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CXCR4 Gene Transfer Enhances The Distribution of Dermal Multipotent Stem Cells to Bone Marrow in Sublethally Irradiated Rats

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Irradiation/Dermal multipotent cells/CXC chemokine receptor 4/Gene transfer.

Our previous study indicated that systemically transplanted dermal multipotent cells (DMCs) were recruited more frequently to bone morrow (BM) of rats with sublethal irradiation than that of normal rats, and the interactions between stromal-derived factor (SDF-1) and its receptor (CXC chemokine receptor 4, CXCR4) played an important role in this process. In the present study, we aimed to investigate whether CXCR4 gene transfer could promote the distribution of DMCs into irradiated BM and accelerate its function recovery. Firstly, adenovirus vector of CXCR4 (Adv-CXCR4) and green fluorescent protein (Adv-GFP) were constructed. Then male DMCs infected by Adv-CXCR4 (group A), or infected by Adv-GFP (group B), and non-infected DMCs (group C) were transplanted into irradiated female rats, and real-time polymerase chain reaction for the sex-determining region of Y chromosome was employed to determined the amount of DMCs in BM. The functional recovery of BM was examined by hematopoietic progenitor colonies assay. The results showed that the amount of DMCs in BM of group A was greater than that in group B and group C from day 5 after injury ($P < 0.05$), and the amount of CFU-F, CFU-E and CFU-GM were greater than that in group B and group C from day 14 after injury ($P < 0.05$). These findings suggest that DMCs infected by Adv-CXCR4 distributed more frequently to the bone marrow of sublethally irradiated rats and could accelerate hematopoiesis function recovery.

INTRODUCTION

Chemokines, small pro-inflammatory chemoattractant cytokines, are initially well-known for their role in immune system function.[1)] More recently, chemokines were found to participate in many physiological and pathological processes including development, angiogenesis, tumor metastasis, and adjustment the distribution of stem cells.[2–5)] Until now, more than 50 different chemokines and 20 different chemokine receptors have been discovered. Based on the relative position of the cysteine residues in the protein sequence, chemokines are classified into four subfamilies: CXC (α) and CC (β) chemokines, fractalkine (CXXXC), and lymphotactin.[1–5)] Chemokines usually bind to multiple receptors, and the same receptor may bind to more than one chemokine.[1–5)] However, there is one exception to this rule: the α-chemokine stromal-derived factor-1 (SDF-1), which binds exclusively to CXC chemokine receptor 4 (CXCR4) and has CXCR4 as its only receptor. Besides its role in development and tumor metastasis,[6,7)] SDF-1/CXCR4 interaction has recently been proved to be essential in regulating the distribution stem cells and participate in the repair of injured tissue.[8–10)] After left hypoglossal nerve injury in rats, systemically transplanted mesenchymal stem cells migrated to the avulsed hypoglossal nucleus under the direction of SDF-1/CXCR4 interaction.[8)] Similarly, SDF-1/CXCR4 interaction recruited transplanted bone marrow (BM)-derived stem cells to infracted heart muscle[9)] and endothelial progenitor cells to ischemic hindlimb muscle as well.[10)]

Due to its important role in regulating the distribution of stem cells, SDF-1/CXCR4 interaction is manipulated in many ways to enhance the repair effect of stem cells.[11–13)] When the serum levels of SDF-1 was elevated either by

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intravenous injection of small peptide analogs to SDF-1 (CTCE0021 and CTCE0214)[11] or an adenovector expressing the SDF-1 gene,[12] hematopoietic cells were significantly recruited to the peripheral blood. By using a lentiviral gene transfer technique, CXCR4 was over-expressed on human $CD34^+$ progenitors, and could help navigate these cells to the murine BM and spleen in response to SDF-1 signaling.[13]

Previously, our lab reported that dermal multipotent cells (DMCs) isolated from newborn rats could help the functional recovery of hematopoiesis in subletally irradiated rats.[14] Transplanted DMCs were recruited more frequently to irradiated BM than normal BM and the interactions of SDF-1/CXCR4 played an important role in this process.[15] In order to increase the number of DMCs recruited to the irradiated BM and enhance the functional recovery of hematopoiesis, we hypothesize that the recruitment of transplanted DMCs into BM can be enhanced by up-regulating the expression of CXCR4 in DMCs. In the present study, we firstly constructed adenovirus vector of CXCR4 (Adv-CXCR4) and green fluorescent protein (Adv-GFP). Then we observed whether DMCs infected by Adv-CXCR4 could distribute more frequently into irradiated BM, and promote functional recovery of injured BM more quickly than DMCs infected by Adv-GFP or non-infected DMCs.

