

# Mesenchymal stem cells and CXC chemokine receptor 4 overexpression improved the therapeutic effect on colitis via mucosa repair

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**Abstract.** The present study intended to observe the homing capability and therapeutic effect of CXC chemokine receptor 4 (CXCR-4) gene overexpressed bone marrow mesenchymal stem cells (BMSCs) on colitis, and to study the possible mechanisms involved. BMSCs were derived from male BALB/c mice and CXCR-4 gene was transfected into BMSCs by the utilization of the lentiviral vector. The expression of CXCR-4 gene was analyzed and the biological characteristics, and vitality of BMSCs and CXCR-4 gene overexpressed BMSCs (CXCR-BMSCs) were detected. The chemotaxis assay was performed to investigate migration *in vitro*. Colitis was induced by TNBS in female BALB/c mice. BMSCs and CXCR-BMSCs were injected into experimental models intravenously. The homing of cells was confirmed by fluorescence observation and Sry gene detection. Clinical manifestation and histological changes were also evaluated. The expression levels of occludin and vascular endothelial growth factor (VEGF) were detected to measure mucosal repair. Furthermore, CXCR-4 gene was successfully transfected into BMSCs by the utilization of lentiviral vector. Results indicated that overexpression of CXCR-4 gene did not influence the biological characteristics and vitality of BMSCs, but enhanced the capability of migration and homing of BMSCs *in vitro* and *in vivo*. Notably, CXCR-BMSCs had an improved effect on treating colitis, and the expression levels of Occludin and VEGF were higher. The results suggested that overexpression of CXCR-4 gene may enhance BMSC homing to damaged intestinal mucosa and their curative effect on colitis. The present findings indicated that using lentiviral vector to transfect CXCR-4 gene into BMSCs may be a potential method to improve the outcomes of BMSCs treatment on inflammatory bowel disease.

## Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), are common diseases in Europe and North America. However, according to a recent epidemiologic data (1), IBD is becoming increasingly common in Asia. Therefore, more attention and health care resources on IBD would be required around the world.

The mechanism of IBD is considered to be closely related to genetic, immunological and environmental factors. Several therapies, including novel medicine, biologic treatment, stem cell transplantation etc., have treatment effects since they are relevant to disease initiation, progression or both. Stem cell transplantation has been considered as an effective therapy of IBD, especially in refractory patients with CD who have no other therapeutic options (2).

Mesenchymal stem cells (MSCs) are undifferentiated cells that have the unique potential to develop into many different cell types in the body as well as the function of immunomodulation. Therefore, these cells have emerged as leading candidates for regenerative treatment and shown great promise in numerous clinical trials (3,4). The key process of tissue repair is as follows: Activated by several inflammatory cytokines and chemokines, MSCs migrate to sites of damaged location (5,6), and then modulate the immunological function and repair the damaged part.

However, a significant barrier to the effective implementation of MSCs therapy is the inability to target these cells to tissues of interest with high efficiency and engraftment (7). Therefore, researches on promoting migration and engraftment of MSCs to target tissue have been the focus of attention. Several methods, including cell surface modification, nanoparticles and advanced biomaterials (8) packing, have been used to enhance the ability to migrate.

The stromal-derived factor-1 (SDF-1) plays an essential role in stem cell homing by recruiting the progenitor cells that express its cognate receptor, CXC chemokine receptor 4 (CXCR-4). This role of SDF-1 has been confirmed in several researches (9-12). It is the theoretical foundation of CXCR-4 gene overexpressed MSCs that improve their homing capacity. As more MSCs migrate to the damaged location, the effect of repair becomes more remarkable. In this study, we used a lentiviral gene vector as a carrier to deliver CXCR-4 gene into MSCs to make this gene overexpress further. We examined the

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effects of CXCR-4 overexpressed MSCs migration *in vitro* as well as the capability of homing and repairing after transplantation of these cells *in vitro*.

## Materials and methods

**Mice.** Four- to eight-week-old male BALB/c mice provided by the Research and Technology Service Center, 302 Hospital of PLA (Beijing, China) were group-housed under controlled temperature (26°C) and a 12-h light/dark cycle, fed standard mouse chow and clean water. The mice were maintained under specific pathogen-free conditions and allowed to acclimatize for 1 week prior to the commencement of experimental work. All animal experiments were approved by the Animal Ethics Committee of the Animal Facility of Chinese PLA General Hospital (Beijing, China).

**Isolation and culture of mice bone marrow mesenchymal stem cells (BMSCs).** Bone marrow (BM) cell suspension was obtained by flushing marrow cavity of four- to five-week-old male mice with  $\alpha$ -minimum essential medium ( $\alpha$ -MEM; HyClone; GE Healthcare Life Sciences, Logan, UT, USA). Then, the BM cells were cultured with  $\alpha$ -MEM containing 10% heat-inactivated fetal bovine serum (FBS; HyClone; GE Healthcare Life Sciences) and penicillin-streptomycin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany). Cells were cultured at 37°C in a 5% CO<sub>2</sub> humidified incubator. To acquire putative BMSCs, non-adherent cells and tissue debris were removed by phosphate-buffered saline (PBS) washing after 72 h (13). The culture medium was changed every 3 days until the cell density reached about 90%. BMSCs were detached by 0.25% trypsin containing 0.02% EDTA (HyClone; GE Healthcare Life Sciences) and expanded. All BMSCs were utilized for subsequent experiments.

To better observe the BMSCs, cells were labeled by cell tracker. The methods were as follows: BMSCs were suspended at a density of  $3 \times 10^6$ /ml with  $\alpha$ -MEM. Every  $1 \times 10^6$  cells were incubated with 3  $\mu$ l CM-Dil (1  $\mu$ g/ml) (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37°C for 5 min then 4°C for 15 min. Cells were washed twice and suspended by  $\alpha$ -MEM.

**Transfection of CXCR-4 gene.** A lentiviral vector with both Mice CXCR-4 gene and Luciferase report gene was used. All operation associated with this lentiviral vector, including the design, construction and detection, were performed by Shanghai Genechem Co., Ltd. (Shanghai, China). Virus aliquots were suspended in PBS and stored at -80°C until needed. Samples were diluted 1:1 before viral titers were measured.

