Cytokine xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Stromal cell-derived factor 1α facilitates aneurysm remodeling in elastase-induced rabbit saccular aneurysm

Zi-Fu Li[a,1], Xing-Gen Fang[b,1], Rui Zhao[a], Peng-Fei Yang[a], Qing-Hai Huang[a,*], Jian-Min Liu[a,*]

[a] Neurosurgery Department, Changhai Hospital, Second Military Medical University, Shanghai 200433, China
[b] Neurosurgery Department, Yijishan Hospital, Wuhu 241001, Anhui Province, China

ARTICLE INFO

Keywords:
Stromal cell-derived factor 1α
Saccular aneurysm
Inflammation
Rabbit

ABSTRACT

Aims: Inflammation plays a crucial role in aneurysm wall remodeling, which could lead to the rupture of intracranial aneurysms. Stromal cell-derived factor 1α (SDF-1α), a vital inflammation cytokine, is also related to aneurysm pathogenesis. However, the characteristics of SDF-1α expression and its role in aneurysm remodeling remain largely unknown. In this study, we aimed to investigate the expression dynamics of SDF-1α and its correlation with aneurysm remodeling.

Methods: Saccular aneurysms were induced by porcine pancreatic elastase in New Zealand White rabbits. Aneurysm size was measured by digital subtraction angiography. Endothelial-like cells on the aneurysm wall were assessed on postoperative days 1, 3, 7, 14, 21, and 30. SDF-1α levels in the aneurysmal wall and serum were examined at several follow-up time points. Adherent molecule expression was examined, and migration assays were performed *in vitro*. After SDF-1α stimulation, the mobilization of endothelial-lineage cells and its role in the reendothelialization of the aneurysm wall were investigated in a saccular aneurysm rabbit model.

Results: After the creation of saccular aneurysms in rabbits, the aneurysm sacs were filled with acute thrombosis within 3 days, followed by a significant enlargement on day 14 and maturation on day 21. Serum SDF-1α levels increased in a bimodal fashion on day 1 and day 14, whereas SDF-1α expression in the aneurysm wall reached its maximum on day 14. VE-cadherin was up-regulated after SDF-1α stimulation and down-regulated by the SDF-1α ligand blocker AMD3100. Endothelial progenitor cell migration was enhanced by SDF-1α and blocked by AMD3100. The *in vivo* administration of SDF-α to rabbits with saccular aneurysms promoted endothelial-lineage cell mobilization into the peripheral blood and reendothelialization of the aneurysm wall.

Conclusions: The SDF-1α expression level in the peripheral blood and local aneurysm wall correlated with the aneurysm remodeling process in rabbits with elastase-induced saccular aneurysms. We conclude that SDF-1α may facilitate aneurysm wall remodeling by up-regulating VE-cadherin expression and mobilizing endothelial-lineage cells.

1. Introduction

The rupture of an intracranial aneurysm results in subarachnoid hemorrhage, leading to catastrophic consequences of high mortality and morbidity [1]. However, the underlying mechanisms responsible for aneurysm pathogenesis remain largely unknown. Accumulating evidence indicates that inflammation plays a crucial role in aneurysm wall remodeling, which can lead to aneurysm rupture [2].

Stromal cell-derived factor-1α (SDF-1α, also known as CXCL12) belongs to the CXC chemokine ligand superfamily. SDF-1α is secreted by platelets, fibroblast cells, dendritic cells, and endothelial cells [3–5]. SDF-1α is a key cytokine in aneurysm inflammation, which contributes to cell proliferation and migration [6]. In addition, SDF-1 plays a crucial role in mobilizing progenitor cells and stem cells, including endothelial or smooth muscle progenitor cells and mesenchymal stem cells, into injured tissues [7–9]. It was reported that SDF-1 exerts its functions via binding its ligand and triggering SDF-1/CXCR4 signaling [10,11]. Hoh et al. [2] also reported that SDF-1 promotes endothelial cell and macrophage migration into the aneurysm wall.

It has been shown that the expression level of SDF-1α in serum and tissue reflects the status of aneurysm wall inflammation. SDF-1α expression is elevated in the first hour after myocardial infarction [12–14], and its level is associated with ischemia severity [7]. SDF-1 expression is regulated by transcription factor hypoxia-inducible factor-

* Corresponding authors.
E-mail addresses: ocinhqh@163.com (Q.-H. Huang), chstroke@163.com (J.-M. Liu).
[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.cyto.2017.07.020
Received 21 October 2016; Received in revised form 1 July 2017; Accepted 26 July 2017

1 (HIF-1) in endothelial cells, resulting in SDF-1 expression under ischemic conditions. The surface expression of SDF-1α on platelets is also enhanced during ischemic events [15]. However, its expression pattern in aneurysms under an inflammatory environment is unclear.

In this study, we used an elastase-induced saccular aneurysm rabbit model to simulate the process of aneurysm wall formation and inflammation conditions to investigate SDF-1α expression levels at different stages and their correlation with aneurysm reendothelialization.

2. Materials and methods

2.1. Experimental animals

This study was approved by the institutional animal care and use committee of Second Military Medical University. The saccular aneurysm model was established using 62 New Zealand White male rabbits (average body weight, 2.8 ± 0.8 kg; range, 2.7–3.5 kg). The rabbits were randomly divided into groups for subsequent experiments. Thirty animals were used for examinations of serum levels of SDF-1α, local expression of SDF-1α, and endothelial cells in the aneurysm sac. Twelve animals were used as a control group. The remaining animals were used for morphological and histological studies.

2.2. Animal models of saccular aneurysm

All surgeries were performed with combined anesthesia using 1% sodium pentobarbital (intravenous injection, 1 ml/kg) and xylazine (intramuscular injection, 0.1 mg/kg). A saccular aneurysm in the right common carotid artery was induced with porcine pancreatic elastase using a previously described technique [16]. The right common carotid artery was ligated 2 cm proximal to the origin after clipping the origin and partial wall of the subclavian artery with a temporary arcuate aneurysm clip. The inner lumen of the proximal segment was incubated for approximately 20 min with 75 units of porcine pancreatic elastase delivered via a 22-gauge catheter and saline was used in the control group. The aneurysm clip was removed after ligation of the catheter puncture point. After model establishment, the rabbits were caged with adequate water and food.

2.3. Serial angiography for aneurysm morphology assessment

Serial intravenous digital subtraction angiography (WINMEDIC2000, Lepu, Beijing, China) was performed in 30 rabbit models via the ear margin vein with a 22-Gauge sheath under combined anesthesia on postoperative days 3, 7, 14, 21, and 30. Angiography was performed using the following parameters: contrast flow speed, 1.5–2 ml/s; total volume, 6–8 ml; psi, 150–200; and 7.5 frames/s. The aneurysm neck, length, and width were measured with workstation software.