MATERIALS AND METHODS

Materials and reagents

Wistar rats were purchased from the Center of Laboratory Animals of the Third Military Medical University. All materials used in cell isolation, culture and differentiation tests were bought from Hyclone (UT, USA). Reagents for PCR, RT-PCR, and Real-time PCR were obtained from TaKaRa BIO INC. (Kyoto, Japan), and all the primers and probes used were synthesized by Sangon Biological Engineering & Technology services Co., Ltd., (ShangHai, China). Tripure™ used to isolate RNA was purchased from Promega Corp. (Madison, Wisconsin, USA). Anti-CXCR4 rabbit polyclonal antibody was from Santa Cruz Biotech, Inc. (Santa Cruz, California, USA). Phycoerythrin-conjugated anti-rabbit secondary antibody was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Reagent for immunostaining was from Boster Corp. (Wu Han, Hu Bei Province, China). All restriction endonucleases used in the experiment and DNA ligation kit were purchased from New England Biolabs (Beberly, MA, USA), and lipofectamin™ 2000 was bought from Invitrogen (Carlsbad, California, USA).

Isolation and culture of male DMCs

Isolation and culture of DMCs were carried out as described previously.[14,15] Briefly, full-thickness skin was obtained from newborn male rats and then transferred to phosphate buffered saline (PBS) containing 0.25% trypsin and enzymatically dissociated at 4°C overnight. Next day the dermis layer was dissociated and sheared, and the suspension was filtered though nylon meshes to remove cellular debris. After centrifugation, cell pellet was then re-suspended and cultured in Iscove's Modified Dulbecco's Medium (IMDM) at a density of 10^6 cells/ml. Six hours later, the non-adherent cells were removed, and the adherent cells were harvested and serially diluted into cultured medium and then seeded in 96-well plastic culture plate, with each well contained 200 µl IMDM and single cell. Four weeks later, single sorted colonies were isolated, expanded and were studied as candidates of DMCs. The test of DMCs candidate's differentiation capacity was performed by culturing cells in various induction media (osteogenic medium, chondrogenic medium, neural medium and adipogenic medium) for proper period, followed by histological staining for alkaline phosphatase, collagen II, nestin and NF-2000, etc.[14]

Construction of Adv-CXCR4 and Adv-GFP

The AdEasy adenoviral vector system used to construct Adv-CXCR4 and Adv-GFP was a kind gift of Dr Bert Vogelstein, Howard Hughes Medical Institute, Johns Hopkins Oncology Center, Baltimore, USA, and methods used to construct Adv-CXCR4 was similar to theirs.[16] Firstly full-length cDNA of CXCR4 was amplified and then cloned into pAdTrack-CMV shuttle vector. Once constructed, the shuttle vector containing CXCR4 cDNA was linearized with Pme I and contransformed into BJ5183 together with the backbone vector. Transformants are selected for kanamycin resistance, and recombinants were subsequently identified. Once identified, recombinant was amplified, purified and then digested with Pac I to expose its inverted terminal repeats, and then used to transfect HEK 293 cells where deleted viral assembly gene were complemented, that is the complete virus was constructed.

The amplification of full-length cDNA of CXCR4. The expression of CXCR4 is abundant in the cells from BM,[8] so CXCR4 cDNA were amplified from rat's bone marrow. The whole BM plugs were obtained by flushing the BM cavity of the femurs with a 21-gauge syringe filled with PBS, and then the total RNA was isolated form cultured DMCs using Tripure according to the manufacture's instructions. The cDNA was prepared from 1.0 µg RNA in the presence of 2.5 µM oligo (dT) primer and 200 U reverse transcriptase in a total volume of 10 µl. The reaction mixture was incubated for 1 hour at 42°C and stopped by heating for 5 minutes at 99°C. Aliquots (1 µl) of cDNA were subsequently amplified using specific primers for CXCR4. The PCR cycles consist of de-naturation at 94°C for 45 s, annealing at 56°C for 45 s, and extension at 72°C for 45 s. The forward primer was 5'-**GA**_*AGATCT* ATGGAA ATATAC ACTTCG GAT-3'and the reverse primer was 5'-**TGG** *GATATC* TTAGCTG-GAGTG AAAACTTGAG-3'. In the sequence of primers, the italicized bases were BglII and EcoRV restriction endonucleases sites, and the bold bases were protected bases. The

PCR product was 1079 bp.

Generation of recombinant pAd-CXCR4. Restriction enzyme reactions using Bg1II and EcoRV were performed to cut pAdTrack-CMV shuttle vector and the full-length CXCR4 cDNA, respectively. Subsequently, the digested products were ligated with solution 1 overnight at 16°C. The region between multiple cloning sites of the pAdTrack-CMV was removed, and was replaced by the full-length CXCR4 cDNA. The resultant vector was named as pAdTrack-CXCR4, and was amplified by transformation of E. coli strain DH5α and testified by restriction endonuclease digestion, PCR and sequence analysis. Totally four combinations of restriction endonuclease were used: Pac**I**, BamH1 + EcoRV, BamH1 + BglII and BglII+ EcoRV.

