

Effects of culture conditions on the proliferation and differentiation capacities of human fetal bone cells

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INTRODUCTION: Human primary fetal bone cells (hFBC) display a high capacity of replicating, differentiating and forming bone tissue. Even though growth factors that regulate bone formation and osteoblastogenesis have been identified, the environmental factors influencing the rapid growth rate and the responsiveness to differentiation of human fetal bone cells have not been characterized to date. In this study, we aimed to optimize environmental conditions for proliferation and differentiation of bone fetal cells.

METHODS: hFBC, obtained from our dedicated, consistent banks of bone cells comprising several fetal donors (from 12 to 16 week gestation), were studied for their ability to proliferate and differentiate into mature osteoblasts *in vitro*.

RESULTS: Proliferative capacities of fetal cells were assessed with three defined growth conditions.

hFBC proliferated more rapidly in MEM alpha medium, compared to other media (0.5×10^6 cells in DMEM; 0.5×10^6 cells in F-12 Ham's/DMEM and 1.0×10^6 cells in MEM alpha after 19 days of proliferation). Then, we were interested in growth factors that could stimulate cell proliferation rate. Platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF) had positive effects on the cell growth of hFBC regardless of media.

Differentiation state was measured by alkaline phosphatase (ALP) enzymatic activity. We demonstrated a three-fold increase of ALP activity induced by a complete osteogenic mix prepared in one of the three media (DMEM, F-12 Ham's DMEM or MEM alpha) containing dexamethasone. Wnt3a, a secreted lipid-modified signaling protein involved in the development of mesenchymal stem cells into osteoprogenitors, was observed to induce an

enhancement of cell proliferation, whereas it reduced ALP activity. Finally Wnt5a protein, a non-canonical Wnt, was shown to enhance ALP activity in all media tested, suggesting that this protein act as a regulatory factor for fetal bone cell differentiation *in vitro*.

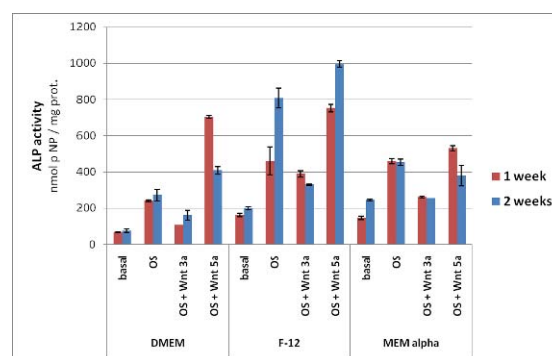


Fig.5. Effect of growth factors Wnt3a and Wnt5a on the ALP activity in bone fetal cells. Cells were initially seeded at 6×10^3 cells/cm². During 15 days, cells were cultured in one of the three media to promote growth before cells begin to differentiate. Cells were then switched to osteogenic (OS) media (+ dex 100nM) with Wnt3a or Wnt5a and harvested at indicated time. As observed for three media, Wnt3a had an inhibitory effect on ALP activity, whereas Wnt5a induced cells to stimulate ALP activity already after one week.

DISCUSSION & CONCLUSIONS: The association of appropriate culture medium with a selection of growth factors could be interesting for human fetal bone cell proliferation and differentiation *in vitro*.

REFERENCES: 1. Montjovent MO, Bone 2004; 2. Montjovent MO, Bone 2008; 3. Hohlfeld J, Lancet 2005, 4. Hirt-Burri N, Pediatr. Surg. Int. 2008.