2.4. Histology and immunohistochemistry of the aneurysm sac

To observe the morphological changes after model induction, the histology of the aneurysm sac was evaluated by two separate observers. Within 6 h and on days 3, 7, 14, and 21 after model establishment, 10 aneurysms were harvested and fixed in paraffin. Gross specimens were observed by microscopy. Aneurysm sac sections were stained with Victory blue to visualize elastic fibers and with HE to examine intraaneurysmal thrombosis.

2.5. Scanning electron microscopy of the aneurysm sac

To investigate the reendothelialization process of the aneurysm sac, on days 1, 3, 7, 14, and 21 after model establishment, 10 aneurysms were harvested and fixed in 4% paraformaldehyde. Endothelial-like cells were observed and counted under scanning electron microscopy (SU-8010; Hitachi, Tokyo, Japan).

2.6. Examination of the expression of SDF-1α in the local aneurysm wall

Local SDF-1α expression was also examined on days 1, 3, 7, 14, and 21 after model establishment. Immunohistochemical staining of SDF-1α was performed using a polyclonal SDF-1 antibody (Abcam, Cambridge, MA, USA) at a dilution of 1:100. Total RNA was extracted, and quantitative RT- PCR was performed to evaluate the SDF-1α RNA level in the aneurysm sac. The primers used were as follows: SDF-1α forward, 5′-TGTCTCAGCGATGGGAAACC-3′, and reverse, 5′-TTGGGCGTGTTGAG GATCTT-3′ (amplicon size: 113 bp), and GADPH forward, 5′-CGCCTGGAGAAAGCTGCTA-3′, and reverse, 5′-ACGACCTGGTCCTC GGTGTA-3′ (amplicon size: 104 bp). Gene expression was calculated using the relative quantification method with the following equation: $2^{-\Delta\Delta CT}$, where ΔΔCT = CT [(Average target gene, sample) − (Average reference gene, sample)] − CT [(Average target gene, control) − (Average reference gene, control)], where GAPDH was the reference gene and the normal common carotid artery was the control. Western blot analysis was performed to investigate the SDF-1α expression in the local aneurysm wall.

2.7. Quantification of serum SDF-1α by ELISA

To examine the peripheral serum level of SDF-1α at different times points after model establishment, the concentration of SDF-1α was measured before and immediately after model establishment on days 1, 3, 7, 14, and 21 using an ELISA kit (R & D Systems, Minneapolis, MN). Peripheral blood (2 ml), collected via the ear central artery, was centrifuged at 1000*g* for 15 min at 25 °C, and the supernatant was then recentrifuged at 10,000*g* for 10 min at 4 °C. Each sample was tested in duplicate and the average values were recorded.

2.8. Migration assay

To investigate the role of SDF-1α in aneurysm sac remodeling, migration assays were performed. Bone marrow-derived endothelial progenitor cells (EPCs) were cultivated, expanded as previously described [16], and suspended in endothelial growth medium-2 microvascular (EGM-2MV, Lonza, Basel, Switzerland) supplemented with 0.5% bovine serum albumin (Sigma Aldrich, St Louis, MO, USA). A Boyden chamber with an 8-μm pore-size transwell membrane was used. Then, 1×10^4 cells in 150 μl medium were placed in the upper compartment, and SDF-1α protein at concentrations of 0–500 ng/ml diluted in a volume of 700 μl phosphate buffered saline was placed in the lower compartment. AMD3100, a CXCR4 ligand blocker, was added to a lower chamber that contained SDF-1α at a concentration of 100 ng/ml. After incubation for 12 h at 37 °C and 6% CO_2, migrated cells were fixed, stained with crystal violet, and counted under microscopy.

2.9. Expression of adhesion molecules

To further investigate the role of SDF-1α in EPC adhesion, the expression of adhesion molecules was examined. Approximately 1×10^5 cells were suspended in 2 ml EGM-2MV in each well of a 6-well plate. EPCs were stimulated for 24 h with SDF-1α at concentrations of 0, 10, 100, and 500 ng/ml and with SDF-1α (100 ng/ml) and AMD3100 at concentrations of 10 ng/ml and 50 ng/ml. Western blotting was used to quantify the adhesion molecules, VE-cadherin, P—selectin, and E-selectin on EPCs.

2.10. Endothelial-lineage progenitor cell mobilization after SDF-1α administration

To determine whether SDF-1α stimulated the mobilization of peripheral endothelial-lineage progenitor cells, SDF-1α (50 μg/kg; Abcam, Cambridge, MA, USA) was intravenously injected via the ear marginal vein (n = 8). Before administration and 4, 8, 12, and 24 h after administration, 0.5 ml of blood was drawn from the ear central artery and