Then, 120 μl of newly constructed shuttle vector was linearized with Pme1 and mixed with competent E. coli strain BJ5183 cells, which contains transformed the pAdEasy-1 backbone vector, in a total volume of 140 μl. The recombinant vector was amplified and testified by restriction endonuclease digestion, PCR and sequence analysis. Two group of restriction endonuclease were used here: Pac1, BglII + EcoRV. The testified vector was named as pAd-CXCR4.

Construction adenovirus vector of CXCR4. HEK 293 cells were plated in 25 cm^2 flasks. About 36 hours later, HEK 293 cells reached a confluency of approximately 60%–70%. A transfection mixture was prepared by adding linearized plasmid pAd-CXCR4 (digested with Pac1) and Lipofectamin™ 2000 to HEK 293 cells according to the manufacture's manual, and subsequently the transfection mixture was incubated at 37°C in a CO_2 incubator. Cytopathogenic effect was observed 72 hours after transfection, suggesting the generation of adenovirus of CXCR4, which was named as Adv-CXCR4. Transfected cells were monitored for GFP expression under a fluorescence microscope and collected 7 days post-transfection. For virus collection, the cells were lysed with three consecutive freeze-thaw cycles, and Adv-CXCR4 was collected from supernatant. Part of the crude lysate was used repeatedly to infect the 293 cells until the desired titer was achieved. After being repeated for 5 times, viral titers were determined by plaque assays.[16,17]

Construction adenovirus vector of GFP. The construction of adenovirus vector of GFP was relatively easier because the pAdTrack-CMV vector itself contains an incorporating GFP gene. Firstly the pAdTrack-CMV vector was linearized by Pme1 and mixed with BJ5183 to allow recombination occur. Then the recombinant adenoviral plasmid was digested with Pac1 and transfected HEK 293 cells to package adenovirus of GFP, which was named as Adv-GFP.

The effect of Adv-CXCR4 infection on the expression of CXCR4 in DMCs

Adv-CXCR4 of fifth generation was used to infect DMCs at the titer of 2.5×10^{10} PFU/ml (here PFU stands for plaque-forming unit). Then RT-PCR, fluorescent immunohistochemistry and fluorescent activated cell sorter (FACS) were employed to identify the expression of CXCR4 in DMCs. DMCs infected by Adv-GFP and non-infected DMCs served as control.

RT-PCR. Total RNA was extracted DMCs and 1.0 μg RNA was used to amplify CXCR4. The primers used here were the same as that used in the amplification of CXCR4 in the above-mentioned step. Also, primers (forward primer was 5'-TCA TCA GCG AAA GTG GAA A-3' and reverse primer was 5'-TGT CTG TCT CAC AAG GGA AGT-3') to detect hypoxanthine guanine phosphoribosyl transferase (HPRT) were added to each reaction tube as internal control. The RT-PCR product of HPRT was 270 bp.

Fluorescent immunohistochemistry. Immunostaining was performed on the DMCs plated on ploy-L-lysine-coated coverslips. The coverslips were blocked with normal serum for 30 minutes, and then incubated with anti-CXCR4 rabbit polyclonal antibody at 4°C overnight. Subsequently, coverslips were incubated in secondary antibodies for 1 hour, and the reaction products were visualized under fluorescent reverse microscope. Between all the steps, the slides were fully rinsed by PBS.

FACS. As the infected DMCs reached a confluency of approximately 70%, the culture medium was discarded. One hundred μl rabbit polyclonal anti-CXCR4 was applied and incubated for 30 minutes at 37°C. After the cells were fully rinsed by PBS, secondary antibody (Anti-rabbit-R-Phycoerythrin) was added and incubated for 30 minutes at 37°C. Then the cells were harvested and suspended with 500 μl PBS and was examined by FACS for the expression of CXCR4.

Animal group and transplantation procedure

One hundred and eight female Wistar rats, six-week-old and weighing about 150 g, were divided randomly into three groups: group A (sublethally irradiated rats accepted Adv-CXCR4 infected DMCs transplantation, n = 36), group B (sublethally irradiated rats accepted Adv-GFP infected DMCs transplantation, n = 36) and group C (sublethally irradiated rats accepted non-infected DMCs transplantation, n = 36). Rats were irradiated over the whole body with a sublethal dose of 5 Gy of gamma rays from a ^{60}Co source, and the absorption rate was 31.02–31.98 cGy/min. Thirty minutes after irradiation, rats were anaesthetized by 1% (w/v) pentobarbital sodium (40 mg/kg body weight) and DMCs were infused at a dose of 2×10^6 cells in 0.5 ml physiological saline by tail vein injection. In group A and group B, DMCs were infected by Adv-CXCR and Adv-GFP respectively 48 h before transplantation. All animals were fed with commercial laboratory food and purified tap water and housed under standard conditions. Six rats from each group were sacrificed to examine the amount of DMCs in BM at each time point of 3 d, 5 d, 1 w, 2 w, 3 w and 4 w. Also, at each time point the BM in each group were harvested and

used to assess the hemotopoietic progenitor colonies assay. Additional six rats received no irradiation served as normal control in hemotopoietic progenitor colonies assay.

