Original Research

Human umbilical cord mesenchymal stem cells attenuate cisplatin-induced acute and chronic renal injury

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Abstract

Mesenchymal stem cell is becoming a promising candidate in acute kidney injury (AKI). We first reported that human umbilical cord mesenchymal stem cells (hucMSCs) could ameliorate renal function in ischemic/reperfusion AKI rats, but the role of hucMSCs in cisplatin-induced acute and chronic injury has been demonstrated. More specifically, it is still unknown whether hucMSCs halt renal interstitial fibrosis. In this study, we investigated the effect of hucMSCs in cisplatin-induced kidney injury and explored the mechanism of action. Blood urea nitrogen (BUN) and creatinine (Cr) analyses showed amelioration of functional parameters in hucMSC-treated rats at early damage. Transplantation with hucMSCs promoted renal cell regeneration, inhibited cell apoptosis, abrogated inflammatory responses and protected mitochondria. Moreover, Masson's trichrome staining demonstrated reduced levels of fibrosis in kidney tissues of hucMSC-treated rats at six and eight weeks after cisplatin injection. These results were corroborated by reduced collagen deposit, the ratio of Bax to Bcl-2 and transforming growth factor β mRNA expression. Furthermore, hucMSCs prevented the epithelial-mesenchymal transition (EMT) in injury renal tissues, leading to the attenuation of chronic renal interstitial fibrosis. Taken together, our findings suggested that hucMSCs could decrease the kidney from development of later renal interstitial fibrosis by amelioration of early AKI.

Keywords: Human umbilical cord mesenchymal stem cells, transplantation, acute kidney injury, renal interstitial fibrosis, epithelial-mesenchymal transition

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Introduction

Despite recent advances in dialysis and renal replacement therapy, the mortality of patients with acute kidney injury (AKI) remains high.[1,2] The patients who have normal or near normal renal function indexes after AKI may still have the risk of progressive chronic kidney disease (CKD), such as renal interstitial fibrosis, in the months to years after hospital discharge.[3] AKI is a major cause of subsequent CKD and also independently contribute to mortality because of its poor early outcome and pathophysiological state. Therefore, there is an urgent need to develop new therapy strategy for AKI.

Bone marrow-derived mesenchymal stem cells (BM-MSCs) have been used for treating AKI.[4–6] BM-MSCs infused into AKI animals promoted the recovery of renal function and structure through the release of growth factors, such as insulin-like growth factor-1 (IGF-1).[7] BM-MSCs of human origin could exert pro-survival effects on renal cells *in vitro* and *in vivo*.[8–10] Due to the limited number of BM-MSCs, it is of great need to seek for alternative MSC sources. Human umbilical cord provides a novel source for MSC-based therapies. Human umbilical cord MSCs (hucMSCs) possess the similar characteristics to BM-MSCs[11] and also have a higher proliferative potency and lower immunogenicity.[12] HucMSCs are expected to be the ideal cells for the therapy of various diseases including nervous system disorders,[13] diabetes,[14] cancer,[15] as well as AKI.[16]

We have previously reported that hucMSCs and hepatocyte growth factor (HGF)-modified hucMSCs can accelerate the recovery of renal function in ischemic AKI rats by anti-inflammatory and pro-proliferative effects.[16,17] Besides ischemia/reperfusion, drug is another high-risk factor for AKI. Cisplatin is a drug commonly used to develop

The first three authors contributed equally to this work.

experimental renal injury animal models. In the present study, we investigated the effects of hucMSCs on cisplatin-induced acute and chronic renal injury and further explored the underlying mechanism of action.

Materials and methods

HucMSCs isolation and identification

Human umbilical cords were collected from full-term births after either caesarean section or normal vaginal delivery. Informed consent was obtained from all pregnant women. The fresh umbilical cords were collected at 4°C and processed within 6 h as previously described.[18] The tissues were incubated at 37°C with 5% CO_2 in Dulbecco's modified Eagle's medium containing 1000 mg/mL glucose (DMEM-LG, Gibco, USA), 10% fetal bovine serum (FBS, Gibco, USA), penicillin and streptomycin. HucMSCs were characterized by flow cytometry (FACS Calibur, Becton Dickinson, USA). Cells were incubated with monoclonal antibodies against CD13, CD29, CD33, CD38, CD44, CD45 (PE-conjugated) and CD34, CD71 and human leukocyte antigen-DR (HLA-DR) (FITC-conjugated) (Becton Dickinson, San Josè, CA, USA). Isotype PE-IgG1 and FITC-IgG1 were used as negative controls. The adipogenic and osteogenic differentiation potential of hucMSCs was examined as described before.[18] Adipogenic differentiation was determined by Oil Red O staining and osteogenic differentiation was assessed by Alizarin red staining.

Cell labelling

HucMSCs were labelled with the cross-linkable membrane dye, CM-Dil (Molecular Probes, USA), according the manufacturer's protocol. Cells (1×10^6/mL) were incubated with 5 μL of the CM-Dil cell-labelling solution at 37°C for 30 min. The unbound dye was removed by washing with PBS, and the labelled cells were resuspended in serum-free medium.

Cell co-culture

Rat renal tubular epithelial cell lines (NRK 52E) were purchased from Cell Bank, Type Culture Collection Committee, Chinese Academy of Sciences. The cells were cultured in DMEM supplemented with 10% FBS, penicillin (100 U/mL) and streptomycin (100 μg/mL) at 37°C in 5% CO_2. When reached 40–50% confluence, cells were pre-exposed to cisplatin (5 μM) for 6 h, followed by co-culture with hucMSCs in a transwell system (pore size 0.4 μm, Corning Costar, USA) for 48 h.

Cisplatin-induced renal injury rat model

The experiments were approved by the Institutional Animal Care Committee and conducted under the guidelines for animal care and use of Jiangsu University. Adult female Sprague-Dawley rats weighing 200 ± 20 g (Animal Centre of Chinese Academy of Sciences, Shanghai, China) were housed at a constant temperature and humidity, with a 12–12 h light-dark cycle and unrestricted access to a standard diet and water. To establish the injury model, cisplatin (Dezhou Pharmaceutical, Shandong, China) of 6.0 mg/kg was intraperitoneally injected into the rats. The animals were sacrificed at different time points (5 days, 10 days, 4 weeks, 6 weeks and 8 weeks) after cisplatin injection for histological and pathological analyses.

HucMSCs transplantation

To evaluate the effects of hucMSCs transplantation, HFL-1 cells (human lung fibroblast-like cells) were used as a cell control. Rats were randomly divided into five groups: control group (non-cisplatin injected rats) ($n = 6$), cisplatin group (cisplatin-treated rats receiving saline, $n = 24$), hucMSCs group (cisplatin-treated rats receiving hucMSCs, $n = 24$), labelled hucMSCs group (cisplatin-treated rats receiving CM-Dil labelled-hucMSCs $n = 6$) and HFL-1 group (cisplatin-treated rats receiving HFL-1 cells, $n = 6$). HucMSCs (2×10^6) or HFL-1 cells (2×10^6) suspended in 500 μL saline solution were injected via the tail vein at 24 h after cisplatin injection. Blood samples were collected before (0 day) and at different time points after cisplatin injection. Rats were sacrificed according to the needs of the experiments. In-Vivo Imaging System (Maestro™ GNIR-Flex, CRI, USA) was used to observe the location of transfused hucMSCs at four weeks after cisplatin injection.

