

HUMAN DENTAL FOLLICLE CELLS ACQUIRE CEMENTOBLAST FEATURES UNDER BMP-2/-7 AND ENAMEL MATRIX DERIVATIVES (EMD) STIMULATION IN VITRO.

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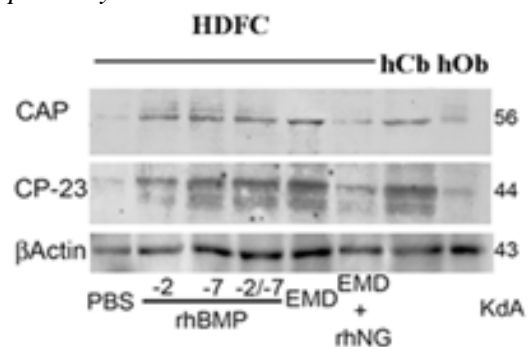
INTRODUCTION: The dental follicle (DF) surrounding the developing tooth germ is an ectomesenchymal tissue composed of various cell populations derived from the cranial neural crest. It is believed that human dental follicle cells (HDFC) contain precursor cells for cementoblasts, periodontal ligament cells, and osteoblasts¹. Bone morphogenetic proteins (BMPs) produced by Hertwig's epithelial root sheath or present in enamel matrix derivatives (EMD)² seem to be involved in the control of DF cells differentiation, but their precise function remains largely unknown. In this study, mesenchymal progenitor properties of human DF cells were first investigated and their ability to be triggered by BMPs evaluated. To test the potential of HDFC to undergo cementoblastic differentiation under EMD and BMP treatments, we assessed the mineralized-cell and specific markers expression in both *in-vitro* and *ex-vivo* culture systems.

METHODS: HDFC isolated from whole DF surrounding third molars before the onset of root development, were submitted to multilineage differentiation culture systems and to FACS analysis for STRO-1, a marker of multipotential mesenchymal progenitor cells. DF tissues and cells were then stimulated by rhBMP-2 and -7 or EMD +/-rhNoggin. Immunostaining were used to localize STRO-1, BMP receptors (BMPR), and cementoblast markers on cell and tissue cultures. Alkaline phosphatase activity was quantified. Expression of two markers, highly expressed in cementoblasts, the Cementum Attachment Protein (CAP) and Cementum Protein 23 (CP-23)³⁻⁴, were also analyzed by Western-blot and Immunohistochemistry.

RESULTS: STRO-1 and BMPR were immunolocalized in the DF *in vivo*. In culture, a mean of 10.75% of HDFC expressed STRO-1 and HDFC exhibited multilineage properties. Cells submitted to EMD demonstrated increased differentiation rates, effects largely dependent on the presence of BMP-2 and -7 in it. EMD and BMP-2 and -7 significantly increased the CAP and

CP-23 expression by EMD- stimulation (fig.1), suggesting a specific effect of these compounds to commit HDFC towards the cementoblast phenotype. In addition, rhNoggin partially inhibited the effects of EMD implying that they also exert BMP-independent effects on HDFC.

Fig. 1: EMD, BMP-2 and/or -7 -stimulated HDFC strongly expressed CAP and CP-23 proteins, two putative cementoblast markers. Their expression decreased when rhNoggin was added to EMD. Both markers were strongly expressed in hCementoblast (hCb), but weakly in hOsteoblast (hOb), used as positive and negative controls, respectively.



DISCUSSION & CONCLUSIONS: Our results regarding the effect of EMD on periodontal progenitor cells point out the potentiality of using HDFC or other mesenchymal precursors as cementoblast progenitors, offering new perspectives in periodontal regeneration.

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