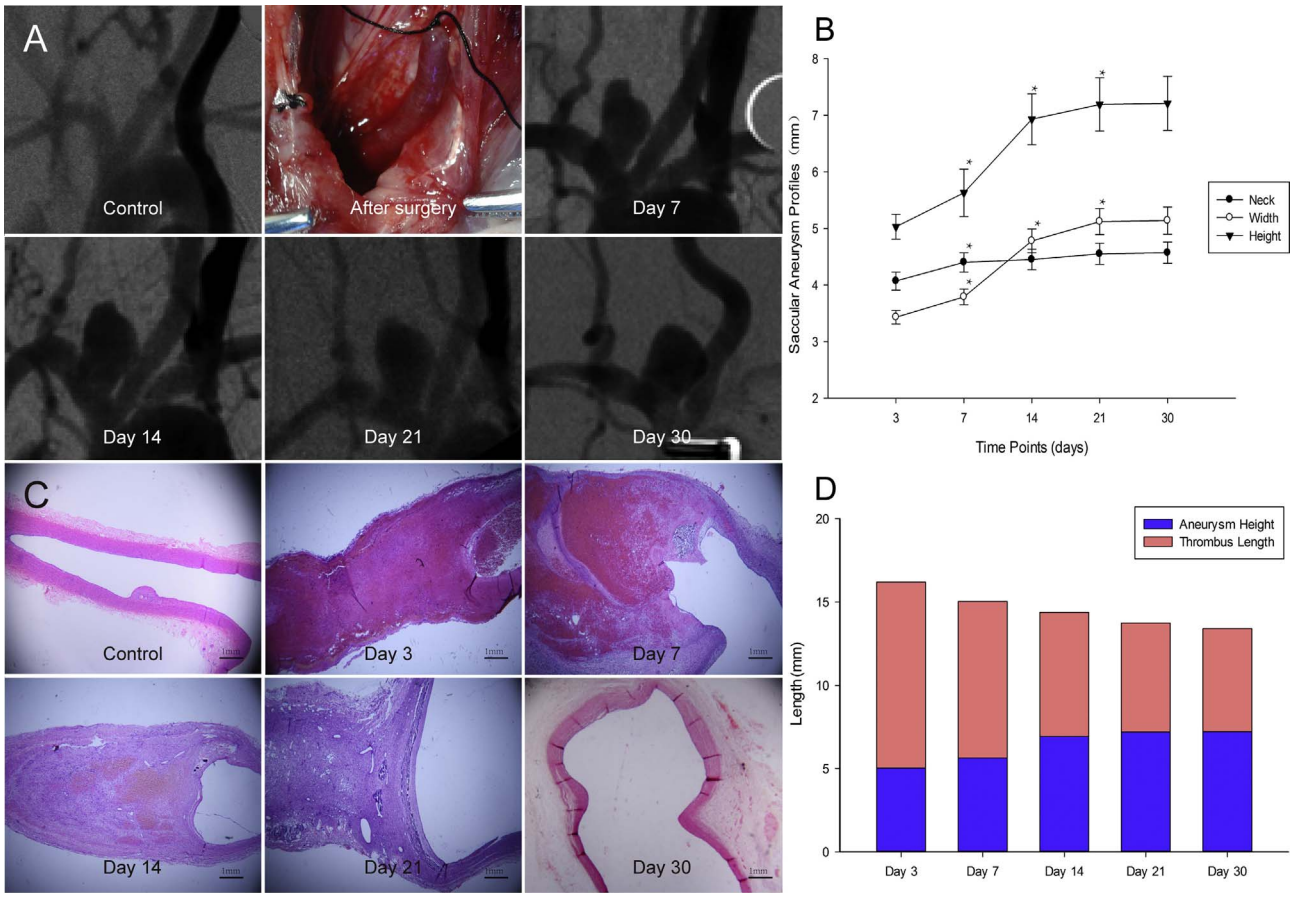

Fig. 1. Morphological changes in saccular aneurysms after model establishment in rabbits. A: Angiography showed the small carotid stump without aneurysmal dilatation in the control group (Panel A upper left), The carotid stump was immediately dilated after incubation with pancreatic elastase (Panel A upper middle) and serial intravenous angiography showed that the aneurysm sac was small, became larger on day 7 (Panel A upper right), was significantly enlarged on day 14 (Panel A lower left), and became morphologically mature on day 21 (Panel A lower middle) and day 30 (Panel A lower right). B: Dynamic changes in aneurysm neck, width and height. C: HE staining displayed the carotid stump with normal elastic layer in the control group (Panel C upper left), and in the experiment group displayed the disappearance of elastic layer, the retardation of the intra-aneurysmal thrombosis from acute thrombosis on day 3 (Panel C upper middle) and the growth of the aneurysm sac on day 7 (Panel C upper right) to organized thrombosis on day 14, 21 and 30 (Panel C lower) (magnification ×40). D: Dynamic changes in the aneurysmal thrombosis length and aneurysm height. $^{*}P < 0.05$.

analyzed on a FACS Calibur flow cytometer (BD Biosciences, Mountain View, CA). FITC-conjugated anti-human KDR (vascular endothelial growth factor receptor-2), APC-conjugated anti-human CD34 (both from R & D, Minneapolis, USA), and PE-conjugated CD133 (Miltenyi Biotec, Auburn, CA) were used. Isotype-matched IgG1 was used as a control.

2.11. Aneurysm wall reendothelialization after SDF-1α administration

To further observe the direct role of SDF-1α in aneurysm remodeling, recombinant human SDF-1α at a dose of 50 μg/kg/day (ChinaPeptides Corporation, Shanghai, China) was intravenously injected via the margin vein of the ear for seven consecutive days after aneurysm model establishment, and the same dose of saline was used in the control group. Endothelial-like cells were counted under scanning electron microscopy on days 14 and 28. Endothelial markers in the aneurysm sac, including VE-cadherin, KDR, Tie-2, and E-selectin, were examined with quantitative RT- PCR.

2.12. Statistical analysis

The results are expressed as the mean ± SD. Statistical analysis between two groups was performed with unpaired Student's *t*-test if quantitative data fit a normal distribution. Otherwise, a non-parameter test was used. Analysis involving more than two groups was performed with analysis of variance and a post hoc test. *P* values < 0.05 were considered significant.

3. Results

3.1. Changes in saccular aneurysm length and width

The serial intravenous angiography results from days 3, 7, 14, 21, and 30 revealed that the aneurysm height reached 5.03 ± 0.22 mm, 5.63 ± 0.42 mm, 6.93 ± 0.45 mm, 7.19 ± 0.47 mm, and 7.21 ± 0.48 mm, respectively; the aneurysm width reached 3.43 ± 0.12 mm, 3.79 ± 0.14 mm, 4.78 ± 0.21 mm, 5.12 ± 0.23 mm, and 5.14 ± 0.24 mm, respectively; and the aneurysm neck reached 4.07 ± 0.16 mm, 4.40 ± 0.17 mm, 4.45 ± 0.18 mm, 4.55 ± 0.19 mm, and 4.57 ± 0.19 mm, respectively. Compared with the results on day 7, the aneurysm sacs became increasingly apparent on day 14 ($P < 0.05$). There were further increases in aneurysm length and width on days 14 and 21 ($P < 0.05$), but they stopped on day 30 ($P > 0.05$) (Fig. 1A-B). In the control group, the morphology of the right carotid stump remained the same during the angiographic follow-up. The average length and width of the stump respectively reach 3.35 mm and 1 mm, and aneurysmal morphology was not observed.

3.2. Degradation of intra-aneurysmal thrombosis

Hematoxylin and eosin (HE) staining showed that the aneurysms were filled with acute thrombosis within 6 h after model establishment in all 10 rabbits. The elastic layer of the arterial wall in the right common carotid artery stump was disrupted and dissolved in all

