Transplantation of endothelial progenitor cells alleviates renal interstitial fibrosis in a mouse model of unilateral ureteral obstruction

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Abstract

Aims: The present study investigated whether transplantation of bone marrow-derived endothelial progenitor cells (BM-EPCs) in renal capillary network improves renal interstitial fibrosis in unilateral ureteral obstruction (UUO) model in mice.

Keywords: Endothelial progenitor cell Transplantation Renal interstitial fibrosis Unilateral ureteral occlusion Peritubular capillary density

Introduction

Renal fibrosis is regarded as the final common pathway for most forms of progressive renal disease, and involves glomerular sclerosis and/or interstitial fibrosis. Increasing evidence shows that the microvasculature plays a critical role in progressive renal disease (Kang et al. 2001a,b, 2002; Sun et al. 2006), and loss of capillaries would result in impaired delivery of oxygen and nutrients to the tubules and interstitial cells, which will induce chronic ischemia (Kang et al. 2001a). We hypothesized that the use of some potent angiogenic factors might represent a novel therapeutic strategy to alleviate the development of renal fibrosis and combat progressive renal disease.

In 1997, Asahara et al. (1997) identified endothelial progenitor cells (EPCs). EPCs are thought to be derived from the bone marrow and circulate in the peripheral blood, and to play a crucial role in the repair or formation of blood vessels (Asahara et al. 1997; Rosenzweig 2003). On the basis of the observation that EPCs are mobilized in response to tissue injuries, it is believed that they have therapeutic potential by aiding in the neovascularization of ischemic tissue (Shintani et al. 2001; Wojakowski et al. 2004; Massa et al. 2005). We hypothesized that neovascularization in the kidney could result in an increasing microvessel density in ischemic renal tissue and reduce the development of renal disease. Thus, neovascularization therapy in the kidney has the potential to be a new therapeutic option or supplemental therapy for renal fibrosis.

Unilateral ureteral obstruction (UUO) is a well-established experimental model for the study of the mechanisms of renal interstitial fibrosis and for evaluation of potential therapeutic approaches to ameliorate the fibrosis (Klahr and Morrissey 2002). UUO in rats and mice produces tubulointerstitial inflammation and fibrosis, which duplicates the human conditions produced by obstructive nephropathy (Hruska 2002; Moller et al. 1984). In the current study, we investigated whether transplantation of bone marrow-derived endothelial progenitor cells (BM-EPCs) in renal capillary network improves renal interstitial fibrosis in UUO model in mice.
Materials and methods

Culture of EPCs

Isolation of EPCs was performed mainly according to the method previously reported (Asahara et al. 1997). Briefly, BM-EPCs obtained by flushing the femurs of mice were isolated by density gradient method using Ficoll-Paque Plus (Buckinghamshire, UK). Cells were plated in disposable culture flasks coated with fibronectin and cultured in endothelial cell basal medium-2 (EBM-2) supplemented with microvascular endothelial cell growth medium-2 (EGM-2MV). The initial seeding density was standardized at 1 × 10^4/ml. After 3 days of culture, nonadherent cells were removed and new medium was supplied. The culture was maintained until 70–80% cell confluence. After 14 days culture, EPCs recognized as attaching spindle-shaped cells, were assayed by co-staining with 1, 1′-dioctadecyl-3, 3′, 3′-tetramethylindocarbocyanine perchlorate-labeled acetylated low-density lipoprotein (Dil-Ac-LDL) and fluorescein isothiocyanate-conjugated ulex europaeus lectin (HTC–UEA-I), characteristics of endothelial lineage. To confirm endothelial cell phenotype of EPCs, cells were also simultaneously stained with the following primary antibodies: vascular endothelial growth factor receptor (KDR, 1:20, R&D), platelet endothelial cell adhesion molecule-1 (CD31, 1:100; BosterBiotechnology) and platelet/endothelial cell adhesion molecule (CD34, 1:100, BosterBiotechnology). FITC and biotin-linked secondary antibodies were used. Label with carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) was used to track the fate of injected cells. Renal tissues were harvested at 1, 3, 7 and 14 days after EPCs transplantation. Frozen renal sections (5 µm each) were examined under fluorescence microscope.

