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Influence of hepatocyte growth factor-transfected bone marrow-derived mesenchymal stem cells towards renal fibrosis in rats

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***Background & objectives*: Hepatocyte growth factor (HGF) produced by endothelial cells, fibroblasts, fat cells and other interstitial cells, can promote angiogenesis, repair damaged tissues and resist fibrosis. Mesenchymal stem cells (MSCs) are located in bone marrow and secrete a variety of cytokines and are often used in the repair and regeneration of damaged tissues. This study was aimed to investigate the influence of HGF-transfected bone marrow-derived MSCs towards renal fibrosis in rats.**

***Methods*: The HGF gene-carrying adenoviral vector (Ad-HGF) was transfected into MSCs, and the Ad-HGF-modified MSCs were transplanted into rats with unilateral ureteral obstruction (UUO). The localization of renal transplanted cells in the frozen section was observed with fluorescence microscope. The Masson's trichrome staining was performed to observe the renal collagen deposition, and the immunohistochemistry was performed to detect the expressions of α-smooth muscle actin (α-SMA) and HGF in renal tissues. Reverse transcription (RT)-PCR was used to detect the mRNA expressions of α-SMA, HGF and fibronectin (FN).**

***Results*: Ad-HGF-modified MSCs could highly express HGF *in vitro*. On the post-transplantation 3rd, 7th and 14th day, the 4',6-diamidino-2-phenylindole (DAP)-labelled transplanted cells were seen inside renal tissues. Compared with UUO group, the renal collagen deposition in transplantation group was significantly reduced, and the expressions of α-SMA mRNA and protein were significantly decreased, while the expressions of HGF mRNA and protein were significantly increased, and the expression of FN mRNA was significantly decreased ($P<0.001$).**

***Interpretation & conclusions*: Trans-renal artery injection of HGF-modified MSCs can effectively reduce the renal interstitial fibrosis in UUO rat model.**

Key words Hepatocyte growth factor - mesenchymal stem cells - renal fibrosis - transplantation - unilateral ureteral obstruction

Epidemiological studies have shown that chronic kidney disease (CKD) has become a global public health problem and its prevalence continues to increase worldwide[1]. Renal interstitial fibrosis is the common pathological outcome of all CKDs when progressing to the end-stage renal failure. Hepatocyte growth factor (HGF) is one multifunctional factor produced by endothelial cells, fibroblasts, fat cells and other interstitial cells, and it can promote angiogenesis, repair damaged tissues and resist fibrosis[2-4]. Experimental studies have indicated that HGF can promote the repair of acute kidney injury[5] and improve the renal

fibrosis[6,7]. After acute kidney injuries, mesenchymal stem cells (MSCs) can be differentiated into the renal tubular epithelial cells, thus participating in the repair of damaged tubules[8]. The *in vitro* studies have shown that in acute kidney injury, MSCs can be induced to differentiate into the renal tubular-like cells[9,10]. Unilateral ureteral obstruction (UUO) animal model is a classic model to study the renal interstitial fibrosis. In the present study the exogenous HGF gene-carrying adenoviral vector (Ad-HGF) was transfected into MSCs, then the Ad-HGF-modified MSCs were transplanted into the rats with UUO, to explore the distribution of HGF-modified MSCs in the renal tissues as well as their impact on renal fibrosis.

Material & Methods

This study was conducted in the department of Nephrology, The First Affiliated Hospital of Fujian Medical University, Fujian, PR China, after obtaining approval from the Institutional Animals Ethics Committee.

Isolation and identification of mesenchymal stem cells (MSCs): Bone marrow was drawn from the femur and tibia of 4 wk old male Sprague Dawley rats (Slaccas Laboratory Inc., Shanghai, China), mixed with L-Dulbecco's modified Eagle's medium (L-DMEM) culture fluid (Gibco Co., MA, USA), followed by filtering with 150 micron copper mesh. The cell suspension was centrifuged at 256 ×g. After discarding the supernatant, the residue was cultured with L-DMEM medium with 10 per cent foetal bovine serum (FBS) (Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, PR China) for 2-3 days. When MSCs grew almost confluent, 0.25 per cent trypsin-0.02 per cent EDTA (Fuzhou Maixin Biotechnology Development Co., Ltd.,) was added for digestion, followed by subculture. The 3rd generation cells with 80-90 per cent confluency were taken and washed with phosphate buffer saline (PBS). The cell suspension (2×10^6 cells/ml) was prepared and filtered through 300 mesh nylon net. The cell suspension was dispensed into ten 1.5 ml Eppendorf tubes, and the fluorescence-labelled mouse anti-rat monoclonal antibodies (CD90-PE, CD34-FITC, CD44-PE, CD11b/c-FITC and CD29-FITC; Shanghai SANGON Biological Engineering Co., Ltd., Shanghai, PR China) and the isotype control were added, respectively. The expressions of CD90, CD34, CD44, CD11b/c and CD29 were detected by flow cytometry according to manufacturer's instructions (Fuzhou Maixin Biotechnology Development Co., Ltd.).

Determination of MSCs viability by trypan blue staining: Nucleated cell suspension (10 µl) and 0.49 ml PBS were added to the EP tube. After mixing well, 100 µl 0.5 per cent trypan blue staining solution was added, followed by mixing. Two minutes later, the cell smears were prepared, and the cells were counted under microscope. The membrane of dead cells was damaged; thus, it could absorb the dye, while the alive cells could not be stained.

