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ORIGINAL ARTICLE

Expression of chemokine receptor-4 in bone marrow mesenchymal stem cells on experimental rat abdominal aortic aneurysms and the migration of bone marrow mesenchymal stem cells with stromal-derived factor-1

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Abstract This study investigated the expression and role of chemokine receptor-4 (CXCR4) in bone marrow mesenchymal stem cells (BMSCs) from experimental rats with abdominal aortic aneurysms (AAA) for migration of BMSCs. Sprague–Dawley rats were divided into an experimental group and control group ($n = 18$ each). AAA was induced with 0.75 M solution infiltrate for 30 minutes, after which the abdomen was rinsed and closed. Saline was used in place of $CaCl_2$ in the control group. CD34 and CD29 were detected by flow cytometry, the gene and protein expression of CXCR4 were detected by real-time polymerase chain reaction and western blot, respectively. The migration of BMSCs with stromal-derived factor-1 was detected by Transwell chamber. CD34 expression was negative and CD29 expression was positive. The gene and protein expression of CXCR4 were significantly higher in experimental group than them in control group ($p < 0.05$), the migration ability of BMSCs from the experimental group was significantly higher than that from the control group ($p < 0.05$). Stromal-derived factor -1/CXCR4 can enhance the migration of BMSCs *in vitro* in a rat AAA model.

Conflicts of interest: All authors declare no conflicts of interest.

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Introduction

Bone marrow mesenchymal stem cells (BMSCs) are multipotential differentiated stem cells derived from mesoderm [1]. When injected intravenously, BMSCs can preferentially migrate and aggregate to wounded tissue and organs in animals under some signals produced by ischemic microenvironment, which suggests that damaged tissue can attract BMSCs specifically [2]. It is generally believed that expression of some cytokines and chemokines increases in damaged tissue. BMSCs expressing chemokine receptors migrate directionally to the damaged area, and differentiate into mature tissue to promote organ reconstruction under the influence of the local microenvironment [3–5]. In recent years, the biological axis of stromal-derived factor-1 (SDF-1) and its receptor chemokine receptor-4 (CXCR4) has received more attention [6,7].

Turnbull et al. [8] proved that after intravenous injection, BMSCs are detected in vessel wall of abdominal aortic aneurysms (AAA) in swine, suggesting that BMSCs have the potential to repair the vessel wall and avoid further development of AAA. The specific mechanism is still unclear, however, and the role of SDF-1/CXCR4 in AAA has not been reported.

In our study, an experimental AAA model was established in rats [9], the expression of CXCR4 in BMSCs was detected, and the migration of BMSCs with SDF-1 was observed, which provides a basis for BMSC homing mediated by the SDF-1/CXCR4 biological axis in AAA.

Materials and methods

Animals

Thirty-six male Sprague–Dawley rats (200–250 g) were provided by the Experimental Animal Center, School of Medicine, Sun Yat-Sen University (Guangdong, China). Animals were divided into an experimental group and control group ($n = 18$ each). The experimental AAA model was established by $CaCl_2$ infiltration, whereas saline was used in the control group. Animals were kept in a light–dark cycle of 12 hours, and the room was maintained at 23–25°C. All the protocols were approved by the animal care and use committee, and guidelines for the care and use of laboratory animals were based on the policy published by the National Academy Press.

Animal models

Rats were anesthetized by intraperitoneal injection of 3% sodium pentobarbital (30 mg/kg), shearing and disinfected in the supine position. An incision of about 5 cm was made on the abdominal median, then the intestine was pushed to the right side of the abdominal cavity and covered with saline gauze, in order to ensure a clear operative field. About 1 cm of the abdominal aortic below the renal artery and above the iliac artery was dissociated out, and encapsulated by a polyethylene sponge containing 0.75 M $CaCl_2$ solution or saline in the control group; at the same time, the sponge was encapsulated with the same width of sterile rubber strips to protect the surrounding tissue from the calcium salt infiltration. After 30 minutes, the sponge and rubber strips were removed and the peritoneal cavity was washed with saline three times. The incision was sutured layer by layer. Six weeks after the operation, animals were sacrificed and bone marrow was isolated from the bilateral femur and tibia.

Isolation and culture of BMSCs

Mononuclear cells were isolated with 1.077 g/mL Ficoll separation solution (Bio-chromfor) at 400 rpm for 20 minutes, and were washed with phosphate buffered saline (PBS) twice, cells were cultured with Dexter complete medium (Invitrogen) at a density $3 \times 10^5/cm^2$ at 37°C, 5% CO_2 incubator. Nonadherent cells were removed after 72 hours, and medium was changed twice weekly.

Flow cytometry

BMSCs (0.5×10^6) were collected and washed with fluorescence-activated cell sorting (FACS) solution (2% bovine serum albumin + PBS), and were incubated with 10 μL monoclonal antibodies CD34-PE or CD29-PE in 500 μL FACS solution at room temperature for 20 minutes. At the same time, a blank control and negative control was set (10 μL IgG-PE). Then BMSCs were washed twice with PBS, and the cells were detected by flow cytometry (BD Biosciences, San Jose, CA, USA).

Real-time polymerase chain reaction

After isolation of total RNA with Trizol (Invitrogen) from BMSCs in two groups, cDNA was synthesized by reverse transcription of total RNA. The upper primer of CXCR4 gene was 5′-GCCTGAGCTACAGATGCCCA-3′, and the antisense primer was 5′-TTCGGGTCAATGCACTTGT-3′. The glyceraldehyde phosphate dehydrogenase gene was used as an internal reference and the primers were: 5′-GAAGGTGAAGGTCGG AGTC-3′ and 5′-GAAGATGGTGATGGGATTTC-3′. The real-time polymerase chain reaction (RT-PCR) was performed on an ABI Prism 7700 PCR instrument (Applera Corporation Norwalk). The amplification conditions were: 95°C for 5 minutes; 35 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute; and a terminal extension step at 72°C for 10 minutes. After the reaction, agarose gel electrophoresis was performed.

Western blot

Protein was extracted from BMSCs. The protein concentration was measured by the Brandford method. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed, then polyvinylidene difluoride membrane was blocked with 5% defatted milk, then incubated with mouse anti-CXCR4 monoclonal antibody (1:1000) at 4°C overnight and horseradish peroxidase labeled goat anti-mouse immunoglobulin (Ig)G (1:5000) at 37°C for 45 minutes, followed by enhanced chemoluminescence development.