Animal model

All animal procedures were conducted after approval of the protocol by the Xuzhou medical college Animal Centre Committee. Kunming mice (25–40 g) of both sexes were kept at constant temperature and light conditions and fed standard mouse chow. They had free access to water and food, at 22 ± 1 °C under a 12:12 h light: dark cycle. The mice were randomized into three groups: normal control group (saline solution, n = 24), UUO group (UUO + saline solution, n = 24) and EPCs group (UUO + EPCs, n = 24). The UUO model (UUO and EPCs group) was performed using an established procedure (Masaki et al. 2003). In brief, before pentobarbital-induced anesthesia, the left ureter was ligated with silk (4/0) at two locations and cut between ligatures to prevent urinary tract infection. Mice in control group underwent no surgical manipulation.

Recipient mice were irradiated with a nonlethal dose (5 Gy) before surgery. After undergoing surgery, mice in EPCs group received a vena caudalis injection of 2 × 10^6 EPCs labelled with CFDA-SE. The other animals received a vena caudalis injection of the same volume of 0.9% saline solution. Six mice were killed at 1, 3, 7 and 14 days after vena caudalis injection in each group, and blood was collected for measurement of blood urea nitrogen (BUN) and serum creatinine (Scr), respectively. The left kidney was removed and dissected into two parts, and the right kidney from EPCs group was also removed as the contralateral control. One part was fixed in 10% formaldehyde for pathological examination and immunohistochemical detection, and the other part was reserved in liquid nitrogen for western blotting assay.

Histology and immunohistochemical staining

Formalin-fixed and paraffin-embedded kidney tissues were cut into 4-µm sections. Then sections were stained with haematoxylin–eosin and Masson's trichrome stains (Moriyama et al. 1998). Mouse anti-vascular endothelial growth factor (VEGF) monoclonal antibody (1:100, Santa Cruz), Rabbit anti-hypoxia inducible factor-1α (Hif-1α) polyclone antibody, Rabbit anti-connective tissue growth factor (CTGF) polyclone antibody (1:50, Boster Biotechnology), Rabbit anti-CD31 polyclone antibody (1:50, Boster Biotechnology), Rabbit anti-transforming growth factor-β1 (TGF-β1) polyclone antibody (1:50, Boster Biotechnology) were used for immunohistochemical procedures and the procedures were performed using the PV6001/PV6002 and DAB Kit (Zhongshan, China) according to manufacturer's instructions. Nonspecific staining was determined by excluding the primary antibodies. Haematoxylin was used for counterstaining procedures. Ten discontinuous visual fields of kidney tissues were randomly selected in the section. The integrated optical density (IOD) total of each visual field was determined using Image 6 Pro Plus system (Media Cybernetics, USA) (Sun et al. 2006). The area of fibrotic lesion in kidney was determined on sections stained by Masson trichrome to stain the collagenfiber (stained in mark blue). Under higher-power magnification (× 40), 10 discontinuous visual fields of kidney tissues were randomly selected in the section. The fibrotic area stained in mark blue were picked up on the digital images, and the percentage of the fibrotic area relative to the whole area of the field was calculated, glomeruli and large vessels were not included for image analysis.

Capillary density

Tissue samples stained for CD31 expression were examined with light microscopy at 40× magnification. Peritubular capillary density was estimated using an adaptation of previously published methods (Ohashi et al. 2000). Peritubular capillaries (PTCs) positively stained for CD31 (endothelial cell-specific marker) were counted in five randomly chosen microscopic fields on each slide, and capillary density was presented as the average number of capillaries/0.065 mm².

Western blotting assay

Renal tissues lysed in RIPA (Bytotime, China) on ice were centrifuged at 10,000 rpm for 10 min at 4 °C. Protein samples (100 µg protein/lane) were electrophoresed through a 7% polyacrylamide gel and then transferred to nitrocellulose membranes (Amersham SA, France). The membranes were blocked in 5% skim milk powder for 1 h, and then incubated overnight at 4 °C with primary monoclonal mouse anti-VEGF (1:100), rabbit anti-CTGF antibody (1:100) or rabbit anti-Hif-1α antibody (1:100). The immune complexes were detected by using an alkaline phosphatase conjugated second antibody (Zhongshan, China) and a BCIP/NBT Alkaline Phosphatase Color Development Kit (Beyotime, China). Positive immunoreactive bands were quantified densitometrically and normalized by actin.

Statistics

Data are expressed as mean ± SD, and statistical analyses were performed with SPSS 13.0. Comparisons between groups were analyzed via ANOVA followed by a Dunnett's post hoc test and p < 0.05 was considered as statistically significant.