Tissue homogenate preparation and mitochondria isolation

Rats were sacrificed at five and ten days after cisplatin injection, and kidneys were removed and washed in cold saline solution. The kidneys were grinded in the pre-cooled homogenization medium (0.01 mol/L sucrose, 0.0001 mol/L EDTA and 0.01 mol/L Tris–HCl in a 0.8% saline solution, pH 7.4). The tissue homogenate was centrifuged at 1000 g for 5 min, and the supernatant was collected for malondialdehyde (MDA) determination. Mitochondria and cytoplasmic fraction were isolated using Cell Mitochondria Isolation Kit (Beyotime Institute of Biotechnology, Jiangsu, China) and stored at −80°C until use.

Renal function and MDA determination

Blood urea nitrogen (BUN) and creatinine (Cr) levels were examined by using a Biochemistry Autoanalyzer (Olympus). The level of MDA was measured using the spectrophotometric method (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China).

Histopathology and Masson's trichrome staining

Renal tissues were fixed in 4% paraformaldehyde (pH 7.4), dehydrated in graded alcohol and then embedded in paraffin. Sections were cut into 4 -μm-thick and used for staining with hematoxylin & eosin (H&E) and Masson's trichrome. For H&E staining, sections were stained with Mayer's hematoxylin solution for 4 min followed by Eosin Y for 2–3 min. For Masson's trichrome staining, sections were placed in 3% mercuric chloride solution for 1–2 h and stained according to the manufacturer's protocol (Yuanye Biotechnology, Shanghai, China). Briefly, sections were placed consecutively in hematoxylin solution for 5 min, Biebrich Scarlet-acid fuchsine solution for 20 min,

aniline blue for 15 min and 0.2% acetic acid solution for 1 min.

TUNEL staining and immunohistochemistry

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL) staining was used to detect apoptotic cells (Roche Diagnostics). To perform immunohistochemical analyses of proliferating cell nuclear antigen (PCNA), IL-1β, TNF-α, Bcl-2, Bax and Vimentin, renal tissue slides were incubated with the primary antibodies against PCNA (1:100, Bioworld, Louis Park, USA), IL-1β (1:100, Bioworld, Louis Park, USA), TNF-α (1:50, Bioworld, Louis Park, USA), Bcl-2 (1:100, Bioworld), Bax (1:100, Bioworld) and Vimentin (1:50, Santa Cruz, Minneapolis, USA) followed by biotinylated sheep anti-rabbit or mouse IgG (Bostar, Wuhan, China). The signal was developed by staining with DAB, and the nuclei were slightly counterstained with hematoxylin. TUNEL positive and PCNA positive cells were counted respectively in 10 consecutive fields in the tissue sections.

Quantitative RT-PCR

Total RNA was isolated from rat kidney tissues using the Trizol reagent (Invitrogen) and first strand cDNA was synthesized from 5 μg of total RNA according to the manufacturer's instructions (MBI, USA). Quantitative RT-PCR (qRT-PCR) was used to detect the expression of β-actin, IL-1β, TNF-α, TGF-β1, Vimentin, Slug and E-cadherin genes. All samples were run in triplicate, and all reactions were repeated three times independently using the Rotor-Gene Real-time analyzer (Rotor-Gene 6000, CR, Australia).

Table 1 Primer sequences of target genes for rat

Genes	Primer sequence (5′–3′)	Amplicon size (bp)	Annealing temp. (°C)
IL-1β	For: AGCCACAATGAGTGACACTG Rev: CTTCCTTGTGCAAGTGTCTG	235	58
TNF-α	For: CCAACAAGGAGGAGAAGT Rev: TACCACCAGTTGGTTGTC	215	53
Slug	For: GTCCGAATGTGCATCTTCAG Rev: CCTGCCTTCATCTGACACTT	276	59
E-cadherin	For: AAGATCCTGAGCTGCCTCAC Rev: ACCTTGAGTGTGGCAATACG	274	60
Vimentin	For: CAGGAACAGCATGTCCAGAT Rev: GGCCAATAGTGTCCTGGTAG	343	59
TGF-β1	For: GCAACAACGCAATCTATGAC Rev: CCCTGTATTCCGTCTCCTT	301	59
β-actin	For: CACGAAACTACCTTCAACTCC Rev: CATACTCCTGCTTGCTGATC	265	56

