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*Original Article*

# Transforming growth factor-β1 promotes homing of bone marrow mesenchymal stem cells in renal ischemia-reperfusion injury

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**Abstract:** Backgrounds: Acute ischemia reperfusion-induced kidney injury is a common cause of acute renal failure, and it is also an important cause of delayed recovery of transplanted kidney functions and even loss of function. However, there is no effective treatment method in clinical applications presently. Objective: The objective was to investigate effects of transforming growth factor-β1 on homing of bone marrow mesenchymal stem cells in renal ischemia-reperfusion injury. Methods: Effects of TGF-β1 over-expression in MSCs on expression of CXCR4 and chemotactic effect to SDF-1 were investigated by *in vitro* transmembrane chemotaxis. Anti-TGF-β1 antibody was incubated with ischemia reperfusion injury renal tissue homogenate and effects of anti-TGF-β1 antibody were observed. In addition, effects of TGF-β1 gene transfection and anti-CXCR4 antibody treatment in MSCs on expression of SDF-1/CXCR4 axis of renal tissues and damage repair were further explored. Results: Expression of TGF-β1 mRNA in the IRI group increased significantly, and MSCs transplantation could enhance expression of CXCR4 mRNA in rats of the IRI group, the expression of CXCR4 can be decreased by the anti-TGF-β1 antibody and the anti-CXCR4 antibody. TGF-β1 induced homing of MSCs in repair of renal ischemic reperfusion injury by regulating expression of CXCR4 on cell membranes. Blue fluorescence of DAPI-positive MSCs cells of renal parenchyma in the IRI+MSC group was enhanced significantly, which was significantly inhibited by anti-TGF-β1 and anti-CXCR4 antibody, and the inhibitory effect of anti-CXCR4 antibody was more obvious than that of anti-TGF-β1 antibody. Conclusion: Transforming growth factor-β1 promotes homing of bone marrow mesenchymal stem cells in renal ischemia-reperfusion injury, which will provide useful data on role of TGF-β1 in regulating SDF-1/CXCR4 axis-induced MSCs homing.

**Keywords:** Transforming growth factor-β1, homing, bone marrow mesenchymal stem cells, renal ischemia-reperfusion injury, SDF-1/CXCR4 axis

## Introduction

Acute ischemia reperfusion-induced kidney injury is a common cause of acute renal failure, and it is also an important cause of delayed recovery of transplanted kidney functions and even loss of function. However, there is no effective treatment method in clinical applications presently [1, 2].

Expression of local CXCR4 plays an important role in promoting stem cell migration, proliferation and injury recovery. Bone marrow mesenchymal stem cells (MSCs) can migrate directly into kidney after renal ischemia-reperfusion injury by SDF-1/CXCR4 axis, but the lower migrating rate restricted the application. CXCR4 is mainly expressed in cytoplasm of cells, and only a little CXCR4 is expressed on cell membrane [3]. Furthermore, expression of CXCR4 is decreased with prolonged cell culture time [4].

A variety of cytokines can stimulate the expression of membrane CXCR4. Our previous study has shown that TGF-β1 antibody can affect surface expression of CXCR4 in MSCs of homogenate of renal ischemia-reperfusion injury and reduced its tendency to SDF-1, and inhibited CXCR4 expression in renal tissues of rats, suggesting that TGF-β1 may promote MSCs homing

by influencing the expression of CXCR4, which has not been reported.

Bone marrow mesenchymal stem cells (MSCs) can play protective roles by homing directly to kidneys, in which SDF-1/CXCR4 axis is closely related to MSCs migration and homing [1]. SDF-1, known as CXCL12, is also called stromal cell derived factor-1, and it is a kind of chemoattractant protein produced by bone marrow stromal cells. CXCR4 is currently the only known receptor of SDF-1. CXCR4 is highly conserved with 7 transmembrane α-helixes, and it is also known as Fusin [2]. In recent years, it has been confirmed that there is existence of CXCR4 receptor on bone marrow MSCs, and MSCs can migrate along SDF-1 concentration gradient transfer and realize homing.

Although rapid progress has been made in repair of renal reperfusion injury ischemia by MSCs [5], lower survival rate of transplanted cells in ischemic tissues restricted its clinical application [6]. Particularly, low migration problem of MSCs in the transplanted tissues remains to be resolved. Due to the important role of SDF-1/CXCR4 axis in MSCs migration and homing, intervention of SDF-1/CXCR4 axis and promotion of MSCs homing are potential treatment methods. Local over-expression of CXCR4 improves mobilization and proliferation of stem cells, which is conducive to "navigation" and proliferation of hematopoietic stem cells. Expression of CXCR4 receptor is also important in improving stem cell migration and proliferation, and promoting damage repair [7]. Secreted SDF-1 by normal tissues is less. The level of SDF-1 is up-regulated greatly and induces homing of MSCs under conditions of inflammation, ischemia and hypoxia. Therefore, cell surface expression of CXCR4 may directly affect migration of MSCs [8, 9].

Although level of CXCR4 of MSCs in bone marrow is high, cell surface expression of CXCR4 on MSCs will decrease gradually with time going, and migration ability of MSCs to SDF-1 also is reduced [10]. Further researches in recent years have demonstrated that cell surface levels of CXCR4 on MSCs were enhanced by stimulation with insulin-like growth factor 1 and hypoxia inducible factor 1 and other cytokines. Correspondingly, migration ability of MSCs increases along with SDF-1α concentration gradients [11]. Therefore, induction and mobilization of cell membrane expression of CXCR4 on MSCs is more meaningful than that of the transgenic method.

