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Original research paper

Therapeutic effect of umbilical cord mesenchymal stem cells on renal ischemia-reperfusion injury

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ABSTRACT

Acute kidney injury (AKI) is a growing global health issue with no effective treatments. This study evaluates the therapeutic effects of umbilical cord mesenchymal stem cells (UC-MSCs) on AKI caused by ischemia-reperfusion injury (IRI) in mice. Thirty mice were divided into a sham group, an IRI group, and an MSC-treated group. Renal function was assessed, and histological analysis, immunofluorescence, and real-time PCR were used to evaluate renal damage, inflammatory cell presence, and cytokine expression (TNF-α, IL-6, IL-10). Results showed that MSC treatment reduced renal damage, decreased pro-inflammatory cytokines (TNF-α, IL-6), increased anti-inflammatory IL-10, and promoted kidney repair by homing to injury sites. Thus, umbilical cord MSCs may mitigate AKI by reducing inflammation and enhancing renal repair.

Keywords: umbilical cord mesenchymal stem cells, inflammation, renal ischemia-reperfusion, acute kidney injury

INTRODUCTION

Acute kidney injury (AKI) is a clinical syndrome characterized by decreased glomerular filtration rate and urine output, posing a significant threat to patients' lives. Approximately 2 million people die from AKI worldwide each year (1). Causes of AKI include: ischemia-reperfusion (IR) (2), drug-induced (3), sepsis (4), *etc*. The kidneys, due to their high perfusion characteristics, are extremely sensitive to fluctuations in blood flow, making ischemia-reperfusion injury a primary cause of damage (5). Despite the latest advances in dialysis, fluid therapy, and transplantation, there has been a lack of effective drugs for the treatment of AKI (6, 7). The key to recovering renal function after AKI is the repair of the tubular basement

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membrane through the proliferation and differentiation of tubular epithelial cells. Compared with the above treatment options, stem cell therapy effectively modulates immune function and shows good potential repairment function (8, 9).

Mesenchymal stem cells (MSCs), pluripotent stem cells with strong proliferation and differentiation potential, attract significant attention as immunomodulatory treatments (10). Research has demonstrated that MSCs significantly improve renal histology and function in experimental models. For instance, Iseri *et al.* (11) found that bone marrow-derived MSCs conditioned medium can alleviate experimental anti-glomerular basement membrane glomerulonephritis through M2 macrophage-mediated anti-inflammatory effects. Furthermore, urine-derived MSCs were effective in treating cisplatin-induced AKI in rats by inhibiting apoptosis (12). Based on these findings, transplantation of MSCs appears to be a promising method for treating AKI. MSCs derived from a variety of tissues, including fat, muscle, umbilical cord blood, peripheral blood, liver, placenta, skin, amniotic fluid, breast milk, synovium, tooth roots, *etc.* (13) The most common bone marrow MSCs are obtained from human bone marrow, however, bone marrow aspiration is a highly invasive procedure (14). Embryonic MSCs are derived from the fetus, which has considerable ethical issues in human applications, so it is difficult to obtain these cells.

Umbilical cord mesenchymal stem cells (UC-MSCs) can be isolated from discarded umbilical cords, and the extraction process is relatively simple and has the advantages of high cell quality, low immunogenicity, and superior differentiation potential (15). For example, in a comparative study of UC-MSCs with dental pulp and menstrual blood mesenchymal stem cells, UC-MSCs had a higher population doubling rate and stronger cell proliferation capacity with low immunogenicity and high differentiation potential (16). Therefore, compared with fetal MSCs and bone marrow MSCs, UC-MSCs are a safe and available source of large amounts of stem cells with great potential for treating diseases. Currently, MSC therapy is mainly focused on ischemia-reperfusion, chemotherapy, and renal transplantation-induced AKI (17), of which research on the use of UC-MSCs for treating AKI is limited (18, 19). However, previous studies lacked detailed inflammatory markers and visualization of the pathological treatment process to investigate the role of UC-MSCs in treating IR-induced AKI. Therefore, this study focused on exploring changes in inflammatory cells and cytokines in renal tissue of mice during UC-MSC-mediated treatment of IR-induced AKI and visualizing the homing of UC-MSCs to the site of kidney injury and possible repairment process.

In this study, an IR-induced AKI model was established in mice, and treated by tail vein injection of UC-MSCs. The therapeutic impact of UC-MSCs on renal function was assessed through both functional and histological evaluations. Additionally, the study analyzed the modulation of pro-inflammatory and anti-inflammatory factors in the kidneys to elucidate the immunomodulatory effects of UC-MSCs in treating acute kidney disease. Our findings provide strong evidence that UC-MSCs show potential for treating IR-induced AKI in mice, supporting future clinical application.