Quantitative real-time TaqMan PCR

The amount of transplanted male DMCs in BM was determined by quantitative real-time TaqMan PCR for the sex-determining region of Y chromosome (SRY) as previous reported.[15] Briefly, DNA of DMCs or tissues was prepared and dissolved in water. And then real-time PCR was carried out with the primers used for SRY were 5'-ATA CTG GCT CTG CTC CTA CCT-3' (forward) and 5'-GCT GTT TGC TGC CTT TGA-3' (reverse), and the TaqMan probe was 5'-FAM- TGC CAA CAC TCC CCT TGC TGC TGT AAT T-TAMRA-3'. The thermal cycle consists of incubation at 95°C for 5 min, 40 cycles of de-naturation at 95°C for 30 s, and annealing and extension at 60°C for 60 s. The standard curves was generated by serially diluting male DMCs DNA into female rat genomic DNA. The amount of DMCs in BM was determined by cycle number in the standard curve.

Examination of the BM functional recovery

Colony forming unit of fibroblast (CFU-F), colony forming unit of erythrocyte (CFU-E) and colony forming unit of granulocyte/macrophage (CFU-GM) of bone marrow were employed to evaluate the functional recovery of BM as previously reported.[14] Briefly, BM was harvested and suspended in IMDM medium. To determine the CFU-GM derived colonies, 2×10^5 BM nucleated cells were cultured in triplicate in the presence of methyl-cellulose (3.0%) in RPMI 1640, L-glutamine (2.0 mM), fetal bovine serum (15%), and 2-mercaptoethanol (0.1 mM). The cells were incubated for 7 days and the colonies containing 50 or more cells were recognized as a colony. To determine the CFU-F derived colonies, 10^6 BM nucleated cells were planted in triplicate in Dulbecco's Modified Eagle Media containing 10% fetal bovine serum, 10^{-6} M hydrocortisone, and 50 μg/ml ascorbic acid. The colonies were counted after 28 days and colonies