As a secretory polypeptide signal molecule, TGF-β1 has extensive biological activity. In ischemia reperfusion renal injury, hypoxia directly activates TGF-β1, and expression of TGF-β1 in renal tissues is enhanced gradually with aggravation of damages. The expression of TGF-β1 will decrease gradually after repair. These data suggest that TGF-β1 is involved in repair of damage and it might be one of the most important mechanisms of repair and protection by organism itself [12]. Due to the similar biological characteristics of MSCs, tumor cells and mesenchymal cells and the overlapped molecular mechanism, it is hypothesized that TGF-β1 may mediate SDF-1/CXCR4 axis-induced MSCs homing.

In the present study, effects of TGF-β1 overexpression in MSCs on expression of CXCR4 and chemotactic effect to SDF-1 were investigated by *in vitro* transmembrane chemotaxis. Anti-TGF-β1 antibody was incubated with ischemia reperfusion injury renal tissue homogenate and effects of anti-TGF-β1 antibody were observed. In addition, effects of TGF-β1 gene transfection and anti-CXCR4 antibody treatment in MSCs on expression of SDF-1/CXCR4 axis of renal tissues and damage repair were further explored, which will provide useful data on role of TGF-β1 in regulating SDF-1/CXCR4 axis-induced MSCs homing.

## Materials and methods

### *Animals*

SPF male SD rats with bodyweight of about 180 g were purchased from experimental animal center of Wuhan University (Animal Certificate No.: SCXK (E) 2008-0004). For experiments involving animals, approval was obtained from the institutional review board of Zhongnan Hospital of Wuhan University.

### *Reagents*

Antibodies including anti-CD34-FITC, anti-CD29-FITC, anti-CD45-FITC and anti-CD105-FITC were from Bioled. Antibodies such as anti-Actin (15596-026) and anti-CXCR4 (C28025-011) were purchased from Invitrogen.

*Isolation and culture of bone marrow MSCs*

Adherent cell separation method and density gradient centrifugation method were used in the isolation of MSCs. Under aseptic conditions, bilateral femur was obtained from healthy male SD rats with 3 weeks. Osteoepiphysis in one side was cut off and washed twice with PBS. Bone marrow was washed with serum free L-DMEM culture medium and was poured into Percoll lymphocyte isolation liquid at a ratio of 1:1. After centrifugation, interface layer mononuclear cells were collected and cultured in 37°C, 5% $CO_2$ cell culture incubator for static culture. First time medium changing was conducted in 48 h to 72 h after inoculation. Cell suspension was discarded and cell culture medium was changed every other day. When cells grew to 80% confluence, cells were passaged.

MSCs were induced and differentiated into osteoblasts and adipocytes. Osteogenic and adipogenic induction was conducted on the P3 generation cells.

The separated MSCs in different stages were identified by flow cytometry with antibodies against surface markers such as CD34, CD45, CD29 and CD105.

*Preparation of ischemia reperfusion injury kidney rat models*

3% pentobarbital sodium (30 mg/Kg) intraperitoneal injection was used in anesthesia of male Wistar rats. Abdominal transverse incision was used to produce abdominal cavity in 8 week old male SD rats. Both renal pedicle was separated and was closed with no damage artery clip clamping for 40 min in both sides, and then the artery clip was opened. Reperfusion for 60 min was conducted for sterile nephrectomy. Renal cortex was cut into pieces in the clean workbench.

*Construction of TGF-β1 lentiviral vectors and gene transfection*

In order to amplify TGF-β1 gene, polymerase chain reaction products of pGC-FU and TGF-β1 plasmids were digested by restriction enzyme Agiv, and the digested products were ligated with T4 ligase. The ligated products were transformed into competent *Escherichia coli* cells. The main plasmid (lenti-CMV-TGF-β1-EGFP), helper plasmids (pHelper 1, pHelper 2) with the same volume of Lipofectamine 2000 were mixed and transfered to 293T cells to construct TGF-β1 lentiviral vector. The third generation MSCs grew close to 70-80% fusion was divided into two groups, the experimental group (MSCs-TGF-β1) with 10 μL lentiviral plasmid containing TGF-β1 and EGFP genes and the control group (MSCs-neo) with 10 μL lentiviral plasmid carrying EGFP gene. After transfection in 48 h, cells in each group were taken for determination of transfection efficiency by a fluorescence method. Expression of TGF-β1 in MSCs was detected by Western blot. Content of TGF-β1 was measured by ELISA.

*Grouping*

The present study included the following groups: the normal control group, the IRI group with tail intravenous infusion of 1 ml saline, the IRI+MSCs transfusion group in which the constructed IRI model was tail intravenous infused with 1 ml saline containing $4\times10^6$ MSCs 6~8 h after infusion, the IRI+MSCs+TGF-β1 neutralizing antibody infusion group in which the constructed IRI model was tail intravenous infused with 1 ml saline containing $4\times10^6$ MSCs 6~8 h after infusion and intraperitoneally injected with TGF-β1 antibody (mouse Monoclonal Anti-TGF-β1 Antibody, R&D Systems, Inc, Minneapolis, MN USA, 20 μg/day for continuous 5 days) and the IRI+MSCs+CXCR4 antibody group. In the IRI+MSCs+CXCR4 antibody group, the anti-CXCR4 monoclonal antibody (AMD3100, Sigma, Saint Louis, Missouri, USA, 10 μg/mL) was incubated with MSCs in 5% $CO_2$ incubator at 37°C for 2 h, and then the model IRI was tail intravenous infused with 1 ml saline containing $4\times10^6$ MSCs 6~8 h after infusion and intraperitoneally injected with CXCR4 antibody. The rats were killed at the third day and the seventh day for further analysis.