EXPERIMENTAL

Animals

Thirty 8-week-old BALB/C male mice were purchased from Guangzhou Maisi Biotechnology Co., Ltd. and housed in the animal facility of Shenzhen Zhongjia Biomedical

Technology Co., Ltd. The mice were maintained at a temperature of 25 ± 3 °C with a 12-hour light/dark cycle and were provided with food and water ad libitum. All experimental procedures were conducted in compliance with ethical principles for the welfare of experimental animals and adhered to the relevant regulations outlined in the "Administrative Measures for Laboratory Animal Permits" and "Regulations on the Administration of Laboratory Animals". This experiment received approval from the Animal Ethics Committee, with the ethical approval number SYSU-IACUC-2024-002238.

Reagents and instruments

Dil cell fluorescent dye, urine protein detection kit, 4 % paraformaldehyde, and hematoxylin-eosin staining kit were purchased from Solarbio (China). The urea nitrogen detection kit and creatinine detection kit were obtained from Enzyme-Linked Biology (China). TRIzol reagent was sourced from Thermo Fisher Scientific (USA). F4/80 and GR-1 immunofluorescent antibodies were provided by Cell Signaling Technology (USA). TNF-α, IL-6, IL-10, and GAPDH primers were acquired from Sangon Biotech (China). EasyScript® First-Strand cDNA Synthesis SuperMix and TransStart® TopGreen qPCR SuperMix were supplied by TransGen Biotech (China). Anti-fluorescence quencher and DAPI were purchased from MedChemExpress (USA).

The A1HD25 confocal microscope was obtained from Nikon (Japan). The Cryostar NX70 Cryoslicer, NanoDrop Microvolume Spectrophotometer, Multiskan SkyHigh Microplate Spectrophotometer, and PCR machine were provided by Thermo Fisher Scientific (USA). The slide scanner was purchased from Shengqiang Technology (China).

Cell culture

Human umbilical cord mesenchymal stem cells (UC-MSCs) were obtained from Pricella Biotechnology Co., Ltd. (China). UC-MSCs cells were cultured in MesenPRO RS™ medium (Thermo Fisher Scientific) and incubated in a thermostatic incubator (37 °C with 5 % CO_2). Once about 80 % confluence, cells were harvested and resuspended in physiological saline to prepare a cell suspension with a concentration of 1.5×10^7 cells per mL, ready for injection.

Mesenchymal stem cell staining

Dil fluorescent dye was used to label UC-MSCs cells. Briefly, 10 mL of 10 μg mL^{-1} Dil red cell membrane fluorescent dye solution was prepared. Subsequently, 10^7 cells were added to the dye solution and incubated for 10 minutes, with gentle mixing every 5 minutes. After staining, the cells were centrifuged at 500 *g* for 5 minutes, the supernatant was discarded, and the cells were resuspended in normal saline. The cells were then centrifuged again for washing and resuspended in normal saline to prepare a cell suspension with a concentration of 1.5×10^7 cells per mL.

Establishment of the AKI mouse model

Thirty mice were randomly divided into three groups: the sham operation group (Sham), the renal ischemia-reperfusion group (IRI), and the umbilical cord stem cell treat-

ment group (IRI + MSCs), with 10 mice in each group. Based on their actual body weight, the mice were anesthetized with an intraperitoneal injection of 1 % sodium pentobarbital solution at a dose of 50 mg kg^{-1}. After successful anesthesia, the mice were positioned on a 37 °C constant temperature operating table. The skin was prepared for surgery 2 cm below the neck and 2–3 cm beside the spine, using tweezers for hair removal and alcohol--soaked cotton balls for disinfection. The mice were placed in a lateral position, and their limbs were secured with tape. A 0.5 cm incision was made along the spine and 0.5 cm below the ribs on the left side (approximately 0.8 cm). The incision was carefully placed to avoid nerves and blood vessels, the muscle layer was incised, and the kidney was gently exposed using a blunt glass needle. The renal blood vessels were separated by gently squeezing with forceps.

In the renal ischemia-reperfusion group (IRI) and the umbilical cord stem cell treatment group (IRI + MSCs), ischemia was induced by clamping the left renal artery with an artery clamp for 45 minutes (20). In the MSCs group, 200 μL of a suspension containing 3 × 10^6 UC-MSCs were injected into the tail vein on the 1st, 3rd, and 5th days post-surgery. The sham group received an equal volume of saline *via* tail vein injection on the 1st, 3rd, and 5th day post-surgery. The sham group underwent the surgical incision and suturing procedures but without any further treatment. All mice were euthanized seven days later, and their blood, urine, kidneys, and other tissues were collected for subsequent experiments and related measurements.

Serum creatinine and urea nitrogen testing

After the ischemia-reperfusion surgery, 5 mice from each group were selected and euthanized on the seventh day. Blood was collected from the eyeball and allowed to clot at room temperature for 30 minutes. The blood samples were then centrifuged at 4 °C at 1000 *g* for 15 minutes, and the supernatant was carefully collected. The levels of creatinine and urea nitrogen in the supernatant were measured using a kit, following the manufacturer's instructions (21). In short, quantitative analysis of the sample includes reagent standardization, sample addition, incubation, washing, color development, stopping, and measurement using a microplate spectrophotometer.