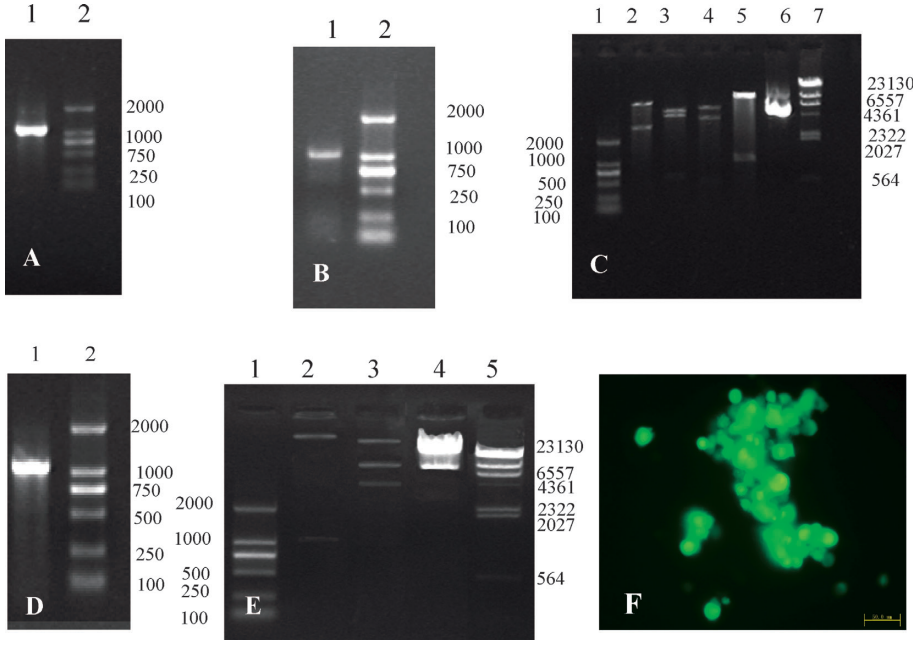

Fig. 1. Construction of Adv-CXCR4. (A) Lane1: RT-PCR amplification of CXCR4. Lane 2: DNA marker DL2000. (B) PCR confirmation of pAdTrack-CXCR4. Lane 1 showed the positive product of CXCR4 (1079 bp) and lane 2 was DNA marker DL2000. (C) Restriction digestion verification of pAdTrack- CXCR4. Lane 1: DNA Marker (DL2000); Lane 2: restriction endonuclease digestion with Pac1 resulted in two expected segments (7.3 kb and 2.9 kb); Lane 3: restriction endonuclease digestion with Pac1 plus EcoRV resulted in two expected segments (5.5k and 4.8kb); Lane 4: restriction endonuclease digestion with BamH1 plus BglII resulted in two expected segments (6.5kb and 3.8kb); Lane 5: restriction endonuclease digestion with BglII plus EcoRV resulted in two expected segments (9kb and 1kb); Lane 6: pAdTrack-CMV-CXCR4 not been digested by restriction endonuclease; Lane 7: DNA Marker (λ Hind III digest). (D) PCR confirmation of pAd-CXCR4. Lane 1 showed the expected product of CXCR4 (1079 bp) and Lane 2 was DNA marker DL2000. (E) Restriction digestion verification of pAd-CXCR4. Lane1: DNA Marker (DL2000); Lane 2: restriction endonuclease digestion with BglII plus EcoRV resulted in two expected segments (1 kb and 32 kb); Lane 3: restriction endonuclease digestion with Pac1 resulted in two expected segments (3 kb and 30 kb); Lane 4: pAd-CXCR4 not been digested by restriction endonuclease; Lane 5: DNA Marker (λ Hind III digest). (F) Cytopathogenic effect 72 h after HEK 293 transfection.

containing 50 or more cells were recognized as a colony. As for the assay of CFU-E, about 2×10^5 marrow nucleated cells were incubated at 37°C in RPMI-1640 medium with 0.2 ml 10^{-4} M dimercaptoethanol, 0.2 ml 3% L-glutamine, 0.5ml horse serum, 0.2 ml 10 U/ml erythropoietin, and 0.7ml 2.7% methyl cellulose. Colony formation was identified by dimethylbenzidine staining. On the third day of incubation, a colony containing no less than 8 cells was considered as one CFU-E colony.

