proximal convoluted tubule in a cell model of renal fibrosis, and lncRNA Meg3 overexpression inhibits TGF-β1-induced EMT and relieves renal fibrosis progression [24]. Unfortunately, the mechanism of lncRNA Meg3 in renal fibrosis induced by UUO has not been reported yet. This study constructed a classic renal fibrosis model caused by UUO. Based on the HE, Masson, and renal function index detection outcomes, a significant difference existed between the UUO and Sham groups, indicating the model's successful establishment. Next, the expression levels of lncRNA Meg3, Col I, a-SMA, and TGF-β1 in the renal tissue and clinical blood samples of the model were detected by qPCR. The detection result revealed significantly increased lncRNA Meg3 in renal tissue of the UUO group rats and blood of patients with CKD. Furthermore, western blot analysis showed that

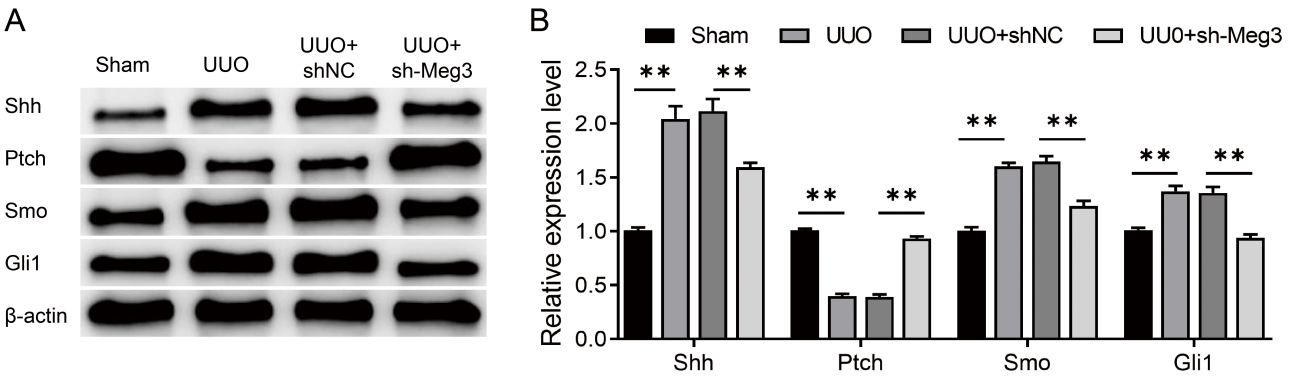

Fig. 5. Knockdown of lncRNA Meg3 inhibits the hedgehog pathway in renal tissues of rats receiving unilateral ureteral obstruction. (A) Western blot to measure the protein expression levels of Shh, Ptch, Smo, and Gli1 in renal tissues in each group (n = 6). (B) Statistical analysis of the protein expression levels of Shh, Ptch, Smo, and Gli1 in each group (n = 6). $^{**}p < 0.01$. Shh, sonic hedgehog; Ptch, patched; Smo, smoothened; Gli1, glioma-associated oncogene homolog 1.

the renal fibrosis marker levels (Col III, α-SMA, and fibronectin) remarkably increased. Still, the protein level of E-cadherin notably decreased in the UUO group. LncRNA Meg3 may be related to the occurrence of renal fibrosis in the UUO model. For further examining the function of lncRNA Meg3 in the UUO model of renal fibrosis, lncRNA Meg3 knockdown rat models were constructed by intraperitoneal injection of lncRNA Meg3 ShRNA adenovirus after UUO. The lncRNA Meg3 knockdown rat models presented benign changes in renal lesions, renal fibrosis, and renal functions, further verifying the promoting role of lncRNA Meg3 in renal fibrosis in the UUO model. LncRNA Meg3 knockdown could alleviate renal fibrosis, indicating that lncRNA Meg3 may serve as a potential target for renal fibrosis treatment.

As a classical signaling pathway, hedgehogs contain many mRNAs, such as Shh, Ptch1, Gli1, and Smo [25]. The hedgehog pathway affects the growth and repair of tissues or organs, particularly in regulating hepatic fibrosis, renal fibrosis, and pulmonary fibrosis. Studies have claimed that the Shh, Ptch 1, Gli1, and Smo expression levels in the hedgehog signaling pathway are up-regulated in lung fibroblasts, which promotes EMT, proliferation, and migration of fibroblasts and increases the number of collagen and fibronectin, thereby promoting the process of lung fibrosis [26]. Moreover, the hedgehog signaling pathway has key functions in hepatic fibrosis. Specifically, abnormal hedgehog signaling pathway activation activates hepatic stellate cells to activate mRNAs (like Shh, Ptch 1, Gli 1, and Smo) and inhibit apoptosis [27]. In addition, the hedgehog pathway is activated when renal fibrosis occurs in the body, significantly increasing the key component expression in the signaling pathway, thereby further promoting renal fibrosis [28]. In short, the hedgehog signaling pathway is notably correlated with fibrosis in the lung, kidney, liver, and other organs.

Previous articles have found that lncRNA Meg3 overexpression inhibits the activation of the Hedgehog pathway, leading to increased autophagy activity and reduced collagen formation, thereby inhibiting nickel oxide nanoparticles-induced pulmonary fibrosis [29]. In addition, overexpression of lncRNA Meg3 inhibits the Hedgehog pathway-mediated liver fibrosis EMT process by down-regulating Smo protein and miR-212 [30]. Although the expression levels of lncRNA Meg3 vary in different organ injuries, they all exert therapeutic effects by inhibiting fibrosis through the Hedgehog pathway. In this article, the Shh, Smo, and Gli1 protein expression levels significantly increased, while the Ptch protein level was remarkably down-regulated in the UUO group. However, after the knockdown of lncRNA Meg3 expression, the Smo, Shh, and Gli1 protein expression levels in rat renal tissue were markedly reduced, while the Ptch protein level remarkably increased. Collectively, knocking down the expression of lncRNA Meg3 might alleviate UUO-induced rat renal fibrosis by inhibiting the hedgehog pathway.

Still, many problems with this study need to be further clarified. First, we did not explore the non-coding RNAs targeted by lncRNA Meg3 and the function of non-coding RNAs in the Hedgehog signaling pathway. Second, we did not add Hedgehog pathway inhibitors or overexpress lncRNA Meg3 to verify our results.

Conclusions

To summarize, lncRNA Meg3 is highly expressed in CKD patients and UUO rats. Knocking down lncRNA Meg3 can improve renal function and pathological damage and reduce renal fibrosis and related protein levels of UUO rats by inhibiting the hedgehog pathway. All in all, lncRNA Meg3 can be a potential biomarker for preventing and treating renal fibrosis and kidney diseases.

Availability of Data and Materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

PD and LL designed the research study. PD and LL performed the research. SZ, DH and RH provided help and advice on the experiments. SZ, DH and RH analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Second People's Hospital of the Three Gorges University (202310). All animal protocols were approved by the Experimental Animal Ethics Committee of the Guangdong Medical Experimental Center (approval number: C202304-3) and complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Acknowledgment

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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