Urine protein test

On the seventh day after the ischemia-reperfusion surgery, the mice in each group were euthanized, and urine samples were collected to measure urine protein levels. The procedure was as follows: Protein precipitant and Ponceau red dye were added to the urine samples. The mixture was then centrifuged to obtain the protein-dye complex, which was dissolved in an alkaline solution. The absorbance of the resulting solution was measured at 560 nm using a microplate spectrophotometer to calculate the protein content in the sample (22).

Renal tissue immunofluorescence staining

To validate the homing ability of UC-MSCs, after the ischemia-reperfusion model was established, an additional 5 mice were selected from each group. On the first day post-surgery, 200 μL of red fluorescent-labeled UC-MSCs (3 × 10^6 cells) were injected *via* the tail

vein. The mice were euthanized on the 3rd day, and their kidney upper kidney tissues were harvested and fixed in 4 % neutral buffered paraformaldehyde for 10 hours. Following fixation, the tissues were transferred to a 4 °C solution of 40 % sucrose for dehydration. Once dehydration was complete, the tissues were embedded in an optimal cutting temperature compound and then frozen in a –20 °C cryotome. After the optimal cutting temperature compound was fully frozen, 6 μm thick sections were cut for immunofluorescence staining.

The sections were first fixed by adding 200 μL of 4 % paraformaldehyde for 15 minutes. They were then washed three times with PBS-T (PBS containing 0.1 % Tween 20) for 3 minutes each time. The sections were blocked with 10 % goat serum for 30 minutes. After discarding the blocking solution, the sections were incubated with primary antibodies Gr-1 (1:200 dilution) or F4/80 (1:200 dilution) at room temperature for 60 minutes. Following incubation, the sections were washed three times with PBS-T and then stained with 10 % DAPI. Finally, the sections were sealed with an anti-fluorescence quencher to preserve the fluorescent signal. Fluorescence images were captured by confocal laser scanning microscopy (Nikon, Japan).

Renal histology

The middle and lower kidney tissue from the mice in each group were partially used for HE (Hematoxylin and Eosin) staining (23). The main steps were as follows: The kidney tissue samples were first fixed in 4 % neutral buffered paraformaldehyde for 24 hours. After fixation, the samples were processed using a tissue dehydrator and then embedded in paraffin using a tissue embedding machine. The paraffin-embedded tissues were sliced into 4 μm thick sections using a paraffin microtome and placed on slides. The sections were stained with hematoxylin for 4 minutes and 30 seconds, then rinsed with tap water for 1 minute. Following the rinse, the sections were differentiated with hydrochloric acid for 10 seconds and then neutralized (blued) with ammonia for 10 seconds. The sections were subsequently stained with eosin for 2 minutes. After staining, the sections were dehydrated through a series of graded alcohol solutions and then cleared with xylene. Finally, the slides were sealed with neutral resin and scanned using a slide scanner for further analysis.

Fluorescence real-time quantitative detection

The tissues at both ends of the kidney from the mice in each group were used for real-time fluorescence quantitative PCR (qPCR) analysis (24). The main steps were as follows: An appropriate amount of kidney tissue was placed in a 1.5 mL centrifuge tube containing 1 mL of Trizol reagent. The tissue was then homogenized using a high-speed tissue grinder. Following homogenization, RNA was extracted using chloroform and isopropanol. For RNA reverse transcription, the EasyScript® First-Strand cDNA Synthesis SuperMix Kit was used. The reverse transcription reaction system and program settings were followed according to the kit's manual. The resulting cDNA was then subjected to real-time fluorescence quantitative PCR using the TransStart® TopGreen qPCR SuperMix Kit. qPCR was performed using an Applied Biosystems™ QuantStudio™ 3 Real-Time PCR System following the manufacturer's instructions to quantify the relative expression levels of the target genes *via* the $2^{(-\Delta\Delta Ct)}$ method.

Statistical analysis

GraphPad Prism 8.0 statistical software was used for statistical analysis. The levels of creatinine, urea nitrogen, urine protein, and gene expression of TNF-α, IL-6, and IL-10 in each group of mice were in accordance with normal distribution (supplementary file), expressed as the mean ± standard deviation (SD). The mean values of samples among multiple groups were compared by one-way analysis of variance, and the SNK-q test was used for pairwise comparisons between groups. $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

