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Upregulation of CC Chemokine Receptor 7 (CCR7) Enables Migration of Xenogeneic Human Adipose-Derived Mesenchymal Stem Cells to Rat Secondary Lymphoid Organs

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Background: CC chemokine receptor 7 (CCR7) expression is vital for cell migration to secondary lymphoid organs (SLOs). Our previous work showed that inducing CCR7 expression enabled syngeneic mesenchymal stem cells (MSCs) to migrate into SLOs, resulting in enhanced immunosuppressive performance in mice. Given that human adipose-derived stem cells (hASCs) are widely used in clinical therapy, we further investigated whether upregulation of CCR7 enables xenogeneic hASCs to migrate to rat SLOs.

Material/Methods: hASCs rarely express CCR7; therefore, hASCs were transfected with lentivirus encoding rat *CCR7* (*rCCR7*) plus green fluorescence protein (*GFP*) or *GFP* alone. CCR7 mRNA and cell surface expression of rCCR7-hASCs and GFP-hASCs were examined by reverse transcription-polymerase chain reaction (RT-PCR) and flow cytometry (FCM), respectively. The phenotype, differentiation, and proliferation capacity of each cell type was also determined. To examine migration, rCCR7-hASCs and GFP-hASCs were injected intravenously into Lewis rats, and the proportion of GFP-positive cells in the spleen and lymph nodes was determined with FCM.

Results: mRNA and cell surface protein expression of CCR7 was essentially undetectable in hASCs and GFP-ASCs; however, CCR7 was highly expressed in rCCR7-ASCs. rCCR7-hASCs, GFP-hASCs, and hASCs shared a similar immunophenotype, and maintained the ability of multilineage differentiation and proliferation. In addition, the average proportion of GFP-positive cells was significantly higher following transplantation of rCCR7-hASCs compared with GFP-hASCs ($p<0.01$).

Conclusions: These results suggest that upregulation of rat CCR7 expression does not change the phenotype, differentiation, or proliferation capacity of hASCs, but does enable efficient migration of hASCs to rat SLOs.

MeSH Keywords: Cell Migration Assays • Mesenchymal Stromal Cells • Receptors, CCR7

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Background

Secondary lymphoid organs (SLOs), such as the spleen, Peyer's patches (PPs), and lymph nodes (LNs), are major niches for priming the immune response [1,2]. Specifically, naïve T and B cells, accumulated within SLOs, are able to transform into effectors by interacting with antigen-presenting cells, and then initiate immune reactions. CC chemokine receptor 7 (CCR7) is a G protein-coupled receptor normally expressed in various subsets of immune cells (e.g., mature dendritic cells, naïve T/B lymphocytes, and T regulatory cells), which enables those cells to migrate towards the ligands (CCL19 and CCL21) of CCR7, facilitating their location in SLOs [2,3].

Mesenchymal stem cells (MSCs), originally isolated from the bone marrow, are characterized by their multipotency and ability for self-renewal [4–6]. MSCs also exhibit immunomodulatory activity. Specifically, MSCs have been shown to suppress activation and proliferation of many types of immune cells, including T/B lymphocytes, dendritic cells, and natural killer cells (NK cells) cells *in vitro* [6–8]. However, when MSCs were delivered systematically in clinical trials, the observed immunosuppressive effects were not nearly as dramatic as those shown *in vitro*, partly because after systemic administration, the MSCs accumulated primarily in other organs (e.g., gastrointestinal tissues, kidney, skin, lung, and liver) [9]. Furthermore, some studies have demonstrated that patients who have taken multiple infusions of intravenous autologous MSC therapy experienced adverse effects such as pulmonary embolism and infarct [10]. To investigate strategies to increase the homing ability of systemically delivered MSCs, we induced CCR7 expression in murine bone marrow-derived stem cells by viral transduction and discovered a targeted migration to SLOs, which increased the immunomodulatory effect of MSCs in graft-versus-host disease mouse models [11]. These findings suggest a way to improve MSC distribution in immune disease models that may lead to improved immunomodulation and reduced dose of MSCs in clinical trials, possibly alleviating the adverse effects of a large MSC infusion.

Adipose-derived stem cells (ASCs), a type of MSCs, have garnered attention as a result of their increased immunomodulatory capacity compared with bone marrow-derived stem cells or other MSC sources [12]. Moreover, hASCs harvested from human subcutaneous lipoaspirates are advantageous because they are easily isolated, exhibit low morbidity and high yields, and have been used in many preclinical and clinical trials [4,12,13] Thus, hASCs are a reasonable alternative to bone marrow-derived MSCs. Since it is not feasible to test hASCs in clinical trials on a large scale, we performed our study in rats. We determined the effect of expression of the rat *CCR7* gene on the phenotype, differentiation, and proliferation of hASCs, and whether the rat *CCR7* gene enables targeted migration of hASCs to rat SLOs.

Material and Methods

Animals

This study was performed according to the guidelines of the Institutional Animal Care Committee of the Chinese PLA General Hospital. Male Lewis rats (LEW; 100–120 g) were obtained from the Experimental Animal Center of the Chinese PLA General Hospital. All animals were housed under pyrogen-free conditions at a controlled temperature, with water and commercial rat chow freely available.

hASC isolation and culture

hASCs were isolated from liposuction aspirates provided by healthy donors who had signed informed consent, as previously described [14,15]. Briefly, adipose tissue was washed with phosphate-buffered saline (PBS), minced, and digested with 0.05% hyaluronidase (Hyclone, USA) and 0.1% type I collagenase (Hyclone, USA) for 45 min at 37°C. The supernatant was discarded after centrifugation at 1500 rpm for 10 min, and the cells were suspended in low-glucose Dulbecco's modified Eagle's medium (DMEM; Hyclone, USA) supplemented with 100 U/mL penicillin, 100 U/mL streptomycin (Hyclone, USA), and 10% fetal bovine serum (FBS; Gibco, USA) in a humidified atmosphere of 5% CO_2 at 37°C. Adherent cells were washed with PBS 24 h later and incubated in the medium described above. The medium was replaced every 2–3 d.

Lentiviral transduction and drug screening

hASCs (P1) were seeded in culture medium at 20% confluence. When the cells reached 30–40% confluence the following day, the medium was replaced with DMEM supplemented with 5 μg/mL polybrene (Sigma, USA). Rat CCR7-transduced recombinant lentivirus (*rCCR7-eGFP: pLV-CMV-CCR7-2A-EGFP-3FLAG-PGK-PURO*) or control green fluorescence protein (GFP)-transduced lentivirus (*eGFP: pLV-CMV-EGFP-3FLAG-PGK-PURO*) (Obio Technology Corp. Ltd., China) were added to the new medium at a multiplicity of infection of 30. The medium was replaced with the original medium 24 h later, and GFP expression was observed with a fluorescence microscope 48 h later.