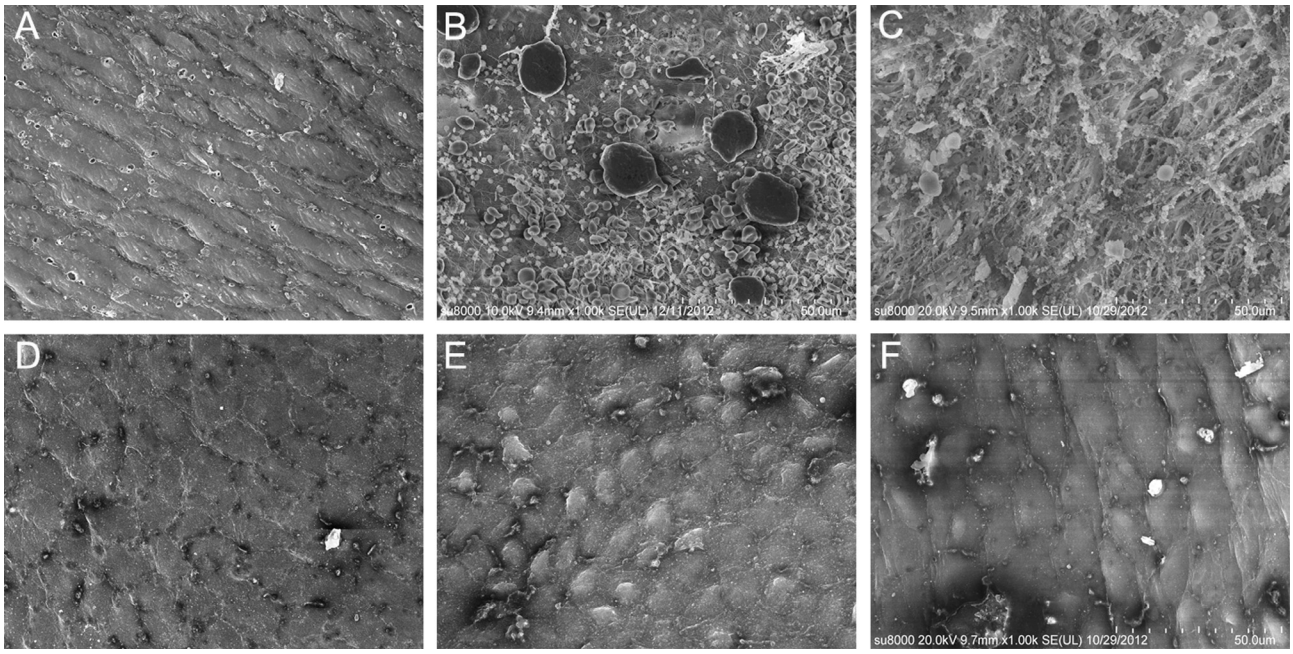

Fig. 2. Reendothelialization of the aneurysm wall after saccular aneurysm induction observed under scanning electron microscopy (magnification ×1000). A: Endothelial cells in a normal artery. B: Six hours after the operation, inflammatory components, mainly platelets, were attached to the aneurysm wall. C: On day 3, inflammatory components, mainly fibrinogen, covered the aneurysm wall. D: On day 7, cells with a round shape appeared on the aneurysm wall, and inflammatory components were diminished. E: On day 14, increased numbers of cobblestone-like cells were observed on the aneurysm wall. F: On day 21, the aneurysm wall presented a mature morphology of complete reendothelialization.

models. On postoperative days 3, 7, 14, and 21, the mean aneurysmal thrombosis length reached 11.6 ± 1.09, 9.40 ± 0.97, 7.45 ± 0.53, 6.55 ± 0.47, and 6.20 ± 0.46 mm, respectively. On day 3, a small aneurysm sac was observed, and the aneurysm sac volume was filled with acute thrombosis (Fig. 1C and D). On day 7, fibrous tissue began to cover the surface of the thrombosis in all aneurysms, and the initially developed intra-aneurysmal thrombosis was disrupted in 3 rabbits, with the sprouting of an additional aneurysm sac. On days 14 and 21, the thrombosis became organized and shrunk at the aneurysm dome in all rabbits. In the control group, elastic fiber layer remained intact and aneurysmal sac was not formed.

3.3. Reendothelialization occurred after inflammation of the aneurysm wall had decreased

Three specimens were evaluated at each time point. Scanning electron microscopy showed that large numbers of platelets, inflammatory cells, and fibers were stacked on the aneurysm wall at postoperative day 3 in all specimens. These inflammatory agents had decreased on day 7, and the surface of the aneurysm wall became exposed; there were scattered inflammatory agents attached to the wall, and cells with a round shape were observed (Fig. 2). On day 14, cobblestone-like cells appeared on the aneurysm wall, and these cells were not the typical spindle-like endothelial cells. Increased numbers of spindle-like endothelial cells were observed on the aneurysm wall on day 21. Reendothelialization was nearly completed on day 21 compared with the normal artery (260 vs 285/high power field, $P > 0.05$).

Expression of SDF-1 α in the local aneurysm wall increased. Data on SDF-1α expression for the 1st day were not obtained because the aneurysm sacs, which were completely filled with acute thrombosis, were not available. Immunohistochemistry showed that SDF-1α expression appeared in the local aneurysm wall on day 3, reached a maximum on day 7, decreased on day 14 after model establishment, and nearly vanished on day 21. The optical density (OD) ratios reached 0.0021 ± 0.00017, 0.0046 ± 0.00033, 0.0029 ± 0.00012, and 0.00020 ± 0.00011 on days 3, 7, 14, and 21, respectively (Fig. 3A and B).

To confirm SDF-1α expression in the aneurysm wall, SDF-1α mRNA levels were examined with quantitative RT-PCR. The average OD values reached 0.05 ± 0.02, 0.32 ± 0.21, 0.70 ± 0.05, and 0.20 ± 0.03 on days 3, 7, 14, and 21, respectively (Fig. 3C-D). The result showed that RNA levels reached the maximum on postoperative day 14 ($P < 0.05$).

3.4. Serum SDF-1α level increased in a bimodal manner

Compared with before model establishment, the level of serum SDF-1α significantly increased on postoperative day 1 (1060.8 ± 502.32 vs 1655.15 ± 329.73 pg/ml, $P < 0.05$). The level decreased on day 3 (1270.98 ± 247.19 pg/ml, $P < 0.05$) and on day 7 (1193.65 ± 300.54 pg/ml, $P > 0.05$), increased again on day 14 (1520.52 ± 181.12 pg/ml, $P < 0.05$), and decreased on day 21 (1446.69 ± 152.63 pg/ml, $P < 0.05$). The expression of serum SDF-1α exhibited the characteristics of a bimodal distribution (Fig. 3E-F). The levels of serum SDF-1α in the control group with healthy rabbits at each time point were 1033 ± 41.49, 1123 ± 36.97, 1090 ± 45.26, 1035 ± 49.99, 1058 ± 36.09, and 1097 ± 38.95 pg/ml.

3.5. EPCs migrated along SDF-1α concentration gradients and were blocked by AMD3100

Migration assays showed that the number of migrated cells increased as the SDF-1α concentration increased and that the number decreased after AMD3100 treatment. At concentrations of 0, 10, 100, and 500 ng/ml, the numbers of migrated cells in the lower chamber were 60 ± 9, 109 ± 12, 150 ± 14, and 300 ± 27, respectively (Fig. 4A-B). The differences between each concentration were significant ($P < 0.05$). After AMD3100 treatment at 10 and 50 ng/ml, (at an SDF-1α concentration of 100 ng/ml), the number of migrated cells decreased to 75 ± 8 and 30 ± 5, respectively ($P < 0.05$).