The transfection processes were modified according to the Ricks' method (14). BMSCs at passage 3 were cultured in 12-well plates at a density of  $5 \times 10^3$  cells in 2 ml of  $\alpha$ -MEM containing 10% FBS per well. After 24 h plating, transfection was carried out at 20 multiplicity of infection (MOI) in the presence of 2  $\mu$ g/ml Polybrene (Sigma-Aldrich; Merck KGaA). Cells were cultured in incubator for 10 h, then the transfection medium was replaced with fresh  $\alpha$ -MEM containing 10% FBS. The absorbance of CXCR-4 gene transfected BMSCs (CXCR-BMSCs) was quantified with a luminometer (Lonza,

Switzerland) according to the manual. To detect the viability and the capability of differentiation about CXCR-BMSCs, cells were sequentially expanded thereafter and were used for the next experiment.

**Analysis of the CXCR-4 gene expression in CXCR-BMSCs.** Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was applied to analyze the expression of CXCR-4 gene in CXCR-BMSCs.

**RNA extraction and RT-qPCR.** Whole RNA was extracted from BMSCs and CXCR-BMSCs by the utilization of Total RNA Kit (SinoGene Scientific Co., Ltd., Beijing, China) according to the manual. The sequences of primers for PCR were as follows: Mice CXCR-4 gene, 5'-CCTCTACAG CAGCGTTCT-3' (forward), 3'-GTTTCCTTGGCCTTT GAC-5' (reverse);  $\beta$ -actin, 5'-CGTTGACATCCGTAAGA CC-3' (forward), and 3'-CTAGGAGCCAGAGCAGTAATC-5' (reverse). The conditions and processes of PCR amplification were as follows: Pre-denature at 95°C for 10 min, then 40 cycles of denature at 95°C for 20 sec, annealing at 60°C for 30 sec, extension at 60°C for 30 sec. qPCR crossing threshold (Ct) values were obtained during the exponential amplification phase and Prizm4 was used to analyze data.

**Identification of biological characteristics of BMSCs.** Flow cytometry analysis and experiment about differentiation of BMSCs were applied to identify BMSCs.

**Flow cytometry analysis.** Two groups of cells, BMSCs group and CXCR-BMSCs group, were trypsinized, inactivated with FBS and washed twice with PBS. These cells were suspended in 100  $\mu$ l PBS and incubated at 4°C for 20 min with PE-conjugated anti-mouse CD90.2 (BD Biosciences, Franklin Lakes, NJ, USA), Alexa Fluor-conjugated anti-mouse CD105 (BD Biosciences), PE Cy<sup>TM</sup>-conjugated anti-mouse CD11b (eBioScience; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and FITC-conjugated anti-mouse CD45 (BD Biosciences) as described previously (13).

**Experiment on differentiation of BMSCs.** The function of BMSCs differentiation into osteogenic and adipogenic lineages was examined to illustrate the biological characteristics of BMSCs (15). Two groups of cells were planted separately in 24-well plates with the concentration of  $2 \times 10^4$  cells/well. All details about experiment and detection of BMSCs differentiation into osteogenic and adipogenic lineages were as previously described (13).

**Cell viability evaluation.** Viability of two groups of cells was confirmed by Trypan Blue staining exclusion. The numbers of live cells and dead cells were counted and the rate of living cell calculated. Living cell rate (%) = (living/total cells) x 100%.

**Migration assay *in vitro*.** To investigate the migration ability of these two groups of cells, a chemotaxis assay was performed by the utilization of 24-well plate transwell chamber with 8  $\mu$ m pore filters (Corning International Co., Tokyo, Japan). Two groups of cells were plated in the upper chambers with  $2 \times 10^5$  cells/ml in 200  $\mu$ l of FPS free  $\alpha$ -MEM

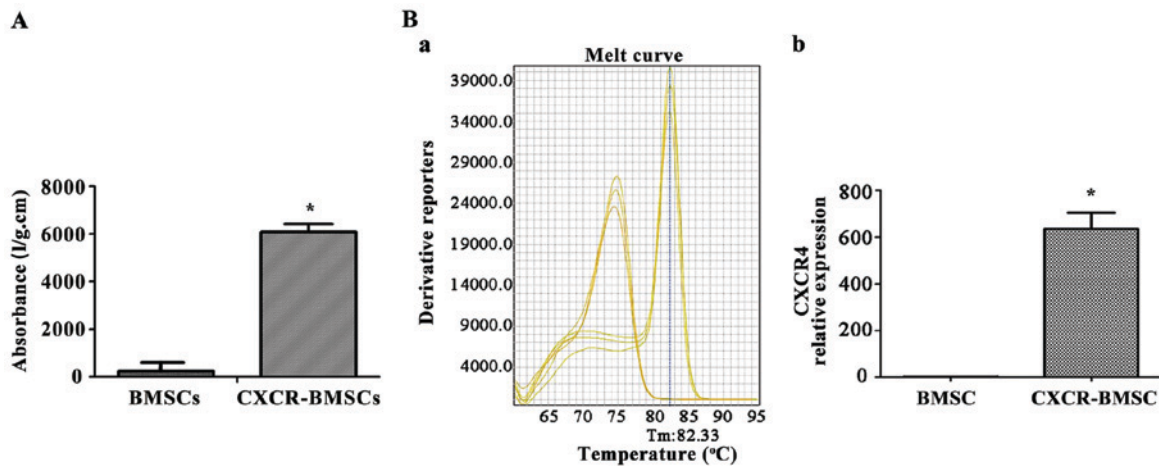


Figure 1. CXCR-4 gene in BMSCs and CXCR-BMSCs. (A) Luciferase report gene absorbance in BMSCs and CXCR-BMSCs; (B-a) The melting curve of CXCR-4; (B-b) The relative expression of BMSCs and CXCR-BMSCs. \*P<0.05 compared with that of BMSCs. CXCR-4, CXC chemokine receptor 4; BMSC, bone marrow mesenchymal stem cell.

containing 0.1% BSA, and the lower chambers with 600  $\mu$ l recombinant mouse SDF-1 $\alpha$  (50 ng/ml). The whole transwell chamber was set at 5% CO<sub>2</sub>, 37°C for 10 h. The migrated cells on the lower side of the filter was stained by crystal violet and their number was determined by two independent observers by counting three random fields per well using an Olympus BH-2 microscope (Olympus, Tokyo, Japan; magnification, x200).

