Research Article

Oct-4 Enhanced the Therapeutic Effects of Mesenchymal Stem Cell-Derived Extracellular Vesicles in Acute Kidney Injury

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Keywords

Extracellular vesicles · Human umbilical cord mesenchymal stem cells · Acute kidney injury · OCT-4

Abstract

Background/Aims: Acute kidney injury (AKI) is a common clinical condition that can lead to chronic kidney failure. Although mesenchymal stem cell-derived extracellular vesicles (MSC EVs) are regarded as a potent AKI treatment, the mechanisms underlying their beneficial effects remain unclear. Oct-4 may play an important role in tissue injury repair. We thus hypothesized that oct-4 overexpression might enhance the therapeutic effects of MSC EVs in AKI treatment. **Methods:** Renal tubular epithelial cells were cultured in a low oxygen environment, then cocultured with MSC EVs or control medium for 48 h. BrdU and transferase-mediated dUTP nick-end labeling (TUNEL) staining were used to assess cell proliferation and apoptosis. Mice subjected to ischemia reperfusion were randomly divided into 4 groups, then injected with either phosphate-buffered saline (vehicle), EVs, EVs overexpressing oct-4 (EVs+Oct-4), and EVs not expressing Oct-4 (EVs–Oct-4). Blood creatinine (CREA) and urine nitro levels were assessed 48 h and 2 weeks after injection. After ischemia reperfusion, renal tissues from each group were stained with TUNEL and proliferating cell nuclear antigen (PCNA) to determine the degree of apoptosis and proliferation. Masson trichrome staining was used to evaluate renal fibrosis progression. Snail gene expression was assessed using polymerase chain reaction (PCR). **Results:** At 48 h after hypoxic treatment, TUNEL and BrdU staining indicated that the EVs+Oct-4 group had the least apoptosis and the most proliferation, respectively. Treatment with EVs overexpressing Oct-4 significantly decreased serum Crea and blood urea

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nitrogen levels and rescued kidney fibrosis, as indicated by the low proportion of Masson staining, high number of PCNA-positive cells, and low number of TUNEL-positive cells. PCR analysis indicated that Snail was most upregulated in the vehicle group and least upregulated in the EVs+Oct-4 group. **Conclusions:** MSC EVs had a pronounced therapeutic effect on ischemic reperfusion injury-related AKI, and Oct-4 overexpression enhanced these therapeutic effects. Our results may inspire a new direction for AKI treatment with MSC EVs.

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Introduction

Acute kidney injury (AKI) is a common clinical disease, which rapidly leads to acute renal dysfunction [1]. Previously, AKI was considered a self-healing condition, but recent studies have shown that AKI may lead to incomplete renal repair, chronic inflammation, and progressive fibrosis, eventually resulting in a chronic loss of organ function [2]. Indeed, about 30–70% of all patients who survive AKI progress to chronic kidney disease (CKD), and about 17% of all AKI patients develop end-stage renal disease within 1 year [3]. Effective clinical treatments for CKD are lacking, and such treatments as exist are far from satisfactory. The kidneys of children are easily affected by low perfusion, a low glomerular filtration rate, strong renovascular resistance, and high renin activity. Therefore, children more often have poor prognoses after renal injury, and the rate of CKD is significantly higher in children as compared to adults, as is the mortality rate [4, 5]. Therefore, the development of appropriate AKI treatment strategies is urgent.