*The positive expression rate of CXCR4 receptor in MSCs*

Anti-CXCR4-PE monoclonal antibody was used to detect positive expression rate of CXCR4 receptor in MSCs of various groups by flow cytometry.

*MSCs CXCR4 mRNA analysis*

Expression of CXCR4 mRNA was detected by real-time quantitative PCR assay in various groups. Briefly, Trizol reagent (Invitrogen Life

**Table 1.** Primer sequences

| Genes | Primer sequences | Length of PCR product/bp |
|---|---|---|
| CXCR4 | 5'-GCTAACACTTACGCAAAGACAT-3' | 232 |
| | 5'-CGTGAAACA GACAAA CAACAG-3' | |
| EGF | 5'-AACACGGAGGGAGGCTACAAC-3' | 200 |
| | 5'-CGGTCCACGGATTCAACATAC-3' | |
| HGF | 5'-CAACGCAGATGGTTTATTACGAG-3' | 221 |
| | 5'-ATCCACGACCAGGAACAATGAC-3' | |
| TGF-β1 | 5'-CGCAACAACGCAATCTATG-3' | 204 |
| | 5'-ACCAAGGTAACGCCAGGA-3' | |
| Actin | 5'-CGTTGACATCCGTAAAGACCTC-3' | 110 |
| | 5'-TAGGAG CCAGGGCAGTAATCT-3' | |

Technologies, Carlsbad, USA) was employed to extract total RNA of MSCs in various groups according to the directions of the kit. 2 μg total RNA served as template, and the Revert Aid H Minus First Strand cDNA Synthesis Kit (Fermentas, Vilnius, Lithuania) was used to synthesize cDNA. SYBR Green PCR Master Mix (ABI, Foster City, USA) was used to conduct real time PCR on Step One Plus Real-Time PCRSystems (ABI). In each PCR reaction, 1 μL cDNA, 2×SYBR Green PCR Master and 100 nmol/L primers were used. Primers used in the study were illustrated in **Table 1**.

The PCR reaction systems were as follows: 94°C for 1 min in pre-denaturation, denaturation at 95°C for 15 s, annealing at 50~60°C for 15 s, and extension at 72°C for 45 s. There were 40 cycles in each PCR reaction. Related gene expressions were calculated by using the difference (ΔCt) of cycle threshold (Ct) of CXCR4 and β-actin RNA. The difference (ΔΔCt) of gene expression between samples was calculated (ΔCt). Multiple expression difference was $2^{-\Delta\Delta Ct}$.

*Transmembrane migration experiment in vitro*

Transwell chambers were put into 24 well plates. 20 μg Matrigel was added above filter membrane of chambers to form matrix layer. Concentrations of cell s with MSCs-TGF-β1 and MSCs-neo were adjusted to $3\times10^4$/mL, and 100 μL cell culture medium was added to upper chamber, and 600 μL 10% FCS-DMEM medium containing SDF-1α (100 ng/mL) was added to lower chamber. In negative controls, 600 μL 10% FCS-DMEM medium was added to lower chamber.

For the anti-CXCR4 antibody blocking group, 1~10 μg/mL anti-CXCR4 monoclonal antibody (MAB170/12G5) was mixed with MSCs-TGF-β1 or MSCs-neo in the 37°C 5% $CO_2$ incubator for 2 h, and then transwell migration experiment was conducted. IgG served as negative controls. After incubation in 37°C 5% $CO_2$ incubator for 12 h, cells on Matrigel and microporous membrane layer were wiped away by cotton swab gently to retain the cells migrated to the lower microporous membrane. The retained cells were fixed with absolute ethyl alcohol for 20 min and stained with DAPI. The cells migrated to the back microporous membranes were counted under fluorescence microscope.

*MSCs homing determination*

For determination of MSCs homing, renal tissues were immersed in 30% sucrose, for dehydration at 4°C for 24 h. The renal tissues were then embedded with Tissue-Tek OCT, and frozen sections were prepared. Fluorescence expression of sections was used to conduct quantitative analysis, to observe migration and colonization of MSCs under fluorescence microscopy.

*Determination of survival and renal function indices*

On the first day, the third day, the seventh day and the fourteenth day, the rats were sacrificed and blood was collected. Urine in metabolism cages was collected for further analysis. Blood urea nitrogen (BUN) and serum creatinine (Scr) were detected by dry chemistry method. Urinary N-acetyl-β-D-Glucosaminidase (NAG) was detected by nitrophenol colorimetric method.

*Renal function and renal tubular necrosis (ATN) determination*

According to degree of renal tubular necrosis, the semi-quantitative pathological score evaluation method of Hauet et al. was used: higher score indicates more severe renal tubular necrosis (maximum 4 points). 0 point indicates normal kidney. 1 point indicates slight damage of kidney (<25% tubular involvement). 2 points indicate mild necrosis of kidney (25%~50% tubular involvement). 3 points indicate moderate necrosis of kidney (50%~75% tubular involvement). 4 points indicate severe necrosis of kidney (>75% renal involvement). More than three different regions were chosen to conduct

