

IMMUNOLocalIZATION OF INTEGRIN α 6 β 4 SUBUNIT DURING THE AMELOGENESIS

Felipe Eltit, Alejandro Oyarzún

Faculty of Dentistry, Finis Terrae University, Santiago-Chile

Introduction

Tooth development involves many interactions between cells and extracellular matrix, these interactions are required for cell survival and differentiation. During early stages of odontogenesis, a basement membrane is located between the inner dental epithelium and the underlying mesenchyma. This basement membrane gives the structural support to the developing ameloblasts and the signals necessary for cell survival and differentiation. However, the basement membrane disappears during the secretory stage. Moreover, the mechanism of adhesion and signaling of ameloblast to enamel matrix is not clearly known.

Integrins are the major adhesion molecules to extracellular matrix, consist of covalently linked subunits α and β . The α 6 β 4 is a hemidesmosome associated integrin, which is expressed in many epithelial cells, in which also transmit mechanical and chemical signals. The main ligand for integrin α 6 β 4 is laminin 332 (also known as laminin 5). Previous reports have described the presence of laminin 332 in the basement membrane of inner dental epithelium and in the junctional epithelium in adult mice. Integrin α 6 β 4 is expressed in mouse junctional epithelium, and α 6 subunit is expressed in presecretory ameloblasts, but the presence of integrin α 6 β 4 during amelogenesis have not been reported.

The present study was designed to probe the hypothesis that integrin β 4 subunit is localized in the interface secretory ameloblasts-enamel matrix during the amelogenesis in the mouse.

Materials and Methods

Ten newborn mice of 0, 2, 4, 6, and 8 days and eight adult mice were used for this study. The animals were killed by decapitation, the maxilar and mandibular segments were immediately fixed in buffered formaldehyde for 24 hours. The specimens were demineralized in 10%

formic acid for three days, dehydrated and embedded in paraffin. An indirect immunofluorescent technique, or a streptavidin-biotin-peroxidase method was applied on serial sections of 5 μ m thickness. A rat monoclonal anti-mouse β 4 integrin antibody was used (346-11A, BD Pharmingen). The observations were made using epifluorescent microscopy and light microscopy.

Results

In mouse incisor basement membrane of outer dental epithelium, and oral epithelium were strongly stained. However, the presecretory ameloblasts were negative for the immunoreaction. Surprisingly the expression of integrin β 4 subunit started in the apical pole of secretory ameloblast, and become more intense in maturative and protective ameloblast. This result was confirmed with the study of mouse molars, which show intense immunolabeling of Tomes processes of ameloblasts during the secretory and maturative phase of amelogenesis. The immunostaining remains in reduced ameloblasts and junctional epithelium, the late phases of ameloblast life cycle.

Discussion

Our results confirm the thesis that the ameloblasts express adhesion molecules in their apical pole, these glycoproteins remained during enamel maturation and tooth eruption. The main ligands for integrin α 6 β 4 are laminin 332 and laminin 511/521, we suggest that these glycoproteins must be present in the enamel matrix, confirming previous reports that proposed that the enamel matrix has an adhesive role during amelogenesis.