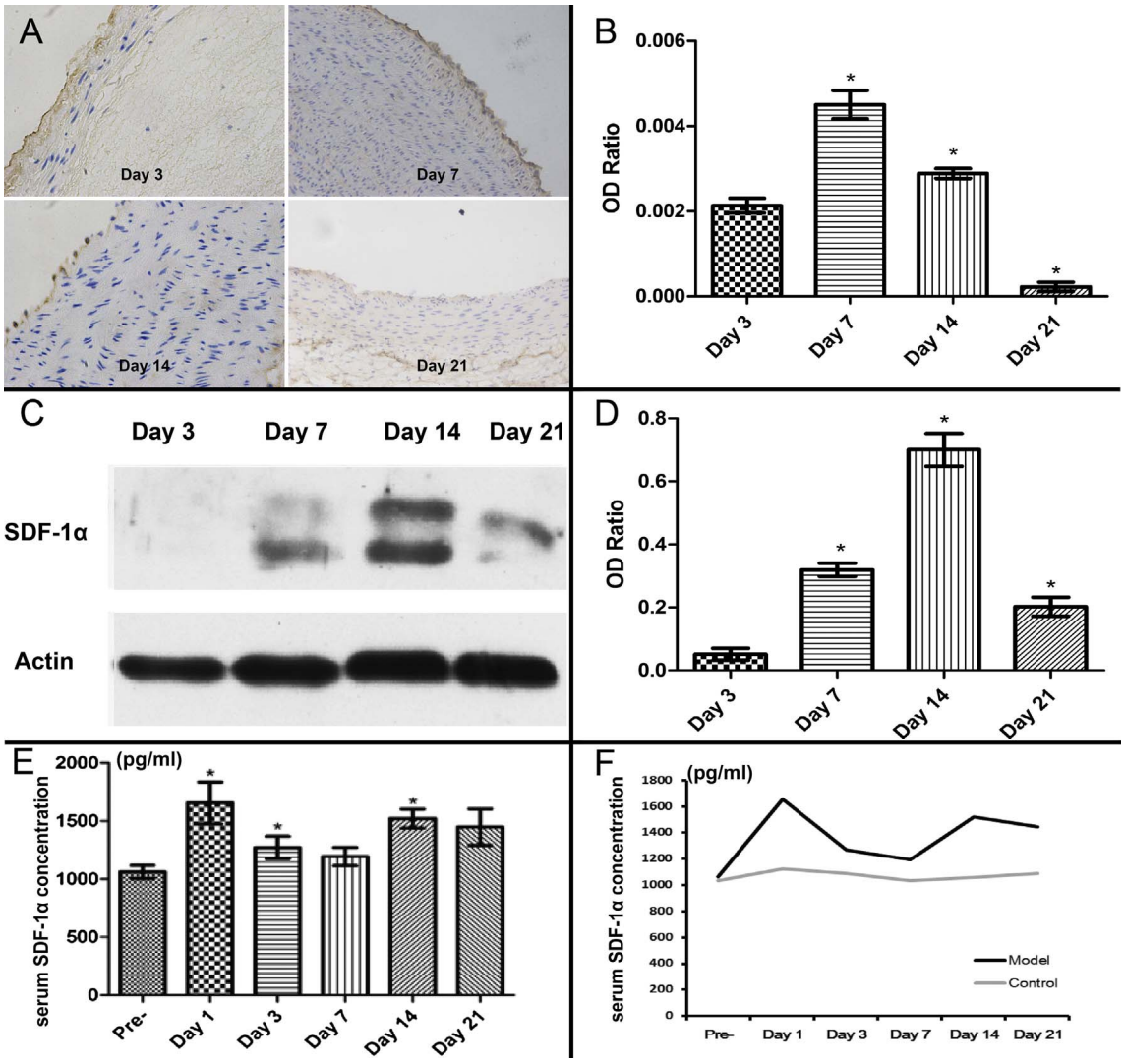

Fig. 3. Dynamic SDF-1α expression in the aneurysm wall and peripheral blood after model establishment. A: SDF-1α expression on IHC (magnification ×100) on day 3, 7, 14, and 21. B: OD ratios indicating that the maximum *in situ* SDF-1α expression level in the aneurysm wall occurred on day 7. C: Western blot analysis of SDF-1α in the aneurysm wall. D: OD ratios indicating that SDF-1α expression peaked in the aneurysm wall on day 14. E: The plasma SDF-1α level significantly increased on day 1 and reached a second peak on day 14. F: SDF-1α expression presents a bimodal distribution. $^{*}P < 0.05$.

3.6. Vascular endothelial (VE)-cadherin was upregulated after SDF-1 α stimulation

Western blot analysis showed that the expression of VE-cadherin in EPCs increased as the SDF-1α stimulus intensity increased at concentrations of 0, 10, 100, and 500 ng/ml in the lower compartment, and the relative OD values reached 0.22 ± 0.04, 0.34 ± 0.03, 0.46 ± 0.03, and 0.63 ± 0.03, respectively. The expression of VE-cadherin decreased at AMD3100 concentrations of 50 ng/ml and 10 ng/ml with 100 ng/ml SDF-1α, and the relative OD values reached 0.47 ± 0.04 and 0.60 ± 0.04, respectively (Fig. 4C-D). The expression levels of P—selectin and E-selectin did not change after treatment with different concentrations of SDF-1α.

3.7. SDF-1α promoted endothelial-lineage cell mobilization in peripheral blood

The flow cytometry results showed that endothelial-lineage cells with the KDR marker were present at levels of 2.4 ± 0.4, 21.4 ± 2.0, 8.8 ± 1.0, 3.3 ± 0.5, and 3.5 ± 0.5 cells per 10^4 cells in peripheral blood after SDF-1α stimulus (Fig. 4E-F), of 2.6 ± 1.8, 2.0 ± 1.4, 1.8 ± 1.3,1.8 ± 1.1 and 1.6 ± 1.3 cells per 10^4 cells with control isotype-matched IgG1 before administration and at 4 time points of 4, 8, 12, and 24 h after administration, respectively. The peak level was achieved approximately 4 h after administration ($P < 0.001$). However, cells with the CD133 and CD34 markers were not observed due to a shortage of rabbit-specific antibodies for CD133 and CD34.