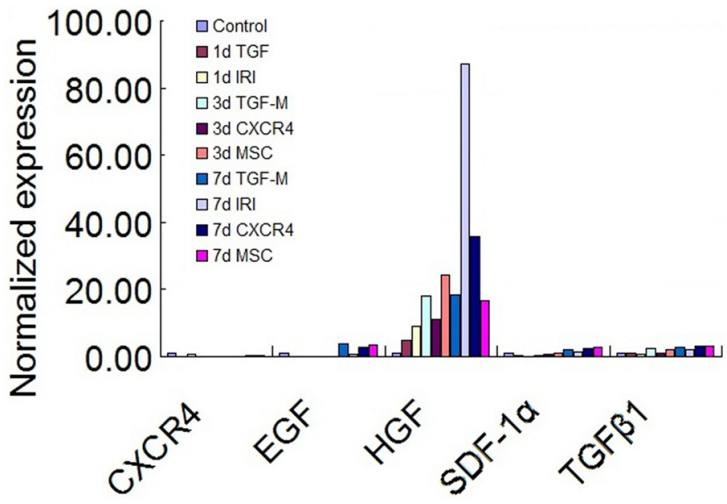


**Figure 1.** Gene expression analysis. Expression of TGF-β1, CXCR4, EGF and HGF mRNA in IRI group increased significantly, which can be inhibited by anti-TGF-β1 antibody and the anti-CXCR4 antibody.

**Table 2.** Expression of TGF-β1 and CXCR4 mRNA in different groups detected by real-time PCR (7d)

| Group | TGF-β1 $2^{-\Delta\Delta Ct}$ | CXCR4 $2^{-\Delta\Delta Ct}$ |
|---|---|---|
| Control | 1 | 1 |
| IRI | 3.26±0.79 | 1.14±0.17 |
| IRI+MSC | 2.82±0.88 | 2.51±0.74 |
| IRI+MSC+TGF | 0.93±0.07 | 1.44±0.36 |
| IRI+MSC+CXCR4 | 2.58±0.63 | 0.84±0.15 |

scoring under optical microscope (400×) in 10 different fields.

*Histological and damage scoring*

Thickness of paraffin section was 2 μm. After conventional dewaxing and hydration, HE and PAS staining were used in sample preparation. Renal tubular damage was determined by percentage score calculation. Numbers of kidney tubules with damage including tubular dilation, atrophy, tubular, exfoliated cells, necrosis and vasculitis in renal cortex and medulla externa of Twenty 400× visual fields in each sample were counted, which were expressed as percentage and average value was taken.

*Real-time quantitative PCR method for detection of expression of EGF and HGF mRNA of renal tissues*

Total RNA of cortex renis was extracted by Trizol agent (Invitrogen Life Technologies, Carlsbad, USA). cDNA was synthesized according to the Revert Aid H Minus First Strand cDNA Synthesis Kit (Fermentas, Vilnius, LTU). 2 μg RNA templates were applied. Using SYBR Green PCR Master Mix (ABI, Foster, USA), real time PCR was carried out and products were detected on Step One Plus Real-Time PCR Systems (ABI, Foster, USA). A concentration of 100 nM for primers was chosen for the PCR system. β-actin was the interior reference. The PCR was performed for 40 cycles at 95°C for 15 s, 58°C for 15 s and 72°C for 45 s. The $2^{-\Delta\Delta Ct}$ method was used for the relative quantity analysis of data.

*Statistical methods*

SPSS 13.0 statistical software was used in statistical analysis. The data obtained were presented as mean ± standard deviation. The relationship between two factors was analyzed by Pearson correlation analysis. A $P<0.05$ indicated that the difference was statistically significant. A $P<0.01$ indicated that the difference was extremely and statistically significant.

## Results

*Changes of TGF-β1 and CXCR4 in nephridial tissues*

As shown in **Figure 1** and **Table 2**, expression of TGF-β1 mRNA in the IRI group increased significantly, which can be prevented dramatically by anti-TGF-β1 antibody. MSCs transplantation could enhance expression of CXCR4 mRNA in rats of the IRI group, which can be decreased by the anti-TGF-β1 antibody and the anti-CXCR4 antibody dramatically.

Real-time PCR results revealed that expression of CXCR4 mRNA in the ischemic reperfusion kidney injury homogenate group was significantly up-regulated. With extension of time, the anti-CXCR4 antibody and anti-TGF-β1 antibody could gradually down-regulate CXCR4 mRNA expression.

Expression of CXCR4 was increased in the IRI group. MSCs transplantation could significantly increase the expression of CXCR4 of rats in the IRI group, and anti-TGF-β1 antibody and anti-

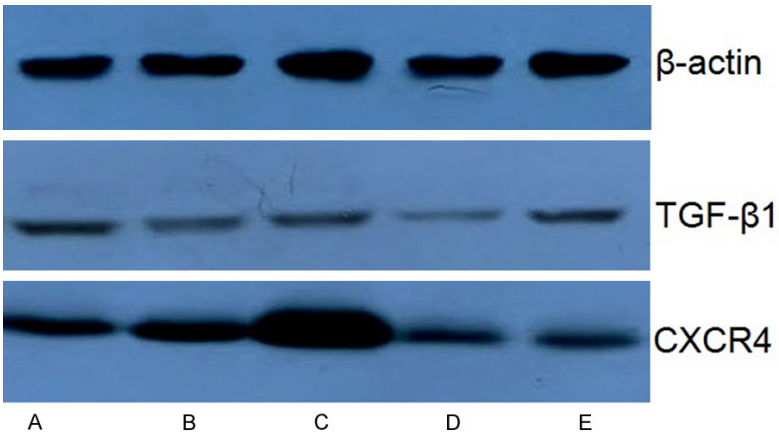


**Figure 2.** Expression of TGF-β1 and CXCR4 of nephridial tissues by Western-blotting method. A: Normal control group; B: IRI group; C: IRI+MSC group; D: IRI+MSC+TGF group; E: IRI+MSC+CXCR4 group. Expression of TGF-β1 and CXCR4 in IRI group increased significantly, which can be inhibited by anti-TGF-β1 antibody and the anti-CXCR4 antibody.

