Mesenchymal stem cell-derived exosome miR-21-3p prevents renal fibrosis

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Research

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Abstract

Background: Renal fibrosis is the pathological result of excessive deposition of extracellular matrix (ECM) in the process of chronic kidney disease, but its mechanism is not clear. Mesenchymal stem cells (MSCs) exerts its therapeutic effect mainly through paracrine effects, such as exosome, to change the cellular microenvironment. Here, we explore the function of exosome derived MSCs in renal fibrosis.

Methods: UUO model was constructed to simulate renal fibrosis in mice. Heat map and RT-PCR were used to explore the differential expression of miRNAs. RT-PCR and western blot were performed to detect the expression levels of fibrosis-associated genes and proteins *in vivo* and *vitro.* Transmission electron microscope and particle size detection were used to confirm the exosome construct. Then we forced expression of miR-21-3p in MSCs and isolated the exosomes. Then the fibrosis-associated genes and proteins were explored after exosomes injection.

Results: In this study, we observed that exogenous miR-21-3p, interacted with smad2, the downstream target of miR-21-3p, which prevented renal fibrosis in UUO mice, and alleviate fibrosis in TGF-β1-induced renal tubular epithelium cells (HK-2). The extractive exosome-miR-21-3p treatment blocked renal fibrosis in UUO mice and alleviated fibrosis in TGF-β1-induced HK-2 cells and renal fibrosis mice.

Conclusion: Taken together, Overexpression of miR-21-3p prevented CKD-induced renal fibrosis via exosome-mediated miR-21-3p transfer. These results suggest possible therapeutic strategies for using exosome delivery of miR-21-3p to treat complications of CKD.

Background

The high incidence of chronic kidney disease (CKD) brings a substantial economic burden to society and patients' families[1]. It is generally believed that the destruction of normal renal parenchyma caused by fibrosis is a common pathogenic factor leading to progressive damage in CKD[2, 3]. Although a serious of studies have found the critical cellular and molecular mediators that lead to fibrosis, they have not been clinically verified. At present, 13% to 16% of patients with CKD need hemodialysis, and they may need kidney transplantation in the long term, and the high risk of cardiovascular disease caused by CKD significantly reduces the survival rate of patients[4-6]. The rapid development of basic scientific research provides a platform for the study of new treatments. Participate in the discovery of the primary mediators of fibrosis and lay the foundation for the development of targeted therapy. However, a continuous in-depth research is still needed to provide more and more reliable basis.

There is a close relationship between miRNA and renal fibrosis. MiRNA has tissue specificity and specificity for the regulation of target genes. Specific miRNA is expected to become an underlying target for anti-renal fibrosis therapy[1]. Meanwhile, the occurrence of renal fibrosis is affected by many factors, and the relationship between miRNA and promoting/anti-fibrosis factors is complicated. At present, only a few preliminary mechanisms of specific miRNA in renal fibrosis have been found, mainly in the TGF-β/Smad signal pathway, and there are differences in the research results[7]. Therefore, the exact

mechanism of miRNA in renal fibrosis and the application of new targets for anti-renal fibrosis treatment need to be further studied.

Some studies have shown that low concentration paclitaxel can prevent and treat renal fibrosis by down-regulating miR-192 in renal injury rats[8, 9]. In the model of local ischemia-reperfusion, the mice with knockout of renal tubules dicer enzyme and decreased expression of miR-192 have a higher survival rate. Members of the miR-29 family include miR-29a, miR-29b, and miR-29c, which contain a common seed sequence, so most of their target genes are the same[10, 11]. At present, more than 40 miR-29 target genes have been confirmed, mainly ECM and migration proteins. MiR-29 can interact with a variety of collagen proteins to inhibit the expression of collagen, including Col1a1, Col2a1, Col3a1, Col4a1, Col4a3, ol4a5, Col5a1, Col5a2, Col6a3 and so on, showing its strong anti-fibrosis potential[12]. In addition, miR-29 target genes also contain a number of fibrosis-related signal pathway proteins[13]. MiR-30 family can participate in podocyte homeostasis and function by regulating the calcium/calcineurin signal pathway. The decrease of miR-30 family expression will lead to podocyte injury[14]. MiR-215 can inhibit the expression of E-cadherin by acting on ZEB2, promote the effect of EMT[15]. In addition, miR-216a can activate the Akt signal pathway and promote Mesangial cell hypertrophy by acting on PTEN[16]. MiR-302d can reduce the expression of TβRII, thus inhibit epithelial-mesenchymal transition induced by TGF-β and improve the deposition of fibronectin and thrombospondin in the mesangial area[17].

Almost all cells release membrane-bound vesicles (extracellular vesicles, ECVs), which are divided into microvesicle, apoptotic bodies, and exosomes. The intracellular vesicle membrane was sunken inward to form a multi-vesicular inclusion body, which released small vesicles after fusion with the cell membrane[18]. Initially, these vesicles were considered "garbage" of cells and later proved to play an essential role in organisms. Various biological cells, including eukaryotes and prokaryotes, release different exosome bodies, which can be detected in blood, semen, saliva, and urine. The exosome is released from specific membrane regions and selectively carries mother cell proteins, mRNA, miRNA, DNA, and other cellular components, with three characteristics of mother cell characteristics, targeting, and strong biological functions[19]. Compared with autocrine or paracrine signals, it is a higher-order intercellular communication signal, which can fully participate in cell metabolism and affect the structure and function of specific receptor cells.

At present, the previous study has verified that MSCs-derived exosome bodies contain a variety of miRNA and micro-molecule, which playan key role in the process of angiogenesis. MiRNA-222, miRNA-21, and let-7f are involved in the control of angiogenesis[20]. Experimental studies have shown that MSCs-derived exosome-induced phenotypic changes can be mediated by their miRNA vectors. Exosomes mediate the transcription of miRNA-410, miRNA-495, and let-7a, and down-regulate several mRNAs, cytoskeletons related to apoptosis, such as CASP3, CASP7, SHCI, and SMAD4[21]. In addition, PTRF/CAVIN1 is related to the decreased expression of SHC1, which promotes cell death by regulating the EGFR pathway.