It is believed that the renal tubular epithelial mesenchymal transition (EMT) is the major cellular mechanism of renal fibrosis in the late stages of renal injury [5, 6]. During the EMT process, many genes that are silent during tissue development are reactivated, disrupting epithelial cell homeostasis, and leading to the reemergence and reactivation of the Snail gene; Snail is a major transcription factor driving EMT [7, 8]. Extracellular vesicles (EVs), which are cystic structures encapsulated by cells, contain functional proteins, mRNAs, and microRNAs. EVs may act as mediators of cell-to-cell communication, as well as key components of the endocrine system [9, 10]. EVs identify target cells by binding between ligands and receptors to transfer bioactive molecules. More importantly, EVs deliver microRNA and mRNA to target cells and transmit genetic information, thus regulating gene expression. Recent studies have shown that EVs derived from mesenchymal stem cells (MSCs) function much like MSCs and can have useful therapeutic effects on various organs and tissues [11, 12]. In our previous study, EVs derived from human umbilical cord MSCs were injected into rats that had received ischemia-reperfusion injury via 1 h of unilateral renal pedicle occlusion. The injection of EVs inhibited the renal fibrosis induced by renal injury [13]. Moreover, neither EVs derived from human fibroblasts nor human umbilical cord MSC-derived microcapsules treated with RNA enzyme had antifibrosis effects [14]. Therefore, we hypothesized that a special RNA, derived from human umbilical cord MSC EVs, must be involved in the regulation of renal fibrosis. In a previous study, viral vectors were used to overexpress 4 transcription factors in mouse fibroblasts, which were then transformed into pluripotent stem cells [15]. Oct-4, one of these 4 transcription factors, induced the transformation of rat fibroblasts into pluripotent stem cells, which then inhibited the pro-EMT signal-activating Snail gene in the epithelial cells [16]. In this study, we aimed to determine whether the verifier of human umbilical cord MSCs could deliver Oct-4 mRNA to damaged renal tubular epithelial cells, promote the homeostasis of epithelial cells, inhibit EMT, and prevent fibrosis. If so, our results might be useful for the future development of new, more effective biological antifibrotic drugs.

Materials and Methods

Cell Culture

Human umbilical cords were aseptically collected from full-term infants delivered by cesarean section at Shanghai Jiao Tong University, which is affiliated with the First People's Hospital in Shanghai, China. Umbilical cords were stored aseptically in cold Dulbecco's modified Eagle medium (DMEM) and subjected to cellular isolation within 4 h of receipt. The isolation of HUMSCs was performed as previously described [17]. Briefly, after the blood cells had been removed from the arteries and veins, each umbilical cord was cut into 3 pieces, each approximately 1–2 mm long. The cord pieces were transferred to a Petri dish containing DMEM and a small amount of glucose (DMED-LG, Gibco) supplemented with 10% fetal bovine serum (FBS, Gibco) and incubated at 37 °C in a humidified atmosphere with 5% CO₂. The medium was changed every 2 days. After 2 weeks, the adherent cells were harvested with 0.25% trypsin (Gibco) and subcultured. Only cells from passages 3 to 6 were used in subsequent experiments.

Renal tubular epithelial cells were isolated from C57BL/6 mouse kidneys. Cortical tissues were minced into pieces, digested with 0.1 collagenase I (Gibco), and centrifuged in 32% Percoll medium to purify proximal tubular cells. The cells were then plated in collagen-coated dishes and maintained in DMEM/F-12 medium supplemented with 10% FBS.

Overexpression and Knockdown of OCT-4 in HUMSCs

The human *OCT-4* gene was amplified using polymerase chain reaction (PCR). The transcription factor was cloned into the lentiviral pGMLV-CEV-MCS-EF1-ZsGreen1 Vector (Genomeditech); cloning was confirmed by sequencing. Lentiviral particles were packaged with 2 other homologous helper plasmids into NIH 293T cells to generate the lentivirus, following the manufacturer's instructions. The shRNA designed to inhibit the expression of endogenous *OCT-4* in the HUMSCs was obtained from Ribo bio.

Isolation of EVs

EVs from normal HUMSCs, oct-4-overexpression HUMSCs, and oct-4-knockdown HUMSCs were obtained from HUMSC supernatants as previously described [18]. Briefly, HUMSCs were cultured overnight in DMEM (without FBS) with 0.5% bovine serum albumin (Sigma-Aldrich). When the cell viability was >99% (as measured by trypan blue exclusion), and no apoptotic cells were identified (by the terminal transferase-mediated dUTP nick-end labeling [TUNEL] assay), the conditioned medium was collected and stored at –80 °C. The medium was centrifuged at 2,000 g for 20 min to remove debris and then ultracentrifuged at 100,000 g in a SW41 swing rotor (Beckman Coulter, Fullerton, CA, USA) for 1 h at 4 °C. EVs were washed once with serum-free M199 (Sigma-Aldrich) containing 25 mL MHEPES (pH = 7.4), then recentrifuged under the same conditions. EVs were stored at –80 °C prior to experimentation. To quantify protein content, we used a Bradford protein assay kit (P0006; Beyotime Institute of Biotechnology, China). We quantified the protein content indirectly, based on 5 × 10⁵ MSCs releasing approximately 100 µg of EVs overnight. RNA was extracted from the EVs using TRIZOL reagent and analyzed with a spectrophotometer.

