

ENDOTHELIAL CELLS MODULATE BONE MARROW STROMAL CELL DIFFERENTIATION INTO OSTEOBLASTS: DEPENDENCY ON ENDOTHELIAL CELL MATURATION

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INTRODUCTION: Osteoblast precursor cells, which are present within the heterogeneous bone marrow stromal cell population, differentiate into mature osteoblasts in a tightly regulated process. It is known that endothelial cells communicate with osteoblast precursors and also with mature osteoblast cells. However, the interaction and the role of endothelial cells on the differentiation process leading to mature osteoblasts are not well understood. Vascular endothelial growth factor (VEGF) is a major factor in vasculogenesis and angiogenesis and is a known endothelial cell mitogen that might be important for endothelial cell maturation. We therefore investigated the effect of human umbilical vein endothelial cells (HUVEC cell line) on human bone marrow stromal cell (BMSC) differentiation towards the expression of the osteogenic phenotype.

METHODS: Two types of co-culture systems were used: indirect contact (providing a 2-way communication system) and EC-conditioned medium (providing a 1-way communication system from HUVEC to BMSC). The cultures were grown with and without the addition of Dexamethasone, a known inducer of osteogenesis *in vitro*. In addition, endothelial cells were stimulated with VEGF (a known EC mitogen and suggested to be an important factor for EC maturation) before being used in the co-culture systems. Using the quantitative real-time-RT-PCR technique, we measured at different culture times mRNA levels of representative genes expressed at various stages during osteoblastic differentiation such as osteopontin, bone sialoprotein II, osteonectin, osteocalcin, collagen I, MMP-13, BMP-2 and cbfa1. Cell proliferation (Hoechst 33258), matrix mineralization (Ca45 isotope incorporation), alkaline phosphatase activity (colorimetric assay) and VEGF levels (ELISA) in the medium were also quantified.

RESULTS: As expected, BMSC cultures stimulated with the steroid dexamethasone differentiate towards the expression of the osteoblastic phenotype, measured by an increased matrix mineralization, an elevated ALP activity and by the expression of specific osteoblastic markers, especially osteopontin, bone sialoprotein II and BMP-2. This differentiation process was delayed independently of the co-culture system used. Furthermore, when HUVEC were stimulated with VEGF before co-culture, the inhibition of osteoblastogenesis was even greater. BMSC proliferation was increased in the presence of HUVEC and even further increased in the presence of VEGF-

stimulated HUVEC. Also this effect was independent of the co-culture type.

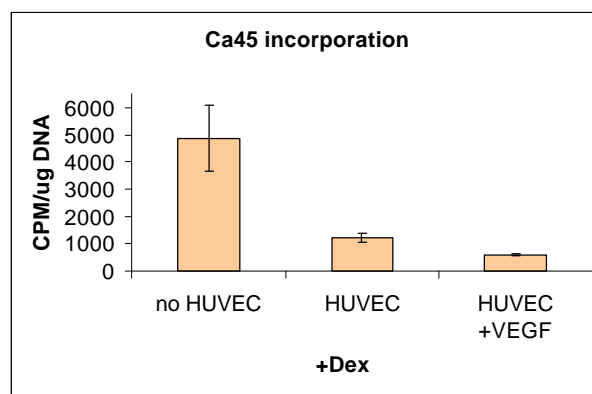


Figure 1: Matrix mineralization by BMSC at day 12

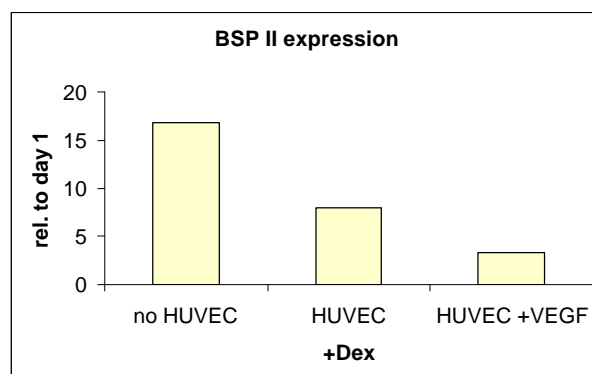


Figure 2: Bone Sialoprotein II expression by BMSC at day 12

DISCUSSION & CONCLUSIONS: These results support the thesis that EC may potentially affect bone precursor cell proliferation and the rate at which BMSC differentiate into osteoblasts. The effect of VEGF suggests that this modulation is dependent on the maturational stage of the endothelial cells.

ACKNOWLEDGEMENTS: We would like to thank Dr. Claude Jaquiere and Dr. Ivan Martin for bone marrow stromal cell supply.