The current research uses MSCs overexpression of miR-21-3p as a therapeutic tool for targeting the renal disease. In the unilateral ureteral obstruction (UUO) mouse model, compared with (NC) MSCs, MSC-miR-

21-3p selectively transferred miR-21-3p to the damaged kidney, with improved renal structure and reduced interstitial collagen. In vitro culture analysis confirmed that miiR-21-3p increased the expression of miR-21-3p in HK-2 through the exosome pathway, and inhibited collagen production, TGF-β, and α-SMA to reduce fibrosis in renal cells induced by TGF-β1.

Materials And Methods

Cell treatment

Human bone marrow mesenchymal stem cells (hMSCs, Shanghai fusheng industrial co. LTD) and HK-2 (human epithelial cells) cell were maintained in DMEM (Hyclone, USA) with 10% FBS, and 1% streptomycin and penicillin (Beyotime, China), placed at 37℃ with 5% CO2. For inducing epithelial-mesenchymal transition (EMT) in HK-2 cell, 5 ng/ml TGF-β1 was treated for 48 h.

Cell transfection

About 5×10^5 cells per well were cultured in 6 well plates, transfection of siRNA into the cells was performed using Lipofectamine 2000 (Thermo Fisher Scientific, USA) according to the manufacturer's recommendations. Cells were added with 20 nmol/L siRNA for 48 h.

Western blot

Total protein was collected from cells with RIPA lysis Mix (Beyotime, China). Briefly, 60 μg protein extraction was loaded via SDS-PAGE and transferred onto nitrocellulose membranes (MILLIPORE, USA), then put them into 5% blocking solution (1× PBS containing 0.1% Tween 20 and 5% w/v nonfat dry milk) for 2 h. The membranes were incubated with primary antibodies at 4 °C for one night. After incubation with the corresponding anti-mouse/-rabbit secondary antibodies, immunoblots were developed using an Odyssey CLx imager (Gene Company Limited, Hongkong, China). Signal intensities were quantified with Odyssey software (LI-COR, USA). The antibody information was shown as follows. TGFβ1 (21898-1-AP, 1:2000, Protientech), TGFβ3 (bs-9454R, 1:1000, Bioss), YY-1(66281-1-Ig, 1:500, Protientech), Fibronectin (15613-1-AP, 1:1000, Protientech), COL1α1 (sc-293182, 1:200, Santa Cruz Biotechnology)， smad2/3 (12570-1-AP, 1:500, Protientech), $P_{Ser465/467}$Smad2 (MA5-15122, 1:400, Monoclonal) and $P_{Ser423/425}$Smad3 (MA5-14936, 1:1000, Monoclonal), α-SMA (55135-1-AP, 1:1000, Protientech), CD63 (25682-1-AP, 1:400, Protientech), CD9 (20597-1-AP, 1:1000, Protientech), GAPDH(60004-1-Ig, 1:2000, Protientech) was used as an internal control.

Real time-PCR

Total RNA was isolated from tissues and cells using Trizol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's protocols. Total RNA (1 μg) was used for synthesizing first-strand cDNA using cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, USA). qRT-PCR was performed with SYBR Green Mix kit (Applied Biosystems) according to the manufacturer's instructions. The relative RNA levels were calculated using the ΔΔCt method. GAPDH levels served as an internal control. miR-21-3p expression was assessed using the qRT-PCR miRNA Detection Kit (Applied Biosystems), with U6 levels as an internal control. The primer sequences used for the amplification are shown as follows.

Human miR-21-3p: sense 5'-CGCGCCAACACCAGTCGATG-3'; antisense 5'-GTGCAGGGTCCGAGGT-3'mouse α-SMA: sense, 5'-GACGCTGAAGTATCCGATAGAACACG-3'; antisense 5'-CACCATCTCCAGAGTCCAGCACAAT-3'; mouse TGF-β1: sense, 5'-AGCGGACTACTATGCTAAAGAGGTCACCC-3'; antisense, 5'-CCAAGGTAACGCCAGGAATTGTTGCTATA-3'; mouse Col1α1: sense, 5'-GGAGGGCGAGTGCTGTGCTTT-3'; antisense, 5'-GGGACCAGGAGGACCAGGAAGT 3'; mouse Col4α1: sense, 5'-TGGTCTTACTGGGAACTTTGCTGC-3'; antisense, 5'-ACCCTGTGGTCCAACGACTCCTCTC-3'; mouse GAPDH: sense, 5'-CGACTTCAACAGCAACTCCCACTCTTCC-3'; antisense, 5'-TGGGTGGTCCAGGGTTTCTTACTCCTT-3'; human GAPDH: sense, 5'-GCTGGCGCTGAGTACGTCGTGGAGT-3'; antisense, 5'-CACAGTCTTCTGGGTGGCAGTGATGG-3'.

Preparation of exosomes

MSCs were transfected with miR-Negative control (miR-NC) or miR-21-3p. After 2 days of incubation, exosomes were isolated from the supernatants of the MSC cultures with the exosome precipitation buffer, ExoQuick (BIOMARS), as previously described[22]. RNA was isolated using a Qiagen RNA extraction kit.

Animal surgery

Male C57BL/6 mice (20-25g) were purchased from Beijing Charles river and fed in experimental Animal Center of Cangzhou Central Hospital with standard conditions. All operations were carried out under 2% isoflurane anesthesia (Sigma, USA) via intraperitoneal injection. UUO surgery was executed as previously described[23], the left ureter was visualized via a flank incision and ligated using double (5-0 surgical silk) for weeks. After UUO surgery, treatment of phosphate-buffered saline (PBS) vehicle, Adenovirus-NC/miR-21-3p (Ad-NC/miR-21-3p, 1×10^{10}/mice) or NC/miR-21-3p MSCs exosomes (200ug) were tail injection. After 3 weeks, mice were intraperitoneally injected with 3% pentobarbital sodium and were killed by excessive anesthesia with a dose of 90 mL/kg, and the organ and tissues were removed for follow-up study. The research protocol of this study was approved by the Animal Care and Use Committee of Cangzhou Central Hospital.

Statistical analysis

All values are expressed as the means ± SEMs. Differences between two groups were determined via Student's t-test. A two-tailed value of $P < 0.05$ was considered statistically significant. Data were analyzed using GraphPad Prism 7.0.