Animal Models of AKI

We developed mouse AKI models by subjecting male C57BL/6 mice to right kidney resection, followed by 45 min of left kidney ischemia. We then administered either 100 µg EVs in 1 mL of vehicle (M199, Gibco BRL Co., USA) or 1 mL of vehicle only into the caudal vein immediately after reperfusion. The animals were separated into 4 groups according to the therapeutic procedure (≥6 mice per group): treatment with vehicle only (vehicle group);

treatment with the EVs from the Oct-4-knockdown HUMSCs (EVs-Oct-4 group); treatment with the EVs from the Oct-4-overexpression HUMSCs (EVs+Oct-4 group); and treatment with normal HUMSC EVs (EV group).

Renal Function

Blood samples were taken 14 days after the procedure, and plasma blood urea nitrogen (BUN) and serum creatinine (CREA) were measured with a Beckman Analyzer II (Beckman Instruments, Inc.).

Immunohistochemistry

One portion of the renal tissue was fixed in 4% paraformaldehyde and embedded in paraffin. We labeled 4- μ m-thick sections with rabbit antibody to mouse proliferating cell nuclear antigen (PCNA; dilution 1:100; Abcam) or with rabbit antibody to mouse α -SMA, dilution (dilution 1:500; Abcam). We then used a horseradish peroxidase-conjugated secondary antibody, with diaminobenzidine reagents as substrates. Specimens were then counterstained with hematoxylin. We estimated the number of PCNA+ cells at the corticomedullary junctions and outer medullas of the kidneys based on the average number of PCNA + cells in 30 randomly selected high-power fields. We estimated the number of α -SMA-positive cells in each mouse from each group by counting the number of positive tubular cell nuclei under 400 $\times$ magnification in 30 random-selected fields across all kidney sections from each animal. Tubular cell apoptosis was assessed with a TUNEL assay, using an in situ cell death detection kit (Roche, Mannheim, Germany). Kidney sections were screened for tubular cells with positive nuclei under 400 $\times$ magnification. The apoptotic score was determined by counting the numbers of positive nuclei in 30 random fields.

After the hypoxic injury, the renal tubular epithelial cells were subjected to immunohistochemistry staining: cells were incubated on chamber slides and exposed to EVs or control medium for 48 h. Subsequently, slides were fixed in 4% paraformaldehyde and permeabilized with HEPES-250 Triton $\times$ 100 buffer (Sigma). Apoptosis of renal tubular epithelial cells was assessed with a TUNEL assay, using an in situ cell death detection kit (Roche, Mannheim, Germany). The proliferation of renal tubular epithelial cells was assessed with a BrdU kit (Sigma). The apoptotic score and proliferation score were calculated by counting the numbers of positive nuclei in 30 random fields.

Real-Time Quantitative PCR Analysis

RNA was extracted with TRIZOL reagent (Invitrogen, Carlsbad, NM, USA), following the standard protocol. Total RNA was quantified spectrophotometrically. We then reverse transcribed 5 ng of RNA with the M-MLV reverse transcriptase kit using Oligo-dT primers (Invitrogen) for 60 min at 42 $^{\circ}$ C. Real-time PCR was performed with TaqMan gene expression assays (Applied Bio-Systems, Foster) to detect the gene expression of mouse Snail and mouse b-actin.

Masson Trichrome Staining

Masson trichrome staining was used to measure the deposition of collagen in the renal interstitium. The degree of interstitial fibrosis was scored semiquantitatively, on a scale of 0–3 (where 0 represented no lesions; 1 represented <33% of the parenchyma affected by lesions; 2 represented 33–67% of the parenchyma affected by lesions; and 3 represent >67% of the parenchyma affected by lesions). The scores were assessed by a blinded observer in 100 random high-power fields (magnification $\times$ 6,400) in the parenchyma of each mouse in each group. The total score was calculated as the sum of all scores for each mouse (i.e., the maximum score per mouse was 300).