When GFP expression was detected, the culture medium was supplemented with puromycin (2 μg/mL) and the medium containing puromycin was replaced every 2–3 d. Cells that survived were digested and re-plated in complete culture medium after 7 d.

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Table 1. Primers used for real-time PCR of target genes.

Primers	Forward	Reverse
HumanCCR7	5'-CCAGACAGGGGTAGTGCGAG-3'	5'-AGGCAGAAGAGTCGCCTATG-3'
Rat CCR7	5'-TGGTCATTTTCCAGGTGTGCT-3'	5'-TACAGGGTGTAGTCCACGGT-3'
humanGAPDH	5'-ATGGGGAAGGTGAAGGTCGGAGTCAA-3'	5'-CGGAGGGGCCATCCACAGTCTTCT-3'
Rat β-actin	5'-GAGAGGGAAATCGTGCGTGAC-3'	5'-CATCTGCTGGAAGGTGGACA-3'
WPRE	5'-CCTTTCCGGGACTTTCGCTTT-3'	5'-GCAGAATCCAGGTGGCAACA-3'

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted with a transcription kit (Applied Biosystems, Thermo Fisher Scientific, USA) and cDNA was amplified by RT-PCR according to the manufacturer's protocol (TOYOBO, Japan).

hASCs (P4) were harvested to determine the expression of human CCR7, and human peripheral blood cells (hPBCs) were collected as a positive control. CCR7-hASCs (P4) and GFP-hASCs (P4) were collected to determine rat CCR7 mRNA expression, and rat peripheral blood cells (rPBCs) were also collected as a positive control. The primer sequences for human CCR7, human glyceraldehyde-3-phosphate dehydrogenase (GAPDH), rat CCR7, and rat β-actin are listed in Table 1.

WoodchUck hepatitis post-transcriptional regulatory element (WPRE), a constructive fragment on the plasmid, was detected for virus infection efficiency. hASCs, rPBCs, GFP-hASCs, and CCR7-hASCs were collected for WPRE mRNA detection. The primer sequences for WPRE are listed in Table 1.

Flow cytometry (FCM)

The FCM FITC channel was used to detect the proportion of GFP-positive cells in GFP-hASCs and rCCR7-hASCs, which were transfected by lentivirus and selected with drug screening, to assess the lentivirus infection efficiency.

To exam human CCR7 expression, hASCs were collected and incubated with human CCR7 polyclonal antibody (353203; BioLegend, USA) for 20 min at 4°C, and washed with PBS. hPBCs were served as positive controls. To exam rat CCR7 expression, hASCs, GFP-hASCs, and rCCR7-hASCs were collected and incubated with CCR7 multiclonal antibody (100712, Novus Biologicals, USA) for 20 min at 4˚C. After being washed twice with PBS, these cells were incubated with Alexa Fluor 647-conjugated anti-CCR7(150135; Abcam, UK). rPBCs were used as positive controls. The samples were tested and analyzed by FCM (BD, Heidelberg, Germany).

Cell immunophenotype examination

hASCs (P2–P5, 2×10^6) were tested. For each passage, cells were divided equally into 10 microcentrifuge tubes. Cells in 1 tube served as a negative control, while the remaining cells were incubated with PE-conjugated anti-CD34 (348057; BD Pharmingen, USA), anti-CD73 (550257; BD Pharmingen, USA), anti-CD105 (560839; BD Pharmingen, USA), and IgG1 control (349043; BD Pharmingen, USA), and FITC-conjugated anti-CD45 (347463; BD Pharmingen, USA), anti-CD90 (2296945; BD Pharmingen, USA), anti-CD11a (347983; BD Pharmingen, USA), anti-HLA-DR (347363, BD Pharmingen, USA), or IgG2a control (349051; BD Pharmingen, USA) for 20 min at 4°C before being washed twice with PBS and analyzed by FCM (BD, Heidelberg, Germany).

CCR7-hASCs/GFP-hASCs (P3; GFP-positive in FITC channel) were incubated with PE-conjugated monoclonal antibodies as hASCs and incubated with APC-conjugated anti-HLA-DR (17-9956e; Bioscience, USA).

Adipogenic and osteogenic differentiation capacity

hASCs (P2), GFP-hASCs (P2), and rCCR7-hASCs (P2) were induced with lineage-specific inductive media (Cyagen, China). For adipogenic differentiation, we used 2 types of media: 1) adipogenic differentiation medium A (175 mL basal medium A with 20 mL FBS, 2 mL penicillin-streptomycin, 2 mL glutamine, 400 µL insulin, 200 µL isobutyl methylxanthine, 200 µL rosiglitazone, and 200 µL dexamethasone) and 2) adipogenic differentiation medium B, which was identical, except for the addition of isobutyl methylxanthine, rosiglitazone, and dexamethasone. hASCs (P2), GFP-hASCs (P2), and rCCR7-hASCs (P2) were digested and seeded in a 6-well plate at 20–30% confluency per well with medium A for 3 d before being switched to medium B, and then changed back to medium A 24 h later. This procedure was repeated 3 times before maintaining the culture in medium B until large fat droplets appeared. Cells were then fixed in 10% formalin and stained with Oil-Red-O.

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For osteogenic differentiation, the induction medium was as follows: 175 mL basal medium with 20 mL FBS, 2 mL penicillin-streptomycin, 2 mL glutamine, 400 µL ascorbate, 2 mL glycerol phosphate, and 200 µL dexamethasone. The medium was changed every 2–3 d for 12 d. Cells were then fixed in 10% formalin and stained with alkaline phosphatase.

Detection of cell proliferation by cell counting kit-8 (CCK-8)

hASCs, rCCR7-hASCs, and GFP-hASCs (P3) were seeded in three 96-well plates (35 wells for each cell type with 1000 cells/well). The following day, supernatants in the first 5 wells of each plate were discarded and replaced with 1 mL of culture medium containing 100 µL CCK-8. The same medium added to blank wells was used as control. After incubation for 3 h in a humidified atmosphere of 5% CO_2 at 37°C, the supernatants were collected in another 96-well plate and a microplate reader was used to detect the absorbance at 450 nm.

