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Adipose-derived stem cells ameliorate renal interstitial fibrosis through inhibition of EMT and inflammatory response via TGF-β1 signaling pathway

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ABSTRACT

Adipose-derived stem cells (ADSCs) have been successfully used to treat acute kidney injury or acute renal failure. However, the effect of ADSCs on treating renal interstitial fibrosis remains unknown. Here, we assessed the therapeutic efficacy of ADSCs on renal interstitial fibrosis induced by unilateral ureter obstruction (UUO) and explored the potential mechanisms. After 7 days of UUO, rats were injected with ADSCs (5×10^6) or vehicle via tail vein. We found that ADSCs administration significantly ameliorated renal interstitial fibrosis, the occurrence of epithelial-mesenchymal transition (EMT) and inflammatory response. Furthermore, ADSCs administration could inhibit the activation of transforming growth factor-β1 (TGF-β1) signaling pathway, which might play a crucial role in renal interstitial fibrosis of the UUO model rats. These results suggested that ADSCs treatment attenuates renal interstitial fibrosis possibly through inhibition of EMT and inflammatory response via TGF-β1 signaling pathway. Therefore, ADSCs may be an effective therapeutic strategy for the treatment of renal interstitial fibrosis.

1. Introduction

Renal interstitial fibrosis is a common pathological pathway and irreversible process that eventually leads to end-stage renal disease. Interstitial fibrosis is characterized by the destruction of renal tubules and interstitial capillaries as well as by the accumulation of extracellular matrix (ECM). Till now, the underlying molecular mechanisms of renal interstitial fibrosis are still not fully understood, and there is still no effective treatment strategy to prevent and halt the progression of renal fibrosis. Therefore, it is necessary to explore the molecular mechanisms involved in renal interstitial fibrosis progression and to develop effective therapeutic strategies for prognosis improvement.

Adipose-derived stem cells (ADSCs) are a mesenchymal stem cell source with capacities of self-renewal and multipotential differentiation. ADSCs can differentiate into a variety of cell types including adipocytes [1], chondrocytes [2], osteoblasts [3], myocytes [4], neurocytes [5], endothelial cells [6], and other cell types [7]. ADSCs also have the potential to treat a variety of diseases, such as autoimmune-induced diseases [8], diabetes mellitus [9], graft-versus-host disease [10], tracheomediastinal fistulas [11], and multiple sclerosis [12]. Compared with bone marrow-derived stem cells, ADSCs have two main advantages. First, ADSCs are easily accessible in large quantities from subcutaneous adipose tissue with less donor site lesion [13]. Second, ADSCs are nonimmunogenic and avoid the ethical and political concerns compared to use of embryonic stem cell, because they can be derived from autologous fat [14]. These two characteristics make ADSCs a more ideal source for tissue and organ transplantation in regenerative medicine and clinical studies.

Recently, several reports had demonstrated that ADSCs therapy could reduce kidney injury and improve kidney function by attenuating inflammation and immune responses. For example, in mice, ADSCs were demonstrated to reduce acute kidney damage through inhibiting the expression of tissue chemokine and cytokine [15]. ADSCs therapy could protect against kidney injury in sepsis syndrome caused by cecal ligation puncture in rats [16,17]. ADSCs could improve protection against acute kidney ischemia–reperfusion injury by reducing the protein expressions of oxidative stress, apoptosis and inflammatory reaction in rats [18,19]. However, the impacts of ADSCs on renal interstitial fibrosis have not been elucidated. This study aimed to evaluate the

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effects of ADSCs treatment on the improvement of renal interstitial fibrosis in a rat model of renal interstitial fibrosis induced by UUO. Furthermore, we investigated whether ADSCs treatment was able to ameliorate EMT and inflammatory response via TGF-β1 signaling pathway in the rat UUO models.

2. Materials and methods

2.1. Animals and ethics

Male Wistar rats weighing 350 to 400 g were obtained from animal experimental center of Shandong University, China. All animal experimental protocols were approved by the Animal Ethics Committee of Shandong University Second Hospital, and the investigation conformed to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2. ADSCs preparation

ADSCs were collected from the adipose tissue surrounding the epididymis of 6-week-old male Wistar rats as described previously [19] and maintained in Dulbecoo's modified Eagle's medium-low glucose (DMEM, Gibco, USA) containing 10% fetal bovine serum (Gibco, USA), 0.3 g/l L-glutamine, 100 μ/ml penicillin and 100 mg/ml streptomycin. The primary ADSCs were grown for about 7–10 days and then passaged in 2–3 days. The third passage of ADSCs were used for the following experiments. The characteristics of membrane receptor phenotyping and differentiation assays were used to identify ADSCs as reported previously [18,19]. The markers of ADSCs were detected through flow cytometric analysis for CD34, CD44, CD45, CD29, CD90 and CD 11b by FACSCalibur flow cytometer (BD Biosciences, San Jose, California, USA). The multipotential differentiation of ADSCs was assessed through culture osteogenesis and adipogenesis under specific differentiation medium stimuli. Adipogenic differentiation was induced with high glucose DMEM medium supplemented with 1 μM dexamethasone (Sigma, USA), 0.5 mM isobutylmethylxanthine (Sigma, USA), 10 μg/ml insulin (Sigma, USA), and 100 μM indomethacin (100 μM) (Sigma, USA) for 14 days. Osteogenic differentiation was induced with low glucose DMEM medium supplemented with 0.1 μM dexamethasone, 50 μM ascorbic acid (Sigma, USA), 10 mM β-glycerophosphate (Sigma, USA) for 21 days. The Oil Red staining or Alizarin Red staining were used to determine the adipocyte lipid and osteocyte calcium deposits, respectively.

