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Mesenchymal stem cells participating in ex vivo endothelium repair and its effect on vascular smooth muscle cells growth

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Abstract

Background: Previous studies have shown that mesenchymal stem cells (MSCs) transplantation can promote neovascularization and regenerate damaged myocardium. However, it remains unknown whether MSCs seeding can be used to repair injured cellular components in vascular diseases. In this study we explored the feasibility of applying MSCs to endothelium repair in endothelial damage and vasoproliferative disorders.

Methods: Ex vivo model of endothelium repair was developed in which rabbit vascular smooth muscle cells (SMCs) were inoculated into the upper chamber and rabbit endothelial cells (ECs)/human MSCs into the lower chamber of a co-culture system. ^{3}H-TdR incorporation and PCNA protein expression were assayed and migrated number of SMCs was calculated to evaluate the effect of MSCs seeding on SMCs growth. Flk-1 and vWF protein expressions were observed to analyze the plasticity of the seeded MSCs along endothelial lineage.

Results: In this co-culture system, no vWF protein but Flk-1 protein was observed in the 25.71% of MSCs after having been co-cultured with mature rabbit ECs for 5 days. Compared with the control group, the proliferation and migration of SMCs was significantly increased by proliferative ECs but decreased by confluent ECs ($n=6$, $P<0.01$). MSCs seeding decreased the proliferation and migration of SMCs compatible with the effect of proliferative ECs ($n=6$, $P<0.001$). However, no inhibition on SMCs growth was observed with MSCs seeding in comparison to the effect of confluent ECs.

Conclusions: MSCs seeding can inhibit the proliferation and migration of SMCs. MSCs co-cultured with mature ECs have the ability to undergo milieu-dependent differentiation toward ECs.

Keywords: Endothelium; Repair; Stem cells; Cell therapy

1. Introduction

Vascular endothelial cells (ECs) line the intima of the vessel wall and may be easily damaged by various metabolic conditions or mechanical manipulations. Endothelium damage is often regarded to be associated with the proliferation and migration of vascular smooth muscle cells (SMCs), which ultimately results in some vasoproliferative disorders, such as atherosclerosis and restenosis [1,2].

Accelerating intact endothelium repair with stem cell transplantation is a novel way in restenosis prevention. Marrow stromal cells had been originally identified because of their critical roles in the formation of the hematopoietic microenvironment (HME) [3]. They later came to the center stage with the growing recognition of the mesenchymal stem cells (MSCs), a subpopulation of rapidly self-renewing adult stem cells with unexpected differentiation potential [4–6]. MSCs may be a good candidate for tissue repair. Although several studies have shown that MSCs transplantation can promote neovascularization and regenerate damaged myocardium [7], it remains unknown whether MSCs seeding can be used to repair injured cellular components in vascular diseases.

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Accumulating evidence indicates that the peak of ECs regeneration parallels the peak of SMCs proliferation and the growth stimulating cytokines secreted by proliferative ECs are also up-regulated [8,9]. Therefore, we hypothesized that the growth status of ECs influence the proliferative features of SMCs and MSCs seeding may be effective in arterial healing by modulating the proliferation and migration of SMCs. To investigate the feasibility of applying MSCs to endothelium repair in ECs damage and vaso-proliferative disorders, an ex vivo model of endothelium repair in a co-culture system was developed. The effect of MSCs seeding on the proliferation and migration of SMCs as well as the plasticity of the seeded MSCs toward endothelial lineage were investigated.

2. Methods

2.1. Cell culture

2.1.1. Animals

New Zealand white rabbits were purchased from the Experimental Animal Center of Xin Qiao Hospital. All animals received humane care and the animal protocols comply with the institution's guidelines.

2.1.2. Rabbit ECs primary culture

ECs were enzymatically isolated from the rabbit aorta and cultured as previously described [10]. The cells of passage 3–5 were used for experiments. More than 95% cells were positive by immunohistochemical staining with the antibody against vWF-related antigen.

