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Original Article

Human mesenchymal stem cell homing induced by SKOV3 cells

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Abstract: Human mesenchymal stem cell (hMSC) homing is the migration of endogenous and exogenous hMSCS to the target organs and the subsequent colonization under the action chemotaxic factors. This is an important process involved in the repair of damaged tissues. However, we know little about the mechanism of hMSC homing. Stromal cell derived factor-1 (SDF-1) is a cytokine secreted by stromal cells. Its only receptor CXCR4 is widely expressed in blood cells, immune cells and cells in the central nervous system. SDF-1/CXCR4 signaling pathway plays an important role in hMSC homing and tissue repair. Human cbll1 gene encodes E3 ubiquitin-protein ligase Hakai (also known as CBLL1) consisting of RING-finger domain that is involved in ubiquitination, endocytosis and degradation of epithelial cadherin (E-cadherin) as well as in the regulation of cell proliferation. We successfully constructed LV3-CXCR4 siRNA lentiviral vector, LV3-CBLL1 RNAi lentiviral vector and the corresponding cell systems which were used to induce hMSC homing in the presence of SKOV3 cells. Thus the mechanism of hMSC homing was studied.

Keywords: LV3-CXCR4 siRNA lentiviral vector, LV3-CBLL1 RNAi lentiviral vector, real-time fluorescent quantitative PCR, human mesenchymal stem cells (hMSCs)

Introduction

Human mesenchymal stem cells (hMSCs) are pluripotent stem cells derived from bone marrow. With multiple differentiation potential, hMSCs can differentiate into bone cells, chondrocytes, endothelial cells and myocardial cells. In the meantime, a large amount of cytokines secreted by hMSCs will be involved in tissue repair. Because of these features, hMSCs are believed to be most suitable for treating a variety of diseases. hMSC homing is the migration of endogenous and exogenous hMSCS to the target organs and the subsequent colonization under the action of chemotaxic factors; it can be utilized as a new method of tissue repair [1, 2]. hMSC homing shares some similarities with the directional migration of leukocytes to the inflammatory tissues in terms of mechanism. However, the former is more complex in that it involves the participation of several chemotaxic factors, receptors and adhesion molecules [3, 4]. SDF-1 (stromal cell derived factor-1) was first discovered as a cytokine secreted by mouse bone marrow stromal cells. Consisting of 68 amino acids, SDF-1 has 4 conservative cysteines in its sequence and belongs to CXC subfamily, from which the name CXCLl2 is derived [5]. CXCR4 is the only receptor of SDF-1 and expressed widely in blood cells, immune cells and cells of the central nervous system. By binding to N-terminal amino acids at position 12-17, CXCR4 promotes the conformational changes of SDF-1 and CXCR4. The N-terminal amino acids at position 1-11 of SDF1 will then fold into a specific conformation and bind to the groove formed by the helices of CXCR4. This causes the conformational changes of CXCR4 transmembrane helical domains, thereby triggering the G-protein coupled receptor signaling [6, 7]. SDF-1/CXCR4 signaling pathway plays an important role in hMSC homing and tissue repair. Tissue damage will prompt the upregulation of SDF-1 and the increase of local SDF-1 concentration. The hMSCs expressing CXCR4 will migrate along the concentration gradient of SDF-1 to the site of damage for tissue repair [8-10]. Abbott et al.

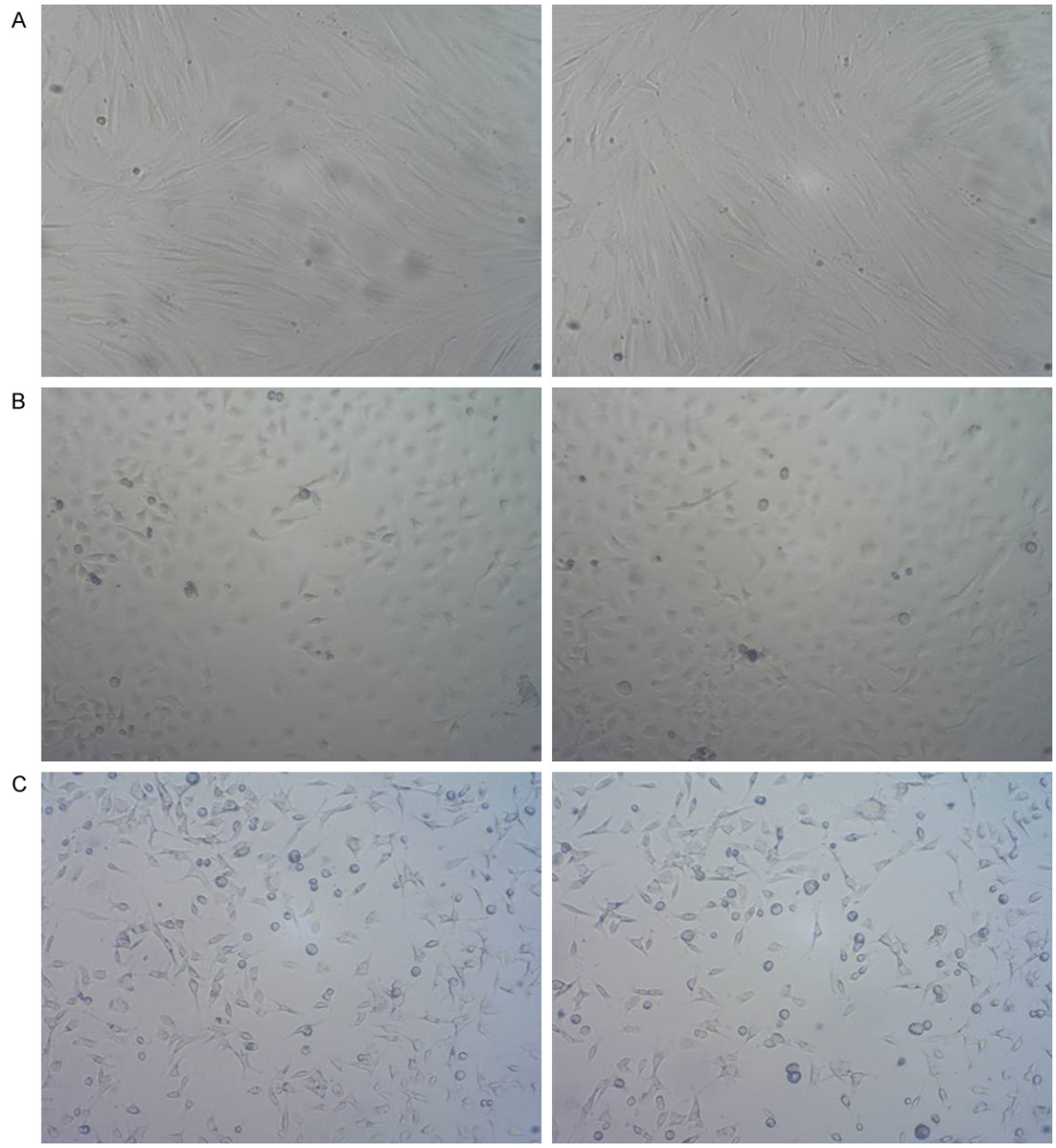


Figure 1. Characteristics of cell culture (100×). A: hMSCs after culture; B: Hela cells; C: SKOV3 cells.

performed transplantation of stem cells to the region of infarct in rats and administered AMD3100 to block the expression of CXCR4, the receptor of SDF-1. As a result, the stem cells migrating to the region of infarct reduced by (64.2±5.5)%. However, the injection of adenovirus carrying SDF-1 gene into the region of infarct caused the massive upregulation of SDF-1, with the number of homing stem cells increased by nearly 100% [11]. Bhakta et al. found that the migration rate of CXCR4-transfected MSCs in Transwell migration assay using 30 g/L SDF-1 was about 3 and 5 times of that in the control group at 3 h and 6 h, respectively. This indicated that SDF-1 promoted the homing of MSCs [12]. Human cbll1 gene encodes E3 ubiquitin-protein ligase Hakai (also known as CBLL1) consisting of RING-finger domain that is involved in ubiquitination, endocytosis and degradation of epithelial cadherin (E-cadherin) as well as in the regulation of cell proliferation [13-15].