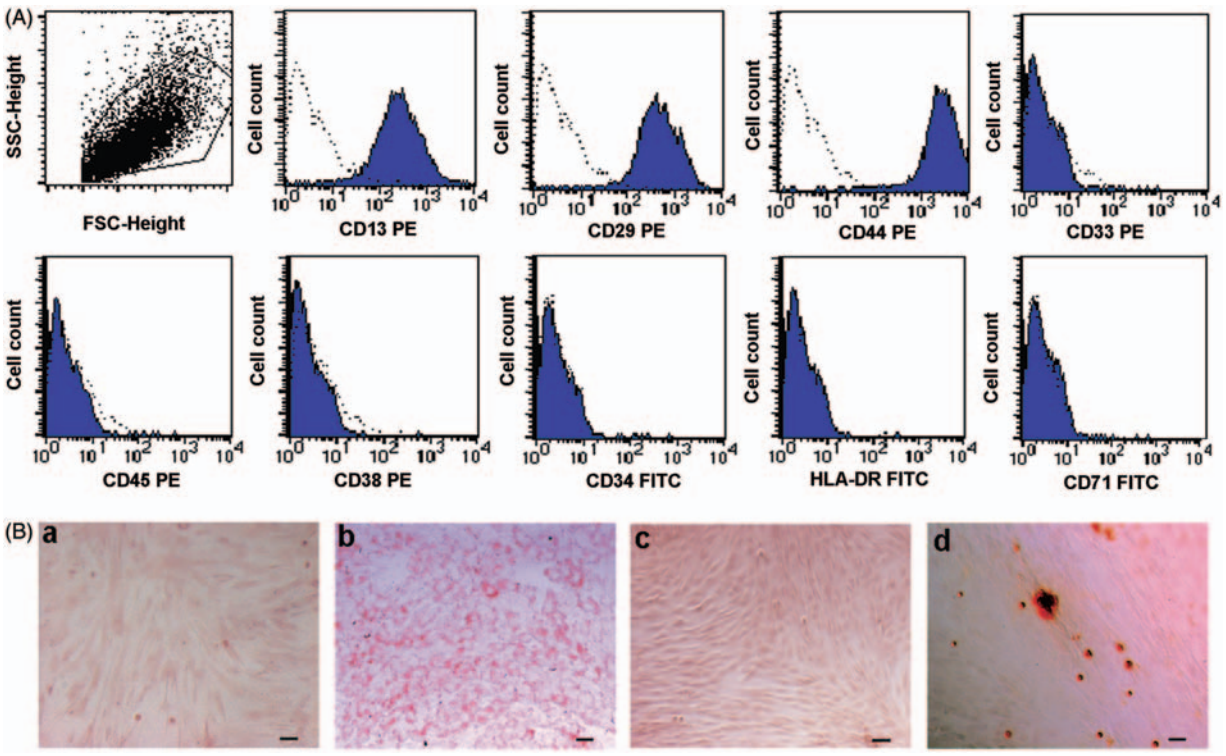

Figure 1 Characterization of hucMSCs. (A) Flow cytometric characterization of hucMSCs. Representative blue dot plots showing the positive expression of CD13, CD29 and CD44 in hucMSCs at passage 3. The respective isotype control is shown as a black dotted line. (B) Adipogenic and osteogenic differentiation of hucMSCs. (a, b) Oil-Red-O staining of control (a) and induced (b) hucMSCs for adipogenic differentiation. (c, d) Alizarin red staining of control (c) and induced (d) hucMSCs for osteogenic differentiation (magnification × 100; Scale bars: 50 μm). (A color version of this figure is available in the online journal)

β-actin was used as a loading control. The sequences of all primers are listed in Table 1.

Western blot

Western blotting assay was performed to analyse the expression of Bcl-2 (1:500, Bioworld), Bax (1:500, Bioworld), cytochrome C (1:500, Abcam, Cambridge, England), caspase-3 (1:500, Bioworld), E-cadherin (1:200, Santa Cruz), N-cadherin (1:200, Santa Cruz), Vimentin (1:200, Santa Cruz) and GAPDH (1:5000, Kangcheng Biotech, Shanghai, China) proteins. Samples were subjected to SDS-PAGE, transferred to the PVDF membrane and blotted with specific antibodies. Blots were developed and detected by enhanced chemiluminescence.

Statistical analysis

Data were shown as means ± SD. Statistical analysis was performed by ANOVA for multiple comparisons using Prism software (Graph Pad, San Diego, USA). $P < 0.05$ was considered significant.

Results

The characteristics of hucMSCs

The expression of cell surface antigens in hucMSCs was evaluated by flow cytometry. As shown in Figure 1(A), hucMSCs expressed CD13, CD29 and CD44, but were negative for CD33, CD45, CD38, CD34, CD71 and HLA-DR. HucMSCs cultured in adipogenic induction medium for two weeks showed numerous Oil-Red-O-positive intracellular lipid droplets (Figure 1B–b). In addition, part of the hucMSCs became Alizarin red staining positive after two weeks of incubation (Figure 1B–d). Control cells growing in the regular medium were negative (Figure 1B–a, c).

HucMSCs accelerate the recovery of renal function in cisplatin-induced AKI rats

HucMSCs were transplanted into cisplatin-induced AKI rats and HFL-1 cells were used as the cell control. BUN levels in cisplatin group markedly increased at three days and reached the peak at five days after cisplatin injection

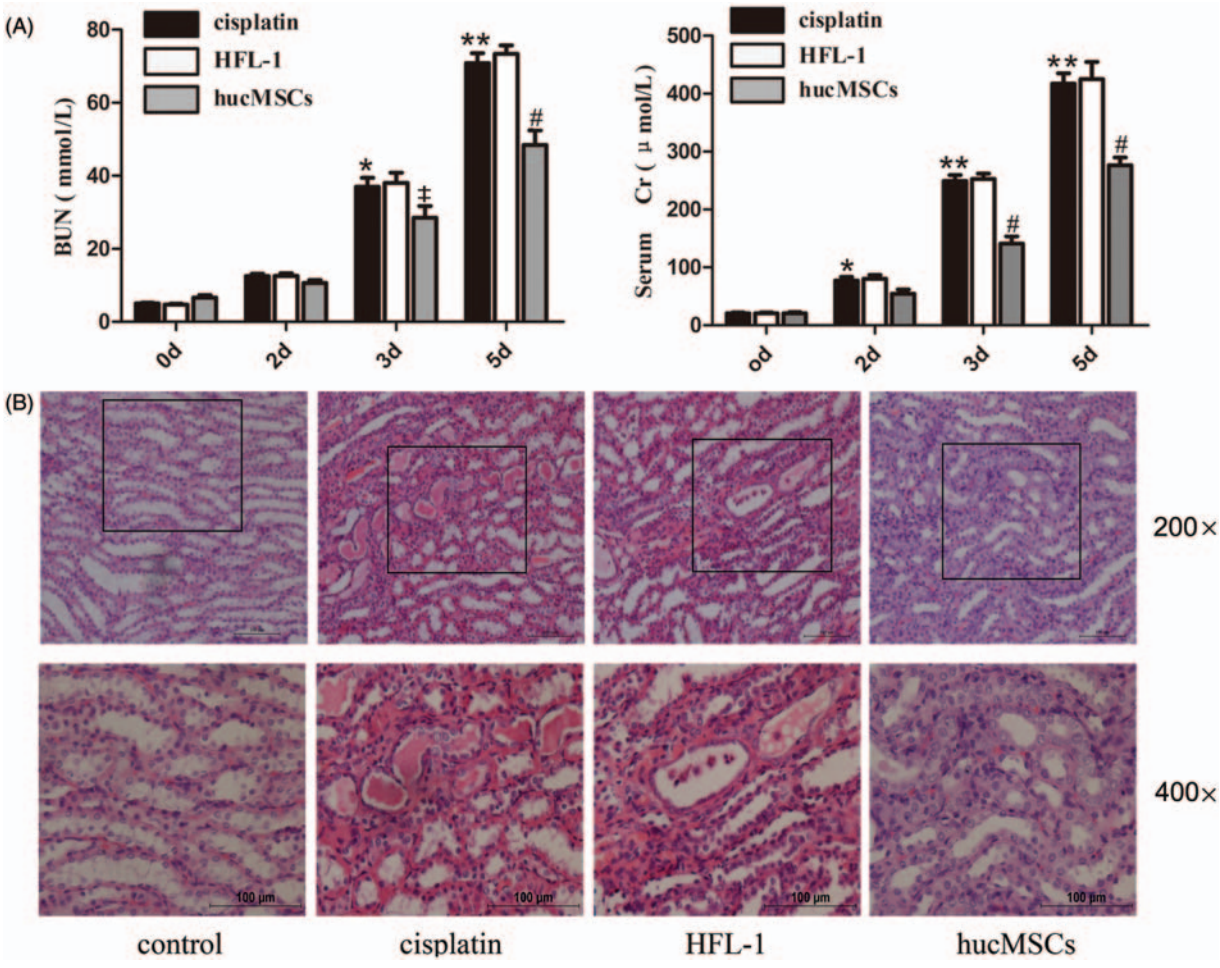

Figure 2 Effects of hucMSCs on rat renal function indexes and histology. (A) The serum Cr and BUN levels in cisplatin group (cisplatin-induced AKI rats receiving saline, $n = 6$), HFL-1 group (cisplatin-induced AKI rats receiving HFL-1 cells, $n = 6$) and hucMSCs group (cisplatin-induced AKI rats receiving hucMSCs, $n = 6$). $^{*}P < 0.05$ and $^{**}P < 0.01$ versus 0 day (before cisplatin treatment). $^{\ddagger}P < 0.05$ and $^{\#}P < 0.01$ versus cisplatin group. (B) Histological estimation of kidney sections at five days after cisplatin injection (magnification × 200 and ×400; Scale bars: 100 μm). Control: non-cisplatin injection rats; cisplatin: cisplatin group; HFL-1: HFL-1 group; hucMSCs: hucMSCs group. (A color version of this figure is available in the online journal)

