Differentiation of isolated porcine mesenchymal bone marrow stem cells into endothelial-like cells by application of endothelial growth factor

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Abstract

**Question:**
This study was induced to investigate the possibility of reprogramming mesenchymal bone marrow stem cells (BMSCs) into endothelial cells by application of growth factors into culture media.

**Methods:**
Isolation of BMSCs were performed by bone marrow aspiration of the mini-pig femur. Bone marrow was filtered with a 100µm cell strainer to eliminate bone remains and tissue fragments. After centrifugation pellet was dissolved in PBS-EDTA solution and the mononuclear cells were isolated by density gradient centrifugation with histopaque. Additionally, cells were washed with media and centrifuged further for 5min. The pellet was solved in medium containing endothelial cell growth factor supplement (10%). Medium was changed every second day. Confluent cells were analysed for the expression of eNOS by immunofluorescence staining and Western blot. Furthermore, the functionality of cells to release NO was examined by spectrophotometric investigation.

**Results:**
Isolated BMSCs cultured with endothelial cell growth factor exhibited a typical cobblestone-like endothelial cell phenotype. Immunofluorescence staining and Western blot analysis with eNOS antibody showed eNOS expression in the same manner than venous endothelial cells. Investigations of the NO release exhibited also the functionality of endothelial-like BMSCs to liberate NO, which is an essential feature for the functionality of endothelial cells.

**Conclusion:**
The reprogramming of isolated pig BMSCs into endothelial-like cells with endothelial characteristics was possible by adding of endothelial cell growth factor. In future studies BMSCs could be a source for production functional endothelial cells.