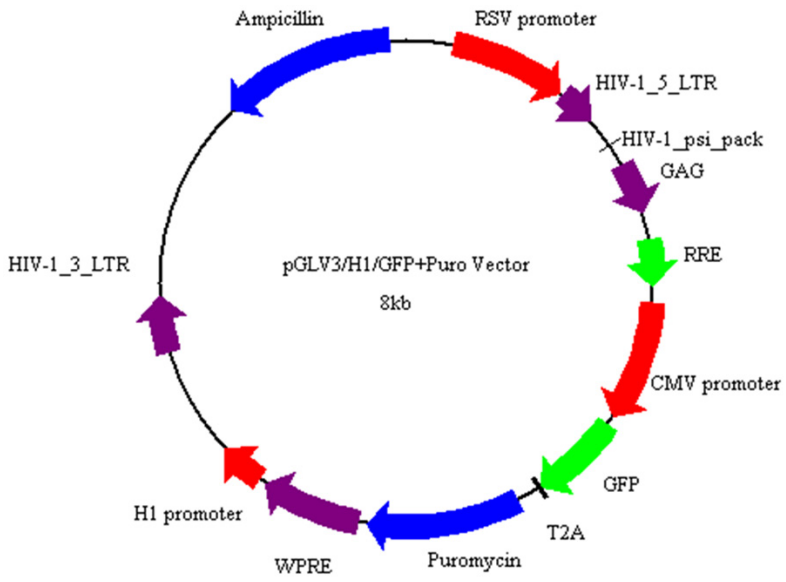


Figure 2. Sequence of LV3 vector.

To investigate the mechanism of hMSC homing, we constructed LV3-CXCR4 siRNA lentiviral vector, LV3-CBLL1 RNAi lentiviral vector and the corresponding cell systems for inducing hMSC homing in the presence of SKOV3 cells.

Materials and method

Cells

hMSCs were purchased from Cyagen Biosciences (Guangzhou) Inc.; SKOV3 human ovarian cancer cells, Hela human cervical cancer cells, and 293T cells were purchased from Shanghai Institutes for Biological Sciences, CAS.

Method

Primary culture and identification of hMSCs: The hMSCs (Cyagen Biosciences (Guangzhou) Inc.) were cultured after resuscitation by the following method. The temperature of water bath was first raised to 37°C, and the cryogenic tube containing hMSCs was taken out from the liquid nitrogen tank and quickly placed into the water bath. The tube was gently shaken to fully dissolve the contents, and the tube was taken out from the water bath with more shaking. After disinfection with 75% alcohol, the biosafety cabinet was opened. The cell suspension was drawn using a pipette, loaded into a centrifuge tube and added with 5 mL of cell culture medium containing 15% fetal bovine serum (FBS). The cells were centrifuged at 1000 rpm for 5 min with supernatant discarded inside the biosafety cabinet. With the addition of 1 mL culture medium, the cells were gently blown to form a suspension and diluted with culture medium containing 20% FBS. Then the cells were inoculated to the culture flask and cultured at 37°C in a humidified 5% CO_2 incubator. The culture medium was replaced after 24 h. After that, the medium was replaced at the frequency depending on cell growth and the color of the culture medium.

After the cells grew to 70%-80% confluence under the inverted microscope, cell passage was performed. The culture medium in the flask was first removed, and the cells were washed with PBS buffer 2-3 times. Then approximately 2 mL of trypsin digestion fluid was added into a 50 mL culture flask and cultured for about 3 min at 37°C in an incubator. After the cells shrank and became rounded under the microscope, the flask was gently shaken to cause the cells to detach from the bottom of the flask. Next 3 ml of complete culture medium was added, and the cells were gently blown. The cells were collected into a centrifuge tube for centrifugation at 1000 rpm for 5 min, with supernatant discarded. The culture medium was added, and the cells were gently blown to form a suspension. Cell passage was performed according to the proportion of 1:3 to a sterilized culture flask, and culture medium was added for further incubation. **Figure 1A** shows the hMSCs after culture.

The cells were identified by immunohistochemistry. Coloration was performed by adding anti-CD3 antibody, anti-CD44 antibody, anti-CD90 antibody in combination with HRP-conjugated streptavidin and biotin-conjugated secondary antibodies to determine whether the cells were hMSCs.

Culture of SKOV3 cells and Hela cells: The SKOV3 cells and Hela cells were resuscitated, cultured and passaged by using the same

Table 1. Primers sequences

Sense strand	5'-GATCC-(GN_{18})-(TTCAAGAGA)-($N_{18}C$)-TTTTTTG-3'
Antisense strand	3'-G(CN_{18})-(AAGTTCTCT)-($N_{18}G$)-AAAAAACTTAA-5'
Target sequence	GCAAGGCAGTCCATGTCATCT
Sense	GATCCGCAAGGCAGTCCATGTCATCTTTCAAGAGAAGATGACATGGACTGCCTTGCTTTTTTG
Antisense	AATTCAAAAAAGCAAGGCAGTCCATGTCATCTTCTCTTGAAAGATGACATGGACTGCCTTGCG

method as above. **Figure 1B-D** showed the growth of Hela cells and SKOV3 cells.

Construction of LV3-CXCR4 siRNA lentiviral vector: The PCR primers were designed according to the sequence of the CXCR4 gene. Restriction enzyme digestion was performed using LV3 vector, and the purified product was ligated and transformed into competent cells. The positive clones were identified by PCR amplification and enzyme digestion. After it was confirmed that the target gene was ligated to the vector, the positive clones were sequenced. The sequencing results were aligned to the reference sequence, and LV3-CXCR4 RNAi lentiviral vector was deemed as constructed successfully if the alignment was correct. **Figure 2** shows the sequence of LV3 vector.

Enzyme digestion of the vector: The vector was digested with BamHI and EcoRI using the system including 5.0 μl of EcoRI (10 U/μl), 5.0 μl of BamHI (10 U/μl), 10.0 μl of 10× Buffer, 10.0 μl of LV3 Plasmid DNA (500 ng/μl), and 70.0 μl of dH_2O, the total volume is 100.0 μl.

Dephosphorization of the vector fragment: Vector fragment obtained by enzyme digestion was dephosphorized using the following system including 5.0 μl of 10× SAP Buffer, 25.0 μl of Product of enzyme digestion of LV3 vector, 1.0 μl of SAP, and 19.0 μl of dH_2O, the total volume is 50.0 μl.

Sequence design: Considering the loop structure of LV3-CXCR4 template, the sequence TTCAAGAGA was designed to avoid the termination signal. To the 5'-end of the sense strand, the sequence GATCC was added so that it would be complementary with the cohesive end after digestion with BamHI; to the 5'-end of the antisense strand, the sequence AATTC was added so it would be complementary with the cohesive end after digestion with EcoRI. The primers sequences were shown in **Table 1**.

Annealing of LV3-shDNA template: DNA oligo was dissolved in TE (pH 8.0) to obtain the concentration of 100 uM. The sense strand and antisense strand oligo solutions were used to prepare the following anneal system including 5 ul of 10× shDNA Annealing Buffer, 5 ul of sense strand (100 uM), 5 ul of antisense strand (100 uM), and 35 ul of ddH_2O, the total volume is 50 ul. Reaction conditions: After reaction at 37°C for 25 min, the enzyme was deactivated at 65°C for 15 min. The target vector was recovered by gel extraction kit (Biotech, Beijing).