Results

miR-21-3p and fibrotic gene expression in vitro and in vivo

In exploring new therapeutic targets for treating renal fibrosis associated with CKD, we performed heat map and RT-PCR to detect expression of miRNA in CKD mice. The data revealed that miR-21-3p was altered in CKD mice compared with sham mice **(Fig. 1A&B)**. Meanwhile, an in bioinformatics website analysis of the 3' UTR of Smad2 predicted a conserved binding site for miR-21-3p. To verify that miR-21-3p interacts with the Smad2 mRNA, luciferase activity was performed in HEK293T cells. Cells transfected with miR-21-3p mimic decreased the luciferase activity in Smad2-WT, not in Smad2-mutant, which indicated the relationship pf miR-21-3p with Smad2 **(Fig. 1C)**, We speculated that miR-21-3p may involve in fibrosis progress by binding with Smad2.

To explore the role of miR-21-3p in renal fibrosis, we created a fibrosis model *in vivo* and *vitro.* UUO model was performed in C57BL/6 mice. Human tubular epithelial cells (HK-2) were treated with 5 ng/ml TGF-β1 for 48h. Then Collage1α1 (Col1α1), Collage4α1 (Col4α1), Alpha-smooth muscle actin (α-SMA) and Fibronectin (Fibro) were detected by RT-PCR for confirming the fibrosis model. As **Fig. 1D** shown, Col1α1, Col4α1,α-SMA and Fibro were up-regulated in UUO mice, which verified the success of the renal fibrosis model. In **Fig. 1E**, compared with the control group, TGF-β1 treatment induced the expression of Col1α1, Col4α1,α-SMA and Fibro in HK-2 cells. Then we evaluated the expression of miR-21-3p in fibrosis tissues and cells, the down-regulated miR-21-3p was observed *in vivo* and *vitro*. The abnormal expression of miR-21-3p may be a novel target for renal fibrosis therapy

Adenovirus over-expression miR-21-3p prevents fibrosis in vivo

To confirm the effect of miR-21-3p on renal fibrosis, we injected an adenovirus vector containing miR-21-3p/NC, one week later, the UUO procedure was conducted. Afterward, miR-21-3p was assessed in a different group **(Fig. 2A**). Then the mice renal were removed and evaluated renal fibrosis with Masson's trichrome staining. Comparison of the deposition of the collagen in the UUO renal, Ad-miR-21-3p was significantly decreased deposition of the collage **(Fig. 2B**). Next, The fibrosis markers were detected in different groups. The up-regulated of Col1α1, Col4α1,α-SMA and Fibro in UUO mice were blocked by Ad-miR-21-3p injection **(Fig. 2C**).

The upregulation of TGF-β is the main performance of fibrosis. Compared with the sham group, the levels of TGF-β1, TGF-β3, Col1α1, Col4α1,α-SMA, and Fibro were significantly increased in UUO mice. Force of

miR-21-3p markedly inhibited the increase of fibrosis-related proteins, and the rise of YinYang1 (YY1), which was transcription factor of TGF-β, also inhibited by Ad-miR-21-3p **(Fig. 2D)**. These results indicated that exogenous miR-21-3p injection could delay the progression of renal fibrosis in UUO mice

Exogenous miR-21-3p attenuates fibrosis in HK-2 cells

To detect the transfection efficacy of miR-21-3p mimic/miR-NC, RT-PCR was employed to evaluate the expression level of miR-21-3p in HK-2 cells with transfection and TGF-β1 treatment **(Fig. 3A).** Then we assessed fibrosis-associated essential genes in HK-2 cells in response to TGF-β1. After the treatment of TGF-β1, the expression of Col1α1 and Col4α1 in HK-2 cells was dramatically higher than that in the untreated cells group. After transfecting with miR-21-3p, the expression of Col1α1 and Col4α1 were significantly decreased. The fibrosis marker α-SMA was detected by RT-PCR and western blot, the expression of α-SMA and Fibro decreased after miR-21-3p mimic transfection in TGF-β1 treated-HK-2 cells **(Fig. 3B&3C).**

After TGF-β1 binds and activates its receptor, its signal transduction is carried out by the post-receptor signal molecule Smad protein. Smad2 and Smad3 can be directly activated by the TGF-β1 receptor. The activated Smad2/Smad3 and Smad4 form a complex to transfer to the nucleus and activate the transcription of the TGF-β1 target gene. It has been confirmed that activated TGF-β1/Smad signal pathway can participate in all aspects of renal fibrosis, activate renal interstitial fibroblasts, promote ECM synthesis and inhibit its degradation, resulting in excessive accumulation of ECM, resulting in extensive renal fibrosis. In this study, we determined the protein level of Smad, $P_{Ser465/467}$Smad2, and $P_{Ser423/425}$Smad3 in HK-2 cells. The phosphate Smad2/3 was upregulated with TGF-β1 treatment. After transfection with miR-21-3p, all these changes were blocked **(Fig. 3C**

The cultured and isolated exosomes in MSCs

Exosomes play an essential role in the microenvironment. It mainly transmits the signal material to the target by paracrine. MiRNA in exosomes has attracted much attention because of its membrane protection, which is not easy to degrade, easy to obtain, high abundance, and concentration. In our study, MSCs were transfected with miR-21-3p mimic or miR-NC, and the expression level of miR-21-3p was detected by RT-PCR **(Fig. 4A)**. Exosomes were identified by particle size analysis (about 30-150 nm, **Fig. 4B**), biomarkers (CD63 and CD9, **Fig. 4C**), transmission electron microscopy (TEM, **Fig. 4D**). Then exosomes were extracted from the treatment MSCs and assessed the expression of miR-21-3p **(Fig. 4E&F)**. In summary, miR-21-3p can enter into the exosome secreted by MSCs.