Fig. 1. TUNEL and BrdU staining of renal tubular epithelial cells after hypoxia. **A** Representative images of TUNEL-stained renal tubular epithelial cells treated with vehicle only (no EVs), EVs, EVs-Oct-4, and EVs+Oct-4. **B** BrdU staining. Quantification of **(C)** TUNEL staining and **(D)** BrdU immunostaining in each group. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.00001$. EVs, extracellular vesicles; HPF, high-power field; TUNEL, transferase-mediated dUTP nick-end labeling.

Statistical Analysis

All experiments were performed at least 3 times. The qRT-PCR data were log 10 transformed, and data were expressed as means $\pm$ one SD of the mean. Statistical significance was determined using paired t tests in SPSS version 19.0. We considered $p < 0.05$ statistically significant.

Results

HUMSC-EVs reduced apoptosis and increased proliferation of renal tubular epithelial cells after hypoxia injury.

We visualized the apoptosis and proliferation of renal tubular epithelial cells after hypoxia using TUNEL and BrdU staining (Fig. 1). After 48 h, there were different degrees of apoptosis in the 4 groups of renal tubular epithelial cells. The most severe level of renal tubular epithelial cell apoptosis was observed in the vehicle group, followed by the EVs–Oct-4 group, the EV group, and the EVs+Oct-4 group. We observed significantly more apoptosis in the vehicle group, as compared to the other 3 groups. In addition, there was significantly more apoptosis in the EVs–Oct-4 group as compared to the EV and EVs+Oct-4 groups.

BrdU can be embedded in the DNA during cell division, thus causing the proliferating cells to fluoresce. At 48 h after hypoxia, we observed the most renal tubular epithelial cell proliferation in the EVs+Oct-4 group, followed by the EV group, the EVs–Oct-4 group, and the vehicle group. There was significantly more renal tubular epithelial cell proliferation in the EVs+Oct-4 group as compared to the EV group and significantly more renal tubular epithelial cell proliferation in the EV group as compared to the EVs–Oct-4 group.

HUMSCs-EVs Rescued Apoptosis and Increased Proliferation of Renal Cells after AKI

There were significantly more TUNEL-positive cells in the vehicle group 48 h and 2 weeks after AKI, as compared to the other 3 groups (Fig. 2). In addition, there were more TUNEL-positive cells in the kidneys of the vehicle group, as compared to the other 3 groups (Fig. 2). There were significantly fewer PCNA+ cells in the vehicle group than in the EV, EVs+Oct-4, and EVs–Oct-4 groups 48 h and 2 weeks after AKI. In addition, the EVs+Oct-4 group had significantly more PCNA-positive cells than the EV and EVs–Oct-4 groups (Fig. 3).

Preservation of Renal Function BY HUMSCs-EVs after AKI

To identify changes in renal function after renal injury, we measured serum BUN and CREA levels in mice 2 weeks after AKI (Fig. 4). We found that serum BUN and CREA were highest in the vehicle group, followed by the EVs–Oct-4 group. Serum BUN and CREA levels in the EVs+Oct-4 and EV groups were significantly lower than in the vehicle and EVs–Oct-4 groups, suggesting that renal function was preserved in the EVs+Oct-4 and EV groups. In addition, the EVs+Oct-4 group had significantly lower serum BUN and CREA levels than did the EV group.

IRI-Induced Fibrosis was Abrogated by EVs Therapy

We assessed renal fibrosis progression after renal injury based on α -SMA expression and Masson staining (Fig. 5). Masson trichrome staining showed that, 2 weeks after AKI, mice from the vehicle group exposed to IRI exposure developed of significant fibrotic lesions in the renal interstitial area. In contrast, fibrogenesis was slight in mice from the EVs+Oct-4 group. In addition, α -SMA expression in tubular cells, which is an indicator of EMT, was observed mainly in the mice from the vehicle group. We observed little α -SMA expression in the tubular cells of mice from the EVs+Oct-4 group.

Oct-4 mRNA Suppressed the Expression of the Snail Gene

To explore the relationship between oct-4 and the Snail gene in kidney tissue, we used qRT-PCR to quantify snail gene expression, postrenal injury, in the kidney tissues of the mice from the 4 groups (Fig. 6). The Snail gene was highly expressed in all 4 groups, but other groups did not express Snail as highly. Snail gene expression was significantly lower in the EVs+Oct-4 group as compared to the EV group and significantly lower in the EVs group as compared to the EVs–Oct-4 group.

