IDENTIFICATION OF PEPTIDES RESULTING FROM DMP1 DEGRADATION BY MMP-2 TO PROMOTE STEM CELL DIFFERENTIATION.

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INTRODUCTION: Historically, matrix metalloproteinases (MMPs) are multidomain proteins that degrade structural components of the ECM. It is now broadly acknowledged that MMPS can produce specific substrate-cleavage fragments that have different biological activities from their precursors. Several MMPs have been identified in dentin and pulp [1]. Among NCPs of dentin, DMP1 has been shown to play a central role in dentin mineralization. The C-terminal domain rich in glutamic acid and serine has been implicated to have a functional role in the nucleation of HAP [2]. DMP1 also acts as a signaling molecule that might induce cytodifferentiation of undifferentiated pulp cells after an insult or trauma of the tooth[3]. Recent data had shown that DMP1 could act as a transcription factor when transported to the nuclear compartment. Furthermore, DMP1 has the capacity to regulate the DSPP gene transcription during early odontoblast differentiation by binding to the promoter of this gene through its carboxyl end [3].
The objective of our work was (1)- to study the cleavage of DMP1 by MMP-2 - and (2) -to analyze if the resulting peptide (s) could act as signaling molecules on dental pulp stem cells (DPSCs).

METHODS: Activated MMP-2 (1 µg/ml) was added to recombinant rat DMP1 at 37°C for 0 to 48 h in 100 mM Tris buffer pH 7.2. The resulting supernatant was run on 12% SDS-PAGE for visualizing peptides by Coomassie blue or stains-all. The bands were excised from the gel and analyzed by mass spectrometry (MS) after trypsin digestion. Human DPSCs were cultured with N and C recombinant polypeptides closely matching limited cleavage products of DMP1 by MMP-2. Proliferation and differentiation were assessed.

RESULTS: MMP-2 at short time points (1 to 6 h) and for both concentrations cleaved DMP1 into two fragments; a major fragment (residues 1-382, 35 kDa) and a second one corresponding to the C terminus (residues 383-489) which could not be visualized neither on various gels nor by western blot. At longer time points (24 to 48 h), the N fragment was processed into 3 smaller fragments and all could be identified by MS. Results from the activity of 2 recombinant polypeptides mimicking the limited cleavage of DMP1 demonstrate that the N polypeptide did not display any significant effect on DPSCs, whereas the C polypeptide enhanced cell proliferation initially. This fragment also had an effect on cell differentiation with an increased expression of Dentin sialoprotein (DSP) and DMP1.

DISCUSSION: Future work including the synthesis of smaller polypeptides with respect to the MMP-2 cleaved fragments will be necessary to determine the precise region within the DMP1 molecule responsible for its cell proliferation and differentiation activity

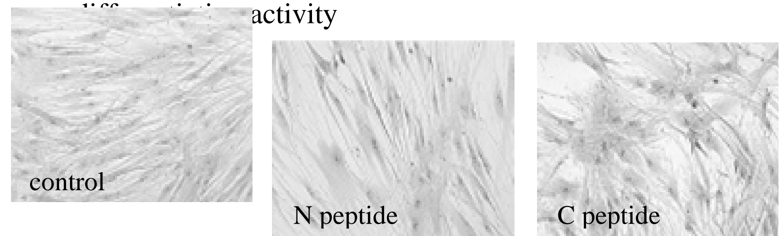

Fig: 1 Effect of DMP1 polypeptides on cell morphology at 48 h

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