Detection of SLO migration *in vivo*

Fifteen Lewis rats were randomly divided into 3 groups (n=5) designated as C-A injection, G-A injection, and PBS injection. rCCR7-hASCs (1.5×10^4/g), GFP-hASCs (1.5×10^4/g), or PBS alone were injected into the tail vein of rats. On the third day post-injection, the spleen and inguinal lymph nodes were harvested and pressed on a 40-µm nylon net to collect single cells. Following centrifugation, cells were washed once with PBS before the supernatant was discarded. After erythrocyte lysis and washing with PBS, the proportion of GFP-positive cells was determined by FCM (BD, Heidelberg, Germany).

Statistical analysis

Statistical analysis was performed using SPSS 17.0 software. All data are presented as the mean±standard deviation (SD). Significant differences between 2 samples were analyzed using Student's t-tests; significant differences among 3 or more samples were analyzed using one-way analysis of variance (ANOVA). For all analyses, the statistical significance level was set at $p<0.05$.

Results

hASCs were successfully isolated

The cells isolated from human liposuction aspirates were adhered to plastic surfaces and displayed spindle-shaped, fibroblast-like phenotypes when cultured (Figure 1A). In addition, immunophenotype examination of these cells by FCM showed that they expressed CD73, CD90, and CD105, but did not express CD45, CD34, CD11a, or HLA-DR (Figure 1B–1H).

CCR7 is not expressed in hASCs

CCR7 expression is vital for immune cells migrating to SLOs, a place for immune cells accumulation and immune reaction initiation. [1,16]. Moreover, severe decrease of T cells in SLOs has been shown in $CCR7^{-/-}$ mice [2]. Therefore, we determined the expression of CCR7 in hASCs at the beginning of this research. RT-PCR results indicated that human CCR7 was scarce in hASCs at the mRNA level (mRNA/GAPDH×1000: 0.043±0.02), but was highly expressed in human peripheral blood cells (hPBCs) (mRNA/GAPDH×1000: 2,658.63±776.46), which served as a positive control (mRNA level measured by mRNA/GAPDH×1000; Figure 2A). Flow cytometric analysis of the CCR7 protein determined the levels to be 44.2±2.5% on hPBCs and 0.47±0.04% on hASCs (Figure 2B).

Rat CCR7 was successfully introduced into hASCs by lentivirus infection

Given the role of CCR7 in the migration of immune cells to SLOs, hASCs were transduced with lentivirus containing the rat *CCR7* gene with *GFP* or *GFP* alone. In this study, we have defined these cells as rCCR7-hASCs and GFP-hASCs, respectively.

To investigate the viral transduction, WPRE mRNA levels in rCCR7-hASCs and GFP-hASCs were detected by RT-PCR, with rat peripheral blood cells and hASCs as negative controls. WPRE is a genetic element in the plasmid that is exploited for packaging of the lentivirus system, which can be seen in both *GFP* (Figure 3A) and *rCCR7* (Figure 3B) plasmid structures. As a result, WPRE levels are high in hASCs infected with lentivirus, (WPRE/GAPDH×1000: 7893.6±721.1 for GFP-hASCs and 4461.4±868.1 for rCCR7-hASCs), but were nearly undetectable in rat blood cells and hASCs ($p<0.001$; Figure 3C).

Both rCCR7-hASCs and GFP-hASCs exhibited GFP expression under a fluorescent microscope (Figure 4B, 4E). The infection efficiency was tested twice. The first test was performed after transduction, and the average proportion of rCCR7-hASC and GFP-hASCs GFP-positive cells was 30.5±2.1% and 80.4±6.2%, respectively (data are not shown). The second test was performed following puromycin selection, and the average proportion of GFP-positive cells in rCCR7-hASCs and GFP-hASCs was 89.6±3.4% and 94.3±2.5%, respectively (Figure 4C, 4F).

We then determined mRNA and cell surface protein levels of rat CCR7 in hASCs, rCCR7-hASCs, and GFP-hASCs. rPBCs served as positive controls. Our data indicated that rCCR7 mRNA and cell surface rCCR7 protein were negligible in hASCs and GFP-hASCs, but were highly expressed in rCCR7-hASCs. (Figure 3D, 3E).

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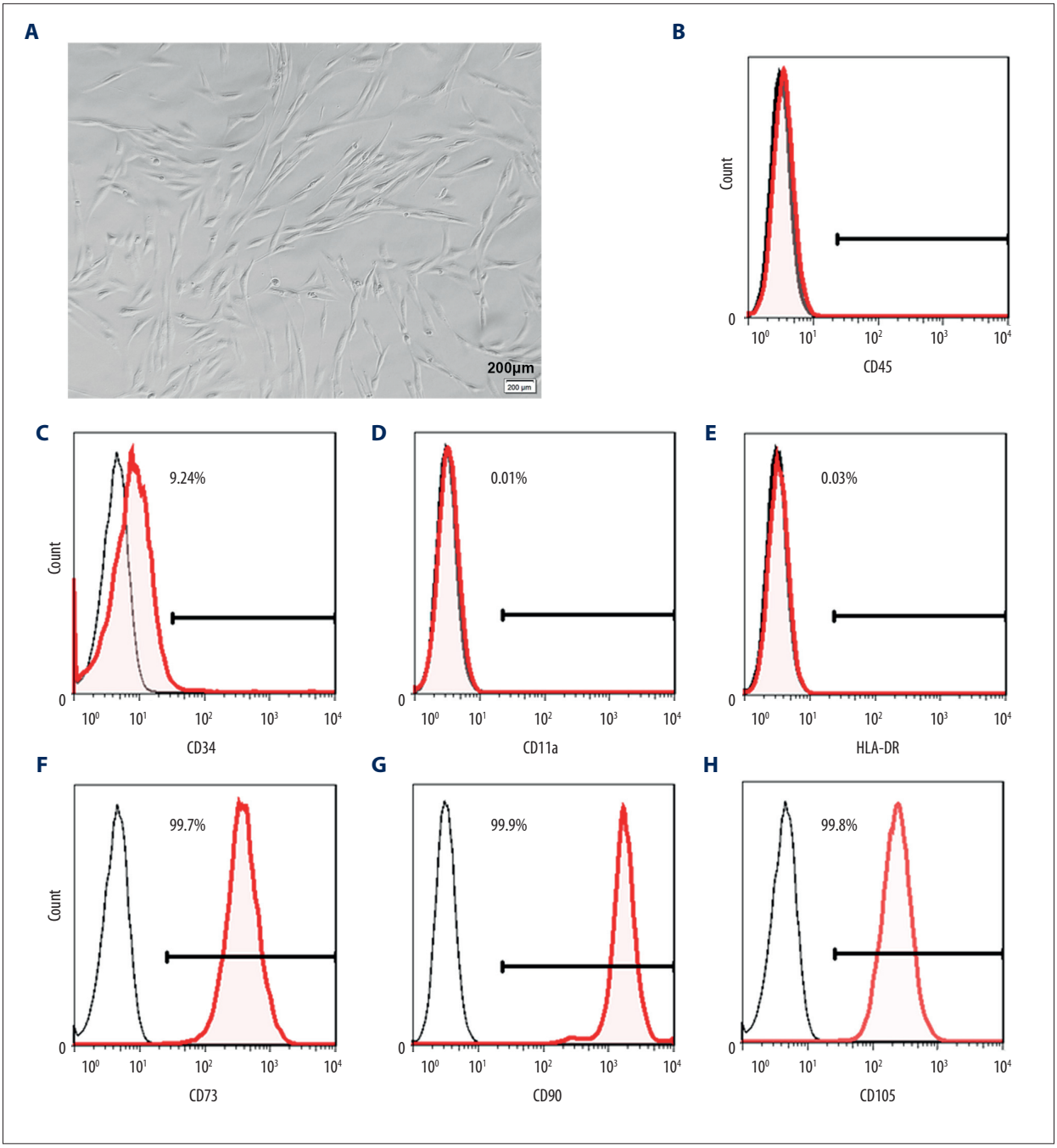