Cell viability=(total cell number − dead cell number)/total cell number×100%

If the cell viability was >90 per cent, the next experiment was performed.

Transfection of MSCs into recombinant adenovirus: The 293A cells (gifted by Dr. Xu Lin, Research Center of Molecular Medicine, Fujian Medical University, Fuzhou, PR China) were used to amplify Ad-HGF (Academy of Military Medical Sciences, Beijing, PR China). Adenovirus containing green fluorescent protein (Ad-GFP) was added with different MOI (multiplicity of infection) (MOI was 25, 50, 100 and 150 pfu/cell, respectively). The GFP expression was observed every 12 h by inverted fluorescence microscope (Leica Science Lab, Berlin, Germany) with DMEM as the control. The transfection efficiency was calculated as follows:

Transfection rate=number of cell with GFP expression/total cell number×100%

The transfection rates were 20, 50, 75 and 100 per cent towards MOI 25, 50, 100 and 150 pfu/ cell, respectively. In this study, Ad-HGF was used to transfect MSCs with MOI 150.

ELISA assay: MSCs were equally seeded in 6-well culture plates (3×10^5 cell/well), followed by culturing at 37°C for 24 h. MOI-150 Ad-HGF was used to transfect MSCs. After one hour of culture, the virus solution was discarded, and 1 ml low-serum DMEM was added, followed by culturing at 37°C. The supernatant was collected at the 1st, 3rd, 5th, 7th, 9th, 11th, 13th and 15th day, respectively. The content of HGF was detected by ELISA according to the manufacturer's instructions (Fuzhou Maixin Biotechnology Development Co., Ltd.).

Labelling of transplanted cells: The 3rd-5th generation rat MSCs were incubated with 50 µg/ml DAPI (Sigma-Aldrich Corp., USA) for 24 h and washed

with PBS to clear the unbound DAPI. The cells were digested with 0.25 per cent trypsin (Sigma-Aldrich), and the reaction was terminated with 10 per cent FBS L-DMEM. After centrifuged at 256 × *g* for five minutes, the residue was resuspended in PBS at a concentration of 10^7 cell/ml. Before cell transplantation, Ad-HGF was used to transfect MSCs, and the DAPI-labelling was performed 48 h later.

Reverse transcription (RT)-PCR assay: One ml triol cell lysate (Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, PR China) was added to 100 mg rat renal tissues of each group. The total RNA extraction and cDNA synthesis of cells and tissues were performed according to the manufacturer's instructions (Fuzhou Maixin Biotechnology Development Co., Ltd.)[11]. The 101 bp α-smooth muscle actin (α-SMA) mRNA PCR products were amplified using the sense primer (5'-GATGGATGGGAAAACAGCC-3') and antisense primer (5'-AAGAGGAAGACAGCACAGCTC-3') (Fuzhou Maixin Biotechnology Development Co., Ltd.). Amplifications were done at 94°C for 30 sec, 55°C for 30 sec, and 72°C for 45 sec for 35 cycles. The 218 bp HGF mRNA PCR products were amplified using the sense primer (5'-TCTCCTTCCTCGCTCAGTTCGT-3') and antisense primer (5'-TCGAACAAAAATA CCAGGAC-3') (Fuzhou Maixin Biotechnology Development Co., Ltd.). Amplifications were done at 94°C for 30 sec, 57°C for 30 sec, and 72°C for 45 sec for 30 cycles. The 205 bp fibronectin (FN) mRNA PCR products were amplified using the sense primer (5'-TTATGGGGAATGAAATGC-3') and antisense primer (5'-TGACTCGCTTTGACTTCACCAC-3') (Fuzhou Maixin Biotechnology Development Co., Ltd.). Amplifications were done at 94°C for 30 sec, 53°C for 30 sec, and 72°C for 45 sec for 35 cycles. The 452 bp glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was used as an internal control using the sense primer (5'-ACCACAGTCCATGCCATCAC-3') and antisense primer (5'-TCCACCACCCTGTTGCTGTA-3') (Fuzhou Maixin Biotechnology Development Co., Ltd.). Amplifications were done at 94°C for 30 sec, 58°C for 30 sec, and 72°C for 45 sec for 30 cycles. The agarose gel electrophoresis was used for detection and analysis of PCR products. The integrated optical density values of the images were analyzed using white/ultraviolet transilluminator system and were corrected with the optical density value of internal reference GAPDH, and the ratio was used to express the relative content.

Establishment and grouping of unilateral ureteral obstruction (UUO) rat model: Seventy eight 6-8 wk old Sprague Dawley male rats were randomly divided into sham-operation group (control group, 18 rats), UUO group (18 rats), MSC transplantation group (MSC group, 18 rats) and Ad-HGF-modified MSC transplantation group (HGF group, 24 rats). For UUO group, the SD rats were intraperitoneally anesthetized with 4 ml/kg chloral hydrate (Sigma-Aldrich). Then, one incision was made in the left abdominal kidney area. The left ureter was separated, and the ureter was ligated with No. 5 surgical thread at kidney calices and renal lower pole, respectively. The kidney back was placed to the original place after surgery. For control group, the rest of the steps were the same as the UUO group except for the ureteral ligation. For MSC group and HGF groups after modelling, 0.1 ml MSC suspension and Ad-HGF-modified MSC suspension were infused through tail vein, respectively. Control group and UUO group were injected with normal saline. Six rats in each of control group, UUO group and MSC groups were killed at the 7th, 14th and 28th day after modelling, respectively, and the renal specimen was sampled. Six rats in the HGF group were killed at the 3rd, 7th, 14th and 28th day after modelling, and the renal specimens were sampled.