(Figure 2(A), left panel, $P < 0.05$ at three days and $P < 0.01$ at five days). Serum Cr levels increased at two days and reached the highest at five days after cisplatin injection (Figure 2(A), right panel, $P < 0.05$ at two days and $P < 0.01$ at three and five days). The levels of Cr and BUN remarkably reduced at both three and five days after hucMSCs transplantation (BUN: $P < 0.05$ at three days and $P < 0.01$ at five days; Cr: $P < 0.01$ at three and five days). However, HFL-1 cells had minimal effects on the BUN and Cr levels.

We next investigated the effect of hucMSCs transplantation on the histology of AKI rats at five days after cisplatin injection. Compared with the cisplatin group, after hucMSCs infusion, the renal injury was significantly alleviated. As shown in Figure 2(B), fewer necrosic renal tubular epithelial cells, lower inflammatory cell accumulation and preservation of the integrity of the tubules. However, HFL-1 cell treatment did not lead to any recovery effects on AKI rat kidney tissues.

HucMSCs promote proliferation of and inhibit apoptosis in the renal tubular cells of cisplatin-induced AKI rats

Cisplatin-induced AKI is associated with tubular cell apoptosis and inflammation.[19] We performed a TUNEL assay to evaluate the anti-apoptotic effects of hucMSCs on AKI rats at five days after cisplatin injection. Compared with the control group, the number of TUNEL-positive cells significantly increased in renal tissue sections at five days in cisplatin group. HucMSCs treatment significantly decreased the number of TUNEL-positive cells (Figure 3(A)). The quantitative results showed that the number of TUNEL-positive cells in cisplatin group was more than control group but was reduced apparently in hucMSCs group (Figure 3(B), $P < 0.01$). The effect of hucMSCs on tubular cell proliferation was analysed by immunohistochemical staining of PCNA. The renal sections from the hucMSCs group showed a significant increase in the number of PCNA-positive cells compared to that in control and

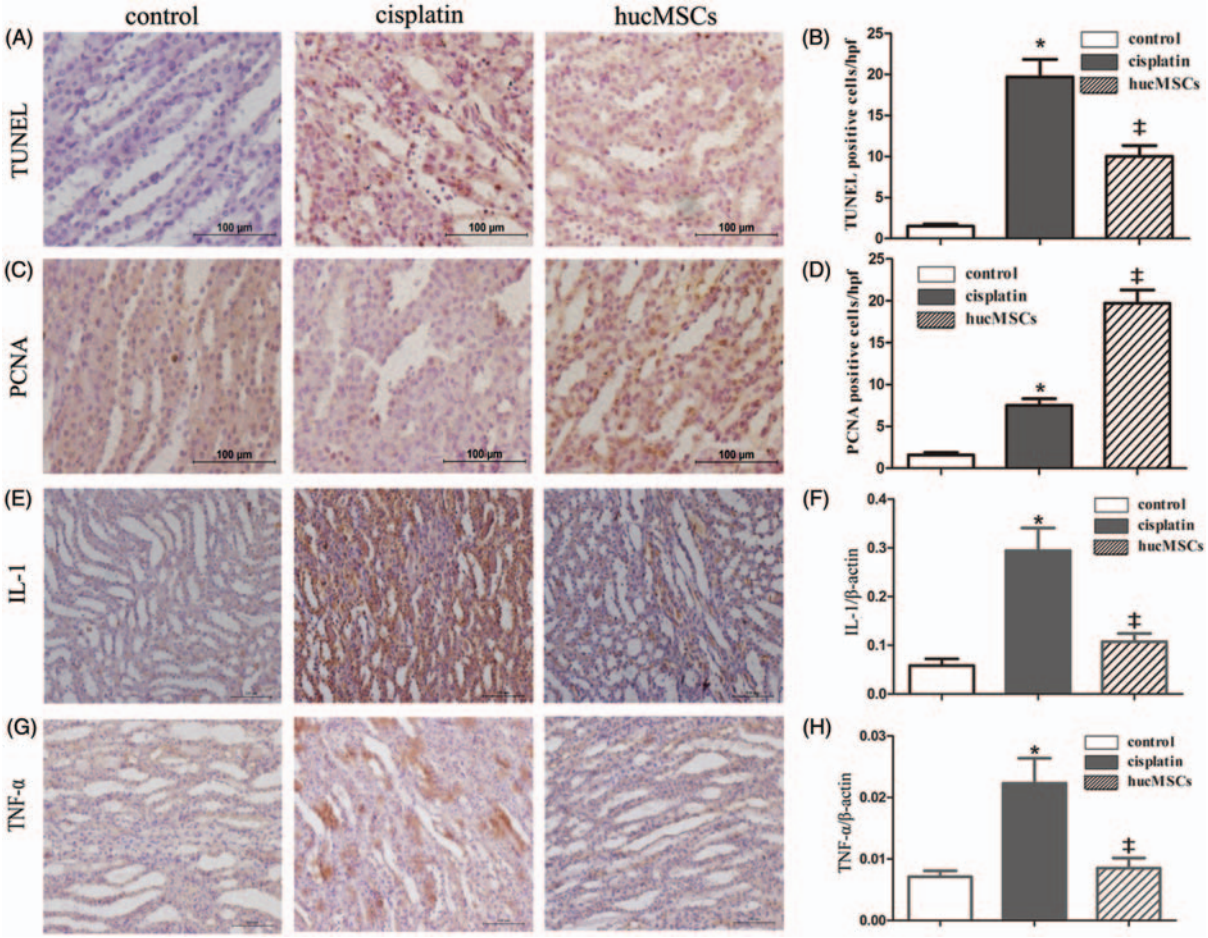

Figure 3 Changes of renal cell proliferation, apoptosis and expression of inflammatory cytokines after hucMSCs injection. (A, C) Representative images of terminal deoxynucleotidyl transferase-mediated dUTP nick end-labelling (TUNEL) and proliferating cell nuclear antigen (PCNA) staining in renal tissue sections at five days after cisplatin injection (magnification ×400; Scale bars: 100 μm). (B, D) Quantification of TUNEL and PCNA positive cells/high power field (hpf) in renal tissue sections from different groups. *$P < 0.01$ versus control group; #$P < 0.01$ versus cisplatin group. (E, G) Immunohistochemical analysis of IL-1β and TNF-α in renal tissues at day 5 after cisplatin injection (magnification ×200; Scale bars: 100 μm). (F, H) The expression of IL-1β and TNF-α in renal tissues at five days after cisplatin injection was determined by qRT-PCR. *$P < 0.05$ versus control; ‡$P < 0.05$ versus cisplatin group. (A color version of this figure is available in the online journal)

cisplatin groups (Figure 3(C)), which was also confirmed by quantitative analysis (Figure 3(D), $P < 0.01$).