Results

Culture, characterization and transplantation of EPCs

Culture of total BM-EPCs resulted in the emergence of characteristic spindle-shaped cells (Fig. 1A) and colonies consisting of peripheral spindle-shaped cells emanating from round central (Fig. 1B). These colonies gradually disappeared and were replaced by another cell population displaying a cobblestone morphology. These cells were expanded from a few cells to a monolayer and showed multiple population doublings without senescence, typical characteristics for late EPCs (Fig. 1C). After 14 days of culture, these
cells were positively stained with CD31, KDR and CD34, characteristic markers of endothelial cells (Fig. 1D, E, F). We further defined that double positive cells for Dil-ac-LDL and FITC-UEA-I, which were reported as endothelial cell lineage markers, were EPCs (Fig. 1G, H, I). Thus, the immunohistochemical characterization clearly revealed that the isolated EPCs are endothelial lineage cells. In order to track the fate, we labelled administered cells with CFDA-SE (Fig. 1J). After transplantation, the frozen sections of renal tissues were observed under fluorescence microscope (Fig. 1K, L). CFDA-SE-positive cells were found in the tubular epithelial lining in the obstructed kidney, but not in the contralateral unobstructed kidney. CFDA-SE-positive cells could also be seen at 14 days after BM-EPCs transplantation (Fig. 1L). This finding indicated that injected BM-EPCs transplanted properly into the renal tissues.

Changes in the renal morphology and renal function

The present study showed that there was no significant histological abnormality in control group (Fig. 2A, D). Cellular swelling could be observed in part of the tubular epithelial cells at 1 day after surgery and vacuole degeneration, tubular expansion, and leucocyte infiltration could be detected at day 3 after surgery in UUO group. Tubular atrophy,
more infiltration of leucocytes, the expanded tubular interstitial volume, and the over accumulation of the extracellular matrix occurred at 7 days after the surgery. The interstitial area in UUO group was significantly higher than that in control group and in EPCs group (Fig 2G), respectively. Fibrosis could be detected in the renal interstitial area at day 7 and was more severe at day 14. EPCs transplantation could significantly inhibit tubulointerstitial fibrosis ($p<0.05$). A–C, HE staining; D–F, Masson staining. Values are means±SD. *$p<0.05$ vs control group. #$p<0.05$ vs UUO group.

**Changes in the PTCs**

PTCs were easily and accurately identified in control group because immunostaining for CD31 clearly delineated their endothelial lining. The PTCs of control group were patent, displayed uniform size and shape, and were regularly arranged in most of the interstitium (Fig. 3A). There was a significant reduction in the density of PTC from day 3 after surgery and this reduction was more prominent at day 14 in UUO group. The reduction in capillaries was particularly prominent in areas of interstitial expansion and tubular atrophy (Fig. 3B). There was no significant difference in the density of PTC between UUO group and EPCs group at day 1 after EPCs transplantation, however,

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>BUN (mmol/l)</th>
<th>UUO (n=6)</th>
<th>EPCs (n=6)</th>
<th>Scr (μmol/l)</th>
<th>UUO (n=6)</th>
<th>EPCs (n=6)</th>
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<tr>
<td></td>
<td>Con (n=6)</td>
<td>UUO (n=6)</td>
<td>EPCs (n=6)</td>
<td>Con (n=6)</td>
<td>UUO (n=6)</td>
<td>EPCs (n=6)</td>
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<tr>
<td>1</td>
<td>5.61 ± 0.58</td>
<td>6.00 ± 0.10</td>
<td>5.80 ± 0.18</td>
<td>35.18 ± 1.59</td>
<td>37.41 ± 1.31</td>
<td>35.5 ± 1.61</td>
</tr>
<tr>
<td>3</td>
<td>5.61 ± 0.49</td>
<td>5.90 ± 0.16</td>
<td>5.68 ± 0.50</td>
<td>34.20 ± 2.29</td>
<td>35.26 ± 2.94</td>
<td>36.57 ± 1.90</td>
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<tr>
<td>7</td>
<td>5.68 ± 0.37</td>
<td>5.54 ± 1.73</td>
<td>5.63 ± 0.28</td>
<td>35.41 ± 1.83</td>
<td>36.15 ± 2.69</td>
<td>34.66 ± 2.75</td>
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<td>14</td>
<td>5.57 ± 0.16</td>
<td>5.80 ± 0.33</td>
<td>5.96 ± 0.12</td>
<td>33.68 ± 3.88</td>
<td>35.94 ± 3.50</td>
<td>34.19 ± 1.04</td>
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There were no significant differences among three groups in BUN and Scr at each time point, respectively ($p>0.05$).
the PTC density in EPCs group was significantly increased at 3, 7 and 14 days, respectively (Fig. 3C, D). The results showed that BM-EPCs transplantation improved angiogenesis in obstructive nephropathy in mice.