UC-MSCs alleviate renal ischemia reperfusion-induced AKI

The kidneys of the mice in each group were excised and subjected to histological examination. The results showed that in the IRI group, the cross-section of the left kidney exhibited a reddish appearance in the renal medulla (Fig. 1a), likely due to tissue color changes caused by recovery after hypoxia. During ischemia, the kidney tissue experiences a sudden drop in energy supply due to hypoxia and metabolic disturbances, resulting in a darkened color. Upon reperfusion, as blood flow and oxygen are restored, the tissue color gradually becomes a lighter red (25, 26). Pathological sections of the kidneys from each group were also examined. The sham group showed orderly arranged renal tubules with intact lumens and walls, and no significant pathological damage was observed (Fig. 1b). In contrast, the IRI group exhibited enlarged renal tubule lumens, thinned walls, and substantial deposition of abnormal protein-like substances. Additionally, some nuclei showed signs of dissolution or absorption (Fig. 1c). Tubular vacuolation refers to the formation of vacuole-like structures within the epithelial cells of the renal tubules, which typically occurs due to cellular damage, leading to fluid accumulation, abnormal swelling of organelles, and thinning of the cell wall. The kidneys are crucial in protein metabolism within the body, and when kidney damage occurs, proteins and their metabolites may not be properly metabolized and excreted, leading to their accumulation in the kidneys. Consequently, the enlargement of the tubular lumen, thinning of the walls, and the deposition of abnormal protein-like substances are often indicative markers of kidney injury (27). This demonstrates that the IRI group's kidneys suffered damage under ischemia--reperfusion, successfully establishing an AKI model. In the MSCs group, where mice were treated with UC-MSCs, only slight enlargement of the tubular lumens was observed, with no significant thinning of the walls, showing marked improvement compared to the IRI group. This may be because UC-MSCs have the ability to home to the site of kidney injury and differentiate into renal intrinsic cells, such as tubular epithelial cells, thereby facilitating the repair of damaged tissue (28), which indicates that UC-MSCs can mitigate the structural damage to the kidneys caused by ischemia-reperfusion in mice.

UC-MSCs can restore renal function after ischemia-reperfusion injury

Blood urea nitrogen (BUN) and creatinine (CR) are crucial indicators for assessing kidney function, reflecting the extent of glomerular filtration impairment. Under normal

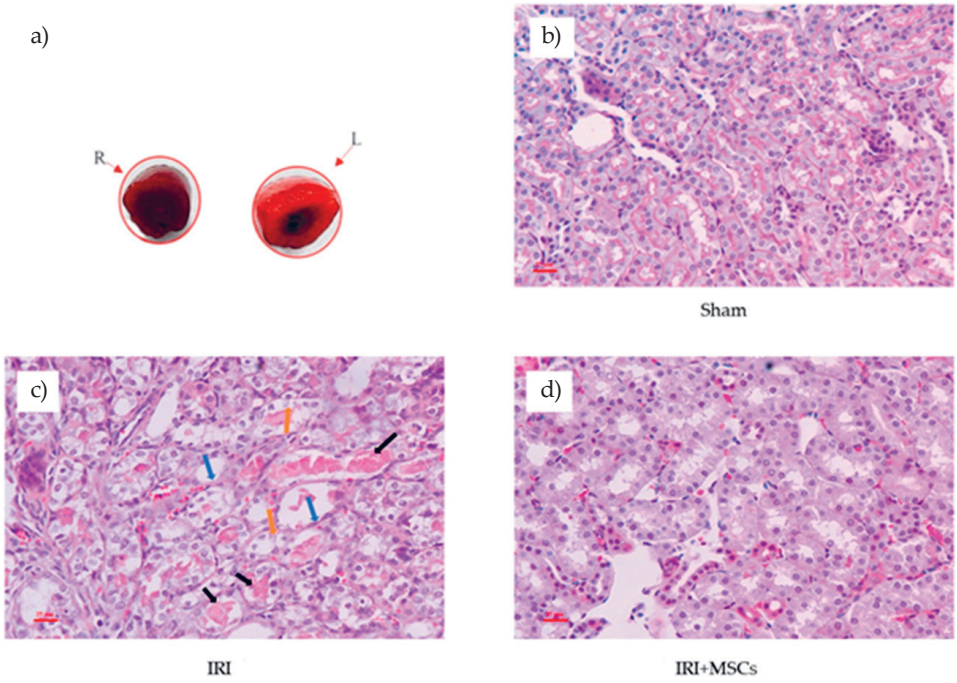

Fig. 1. The effects of UC-MSCs on renal IRI: a) gross characteristics of the cross-sections of the left and right kidneys in mice with acute kidney injury (R: right kidney; L: left kidney); b) to d) hematoxylin and eosin (HE) staining of the mice left kidneys of each group (200×); b) HE section of the normal kidney from the Sham group; c) HE section of the kidney from the IRI group, showing enlarged renal tubule lumens, thinned renal tubule walls, and a large accumulation of abnormal protein-like substances; d) HE section of the kidney after MSC intervention, showing significant improvement in both the renal tubule lumen and renal tubules. Black arrows – proteinaceous material deposition, blue arrows – thinning of renal tubular walls, orange arrows – nuclear dissolution and absorption, Sham – sham operation group, IRI – the renal ischemia-reperfusion group; IRI+MSCs – the umbilical cord stem cells treatment group.