2.1.3. Rabbit SMCs primary culture

SMCs were obtained from the rabbit aorta by the explant method [11]. The cells of passage 3–5 were used for experiments. The cells cultured by this method contained at least 95% SMCs, which were confirmed by positive staining with the antibody against α-SM-actin.

2.1.4. Isolation and culture of human MSCs [5]

MSCs were isolated from bone marrows of volunteers. Informed consent was obtained from each volunteer and the study protocol conforms to the ethical guidelines of Declaration of Helsinki. Nucleated cells were isolated with a density gradient (Ficoll-Paque,1.077, Pharmacia, USA), and re-suspended in 20 ml Dulbecco's Modified Eagle medium (DMEM, Hyclone, Logan, UT USA) with the supplements of 10% fetal bovine serum (FBS, Hyclone), 100 U/ml penicillin, and 100 μg/ml streptomycin. All of the cells were plated in the plastic culture dish and incubated at 37 °C in a humidified atmosphere with 5% CO_2. Forty-eight hours later, non-adherent cells were discarded and the adherent cells were further incubated for 5–7 days. The cells were subcultured, harvested, and re-plated at about 3 cells/cm^2 in 6-well plates (Corning, New York, USA). Then, the single-cell-derived colonies were further expanded and used for the experiments.

2.2. Fluorescence-activated cell sorter (FACS) analysis

Using flow cytometry, the surface markers of MSCs were evaluated based on the expression of CD105, CD166, CD34, Flk-1 and vWF. The cells were stained, respectively with primary antibodies (mouse antihuman CD105, CD166 and CD34, Pharmingen, San Diego, USA; rabbit antihuman Flk-1 1:50, vWF 1:100, Santa Cruz, California, USA). Negative control was incubated in 0.01 mol/L phosphate buffered saline (PBS). The cells were subsequently reacted with a FITC conjugated goat anti mouse or rabbit secondary antibody (Zymed, South San Francisco, USA). The stained cells were then fixed with 1% paraformaldehyde and immediately analyzed with flow cytometry (FACSCalibur, Becton Dickinson, USA).

2.3. ECs conditioned medium

Rabbit ECs were cultured in DMEM supplemented with 10% FBS (Hyclone) in 6-well plates. When they grew to about 60% confluence, the medium was changed with fresh serum-free DMEM. After the cells were incubated for another 24 h, the conditioned medium was collected, centrifuged at 15,000 $\times g$ for 3 min, and filtered through 0.22 μm filters.

2.4. Fluorescence immunocytochemistry

To investigate the role of vascular endothelial growth factor (VEGF) or ECs conditioned medium in MSCs differentiation, human MSCs were inoculated onto the coverlips. When the MSCs grew to about 60% confluence, the medium was changed with DMEM containing 50 ng/ml VEGF (Sigma, St. Louis, MO, USA) or with ECs conditioned medium. To evaluate the milieu-dependent plasticity of MSCs, rabbit ECs were inoculated onto the coverlips. When the ECs grew to about 60% confluence, MSCs were seeded onto the coverlips with ECs. Six hours later, the non-adherent MSCs were discarded and the adherent cells were further cultured in fresh DMEM with different concentrations of neutralizing anti-VEGF antibody (0, 50, 100, and 500 ng/ml, R&D Systems, Minneapolis, MN, USA). MSCs were either cultured in DMEM with VEGF, or in ECs conditioned medium, or co-cultured with mature ECs for 5 days. Then, the coverlips were taken out. The cells were fixed in 4% paraformaldehyde for 5 min at room temperature and then incubated with the primary antibody (rabbit antihuman Flk-1 1:50, vWF 1:100, Santa Cruz; mouse antihuman CD105 and CD166, 1:100, Pharmingen) overnight at 4 °C. Negative control was incubated with 0.01 mol/L PBS in the same manner. Subsequently, the samples were stained with goat anti rabbit or mouse secondary antibody (1:100, Zymed) for 30 min at