Figure 1. hASCs were successfully isolated. (**A**) The morphology of hASCs at passage 2. Bar=200 μm. (**B–H**) The phenotype examination of hASCs. Cell surface antigen of CD45 (**B**), CD34 (**C**), CD11a (**D**), HLA-DR (**E**), CD73 (**F**), CD90 (**G**), and CD105 (**H**) were examined by FCM technique. hASCs – human adipose-derived mesenchymal stem cells; FCM – flow cytometry.

hASCs, rCCR7-hASCs, and GFP-hASCs share similar characteristics

We examined whether the transduction had altered the intrinsic characteristics of hASCs. rCCR7-hASCs and GFP-hASCs were adhered to plastic surfaces and displayed spindle-shaped, fibroblast-like phenotypes as hASCs (Figure 4A, 4D). Both CCR7-hASCs and GFP-hASCs were positive for CD73, CD105, but negative for CD34 and HLA-DR (Figure 4G, 4H).

Furthermore, the proliferation condition in hASCs, GFP-hASCs, and rCCR7-hASCs was assessed by CCK-8 for 7d (Figure 5A) and no significant differences were found ($p>0.05$). In addition, the 3 cell groups mentioned above differentiated into adipocytes

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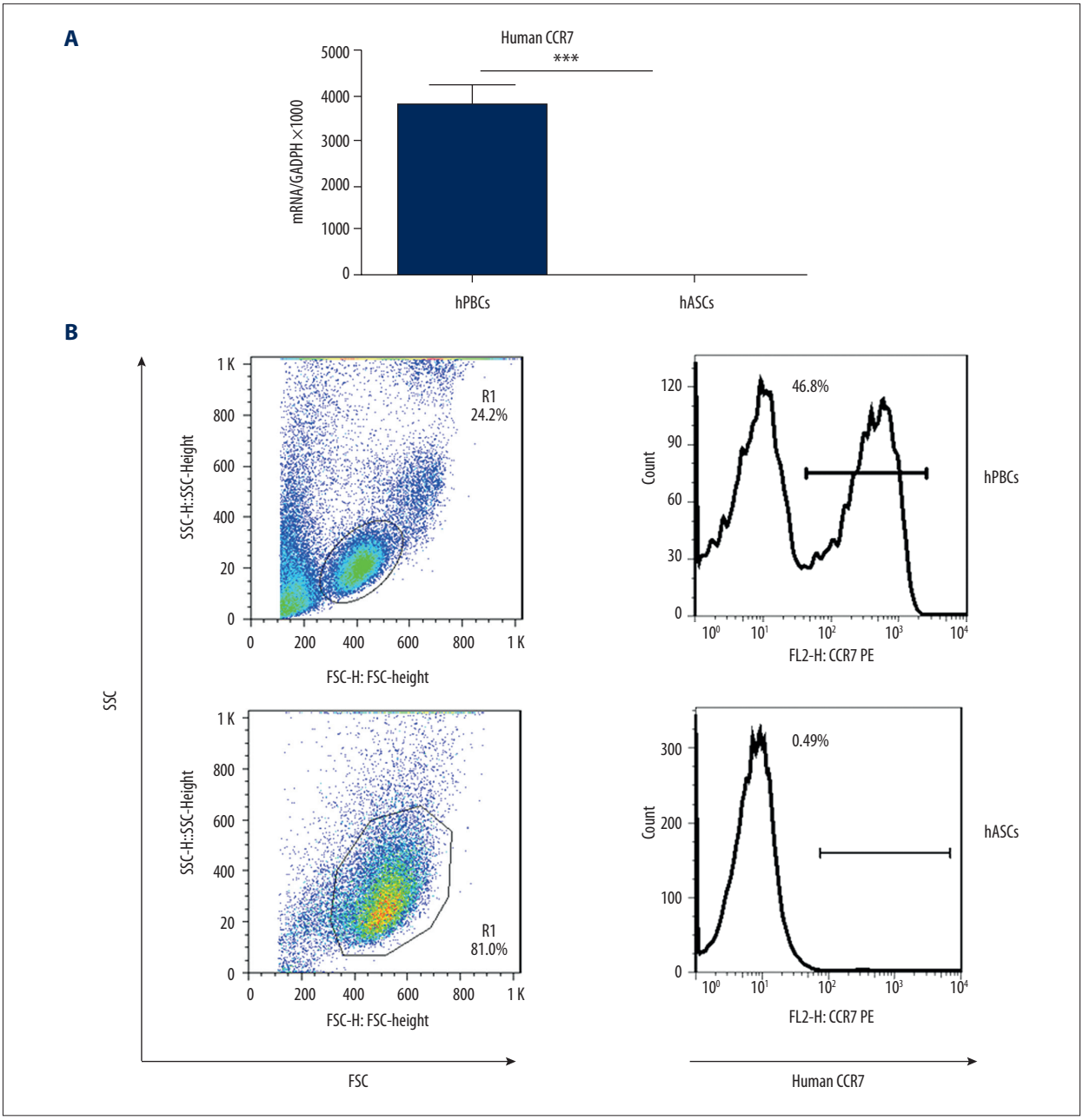

Figure 2. hASCs rarely express CCR7. (**A**) Human CCR7 mRNA expression level in hASCs was analyzed by RT-PCR. hPBCs served as positive control. (**B**) Human CCR7 protein on cell surface of hASCs was detected by FCM technique, and hPBCs served as positive control. hASCs – human adipose-derived stem cells; hPBCs – human peripheral blood cells; CCR7 – CC chemokine receptor 7; RT-PCR – reverse transcription-polymerase chain reaction; FCM – flow cytometry; *** $p<0.001$.