**Table 3.** Expression of EGF and HGF mRNA in different groups detected by real-time PCR (7d)

| Group | EGF $2^{-\Delta\Delta Ct}$ | HGF $2^{-\Delta\Delta Ct}$ |
|---|---|---|
| Control | 1 | 1 |
| IRI | 2.61±0.92 | 9.06±1.57 |
| IRI+MSC | 3.69±1.33 | 11.03±2.74 |
| IRI+MSC+TGF | 1.58±0.67 | 4.99±1.82 |
| IRI+MSC+CXCR4 | 1.22±0.59 | 2.78±0.86 |

CXCR4 antibody could dramatically inhibit the expression of CXCR4.

*Western-blotting results*

The western-blotting results were shown in **Figure 2**. It showed the similar results as that of RT-PCR. CXCR4 was increased in the IRI group. MSCs transplantation could significantly increase CXCR4 in the IRI group, and anti-TGF-β1 antibody and anti-CXCR4 antibody could dramatically inhibit the expression of CXCR4. These results suggested that TGF-β1 induced homing of MSCs in repair of renal ischemic reperfusion injury by regulating expression of CXCR4 on cell membranes, which can be inhibited by the anti-CXCR4 antibody.

*Expression of EGF and HGF mRNA in different groups*

The expression levels of EGF and HGF mRNA in different groups were shown in **Table 3**. It showed that expression of EGF and HGF mRNA in IRI group increased significantly especially HGF, which can be inhibited by anti-TGF-β1 antibody and the anti-CXCR4 antibody.

*Histological and damage scoring*

The immuhistochemical results were shown in **Figures 3** and **4**. Renal cortical tubular structures in the IRI group almost disappeared. Renal tubular epithelial cell demonstrated brush border flat, shrinkage, shedding, hyperchromatic nuclei and bare morphology. There was necrosis, shedding in the cells, and basement membrane was not complete. Renal tubular structure in distal parts was completely destroyed. There were lots of inflammatory cell infiltrations in renal interstitium. MSCs cell transplantation could significantly improve damage of renal tubules, which was delayed dramatically by the anti-TGF-β1 antibody and the anti-CXCR4 antibody.

*MSCs homing*

Nuclei of DAPI-positive MSCs cells showed blue fluorescence (**Figure 5**). There was blue fluorescence phenomenon around renal vascular walls in the IRI group in 3 days, indicating there was MSCs migration. Blue fluorescence of renal parenchyma in the IRI+MSC group was enhanced significantly, indicating that MSCs migration increased dramatically, which was significantly inhibited by anti-TGF-β1 and anti-CXCR4 antibody, and the inhibitory effect of anti-CXCR4 antibody was more obvious than that of anti-TGF-β1 antibody.

*Renal function and renal tubular necrosis (ATN) determination*

The results were shown in **Table 4**. It showed that BUN and Scr increased in IRI group while they were inhibited by the anti-TGF-β1 antibody and the anti-CXCR4 antibody.

## Discussion

Acute ischemia reperfusion-induced kidney injury is a common cause of acute renal failure,