conditions, the levels of BUN and CR in the body remain stable. However, in cases of renal insufficiency, BUN and CR levels significantly increase, indicating a severe impact on the filtration function, leading to the accumulation of metabolic waste and kidney function impairment (29). We evaluated various kidney function indicators in the different groups of mice using assay kits, as shown in Fig. 2. Compared to the Sham group, the IRI group exhibited a significant increase in BUN and CR levels ($p < 0.01$, $p < 0.001$), indicating that ischemia-reperfusion caused kidney function damage. After intervention with UC-MSCs, the MSCs group showed significantly lower levels of BUN and CR compared to the IRI group ($p < 0.05$, $p < 0.05$). In contrast to the Sham group, BUN levels showed no significant change, whereas CR levels remained significantly elevated ($p < 0.01$), indicating that UC-MSCs did not fully restore renal function but were able to mitigate the kidney function damage caused by ischemia-reperfusion.

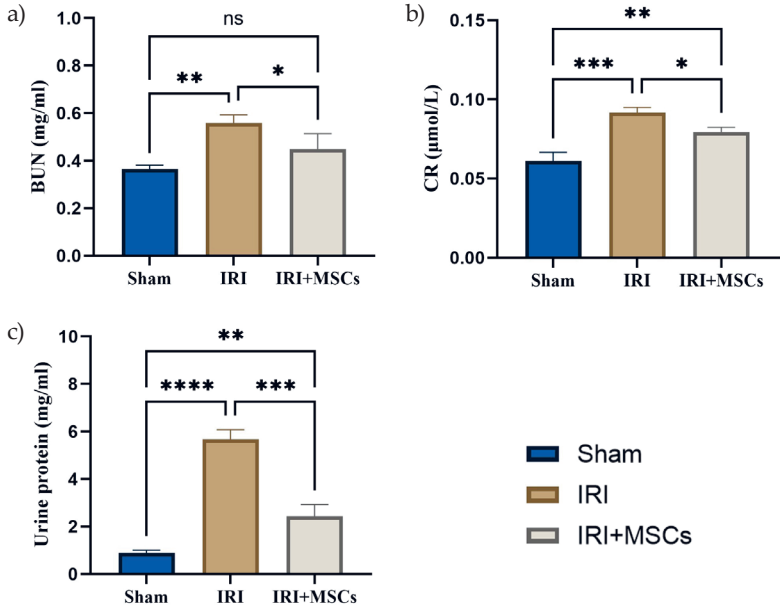

Fig. 2. Effects of UC-MSCs on kidney function: a) BUN levels; b) CR levels; c) urinary protein content. Mean ± SD, $n = 5$. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Sham – sham operation, IRI – ischemia-reperfusion, IRI+MSCs – MSCs treatment.

Tubular cell apoptosis and tubular dilation are important characteristics of IRI (30, 31). Plasma passing through the kidneys forms primary urine, and renal tubular epithelial cells reabsorb proteins from the primary urine. If the kidneys are damaged, the filtered proteins cannot be fully reabsorbed by the renal tubular epithelial cells, resulting in proteinuria, which is a common clinical sign of kidney disease and serves as an auxiliary diagnostic indicator. In summary, abnormalities in the glomerular filtration barrier and/or the structure and function of the renal tubules are the main causes of proteinuria. The proteinuria results for the different groups of mice are shown in Fig. 2c. Compared to the Sham group, the urine protein levels in the IRI group and MSCs group were significantly increased ($p < 0.0001$, $p < 0.01$). After MSC treatment, urinary protein levels significantly decreased ($p < 0.001$), indicating that intravenous infusion of UC-MSCs can effectively alleviate AKI caused by ischemia-reperfusion. This process may be related to the involvement of UC-MSCs in the repair of the damaged kidney.

UC-MSCs reduce macrophage and neutrophil infiltration after ischemia-reperfusion injury

In the renal ischemia-reperfusion model, apoptosis and necrosis of renal tubular epithelial cells are considered the primary pathological changes, closely linked to the development of inflammation (32). To further explore whether UC-MSCs can reduce kidney damage by mitigating the inflammatory response, we examined the expression of