(red oil droplets) and osteocytes (alkaline phosphatase-positive) (Figure 5B). rCCR7-hASCs and GFP-hASCs exhibited similar adipogenic and osteogenic differentiation capacity as the hASCs.

rCCR7-hASC-targeted homing to rat SLOs

SLOs are vital for the induction of adaptive immune responses, especially with respect to the collection of antigens and the priming of lymphocytes [1,2]. To test whether rCCR7-hASCs effectively migrate to SLOs, we determined the proportion of GFP-positive cells in suspension from SLOs in rats injected with rCCR7-hASCs and GFP-hASCs by FCM. Previous reports have shown that the spleen and LNs are SLOs [2,16]. Thus, rat spleen and inguinal LNs, which can be harvested easily, were analyzed. The spleen and LNs from rats injected with PBS served as a control. In the spleen, the average

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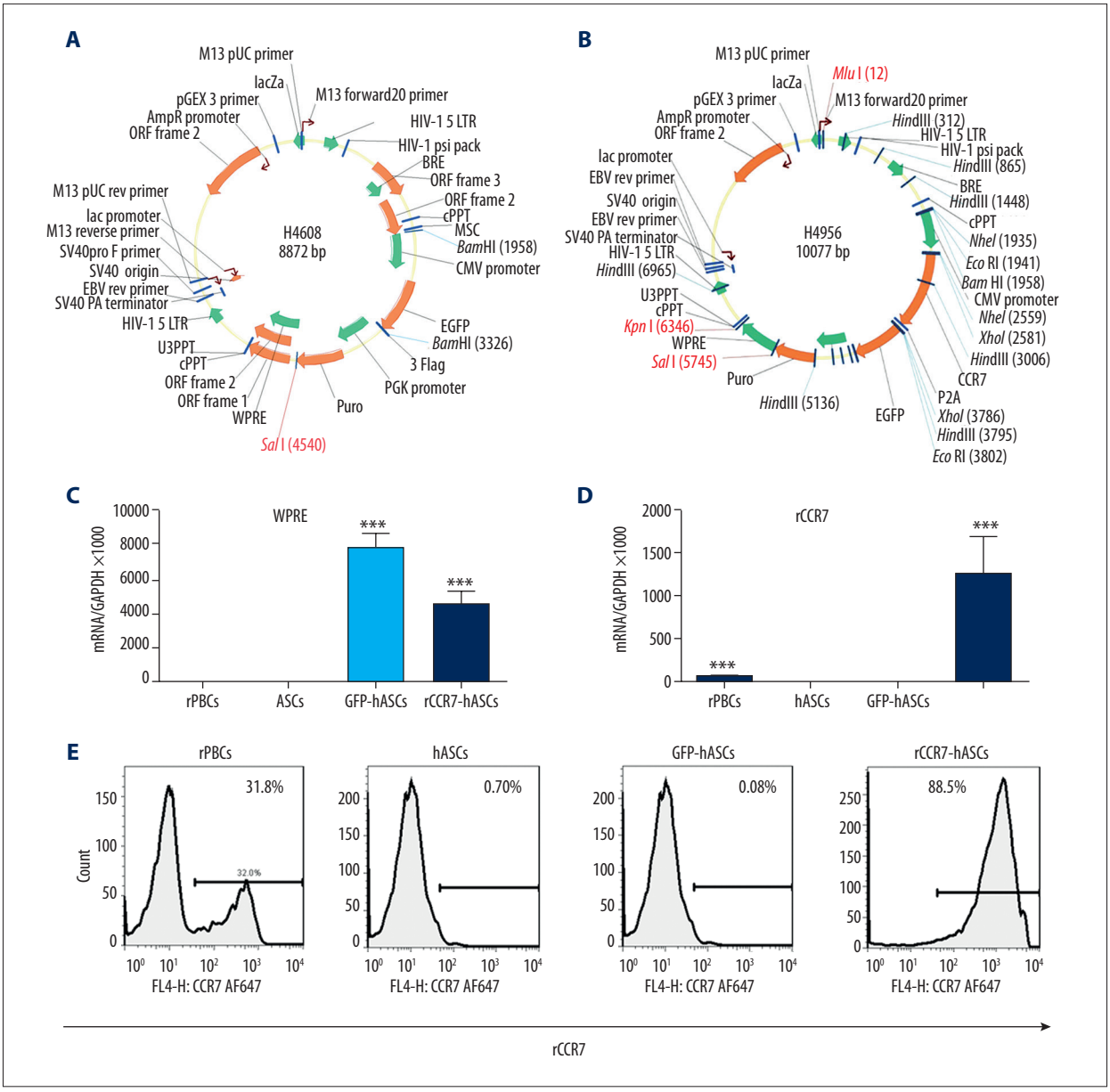

Figure 3. Rat *CCR7* gene was introduced into hASCs. (**A**) Plasmid structure exploited for packaging lentivirus and *WPRE* is an element in the plasmid. (**B**) The rat *CCR7* gene was inserted into the control plasmid of (**A**). (**C**) WPRE mRNA level in rCCR7-hASCs and GFP-hASCs was examined by RT-PCR, rPBCs and hASCs served as negative control. (**D**) Rat CCR7 mRNA level in GFP-hASCs and rCCR7-hASCs. rPBCs served as positive control and hASCs served as negative control. (**E**) Rat CCR7 protein expression on cells detected by FCM technique. hASCs – human adipose-derived stem cells; rPBCs – rat peripheral blood cells; CCR7 – CC chemokine receptor 7; GFP – green fluorescent protein; WPRE – WoodchUck hepatitis post-transcriptional regulatory element; RT-PCR – reverse transcription-polymerase chain reaction; FCM – flow cytometry; *** $p<0.001$.

rate of GFP-positive cells was 5.23±0.89% in the rCCR7-hASC group, 1.33±0.26% for the GFP-hASC group, and 0.31±0.11% for the control group (Figure 6A, 6C). The average rate in the LNs was 2.25±0.29%, 0.22±0.04%, and 0.37±0.08%, respectively ($p<0.01$) (Figure 6B, 6C).

