

MODULATION OF BMP ACTIVITY AFFECTS SIZE AND SHAPE OF INCISOR MORPHOGENESIS

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INTRODUCTION: Teeth are typical examples of epithelial appendages, where sequential and reciprocal interactions between oral epithelium and neural crest derived mesenchyme play a pivotal role in their development. The repeating crosstalk via different members of the BMP, FGF, Wnt and Hedgehog families is responsible for their patterning, morphogenesis and differentiation. Mutations in critical components of these pathways profoundly affect tooth development. The importance of different BMP inhibitors for tooth developmental program has been illustrated earlier, and it is possible that they have redundant functions. Therefore the purpose of this study was to determine how the collective absence of two inhibitors i.e. *ectodin* and *follistatin* affects incisor morphogenesis and differentiation.

MATERIALS & METHODS: All procedures using animals were approved by the Animal Care and Use Committee of University of Helsinki and tissues were obtained in accordance with the guidelines. The generation of *follistatin* and *ectodin* mice lines have been reported earlier and double knockout mice were generated in-house by mating heterozygous *ectodin* and *follistatin* mice. Wild-type, heterozygous, and homozygous *ectodin* and *follistatin* screening was done by PCR. In situ hybridization and immunohistochemistry have been performed on serial sagittal sections obtained either from E14 to E17,5 stage of development or from kidney transplanted incisor. For organ culture experiments, the lower incisor teeth from day 14 mouse embryos were dissected and cultured. Cell proliferation assays have been also applied to define the cell proliferation index.

RESULTS & DISCUSSION: In all compound null mutants overgrown lingual cervical loops with numerous BrdU positive cells were obvious together with an extra coronal invagination, formed by the inner enamel

epithelium. As it shown earlier Fgf3, Fgf9 participate PEK regulation, BMP down-regulate Fgf3, indirectly Fgf9 and both *follistatin* and *ectodin* is a negative regulator of BMP. Taken together *follistatin* and *ectodin* may effect -inhibit- incisor enamel knot function, indirectly resulting the observed invagination. In situ hybridization (MMP20, Shh, TBX-1) and immunohistochemical analysis (amelogenin) indicated that these cells later differentiated into enamel secreting ameloblasts suggesting that *follistatin* and *ectodin* not only regulates the early morphological event of incisor development but also effect ameloblast differentiation. Tissue culture experiments indicated that similar to molar morphogenesis, the development of wild type incisor is relatively robust against excess BMP in contrast to inhibitor knockout, where the sensitivity of tooth germs to BMP resulted in a more advanced stage of ameloblast differentiation. Moreover, when the compound mutant tooth germs were cultured as transplants under adult mouse kidney capsules, a dramatic stimulation of matrix secretion and abnormal incisor phenotype was evident.

CONCLUSIONS:

Our study further supports the hypothesis that BMP activity is regulated at different molecular levels and by several inhibitors, which play an important role in ameloblast maturation and matrix formation.

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