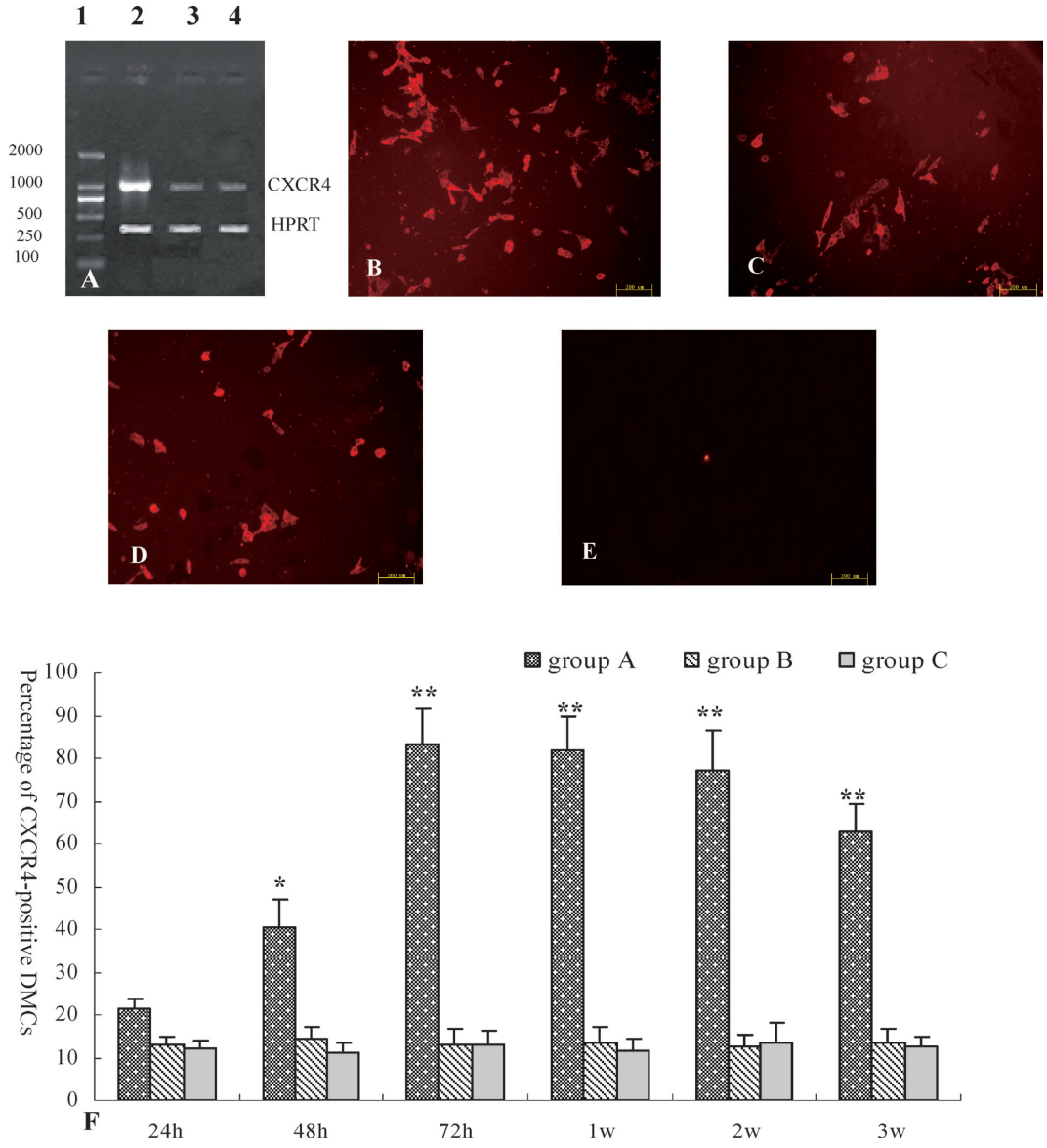

Fig. 2. The effect of Adv-CXCR4 infection on the expression of CXCR4 in DMCs. About 48 hours after infection, DMCs were harvested for RT-PCR analysis or were fixed for immunostaining. Twenty-four hours, 48 h, 72 h, 1 w, 2 w and 3 w after infection, DMCs were harvested for FACS analysis. The passage and harvest of non-infected DMCs were performed at the same time as infected DMCs. A: RT-PCR showed that stronger signal could be detected in group A (DMCs infected by Adv-CXCR4, Lane 2) than that of group B (DMCs infected by Adv-GFP, Lane 3) and group C (non-infected DMCs, Lane 4). The results of fluorescent immunohistochemistry showed that the CXCR4-positive DMCs demonstrated red color under fluorescence microscope. The number of CXCR4-positive DMCs in group A (Fig. 2 B) was significantly greater than that in group B (Fig. 2 C) and group C (Fig. 2 D). No CXCR4-positive DMCs were found when primary antibody was not added (Fig. 2 E). Fig. 2F demonstrated the results of FACS. Twenty-four hours after infection, the percentage of CXCR4-positive DMCs in group A was greater than that of group B and group C ($P < 0.05$), and the difference continued at the followed time points ($P < 0.01$). *: $P < 0.05$, **: $P < 0.01$, compared with group B and group C.

Statistical analysis

All data were expressed as means ± standard deviation. Statistical significance was evaluated with an unpaired Student's t test for comparison between two groups or by ANOVA for multiple comparisons. A value of $P < 0.05$ was considered significant.

The experimental protocol was reviewed and approved by the Animal Ethical Committee of the Third Military Medical University, P. R. China.

RESULTS

The Construction of Adv-CXCR4 and Adv-GFP

With total RNA from BM as template, the full length cDNA of CXCR4 (containing BglII and EcoRV restriction endonucleases sites) was successfully amplified from the rat's BM total RNA (Fig. 1A). Then the CXCR4 cDNA and pAdTrack-CMV shuttle vector were digested with BglII and EcoRV, followed by ligation with solution 1. As confirmed by PCR (Fig. 1B), restriction endonuclease digestion (Fig. 1C) and sequence analysis (data not shown), CXCR4 cDNA was cloned into pAdTrack-CMV shuttle vector correctly.

The shuttle vector containing CXCR4 cDNA was linearized with Pme 1 and transfected BJ5183 cells to allow recombination occur. PCR (Fig. 1D), restriction endonuclease digestion (Fig. 1E) and sequence analysis (data not shown) proved a successful recombination. The recombinant vector was named as pAd-CXCR4.

Then pAd-CXCR4 was amplified, linearized by Pac 1 digestion and was used to transfect HEK 293 cells to construct primary adenovirus stock. About 30 h after transfection, GFP expression could be found under a fluorescence microscope, and 72 hours after transfection, cytopathogenic effect was observed (Fig. 1F), suggesting the generation of adenovirus of CXCR4. The adenovirus was named as Adv-CXCR4 and its titer determined by plaque assays was about 8.0×10^{10}–3.3×10^{11} PFU/ml.

With similar strategy, we obtained Adv-GFP (data not shown here).