Renal pathological examination: The obstructed kidney was fixed with four per cent formaldehyde (Sigma-Aldrich), followed by ethanol dehydration and paraffin embedding; 2 μm-thick paraffin sections were prepared for the hematoxylin-eosin (HE) staining and Masson's trichrome staining. For semi-quantitative analysis of Masson's staining under 400-fold magnified view field, 10 non-overlapped tubulointerstitial vision of each section was randomly selected. The semi-quantitative analysis was performed based on the percentage of positive collagen staining area to the entire vision field: 0 point, positive area <2 per cent; 1 point, positive area 2-10 per cent, mild lesion; 2 points, positive area 11-20 per cent, moderate lesion; 3 points, positive area 21-30 per cent, severe lesion and 4 points, positive area >30 per cent, very severe lesion. The frozen sections were used to determine the number and location of DAPI-labelled transplanted cells in renal tissues under BX61 fluorescent microscope (Olympus Corp., Tokyo, Japan).

Immunohistochemistry assay: Paraffin sections (2 µm thick) were deparaffinized and washed with distilled water. After soaking in PBS for five minutes, these were incubated with three per cent H_2O_2 at room temperature for 15 min. After washing with PBS, the appropriate proportion of primary antibodies (rabbit anti-rat α-SMA, dilution 1:4000; goat anti-rat HGF, dilution 1:200; Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, PR China) were added for incubation at 37°C for 1-2 h, followed by washing with PBS. The corresponding secondary antibodies were added, followed by incubation at 37°C for 30 min. After washing with PBS, the 3,3'-diaminobenzidine (DAB) staining was performed, followed by flushing with water, restaining, dehydration and mounting. The sites with positive reactions exhibited brown or tan particles, and the nuclei were stained blue. The semi-quantitative analysis was performed with IPP-6 image analysis software (Media Cybernetics, Inc., MD, USA).

Statistical analysis: The data were expressed as mean±standard deviation (SD). SPSS 15.0 statistical analysis software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The intergroup difference was analyzed with ANOVA, and Dunnett's *t* test was used for intergroup pairwise comparison.

Results

Isolation and identification of MSCs: The cultured MSCs exhibited dispersed and clone-mode proliferation, with fusiform, radial or fence-like growth manner. The test towards the 3rd generation cells showed that the positive cellular expression rates of CD11b/c, CD34, CD44, CD90 and CD29 were 13.2±0.6, 1.2±0.5, 97.8±0.9, 96.8±1.4 and 97.6±2.4 per cent, respectively. This indicated that the 3rd generation cells were relatively purified MSCs.

HGF expression in Ad-HGF-transfected MSCs: Compared with the non-transfected MSCs, the Ad-HGF-transfected MSCs showed significantly (P<0.001) increased HGF level compared to 0 day, which reached the peak on the 8th day, and then gradually reduced but still remained significantly higher (P<0.001) than