**Mice colitis model and BMSCs transplantation.** Six- to eight-week-old female BABL/C mice were randomly assigned into four groups: Control group (Ctr group), colitis model group (T group), BMSCs transplantation group (MT group) and CXCR-BMSCs transplantation group (CMT group), n=12/group. Colitis was induced by 2.0 mg TNBS/50% ethanol enema (Sigma-Aldrich; Merck KGaA), as previously described (13). At 24 h after the colitis model was established, mice in MT group and CMT group were injected 1x10<sup>6</sup> cells in 100  $\mu$ l PBS via tail vein. Mice in Ctr group and T group were given pure PBS in the volume equivalent to the PBS of MT group and CMT group. The body weight and Disease Activity Index (DAI) of mice in each group were recorded and calculated. Mice were respectively sacrificed at the 3rd, 5th, 9th, 13th day after BMSCs transplantation. The sections of tissues were H&E-stained to assess the severity of inflammation in colitis.

**Identification of homing of BMSCs.** Observation of BMSCs with red fluorescence and detection of mouse Y-chromosome (Sry) gene in colon mucosa were applied to identify the homing of BMSCs.

**Fluorescence observation.** The sections of colonic specimens were observed with fluorescent microscopy (Olympus).

**Sry gene detection.** Whole DNA was extracted from colon tissues in each group by the utilization of Total DNA Kit (SinoGene Scientific Co., Ltd., Beijing, China) according to the manual. The sequences of primers for PCR were as follows: Mouse Sry, 5'-TCGGAGGGCTAAAGTGTC-3' (forward),

and 3'-TCTTGCCTGTATGTGATGG-5' (reverse). The conditions and processes of PCR amplification were as follows: Pre-denature at 94°C for 3 min, then 30 cycles of denature at 94°C for 20 sec, annealing at 58°C for 20 sec, and extension at 72°C for 30 sec. qPCR crossing threshold (Ct) values were obtained during the exponential amplification phase and Prizm4 was used to analyze data.

**Immunohistochemistry.** Immunohistochemistry was performed to detect the expression of occludin and vascular endothelial growth factor (VEGF) in colon mucosa. The conditions and processes of immunohistochemistry were as follows: The slices of 4  $\mu$ m formalin-fixed and paraffin-embedded colon sections were respectively incubated with a rabbit anti-mouse primary antibody to Occludin (1:200, ab64482; Abcam) and a rabbit anti-VEGF antibody (1:200, bs-0279R; Bioss, Beijing, China) at 4°C overnight, after washed with PBS 3 times, the slices were incubated with a goat anti-rabbit secondary polyclonal antibody (1:1,000, cat. no. ZDR-5306; ZSGB-Bio, Beijing, China) at 37°C for 30 min, the samples were finally lightly stained with H&E and examined.

**Statistical analysis.** Data were represented as mean  $\pm$  standard deviation. The t-test was used for comparison between two groups. Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test by SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

## Results

**Transfection of CXCR-4 gene in BMSCs.** The absorbance of samples from BMSCs and CXCR-BMSCs was quantified to illustrate the existence of CXCR-4 gene in these cells (Fig. 1A). The luciferase report gene absorbance was low in BMSCs, but significantly high in CXCR-BMSCs (P<0.05).

RT-qPCR was performed to detect CXCR-4 gene expression in BMSCs. The mRNA transcription level in BMSCs (P4) was low, but significantly high in CXCR-BMSCs. The result indicated that the expression of CXCR-4 gene in

Table I. Clinical scoring standard.

Stool consistency	Fecal blood	Body weight loss
0: Normal	0: No bleeding	0: None
1: Wet stools		1: 0-5% weight loss
2: Soft stools with sticking surface	2: Slight bleeding	2: 5-10% weight loss
3: Loose stools	3: Moderate bleeding	3: 10-15% weight loss
4: Watery diarrhea	4: Severe bleeding	4: >15% weight loss

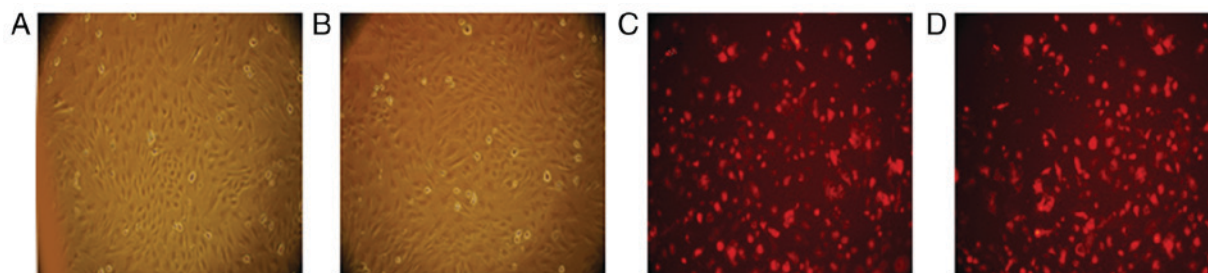


Figure 2. Morphological characteristics of BMSCs and CXCR-BMSCs. (A) The morphology of BMSCs; (B) the morphology of CXCR-BMSCs; (C) CM-Dil staining on the cell membrane of BMSCs; and (D) CM-Dil staining on the cell membrane of CXCR-BMSCs (magnification, x400). CXCR, chemokine receptor; BMSC, bone marrow mesenchymal stem cell.

CXCR-BMSCs (Fig. 1B) was significantly higher than that in BMSCs ( $P < 0.05$ ).

*Biological characteristics of BMSCs and CXCR-BMSCs.* Comparing the morphological characteristics of BMSCs and CXCR-BMSCs on the same passage, we found that all cells were long spindle and arranged closely (Fig. 2A and B). The cell membrane of both BMSCs and CXCR-BMSCs presented red under the green fluorescence observation after being marked by CM-Dil (Fig. 2C and D).

Cell phenotype of the BMSCs and CXCR-BMSCs were authenticated by flow cytometry. The expression of bone marrow progenitor cell marker CD90 and CD105 were positive, while the expression of monocyte/macrophage marker CD11b and pan leukocyte marker CD45 were negative. Details were as follows: The expression of CD90 (92.29%) and CD105 (99.30%), CD11b (19.07%) and CD45 (8.86%) in BMSCs; the expression of CD90 (88.79%) and CD105 (99.68%), CD11b (17.87%) and CD45 (10.37%) in CXCR-BMSCs (Fig. 3). It demonstrated that BMSCs were cultured successfully and transfection of CXCR-4 gene had no impact on the cell phenotype.