Adv-CXCR4 infection up-regulate the expression of CXCR4 in DMCs

We tested whether Adv-CXCR4 infection could up-regulate the expression of CXCR4 in DMCs by RT-PCR, fluorescent immunohistochemistry and FACS. The results of RT-PCR showed that the expression of CXCR4 in Adv-CXCR4 infected DMCs was higher than that of Adv-GFP infected DMCs and non-infected DMCs (Fig. 2A). The results of fluorescent immunohistochemistry showed that the number of positive DMCs (red colored under fluorescence microscope) in group A was significantly higher than that of the control groups (Fig. 2B–D). Before infection with Adv-CXCR4, the percentage of CXCR4-positive DMCs was about 13.25% as determined by FACS, increased to 21.36% 24 hours later, reached the peak of 83.2% 60 hours after infection, and still remained at a high level of 62.58% 3 weeks after infection. The percentage of CXCR4-positive DMCs infected by Adv-GFP and non-infected DMCs were 12.59% and 13.45% respectively, which were significantly less than that of Adv-CXCR4 DMCs ($P < 0.05$) (Fig. 2F). Totally, all these findings showed that Adv-CXCR4 infection could increase the expression of CXCR4 in DMCs.

Adv-CXCR4 infected DMCs distributed more frequently to irradiated BM

Three days after transplantation, the amount of DMCs in BM of group A rats was greater than the control group, but without statistics significance ($P > 0.05$). Five days after transplantation, the amount of DMCs in BM of group A rats was $(2.63 \pm 0.25) \times 10^4$, significantly greater than that of group B ($(1.57 \pm 0.19) \times 10^4$) and group C ($(1.65 \pm 0.15) \times 10^4$) ($P < 0.05$). One week after transplantation, the amount of DMCs in BM in each group reached its peak, with the amount in group A was $(3.77 \pm 0.45) \times 10^4$, which was about

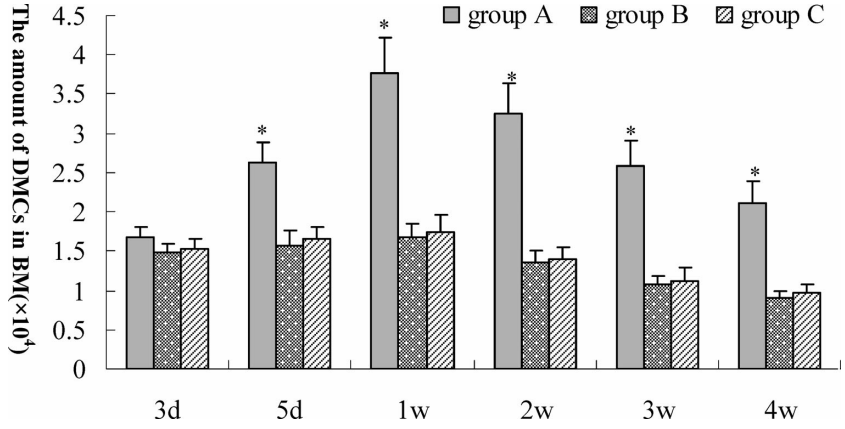

Fig. 3. The amount of DMCs in BM in each group. *: $P < 0.05$, compared with group B and group C.

Table 1. The number of CFU-E colonies in each group

	3 d	5 d	1 w	2 w	3 w	4 w
Group A	55 ± 9.4▲▲	64 ± 7.8▲	83 ± 112.4▲	94 ± 7.9*	99 ± 11.4*	108 ± 11.5*
Group B	57 ± 8.8▲▲	58 ± 6.5▲▲	63 ± 9.3▲	69 ± 8.7▲	74 ± 6.9▲	79 ± 8.5▲
Group C	53 ± 8.1▲▲	59 ± 10.3▲▲	65 ± 10.6▲▲	72 ± 8.9▲	76 ± 9.5	82 ± 9.0▲

The value for normal control was 112 ± 13.4.
*: $P < 0.05$, compared with group B and group C.
▲: $P < 0.05$, : $P < 0.01$, compared with normal control.

Table 2. The number of CFU-F colonies in each group

	3 d	5 d	1 w	2 w	3 w	4 w
Group A	49.6 ± 5.9▲▲	61.4 ± .75▲▲	69.5 ± 7.8▲	79.2 ± 10.6*▲	92 ± 8.7*	101.4 ± 10.9*
Group B	43.8 ± 6.7▲▲	48.4 ± 6.89▲▲	55.6 ± 5.3▲▲	61.3 ± 8.5▲▲	68.8 ± 7.6▲	75.6 ± 8.1▲
Group C	47.4 ± 5.8▲▲	51.2 ± 5.3▲▲	59.2 ± 7.3▲▲	65.3 ± 7.2▲▲	71.4 ± 8.4▲	78.8 ± 8.2▲

The value for normal control was 107 ± 13.2.
*: $P < 0.05$, compared with group B and group C.
▲: $P < 0.05$, : $P < 0.01$, compared with normal control.

