RESULTS IN SECRETION OF TROPHIC, BIOLOGICALLY ACTIVE, GROWTH FACTORS

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Introduction. Human mesenchymal stromal cells (hMSCs) are an interesting cell source for tissue engineering (TE) applications and cell therapies, both for their ability to differentiate into various cell types and for their secretome, which has immunosuppressive, anti-apoptotic and pro-angiogenic effects. For bone TE, the direct differentiation of hMSCs into osteoblasts is generally believed to account for new bone formation, but secreted growth factors could also act as chemokines for host cells and attract cells that contribute to bone formation but also to the vasculature. Here, we treated hMSCs with the small molecule db-cAMP, which enhances bone formation, to adjust growth factor secretion for bone TE, and investigated the effect of secreted factors.

Materials and Methods. Conditioned medium (CM) was prepared by culturing hMSCs with or without 1 mM db-cAMP for 3 days after which fresh medium was added for 2 more days. To test the biological activity of growth factors in cAMP-CM, various cell types were cultured in either non-CM, basic-CM or cAMP-CM, after which proliferation and gene expression were analyzed.

Results. ELISAs demonstrated an increase in bone-specific growth factors in cAMP-CM; Il-8, Il-11, BMP-2 and IGF-1. Culture of MG-63s, C2C12s and HUVECs in cAMP-CM resulted in increased proliferation as compared to non-CM and basic-CM, whereas proliferation of primary hMSCs was not affected. cAMP-CM also increased differentiation of C2C12s, as demonstrated by an increase in ALP expression. Primary hMSCs demonstrated an enhanced osteogenic gene profile in cAMP-CM after 6 hours, but gene expression was reduced to basal level after 72 hours. We found paracrine signaling via BMPs, but no mitogenic effect of IGF-1. A currently performed microarray should reveal additional mechanisms.

Conclusions. We conclude that treatment with db-cAMP results in increased secretion of specific trophic factors that are biologically active and influence both proliferation and differentiation of various cell types.

Keywords. Mesenchymal stromal cells, bone tissue engineering, trophic effect

(24.O2) THE NEOVASCULARIZATION EFFECT OF BONE MARROW STROMAL CELLS IN TEMPORAL MUSCLE AFTER ENCEPHALOMYOSYNANGIOSIS

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Objective. Patients with Moyamoya disease are in a state of chronic cerebral ischemia,and the primary goal of treatment is to improve collateral circulation through angiogenesis. During angiogenesis, the expression of vascular endothelial growth factor (VEGF) plays the most important role. In the present study, we obtained and sub-cultured bone marrow stromal cells (BMSCs) from rats and injected the labeled BMSCs directly into adjacent temporal muscle during encephalomyosynangiosis (EMS).

Materials and Methods. We divided 20 rats into a BMSCs transplantation group (n=12) and a control group (n=8). Seven days after induction of chronic cerebral ischemia, an EMS operation was performed on the right side, and labeled BMSCs (1X106/100 μl) were injected in the temporal muscle of the right side for the transplantation group, while an equivalent amount of culture solution was injected directly into the right side for the control group. Three weeks after transplantation, temporal muscle and brain tissue were collected for histological examination and western blot analysis.

Results. The capillary/muscle ratio in the temporal muscle was increased in the BMSC transplantation group compared to the control group, showing a greater increase of angiogenesis ($P<0.05$). The injected BMSCs in the temporal muscle were VEGF-positive by immunofluorescence staining. In both temporal muscle and brain tissue, the expression of VEGF by western blot analysis was not much different between the BMSC transplantation group and the control group.

Conclusions. After EMS in a chronic cerebral ischemia rat model, the injection of BMSCs resulted in accelerated angiogenesis in the temporal muscle compared to the control group. The results of this study may be applicable for enhancing revascularization in Moyamoya disease through further study.

Keywords. Bone marrow stromal cell, chronic cerebral ischemia, angiogenesis, Vascular endothelial growth factor

(24.O3) EXPANSION OF ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS MAINTAINING THEIR STEMNESS

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Mesenchymal stem cells (MSCs) are capable of self-renewing and have the multilineage differentiation potential that gives rise to the cells of three germ layers. It is reported that the stemness of MSCs reduces as they are expanded in vitro. Recently, some researches demonstrated that their stemness was maintained in commercial media or antioxidant-supplemented media.

In this study, we tried to find out appropriate culture media for the expansion of MSCs by performing cell culture with three experimental groups: (1) general media, (2) general media with antioxidants, (3) commercial media. The stemness was assessed by analysing cell proliferation, colony-forming activity, osteogenesis, chondrogenesis, adipogenesis, neurogenesis at 5th and 10th passage. As results, MSCs in antioxidant-added group had similar activities to commercial media, but those in general media did not. Therefore, it is strongly supposed that antioxidants play a crucial role in maintaining their stemness.

Keywords. Mesenchymal stem cell, Expansion, Stemness