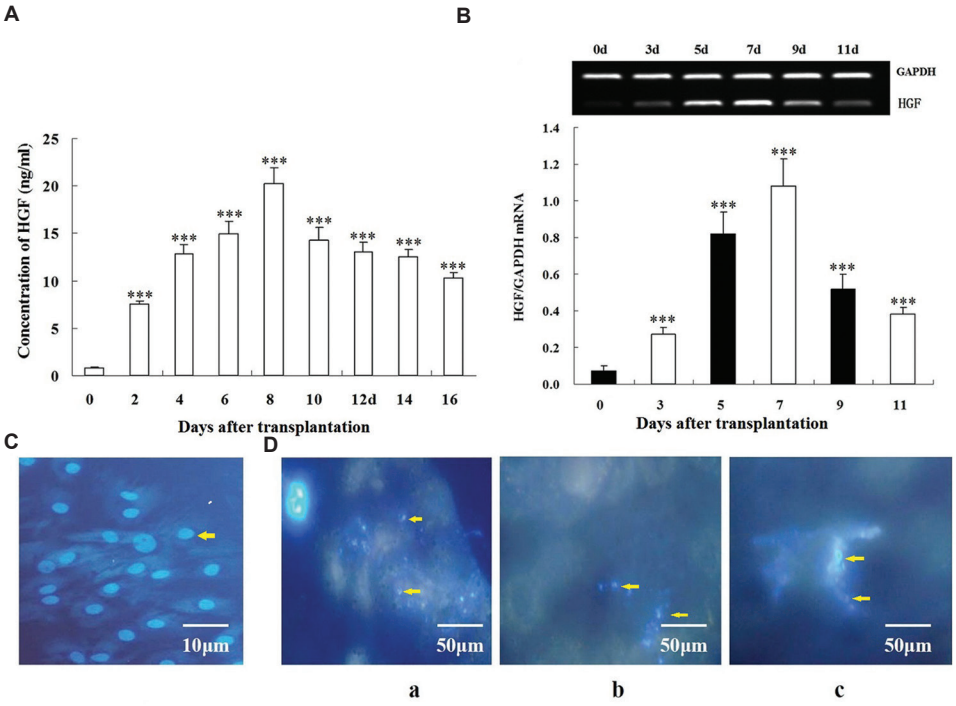

Fig. 1. (**A**) Hepatocyte growth factor (HGF) concentration in the supernatant of mesenchymal stem cell (MSC) transfected by Ad-hepatocyte growth factor (Ad-HGF). ***P<0.001 compared with 0 day (no transfection). (**B**) Expression of hepatocyte growth factor mRNA of MSCs transfected by Ad-HGF. ***P<0.001 compared with 0 day (no transfection). (**C**) Ad-HGF-MSC labelled by 4',6-diamidino-2-phenylindole (DAPI) (×400). The elliptical/oval structures (arrows) are the nucleus of DAPI-labelled MSCs. (**D**) The blue dots shown by the arrows represent DAPI-labelled transplanted Ad-HGF-MSC in the renal tissues (×100). The positions of Ad-HGF-modified MSCs in the obstructed kidneys after 3, 7 and 14 days of transplantation are shown. The DAPI-labelled MSCs were distributed among the expanded tubular epithelial cells. (a) 3rd day after transplantation; (b) 7th day after transplantation; (c) 14th day after transplantation. GADPH, glyceraldehyde-3-phosphate dehydrogenase.

0 day (Fig. 1A). RT-PCR findings revealed that compared with the non-transfected MSCs, the Ad-HGF-transfected MSCs showed significantly (P<0.001) increased HGF mRNA expression, which reached the peak on the 7th day compared to 0 day (Fig. 1B).

Cell transplantation: The DAPI-labelled MSCs under fluorescence microscopy are shown in Fig. 1C. The frozen sections were used to observe the number and location of DAPI-labelled transplanted cells in the renal tissues. The positions of Ad-HGF-modified MSCs in the obstructed kidneys after 3-, 7- and 14-day transplantation are shown in Fig. 1D. The DAPI-labelled MSCs were distributed among the expanded tubular epithelial cells. This indicated that the modified MSC could be targetedly located into the renal tissues.

Renal pathological changes and expression of fibronectin (*FN*): HE staining revealed that with prolonged obstruction time, UUO group exhibited gradually obvious renal tubular atrophy and interstitial fibrosis, while the situations of interstitial fibrosis in MSC group and HGF group were significantly reduced (Fig. 2A). Masson's trichrome staining showed that with prolonged obstruction of time, UUO group exhibited significantly increased content of collagen fibres, while those in MSC and HGF groups were significantly reduced (Fig. 2B). The semi-quantitative analysis showed that on the post-modelling 14th day, the scores of collagen content integral in obstructed kidney in UUO, MSC and HGF groups were 6.5±0.6, 5.8±0.5 and 4.2±0.4, respectively. On the post-modelling 28th day, the scores of collagen content integral in UUO, MSC and HGF groups were 11.8±1.2, 9.5±0.9 and 6.3±0.8, respectively. On the post-modelling 14th day, compared with UUO group, the score in HGF group was significantly reduced (P<0.001). On the post-modelling 28th day, compared with UUO group, the scores in MSC and HGF groups were significantly reduced (P<0.001). Compared with MSC group, the scores in HGF group were significantly reduced on the post-modelling 14th and 28th day (P<0.001). RT-PCR assay showed that with prolonged obstruction time, the expression of FN mRNA in UUO group was gradually increased. On the 28th day after modelling, compared with UUO group, the expression of FN mRNA in MSC group was significantly reduced (P<0.001). Compared with MSC group, the expression of FN mRNA in HGF group was significantly reduced on the 28th day after modelling (P<0.001) (Fig. 2C).