The capability of differentiation into osteoblasts and adipocytes is a crucial biological feature of MSCs. After the culturing with osteogenic medium for 3 weeks and alizarin red staining, mineralized nodules were formed and could be observed in both two groups of cells (Fig. 4A and B). Red fat particles could also be observed after cells were induced with adipogenic medium for 8 days and stained by Oil Red O (Fig. 4C and D).

*Cell viability of BMSCs and CXCR-BMSCs.* The DNA of inanimate cells can be stained by Trypan Blue, but living cells with completed membrane can inhibit this function. By

counting the numbers of live cells and dead cells, the rate of living cell can be calculated. In both groups, rates of living cell were above 93% and no statistical difference (Fig. 5). It demonstrated that transfection of CXCR-4 gene had no influence on the viability of BMSCs.

*CXCR-4 promotes the migration of BMSCs to SDF-1 in vitro.* The cells stained by crystal violet on the lower side of the filter in both groups (Fig. 6) were counted and compared. The results showed that the number of CXCR-BMSCs migrating to SDF-1 was more than that of BMSCs ( $P < 0.05$ ).

*CXCR-4 promotes the homing of BMSCs to damaged intestinal mucosa.* Red-labeled BMSCs on the injured colon were observed in both MT and CMT group (Fig. 7A and B). No red fluorescence was seen in T group.

Sry gene was related to Y-chromosome in male. Through the detection of Sry gene in colon tissue, the situation of BMSCs homing to damaged intestinal mucosa can be reflected. The results showed the existence of Sry gene in both MT and CMT group, and the relative expression of sry gene in CMT group was more than that in MT group ( $P < 0.05$ ). Besides, there was no expression of Sry gene in T group, in which the female mice were not injected the BMSCs isolated from the male mice (Fig. 7C).

*CXCR-4 overexpression BMSCs cure TNBS-induced colitis better.* Clinical manifestation associated with colitis, such as weight loss, diarrhea, loose stools, even gastrointestinal bleeding, were observed in groups in which the mice accepted enema with TNBS. The DAI was calculated according to the Clinical scoring standard (Table I), which was modified from the Cooper's scoring method. All manifestations were improved after injection with two kinds of BMSCs after 2 days

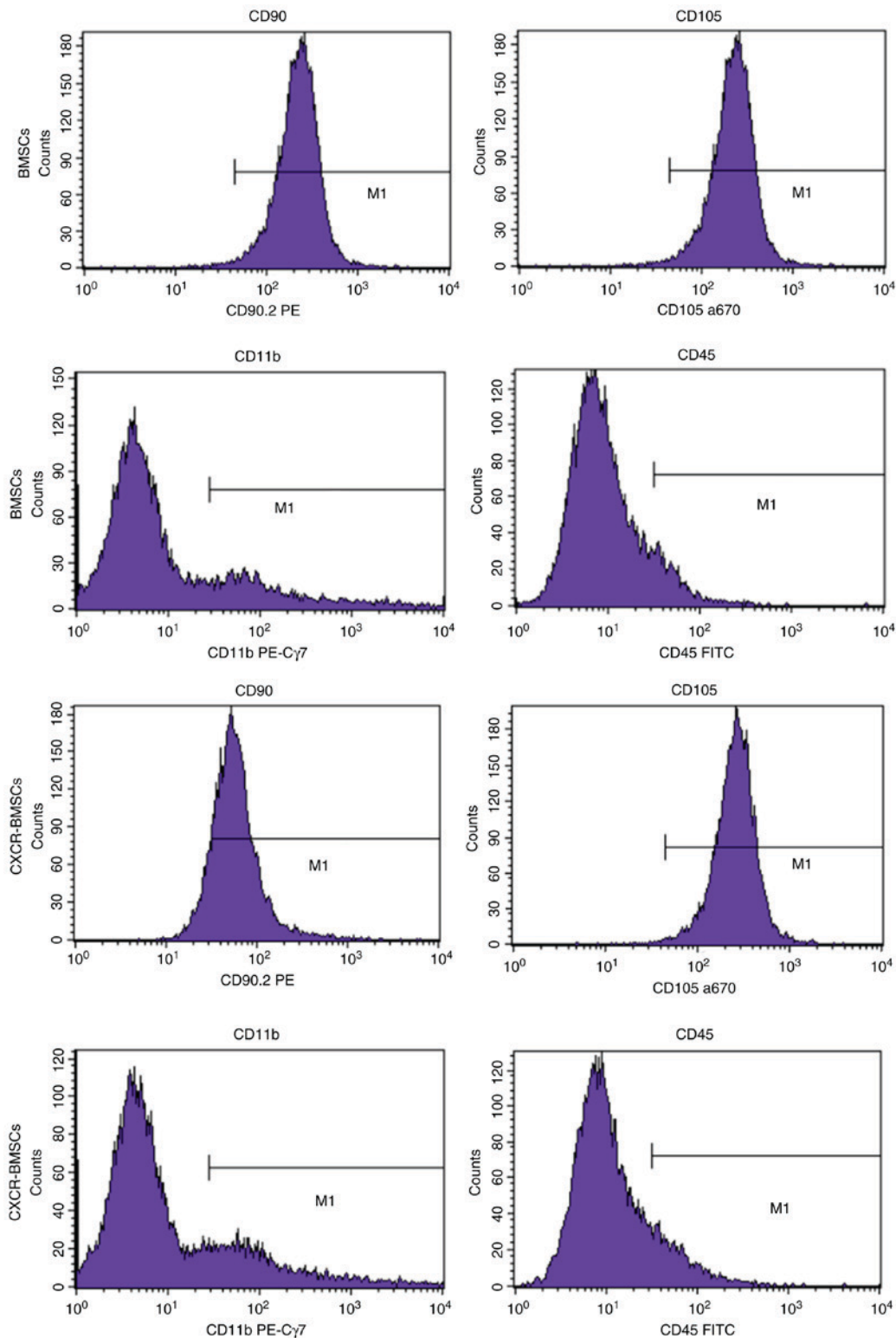


Figure 3. Cell phenotypes of BMSCs and CXCR-BMSCs. The expression of bone marrow progenitor cell marker CD90 and CD105 were positive, while the expression of amonocyte/macrophage marker CD11b and pan leukocyte marker CD45 were negative. CXCR, chemokine receptor; BMSC, bone marrow mesenchymal stem cell.