Fig. 2. TUNEL staining of kidney sections after AKI. Representative images of TUNEL-stained kidneys of AKI mice treated with vehicle only (no EVs), EVs, EVs-Oct-4, and EVs+Oct-4 after **(A)** 48 h and **(B)** 2 weeks. Quantification of TUNEL-stained sections after **(C)** 48 h and **(D)** 2 weeks. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.00001$. TUNEL, transferase-mediated dUTP nick-end labeling; EVs, extracellular vesicles; HPF, high-power field.

Fig. 3. PCNA staining of kidney sections after AKI. Representative images of PCNA-stained kidneys of AKI mice treated with vehicle only (no EVs), EVs, EVs–Oct-4, and EVs+Oct-4 after **(A)** 48 h and **(B)** 2 weeks. Quantification of PCNA-stained sections after **(C)** 48 h and **(D)** 2 weeks. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.00001$. PCNA, proliferating cell nuclear antigen; EVs, extracellular vesicles; HPF, high-power field.

Fig. 4. A BUN and **(B)** CREA levels after AKI. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.00001$. BUN, blood urea nitrogen; EVs, extracellular vesicles; CREA, creatinine.

Discussion

Multiple mechanisms are involved in AKI and the subsequent renal fibrosis: the proliferation of renal tubular cells, the activation of apoptosis, the release of inflammatory cytokines, the generation and release of reactive oxygen species, the activation of TGF- β 1, and renal tubular epithelial DNA damage. These processes eventually lead to the abnormal proliferation of renal tubular epithelial cells and excessive fibrotic repair [19].

Previous studies have shown that MSCs may reduce renal damage via paracrine mechanisms [20, 21], and that EVs may play an important role in this process. We have demonstrated that HUMSC EVs restrict the aggregation of macrophages by inhibiting CX3CL1 [19],

Fig. 5. Masson trichrome and α -SMA staining after AKI. Representative images of **(A)** Masson trichrome staining and **(B)** α -SMA staining of AKI mice treated with vehicle only (no EVs), EVs, EVs-Oct-4, and EVs+Oct-4 after **(A)** 48 h and **(B)** 2 weeks. Quantification of **(C)** Masson trichrome staining and **(D)** α -SMA immunofluorescent staining. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.00001$. EVs, extracellular vesicles.

Fig. 6. Expression of the Snail gene in kidney tissue after AKI, as quantified by real-time PCR. ** $p < 0.01$; **** $p < 0.00001$. EVs, extracellular vesicles.

reduce reactive oxygen species by inhibiting NOX2 [22], and inhibit mitochondrial fission by upregulating microRNA30 [23]. Thus, HUMSC EVs alleviate acute renal injury and reduce renal fibrosis. Evidence indicates that EVs from MSCs may ameliorate acute renal injury via a variety of mechanisms.

Here, we focused on the effects of oct-4 on renal damage. By both upregulating and knocking down oct-4 in EVs, we sought to observe the effects of oct-4 on renal damage after renal injury. We found that the overexpression of oct-4 significantly reduced the hypoxia-induced apoptosis of renal tubular epithelial cells in vitro and increased the proliferation of renal tubular epithelial cells after hypoxia. In animal experiments, EVs overexpressing oct-4 level played a similar role, inhibiting apoptosis and increasing the proliferation of kidney cells after injury. We also characterized fibrosis progression 2 weeks after AKI in each group. We found that EVs overexpressing oct-4 significantly inhibited the fibrosis caused by renal injury, and that oct-4-knockdown EVs inhibited kidney fibrosis only slightly. The effects of the oct-4-knockdown EVs on renal injury were not only weaker than those of the EVs overexpressing oct-4 but were also weaker than those of the normal MSC EVs. This indicated that oct-4 expression in EVs plays an important role in the inhibition of renal injury.