Injection of exosome-miR-21-3p attenuates renal fibrosis in UUO mice

During the UUO operation, we found that miR-21-3p expression in UUO mice with the tail injection of exosome-miR-21-3p (exo-miR-21-3p) was significantly up-regulated compared with exosome-miR-NC (exo-miR-NC) injected UUO mice **(Fig.5A)**, suggesting that exosome may successfully delivery *in vivo*. To verify whether miR-21-3p regulated UUO-induced renal fibrosis, we determined renal fibrosis with Masson's trichrome staining. The density of the collagen showed that exo/miR-21-3p was significantly blocked the UUO renal with exo-miR-NC **(Fig. 5B**). Next, The fibrosis markers were detected in a different group, the up-regulated of Col1α1, Col4α1,α-SMA, and Fibro in UUO mice with exo-miR-NC were blocked by exo-miR-21-3p injection **(Fig. 5C).**

Compared with the sham group, the expression levels of TGF-β1, TGF-β3, Col1α1, Col4α1,α-SMA, and Fibro were increased in UUO mice. Exo- miR-21-3p inhibited the increase of fibrosis-related proteins, and the up-regulated of YY1 also inhibited by exo-miR-21-3p **(Fig. 5D).** These results suggest that exo-miR-21-3p injection can prevent the progression of renal fibrosis in UUO mice

MSCs-miR-21-3p reduces the renal fibrosis in vitro

To assess the efficacy of miR-21-3p delivered from MSCs, RT-PCR was performed to evaluate the level of miR-21-3p in HK-2 cells co-cultured with MSCs after TGF-β1 treatment **(Fig. 6A)**. HK-2 cells were co-cultured using the indirect transwell with miR-21-3p-MSCs, and miR-NC-MSCs were identified as control. The addition of TGF-β1 induced a significant increased Col1α1, Col4α1,α-SMA, and Fibro expression in HK-2 cells compared with control groups. TGF-β1-treated HK-2 cells were co-cultured with miR-21-3p-MSCs, there was a decrease in Col1α1, Col4α1,α-SMA, and Fibro expression, and compared with NC-MSCs co-cultured with TGF-β1-treated HK-2 cells **(Fig. 6B&6C**). The co-culture of HK-2 cells with NC-MSCs also showed induced expression of phosphate Smad2/3 in the presence of TGF-β1, and there was down-regulated of phosphorylated Smad2/3 in TGF-β1-treated HK-2 cells co-cultured with miR-21-3p-MSCs **(Fig. 6C)**.

These data suggest that miR-21-3p derived from MSCs had an amplified antifibrotic effect in the presence of TGF-β1.

Discussion

Renal fibrosis is the common pathological manifestation of most CKD, and it is also the main pathological change that leads to end-stage renal disease[24]. The progress of CKD is affected by many factors, such as persistent inflammatory stimulation, ion disorder, and high glucose environment. 50% of adults over the age of 70 and 10% of the world's population are affected by CKD and renal fibrosis[25].

The main characteristics of renal fibrosis are the activation of fibroblasts and the accumulation of myofibroblasts, the change and loss of inherent cell phenotype in the kidney, and the abnormal deposition of a large amount of extracellular matrix (extracellular matrix, ECM). TGF- β/Smad signal

pathway plays a vital role in renal fibrosis[26]. Here, we found that miR-21-3p could interact with Smad2, which may involve in renal fibrosis progression.

Exosomes are a kind of nano-vesicle secreted by cells, which carry a large number of active substances and transmits communication between cells. Renal exosome participates in renal regeneration, repair, and communication between renal tubular cells, regulates the growth of osteocytes, plays a vital role in the progression and control of nephropathy and renal tumor, and has the potential to be used as a targeted therapeutic agent for the renal disease[27, 28]. Recent studies have shown that, In UUO mice, tail vein injection of exosome secreted by MSCs overexpressing miRNA-let-7c can alleviate renal injury and down-expression in UUO kidney[29]. Another study showed that exosome derived from MSCs reversed the deterioration of renal function in the UUO model. Here, we isolated exosomes from MSCs containing miR-21-3p, which was confirmed by RT-PCR. In UUO mice, tail vein injection of exosome-miR-21-3p derived from MSCs could improve renal fibrosis, which could provide a new basis for clinical treatment of renal fibrosis.

Conclusion

Our research shows that mesenchymal stem cell exosome miR-21-3p can effectively resist fibrosis and efficiently deliver therapeutic miR-21-3p. More importantly, this method allows exosome pathways to transmit miRNAs to produce a functional response. MiR-21-3p exosome was applied to the treatment of renal fibrosis in injured kidneys of patients with renal injury.

Abbreviations

extracellular matrix (ECM)

chronic kidney disease (CKD)

mesenchymal stem cells (MSCs)

miR-Negative control (miR-NC)

phosphate-buffered saline (PBS)

Alpha-smooth muscle actin (α-SMA)

Fibronectin (Fibro)

exosome-miR-21-3p (exo-miR-21-3p)

Declarations

- **Ethics approval and consent to participate**

The research protocol of this study was approved by the Animal Care and Use Committee of Cangzhou Central Hospital.

- **Consent for publication**

Not applicable

- **Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

- **Competing interests**

The authors declare that they have no competing interests

- **Funding**

The authors received no funding for this work.

- **Authors' contributions**

Zongying Li, Shuyi Cao made substantial contributions to the design of the study. They drafted the work or revised it and approved the version to be published.