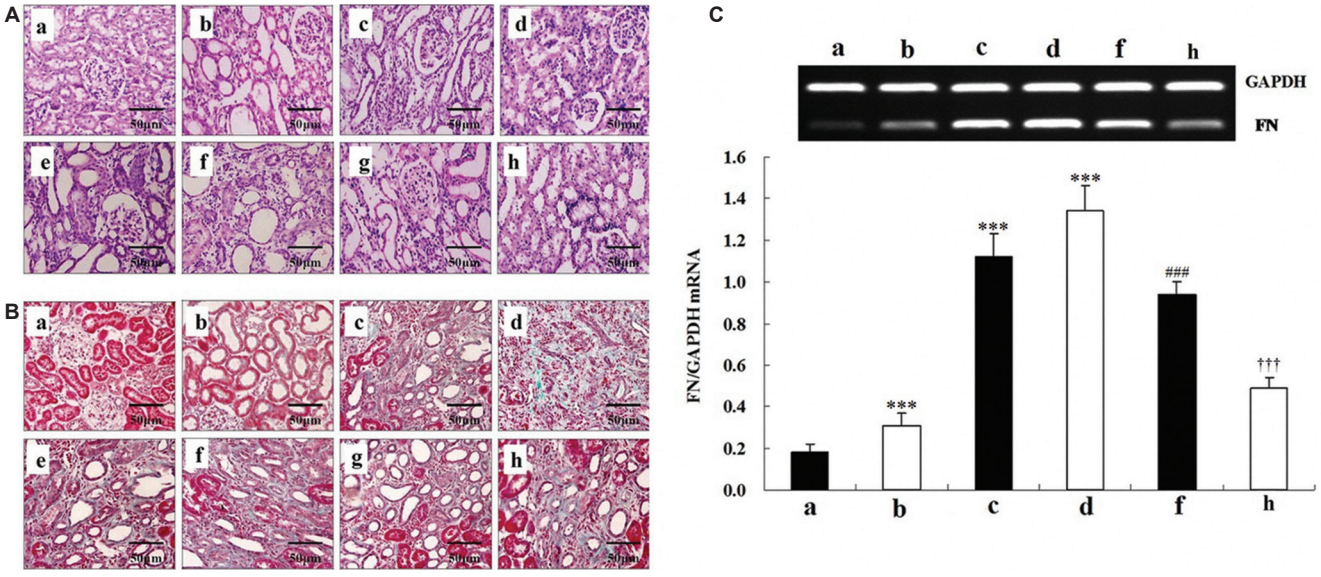

Fig. 2. (**A**) Pathological changes (tubular atrophy and interstitial fibrosis) of renal tissue on obstruction side (H and E, ×400). (a) Control group; (b), (c), (d) 7, 14 and 28 days, respectively after unilateral ureteral obstruction (UUO) operation; (e), (f) 14 and 28 days, respectively after mesenchymal stem cell (MSC) transplantation; (g), (h) 14 and 28 days, respectively after Ad-hepatocyte growth factor-mesenchymal stem cell (Ad-HGF-MSC) transplantation; the following is the same. (**B**) Pathological changes of renal tissue on obstruction side (Masson ×400). (a) Control group; (b), (c), (d) 7, 14 and 28 days, respectively after UUO operation; (e), (f) 14 and 28 days, respectively after MSC transplantation; (g), (h) 14 and 28 days, respectively after Ad-HGF-MSC transplantation. (**C**) Expression of fibronectin (FN) mRNA in renal tissue on obstruction side. $^{***}P$<0.001 compared with control group; $^{\#\#\#}P$<0.001 compared with UUO group (28 days after operation); $^{\dagger\dagger\dagger}P$<0.001 compared with MSC group (28 days after transplantation). GADPH, glyceraldehyde-3-phosphate dehydrogenase.

Expression of α-smooth muscle actin (*α-SMA*): The immunohistochemical assay revealed that the expression of α-SMA in control group was mainly located in the vessel walls and occasionally inside the renal interstitium. With prolonged obstruction time, UUO group showed gradually increased expression of α-SMA, while those in MSC group and HGF group were weakened (Fig. 3A). The semi-quantitative analysis showed that on the 14th and 28th day after modelling, compared with UUO group, the expressions of α-SMA in obstructive renal tissues of HGF and MSC groups were significantly reduced ($P<0.001$). Compared with MSC group, the expressions of α-SMA in HGF group were significantly reduced on the 14th and 28th day after modelling ($P<0.001$) (Fig. 3B). RT-PCR assay showed that with prolonged obstruction time, the expression of α-SMA mRNA in UUO group was gradually increased. On the 28th day after modelling, compared with UUO group, the expression of α-SMA mRNA in MSC groups was significantly reduced ($P<0.001$). Compared with MSC group, the expression of α-SMA mRNA in HGF group was significantly reduced on the 28th day after modelling ($P<0.001$) (Fig. 3C).

Expression of hepatocyte growth factor (*HGF*): The immunohistochemistry assay showed that the expression of HGF in obstructed renal tissues of UUO group was significantly reduced, while those in MSC and HGF groups were significantly increased (Fig. 4A). The semi-quantitative analysis showed that on the 14th and 28th day after modelling, compared with UUO group, the expressions of HGF in HGF and MSC groups were significantly increased ($P<0.001$). Compared with MSC group, the expression of HGF in HGF group was significantly increased on the 28th day after modelling ($P<0.001$) (Fig. 4B). RT-PCR assay revealed that with prolonged obstruction time, the expression of HGF mRNA in UUO group was gradually decreased. On the 28th day after modelling, compared with UUO group, the expression of

