

## REVISITED PHENOTYPE OF BONE AND DENTAL CELLS – THE PHYSIOLOGICAL QUESTION OF SITE-SPECIFIC BIODIVERSITY

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**INTRODUCTION:** Recently, mineral-related genes emerge as specific modules driven by several transcription factors depending on tissue identity. Our laboratory raises another question in cell phenotype, their anatomical diversity. Our goal is to provide some clues on the relationships between anatomical shapes, site-specific homeostasis and regional gene regulation. The strategy used *Msx2* as a candidate developmental gene which would play a part in physiology, based on its established roles at various regulatory levels from organs, to cells and genes.

**METHODS:** Wild-type and transgenic *Msx2* knock-in mouse mandibles were studied. Molar and alveolar bone growth was analysed from birth to 28 days. 3 month-old mice enabled the study of adult homeostasis (basal bone, molars and their alveolar bone). The incisors were used for continuous tooth and alveolar bone growth. Dental (epithelial enamel-related and mesenchymal dentin related) and oral bone secretory cells were studied by real time RT-PCR. Triplicate samples were microdissected from 3 month-old *Msx2* *+/+*, *Msx2* *+/-* and *Msx2* *-/-* mice. Amelogenins (AMG), amelin (AMB) and type I collagen  $\alpha 1$  chain (Col1A1) were analysed. Microradiographical and morphological studies were done at all stages. Guided by RNA measurements, AMG and AMB immunoperoxidase labeling was performed with sequential dilutions. Bone resorption was studied by tartrate-resistant acid phosphatase (TRAP) histoenzymology. RANK, RANK-ligand and osteoprotegerin (OPG) RNAs were also measured by RT-PCR in the same samples than the ones used for matrix protein RNAs.

**RESULTS:** AMG and AMB RNAs were expressed in all mineralized tissues, while Col1A1, only in bone and dentin samples. Their relative abundance was higher in enamel versus all mesenchymal mineralized tissues in wild-type mice. Measurements showed statistically significant site-specific reverse up- or down-regulation of their steady levels in *Msx2* *+/-*,

*Msx2* *-/-* and control mice. *In situ* studies could indicate an “ectopic” expression but sequential dilutions evidenced affected ratios in epithelial dental cells. Supra-ameloblastic and root sheath cells expressing lowest levels in wild-type mice were reversely the ones showing the highest levels in *Msx2* *-/-* mice. An irregular amelogenesis imperfecta in *Msx2* *-/-* and regional osteopetrosis involving exclusively the alveolar region were evidenced on microradiographs, tissue sections and by TRAP assays on growing teeth. This phenotype was related to a RANK-ligand (but not RANK) decrease in dental and in alveolar bone cells where it was statistically significant. OPG variations were not significant.

### DISCUSSION & CONCLUSIONS:

Our data support previous studies on AMG and AMB in bone tissue and cells. Low dilutions in immunoperoxidase assays provide a diffuse pattern in the mandible. They diverged from ameloblast-specific pattern which may be generated by high dilutions but do not correspond to the relative expression levels of RNAs shown here. *Msx2* is instrumental in the regulation of AMG and AMB expression *in vivo*, in a cell-specific manner. *Msx2* *-/-* phenotype is a physiological example of AMG/AMB overexpression in relation with alveolar osteoclast impairment. More refined cell sampling is required to decipher the suspected signaling cascades in physiological cell networks, complex when compared to early development. The most fascinating finding of our strategy was that in physiology and anatomy, there exists molecular fields, delineated by *Msx1* and *Msx2*. Developmental transcription factors could decipher physiopathological pathways, linking specific target-genes (matrix proteins) and -cells within anatomical sites.

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