Table 3. The number of CFU-GM colonies in each group

	3 d	5 d	1 w	2 w	3 w	4 w
Group A	55.6 ± 6.9▲▲	71.7 ± 9.5▲▲	85.8 ± 8.4*▲	98.7 ± 11.4*▲	114.2 ± 9.6*	120.5 ± 13.9*
Group B	53.7 ± 8.9▲▲	59.0 ± 7.8▲▲	66.7 ± 5.8▲▲	74.5 ± 6.7▲	80.4 ± 10.6▲	85.9 ± 7.4▲
Group C	57.2 ± 4.8▲▲	61.4 ± 6.3▲▲	69.3 ± 8.3▲▲	78.3 ± 8.1▲	83.6 ± 5.8▲	90.1 ± 9.2▲

The value for normal control was 135.3 ± 11.7.
*: $P < 0.05$, compared with group B and group C.
▲: $P < 0.05$, : $P < 0.01$, compared with normal control.

2.24 times and 2.18 times of that in group B and group C respectively (Fig. 3).

Adv-CXCR4 infected DMCs accelerate BM function recovery after transplantation

The hematopoiesis function of BM was monitored by hematopoietic progenitor colonies assay. As shown in Table 1, the number of CFU-E colonies in normal control was 112.0 ± 13.4. On day 3 after irradiation, the number of CFU-E colonies in all the three groups decreased significantly when compared with the normal control ($P < 0.01$). Two week after irradiation, the number increased gradually in each group with the fastest recovery in group A. Four week after irradiation, the number in group A nearly reached normal level, and the number in group B and group C was significantly lower than that in group A ($P < 0.05$). Similar tendency were observed in CFU-F and CFU-GM assay, and the detailed data were listed in Table 2 and Table 3. These data suggested that Adv-CXCR4 infected DMCs could promote hematopoiesis function recovery more quickly than controls.

DISCUSSION

The chemokine SDF-1, also termed CXCL12, is widely expressed in many tissues during development and adulthood, including BM, liver, central nervous system, and skin, etc. Its interaction with CXCR4 plays an important role in many biological processes, such as the development of many organs and systems,[6] the pathological process of acquired immune-deficiency syndrome,[18] neoangiogenesis and metastasis of tumor.[7] In recent years, the interaction of SDF-1/CXCR4 has been reported to recruit both endogenous and exogenous stem cells to injured sites and participate in the repair of injured tissues.[8–10] Also, SDF-1/CXCR4 interaction is manipulated in many ways including gene transfer to enhance the repair effect of stem cells.[11–13] In the present study, the expression of CXCR4 was over-expressed in DMCs by Adv-CXCR4 infection. After transplantation, Adv-CXCR4 infected DMCs could distribute more frequently to irradiated BM and accelerate hematopoiesis function recovery.

Why the over-expression of CXCR4 could lead to the increase in recruitment of DMCs? Studies from Chopp's group have demonstrated mesenchymal stem cells administered either intravenously, intra-arterially, or intracerebrally could preferentially and selectively migrate into the region of traumatic or ischemic brain.[19–22] Because mesenchymal stem cells seem to home similarly to pathologic sites derived from disparate etiologies, Ji, *et al*[8] hypothesized that the inflammatory response in the pathologic sites itself guide transplanted stem cells to the injured sites. Their study showed that the expression of SDF-1 and fractalkine increased in the injured sites, and worked as powerful chemoattractant to CXCR-4 and CX_3CR1 positive stem cells.[8] Furthermore, they showed that the chemotactic effect of SDF-1 is more powerful than fractalkine.[8] Taken together, the above-mentioned studies indicated that SDF-1 is the main factor responsible for the recruitment of transplanted stem cell to injured sites. When CXCR4, were over-expressed in human $CD34^+$ progenitors by gene transfer, the infected cells exhibited significant increases in SDF-1–mediated chemotaxis and could distribute more frequently into the injured sites under the direction of SDF-1.[13] So when the expression of CXCR4 was up-regulated in DMCs by gene transfer, it could increase DMCs distribution to irradiated BM under the chemotactic effect of SDF-1.

The percentage of CXCR4-positive DMCs 60 h after Adv-CXCR4 infection was about 6.2 times of the non-infected DMCs, while the amount of Adv-CXCR4 infected DMCs in BM one week after transplantation was only about 2.18 times of non-infected DMCs. It seems that the amount of DMCs recruited to irradiated BM was not proportional to the quantity of CXCR4 in DMCs. We hypothesize that this phenomenon maybe caused by the fact that stem cell recruitment to the injured sites is due to a 2-step process. The transplanted stem cells firstly bind to adhesive complexes in the vasculature around the injured zone, followed by local chemotaxis to the site of engraftment. Increased expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, matrix metalloproteinase-9, vascular endothelial growth factor, monocyte chemoattractant protein-1 and stem cell factor in the injured sites might in concert with SDF-1 to recruit transplanted stem cells to the injured sites.[8,23,24]

In summary, DMCs infected by Adv-CXCR4 distributed more frequently to BM of sublethally irradiated rats and could accelerate the functional recovery of BM.

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