3.8. rhSDF-1α administration accelerated the reendothelialization of the aneurysm sac

At 2 weeks and 4 weeks after aneurysm induction, scanning electron microscopy showed that more endothelial cells were observed in the aneurysm model in the rhSDF-1α group compared with the control group (2 weeks: 58 vs 17/high power field, $P < 0.05$; 4 weeks: 130 vs 72/high power field, $P < 0.05$) (Fig. 5A and B). At 4 weeks, the aneurysm sac was harvested to determine the differences in the expression of endothelial markers between the two groups. The RT-PCR results showed that the aneurysm sac in the SDF-1α group expressed higher levels of endothelial markers. The $2^{-\Delta\Delta CT}$ amplification times for VE-cadherin, Tie-2, KDR, and E-selectin, respectively, reached 0.16, 0.24, 0.04, and 0.47 in the rhSDF-1α group and 0.09, 0.15, 0.02, and 0.13 in the control group (Fig. 5C).

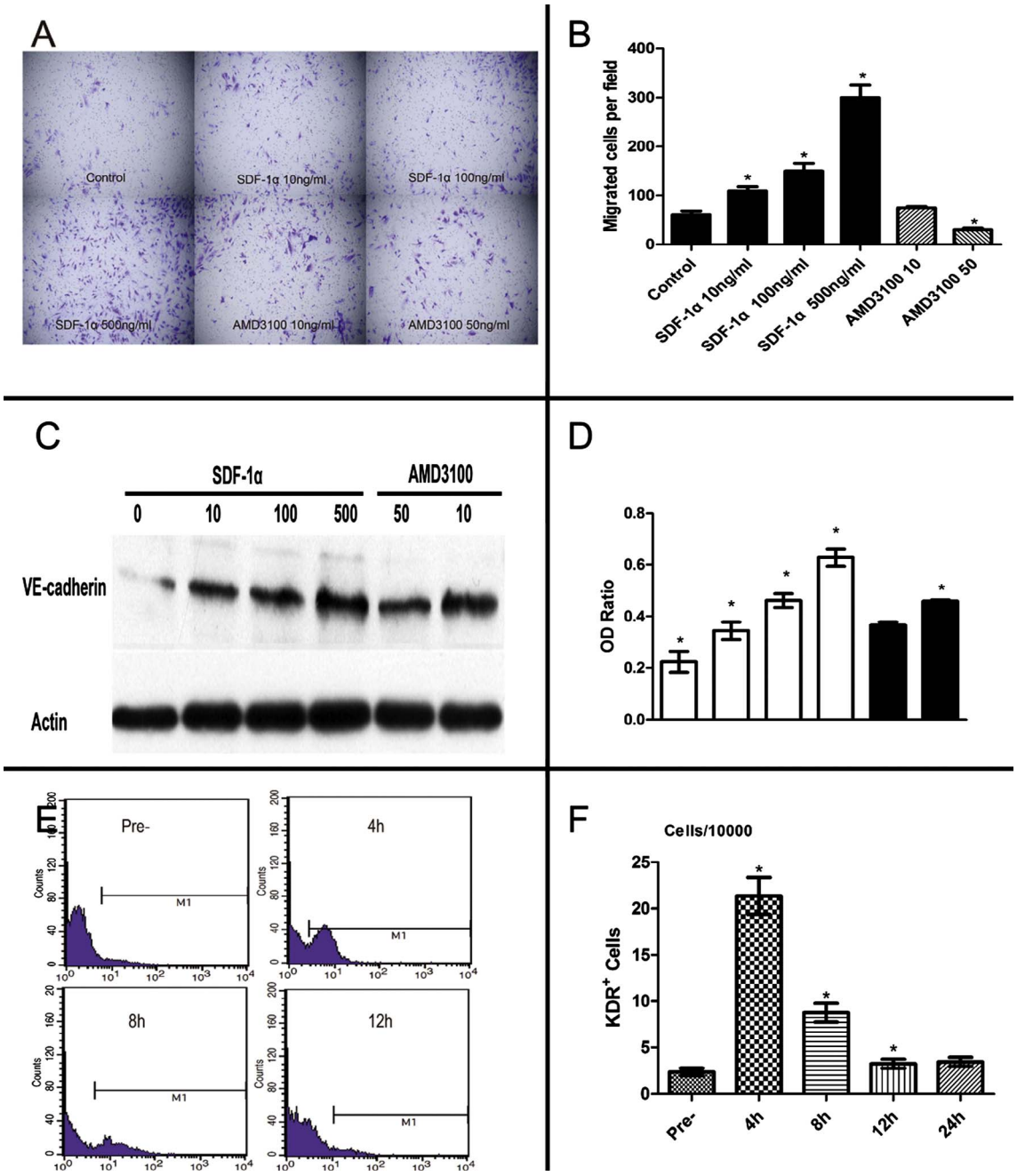

Fig. 4. Migration assay, VE-cadherin expression, and mobilization of endothelial-lineage cells after SDF-1α stimulation. A and B: Migration assay showing an increased migration ability parallel with the SDF-1α concentration gradient, which decreased with AMD3100. C and D: VE-cadherin expression was enhanced as the SDF-1α gradient increased and was blocked by AMD3100. E and F: Flow cytometry showing that the level of cells with the KDR marker in peripheral blood peaked at 4 h and returned to baseline 8, 12, and 24 h after SDF-1α administration *in vivo*.

4. Discussion

The results of the present study demonstrate that SDF-1α expression correlates with the pathophysiological process of aneurysm remodeling and that SDF-1α promotes aneurysm reendothelialization or repair by up-regulating VE-cadherin and mobilizing endothelial-lineage cells into peripheral blood.

SDF-1 expression reflects the inflammation and ischemic status in various tissues. However, its expression pattern in aneurysms under an inflammatory environment is unclear. In this study, SDF-1α expression presented a bimodal expression pattern in saccular aneurysms. SDF-1α was elevated in peripheral blood on day 1 after model induction. These phenomena complied with most previous studies on ischemic or damaged tissue. At the acute stage of a saccular aneurysm, platelets, which release SDF-1α, congregated into a thrombosis, and the damaged endothelial cells and fibroblasts also contributed to the increase in SDF-1α expression.