Ligation: The ligation was performed using the system below: 2 ul of 10× T4 Ligation buffer, 1 ul of LV3 (BamHI+EcoRI), 1 ul of shDNA template (100 nM), 1 ul of T4 DNA ligase (5 weissU/ul), and 15 ul of ddH_2O, the total volume is 20 ul. Annealing was performed on the PCR machine by the following procedures: 95°C for 5 min; 85°C for 5 min; 75°C for 5 min; 70°C for 5 min; preservation at 4°C. After annealing, 10 μM shRNA template was obtained and diluted 50-fold to achieve the final concentration of 200 nM for ligation.

Transformation: (1) To thaw the competent cells, the vial was removed from -80°C and thawed on ice. Then 10 μl of ligation product was added to transform the cells. The content was gently mixed and placed on ice for 30 min. (2) The centrifuge tube was placed into the water bath preheated to 42°C for 60 s, without shaking the tube. (3) The centrifuge tube was rapidly transferred to the ice bat to cool the cells for 5 min. (4) Into each tube 300 μl of LB medium (containing no antibiotics) was added. The tube was transferred to shake culture at 37°C and 220 rpm for 1 h to resuscitate the cells. (5) Onto agar plate with LB medium and 50 μg/ml Ampicillin, 100 μl of the cells were coated. (6) After the liquid coated on the plate was absorbed, the plate was placed upside down in the incubator at 37°C for 16 h. (7) The clonal colonies were picked from the plate. Plasmid extraction was performed and the pos-

itive clones were identified. (8) Four independent, well-growing colonies were picked from the plate and placed into the tube containing 5 ml LB medium and 50 μg/ml Ampicillin. (9) The tube was placed on the shaker at 37°C and 250 rpm for 16 h.

Identification of recombinant plasmid by enzyme digestion: Plasmid was extracted from the bacterial liquid using plasmid extraction kit according to the manufacturer's instruction. The extracted plasmid was identified by digestion with EcoRI alone using the system below: 1 μl of 10× Buffer, 1 μl of plasmi, 1 μl of EcoRI, 7 μl of ddH_2O, the total volume is 10 ul. Reaction condition: 22°C, 1 h.

Sequencing of the recombinant plasmid and plasmid extraction: 200 μl liquid culture of positive clones was sequenced, and the remaining liquid was preserved in glycerol. The sequences were aligned with the target genes. If the sequence was correct, the glycerol-preserved positive clones were inoculated to LB medium, and the recombinant plasmid was extracted.

Packaging and purification of LV3-CXCR4 RNAi lentiviral vector: Lentiviral shuttle plasmid and the auxiliary assemble vector plasmid were prepared. Four plasmid vectors (LV3, PG-p1-VSVG, PG-P2-REV and PG-P3-RRE) were subjected to high-purity endotoxin-free plasmid extraction. The 293T cells were co-transfected with the four plasmid vectors. The medium was replaced by complete medium after co-transfection for 8 h, and cell culture continued for 48 h. The supernatant containing lentiviral particles was collected and condensed into high-titer lentiviral concentration solution. The 293T cells were infected with the lentiviruses, and the viral titer was detected by double-dilution method.

The lentiviruses were purified by CsCl density gradient centrifugation by the following method:

(1) For every 100 ml of supernatant, 50 ml PEG8000 (20% PEG8000, 2.5 M NaCl) was added and then placed on ice to precipitate the viruses. (2) The above mixture was centrifuged at 12000 rpm for 20 min with supernatant discarded. The precipitate was suspended in 10 ml of 1.10 g/ml CsCl solution (20 mM Tris-Hcl as solvent, Ph 8.0) and centrifuged at 4°C and 7000 rmp for 5 min to obtain the viral suspension. (3) CsCl concentration gradient was formed by adding 2.0 ml of CsCl (density 1.40 g/ml, the same solvent as above). Then 3.0 ml 1.30 g/ml CsCl solution was added, followed by the addition of 5 ml of viral suspension. The cells were centrifuged at 20000 rpm at room temperature for 2 h. (4) The viral liquid with density of 1.30-1.40 g/ml was collected into a dialysis bag. Before use, the dialysis bag was boiled in 10 mM EDTA Na2 for 10 min. (5) Dialysis was performed using dialysis buffer at 4°C overnight (the solution containing 50 g sucrose, 10 ml 1 M Tris-HCl (pH 8.0) and 2 ml 1 M $MgCl_2$ was diluted to 1000 ml). The dialysis buffer was changed once during the process. (6) The viruses were collected and the viral titer was detected using hole-by-hole dilution method. The fluorescent cells were counted under the fluorescence microscope. The viral titer was calculated according to the dilation factor.

Screening of the target sequence of the CXCR4 gene lentiviral RNAi vector by fluorescent quantitative PCR: The following primers were designed according to sequence of CXCR4 (GeneID: 7852). hCXCR4F: AATGGGCTCAGGGGACTATG; hCXCR4R: AAGATGATGGAGTAGATGGTGGG; h actin f: TGACGTGGACATCCGCAAAG; h actin r: CTGGAAGGTGGACAGCGAGG.

RNA was extracted using RNA extraction kit and was then reverse transcribed into cDNA using random primers. Fluorescent quantitative PCR was performed using specific primers and sybr green I dye. Reverse transcription system was as follows: 10 ul of 2× RT buffer, 1 ul of 6N random primer (100 pmol/ul), 1 ul of RT-mix, 5 ul of Template (RNA), 3 ul of DEPC-treated water, the total volume is 20 ul. Enzyme digestion was performed at 37°C for 1 h, and the product was analyzed with agarose gel electrophoresis. The positive clones were those corresponding to the bands of correct size.

Transfection of SKOV3 cells and Hela cells by CXCR4 RNAi lentiviral vector: Preparation of cells: Into each well of 24-well plate, about 4×10^4 target cells were inoculated. Transfection was performed after the cell grew to about 70% confluence.

For transfection of SKOV3 cells and Hela cells, the viruses preserved at 4°C were taken and centrifuged with a benchtop centrifuge for 20 s. The amount of lentiviruses calculated by MOI