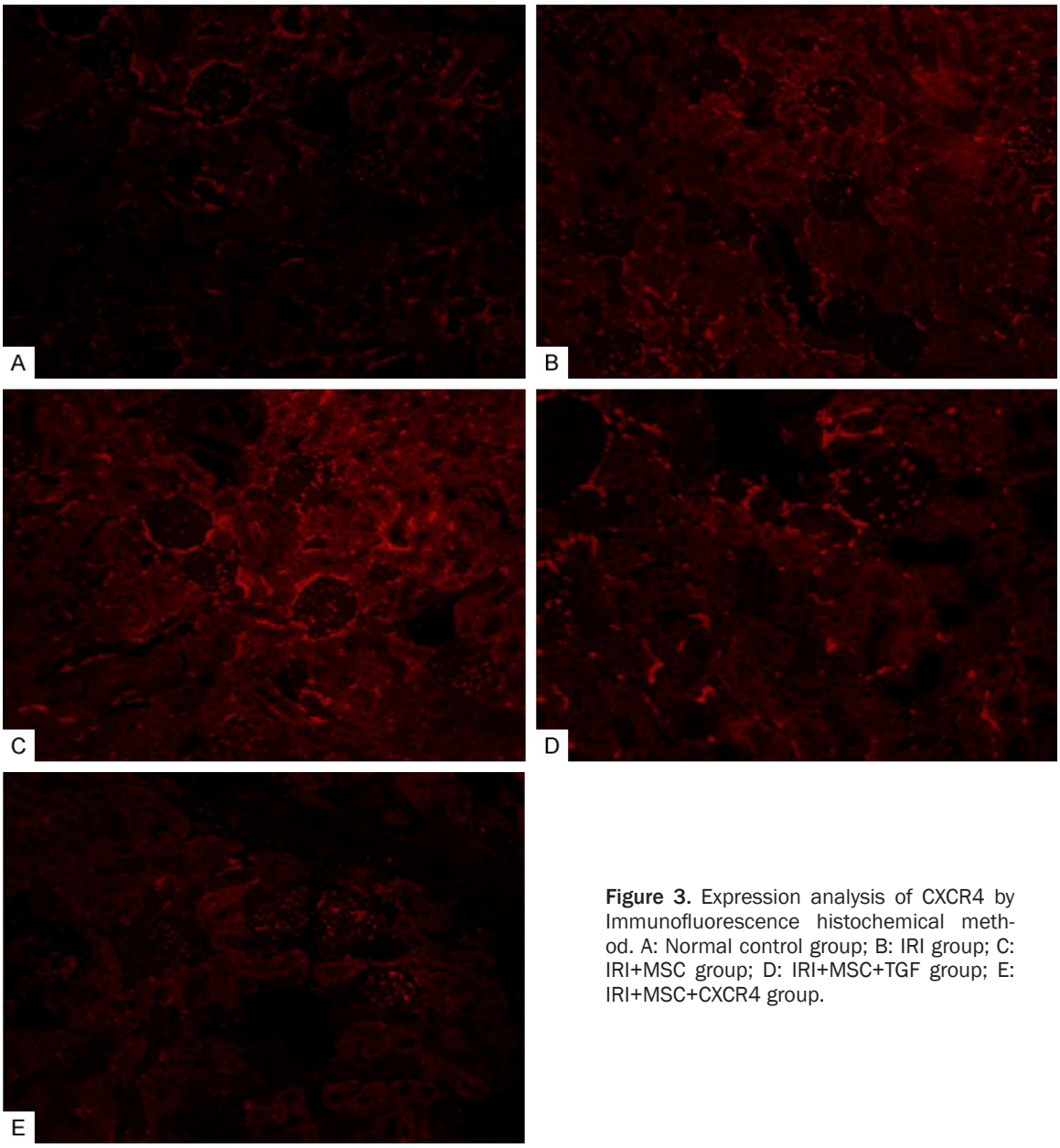


**Figure 3.** Expression analysis of CXCR4 by Immunofluorescence histochemical method. A: Normal control group; B: IRI group; C: IRI+MSC group; D: IRI+MSC+TGF group; E: IRI+MSC+CXCR4 group.

and it is also an important cause of delayed recovery of transplanted kidney functions and even loss of function. However, there is no effective treatment method in clinical applications presently. Effects of TGF-β1 over-expression in MSCs on expression of CXCR4 and chemotactic effect to SDF-1 were investigated by *in vitro* transmembrane chemotaxis. Anti-TGF-β1 antibody was incubated with ischemia reperfusion injury renal tissue homogenate and effects of anti-TGF-β1 antibody were observed. In addition, effects of TGF-β1 gene transfection and anti-CXCR4 antibody treatment in MSCs on expression of SDF-1/CXCR4 axis of renal tissues and damage repair were further explored.

We found in preliminary work that chemotaxis of MSCs to SDF-1 was enhanced in the homogenate of renal injury in ischemia reperfusion, which can be blocked by the anti-TGF-β1 antibody. In renal ischemia reperfusion injury model, the anti-TGF-β1 antibody can inhibit CXCR4 expression in renal tissue. The results suggested that TGF-β1 may be involved in the MSCs homing in repair of acute kidney injury, and may promote MSCs homing by influencing

