IJAE
Vol. 116, n. 1 (Supplement): 109, 2011
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Bone marrow-derived mesenchymal stem cells from early diffuse systemic sclerosis stimulate in vitro angiogenesis and foster revascularization of ischemic limbs

Mirko Manetti[1], Anna Franca Milia[2], Serena Guiducci[2], Eloisa Romano[2], Benedetta Mazzanti[3], Claudia Ceccarelli[2], Riccardo Saccardi[3], Marco Matucci-Cerinic[2], Lidia Ibba-Manneschi[1]

[1] Department of Anatomy, Histology and Forensic Medicine, [2] Department of Biomedicine, Division of Rheumatology, [3]Department of Hematology, University of Florence, Florence, Italy

Systemic sclerosis (SSc) is a chronic connective tissue disorder characterized by widespread microangiopathy, lack of angiogenesis, and fibrosis. Bone marrow-derived mesenchymal stem cells (MSCs) produce and release high amounts of proangiogenic and antiapoptotic factors that inhibit apoptosis of endothelial cells under hypoxic conditions and promote the formation of capillary-like structures in vitro. In the present study, we characterized MSCs from SSc patients for the expression of factors implicated in MSC recruitment at sites of injury, angiogenesis, and fibrosis. We also analyzed whether SSc-MSCs could modulate dermal microvascular endothelial cell (MVEC) angiogenesis in vitro. MSCs were obtained from 5 patients with early diffuse SSc and 5 healthy donors. MSCs were expanded in culture and their phenotype was investigated by flow cytometry, CFU-F assay, and analysis of osteogenic/adipogenic differentiation. MSCs were stimulated with VEGF, TGFβ, or SDF-1. Transcript and protein levels of SDF-1 and its receptor CXCR4, VEGF, TGFβ1 and receptors TβRI/TβRII were evaluated by real-time RT-PCR, Western blotting, and confocal microscopy. VEGF, SDF-1, and TGFβ1 secretion in culture supernatant was measured by ELISA. MVEC capillary morphogenesis was performed on Matrigel with the addition of MSC-conditioned medium. SSc- and healthy-MSCs showed no significant differences in morphology, proliferation rate, CFU-F colony formation or osteogenic/adipogenic differentiation. In SSc-MSCs, the basal expression of proangiogenic SDF-1/CXCR4 and VEGF was significantly increased compared to healthy-MSCs. SSc-MSCs constitutively released higher levels of SDF-1 and VEGF. SDF-1/CXCR4 were upregulated after VEGF stimulation, and CXCR4 redistributed from cytoplasm to cell surface. VEGF was increased by SDF-1 challenge. VEGF, TGFβ and SDF-1 stimulation upregulated TGFβ1, TβRI, and TβRII in SSc-MSCs. SSc-MSC-conditioned medium showed a greater proangiogenic effect on MVECs than healthy-MSCs. Experiments with blocking antibodies demonstrated that MSC-derived cytokines were responsible for this potent proangiogenic effect. One patient who developed gangrene of the upper and lower limbs was treated with 3 intravenous infusions of expanded autologous MSCs. Areas of necrotic skin were reduced after the first MSC infusion. After the third infusion, angiography showed revascularization of the patient's extremities. Skin section analysis revealed cell clusters with tube-like structures, and angiogenic factors were strongly expressed. SSc-MSCs constitutively overexpress and release proangiogenic factors, and potentiate dermal MVEC angiogenesis in vitro. In SSc patients with severe peripheral ischemia, intravenous infusion of expanded autologous MSCs may foster the recovery of the vascular network.

Keywords: Mesenchymal stem cells, angiogenesis, systemic sclerosis, stem cell therapy