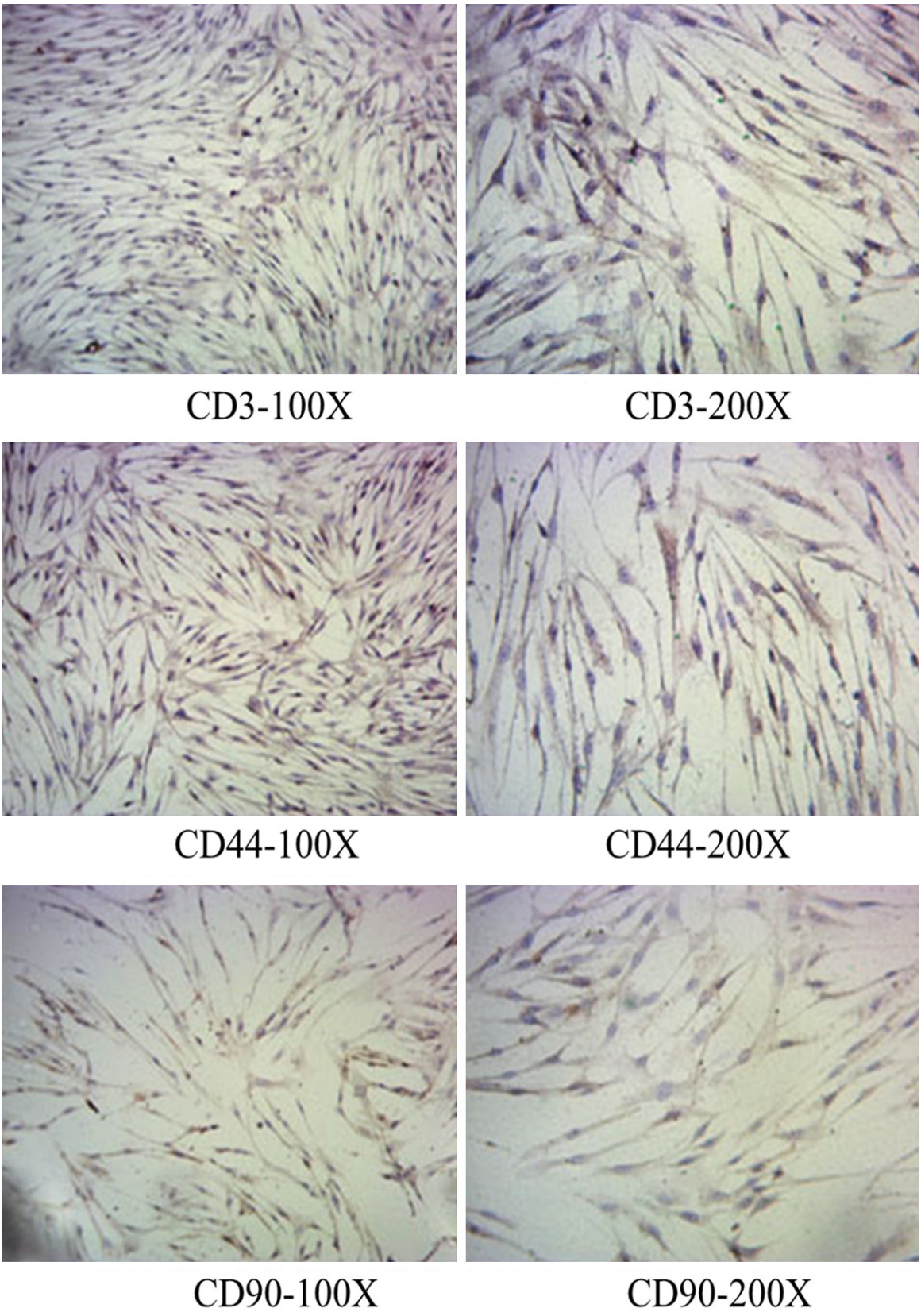


Figure 3. Immunohistochemistry of hMSCs.

was added into the culture medium to make the total volume as small as possible. The purpose was to achieve the best transfection efficiency. After that, the cells were taken out from the incubator. A proper amount of viral fluid was added into the culture medium using a pipette, and the original culture medium in the dish was removed. Viral fluid was added into the target cells and the control cells, respectively. After mixing, the cells were cultured at 37°C in a 5% CO_2 incubator overnight using medium supplemented with 8 μg/ml ploybrene. The culture medium was changed at 8 h and 24 h. Fluorescence was observed under the inverted fluorescence microscope at day 5 after transfection, and the transfection efficiency was detected [17].

Construction of Hakai gene lentiviral RNAi vector and transfection of SKOV3 cells and Hela cells: Hakai gene lentiviral RNAi vector, LV3-CBLL1 RNAi vector, was constructed by referring to the sequence of wild-type CBLL1 gene. The same subsequent procedures were carried out for this vector as with LV3-CXCR4 siRNA lentiviral vector. Then SKOV3 cells and Hela cells were infected with LV3-CBLL1 RNAi vector, and the transfection efficiency was detected using the inverted fluorescence microscope. The results were identified by fluorescent quantitative PCR. Reaction conditions: 25°C for 10 min, 42°C for 50 min, 85°C for 5 min. Fluorescent quantitative PCR reaction system is as follows: 25 ul of 2× PCR buffer; 1ul of each primers (25 pmol/ul), 0.5 ul of Sybr green I (20*), 2 ul of template (cDNA), and 20.5 ul of DEPC-treated water, the total volume is 50.0 ul.

Result

Immunohistochemical detection of hMSCs

Immunohistochemistry is the method for localizing and qualitatively and quantitatively detecting the antigens (polypeptides and proteins) in tissues based on the coloration of antibody labels (fluorecein, enzymes, metal ions and isotopes) upon binding to the antigens. In this study, the hMSCs were identified using anti-CD3 antibody, anti-CD44 antibody, anti-CD90 antibody in combination with HRP-conjugated streptavidin and biotin-conjugated secondary antibodies. The coverslips were first prepared. After cell digestion, the cells were resuspended in complete culture medium to achieve the density of about $2*10^4$/ml. The coverslip was placed on a sterilized 12-well plate. The cell suspension was added to the round coverslip, and the cells were left to grow for about 30 min. Complete culture medium was supplemented into the dish and the cells were cultured at 37°C in a 5% CO_2 incubator. Pretreatment was performed before immunohistochemical detection. The cells were washed with PBS buffer twice and fixed in 4% paraformaldehyde at room temperature for 20 min. Then the cells were washed with PBS for three times, treated with 0.5% Triton X-100 at room temperature for 20 min, and washed again with PBS for three times. After that, the cells were treated with 3% H_2O_2 at room temperature for 15 min and washed with PBS for three times. Immunohistochemical detection was performed by the following method. The cells were blocked for 60 min at room temperature with goat serum. The cells were taken out with the removal of blocking buffer (no washing) and incubated with primary antibodies at 4°C overnight (dilution factor 1:500 for all three antibodies. PBS buffer was used instead of primary antibodies in negative control). The cells were washed with PBS for three times, 3 min each time. Then the cells were incubated with working solution of biotin-labeled secondary antibodies at 37°C for 30 min, and washed with PBS for three times, 3 min each time. After that, the cells were incubated with working solution of HRP-conjugated streptavidin at 37°C for 30 min. This was followed by washing with PBS for three times, 3 min each time. DAB reagent was added for coloration for about 5 min. After washing with distilled water for twice, 5 min each time, the cells were counterstained with hematoxylin for 5 min. The cells were washed with running water and dehydrated through alcohol gradient (75%, 85%, 95%, 100%), 3 min each time, followed by transparentization with xylene twice, 3 min each time. Finally the coverslip was sealed in place with neutral balsam. The results are shown in the **Figure 3**. During the detection of CD3, CD44 and CD90 antibodies, brown precipitate was found in all groups of cells, which indicated positive reaction. Thus the cells were confirmed as hMSCs.

Extraction of target recombinant plasmid

After digestion of LV3 vector with BamHI and EcoRI, DNA fragments were analyzed by agarose gel electrophoresis. As shown in **Figure 4A**, the enzyme digestion was successful.