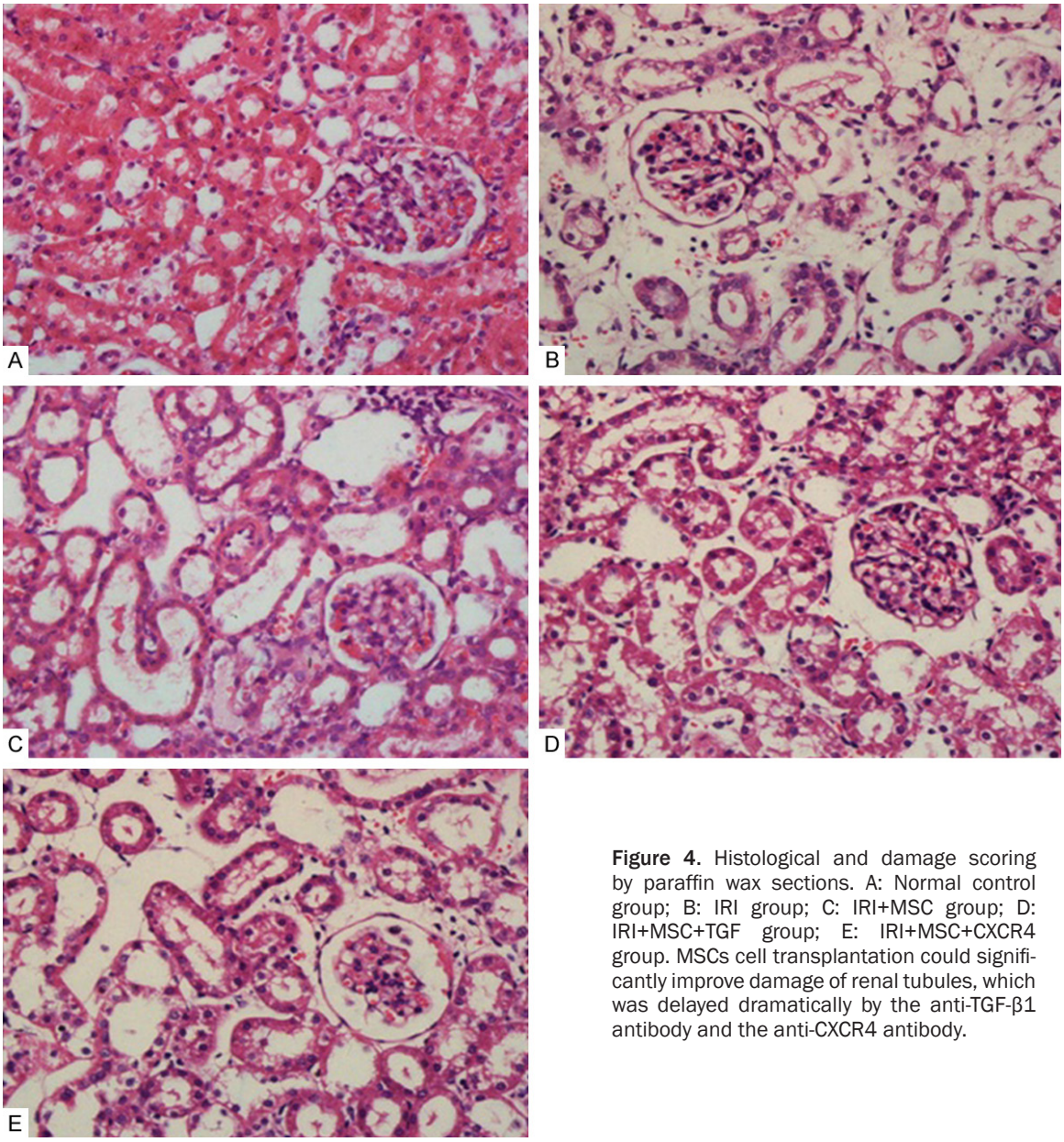


**Figure 4.** Histological and damage scoring by paraffin wax sections. A: Normal control group; B: IRI group; C: IRI+MSC group; D: IRI+MSC+TGF group; E: IRI+MSC+CXCR4 group. MSCs cell transplantation could significantly improve damage of renal tubules, which was delayed dramatically by the anti-TGF-β1 antibody and the anti-CXCR4 antibody.

the expression of CXCR4. However, the exact relationship between TGF-β1 and SDF-1/CXCR4 axis remains to be explored.

Tang [3] observed the effects of different concentrations of SDF-1α on migration of bone marrow MSCs by *in vitro* migration experiments, and they found that the migration ability of MSCs was enhanced with increasing concentration of SDF-1α, which was significantly decreased by CXCR4 inhibitor AMD3100 treatment. Toggle et al. [4] also observed in ischemic acute renal failure model that expression of SDF-1 in ischemic group increased significantly compared with that of the normal rats, at the same time migration of CXCR4-positive bone marrow stem cells into ischemic kidney increased. Yue Zhang [8] transfected CXCR4 into MSCs to increase the surface CXCR4 protein expression, and MSCs chemotaxis was also enhanced. However, under normal circumstances, there was only a small part of MSCs membrane expression of CXCR4, and CXCR4 mRNA and CXCR4 antigen expression (83%~98%) mainly were present in inside of cells [9].

Activation of TGF-β1 showed strong chemotaxis on monocytes, neutrophils, fiber cells, mesen-