( $P < 0.05$ ), but the difference in DAI between MT and CMT group was not significant ( $P > 0.05$ ; Fig. 8A and B).

Compared with the normal colon mucosa in Ctr group, more serious inflammation and mucosa damage were observed in T group (Fig. 8C-a and -b). Damaged mucosa rehabilitated predominantly after BMSCs and CXCR-4 gene

overexpressed BMSCs transplantation, especially in the latter one (Fig. 8C-c and -d).

*CXCR-4 gene overexpressed BMSCs promotes the repair of damaged intestinal mucosa.* Occludin, one of the tight junction proteins, is critical to protecting the integrity of intestinal

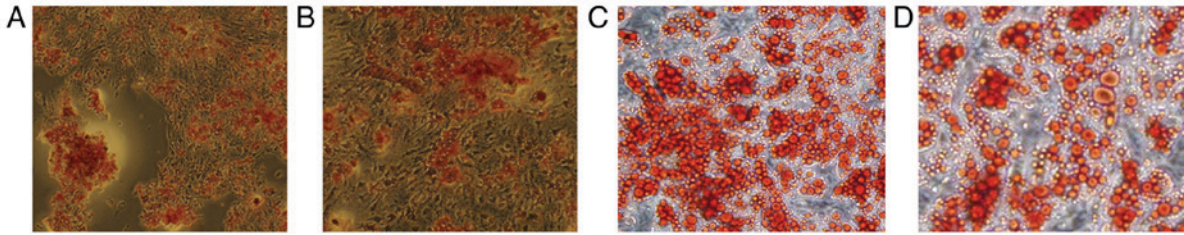


Figure 4. Capabilities of differentiation into osteoblasts and adipocytes of BMSCs and CXCR-BMSCs. (A) Osteogenic differentiation of BMSCs; (B) osteogenic differentiation of CXCR-BMSCs; (C) adipogenic differentiation of BMSCs; and (D) adipogenic differentiation of CXCR-BMSCs (magnification, x400). CXCR, chemokine receptor; BMSC, bone marrow mesenchymal stem cell.

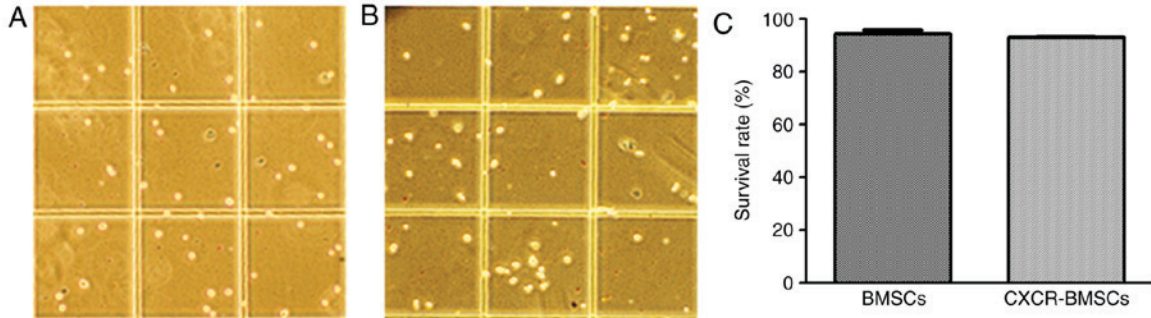


Figure 5. Cell viability of BMSCs and CXCR-BMSCs. (A) Trypan blue staining in BMSCs; (B) Trypan blue staining in CXCR-BMSCs (magnification, x200); and (C) the survival rate of cells in BMSCs and CXCR-BMSCs. CXCR, chemokine receptor; BMSC, bone marrow mesenchymal stem cell.

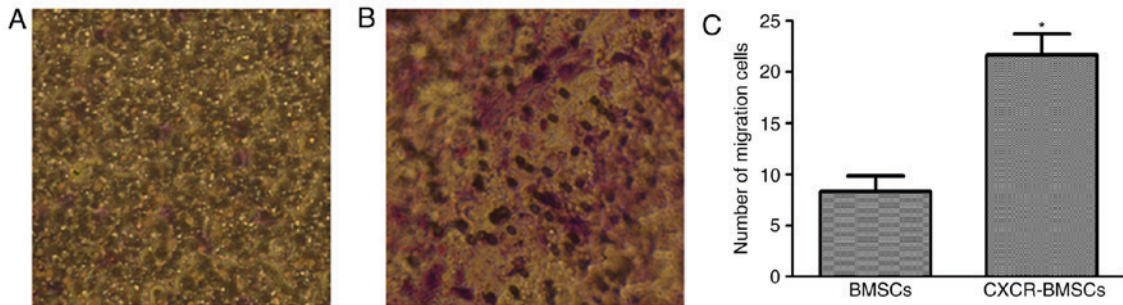


Figure 6. Capabilities of migration into SDF-1 of BMSCs and CXCR-BMSCs. (A) BMSCs migrated to SDF-1; (B) CXCR-BMSCs migrated to SDF-1 (magnification, x200); and (C) the number of migration cells in CXCR-BMSCs and BMSCs. \*P<0.05 compared with that of BMSCs. SDF-1, stromal-derived factor-1; CXCR, chemokine receptor; BMSC, bone marrow mesenchymal stem cell.

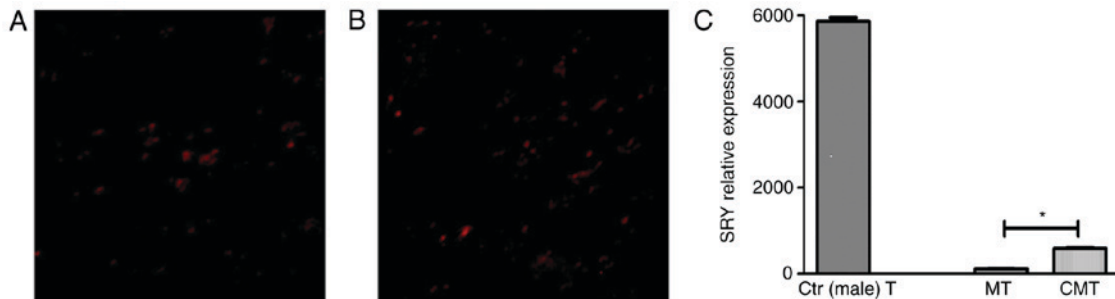


Figure 7. Capabilities of homing to damaged intestinal mucosa of BMSCs and CXCR-BMSCs. (A) Red fluorescence observed on the lamina propria mucosa of colon in MT group; (B) red fluorescence observed on the lamina propria mucosa of colon in CMT group (magnification, x100); and (C) the expression of Sry gene in the colon tissues among Ctrl, T, MT and CMT groups. \*P<0.05 compared with that of MT group. CXCR, chemokine receptor; BMSC, bone marrow mesenchymal stem cell.

mucosa. There were different amounts of Occludin expression in each group (Fig. 9A-a-d), with the least expression in T

group, the most expression in CMT group, and the expression in MT group less than that in CMT group.