Discussion

hASCs are biologically similar to bone marrow-derived MSCs and are thereby thought to be an alternative source of cells for clinical therapy [17–19]. hASCs have been shown to potently suppress rat immune cell activation and proliferation *in vitro* via paracrine and cell-cell contact [20,21]. However, comparable

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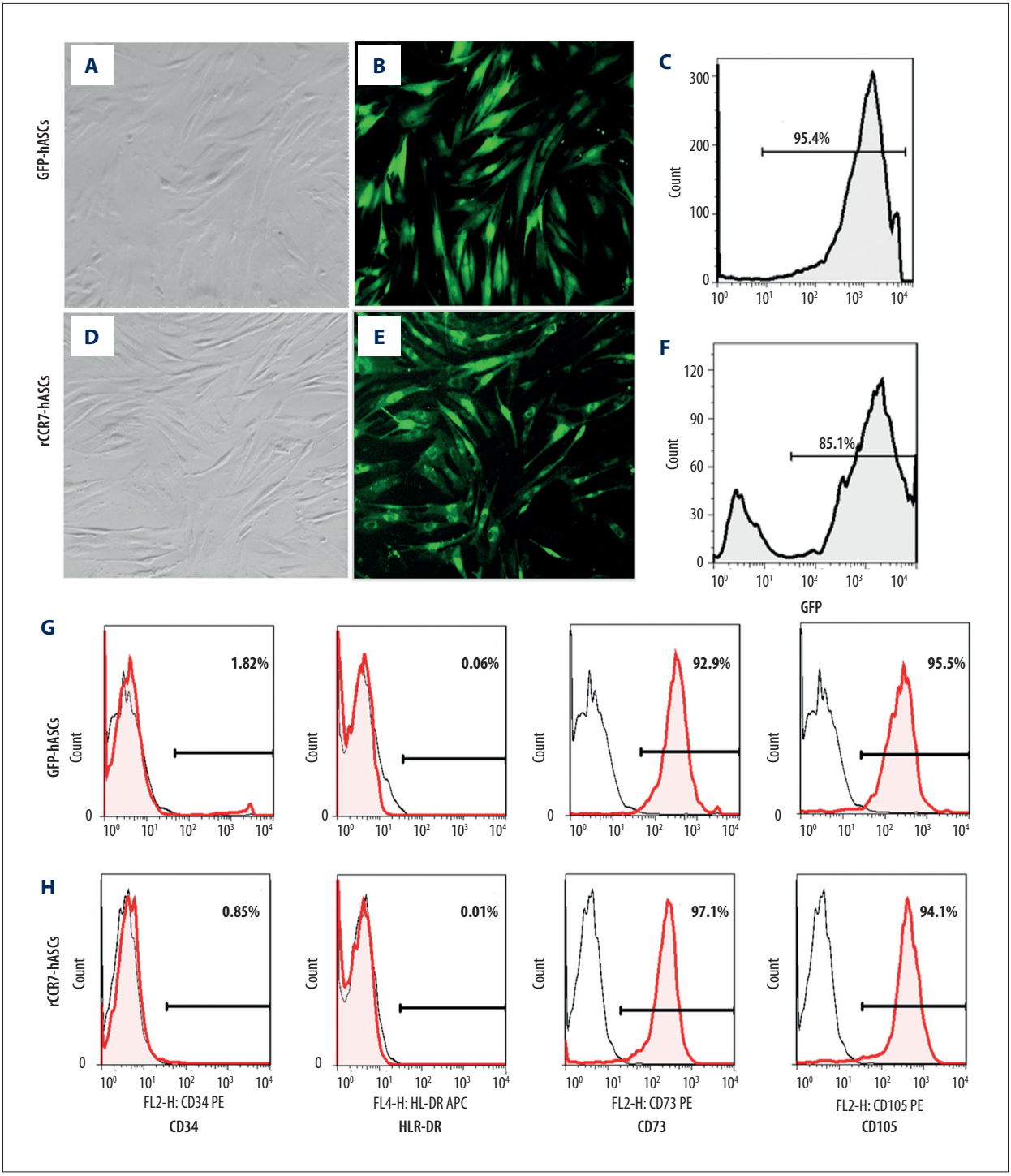

Figure 4. The morphology and phenotype of GFP-hASCs and rCCR7-hASCs were not altered. (**A, D**) GFP-hASCs (**A**) and rCCR7-hASCs (**D**) displayed spindle-shaped, fibroblast-like morphology. (**B, E**) *rCCR7-GFP* gene or *GFP* gene alone were introduced into hASCs. Green fluorescence of GFP-hASCs (**B**) and rCCR7-hASCs (**E**) observed under fluorescent microscope. (**C, F**) The FCM results showed most of the GFP-hASCs (**C**) and CCR7-hASCs (**F**) were GFP-positive. (**G, H**) The representative hASCs markers, CD34, HLR-DR, CD73, and CD105 were detected on the GFP-hASCs (**G**) and rCCR7-hASCs (**H**) by FCM technique. hASCs – human adipose-derived stem cells; CCR7 – CC chemokine receptor 7; GFP – green fluorescent protein; FCM – flow cytometry.

