Co-culture of Mesenchymal Stem Cells with Umbilical Vein Endothelial Cells under Hypoxic Condition

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Summary: By co-culturing humm mesenchymal stem cells (hMSCs) and human umbilical vein endothelial cells (HUVECs) under hypoxia and creating a microenvironment similar to that of transplanted hMSCs for the treatment of avascular femoral head necrosis (ANFH), the effect of hMSCs on survival, apoptosis, migration and angiogenesis of human umbilical vein endothelial cells (HUVECs) under the hypoxic condition were investigated in vitro. hMSCs and HUVECs were cultured and identified in vitro. Three kinds of conditioned media, CdM-CdM NOR, CdM-CdM HYP and HUVEC-CdM HYP were prepared. HUVECs were cultured with these conditioned media under hypoxia. The survival rate, apoptosis rate, migration and angiogenesis of HUVECs were respectively detected by CCK-8, flow cytometry, Transwell and tube formation assay. The content of SDF-1α, VEGF and IL-6 in CdM was determined by ELISA. Our results showed that hMSCs and HUVECs were cultured and identified successfully. Compared with MSC-CdM NOR and HUVEC-CdM HYP groups, the survival rate, migration and angiogenesis of HUVECs in MSC-CdM HYP group were significantly increased while the apoptosis rate was declined (P<0.05). Moreover, the expression of SDF-1α, VEGF and IL-6 in MSC-CdM HYP group was up-regulated. Under hypoxia, the apoptosis of HUVECs was inhibited while survival, migration and angiogenesis were improved by co-culture of hMSCs and HUVECs. The underlying mechanism may be that hMSCs could secrete a number of cytokines and improve niche, which might be helpful in the treatment of femoral head necrosis.

Key words: hypoxia; mesenchymal stem cells; umbilical vein endothelial cells; co-culture; femoral head necrosis

Avascular necrosis of the femoral head (ANFH) is a common condition characterized by necrosis of bone trabeculae and bone marrow. It is mainly caused by the destruction of the blood supply into the femoral head, and the incidence of disability due to the disease is high. Many treatment strategies have been used, including conservative treatment[1-3], such as drugs, electric stimulus, shock-wave and electromagnetic field therapy and surgical treatment[4-6], such as core compression, rotary osteotomy and vascularized bone grafting, but neither of them has definitive effect. Recently, stem cell-based therapies offer promise for the treatment of ANFH. Mesenchymal stem cells (MSCs) from bone marrow are on the leading edge because they are easy to expand in culture while maintaining their multi-lineage potential[7, 8]. A number of studies[9, 10] have demonstrated that the implanted MSCs can not only survive but proliferate in the necrotic femoral head after transplantation, indicating that the in vitro expanded bone-marrow stem cells can serve as a graft material to enhance bone repair and to treat osteonecrosis through their differentiation into osteoblasts and endothelial cells. However, the role of the transplanted MSCs remains poorly understood. In recent years, some studies[11, 12] revealed that, stem cells, particularly those derived from bone marrow, promote tissue repair by secreting factors that enhance regeneration of injured cells, stimulate proliferation and differentiation of endogenous stem-like progenitors found in most tissues. Animal experiments[13, 14] also demonstrated that under hypoxic environment, implanted stem cells can promote neovascularization that effectively increases the blood perfusion into ischemic tissues, and thus stop further necrosis of tissues. Therefore, the ability of such cells to alter the tissue microenvironment may contribute more significantly than their capacity of trans-differentiation to tissue-repair-enhancing effect.

For these reasons, in this study, in vitro indirect co-culture was employed to assess the interaction between MSCs and human umbilical vein endothelial cells (HUVECs) to examine the effects of conditioned medium (CdM) from human MSCs on HUVECs exposed to hypoxia.