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Not applicable

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Figures

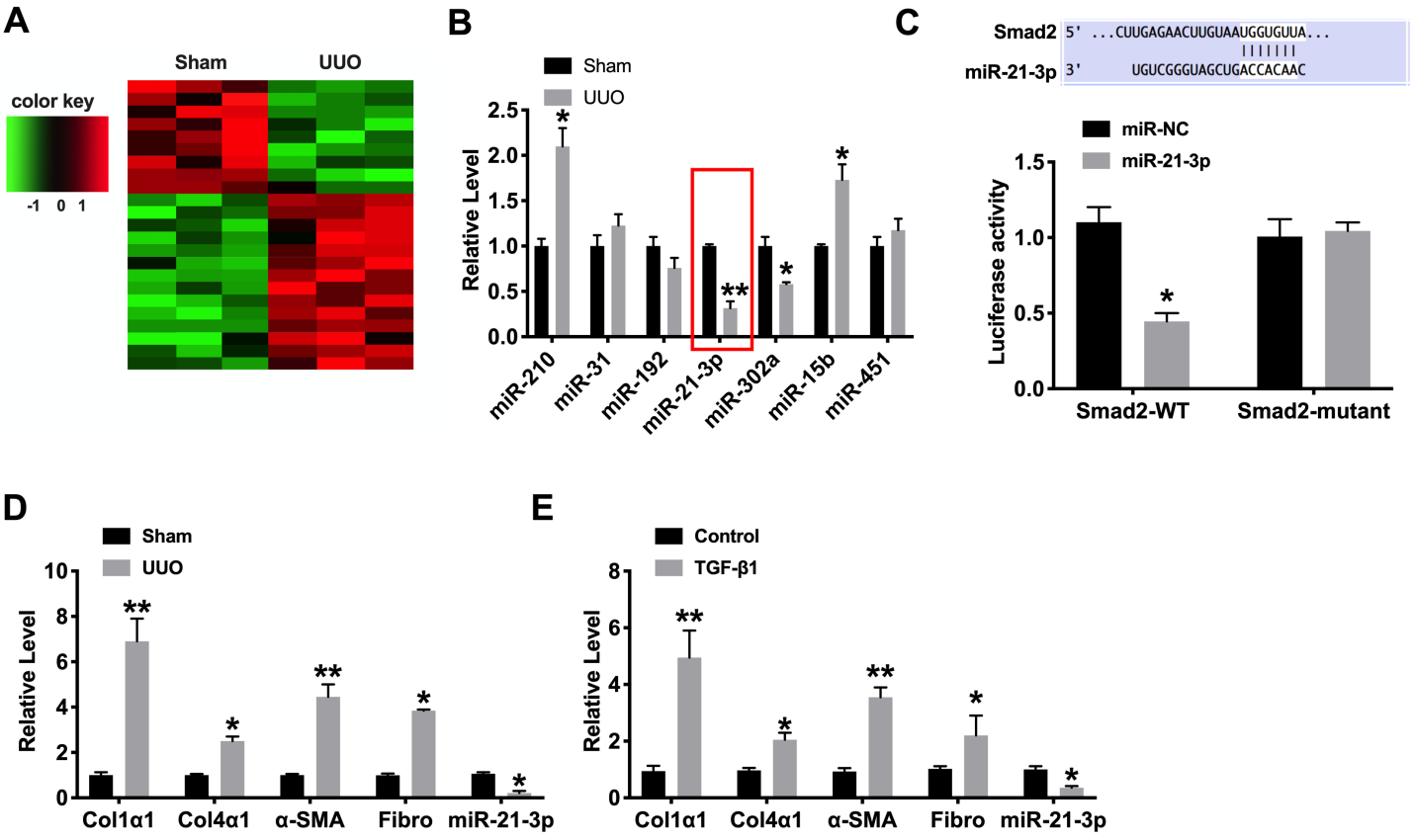

Figure 1

miR-21-3p and fibrotic relative-gene level in vitro and in vivo. A. Heat map representing color coded expression levels of differentially expressed miRNAs in UUO compared to sham n=3, P<0.05. B. The level of different miRNAs in CKD mice. n=8, *P<0.05, **P<0.01. C. The luciferase assay was performed to verify the relationship of miR-21-3p and Smad2. n=3, *P<0.05. D. Mice were subjected UUO or sham operation. The fibrotic-relative genes Col1α1, Col4α1,α-SMA Fibro and miR-21-3p were detected in renal tissue. n=6, *P<0.05. E. HK-2 cells were treated with TGF-β1 for 48h. The fibrotic-relative genes Col1α1, Col4α1,α-SMA Fibro and miR-21-3p were detected in HK-2 cells. n=6, *P<0.05.

