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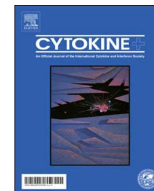

Stromal cell-derived factor 1α facilitates aneurysm remodeling in elastase-induced rabbit saccular aneurysm

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

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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.

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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 intra-aneurysmal 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

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