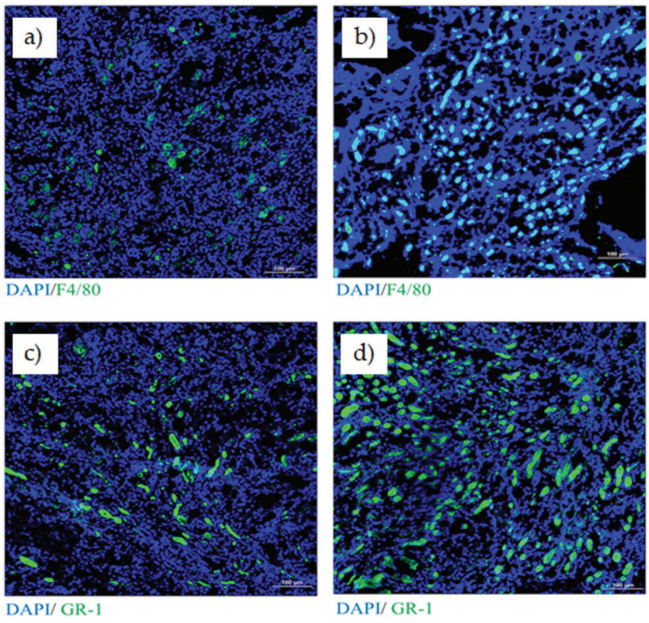

Fig. 3. Macrophage infiltration (F4/80, green expression) in kidneys from: a) IRI-MSCs group; b) IRI group. Neutrophil infiltration (GR-1, green expression) in kidneys from: c) IRI-MSCs group; d) IRI group. IRI – ischemia-reperfusion, IRI+MSCs – MSCs treatment.

macrophages (F4/80) and neutrophils (Gr-1). F4/80, a member of the adhesion G protein--coupled receptor (ADGR) subfamily in the G protein-coupled receptor 2 family, is commonly used as a specific marker for mature mouse macrophages (33). Gr-1 is a protein with specific functions in the immune system, playing a key role in granulocyte function and activation (34). Laser confocal microscopy imaging showed that the expression of F4/80 antibody in the MSCs group (Fig. 3a) was significantly lower than in the IRI group (Fig. 3b). Similarly, the expression of Gr-1 antibody in the MSCs group (Fig. 3c) was markedly lower than in the IRI group (Fig. 3d). In IRI-induced kidney injury, inflammation is considered a key factor in tissue damage and the progression of kidney dysfunction. Following ischemia-reperfusion, endothelial cells in the kidneys undergo apoptosis, which may lead to vascular abnormalities (35). Endothelial damage can increase microvascular permeability, resulting in the accumulation of inflammatory cells at the injury site. This accumulation exacerbates the damage, creating a vicious cycle. High expression levels of F4/80 and Gr-1 are often closely associated with the development of inflammation (36). Therefore, intravenous injection of MSCs can reduce the expression of macrophages and neutrophils in kidney tissue, thereby alleviating inflammation.

UC-MSCs alleviate inflammatory phenomena after ischemia-reperfusion injury

Additionally, we used RT-qPCR to measure the gene expression of pro-inflammatory factors TNF-α and IL-6, as well as the anti-inflammatory factor IL-10 (Fig. 4). Compared to

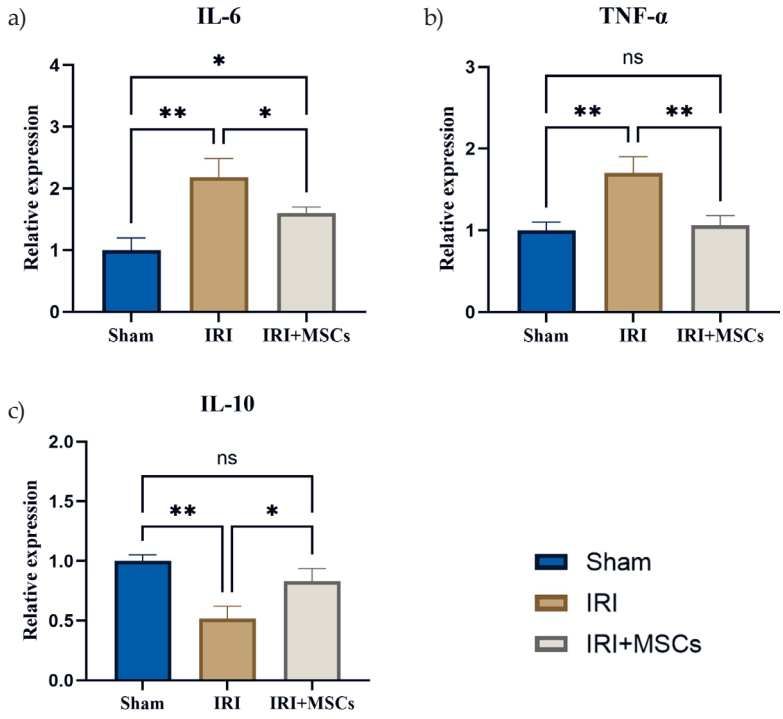

Fig. 4. Inflammatory factor expression (mRNA levels) in kidney tissues: a) IL-6; b TNF-α; c) IL-10. Mean ± SD, $n = 5$. * $p < 0.05$, ** $p < 0.01$. Sham – sham operation, IRI – ischemia-reperfusion, IRI+MSCs – MSC treatment.