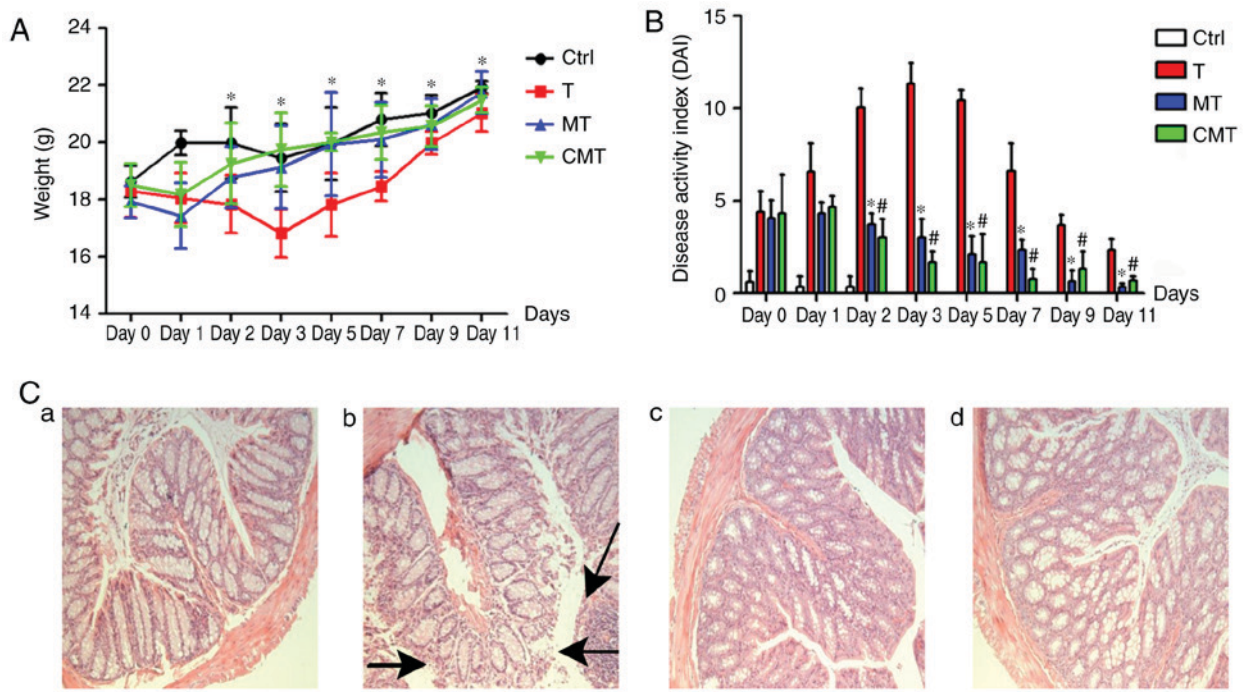


Figure 8. Evaluations of therapeutic effect in all groups. (A) Weight change of mice in all groups from day 0 to 11; (B) the disease activity index (DAI) of mice in all groups from day 0 to 11; (C) histologic character of colonic tissues in all groups (a, control group; b, colitis model group (T group); c, BMSCs transplantation group (MT group); and d, CXCR-BMSCs transplantation group (CMT group) at 3rd day (x200). Arrows indicating regions of damaged mucosa. \*P<0.05, compared with T group; #P>0.05 compared with MT group. CXCR, chemokine receptor; BMSC, bone marrow mesenchymal stem cell.

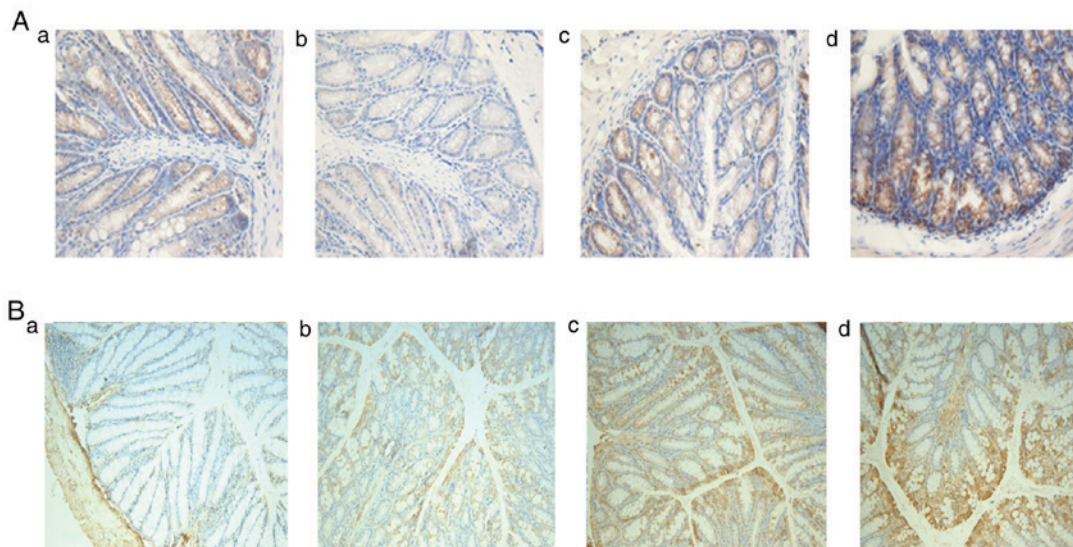


Figure 9. Immunohistochemistry of occludin and VEGF in colonic tissues in all groups. (A) Immunohistochemistry of Occludin in colonic tissues in all groups (a, control group; b, T group; c, MT group; and d, CMT group) magnification, x200. (B) Immunohistochemistry of VEGF in colonic tissues in all groups (a, control group; b, T group; c, MT group; and d, CMT group) magnification, x200. VEGF, vascular endothelial growth factor.