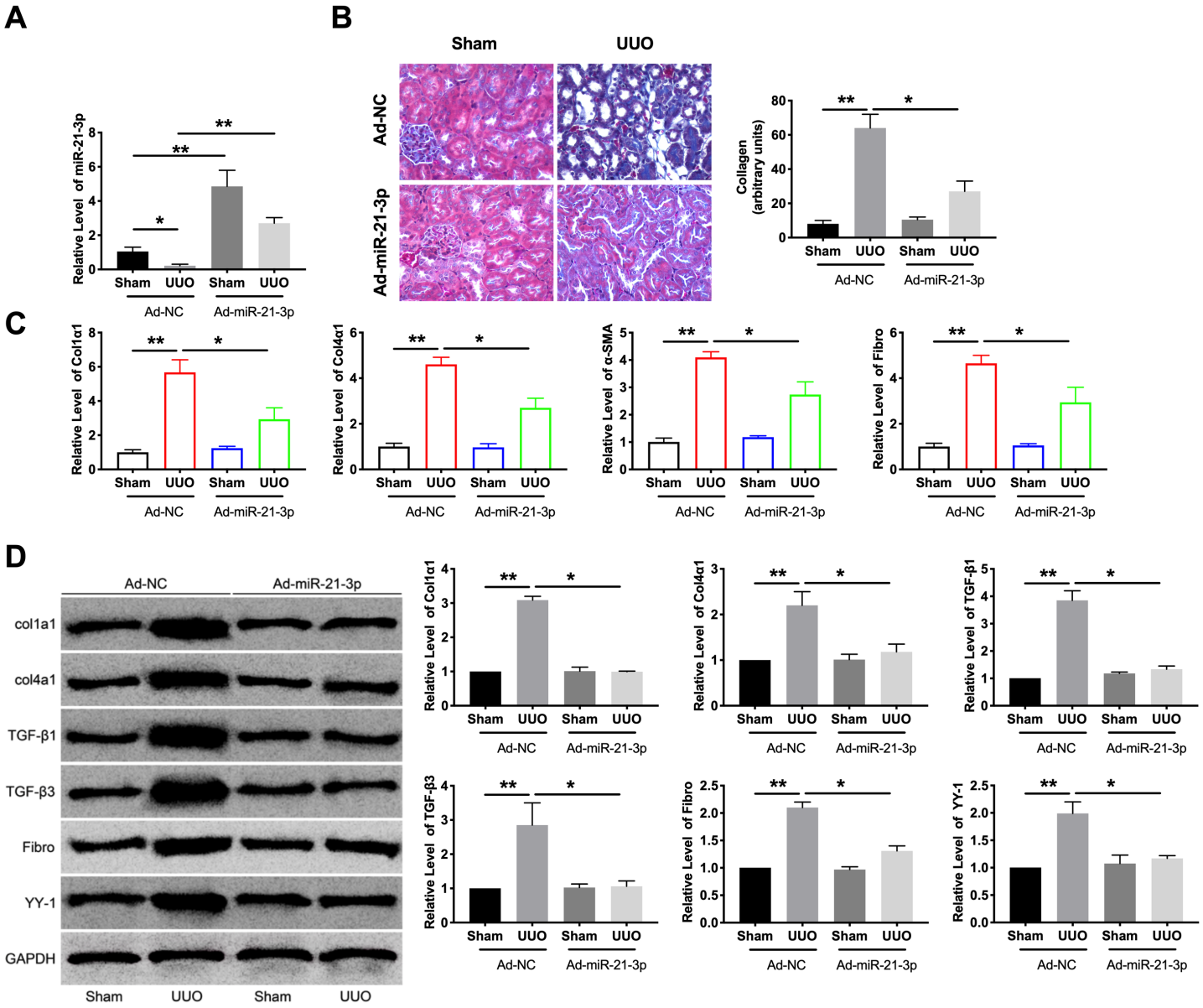

Figure 2

Ad-miR-21-3p prevented fibrosis in kidney. A. The expression level of miR-21-3p was detected in different group. n=6, *P<0.05. B. Representative Masson's trichrome staining of renal sections. n=3, *P<0.05. C. The fibrotic-relative genes Col1α1, Col4α1,α-SMA and Fibro mRNA level were assessed by RT-PCR. n=6, *P<0.05. D. After Ad-miR-21-3p/Ad-miR-NC injection, the protein level of TGFβ1, TGFβ3, YY-1, Fibronectin, COL4α1, COL1α1 were detected in renal tissues. n=6, *P<0.05.

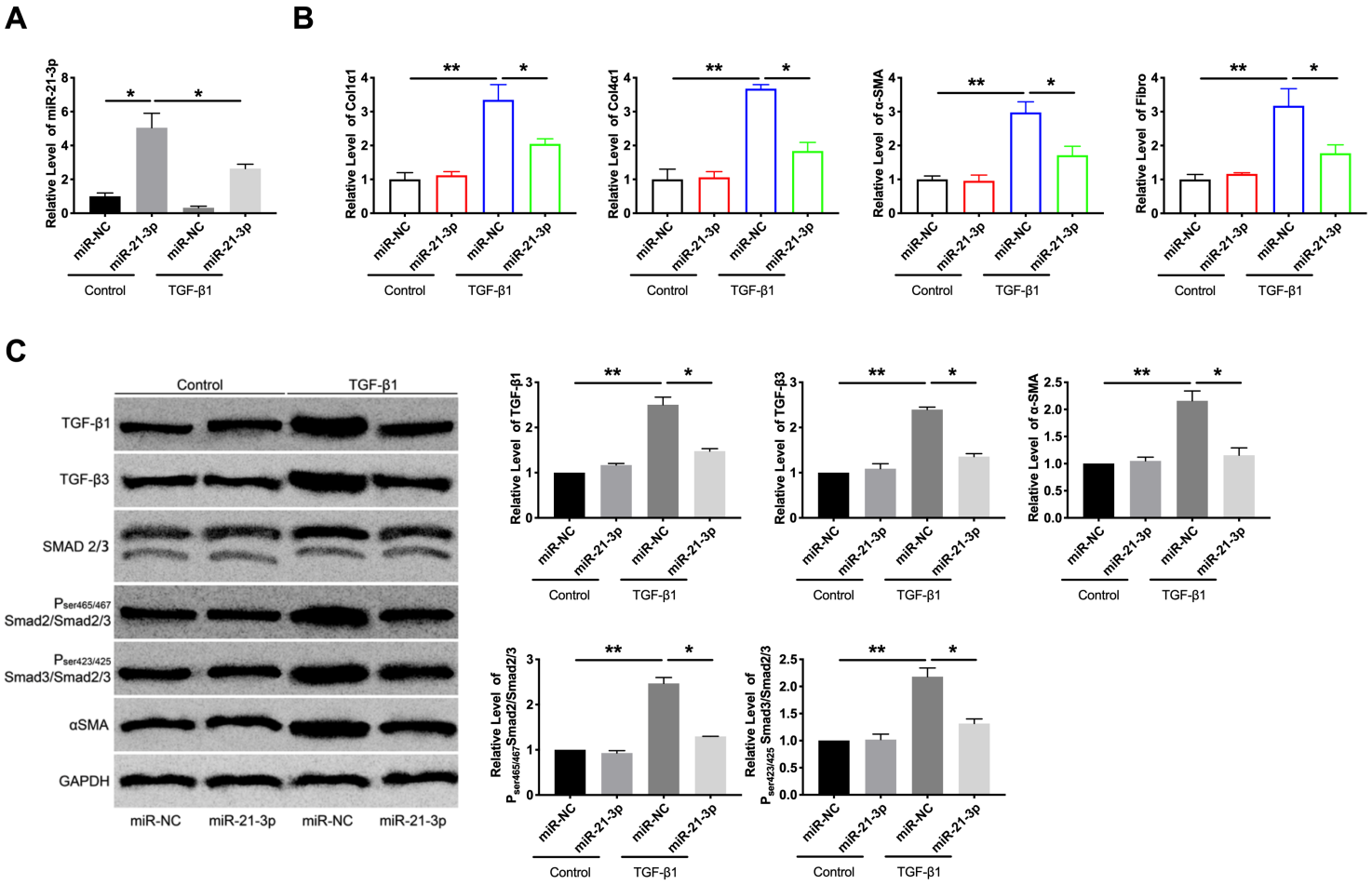

Figure 3

Forced of miR-21-3p blocked fibrosis in HK-2 cells. A. The expression of miR-21-3p was detected in different group. n=6, *P<0.05. B. The fibrotic-relative genes Col1α1, Col4α1,α-SMA and Fibro mRNA level were assessed by RT-PCR. n=6, *P<0.05. C. TGFβ1, TGFβ3, smad2/3, PSer465/467Smad2 and PSer423/425Smad3, α-SMA and COL1A1 were detected in HK-2 cells. n=6, *P<0.05.