**Immunostaining and Western Blotting for HIF-1α**

In control group, there was faint staining of HIF-1α in cortical nuclei, predominantly in distal tubules (Fig. 4A). No nuclear staining was seen in the medulla or papilla. There were no significant differences in the expression of HIF-1α among 3 groups at day 1. In UUO group, the HIF-1α expression was increased in fibrotic areas and tubular epithelial cells at day 3, and further increased at day 7 and 14 (Fig. 4B) than control group and EPCs group, respectively (Fig. 4D). The expression of HIF-1α was induced progressively throughout the time course. After EPCs transplantation, from day 3 onwards, HIF-1α staining was apparently generally decreased in tubules, especially in areas of atrophy and fibrosis (Fig. 4C). Western blotting test showed that the level of HIF-1α protein was decreased in EPCs group at day 3 compared with UUO group and remained low level at day 7 and 14 (Fig. 4E), which was in accord with the results of the immunohistochemistry. These results suggested that the level of HIF-1α protein was decreased and hypoxia was ameliorated after transplantation of BM-EPCs.

**Immunostaining and Western Blotting for VEGF**

VEGF was most prominent in glomerular podocytes and in tubular epithelial cells in control group (Fig. 5A). The VEGF staining was markedly increased at day 1 in UUO group and in EPCs group than in control group, respectively. However, the VEGF staining was gradually reduced, particularly in areas of tubular damage or atrophy and the reduction was significantly more prominent in UUO group than in EPCs group (Fig. 5B, C). There was a significant reduction in VEGF levels in both UUO and EPCs groups at day 3, 7 and 14 compared to control group but the VEGF protein expression in EPCs group was obviously enhanced in mice of EPCs group compared to mice in UUO group (Fig. 5D). The protein expression of VEGF by western blotting showed the similar trends with the results of the immunohistochemistry. These data clearly indicated that the expression of VEGF protein was markedly increased in EPCs group after BM-EPCs transplantation (Fig. 5E).

**Immunostaining and Western Blotting for CTGF and TGF-β1 immunostaining**

In control group, faint CTGF staining could be seen in the glomerular cells and interstitial cells (Fig. 6A). However, markedly increased immunostaining of CTGF was detected in the interstitial cells and in the tubular epithelial cells, interstitial fibrotic regions and glomerulosclerosis in UUO group (Fig. 6B). Expression of CTGF began to increase from 7 days after UUO and reached a higher level at 14 days after UUO. Though there was no significant difference in the expression of CTGF at day 1 and 3 between UUO and EPCs group, the protein expression level of CTGF in EPCs group was reduced significantly at day 7 and 14 after BM-EPCs transplantation than in UUO group, respectively (Fig. 6C, D). CTGF protein levels by western blotting in UUO group began to increase at 7 days and still remained a high level at 14 days, which was in accord with the results of the immunohistochemistry (Fig. 6E). As shown in Fig. 7A, TGF-β1 was slightly detected in kidney of control group (Fig. 7A). TGF-β1 protein was dramatically increased at day 7 and 14 in UUO, consistent with the result of CTGF expression (Fig. 7B). In EPCs group, TGF-β1 was significantly suppressed at day 7 and 14 (Fig. 7C), respectively. Taken
together, the expression of CTGF and TGF-β was reduced in EPCs-injected UUO kidney in mice (Figs. 6E and 7D).

Discussion

Accumulated evidence has shown the contribution of BM-EPCs to neovascularization in animal models, such as hindlimb ischemia, myocardial infarction, retinal ischemia, cutaneous wounds, and tumor growth (Kawamoto et al. 2001, 2003; Walter and Dimmeler 2002; Choi et al. 2004; Suh et al. 2005; Kong et al. 2004). These findings raise the possibility that EPCs transplantation may serve as a new therapy for ischemic diseases (Dzau et al. 2005). EPCs are considered as candidates for vascular regeneration (Gehling et al. 2000; Gunsilius et al. 2001). The present study confirmed our hypothesis that neovascularization therapy can increase capillary density and alleviate the development of renal fibrosis in the ischemic kidney disease.