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*This project was supported by a grant from the National Natural Sciences Foundation of China (No. 30750010).
1 MATERIALS AND METHODS

1.1 Isolation, Culture and Identification of Human MSCs

Bone marrow aspirates were obtained from the healthy adult human bone marrow donors and the study had been approved by the Ethical Committee of Health Science Center of Union Hospital, Wuhan, China. The sample was gently layered onto Percoll separating liquid (density: 1.13 g/mL) (Pharmacia, USA) and centrifuged at 1500 rpm for 15 min at 4°C. The low-density hMSC-rich layer was collected and re-suspended in stem cell growth medium (SGM). SGM was composed of low glucose Dulbecco’s Modified Eagle’s medium (DMEM-L) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA), 100 U/mL penicillin-streptomycin (Gibco-BRL, USA), and 2 mmol/L L-glutamine (Gibco-BRL, USA). Cells were seeded on T75 culture flasks at a density of 2×10^6/cm^2 and incubated in EGM for 24 hours. The medium was replaced with EBM sf and incubated under hypoxic condition, to prepare CdM for MSCs incubated in hypoxic condition. After 48 hours, the medium was harvested as HUVEC-CdM®. During this study, a tri-gas incubator (ThermoForma, USA), set at 37°C, 1% O2, 5% CO2, 94% N2, provided the hypoxic condition. A general incubator set at 37°C, 21% O2, 5% CO2 afforded the normoxic condition.

1.4 Assays for Cell Survival and Apoptosis

HUVECs were plated into a 96-well plate at a density of 2×10^5/well. The cells were randomly sorted into three groups according to the kind of medium. After adherence of cells to the plate, medium was, respectively, replaced by MSC-CdM®®, MSC-CdM® and HUVEC-CdM®. Then they were incubated in the tri-gas incubator. At five checkpoints (t=12, 24, 48, 72 h), Cell counting kit-8 (Dojindo Molecular Technologies, Japan) was used to assay the cell survival according to the kit instructions.

To detect the effect of CdM on HUVECs under hypoxia, HUVECs were plated into a 6-well plate at a density of 1×10^5/well. After adherence of the cells to the plate, medium was, respectively, replaced by MSC-CdM®®, MSC-CdM® and HUVEC-CdM®. Then they were incubated in the tri-gas incubator for 12, 24, 48, and 72 h. The cells in each group and at each time point were harvested and transferred to Eppendorf tubes. By following the instructions of Annexin V FITC apoptosis detection kit (BD Pharmingen™, USA), apoptosis rate of HUVECs was detected by flow cytometry.

1.5 Assessment of Cells Migration by Transwell

Transwell (Corning, USA) inserts with an 8-µm pore size were coated with 0.1% gelatin and allowed to air dry. HUVECs were suspended in EBM, and 100 µL of cell suspension (2×10^5 cells/mL) were added to transwells in triplicate, while 900 µL of medium was added to the wells. After 24 h of incubation in the tri-gas incubator, HUVECs that migrated to the underside of the membrane were fixed, stained in 0.1% crystal violet (Sigma-Aldrich, USA), and sealed on slides. Light microscope was used to count the number of cells on the underside of the insert for each condition. Six fields are evaluated per replicate well to estimate the mean number of cells for each replicate. The mean number of cells for each condition is then estimated by calculating the mean of the three mean replicates. Representative stained filters were photographed as a supplementary record of migration.

1.6 Endothelial Tube Formation Assay

Growth factor reduced matrigel (GFR-matrigel) (BD Biosciences, USA) was diluted by EBM® at a ratio of 1:2. The 96-well plate was evenly coated with diluted GFR-matrigel at 50 µL/well and then was placed at 37°C overnight to allow gel formation. HUVECs were, respectively, suspended in the following media: MSC-CdM®®, MSC-CdM® and HUVEC-CdM®. Then they were added to the 96-well plate coated with GFR-matrigel in triplicate. Each cell group was incubated under hypoxic condition for 48 h. Endothelial cells and tubes were examined by using a light microscope. Several images of each well were acquired, processed and then analyzed by employing the Image Pro Plus software package.

1.7 ELISA of Cytokines

After exposing hMSCs and HUVECs to either hypoxic or control condition for 48 h, CdM was collected, centrifuged at 1500 rpm at 4°C for 10 min, re-collected,