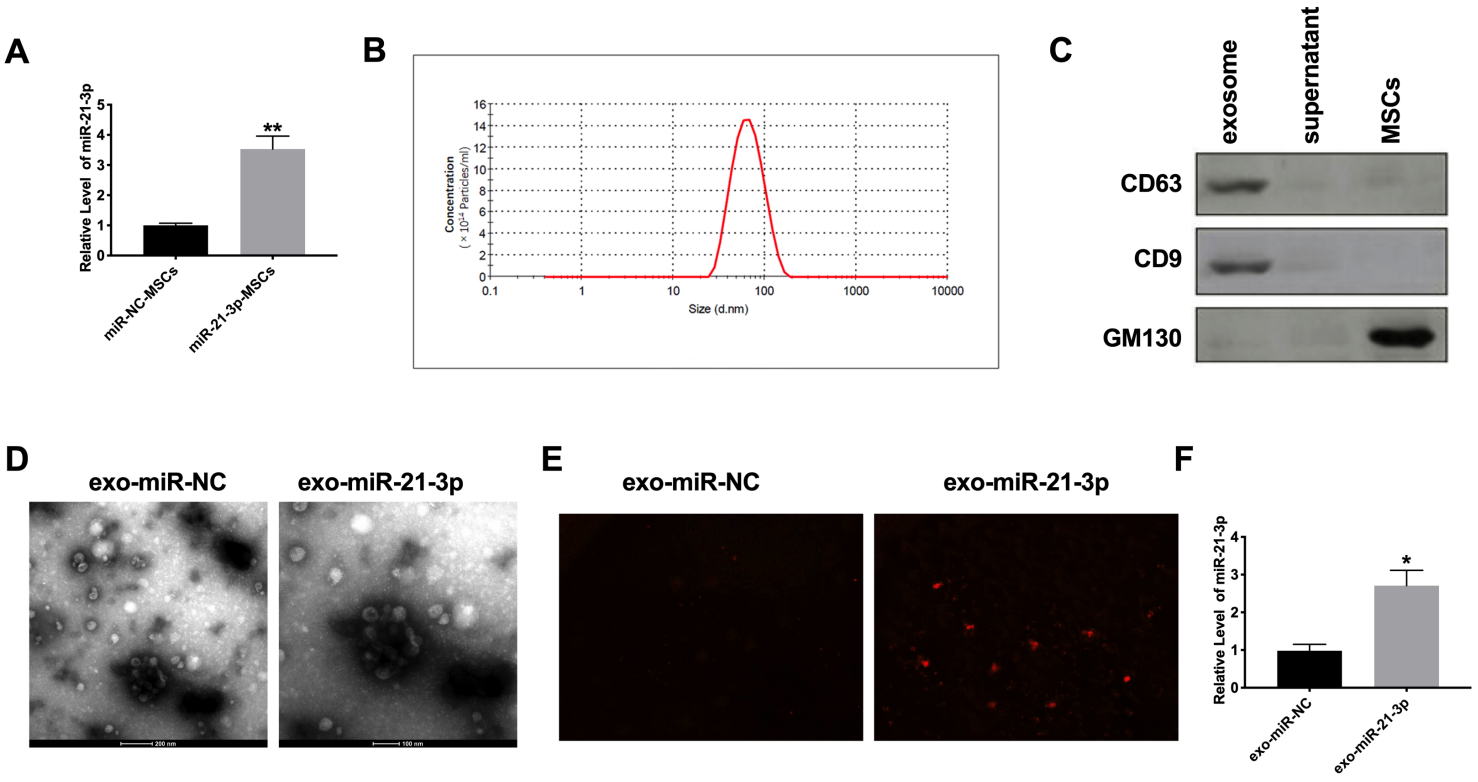

Figure 4

Exo-miR-21-3p was generated from MSCs. A. miR-21-3p/miR-NC were transfected into MSCs, and the expression of miR-21-3p was detected. n=6, *P<0.05. B. particle size analysis of exosome from MSCs. C. Exosome biomarkers (CD63 and CD9) were determined in exosome, supernatant and MSCs. D. transmission electron microscopy (TEM) was used to confirm exosome morphology. E. The level of miR-21-3p in exosomes derived from MSCs. The red fluorescence indicated the miR-21-3p. F. The exosomes were extracted from the treatment MSCs and assessed the expression of miR-21-3p. n=6, *P<0.05.