2.3. rRenal interstitial fibrosis model establishment

Rats were randomly divided into a total of three groups as follows: sham, UUO, UUO + ADSCs (n = 15, each group). UUO or sham operation was conducted as described previously [20]. In the sham group, rats had their left ureters exposed and manipulated without ligation. Rats in UUO and UUO + ADSCs group were obstructed left ureter. Rats in UUO + ADSCs group received tail vein transplantation of 1 mL ADSCs (5×10^6 cells), and rats in UUO and sham group received equal volume Saline at 7 days after operation. Groups of rats were sacrificed at 14 days after the operation, and the obstructed kidneys were collected for tissue analysis.

2.4. cCell labeling for in vivo tracking

To evaluate the intrarenal localization of ADSCs, green fluorescent protein (GFP) was used as a cell tracker in the tracking experiment as previously described [21]. ADSCs which were labeled with GFP through transduction method by lentiviral plasmid (Genechem, Shanghai, China) according to the manufacturer's advised procedure were harvested and named ADSCs^{GFP}. To track the ADSCs intrarenal distribution after ADSCs administration, eight rats with UUO received ADSCs^{GFP} transplantation instead of ADSCs.

2.5. kKidney function measurement

Seven days after treatment, renal function was evaluated by measuring serum creatinine (Scr) and blood urea nitrogen (BUN) levels with automatic biochemistry analyzer (Roche, Cobasc 311, Mannheim, Germany). And kidneys samples were harvested for molecular biological and histopathological analysis as previously described [21].

2.6. hHistologic examination

The kidney tissues were fixed in 10% formalin for 24 h for routine dehydration and paraffin embedding. Paraffin sections (4 μm thick) were stained with hematoxylin-eosin (HE) and Masson's trichrome, and were examined by light microscopy. The index of tubulointerstitial fibrosis was assayed in 10 different Masson's trichrome stained sections through a light microscope on a scale of 0 to 4 (grade 0, normal; grade 1, affected area < 10%; grade 2, affected area 10%–25%; grade 3, affected area 25%–75%; grade 4, affected area >75%) [22].

2.7. Immunohistochemistry

The localizations of alpha-smooth muscle actin (α-SMA, Abcam, Cambridge, MA, ab5694), fibroblast-specific protein1 (FSP-1, Abcam, ab41532), fibronectin (FN, Abcam, ab2413), epithelial cadherin (E-cadherin, Abcam, ab76055), monocyte chemoattractant protein-1 (MCP-1, Abcam, ab25124) and toll-like receptor 4 (TLR4, Abcam, ab22048) in kidney tissues were examined by immunohistochemistry in paraffin embedded sections according to previously published protocols [23]. The immunohistochemistry was also used to determine the localization of ADSCs^{GFP} in kidney using the anti-GFP antibody (Santa Cruz, CA, USA) due to the strong autofluorescence of kidney. Positive tissue staining area was quantified through Image Pro-plus v 6.0 software (Media Cybernetics, Inc.) to analyze the mean optical density. All morphological analyses and cell counting were conducted in a blinded fashion.

2.8. Gene expression analysis

Expression levels of α-SMA, FSP-1, FN, E-cadherin, MCP-1, TLR4, TGF-β1, Smad7, Tumor Necrosis Factor-alpha (TNF-α), IL-1β, IL-6, and GAPDH from kidney tissues were measured by real-time RT-PCR. Total

Table 1
The primers used for real-time RT-PCR.

Gene	Primers	
α-SMA	Forward	5′-CCGAGATCTCACCGACTACC-3′
	Reverse	5′-TCCAGAGCGACATAGCACAG-3′
FSP-1	Forward	5′-ACCTCTCTGTTCAGCACTTCC-3′
	Reverse	5′-GAACTTGTCACCCTCGTTGC-3′
FN	Forward	5′-ACCAAGGCTGGATGATGGTG-3′
	Reverse	5′-TGTCGCTCACACTTCCACTC-3′
E-cadherin	Forward	5′-CACACTGATGGTGAGGGTACAAGG-3′
	Reverse	5′-GGGCTTCAGGAACACATACATGG-3′
MCP-1	Forward	5′-AAAACCTGGATCGGAACCAAA-3′
	Reverse	5′-TGCTTGAGGTGGTTGTGGAA-3′
TLR4	Forward	5′-GGATGATGCCTCTCTTGCAT-3′
	Reverse	5′-TGATCCATGCATTGGTAGGTAA-3′
TNF-α	Forward	5′-ATACACTGGCCCGAGGCAAC-3′
	Reverse	5′-CCACATCTCGGATCATGCTTTC-3′
IL-1β	Forward	5′-CTACCTATGTCTTGCCCGTGGAG-3′
	Reverse	5′-GGGAACATCACACACTAGCAGGTC-3′
IL-6	Forward	5′-ATTGTATGAACAGCGATGATGCAC-3′
	Reverse	5′-CCAGGTAGAAACGGAACTCCAGA-3′
TGF-β1	Forward	5′-ATACGCCTGAGTGGCTGTCT-3′
	Reverse	5′-TGGGACTGATCCCATTGATT-3′
GAPDH	Forward	5′-ACAAGATGGTGAAGGTCGGTG-3′
	Reverse	5′-AGAAGGCAGCCCTGGTAACC-3′