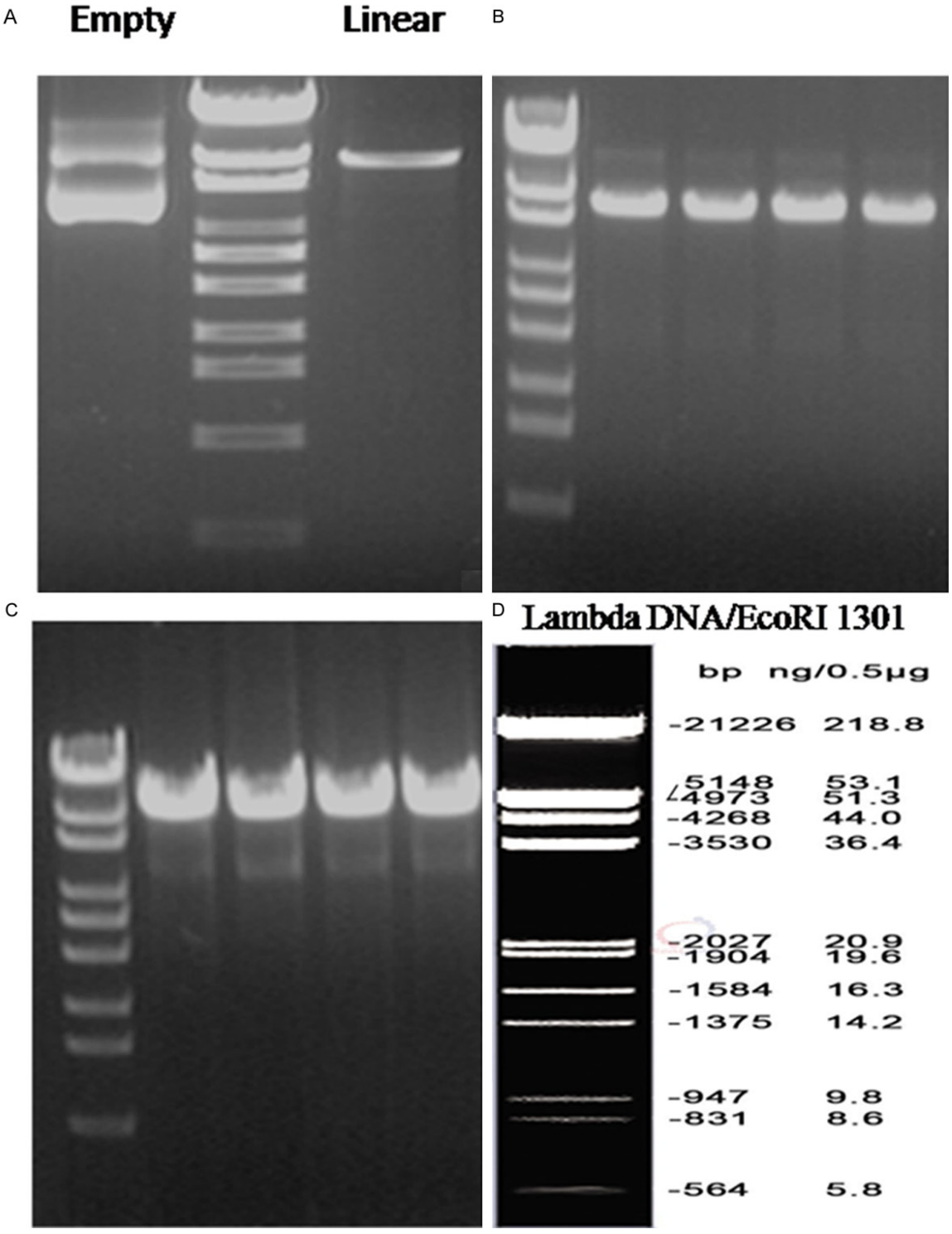


Figure 4. Identification of monoclones by digestion with EcoRI alone. A: Enzyme digestion of LV3 vector; B: Recombinant plasmid; C: Identification of recombinant vector by enzyme digestion; D: Lambda DNA/Eco130I.

Dephorization was performed for the target fragments to prevent self-ligation. Then the target fragments and the annealed CXCR4 shRNA template were subjected to ligation reaction to obtain the complete target fragment which was then transferred to the com-

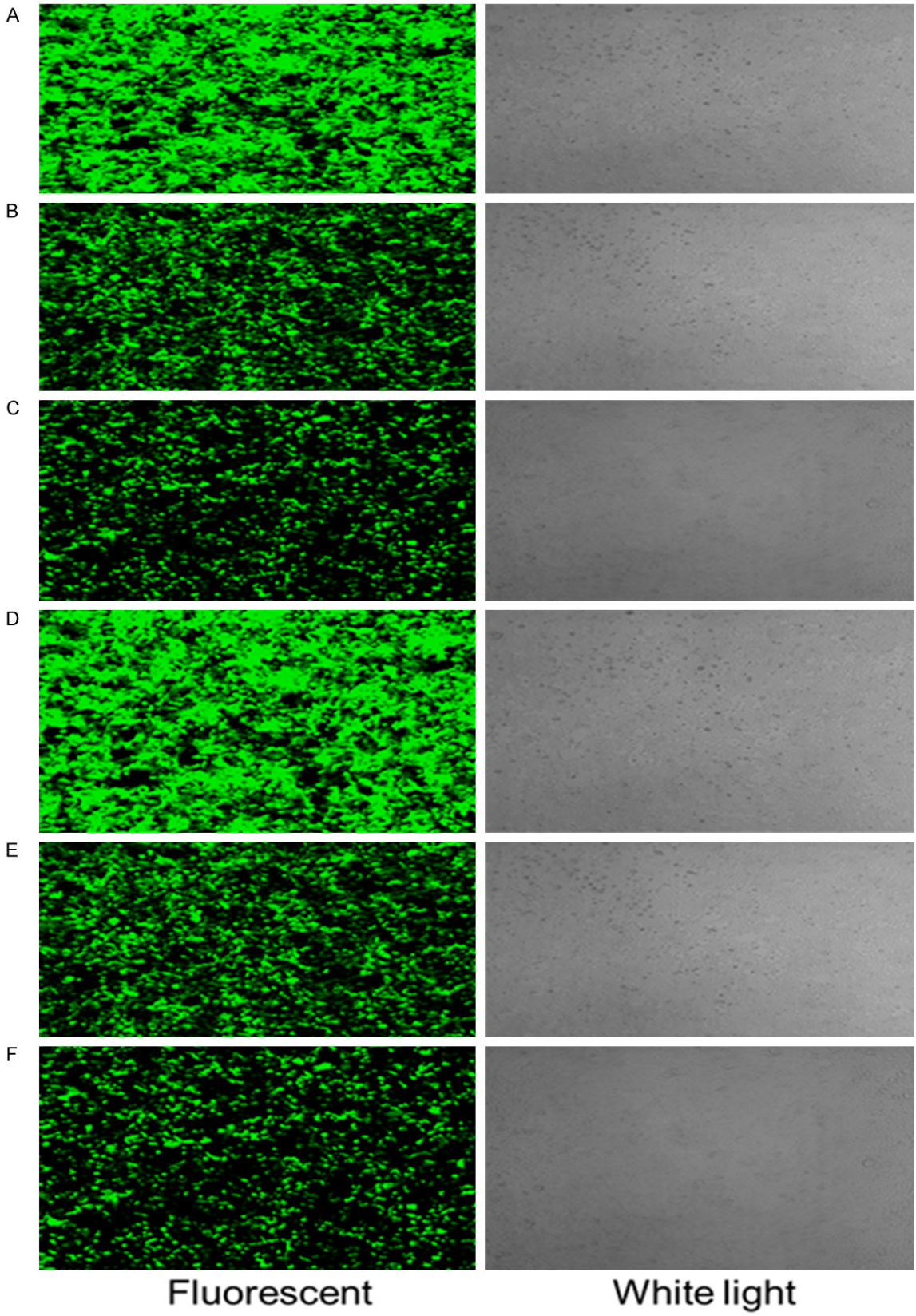
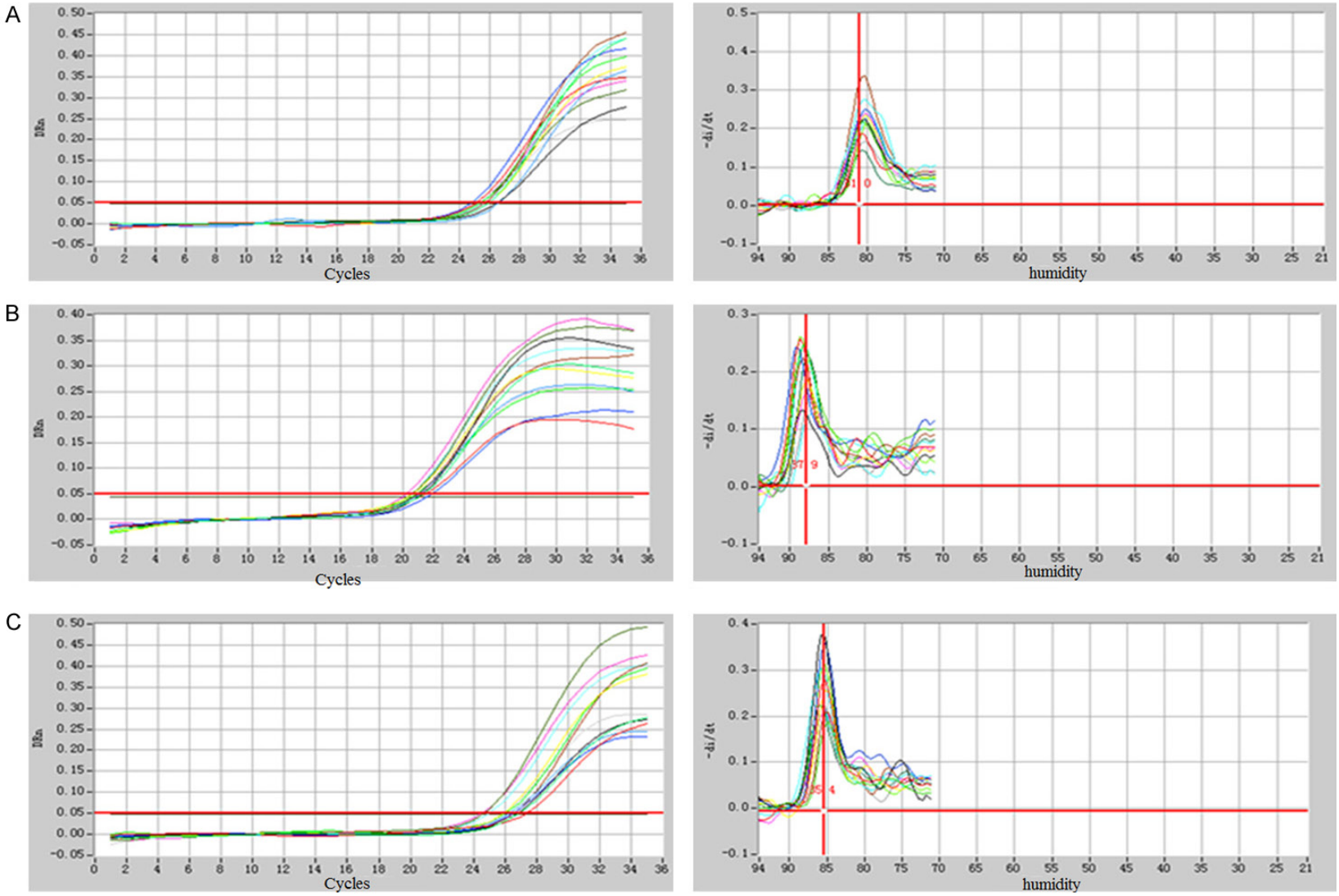