VEGF promotes the repair of damaged intestinal mucosa by accelerating the growth of vessels. There were different amounts of VEGF expression in each group (Fig. 9B-a-d), with the least expression in T group, the most expression in CMT group, and the expression in MT group less than that in CMT group.

**Discussion**

Several studies and clinical trials have been performed worldwide to present the advantages of stem cell therapy in treating

a variety of diseases. MSCs transplantation has also been proposed as a promising strategy for IBD treatment (16,17) and BMSCs have become an ideal candidate for cell transplantation owing to their biological characteristics (18). However, the therapeutic effect of BMSCs transplantation is limited because the efficiency of cell homing to the damaged tissues is low (19). This low efficiency has been found in the previous study (13). Thus, finding a proper method for BMSCs to improve the capacity of homing is crucial to a successful cell therapy.

Several studies have confirmed that SDF-1 can be expressed/secreted by several tissues/organs in the body (9) and up-regulated in stressed or injured tissues (20,21) and the CXCR4 is the specific receptor of SDF-1. Thus, SDF-1/CXCR4 axis plays a key role in promoting the migration and homing of stem cells to target site. In this study, CXCR-4 gene was carried by lentiviral vector and transfected to BMSCs in order to make it overexpress. The CXCR-4 gene overexpression in CXCR-BMSCs was confirmed by qPCR technology.

The function of BMSCs in promoting damaged tissues regeneration is the basis of repairing injured mucosa, which is intrinsically related to the biological characteristics of BMSCs. Therefore, the features of both BMSCs and CXCR-BMSCs were detected by morphology, cell phenotype and capability of differentiation. A series of related experiments have proved that the overexpression of CXCR-4 on BMSCs has no influence on original biological features and viability of BMSCs themselves.

Migration and homing of CXCR-4 gene overexpressed BMSCs were detected and compared with those of common BMSCs *in vitro* and *vivo*. Migration assay was performed and the results demonstrated that CXCR-4 gene overexpressed BMSCs were easier to migrate. *In vivo*, two groups of cells were transplanted into the TNBS-induced colitis model. The number of CXCR-BMSCs homing to the damaged intestinal mucosa was much more than that of BMSCs, which was confirmed by observation of red fluorescence and detection of Sry gene. Besides, clinical manifestation, including the variation of body weight and DAI, also proved that the curative effect of CXCR-4 gene overexpressed BMSCs on colitis was better than that of common BMSCs. At present, there are two methods for colitis animal models construction: One is DSS oral method, and the other is TNBS enema method. Because we used enema method in the experiment, and feces of mice will lead to a greater impact on enema effect. Therefore, mice underwent fasting for 36 h before the enemata and back to eat after enemata. The enema was not conducted every day for 12 days (day 0-12). It was only done once at day 0. For small mammals, eating or not in a short period of time has great impact on the weight, so after enema (back to eating) the weight would rise, which apparently is related to the resumption of eating. In addition, because 'weight' itself is a dynamic index, lack of convincing. In order to better explain the occurrence of symptoms and remission of symptoms after treatment, so we use the disease activity score (DAI). The DAI score prove the successful construction of the model. And we know that tissue damage is able to self-repair. With the body self-healing, even the module DAI score is also showing a downward trend, this is the objective reality.

Available evidence has indicated that BMSCs accumulate in injured colon and there are several potential mechanisms for BMSCs to have the therapeutic effect on colitis, including mucosa repair, immunomodulation and so on. In this study, the expression of Occludin was detected to reflect the tight junctional protein production after BMSCs transplantation, and tight junctional proteins are associated with maintaining the integrity of epithelium (22), which is essential for the physiologic function of intestinal mucosa. Besides, BMSCs can

secrete angiogenic cytokines including bFGF and VEGF (23), which are beneficial to the growth of vessels to promote the repair of damaged mucosa.

In conclusion, the number of BMSCs located at the target site has great influence on the therapeutic effect. Therefore, improving the homing of BMSCs is crucial. SDF-1, a common cytokine presented on the inflamed colonic tissues, can specifically combine with CXCR-4. This provides a theoretical foundation for improving the curative effect by transplantation of CXCR-4 gene overexpressed BMSCs. In this study, we used lentiviral vector to transfect the CXCR-4 gene into BMSCs and made it overexpress. Our study demonstrated that CXCR-4 gene overexpressed BMSCs could enhance BMSCs homing to damaged intestinal mucosa and have better effect on treating colitis. In addition, some studies have used selectin, cell adhesion molecules or peptide ligands to modify stem cells, so as to increase homing ability (24-26). Compared with other methods, CXCR-4 gene overexpressed BMSCs method mediated repair procedure based on BMSCs, which has good operability and repeatability (27). We consider that this decoration to BMSCs might be a potentially novel method to improve the outcomes of BMSCs treatments on IBD.

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#### Availability of data and material

All data generated or analyzed during this study are included in this published article.

#### Authors' contributions

ZC was a major contributor in writing the manuscript and was responsible for isolation and culture of mice BMSCs. QC contributed to manuscript preparation and the conception of the study. HD was a major contributor in performing the migration assay. LX performed the qPCR. JW contributed in the data analysis and manuscript revision. All authors read and approved the final manuscript.

#### Ethics approval and consent to participate

All animal experiments were approved by the Animal Ethics Committee of the Animal Facility of Chinese PLA General Hospital (Beijing, China).

#### Patient consent for publication

Not applicable.

#### Competing interests

The authors declare that they have no competing interests.