HucMSCs alleviate inflammation in cisplatin-induced AKI rats

The expression of IL-1β and TNF-α, two major inflammation-related cytokines, was analysed by qRT-PCR and normalized to β-actin. Our results showed that the expression of IL-1β and TNF-α was significantly higher in cisplatin group at five days after cisplatin injection than that in the control group. The expression of both genes was decreased after hucMSCs transplantation (Figure 3(F) and (H), $P < 0.05$). At the same time, IL-1β and TNF-α protein expression were accordant with message level (Figure 3(E) and (G)).

HucMSCs protect renal cell mitochondria from dysfunction and inhibit the activation of mitochondrial apoptosis signaling

In order to determine the potential mechanisms for the anti-apoptotic effects of hucMSCs, the apoptosis-related molecules such as Bax, Bcl-2, cytochrome C and cleaved caspase-3 in renal tissues were analysed at five days after cisplatin injection. The expression of Bcl-2 and Bax proteins in renal tissues was determined by immunohistochemistry. Bax protein level significantly increased in the renal tissues of cisplatin group, while its expression was markedly decreased after hucMSCs transplantation (Figure 4(A)). We found that the expression of Bcl-2 in cisplatin group was lower than that in control group, but hucMSCs significantly increased its expression (Figure 4(B)). The expression

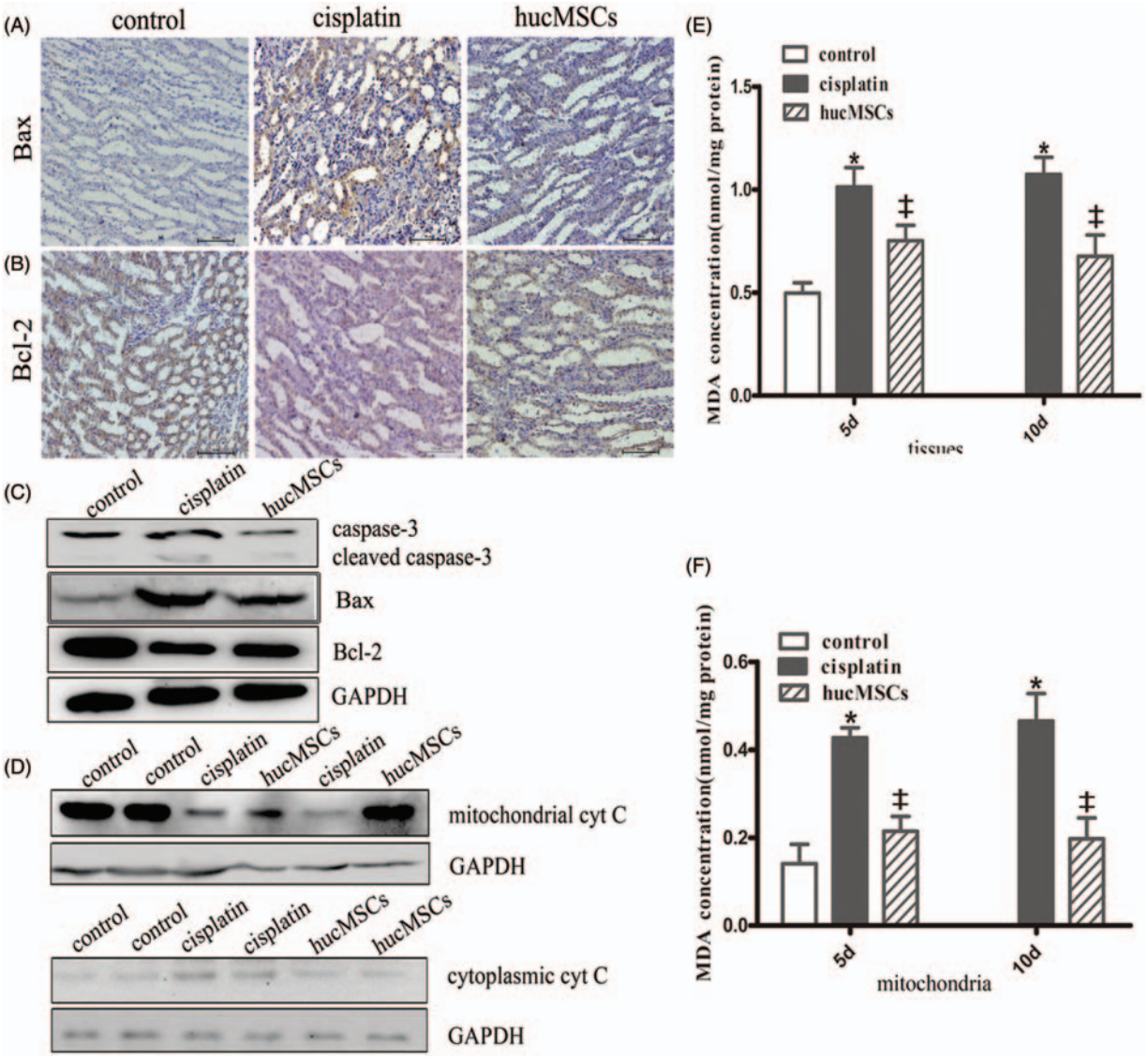

Figure 4 Bax and Bcl-2 expression, cytochrome C release and MDA content after hucMSCs administration. (A, B) Representative images of Bax and Bcl-2 staining in kidney tissue sections at five days after cisplatin (magnification ×200; Scale bars: 100 μm). (C) Western blotting analysis of Bax, Bcl-2 and caspase-3 in renal tissues at five days after cisplatin. (D) Western blotting analysis of cytochrome C in renal cell fractions at five days after cisplatin injection. (E, F) MDA contents in renal tissues and mitochondria at five and ten days after cisplatin injection. *$P < 0.05$ versus control group; ‡$P < 0.05$ versus cisplatin group. (A color version of this figure is available in the online journal)

of Bax and Bcl-2 was further confirmed by Western blotting (Figure 4(C)).