Interestingly, SDF-1α expression peaked again in the peripheral blood on day 14, presenting a bimodal distribution. Wilson et al. [17] observed that serum SDF-1α levels peaked after 8 h of reperfusion and returned to baseline 24 h after ischemia/perfusion in a murine model. The delayed peak in SDF-1α in plasma can be explained by the progressive enlargement of the aneurysm sac and slow decrease in thrombosis. SDF-1α expression was also detected in the local aneurysm wall on day 14. Up to day 14, the aneurysm sac became significantly larger, resulting in more exposure for the aneurysm sac. At this time,

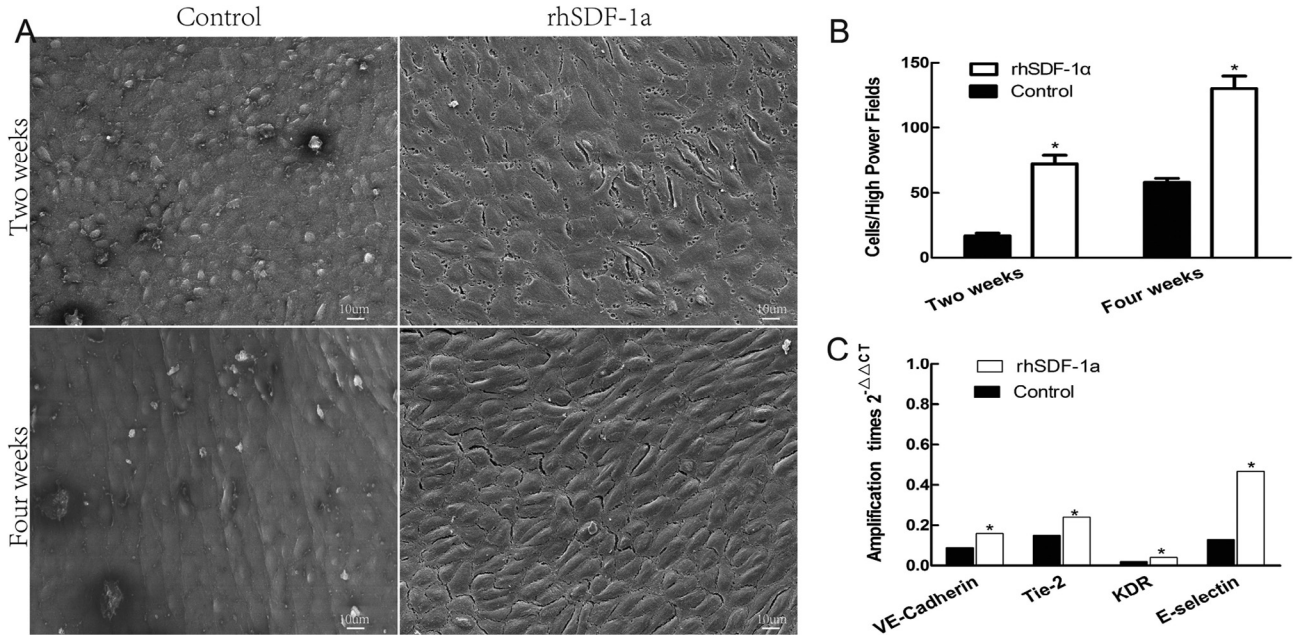

Fig. 5. Effect of SDF-1α on the reendothelialization of the saccular aneurysm wall. A-B: Representative images from scanning electron microscopy showing that endothelial-like cells significantly increased at 2 weeks and 4 weeks in the rhSDF-1α group compared with the control group after SDF-1α administration C: Amplification times showing that the expression of VE-cadherin, Tie-2, KDR, and E-selectin significantly increased at 4 weeks after SDF-1α administration. $^{*}P < 0.05$.

the increase in the number of cells, including injured endothelial cells, fibroblast cells, and platelets, that were exposed on the enlarged sac produced a second SDF-1α expression peak. With the re-endothelialization process of the aneurysm wall, the aneurysm matured, and inflammation in the aneurysm wall also decreased, resulting in a decrease in SDF-1α expression. This expression pattern indicates that the aneurysm size and inflammation status of the aneurysm wall may contribute to the fluctuation in SDF-1α expression.

SDF-1 plays a role in mobilizing progenitor cells and stem cells [10,11]. For example, Hoh et al. [2] reported that SDF-1 promotes endothelial cell and macrophage migration into the aneurysm wall. It remains unclear whether SDF-1α plays a detrimental or beneficial role in aneurysm remodeling. Hiasa et al. [5] found that SDF-1 gene transfer enhanced ischemia-induced vasculogenesis and angiogenesis *in vivo* through a VEGF/eNOS-related pathway. Yamaguchi et al. [18] observed that locally delivered SDF-1 augments vasculogenesis and subsequently contributes to ischemic neovascularization *in vivo* by augmenting EPC recruitment in ischemic tissues. Our previous study also showed that intravenous administration of SDF-1α accelerates aneurysm neck reendothelialization in the rabbit saccular aneurysm after flow diverter treatment [19]. The role of angiogenesis in trafficking stem cells to injured tissues is modulated by the SDF-1/CXCR4 interaction [20,21]. Our study demonstrated that SDF-1α promotes the re-endothelialization of the aneurysm wall and plays a beneficial role in aneurysm remodeling.

The results of our study indicated that multiple mechanisms were responsible for the beneficial role of SDF-1α in aneurysm re-endothelialization. *In vitro*, SDF-1α enhanced EPC migration, and *in vivo*, the number of endothelial-lineage cells increased in peripheral blood after SDF-1α stimulus. This potentially leads to the mobilization of more endothelial-lineage cells and their attachment to the aneurysm wall. Meanwhile, SDF-1α up-regulated VE-cadherin expression. The up-regulation of VE-cadherin in EPCs may facilitate the adherence of SDF-1α to the aneurysm wall. Peled et al. [22] showed that the adhesion of EPCs was mediated by integrin after SDF-1α stimulation under shear stress. This discrepancy implies that versatile mechanisms may be involved under different circumstances.

There were a few limitations to this study. First, the clinical relevance remains unclear because the induced saccular aneurysm in rabbits that developed in the right carotid artery does not resemble clinical aneurysms in humans. Second, the process of endothelization may become sluggish without the support of the inner elastic layer of artery. The dose-independent or time-independent effect also may devote to the endothelization degree. Although SDF-1α contributes to aneurysm wall remodeling, its role in mobilizing EPCs into the blood could not be evaluated due to a shortage of specific antibodies for rabbits and more samples should be used to verify the results. Third, an *in vivo* study using an SDF-1α blocker should be performed to verify its role in the saccular aneurysm remodeling process. However, the SDF-1α blocker AMD3100 has a paradoxical role of promoting the migration of EPCs into peripheral blood and blocking the interactions between EPCs and SDF-1α [23,24]. Fourth, EPC nomenclature remains ambiguous and distinct cell subtypes exist, EPCs was traditionally identified as cells with the phenotype of CD34, CD133, KDR markers. However, these markers were also expressed in hematopoietic progenitors [25].

In conclusion, SDF-1α was dynamically involved in the process of aneurysm wall remodeling. Its expression correlated with the morphological status of the saccular aneurysm and reflected the inflammation status of the aneurysm wall. SDF-1α contributed to aneurysm wall reendothelialization by up-regulating VE-cadherin and promoting endothelial-lineage cell mobilization.

Sources of funding

This study was funded in part by grants from the Chinese National Natural Science Foundation (81171093, 81671137, 81571126 and 81301020).

Disclosure

None.

References

[1] J.L. Brisman, J.K. Song, D.W. Newell, Cerebral aneurysms, N Engl J Med 355 (2006) 928–939.