Some studies of cell transplantation into the kidney have been published (Rookmaaker et al. 2003; Patschan et al. 2006; Abe-Yoshio et al. 2008). These studies used intravenous injection to achieve cell transplantation. Because the method is minimally invasive, BM-EPCs were transplanted with intravenous administration in this study. BM-derived circulating EPCs and progenitor cells have the potential to home at the sites of ischemic tissues to contribute to revascularization (Urao et al. 2008). In this study, CFDA-SE-positive cells were found in the obstructed kidney. BM-EPCs transplanted with intravenous administration are successfully incorporated into the kidney with injury in vivo.

No renal functional disorders could be diagnosed from BUN and Scr in UUO mice. These clinical normal conditions were caused by a compensatory increase in unobstructed kidney functions (Yabuki et al. 2005). Unobstructed-kidney function compensated for the damage of obstructed-kidney.

VEGF is an endothelial-specific growth factor that promotes endothelial cell proliferation, differentiation and survival, mediates endothelium-dependent vasodilation, induces microvascular hyperpermeability and participant in interstitial matrix remodeling (Ferrara 1999). Cytokines and VEGF, which respond to tissue ischemia, have a stimulatory effect on the BM-EPCs and refers to development of new blood vessels from in site differentiating endothelial cells (Tamura et al. 2003). At day 1 VEGF expression in UUO and EPCs group was higher than control group, which may be a compensatory response to the body, a period of repair and recovery of microvascular injury (Wakelin et al. 2004). Then we observed a gradual reduction of VEGF as the disease progressed in UUO and EPCs group. The reduction of VEGF expression was correlated with decreased PTC density and with more severe interstitial fibrosis.

After EPCs transplantation, the expression of VEGF in EPCs group was significantly elevated than in UUO group.

Fig. 4. HIF-1α staining in tissues samples. (A) Normal control group at 14 days after saline solution injection. Weak nuclear staining of occasional distal tubular nuclei was seen in the control kidney. (B) UUO group at 14 days after saline solution injection. Nuclear HIF-1α staining was most apparent in areas of interstitial fibrosis and tubular dilation or atrophy. (C) EPCs group at 14 days after EPCs injection. HIF-1α expression was suppressed in EPCs-treated kidney. Bar = 50 μm (D) The total IOD of HIF-1α in each group. The total IOD in each group reflected the protein level. After EPCs transplantation, the level of HIF-1α protein was decreased from day 3 compared with UUO group, respectively (p < 0.05). (E) Western blotting of HIF-1α protein expression. HIF-1α protein level was significantly reduced from day 3, which was in accord with the results of the immunohistochemistry. Values are means ± SD. *p < 0.05 vs. normal control group. #p < 0.05 vs. UUO group. Con is the normal control group at 14 days. U1, U3, U7, U14 are the UUO group at 1, 3, 7, 14 days, respectively. E1, E3, E7, E14 are the EPCs group at 1, 3, 7, 14 days, respectively.
The amount of PTC could be evaluated by CD31 immunostaining. In this research, PTC density was measured as a surrogate measure of oxygen supply. We found that PTC injury was gradually aggravated and PTC density was significantly reduced at 7 and 14 days after UUO. PTC density in EPCs group was markedly higher than in UUO group at 7 and 14 days, respectively. PTC injury is related to a decreased blood supply to renal tubules (Choi et al. 2000). The net result of diminished oxygen delivery would lead to further tubulointerstitial injury (Sun et al. 2006). After EPCs transplantation, PTC density was significantly increased in UUO mice in this study.

In order to observe the development of hypoxia following PTC loss, we investigated tubulointerstitial expression levels of HIF-1α. HIF-1α is a sensitive hypoxia indicator, since it is stabilized in a low-oxygen cellular milieu (Stravodimos et al. 2009), and HIF-1α is the master hypoxia response regulator, the amount of which is determined by oxygen-dependent degradation (Safran and Kaelin 2003). The expression of HIF-1α in control group was relatively low, but its expression gradually increased in UUO group. After EPCs transplantation, the level of HIF-1α was decreased than UUO group at day 7 and 14, respectively, suggesting hypoxia was ameliorated after EPCs transplantation.