However, 2 weeks after ischemia-reperfusion injury, BUN and CREA levels in mice treated with EVs overexpressing oct-4 were not significantly different from BUN and CREA levels in mice treated with normal EVs. This suggested that the administration of EVs overexpressing oct-4 after ischemia-reperfusion injury would not confer greater long-term renoprotection than treatment with normal EVs. However, this similarity of effects might be due to the degradation of oct-4 mRNA. Thus, we hypothesized that the effects of EVs overexpressing oct-4 might be stronger if administered an appropriate time.

Li et al. [17] found that oct-4 stimulated the MET process by inhibiting the expression of the Snail gene in fibroblasts, leading to the transformation of fibroblasts into iPSCs. To clarify the mechanisms underlying the effects of oct-4 mRNA on fibrosis after renal injury, we explored the relationship between oct-4 and the Snail gene. Snail is a zinc finger transcription protein, which can be combined with e-cadherin promoter e-boxes to inhibit the

expression of e-cadherin. Snail primarily triggers the involvement of EMT in embryonic renal development, fibrosis, and epithelial neoplasm formation. In the adult kidney, the Snail gene is mute, but it is highly expressed in fibrotic kidneys, renal cysts, and renal carcinomas. When Snail triggers EMT, epithelial cell markers (e.g., cadherin-16, E-cadherin, and cytokeratins) are downregulated, while interstitial cell biomarkers (e.g., vimentin and alpha-MA) are markedly upregulated. In addition, the expression levels of the fibrosis-related factors collagen I and fibronectin increase, and the extracellular matrix is overdeposited. This eventually leads to the destruction of tissues and organs, as well as to functional impairment [24].

Our results were consistent with 2 previous studies [20, 21], but incongruent with third, which suggested that MSCs or MSC-derived EVs might promote tumor growth [14]. This might be due to the complex cargo of EVs. On the other hand, this may be due to the similar proliferation and vascularization characteristics between the damaged renal tissue and tumor tissue.

We found that EVs in normal HUMSCs downregulated Snail in renal tissue after injury, and that the overexpression of oct-4 mRNA in EVs amplified the inhibitory effect of EVs on Snail expression and vice versa. Thus, Oct-4 might inhibit fibrosis progression by inhibiting Snail expression after renal injury. Here and previously, we have shown that the potential therapeutic effects of HUMSC-EVs on renal injury may be affected by multiple factors, which are far from fully revealed. Many mechanisms still remain to be explored.

Statement of Ethics

All research involving human participants was approved by the Medical School of Shanghai Jiao Tong University, Shanghai, China. Human subjects gave written informed consent for participation in this research and for the publication of the results. This study was carried out in strict accordance with the recommendations of the *Guide for the Care and Use of Laboratory Animals* of Shanghai Jiao Tong University. The protocol was approved by the Committee on the Ethics of Animal Experiments of Shanghai Jiao Tong University. All protocols were approved by the Committee on the Ethics of Animal Experiments of Shanghai Jiao Tong University. All surgery was performed under sodium pentobarbital anesthesia, and every effort was made to minimize animal suffering.

Disclosure Statement

The authors declare no conflicts of interest.

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Author Contributions

Z.-Y.Z. and X.-Y.Z. carried out the cell culture studies and drafted the manuscript. Y.-P.H. carried out the immunoassays. Z.-Y.Z. and Y.-P.H. contributed equally to this work. X.-Y.X. participated in the PCR tests. G.-Q.J. and L.Z. participated in the design of the study and

performed the statistical analysis. J.S. conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

Consent for Publication

Written informed consent for publication was obtained from all participants.