After hucMSC administration, the active form of caspase-3 (cleaved caspase-3) was markedly depressed (Figure 4(C)). We also detected the expression of cytochrome C in the renal tissues. The results showed that hucMSCs infusion could reduce the release of cytochrome C from mitochondria into the cytoplasm (Figure 4(D)). MDA levels reflect the degree of lipid peroxidation and cell injury. We then measured the concentration of MDA in the renal tissues (Figure 4(E)) and mitochondria (Figure 4(F)) in AKI rats at five and 10 days after cisplatin injection. The MDA levels significantly increased in the cisplatin group while decreased after hucMSCs administration at both time points ($P < 0.05$).

HucMSCs transplantation attenuates renal fibrosis

The renal function indexes of rats in cisplatin and hucMSCs groups had no obvious difference at 10 days after cisplatin injection (Figure 5(A) and (B)). We then want to know whether hucMSCs still have protective role at the late stage of renal injury. We performed histopathological analysis and confirmed that cisplatin-induced chronic renal injury after acute stage. Visibly renal interstitial fibrosis was first found at four weeks after cisplatin injection (data not shown) and the degree of fibrosis became more severe as time progressed. To evaluate the engraftment of hucMSCs in the renal parenchyma of AKI rats, hucMSCs were labelled with CM-Dil and infused into AKI rats. Four weeks after transplantation, the kidneys were removed and examined by using an In-Vivo Imaging system. As shown in

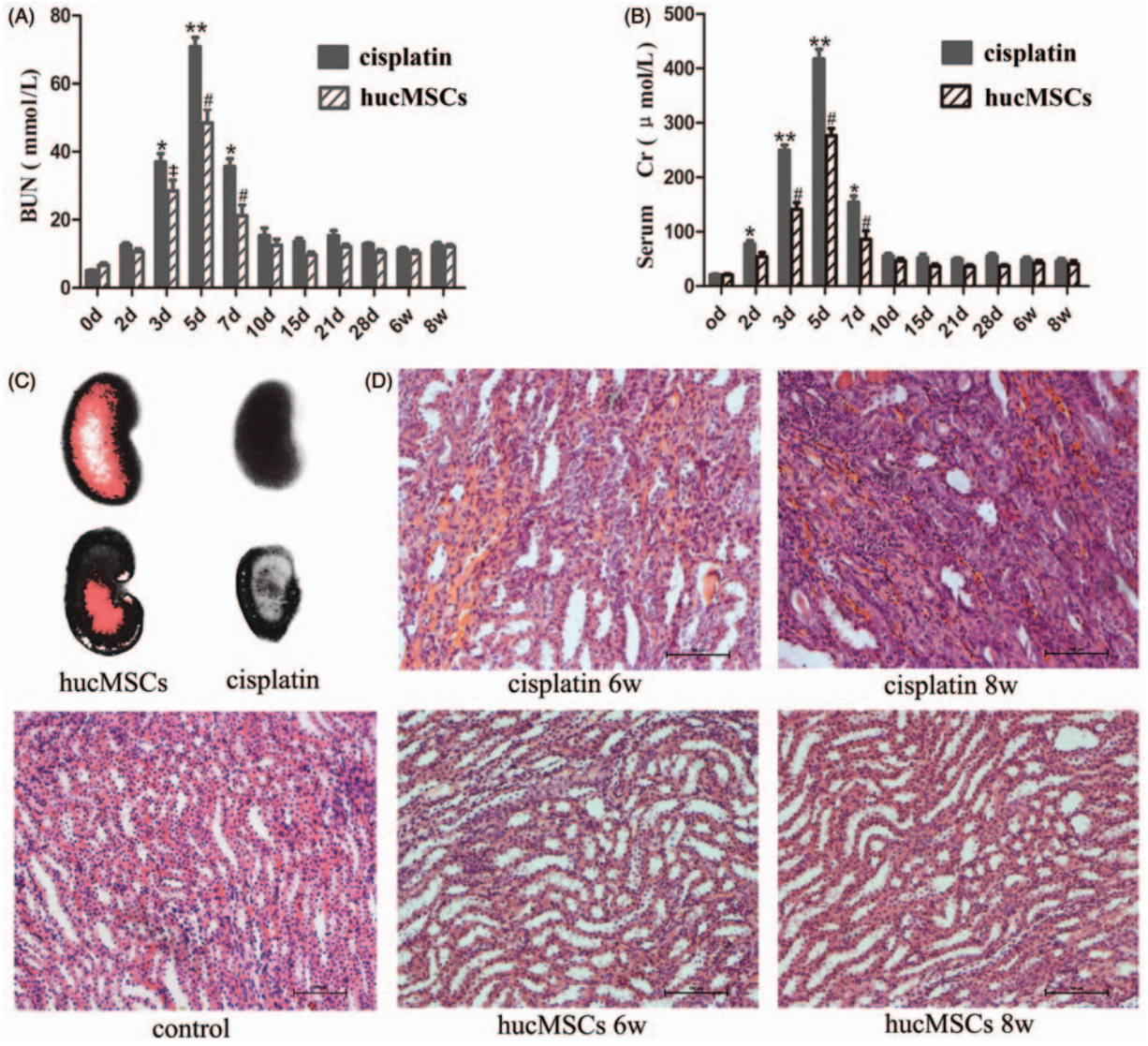

Figure 5 Effects of hucMSC transplantation on histology and renal function indexes at the late stage of renal injury. (A, B) Renal functional indexes, measured as BUN (A) and Cr levels (B) in the serum of rats in cisplatin ($n = 12$) and hucMSCs groups ($n = 12$) from day 0 to eight weeks. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control group; $^{\ddagger}P < 0.05$ and $^{\#}P < 0.01$ versus cisplatin group. (C) The intrarenal localization of CM-Dil labelled-hucMSCs. (Left) Kidneys of HucMSCs group at four weeks after cisplatin injection. (Right) Kidneys of cisplatin group at four weeks after cisplatin injection. Lower tracings are longitudinal section of half kidney. (D) Representative images of H&E staining in renal tissue sections of rats with interstitial fibrosis (magnification ×200; Scale bars: 100 μm). (A color version of this figure is available in the online journal)

Figure 5(C), the homing of labelled-hucMSCs to the renal lesion site was observed. Renal tubular epithelial cell loss, cast formation, renal tubular necrosis and the transformation of fibroblasts or myofibroblasts were also seen at six and eight weeks after cisplatin injection (Figure 5(D)). The structure of kidney tubules was restored, and fibroblasts or myofibroblasts in the renal tubular area were rarely observed in hucMSC-transplanted rats.