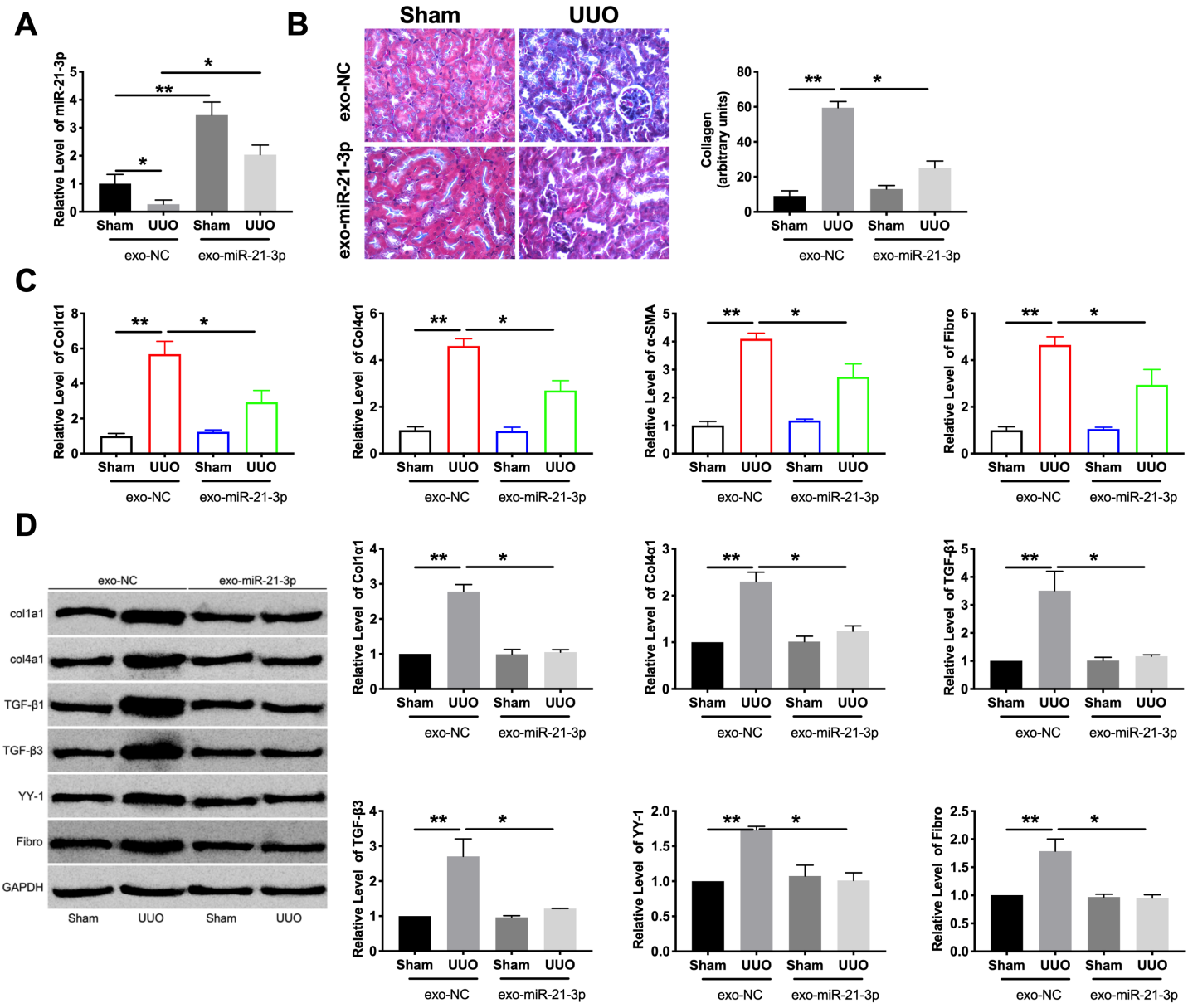

Figure 5

Exo-miR-21-3p prevented fibrosis in UUO mice. A. The expression of miR-21-3p was detected in total renal tissues from different group. n=5, *P<0.05. B. Representative Masson's trichrome staining of renal sections. n=3, *P<0.05. C. The fibrotic-relative genes Col1α1, Col4α1,α-SMA and Fibro mRNA level were determined by RT-PCR. n=6, *P<0.05. D. After exo-miR-21-3p/exo-miR-NC injection, the protein level of TGFβ1, TGFβ3, YY-1, Fibronectin, COL4α1, COL1α1 were detected in renal tissues. n=6, *P<0.05.

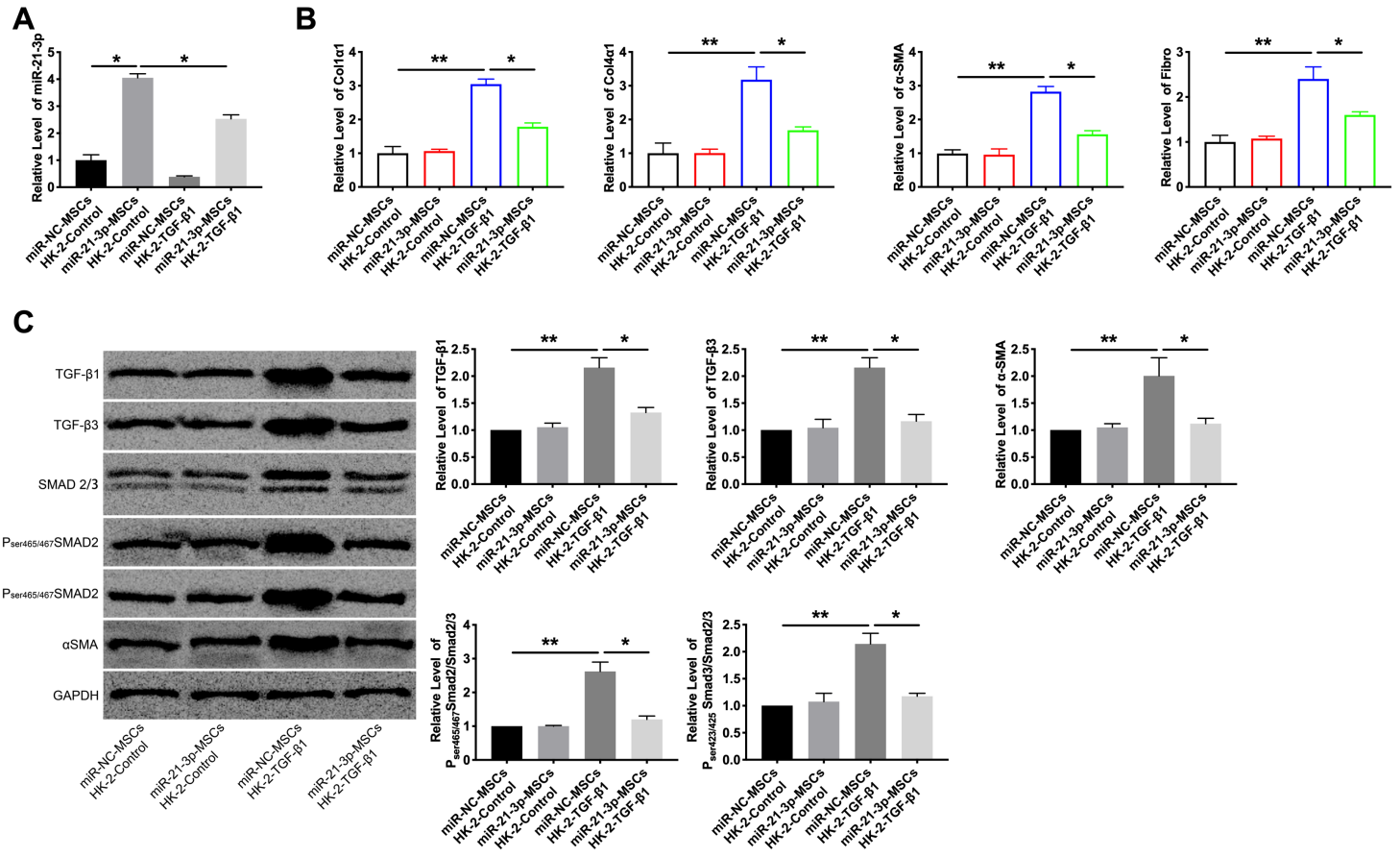

Figure 6

MSCs-miR-21-3p blocked fibrosis in HK-2 cells. A. The expression of miR-21-3p was detected in HK-2 cells co-cultured with MSCs. n=6, *P<0.05. B. The fibrotic-relative genes Col1α1, Col4α1,α-SMA and Fibro mRNA level were determined by RT-PCR. n=6, *P<0.05. C. TGFβ1, TGFβ3, smad2/3, PSer465/467Smad2 and PSer423/425Smad3, α-SMA, and COL1A1 were detected in HK-2 cells. n=6, *P<0.05.