Abstract

Effect of extracellular matrix protein of Epithelial rests of Malassez supernatants during enamel crystallization of tooth germ

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Graduate School of Dentistry,

Health Sciences University of Hokkaido

Dembereldorj Bolortsetseg

Introduction

Enamel covers the surface of the tooth and is the hardest tissue in the body. It is composed of approximately 95–97% hydroxyapatite (HAP), 1% enamel protein, and 2% water. Tooth decay is caused by bacteria, and when enamel is lost, it cannot be fully regenerated. Although lost enamel can be restored with composite resin, glass ionomer cement, or porcelain, it is preferable to replace it with HAP, which has a regular structure like enamel. The importance of Amelogenin (Amelx) is well known, as the self-assembly of Amelx controls the morphology, size, and orientation of the growing enamel crystals.

After root formation, degraded Hertwig's epithelial sheaths (HERS) migrate into the space of the periodontal ligament to form filamentous or reticular aggregates as remnant epithelial cells of Malassez (ERM). ERM was reported to secrete enamel matrix proteins such as Amelx. Clonal ERM cells isolated from porcine dentate ligament (PDL) exhibit high Amelx expression.

This study aims to promote the crystallization of immature enamel in tooth germs using ERM conditional medium (CM) containing Amelx, instead of the recombinant full-length or spliced form of Amelx, which is complex, costly, and requires safety considerations.

Methods

Cloned ERM cell culture

For this study, ERM-2, a clone of CRUDE ERM, was selected based on the highest expression level of Amelx.

RT-qPCR analysis of Enamel matrix proteins in ERM-2 cells

For quantitative real-time reverse transcription-polymerase chain reaction (RT-qPCR), aliquots of total cDNA were amplified with Amelx, Ameloblastin (Ambn), KLK4, MMP-20, and GAPDH primers.

mice

Pregnant female C57BL/6J mice were purchased from The Jackson Laboratory. In this experiment, tooth germs were extracted from the first mandibular molar of a postnatal (PN) 3-day mouse.

Histological and immunohistochemical studies on extracted tooth germs

Mandibular molar tooth germs were dissected from C57BL/6J mice on PN 3. A histological and immunohistochemical study was performed to investigate the detailed distribution of Amelx protein in the tooth germ.

Collection of conditioned mediums

ERM-2 clonal cells and porcine gingival epithelial cells were cultured in KGM. When the confluence of cells reached 5×10^4 cells/ml, the medium was replaced with KBM. After four days of cell culture, the supernatant was collected and used as the ERM-2 CM.

Organ culture with conditioned mediums and analysis by scanning electron microscopy

Mandibular molar tooth germs were employed on postnatal day 3. Using previously stored ERM-2 CM, tooth germs were grown in 24-well dishes. Tooth germs were incubated for 1, 3, 7, and 14 days. The surface layers of the enamel structure were examined with a scanning electron microscope (SEM).

Small interfering RNA-Transfection in ERM-2 cells and small interfering conditional medium collection

ERM-2 cells were transfected with si-RNA targeting negative (scramble) and specific enamel matrix proteins such as Amelx, Ambn, KLK4, and MMP-20. After 2 days of incubation, each CM was collected from each group of cells. RT-qPCR was performed to determine the expression of the target genes in the si-RNA transfection experimental condition.

SEM analysis of organ culture with si-EMPs conditional medium

Tooth germs were cultured in tooth germ at PN 3 mice for 1, 3, 7, and 14 days with supernatants from si-RNA-treated ERM-2 cell CM, including si-negative, si-Amelx, si-Ambn, si-KLK4, and si-MMP-20. The enamel surface of this tooth germ was examined using SEM.

SPSS analysis

All values are expressed as mean ± SE for the respective groups. Statistical analysis was performed using IBM SPSS. The results were compared using the Mann–Whitney U test with a p value < 0.05 accepted as statistically significant

Results:

The hematoxylin-eosin staining of mouse tooth germ sections on PN3 revealed that they were in the bell stage with a thin layer of enamel. Immunohistochemical staining showed that Amelx protein was specifically positive in ameloblast with enamel formation. Tooth germs excised from PN3 mice were observed by SEM. PN3 tooth germ was observed thin enamel crown morphology, in high magnification, the enamel surface showed smooth.

Tooth germs cultured with ERM-2 CM on days 3 and 7 of organ culture showed elongation and densification of the columnar structures in SEM analysis. The columnar structures became denser and aggregated forming a HAP-like hexagonal columnar structure 14 days after culture in ERM-2 CM.

ERM-2 expressed a significantly higher level of Amelx, Ambn, KLK4, and MMP-20 than CRUDE ERM ($p<0.05$).

Tooth germ cultured on days 3 and 7 with si-negative control ERM-2 CM observed elongation and densification of the columnar structures. On 14 days of culture with si-negative control ERM-2 CM, the columnar structures appeared denser and aggregated forming a HAP-like hexagonal columnar structure. In contrast, no clear columnar structures were observed in each si-

EMPs ERM-2 CM.

There was a significant decrease expression of all si-EMP genes compared to the si-RNA negative control in RT-qPCR.

Discussion

The effects of Amelx and other EMPs secreted by ERM-2 were investigated in this study on the enamel surface of the organ-cultured tooth germs. ERM-2 conditioned medium was used to organ culture tooth germs isolated from PN3 mice. On days 4, 6, 10, and 17, the enamel surface of the organ-cultured tooth germ was examined using SEM. These results demonstrate that ERM CM enhances the formation of enamel-like hydroxyapatite hexagonal columnar structures in organ-cultured tooth germs. This acceleration was reduced in ex-vivo tooth germs by si-RNA-mediated inhibition of Amelx and EMPs expression in ERM-2 CM. The results imply that Amelx and EMPs are crucial in the maturation of HAP crystals and support the theory that they govern HAP crystal growth.

Conclusion

ERM clones with a high content of Amelx in their CM were able to accelerate the formation of enamel prisms in an ex vivo tooth germ organ culture. It follows that Amelx promotes the development of enamel prisms. The precise mechanism of the interactions between Amelx, Ambn, KLK4, and MMP-20 molecules, however, remained unknown and will require further investigation.