Consistent with results in other models of chronic renal injury, the expression of VEGF was significantly reduced as the disease progressed. This result may seem contradictory to the finding of increased hypoxia in this model, particularly since VEGF is both induced by hypoxia and transcriptionally activated by HIF-1. There are several explanations for this disparity. First, HIF-1α is not the only known transcriptional activator of VEGF, with experimental evidence suggesting a role for SP-1 as well as the oncogene ras. VEGF transcription is also inhibited by the anti-oncogenes p53 and p73. Also relevant are previous experiments showing that VEGF expression is reduced in cutaneous models of inflammation at a time when HIF-1α expression is increased and the finding that VEGF production in response to hypoxia was reduced in the presence of macrophage-derived cytokines in vitro. It is therefore likely that the finding of reduced VEGF in UUO as well as in other renal disease models (Kairaitis et al. 2005; Sun et al. 2006) are multifactorial in origin.

CTGF is expressed in proliferative and fibrotic glomerular and interstitial lesions of many kinds of human and experimental nephropathies, and related closely with the degree of renal tubulointerstitial fibrosis (Ito et al. 1998). CTGF, an important downstream mediator in the various profibrotic actions of TGF-β (Zhang et al. 2004), plays an important role in pathological fibrosis (Qi et al. 2005). TGF-β1 is a key mediator of renal fibrosis in clinical and experimental kidney disease (Schnaper et al. 2003; Peters et al. 1997). It increases the synthesis of matrix proteins while decreasing that of proteases which could degrade matrix (Vuruskan et al. 2005). In this study, the expression of CTGF and TGF-β1 was significantly upregulated after UUO. While the levels of CTGF and TGF-β1 in EPCs group were lower than in UUO group at day 7 and 14, respectively.
From the morphologic data, we found that renal interstitial fibrosis gradually aggravated after UUO. Both the loss of VEGF expression and the increase in HIF-1α expression were correlated with capillary loss and the development of glomerulosclerosis and interstitial fibrosis. Microvascular injury, with consequent tissue hypoxia and ischemia, may play an important role in progressive renal disease. After EPCs transplantation, the VEGF protein levels and PTC density were increased, and HIF1-α, CTGF and TGF-β1 expression were reduced, and renal interstitial fibrosis was alleviated. Transplantation of EPCs alleviated renal interstitial fibrosis in a mouse model of UUO.

The mechanisms of EPCs for increasing capillary density and ameliorating renal fibrosis have not been fully elucidated. EPCs can provide a source of robust endothelial cells and secrete various angiogenic factors such as VEGF to promote neovasculogenesis (Rehman et al. 2003; Hur et al. 2004). There is growing evidence that BM-EPCs participates in the repair of endothelial dysfunction (Walter et al. 2002; Hill et al. 2003; Rafii and Lyden 2003).

In this study, BM-EPCs were given right after induction of UUO, when the renal fibrosis could not be observed. This approach is a preventive intervention rather than a reversal treatment. The use of animal models of UUO has provided many new insights into the pathogenesis of obstructive nephropathy, and of progressive renal fibrosis in general (Chevalier et al. 2009). BM-EPCs transplantation prevented the progression of renal fibrosis. Although this study did not have sufficient data to prove the therapeutic effect of BM-EPCs transplantation as a reversal treatment, a large study may well be able to demonstrate the effect of the transplantation.

In summary, our findings suggested that transplanted BM-EPCs survived and were successfully incorporated into the capillary network in obstructive nephropathy in vivo and alleviated the development of renal fibrosis.

**Conclusion**

Following significant reductions in VEGF expression and PTC density, HIF1-α expression increased markedly in UUO mice, which contributed to the progressive tubulointerstitial fibrosis. We think PTC loss, with consequent tissue hypoxia and ischemia, may play an important role in obstructive nephropathy. Transplantation of BM-EPCs quantitatively augmented neovascularization. After BM-EPCs transplantation, the PTC density was increased, hypoxia and tubulointerstitial fibrosis were ameliorated. It seemed that the EPCs treatment may represent a new and promising strategy for clinical application designed to block progressive renal fibrosis in patients.
Conflict of interest statement
The authors declare that there are no conflicts of interest.

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