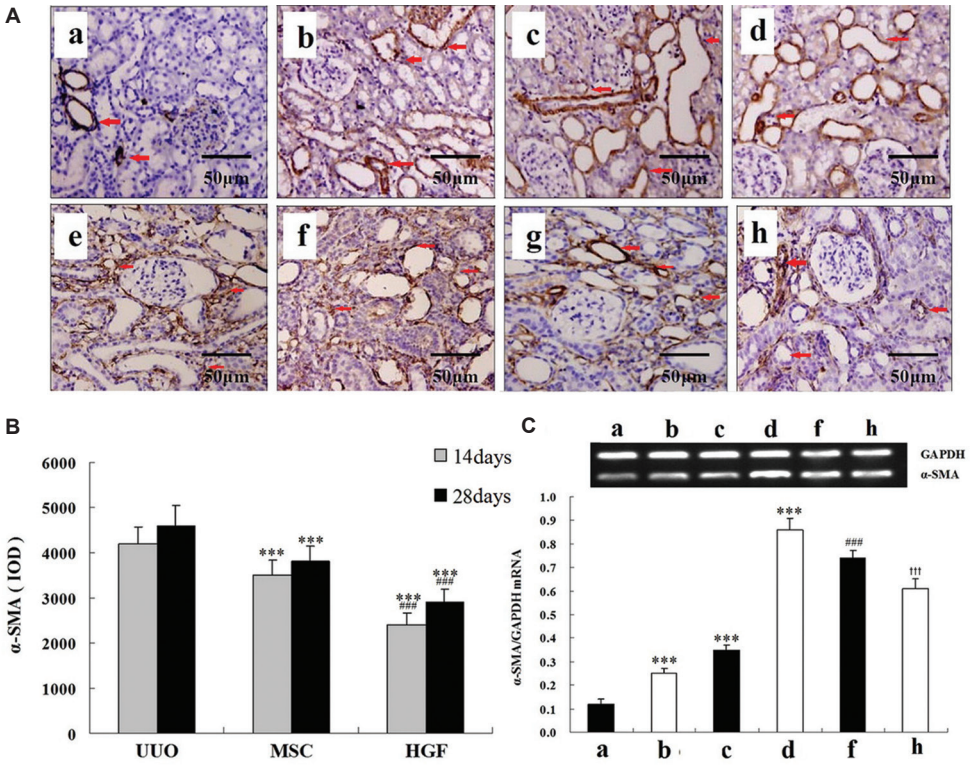

Fig. 3. (**A**) Expression of α-smooth muscle actin (α-SMA) in renal tissue on obstruction side (×400). The brown area (arrows) is an α-SMA positive cell. With prolonged obstruction time, unilateral ureteral obstruction (UUO) group showed gradually increased expression of α-SMA, while those in mesenchymal stem cell (MSC) group and hepatocyte growth factor (HGF) group were weakened. (a) Control group; (b), (c), (d) 7, 14 and 28 days, respectively after UUO operation; (e), (f) 14 and 28 days, respectively after MSC transplantation; (g), (h) 14 and 28 days, respectively after Ad-hepatocyte growth factor-mesenchymal stem cell (Ad-HGF-MSC) transplantation. The following is the same. (**B**) Semi-quantitative analysis of expression of α-SMA in renal tissue on obstruction side. $^{***}P<0.001$ compared with UUO group; $^{\#\#\#}P<0.001$ compared with MSC group. (**C**) Expression of α-SMA mRNA in renal tissue on obstruction side. $^{***}P<0.001$ compared with control group; $^{\#\#\#}P<0.001$ compared with UUO group (28 days after operation); $^{\dagger\dagger\dagger}P<0.001$ compared with MSC group (28 days after transplantation).

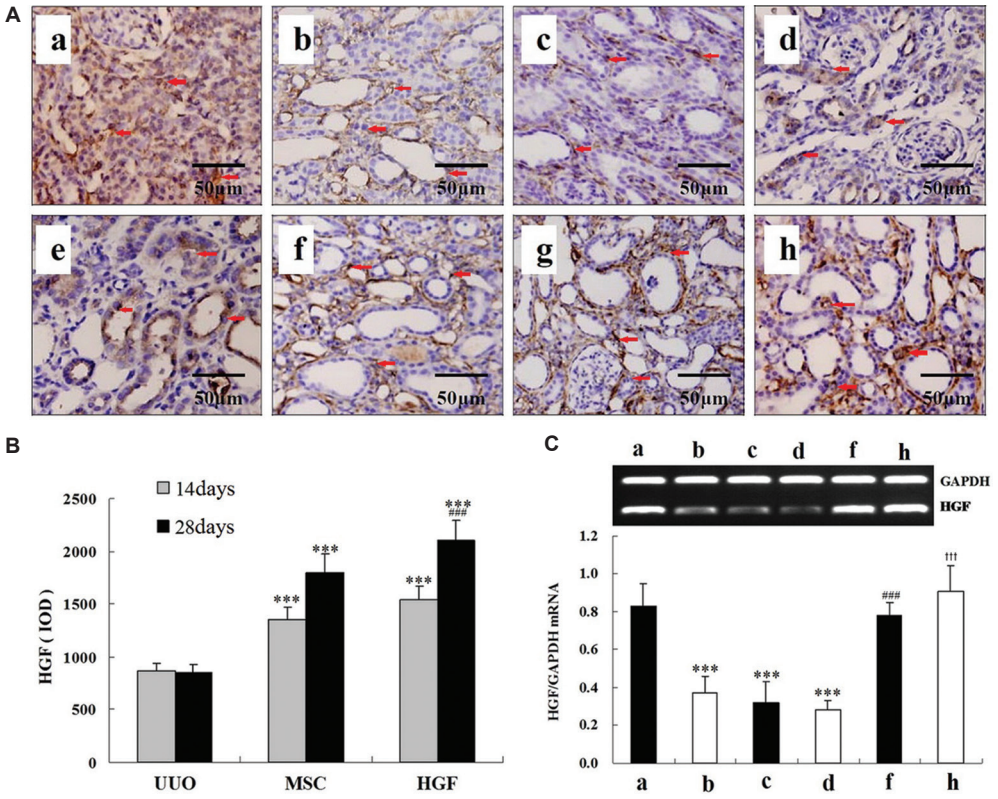

Fig. 4. (**A**) Expression of hepatocyte growth factor (HGF) in renal tissue on obstruction side (×400). The brown area (arrows) is an HGF positive cell. Expression of HGF in obstructed renal tissues of unilateral ureteral obstruction (UUO) group was reduced, while those in mesenchymal stem cell (MSC) and HGF groups were increased. (a) Control group; (b), (c), (d) 7, 14 and 28 days, respectively after UUO operation; (e), (f) 14 and 28 days, respectively after MSC transplantation; (g), (h) 14 and 28 days, respectively after Ad-hepatocyte growth factor-mesenchymal stem cell (Ad-HGF-MSC) transplantation. The following is the same. (**B**) Semi-quantitative analysis of expression of HGF in renal tissue on obstruction side. $^{***}P<0.001$ compared with UUO group; $^{\#\#\#}P<0.001$ compared with MSC group. (**C**) Expression of HGF mRNA in renal tissue on obstruction side. $^{***}P<0.001$ compared with control group; $^{\#\#\#}P<0.001$ compared with UUO group (28 days after operation); $^{\dagger\dagger\dagger}P<0.001$ compared with MSC group (28 days after transplantation). GADPH, glyceraldehyde-3-phosphate dehydrogenase.