Figure 5. Detection of lentiviral titer. A: 1.0 μL of LV3-CXCR4 RNAi; B: 0.1 μL of LV3-CXCR4 RNAi; C: 0.01 μL of LV3-CXCR4 RNAi; D: 1.0 μL of LV3-CBLL1 RNAi; E: 0.1 μL of LV3-CBLL1 RNAi; F: 0.01 μL of LV3-CBLL1 RNAi.


A
B
C
Cycles
humidity
-dI/dt

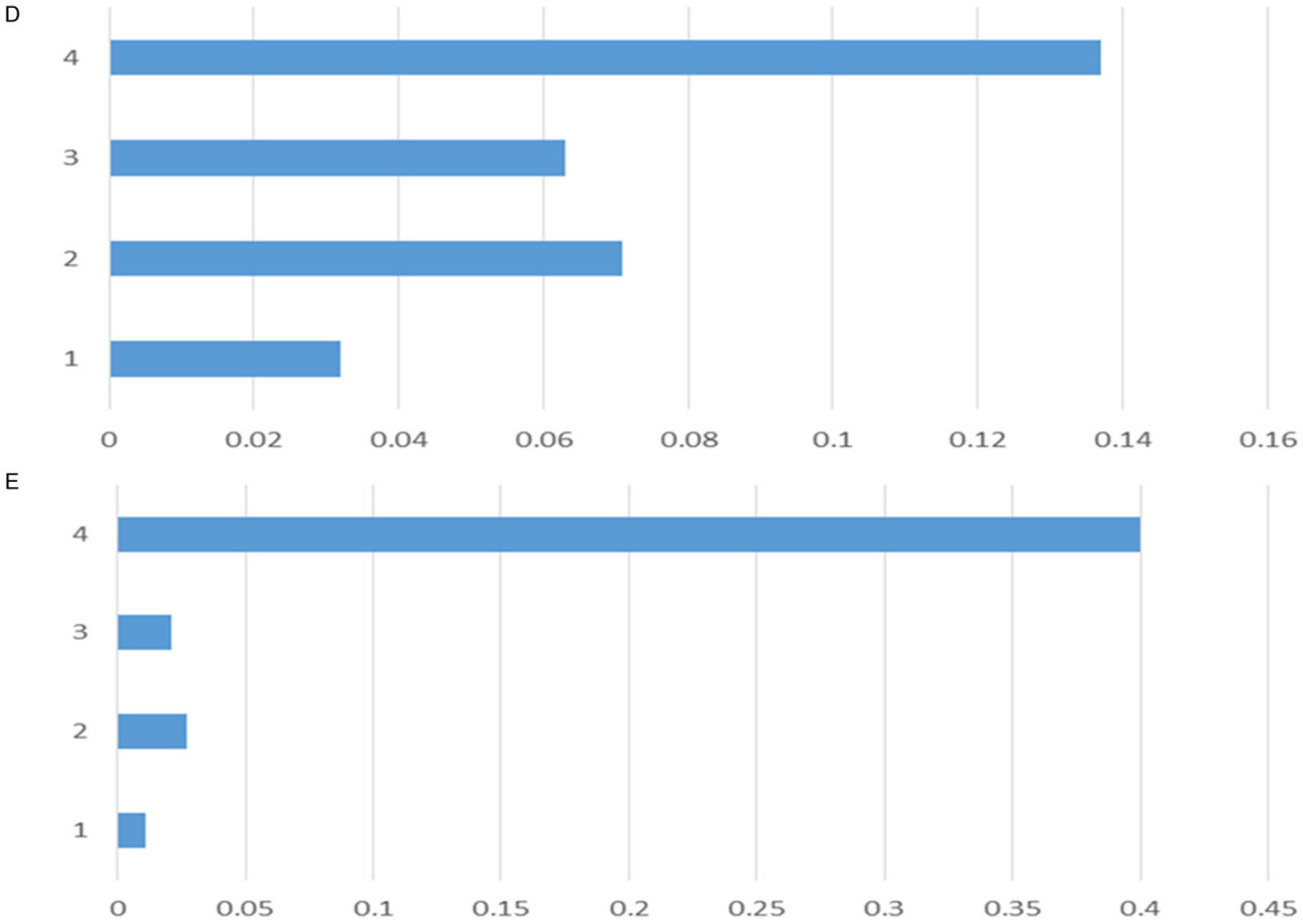


Figure 6. Results of fluorescent quantitative PCR. A: Dissolve curve of CXCR4; B: Dissolve curve of internal reference; C: Dissolve curve of CBLL1; D: Relative quality of CXCR4; E: Relative quality of CBLL1.