the Sham group, the IRI group showed a significant increase in IL-6 and TNF-α levels ($p < 0.01$), while the expression of IL-10 decreased, indicating that ischemia-reperfusion induces an inflammatory response, likely mediated by high levels of pro-inflammatory cytokines and reduced levels of immunosuppressive factors (37, 38). After intervention with UC-MSCs, the levels of IL-6 and TNF-α in the MSCs group significantly decreased compared to the IRI group ($p < 0.05$, $p < 0.01$), and the expression of the anti-inflammatory factor IL-10 significantly increased ($p < 0.05$), further demonstrating that UC-MSCs could reduce inflammation, thereby mitigating kidney damage following ischemia-reperfusion. IL-6, TNF-α, and IL-10 are common inflammatory responses following renal ischemia-reperfusion; however, these indicators were significantly reduced after MSC intervention, a process likely related to the strong immunomodulatory functions and multipotency of MSCs (36). Furthermore, studies have reported that MSCs can induce macrophage polarization from the M1 to the M2 phenotype (39), which also explains why intravenous infusion of UC-MSCs can reduce the inflammatory response in ischemia-reperfusion kidney injury.

UC-MSCs migration to the kidney injury sites

MSCs are considered a promising therapeutic tool in regenerative medicine due to their self-renewal and multipotency. The first step in MSC-mediated tissue regeneration is

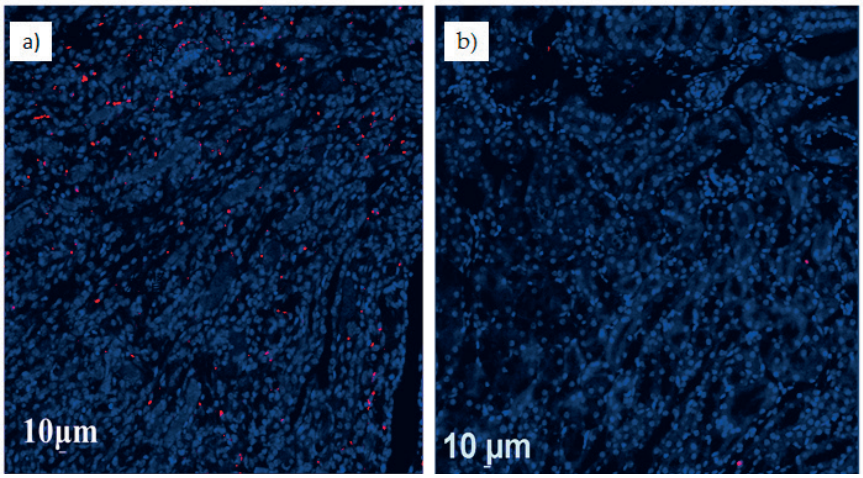

Fig. 5. Distribution of UC-MSCs in the kidneys of MSCs group mice: a) image of injured left kidney from MSCs group mice after intravenous injection of UC-MSCs; b) image of normal right kidney from MSCs group mice after intravenous injection of UC-MSCs. UC-MSCs were indicated by red fluorescent cells, showing that intravenous infusion of UC-MSCs leads to their accumulation at the site of renal injury. MSCs – the umbilical cord stem cells.

their migration to the site of tissue injury (40). To verify whether UC-MSCs can reach the injured left kidney in mice and exert therapeutic effects directly, we used Dil red fluorescence to label the infused cells for cellular localization. Laser confocal microscopy imaging showed that in the MSCs group, the number of red fluorescently labeled cells was significantly higher in the injured left kidney (Fig. 5a) compared to the normal right kidney (Fig. 5b). MSCs possess homing ability, meaning they can migrate to the injury site and have the potential to differentiate into local components of the injury site (41). Additionally, MSCs can secrete chemokines, cytokines, and growth factors that aid in tissue regeneration. This study demonstrated that intravenous infusion of UC-MSCs results in their accumulation at the sites of kidney injury, contributing to kidney repair and exerting anti-kidney injury effects.

Possible mechanisms of UC-MSCs in treating AKI

IRI is an inevitable consequence of organ transplantation. In many clinical scenarios, the duration of ischemia is uncontrollable, necessitating preventive and therapeutic measures to mitigate the harm caused by IRI (42). This study demonstrated that intravenous injection of UC-MSCs can effectively improve renal IRI. UC-MSCs possess multipotency, regenerative capabilities, and immunomodulatory properties, which enhance kidney recovery by homing to the site of renal injury. There is substantial evidence suggesting that in toxic and ischemic rodent models, leveraging the homing ability of MSCs can prevent AKI and accelerate the recovery phase (43, 44). Furthermore, research by Barakat *et al.* (45) indicated that Wharton jelly mesenchymal stem cells (WJ-MSCs) can enhance kidney protection by upregulating the β-catenin/Wnt pathway and reducing apoptosis, inflamma-