[2] B.L. Hoh, K. Hosaka, D.P. Downes, K.W. Nowicki, E.N. Wilmer, G.J. Velat, et al., Stromal cell-derived factor-1 promoted angiogenesis and inflammatory cell

infiltration in aneurysm walls, J Neurosurg 120 (2014) 73–86.
[3] J.J. Lataillade, D. Clay, C. Dupuy, S. Rigal, C. Jasmin, P. Bourin, et al., Chemokine SDF-1 enhances circulating CD34(+) cell proliferation in synergy with cytokines: possible role in progenitor survival, Blood 95 (2000) 756–768.
[4] T. Wurster, R. Tegtmeyer, O. Borst, D. Rath, T. Geisler, M. Gawaz, et al., Platelet expression of stromal cell-derived factor-1 is associated with the degree of valvular aortic stenosis, PLoS One 9 (2014) e97405.
[5] R. Nistala, J. Habibi, A. Aroor, J.R. Sowers, M.R. Hayden, A. Meuth, et al., DPP4 inhibition attenuates filtration barrier injury and oxidant stress in the zucker obese rat, Obesity (Silver Spring) 22 (2014) 2172–2179.
[6] M. Ziegler, C. Hughes, The SDF-1/CXCR4 axis utilizes mTOR signal transduction to promote angiogenesis (278.8), FASEB J. 28 (278) (2014) 278.
[7] D.J. Ceradini, A.R. Kulkarni, M.J. Callaghan, O.M. Tepper, N. Bastidas, M.E. Kleinman, et al., Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1, Nat. Med. 10 (2004) 858–864.
[8] Y. Wang, Y. Deng, G.Q. Zhou, SDF-1alpha/CXCR4-mediated migration of systemically transplanted bone marrow stromal cells towards ischemic brain lesion in a rat model, Brain Res. 1195 (2008) 104–112.
[9] C. Hitchon, K. Wong, G. Ma, J. Reed, D. Lyttle, H. El-Gabalawy, Hypoxia-induced production of stromal cell-derived factor 1 (CXCL12) and vascular endothelial growth factor by synovial fibroblasts, Arthritis Rheum 46 (2002) 2587–2597.
[10] M. Mendt, J.E. Cardier, Role of SDF-1 (CXCL12) in regulating hematopoietic stem and progenitor cells traffic into the liver during extramedullary hematopoiesis induced by G-CSF, AMD3100 and PHZ, Cytokine 76 (2015) 214–221.
[11] E.K. Stuermer, A. Lipenksy, O. Thamm, E. Neugebauer, N. Schaefer, P. Fuchs, et al., The role of SDF-1 in homing of human adipose-derived stem cells, Wound Repair Regen 23 (2015) 82–89.
[12] S.P. Lee, S.W. Youn, H.J. Cho, L. Li, T.Y. Kim, H.S. Yook, et al., Integrin-linked kinase, a hypoxia-responsive molecule, controls postnatal vasculogenesis by recruitment of endothelial progenitor cells to ischemic tissue, Circulation 114 (2006) 150–159.
[13] E. De Falco, D. Porcelli, A.R. Torella, S. Straino, M.G. Iachininoto, A. Orlandi, et al., SDF-1 involvement in endothelial phenotype and ischemia-induced recruitment of bone marrow progenitor cells, Blood 104 (2004) 3472–3482.
[14] C. Yang, Z.H. Zhang, Z.J. Li, R.C. Yang, G.Q. Qian, Z.C. Han, Enhancement of neovascularization with cord blood CD133+ cell-derived endothelial progenitor cell transplantation, Thromb Haemost 91 (2004) 1202–1212.
[15] D. Rath, M. Chatterjee, O. Borst, K. Müller, H. Langer, A. Mack, et al., Platelet surface expression of stromal cell–derived factor-1 receptors CXCR4 and CXCR7 is associated with clinical outcomes in patients with coronary artery disease, J. Thromb. Haemost. 13 (2015) 719–728.
[16] Z.F. Li, X.G. Fang, P.F. Yang, Q.H. Huang, W.Y. Zhao, C. Liang, et al., Endothelial progenitor cells contribute to neointima formation in rabbit elastase-induced aneurysm after flow diverter treatment, CNS Neurosci Ther 19 (2013) 352–357.
[17] G. Wilson, H. Nojima, R. Schuster, J. Blanchard, M. Edwards, A. Lentsch, Blockade of CXC chemokine receptor-4 improves hepatocyte proliferation after ischemia/reperfusion in a murine model (144.2), FASEB J. 28 (144) (2014) 142.
[18] J. Yamaguchi, K.F. Kusano, O. Masuo, A. Kawamoto, M. Silver, S. Murasawa, et al., Stromal cell-derived factor-1 effects on ex vivo expanded endothelial progenitor cell recruitment for ischemic neovascularization, Circulation 107 (2003) 1322–1328.
[19] Z. Li, R. Zhao, X. Fang, Q. Huang, J. Liu, Recombinant human SDF-1α administration accelerates aneurysm neck reendothelialization in rabbit saccular aneurysm after flow diverter treatment, Acta Biochim Biophys Sin (Shanghai) (2017), http://dx.doi.org/10.1093/abbs/gmx001.
[20] Y. Won, F. Silva, K. Lim, S. Kim, D. Bull, A. Patel, Cell surface engineering to enhance mesenchymal stem cell migration towards SDF-1 for ischemic tissue, Cytotherapy 16 (2014) S80-S80.
[21] R.K. Hopman, J.F. DiPersio, Advances in stem cell mobilization, Blood Rev 28 (2014) 31–40.
[22] A. Peled, V. Grabovsky, L. Habler, J. Sandbank, F. Arenzana-Seisdedos, I. Petit, et al., The chemokine SDF-1 stimulates integrin-mediated arrest of CD34(+) cells on vascular endothelium under shear flow, J Clin Invest 104 (1999) 1199–1211.
[23] A.F. Cashen, B. Nervi, J. DiPersio, AMD3100: CXCR4 antagonist and rapid stem cell-mobilizing agent, Future Oncol 3 (2007) 19–27.
[24] K. Jujo, M. Ii, H. Sekiguchi, E. Klyachko, S. Misener, T. Tanaka, et al., CXC-chemokine receptor 4 antagonist AMD3100 promotes cardiac functional recovery after ischemia/reperfusion injury via endothelial nitric oxide synthase-dependent mechanism, Circulation 127 (2013) 63–73.
[25] R.J. Medina, C.L. Barber, F. Sabatier, F. Dignat-George, J.M. Melero-Martin, et al., Endothelial Progenitors: A Consensus Statement on Nomenclature, Stem Cells Transl Med (2017), http://dx.doi.org/10.1002/sctm.16-0360.