Collagen deposition, and the ratio of Bax to Bcl-2, which determines a cell's susceptibility to apoptosis, is associated with the development of renal interstitial fibrosis.[20] To determine the anti-fibrotic effect of hucMSCs, the cisplatin-induced renal injury rats with or without hucMSCs treatment were sacrificed at six and eight weeks after cisplatin injection and the expression of Bax and Bcl-2 proteins was determined by western blot. The results revealed that the expression of Bax was higher while Bcl-2 was lower in cisplatin group than that in the hucMSCs group (Figure 6(A)). The ratio of Bax/Bcl-2 decreased after hucMSCs transplantation (Figure 6(B)). In addition, we found the expression of TGF-β1 mRNA was higher in cisplatin group than that in the hucMSCs group at eight weeks by qRT-PCR (Figure 6(C)). Moreover, Masson's trichrome staining showed large areas of collagen deposit in cisplatin group while little collagen accumulation was observed in hucMSCs group (Figure 6(D)).

HucMSCs inhibit epithelial-mesenchymal transition in renal cells in vivo and in vitro

Epithelial–mesenchymal transition (EMT) has been considered as one of the major mechanisms responsible for renal fibrosis caused by chronic renal injury.[21,22] We next investigated whether hucMSCs attenuated renal fibrosis through the inhibition of EMT. The expression of several EMT-associated genes, such as Slug, Vimentin and E-cadherin, was detected by quantitative RT-PCR. As shown in Figure 7(A), the expression of Slug and Vimentin mRNA increased while that of E-cadherin decreased in cisplatin group ($P < 0.01$). However, the expression of Slug, Vimentin and E-cadherin was restored in hucMSCs-transplanted group ($P < 0.05$). The inhibitory effect of hucMSCs on Vimentin expression was also confirmed by immunohistochemistry (Figure 7(B)). The results of Western blot showed that the expression of N-cadherin and Vimentin proteins, two mesenchymal lineage markers, was significantly higher in cisplatin group, while it was markedly reduced in hucMSCs group at six and eight weeks after cisplatin injection. In contrary, the expression of E-cadherin, the marker of epithelial lineage, significantly increased in hucMSCs group compared to that in cisplatin group (Figure 7(C)). Furthermore, the effect of hucMSCs on EMT-associated gene expression in renal epithelial cells was confirmed *in vitro*. Cisplatin-treated NRK-52 E cells were

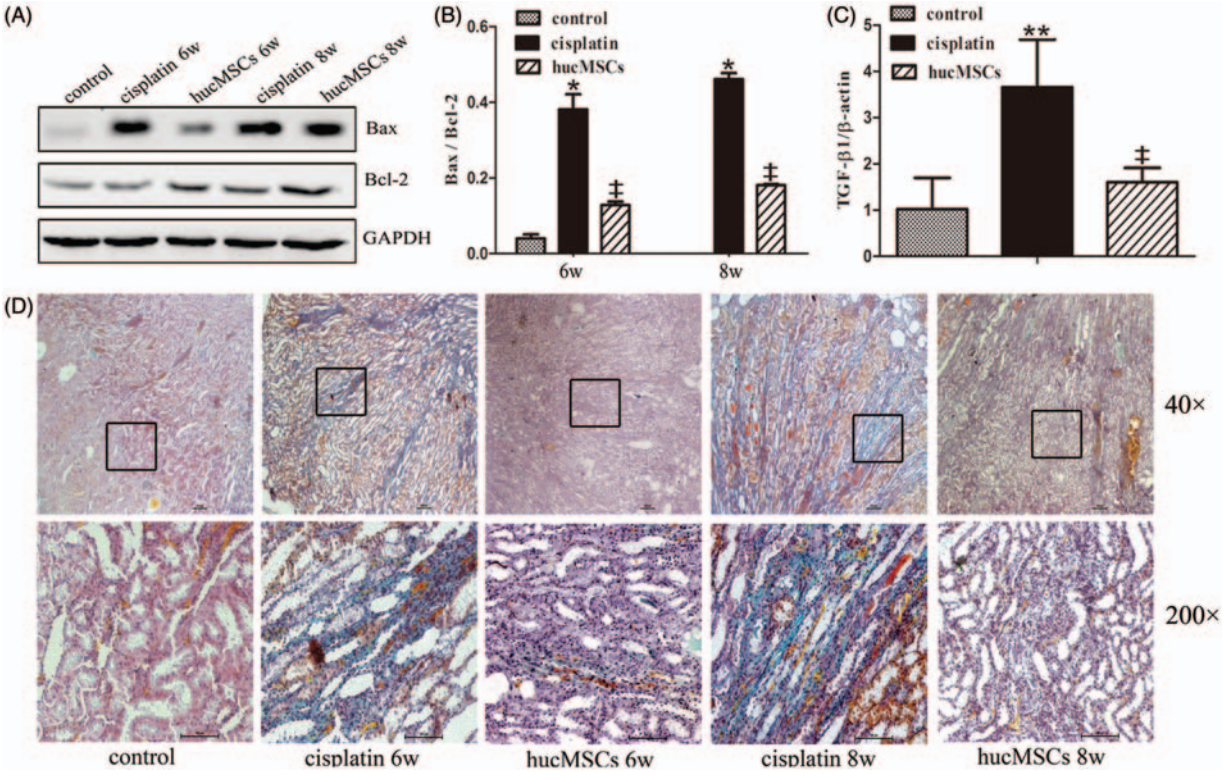

Figure 6 Inhibition of renal interstitial fibrosis by hucMSC injection. (A) Western blotting analyses of Bcl-2 and Bax expression. (B) Gray density analyses of Bax and Bcl-2. *$P < 0.05$ versus control; ‡$P < 0.05$ versus cisplatin group. (C) Quantitative RT-PCR analysis of TGF-β1 mRNA in kidneys with or without hucMSC treatment. The data were normalized to β-actin mRNA: **$P < 0.01$ versus control group; ‡$P < 0.05$ versus cisplatin group). (D) Masson's trichrome staining of renal tissue sections from rats with interstitial fibrosis (magnification ×40 and ×200; Scale bars: 100 μm). (A color version of this figure is available in the online journal)

co-cultured with hucMSCs in a transwell system for 48 h. The results of Western blotting assay showed that cisplatin reduced the expression of E-cadherin and increased the expression of N-cadherin and Vimentin, which was restored by co-culture with hucMSCs (Figure 7(D)).

Discussion

The clinical use of cisplatin is limited by its nephrotoxicity.[23] The mechanism for the side effect of cisplatin is linked to its induction of oxidative damage, reactive oxygen species (ROS) formation and apoptosis.[19,24–26] In addition, the products of lipid peroxidation (LPO) reactions induced by cisplatin may change the membrane fluidity and permeability, leading to changes in renal cell structure and function.

Our results demonstrate that hucMSCs accelerate the recovery of renal function by reducing the production of inflammatory cytokines and promoting the proliferation of renal tubular cells. Furthermore, hucMSCs transplantation decreases the Bax/Bcl-2 ratio and reduces the content of MDA in renal tissues, indicating that hucMSCs can protect renal cells from mitochondrial dysfunction and oxidative damage, and have the ability to inhibit the activation of mitochondrial apoptosis signaling pathway.