Availability of Data and Material

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

References

- 1 Bellomo R, Kellum JA, Ronco C. Acute kidney injury. *Lancet*. 2012 Aug;380(9843):756–66.
- 2 Zuk A, Bonventre JV. Acute Kidney Injury. *Annu Rev Med*. 2016;67(1):293–307.
- 3 Awate N, Wagh V. Incidence and outcome of acute kidney injury in intensive care unit. *J Assoc Physicians India*. 2016;64(1).
- 4 Libório AB, Branco KM, Torres de Melo Bezerra C. Acute kidney injury in neonates: from urine output to new biomarkers. *BioMed Res Int*. 2014;2014:601568.
- 5 Zeisberg M, Duffield JS. Resolved: EMT produces fibroblasts in the kidney. *J Am Soc Nephrol*. 2010 Aug;21(8):1247–53.
- 6 He J, Xu Y, Koya D, Kanasaki K. Role of the endothelial-to-mesenchymal transition in renal fibrosis of chronic kidney disease. *Clin Exp Nephrol*. 2013 Aug;17(4):488–97.
- 7 Boutet A, De Frutos CA, Maxwell PH, Mayol MJ, Romero J, Nieto MA. Snail activation disrupts tissue homeostasis and induces fibrosis in the adult kidney. *EMBO J*. 2006 Nov;25(23):5603–13.
- 8 Boutet A, Esteban MA, Maxwell PH, Nieto MA. Reactivation of Snail genes in renal fibrosis and carcinomas: a process of reversed embryogenesis? *Cell Cycle*. 2007 Mar;6(6):638–42.
- 9 Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. *Kidney Int*. 2010 Nov;78(9):838–48.
- 10 Camussi G, Deregibus MC, Cantaluppi V. Role of stem-cell-derived microvesicles in the paracrine action of stem cells. *Biochem Soc Trans*. 2013 Feb;41(1):283–7.
- 11 Zhu YG, Feng XM, Abbott J, Fang XH, Hao Q, Monsel A, et al. Human mesenchymal stem cell microvesicles for treatment of Escherichia coli endotoxin-induced acute lung injury in mice. *Stem Cells*. 2014 Jan;32(1):116–25.
- 12 Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Agur EN, et al. Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. *Stem Cell Res (Amst)*. 2013 May;10(3):301–12.
- 13 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 Jun;4(3):59.
- 14 Du T, Ju G, Wu S, Cheng Z, Cheng J, Zou X, et al. Microvesicles derived from human Wharton's jelly mesenchymal stem cells promote human renal cancer cell growth and aggressiveness through induction of hepatocyte growth factor. *PLoS One*. 2014 May;9(5):e96836.
- 15 Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell*. 2006 Aug;126(4):663–76.
- 16 Li R, Liang J, Ni S, Zhou T, Qing X, Li H, et al. A mesenchymal-to-epithelial transition initiates and is required for the nuclear reprogramming of mouse fibroblasts. *Cell Stem Cell*. 2010 Jul;7(1):51–63.
- 17 Majore I, Moretti P, Hass R, Kasper C. Identification of subpopulations in mesenchymal stem cell-like cultures from human umbilical cord. *Cell Commun Signal*. 2009 Mar;7(1):6.
- 18 Zou X, Zhang G, Cheng Z, Yin D, Du T, Ju G, et al. Microvesicles derived from human Wharton's Jelly mesenchymal stromal cells ameliorate renal ischemia-reperfusion injury in rats by suppressing CX3CL1. *Stem Cell Res Ther*. 2014 Mar;5(2):40.

- 19 Devarajan P. Update on mechanisms of ischemic acute kidney injury. [J Am Soc Nephrol](#). 2006 Jun;17(6):1503–20.
- 20 Caplan AI, Dennis JE. Mesenchymal stem cells as trophic mediators. [J Cell Biochem](#). 2006 Aug;98(5):1076–84.
- 21 Du T, Cheng J, Zhong L, Zhao XF, Zhu J, Zhu YJ, et al. The alleviation of acute and chronic kidney injury by human Wharton's jelly-derived mesenchymal stromal cells triggered by ischemia-reperfusion injury via an endocrine mechanism. [Cytotherapy](#). 2012 Nov;14(10):1215–27.
- 22 Zhang G, Zou X, Miao S, Chen J, Du T, Zhong L, et al. The anti-oxidative role of micro-vesicles derived from human Wharton-Jelly mesenchymal stromal cells through NOX2/gp91(phox) suppression in alleviating renal ischemia-reperfusion injury in rats. [PLoS One](#). 2014 Mar;9(3):e92129.
- 23 Gu D, Zou X, Ju G, Zhang G, Bao E, Zhu Y. Mesenchymal Stromal Cells Derived Extracellular Vesicles Ameliorate Acute Renal Ischemia Reperfusion Injury by Inhibition of Mitochondrial Fission through miR-30. [Stem Cells Int](#). 2016;2016:2093940.
- 24 Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. [Nat Rev Mol Cell Biol](#). 2014 Mar;15(3):178–96.