Table 2. Sequencing and transfection with LV3-CXCR4 RNAi

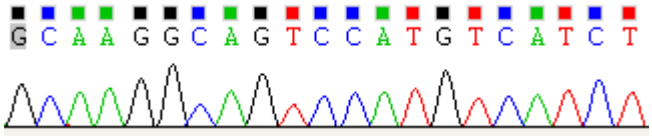
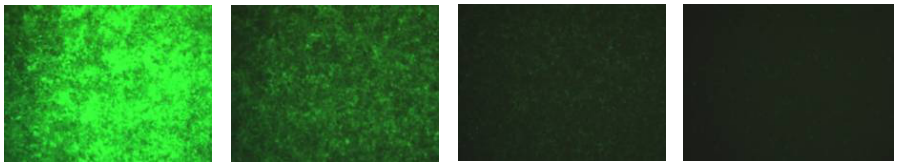
Sequence (5' to 3')	GCAAGGCAGTCCATGTCATCT
Sequencing result	
Total stock	1 ml
Cell for titer detection	293T
Titer detection	10^{-1} 10^{-2} 10^{-3} 10^{-4} 
Result	5×10^{8} TU/ml (transfection time 72 h)

Table 3. Sequencing and transfection with LV3-GFP

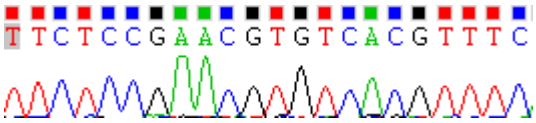
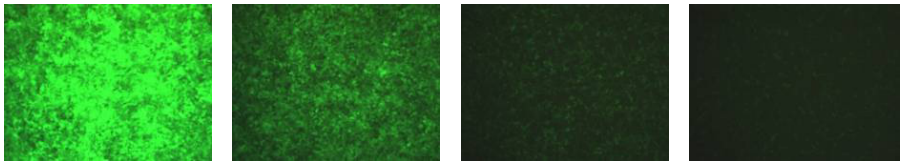
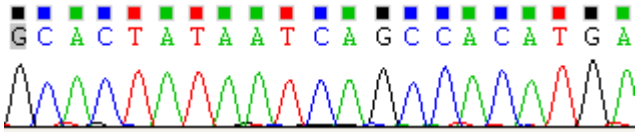
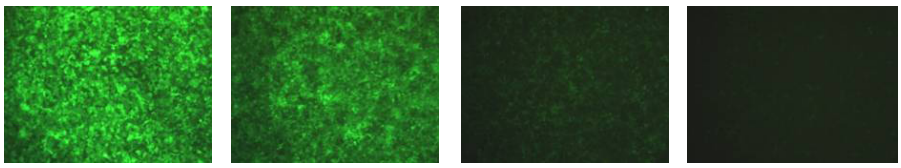
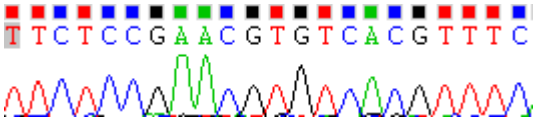
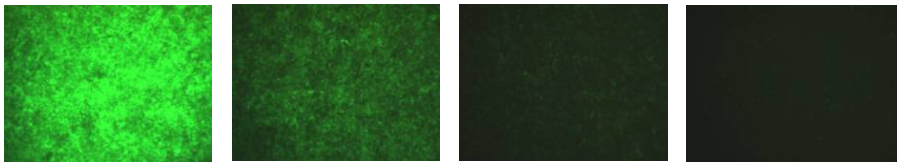
Sequence (5' to 3')	TTCTCCGAACGTGTCACGT
Sequencing result	
Total stock	0.2 mL
Cell for titer detection	293T
Titer detection	10^{-1} 10^{-2} 10^{-3} 10^{-4} 
Result	5×10^{8} TU/ml (transfection time 72 h)

Table 4. Sequencing and transfection with LV3-CBLL1 RNAi

Sequence (5' to 3')	GCACTATAATCAGCCACATGA
Sequencing result	
Total stock	1 ml
Cell for titer detection	293T
Titer detection	10^{-1} 10^{-2} 10^{-3} 10^{-4} 
Result	3×10^{8} TU/ml (transfection time 72 h)

petent cells. After resuscitation, the monoclones were picked for extended culture. Finally the extracted plasmid was identified by digestion with EcoRI alone. As shown in **Figure**

Table 5. Sequencing and transfection with LV3-GFP

Sequence (5' to 3')	TTCTCCGAACGTGTCACGT
Sequencing result	
Total stock	0.2 mL
Cell for titer detection	293T
Titer detection	10^{-1} 10^{-2} 10^{-3} 10^{-4} 
Result	3×10^{8} TU/ml (transfection time 72 h)

4B-D, the picked monoclones were positive clones.

The positive clones were sequenced. The sequences obtained were aligned with the target gene to determine whether the clones were positive. After that, the glycerol-preserved positive clones were inoculated to LB medium. Plasmid was extracted after extended culture to obtain sufficient amount of LV3-CXCR4 shRNA and LV3-CBLLI shRNA. Using the specified method, LV3-CXCR4 shRN was packaged and purified and used to infect 293T cells for 48h. The lentiviral titer was detected using the fluorescence microscope, with results shown in **Figure 5**.

Determination of target sequences of LV3-CXCR4 siRNA lentiviral vector, LV3-CBLL1 RNAi lentiviral vector

Fluorescent quantitative PCR: Fluorescent quantitative PCR was performed for the extracted RNA and the results are shown in **Figure 6**.

Sequencing and transfection with LV3-CXCR4 RNAi lentiviral vector and LV3-CBLL1 RNAi lentiviral vector

The results of sequencing of LV3-CXCR4 RNAi lentiviral vector are shown in **Table 2**. In transfection experiments of 293T cells, CXCR4 produced the best inhibitory effect. The results of transfection with LV3-GFP lentivirus as negative control are shown in **Table 3**. The results of transfection with LV3-CBLL1 RNAi are shown in **Table 4**, and the transfection with LV3-GFP lentivirus as negative control in **Table 5**. It can be seen that the constructed lentiviral vector successfully transfected 293T cells.

Transfection of SKOV3 cells and Hela cells with LV3-CXCR4 RNAi and LV3-CBLL1 RNAi

Fluorescence was observed under the inverted fluorescence microscope on day 6 after transfection of SKOV3 cells and Hela cells with LV3-CXCR4 RNAi and LV3-CBLL1 RNAi. The transfection efficiency was evaluated, with results shown in **Figures 7** and **8**, respectively. It can be seen that the lentiviral vector constructed had high quality and successfully transfected SKOV3 cells and Hela cells. They were qualified for inducing hMSC homing.

Discussion

Haider et al. performed transplantation of hMSCs transfected with SDF-1 gene into the region of infarct and found that SDF-1 expression was greatly upregulated in this region. Thus the homing of transplanted hMSCs was successfully induced, thereby improving the cardiac function [18]. Cheng et al. transfected CXCR4 gene into hMSCs and the chemotaxis to SDF-1 was greatly enhanced. After transplantation of hMSCs via the tail vein into the region of infarct in rats, it was found that the number of hMSCs migrating to the site of injury increased by 1 time. This was accompanied by obvious increase of capillary density, decrease of myocardial collagen content and improvement of cardiac function [19]. MSCs have been shown to possess a strong multiple differentiation potential, but their homing to the target organs is low. The distribution and differentiation of