In the process of AKI, if the disorganized renal structure is not repaired, it would cause irreversible damage and generate chronic interstitial fibrosis. The enhanced apoptosis and decreased proliferation has been observed in a ureteral obstruction model.[27] In the present study, we observed large areas of collagen accumulation, which indicates renal interstitial fibrosis in rats at four weeks after cisplatin injection. The increased Bax/Bcl-2 ratio was also shown in this stage. Four weeks after hucMSCs transplantation, we could trace the transplanted cells in peri-tubular areas and found that hucMSCs transplantation alleviated the deposit of collagen. These lines of evidence suggest that hucMSCs could inhibit renal interstitial fibrosis. To our knowledge, this is the first report to reveal that hucMSC transplantation attenuates renal interstitial fibrosis in cisplatin-induced AKI rats.

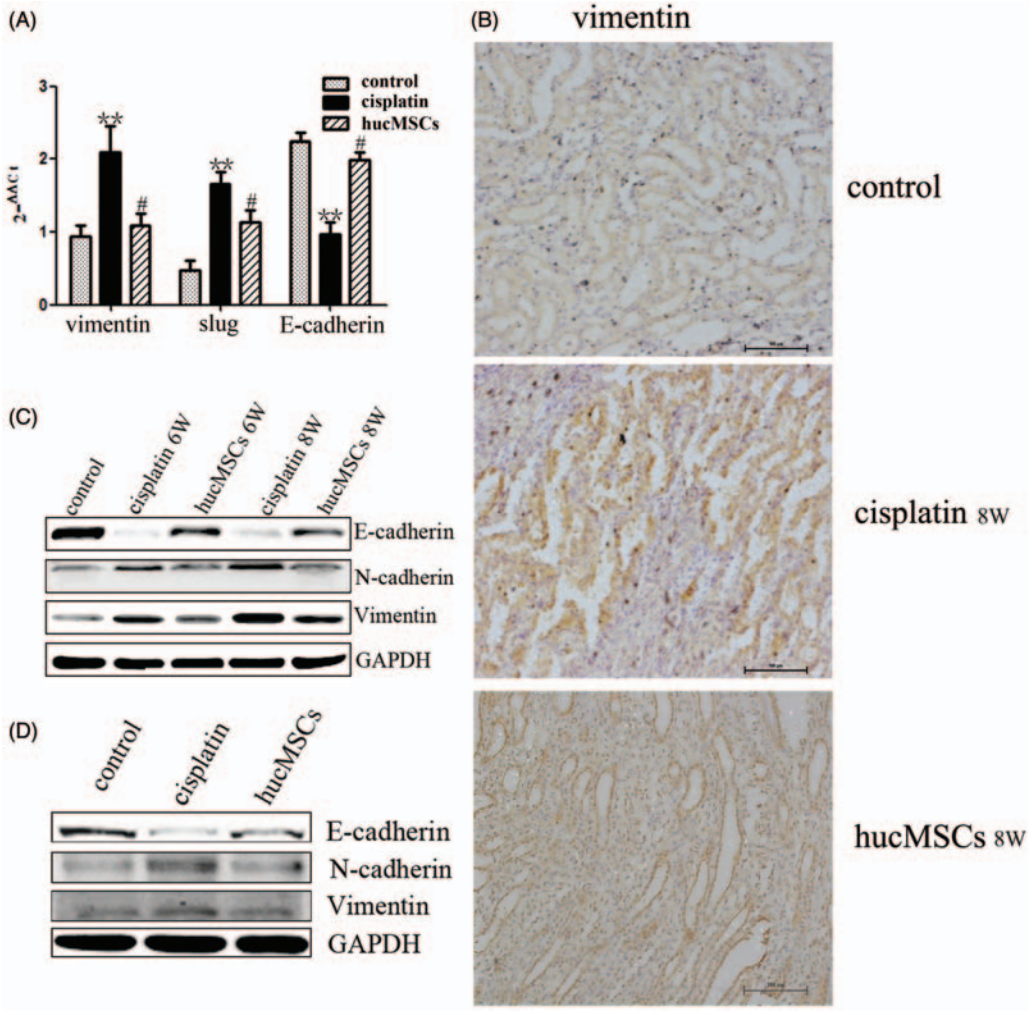

Figure 7 Suppression of EMT by hucMSC transplantation in the late chronic renal interstitial fibrosis stage. (A) Quantitative RT-PCR results of Vimentin, Slug and E-cadherin genes in renal tissues of rats at eight weeks after cisplatin injection (**$P < 0.01$ versus control group; $^{\#}P < 0.01$ versus cisplatin group). (B) Immunohistochemical analysis of Vimentin in renal tissues of rats at eight weeks after cisplatin injection (magnification ×200; Scale bars: 100 μm). (C) Western blotting analyses of E-cadherin, N-cadherin and Vimentin in renal tissues of rats at six and eight weeks after cisplatin injection. (D) Western blotting analyses of E-cadherin, N-cadherin and Vimentin in cisplatin-treated NRK-52E cells with or without hucMSCs treatment. (A color version of this figure is available in the online journal)

Renal fibrosis involving glomerulosclerosis and tubulointerstitial fibrosis is the principal process underlying the progression of CKD.[28] Previous studies have reported that one of the critical steps in the pathogenesis of tubulointerstitial fibrosis is EMT.[21,22] EMT is a process whereby epithelial cells lose their epithelial markers such as E-cadherin and acquire *de novo* mesenchymal markers, including N-cadherin and Vimentin, converting to motile mesenchymal cells.[29,30] EMT has been considered as one of the major reasons responsible for renal fibrosis caused by chronic renal injury.[31] To further elucidate the mechanisms by which hucMSCs inhibit renal interstitial fibrosis, we determined the expression of specific markers for EMT in hucMSCs-treated AKI rats. We found that the expression of Slug, N-cadherin and Vimentin significantly increased in the fibrotic kidneys. However, in the hucMSC-treated kidney tissues, Slug, N-cadherin and Vimentin levels reduced while the expression of E-cadherin increased. In consistent with our *in vivo* data, the inhibition of cisplatin-induced EMT by hucMSCs was also demonstrated by using renal tubular epithelial cells *in vitro*. These results suggest that hucMSCs prevent renal injury by suppressing EMT at the late stage in the cisplatin-induced cell and animal models.

In conclusion, our results demonstrate that hucMSCs attenuate cisplatin-induced acute and chronic renal injury through preventing mitochondria dysfunction, inhibiting the activation of mitochondrial signaling pathway and suppressing the development of EMT. Our findings suggest that hucMSC transplantation is a useful approach for the therapy of cisplatin-induced AKI and the subsequent renal interstitial fibrosis.

Author contributions: XP and HX designed the experiments, performed most of the experiments, analysed the data and wrote the paper; YZ, BW and SG contributed to provide human umbilical cord mesenchymal stem cells and performed the transplantation experiment; MW performed the flow cytometry work and analysed the data; YY, XZ and WZ contributed to analyse the data and write the paper; HQ and WX designed the experiments, analysed the data, wrote the paper and oversaw the research project.

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