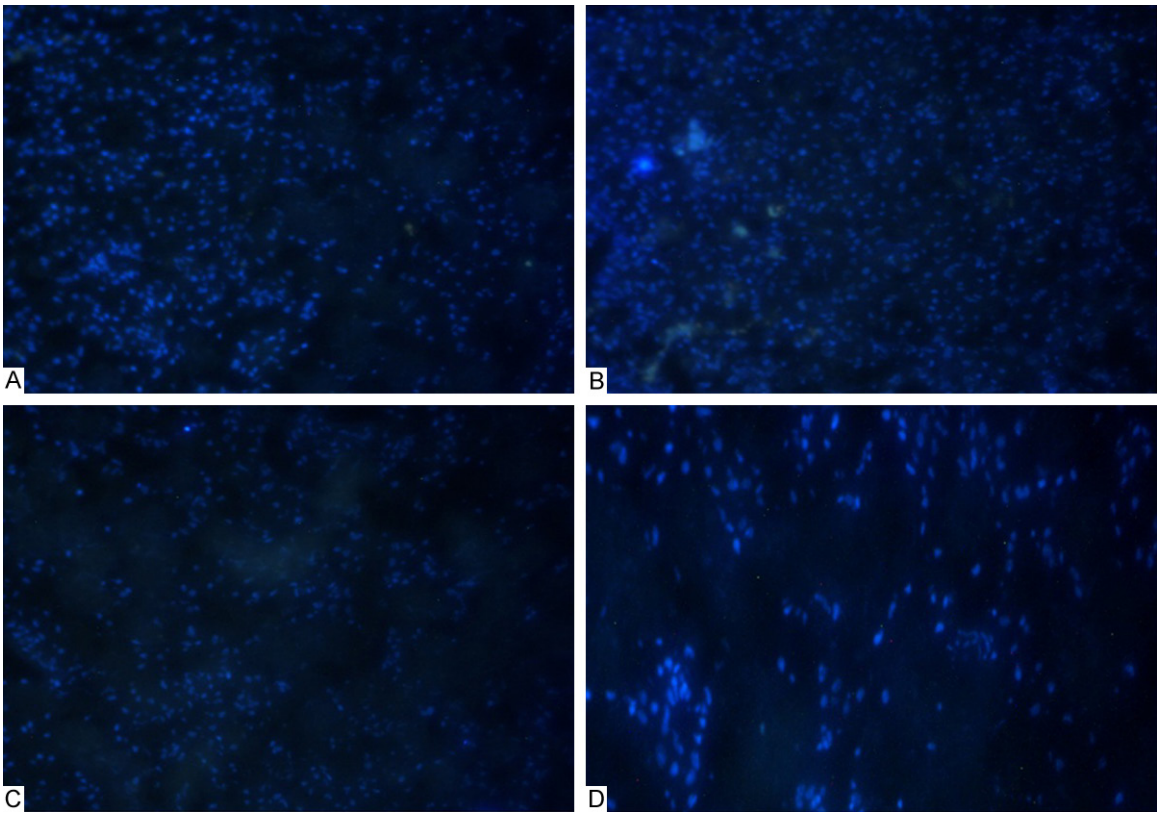


**Figure 5.** MSCs homing determination. A: IRI group; B: IRI+MSC group; C: IRI+MSC+TGF group; D: IRI+MSC+CXCR4 group. Blue fluorescence of renal parenchyma in the IRI+MSC group was enhanced significantly.

**Table 4.** Comparisons of Renal function and ATN scoring

| Groups | BUN | Scr | ATN scoring |
|---|---|---|---|
| Normal control group | 5.84±0.38 | 16.22±5.17 | 0±0 |
| IRI | 20.76±1.79[a,b] | 79.06±11.28[a,b] | 2.75±0.21[a,b] |
| IRI+MSC | 10.60±1.15[a] | 30.57±11.73[a] | 1.08±0.14[a] |
| IRI+MSC+TGF-β1 | 14.47±0.81[a,b] | 57.85±19.53[a,b] | 1.63±0.11[a,b] |
| IRI+MSC+CXCR4 | 15.24±1.30[a,b] | 61.99±20.21[a,b] | 1.76±0.14[a,b] |

Compared with that of the normal control group, [a]$P<0.05$. Compared with that of the IRI group, [b]$P<0.05$.

chymal cells. These cells can produce and secret more TGF-β1, which acted on fiber cells of the tissue damage and enhanced large scale synthesis of I, III collagen, fibronectin, elastin proteins, integrins, proteoglycan and extracellular matrix components, which were main factors in increasing enhancing renal tubular regenerations [13]. Zheng [14] confirmed that TGF-β1 could also induce and chemotaxis MSCs, and enhancement of TGF-β1 on MSCs migration was dose dependent. Low concentration of TGF-β1 (0.5~2 μg/L) can promote the migration of MSCs, while high concentration of TGF-β1 (5~10 μg/L) inhibited MSCs migration. Wright [15] found that the expression of TGF-β1 regulated bone marrow SDF-1 expression in mRNA level and protein level, and affected hematopoietic stem cell migration and adhesion. Jeon ES [16] induced SDF-1 expression of human adipose derived mesenchymal stem cells with lysolecithin acid and found that the expression could be decreased by TGF-β1I receptor inhibitor SB431542 or anti-Smad antibody, suggesting that TGF-β1-Smad signaling pathway may mediate SDF-1 expression of adipose derived mesenchymal stem cells induced by hemolytic lecithin acid. The data indicate that TGF-β1 and SDF-1/CXCR4 axis have similar chemotaxis in the migration and homing of MSCs. Prostate cancer patient fibroblasts over-expressed TGF-β1 and SDF-1, and increased level of TGF-β1 up-regulated surface expression of CXCR4 of prostatic carcinoma cells, suggesting that TGF-β1, SDF-1 and CXCR4 may have synergistic

reactions in enhancing tumorigenesis [17-19]. However, whether TGF-β1 induces homing of MSCs by SDF-1/CXCR4 remains to be investigated. Therefore, the question was addressed in the present study.

The present study demonstrated that expression of TGF-β1 mRNA in the IRI group increased significantly, and MSCs transplantation could enhance expression of CXCR4 mRNA in rats of the IRI group. Expression of CXCR4 was increased in the IRI group. MSCs transplantation could significantly increase the expression of CXCR4 of rats in the IRI group. TGF-β1 induced homing of MSCs in repair of renal ischemic reperfusion injury by regulating expression of CXCR4 on cell membranes. Blue fluorescence of renal parenchyma in the IRI+MSC group was enhanced significantly, which was significantly inhibited by anti-TGF-β1 and anti-CXCR4 antibody, and the inhibitory effect of anti-CXCR4 antibody was more obvious than that of anti-TGF-β1 antibody.

In order to further investigate whether cytokines could influence MSCs migration in injured renal tissues, the relationship between HGF and EGF expression and the migration activity of rab MSCs by using real-time PCR analysis. It has been confirmed that thrombin, insulin growth factor (IGF-I), platelet-derived growth factor (PDGF)-BB, fibroblast growth factor (FGF-2), HB-EGF, transforming growth factor (TGF-α), PDGF-AB, hepatocyte growth factor (HGF) and epidermal growth factor (EGF) increased the migration of rab MSCs under various conditions. Effects of TGF-β1 and CXCR4, and MSCs transplantation on secretion of HGF and EGF were also explored in the present study. Results revealed that both transfection of TGF-β1 and MSCs transplantation played important roles in mRNA expression of HGF and EGF, suggesting that growth factors play critical roles in inducing maximal chemotactic effects, which may also increase MSCs migration by proliferation, indicating that proliferation and migration can play synergistic roles at the same time.

In conclusion, transforming growth factor-β1 promotes homing of bone marrow mesenchymal stem cells in renal ischemia-reperfusion injury, which will provide useful data on role of TGF-β1 in regulating SDF-1/CXCR4 axis-induced MSCs homing.

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## Disclosure of conflict of interest

None.

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