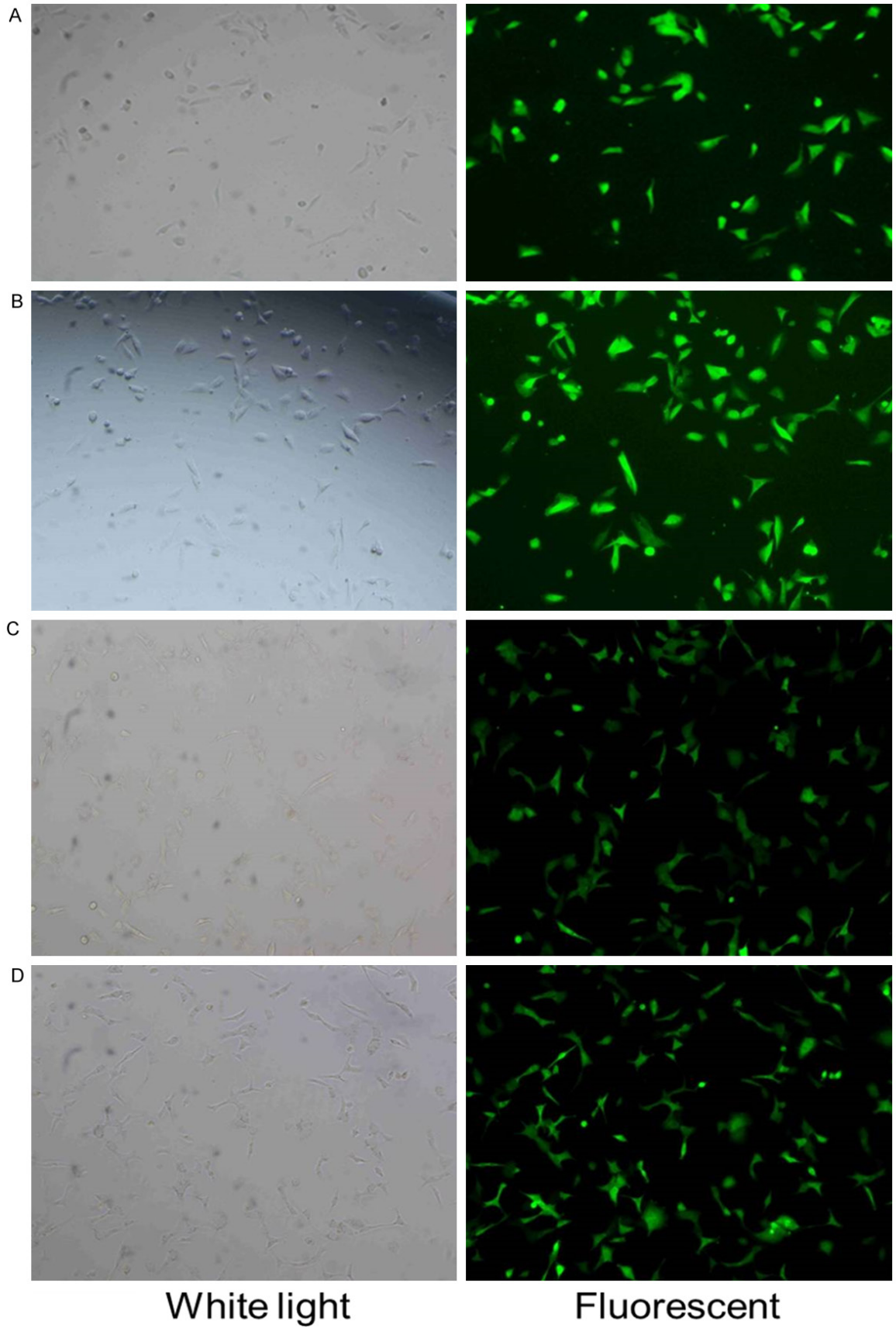

A
B
C
D
White light
Fluorescent

Figure 7. Transfection of SKOV3 cells and Hela cells with LV3-CXCR4 RNAi. A: Hela cells transfected with GFP; B: Hela cells transfected with CXCR4 RNAi; C: SKOV3 cells transfected with GFP; D: SKOV3 cells transfected with CXCR4 RNAi.

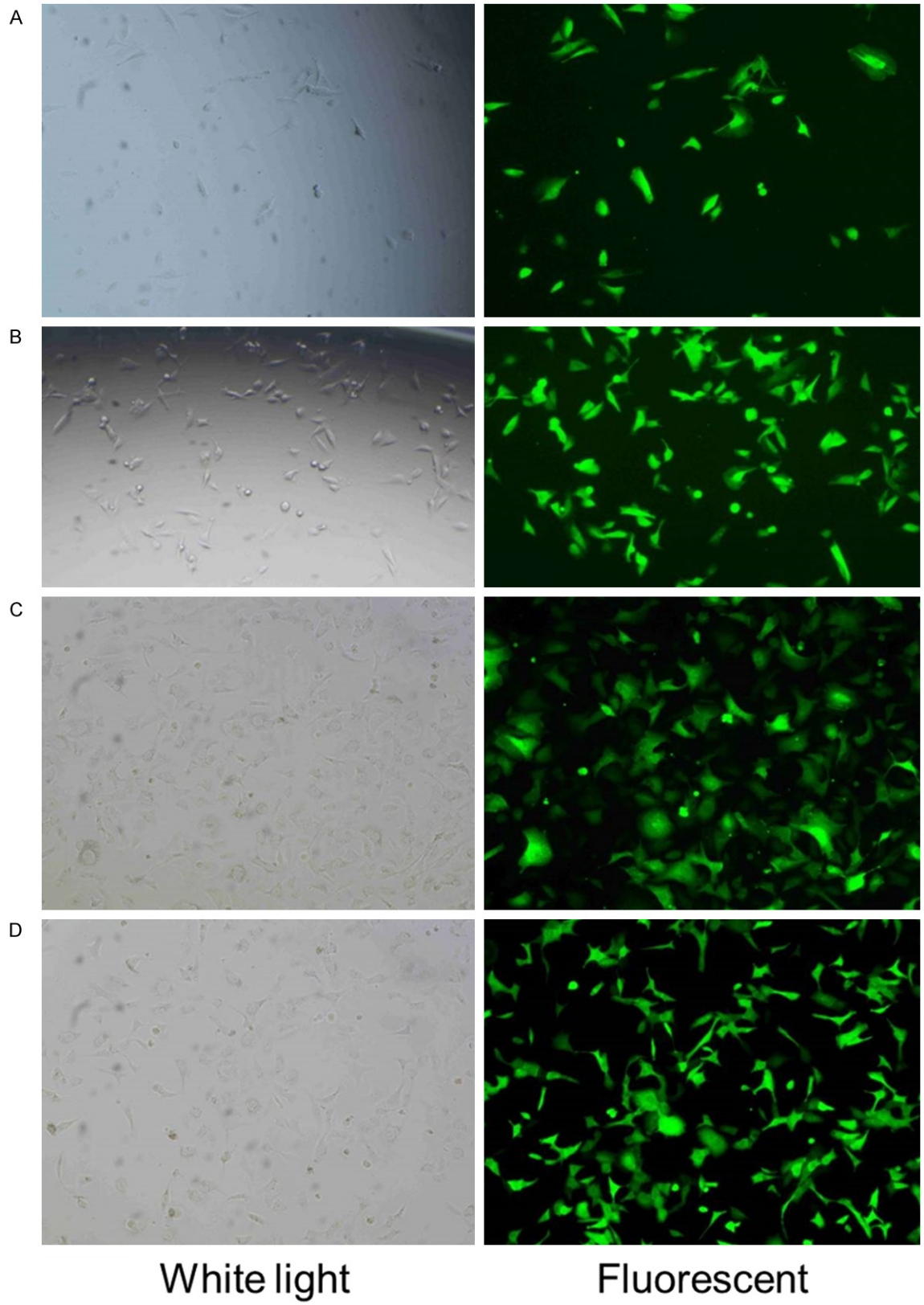


Figure 8. Transfection of SKOV3 cells and Hela cells with LV3-CBLL1 RNAi. A: Hela cells transfected with GFP; B: Hela cells transfected with CBLL1 RNAi; C: SKOV3 cells transfected with GFP; D: SKOV3 cells transfected with CBLL1 RNAi.

MSCs migrating to the sites of injury directly determine the repair effect. However, little is known about the phenotype of MSCs, the mechanism of MSC homing as a multifactorial process and the proliferation and differentiation in vivo. It is of high significance to evaluate the tumorigenicity, uncontrollability and immunosuppressive effect of MSCs in vivo. The immunoregulatory effect and the multiple differentiation potential make MSCs very suitable for clinical treatment [20, 21]. In order to make use of MSCs, we should gain more knowledge of the mechanism of MSC homing and the influence factors. In this study, LV3-CXCR4 shRNA lentiviral vector and LV3-CBLL1 RNAi lentiviral vector were constructed along with the cell system for inducing hMSC homing in the presence of SKOV3 cells. The purpose was to investigate the mechanism of hMSC homing.

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