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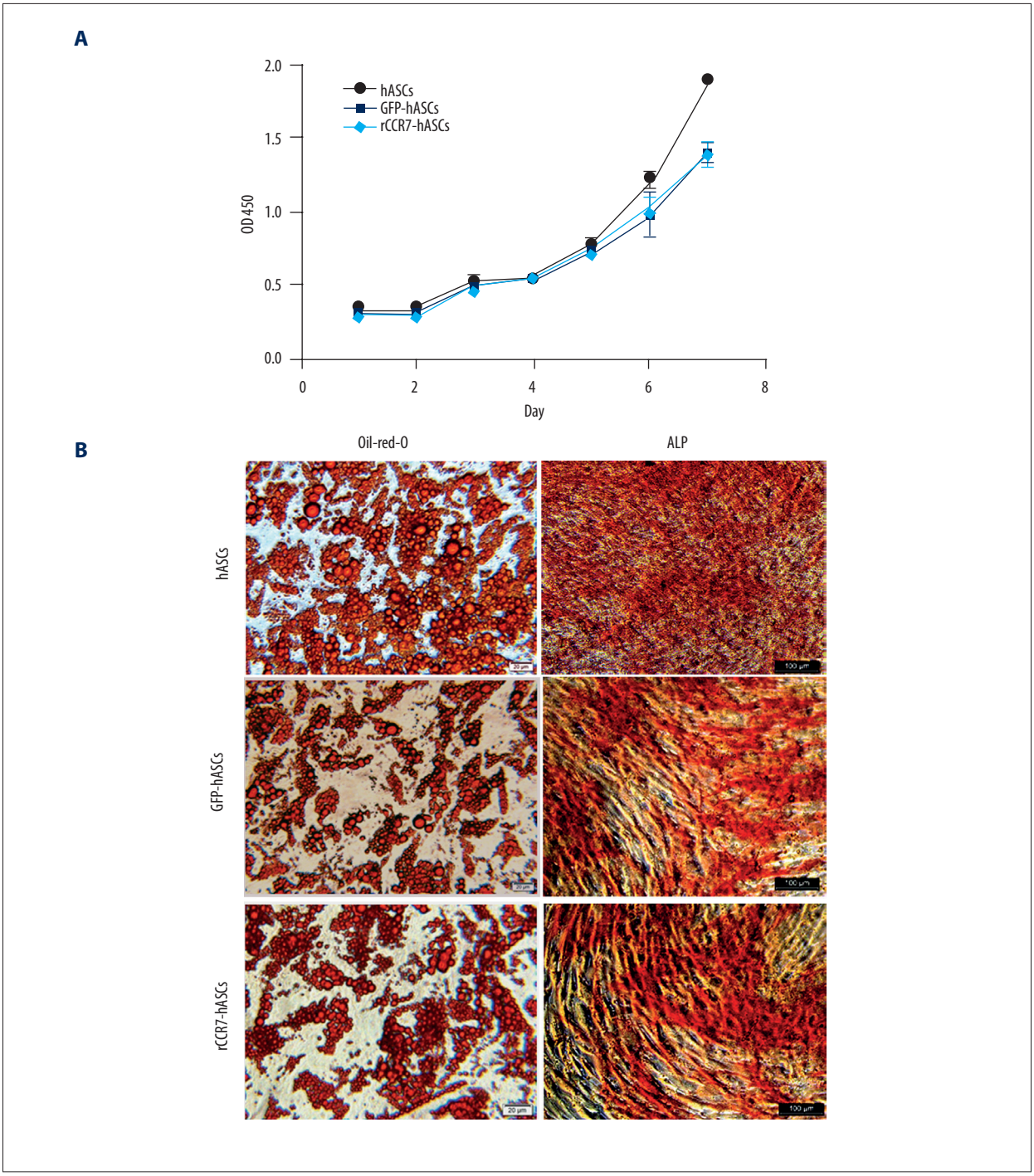

Figure 5. CCR7 expression did not alter the proliferation and differentiation capacity of hASCs. (**A**) The proliferation ability of hASCs, GFP-hASCs, and rCCR7-hASCs cells were examined by CCK-8 experiment. The result showed that there was no significant difference between the 3 types of cells at different time points. (**B**) hASCs, GFP-hASCs, and rCCR7-hASCs cells maintained the osteogenic and adipogenic differentiation capability. The 3 groups of cells were cultured under adipogenic differentiation medium followed by Oil-Red-O stain showing oil droplet-filled adipocyte-like cells (**left panel**). Cells were cultured under osteoblast differentiation medium, and then the cells were stained after 3-week induction followed by ALP staining (**right panel**). hASCs – human adipose-derived stem cells; CCR7 – CC chemokine receptor 7; GFP – green fluorescent protein; CCK-8 – cell counting kit-8.

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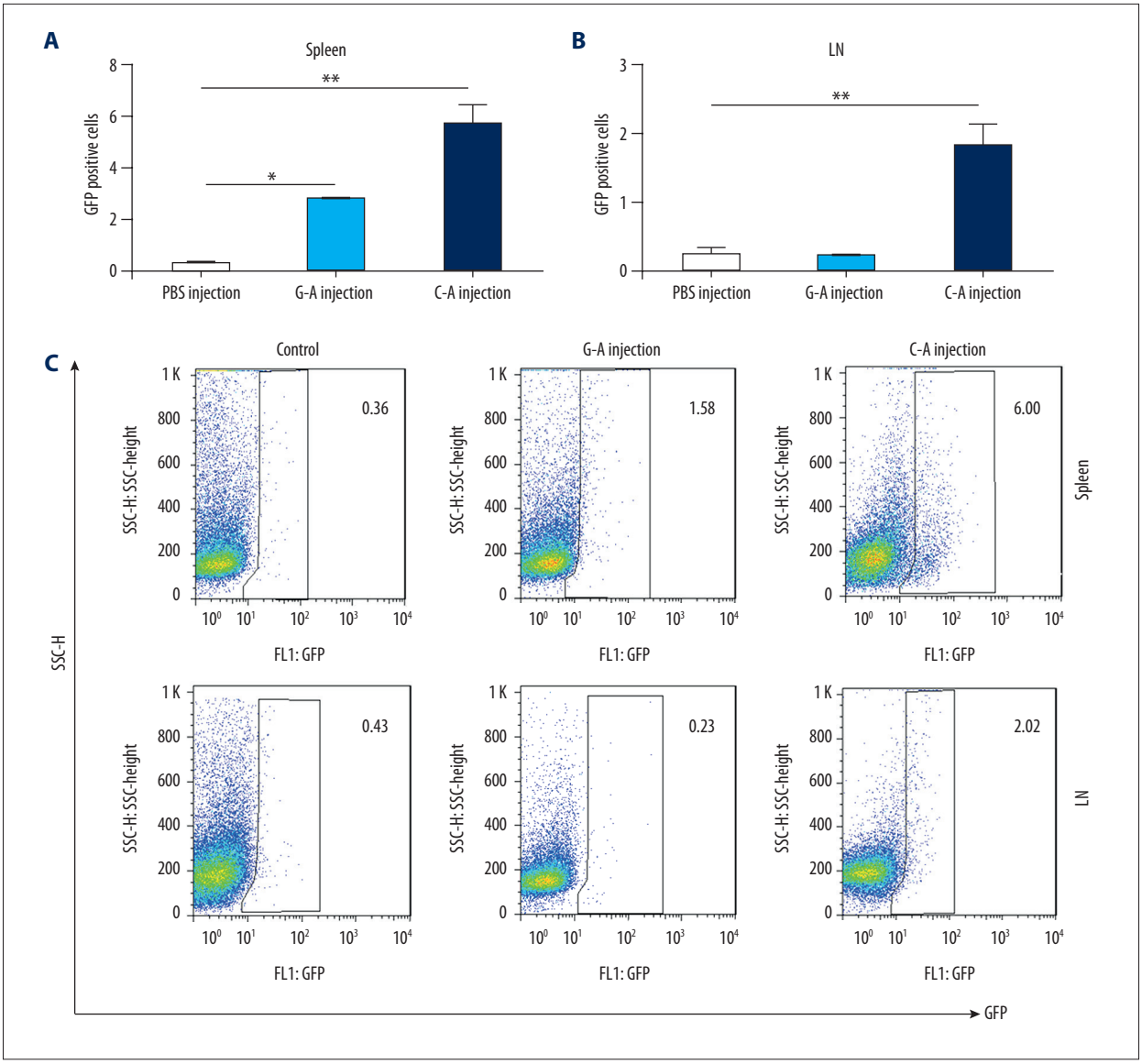

