

REUPTAKE OF EXTRACELLULAR AMELOGENIN BY DENTAL EPITHELIAL CELLS RESULTS IN INCREASED LEVELS OF AMELOGENIN mRNA THROUGH ENHANCED mRNA STABILIZATION

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INTRODUCTION: In this study, we focused on effects of amelogenin on the differentiation of dental epithelial cells. We produced recombinant mouse amelogenin in baculovirus insect cell expression system and administered Amelogenin protein to dental the epithelial cell line (HAT-7). Our results indicated that amelogenin protein induces increases in the quantity of amelogenin mRNA through enhancing mRNA stability. Here, we describe a unique role for amelogenin protein regulating amelogenin mRNA quantity at the post-transcriptional level.

METHODS: The mRNA levels of differentiation-related marker genes were determined by quantitative real-time PCR as described previously (1-3). A rat amelogenin promoter driving luciferase reporter plasmid was constructed as described in previous study (4) according to the genomic database information of rat (NW_048039). Transient tranfection luciferase assays were performed with Lipofectamine 2000 (Invitrogen). 200 µg of Amelogenin protein was precipitated and re-dissolved in 200 µl of 0.1 M bicarbonate buffer (pH 9.5). Then, 20 µl of 1 M bicarbonate buffer (pH 9.0) was added. Next, 20 µl of FITC solution (10 mg/ml) was added and incubated with stirring for 1 h on ice. The reaction mixture was passed through a G-50 column to remove excessive FITC. For the study of uptake and localization of exogenous amelogenin, FITC-Amelogenin was added exogenously to cells at a concentration of 10 µg /ml and followed by a 4-hour culture period. The cells were fixed in paraformaldehyde and the nuclei were stained with Hoechst dye. Coverslips were mounted and cells were observed under laser confocal microscope (Zeiss, LSM 510).

RESULTS: Recombinant mouse Amelogenin enhanced expression of endogenous amelogenin mRNA in a cultured dental epithelial cell line (HAT-7), despite of a lack of increased amelogenin promoter activity. To solve this discrepancy, we analyzed effects of Amelogenin protein on stability of amelogenin mRNA. The half-life of amelogenin mRNA is extremely short, but in the presence of Amelogenin protein its half-life was extended four times longer than control. Furthermore, we showed

entry of exogenous FITC-conjugated Amelogenin protein into the cytoplasm of HAT-7 cells (Fig.1). It follows from our results that exogenous amelogenin increases amelogenin mRNA levels through stabilization of mRNA in the cytoplasm of HAT-7 cells. Here, we speculate that during differentiation, dental epithelial cells utilize a unique mechanism for increasing the production of amelogenin, the reuptake of secreted amelogenin.

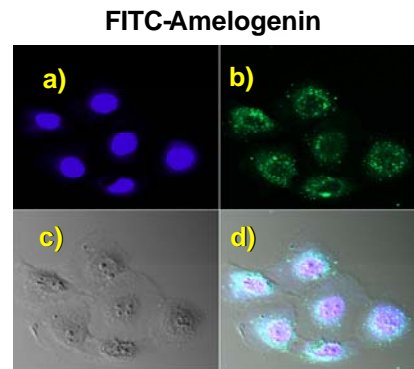


Fig. 1. Uptake and localization of Amelogenin by HAT-7 cells. a) nuclear stain, b) Split XY image of FITC-Amelogenin, c) DIC image and d) composite of the three images, and ortho image of Z-section of composite of FITC-Amelogenin.

DISCUSSION & CONCLUSIONS: In this study, we showed that dental epithelial cells take extracellular amelogenin into the cytoplasm, and increases stability of amelogenin mRNA. It could be speculated that *in vivo*, ameloblasts are able to, in an autocrine fashion dramatically increase production of amelogenin.

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