**References**

1. Ng SC, Tang W, Ching JY, Wong M, Chow CM, Hui AJ, Wong TC, Leung VK, Tsang SW, Yu HH, *et al*: Incidence and phenotype of inflammatory bowel disease based on results from the Asia-pacific Crohn's and colitis epidemiology study. *Gastroenterology* 145: 158-165.e2, 2013.
2. Rogler G: Where are we heading to in pharmacological IBD therapy? *Pharmacol Res* 100: 220-227, 2015.
3. Makridakis M, Roubelakis MG and Vlahou A: Stem cells: Insights into the secretome. *Biochim Biophys Acta* 1834: 2380-2384, 2013.
4. Wagner J, Kean T, Young R, Dennis JE and Caplan AI: Optimizing mesenchymal stem cell-based therapeutics. *Curr Opin Biotechnol* 20: 531-536, 2009.
5. Kawada H, Fujita J, Kinjo K, Matsuzaki Y, Tsuma M, Miyatake H, Muguruma Y, Tsuboi K, Itabashi Y, Ikeda Y, *et al*: Nonhematopoietic mesenchymal stem cells can be mobilized and differentiate into cardiomyocytes after myocardial infarction. *Blood* 104: 3581-3587, 2004.
6. Chang PY, Zhang BY, Cui S, Qu C, Shao LH, Xu TK, Qu YQ, Dong LH and Wang J: MSC-derived cytokines repair radiation-induced intra-villi microvascular injury. *Oncotarget* 8: 87821-87836, 2017.
7. Fan D, Xie X, Qi P, Yang X and Jin X: Human mesenchymal stem cell homing induced by SKOV3 cells. *Am J Transl Res* 9: 230-246, 2017.
8. Xinaris C, Morigi M, Benedetti V, Imberti B, Fabricio AS, Squarcina E, Benigni A, Gagliardini E and Remuzzi G: A novel strategy to enhance mesenchymal stem cell migration capacity and promote tissue repair in an injury specific fashion. *Cell Transplant* 22: 423-436, 2013.
9. Bobis-Wozowicz S, Miekus K, Wybieralska E, Jarocha D, Zawisz A, Madeja Z and Majka M: Genetically modified adipose tissue-derived mesenchymal stem cells overexpressing CXCR4 display increased motility, invasiveness, and homing to bone marrow of NOD/SCID mice. *Exp Hematol* 39: 686-696.e4, 2011.
10. Trotta T, Di Gioia S, Piro D, Lepore S, Cantatore S, Porro C, Castellani S, Petrella A, Fortunato F, Maffione AB and Conese M: Effect of acute lung injury on VLA-4 and CXCR4 expression in resident and circulating hematopoietic stem/progenitor cells. *Respiration* 85: 252-264, 2013.
11. Ratajczak MZ, Kim CH, Abdel-Latif A, Schneider G, Kucia M, Morris AJ, Laughlin MJ and Ratajczak J: A novel perspective on stem cell homing and mobilization: review on bioactive lipids as potent chemoattractants and cationic peptides as underappreciated modulators of responsiveness to SDF-1 gradients. *Leukemia* 26: 63-72, 2012.
12. Kang SK, Shin IS, Ko MS, Jo JY and Ra JC: Journey of mesenchymal stem cells for homing: strategies to enhance efficacy and safety of stem cell therapy. *Stem Cells Int* 2012: 342968, 2012.
13. Chen QQ, Yan L, Wang CZ, Wang WH, Shi H, Su BB, Zeng QH, Du HT and Wan J: Mesenchymal stem cells alleviate TNBS-induced colitis by modulating inflammatory and autoimmune responses. *World J Gastroenterol* 19: 4702-4717, 2013.
14. Pisano F, Mura M, Ciuffreda MC, Calabrò F, Lanzo N and Gneccchi M: Optimized lentiviral transduction of human amniotic mesenchymal stromal cells. *Pharmacol Res* 127: 49-57, 2018.
15. Gneccchi M and Melo LG: Bone marrow-derived mesenchymal stem cells: Isolation, expansion, characterization, viral transduction, and production of conditioned medium. *Methods Mol Biol* 482: 281-294, 2009.
16. Cooper HS, Murthy SN, Shah RS and Sedergran DJ: Clinicopathologic study of dextran sulfate sodium experimental murine colitis. *Lab Invest* 69: 238-249, 1993.
17. Scharl M, McCole DF, Weber A, Vavricka SR, Frei P, Kellermeier S, Pesch T, Fried M and Rogler G: Protein tyrosine phosphatase N2 regulates TNF $\alpha$ -induced signalling and cytokine secretion in human intestinal epithelial cells. *Gut* 60: 189-197, 2011.
18. Boyd AS and Fairchild PJ: Approaches for immunological tolerance induction to stem cell-derived cell replacement therapies. *Expert Rev Clin Immunol* 6: 435-448, 2010.
19. Castelo-Branco MT, Soares ID, Lopes DV, Buongusto F, Martinusso CA, do Rosario A Jr, Souza SA, Gutfilen B, Fonseca LM, Elia C, *et al*: Intraperitoneal but not intravenous cryopreserved mesenchymal stromal cells home to the inflamed colon and ameliorate experimental colitis. *PLoS One* 7: e33360, 2012.
20. Ponomaryov T, Peled A, Petit I, Taichman RS, Habler L, Sandbank J, Arenzana-Seisdedos F, Magerus A, Caruz A, Fujii N, *et al*: Induction of the chemokine stromal-derived factor-1 following DNA damage improves human stem cell function. *J Clin Invest* 106: 1331-1339, 2000.
21. Liu H, Liu S, Li Y, Wang X, Xue W, Ge G and Luo X: The role of SDF-1-CXCR4/CXCR7 axis in the therapeutic effects of hypoxia-preconditioned mesenchymal stem cells for renal ischemia/reperfusion injury. *PLoS One* 7: e34608, 2012.
22. Goto Y and Ivanov II: Intestinal epithelial cells as mediators of the commensal-host immune crosstalk. *Immunol Cell Biol* 91: 204-214, 2013.
23. Kinnaird T, Stabile E, Burnett MS, Shou M, Lee CW, Barr S, Fuchs S and Epstein SE: Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. *Circulation* 109: 1543-1549, 2004.
24. Sackstein R, Merzaban JS, Cain DW, Dagia NM, Spencer JA, Lin CP and Wohlgenuth R: Ex vivo glycan engineering of CD44 programs human multipotent mesenchymal stromal cell trafficking to bone. *Nat Med* 14: 181-187, 2008.
25. Ko IK, Kim BG, Awadallah A, Mikulan J, Lin P, Letterio JJ and Dennis JE: Targeting improves MSC treatment of inflammatory bowel disease. *Mol Ther* 18: 1365-1372, 2010.
26. Kean TJ, Duesler L, Young RG, Dadabayev A, Olenyik A, Penn M, Wagner J, Fink DJ, Caplan AI and Dennis JE: Development of a peptide-targeted, myocardial ischemia-homing, mesenchymal stem cell. *J Drug Target* 20: 23-32, 2012.
27. Nakase H, Matsuura M, Mikami S, Uza N and Chiba T: Role of the CXCL12-CXCR4 axis and CXCL16 in inflammatory bowel disease. *Intest Res* 10: 125-133, 2012.



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