Figure 6. rCCR7 enabled hASCs to migrate to SLOs efficiently. GFP-hASCs (1.5×10^4/g) or rCCR7-hASCs (1.5×10^4/g) were intravenously injected into Lewis rats. Rats injected with PBS only served as negative control. At 3 days after transfusion, the GFP cells in the rat spleen and lymphoid nodes were detected by FCM. (**A**) Rate of GFP-positive cells in spleen. (**B**) Rate of GFP-positive cells in lymphoid nodes. (**C**) Representative FCM result in **A** and **B**. SLOs – secondary lymphoid organs; hASCs – human adipose-derived stem cells; CCR7 – CC chemokine receptor 7; GFP – green fluorescent protein; PBS – phosphate-buffered saline; G-A injection – GFP-hASCs-injected rat; C-A injection – rCCR7-hASCs-injected rat; FCM – flow cytometry; * P<0.05; ** P<0.01.

effects have not been seen in clinical trials, possibly due to wide and non-target distribution character after intravenous delivery [22]. CCR7 and its ligands perform an essential role in lymphocyte homing to SLOs, which are prime sites for lymphocyte accumulation and immune reaction priming [1–3,16]. hASCs have been shown to exert immunomodulation ability via expression of chemokine receptors such as CXCR4, and lead them to the site of injured tissue [23,24]. We did not, however, detect the expression of the chemokine receptor CCR7 in hASCs in this study.

We discovered that hASCs can be made to express rat CCR7 protein on the cell surface by lentivirus infection. WPRE mRNA was detected in both rCCR7-hASCs and GFP-hASCs, indicating that the lentiviral plasmids entered the targeted cells. We did not detect rat CCR7 mRNA or membranous protein expression in any cells other than rCCR7-hASCs and its positive control cells. The transduction efficiency of the lentivirus encoding the rat *CCR7-GFP* gene was lower than the control, which encoded only the *GFP* gene. The efficiency was evaluated by

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assessment of the level of WPRE mRNA and the proportion of GFP-positive cells, as determined by RT-PCR and FCM, respectively. Specifically, the level of WPRE mRNA and the proportion of GFP-positive cells in rCCR7-hASCs were lower than those in GFP-hASCs. These results suggest that less plasmid entered the cells during lentiviral infection. The *rCCR7-GFP* gene is large, and likely not easily integrated into the hASC genome. In addition, the fluorescence intensity of GFP-hASCs was greater than that of rCCR7-hASCs.

We also examined rCCR7-hASCs and GFP-hASCs and found that viral transduction manipulation did not alter the intrinsic capacity of hASCs. To qualify the cells in our study as hASCs, we examined cell surface markers, and evaluated their multipotency and their ability to differentiate into osteoblastic and adipocytic lineages. Concerned with unintended alterations in the characteristics of hASCs by modification of the expression of surface proteins, we performed similar analysis of the rCCR7-hASCs and GFP-hASCs used in this study. Generally, CD73, CD105, CD34, and HLA-DR were detected in virally infected hASCs. Some of the antibodies purchased for hASC cell marker detection were FITC-linked, which overlapped with GFP in the FITC channel. However, we found this was not significant when qualifying rCCR7-hASCs and GFP-hASCs because CD73 and CD105 were the primary positive markers and HLA-DR was identified as a negative marker. Although it has been described as a hematopoietic marker, CD34 has been shown to be a primary unstable positive marker of hASCs [25]. Data have shown that hASCs isolated from the stromal vascular fraction (SVF) are $CD34^+$, but these cells lost CD34 expression during expansion and passaging [26]. Consistently, in our study, hASCs were positive for CD34 in passage 2 (13.72±2.61%), passage 3 (6.57±1.04%), and passage 4 (2.46±0.58%), but negative in passage 5 (0.93±0.05%). Moreover, the rate of proliferation was evaluated by CCK-8 and no statistically significant difference was apparent among the cells, regardless of viral infection.

Finally, we detected the migration ability of hASCs in the rats and found that upregulating rat CCR7 expression in hASCs allowed them to migrate to SLOs. Circulating lymphocytes can roll along high endothelial venules of LNs via interaction between CCR7 and its ligands (CCL21 and CCL19), allowing movement across the venules and recruitment into the LNs [2]. T lymphocytes in particular rely on CCR7 for entering LNs [27]. Many studies have shown that paracrine and cell-cell direct contact are the primary pathways of the immunomodulation ability of hASCs [28–30]. It is assumed that when accumulating around T cells within a given niche, hASCs would give rise to a potent immunosuppressive effect on them. In short, T lymphocytes are important mediators of various pathological conditions such as graft-versus-host disease, autoimmune diseases, and transplant rejection. hASCs that overexpress rat CCR7 may be an alternative option to hASCs alone in rat immune disease models.

Conclusions

This research provides evidence that upregulation of CCR7 expression in hASCs enables targeted migration into SLOs. We also demonstrated that viral transduction does not change the immunophenotype, multipotency, or proliferative capacity. This study provides a new strategy to enhance the *in vivo* immunoregulatory effects of hASCs, thereby improving the clinical therapy results in hASCs-based application in immune disease.

Conflicts of interest

The authors indicate no potential conflicts of interest.

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