HGF mRNA in MSC group was significantly reduced ($P<0.001$). Compared with MSC group, the expression of HGF mRNA in HGF group was significantly reduced on the 28th day after modelling ($P<0.001$) (Fig. 4C).

Discussion

MSCs are the stem cells that have self-renewing function and exhibit strong ability of self-amplification *in vitro*, but still remain the characteristics of stem cells and normal phenotype after 10^7-fold amplification[11]. Therefore, MSCs are considered as the ideal exogenous gene transferring target cells. Adenovirus vector is the most commonly used gene vector[12]. Our results showed that the transfection rate of recombinant adenovirus Ad-HGF towards MSC had the dose-response relationship with viral MOI. With increase of MOI value, the transfection efficiency of recombinant adenovirus towards MSC was also increased. Once transfected with Ad-HGF, MSCs could effectively express HGF.

Ito *et al*[13] transplanted the enhanced green fluorescent protein (EGFP)-labelled bone marrow of transgenic mice into the wild-type mice; 21 days after transplantation, 80 per cent bone marrow and spleen cells in the recipient mice expressed EGFP, while there existed almost no EGFP-positive cells in kidney. This indicated that under normal physiological conditions, the bone marrow stem cells transplanted through the systemic circulation could not be localized in kidney. In the present study, the modified MSCs were infected through the renal artery. The results showed that the kidney-frozen sections in the transplantation group exhibited the DAPI-labelled-modified MSC among the expanded tubular epithelial cells under fluorescence microscope, which could continue until 14 days after

transplantation. This indicated that the modified MSCs injected through the renal artery could be located into the renal tissues.

HGF is the important factor towards the kidney development and function maintenance, mainly expressed by renal interstitial cells, glomerular endothelial cells and macrophages, and specifically acted on the renal tubular epithelial cells through endocrine, autocrine and paracrine system[14]. Yang & Liu[15] reported that the short-term injection of recombinant HGF into UUO model could inhibit the phenotype transformation of tubular epithelial cells and the deposition of matrix proteins, thereby reducing the interstitial fibrosis. Liu *et al*[16] used the adenovirus Ad-HGF-transfected MSC to transplant into the UUO rat model and found that the Ad-HGF-modified MSCs were mainly distributed inside the renal tubular epithelium. It was also found that the renal interstitial fibrosis could be significantly reduced, similar to the results of the present study. The reason might be that the exogenous HGF-modified MSC could continuously express HGF in the homing renal tissues, and MSCs could promote the renal tissues to secrete endogenous HGF. Our previous *in vitro* study[10] showed that the addition of HGF into the cell culture medium promoted the differentiation of MSC into the renal tubular epithelial cells.

During the procedure of renal interstitial fibrosis, epithelial-mesenchymal transition (EMT) was an important mechanism[17]. When EMT occurred, the renal tubular epithelial cells would lose the phenotype of epithelial cells and then get the characteristics of mesenchyme (*e.g.* expressing α-SMA)[18,19]. α-SMA was considered as the marker protein of renal interstitial myofibroblasts (MF). Its amount was directly related to the degrees of interstitial fibrosis[20]. The UUO model established in the present study exhibited a continuous increase in α-SMA expression in renal tubular epithelial cells with the obstruction time prolonged, which also migrated towards the renal interstitium. This indicated that the tubular epithelial cells were transformed into the MF that could express α-SMA. Liu[21] also showed that MF was primarily transformed from tubular epithelial cells through EMT.

The increase of extracellular matrix (ECM) is one major pathological basis for the fibrosis of renal tissues. ECM is composed of collagen, non-collagenous glycoproteins and proteoglycans. The most important component of collagens is collagen IV, and it constitutes the ECM scaffold. The most important component of non-collagenous glycoproteins is FN, which is the adhesion protein among cells, as well as between cells and ECM. The increased synthesis of FN represents the excessive accumulation of ECM to some extent[22]. Local FN and collagen accumulation would promote the proliferation of fibroblasts, as well as the formation of renal interstitial fibrosis. This experiment showed that with prolonged obstruction time, the FN mRNA expression was continuously increased, while the FN mRNA expression in the transplantation group on the 28th day was reduced compared with UUO group. Therefore, the transplanted HGF-modified MSC could help reduce the FN mRNA expression; thus, the degree of renal interstitial fibrosis was reduced.

There were some limitations of this study. UUO rats were observed only for 28 days. Future research should be designed for longer periods of time. In addition, DAPI staining to follow up transplanted cells was not convincing. It would be more persuasive if bone marrow-derived MSCs from GFP transgenic rats are used for transplanted cells' homing and differentiation tests.

In conclusion, the findings revealed that Ad-HGF-transfected MSCs significantly increased HGF expression and that the HGF-modified MSCs injected through the renal artery could be located into the renal tissues. Cell transplantation with Ad-HGF-transfected MSCs can reduce the renal interstitial fibrosis of UUO rats, and its mechanism may be related to attenuated renal EMT.

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***Conflicts of Interest*:** None.