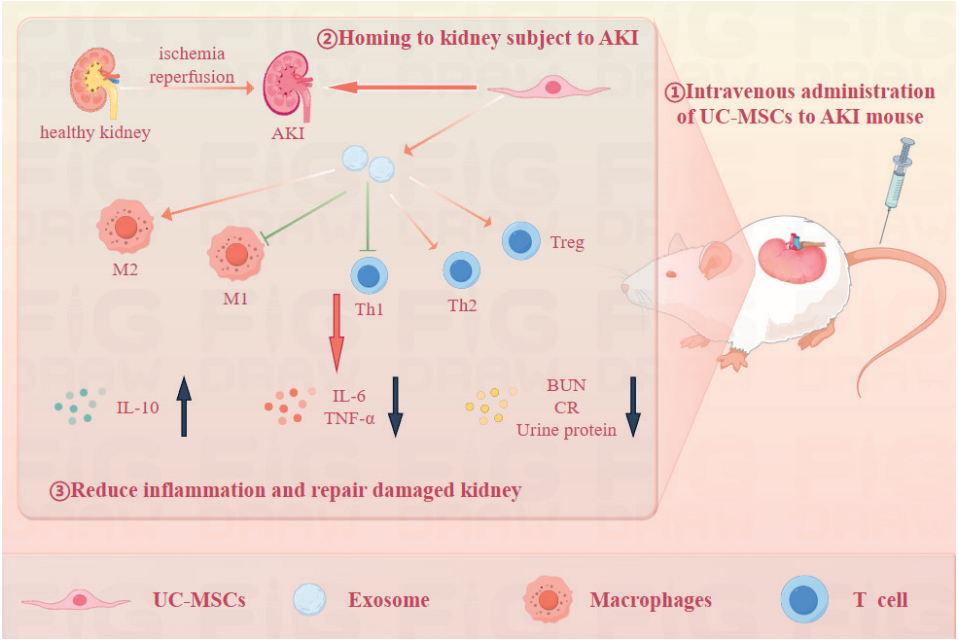

Fig. 6. Proposed mechanisms of AKI treated with UC-MSCs. Homing to the kidney of AKI mice, UC-MSCs may reduce inflammation and repair damaged kidneys through a variety of immunomodulatory effects of exosomes. This figure was drawn on the Figdraw website (ID: URYUU1dfcb). AKI – acute kidney injury, BUN – blood urea nitrogen, CR – creatinine, IL-6 – interleukin-6, IL-10 – interleukin-10, M1 – M1 macrophages, M2 – M2 macrophages, Th1 – T helper 1 cells, Th2 – T helper 2 cells, TNF-α – tumor necrosis factor-alpha, UC-MSCs – umbilical cord mesenchymal stem cells.

tion, and oxidative stress. Kin *et al.* (46) showed that adipose-derived MSC exosomes could activate the ERK 1/2 signaling pathway to treat AKI. In summary, over the past decade, the focus of AKI treatment has shifted towards utilizing MSCs to stimulate innate tissue repair or leveraging their ability to regulate immune responses and cytokine production. This study indicated that UC-MSCs migrate to the site of kidney injury and participate in AKI repair by regulating immune response, and exosomes may also play a role in this process (as shown in Fig. 6). Although most clinical studies are still in the early stages, the continuous improvement in the safety and efficacy of stem cell therapies promises a broad future for the treatment of kidney injuries.

CONCLUSIONS

In this experiment, an AKI model was successfully established in mice using the ischemia-reperfusion method, and the therapeutic effects of UC-MSC preparations were validated through intravenous infusion. Histological and immunofluorescence analysis of kidney tissues indicated that UC-MSCs homed to the site of kidney injury and promoted the repair of damaged tissues. The reduction in serum creatinine, blood urea nitrogen

levels, and urinary protein levels demonstrated the improvement of kidney function by UC-MSCs. Real-time PCR results showed a decrease in pro-inflammatory cytokines IL-6 and TNF-α and an increase in the anti-inflammatory cytokine IL-10, suggesting that UC-MSCs alleviated AKI by reducing inflammation. These findings provide evidence for the function and therapeutic potential of UC-MSCs and offer a basis for evaluating their efficacy and safety in clinical applications. Thus, umbilical cord MSCs can alleviate AKI by reducing inflammation and enhancing kidney repair.

Ethics approval. – All animal handling in this experiment was carried out in strict accordance with the Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Care and Use Committee, Sun Yat-Sen University (No. SYSU-IACUC-2024-002238).

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Conflict of interest. – The authors declare no conflicts of interest.

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Authors contributions. – Conceptualization, L.X., F.X. and J.L.; methodology, L.X., C.H. and S.X.; investigation, L.X., L.X. and X.Z.; data processing, L.X., F.X., H.C. and S.Y.; writing, original draft preparation, L.X., C.H. and S.X.; writing, review and editing, L.X., X.Z., S.W. and J.L.; supervision, J.L.; project administration, S.W. All authors have read and agreed to the published version of the manuscript.

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