References

1. Zhang L, Wang F, Wang L, Wang W, Liu B, Liu J, *et al.* Prevalence of chronic kidney disease in China: A cross-sectional survey. *Lancet* 2012; *379* : 815-22.
2. Iekushi K, Taniyama Y, Kusunoki H, Azuma J, Sanada F, Okayama K, *et al.* Hepatocyte growth factor attenuates transforming growth factor-β-angiotensin II crosstalk through inhibition of the PTEN/Akt pathway. *Hypertension* 2011; *58* : 190-6.

3. Nakamura T, Sakai K, Nakamura T, Matsumoto K. Hepatocyte growth factor twenty years on: Much more than a growth factor. *J Gastroenterol Hepatol* 2011; *26* (Suppl 1) : 188-202.
4. Iekushi K, Taniyama Y, Azuma J, Sanada F, Kusunoki H, Yokoi T, *et al.* Hepatocyte growth factor attenuates renal fibrosis through TGF-β1 suppression by apoptosis of myofibroblasts. *J Hypertens* 2010; *28* : 2454-61.
5. Zhou D, Tan RJ, Lin L, Zhou L, Liu Y. Activation of hepatocyte growth factor receptor, c-met, in renal tubules is required for renoprotection after acute kidney injury. *Kidney Int* 2013; *84* : 509-20.
6. Wang HY, Yang LZ, Gu CM, Chen Y, Zhao Y, Zhao D, *et al.* Pathological changes, TGF-β1 expression, and the effects of hepatocyte growth factor in 5/6 nephrectomized rats. *Ren Fail* 2014; *36* : 393-9.
7. Du T, Zou X, Cheng J, Wu S, Zhong L, Ju G, *et al.* Human wharton's jelly-derived mesenchymal stromal cells reduce renal fibrosis through induction of native and foreign hepatocyte growth factor synthesis in injured tubular epithelial cells. *Stem Cell Res Ther* 2013; *4* : 59.
8. Qian H, Yang H, Xu W, Yan Y, Chen Q, Zhu W, *et al.* Bone marrow mesenchymal stem cells ameliorate rat acute renal failure by differentiation into renal tubular epithelial-like cells. *Int J Mol Med* 2008; *22* : 325-32.
9. Singaravelu K, Padanilam BJ. *In vitro* differentiation of MSC into cells with a renal tubular epithelial-like phenotype. *Ren Fail* 2009; *31* : 492-502.
10. Wan JX, Zou ZH, You DY, Cui J, Pan YB. Bone marrow-derived mesenchymal stem cells differentiation into tubular epithelial-like cells *in vitro*. *Cell Biochem Funct* 2012; *30* : 129-38.
11. Pang Y, Cui P, Chen W, Gao P, Zhang H. Quantitative study of tissue-engineered cartilage with human bone marrow mesenchymal stem cells. *Arch Facial Plast Surg* 2005; *7* : 7-11.
12. Rafi MA. Gene and stem cell therapy: Alone or in combination? *Bioimpacts* 2011; *1* : 213-8.
13. Ito T, Suzuki A, Imai E, Okabe M, Hori M. Bone marrow is a reservoir of repopulating mesangial cells during glomerular remodeling. *J Am Soc Nephrol* 2001; *12* : 2625-35.
14. Dai C, Saleem MA, Holzman LB, Mathieson P, Liu Y. Hepatocyte growth factor signaling ameliorates podocyte injury and proteinuria. *Kidney Int* 2010; *77* : 962-73.
15. Yang J, Liu Y. Blockage of tubular epithelial to myofibroblast transition by hepatocyte growth factor prevents renal interstitial fibrosis. *J Am Soc Nephrol* 2002; *13* : 96-107.
16. Liu X, Shen W, Yang Y, Liu G. Therapeutic implications of mesenchymal stem cells transfected with hepatocyte growth factor transplanted in rat kidney with unilateral ureteral obstruction. *J Pediatr Surg* 2011; *46* : 537-45.
17. Lee JH, Kim JH, Kim JS, Chang JW, Kim SB, Park JS, *et al.* AMP-activated protein kinase inhibits TGF-β-, angiotensin II-, aldosterone-, high glucose-, and albumin-induced epithelial-mesenchymal transition. *Am J Physiol Renal Physiol* 2013; *304* : F686-97.
18. Yang J, Liu Y. Dissection of key events in tubular epithelial to myofibroblast transition and its implications in renal interstitial fibrosis. *Am J Pathol* 2001; *159* : 1465-75.
19. Yang J, Dai C, Liu Y. A novel mechanism by which hepatocyte growth factor blocks tubular epithelial to mesenchymal transition. *J Am Soc Nephrol* 2005; *16* : 68-78.
20. Yang J, Shultz RW, Mars WM, Wegner RE, Li Y, Dai C, *et al.* Disruption of tissue-type plasminogen activator gene in mice reduces renal interstitial fibrosis in obstructive nephropathy. *J Clin Invest* 2002; *110* : 1525-38.
21. Liu Y. New insights into epithelial-mesenchymal transition in kidney fibrosis. *J Am Soc Nephrol* 2010; *21* : 212-22.
22. Yuan Q, Wang R, Peng Y, Fu X, Wang W, Wang L, *et al.* Fluorofenidone attenuates tubulointerstitial fibrosis by inhibiting TGF-β(1)-induced fibroblast activation. *Am J Nephrol* 2011; *34* : 181-94.

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