P9-REDUCED EXPRESSION OF TIGHT JUNCTION PROTEINS ZO-1 AND CLAUDIN-1 IN AMELOBLASTS AND ODONTOBLASTS OF EPIPROFIN/SP6 DEFICIENT MICE

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Introduction

Odontoblasts and ameloblasts are columnar cells that characterize by a highly polarized distribution of organelles. Junctional complexes and the cytoskeleton are important in maintaining ameloblast and odontoblast cell polarity and cell-cell interactions. In addition, cell adhesion is a key regulator of gene expression and cell differentiation. Tight junction binding proteins proteins such as Zonnula Occludens-1 (ZO-1) and the Y-box transcription factor ZONAB (ZO-1 associated Nucleic Acid Binding protein) can be either recruited to tight junctions or alternatively translocated to the nucleus, where they act to upregulate expression of genes involved in cell proliferation and differentiation (1).

We recently reported that mice deficient in the transcription factor Epiprofin/Sp6 present a highly aberrant dental phenotype, including formation of multiple supernumerary incisor and molar teeth. Teeth form *Epiprofin / Sp6* knock-out (KO) mice also present big disturbances in ameloblast and odontoblast differentiation, reflected by a complete absence of enamel development and structural defects of dentin (2,3).

Materials and Methods

We studied odontoblast and ameloblast polarization and differentiation in incisor and molar teeth of *Epiprofin / Sp6* KO mice at different developmental stages, and its correlation with the expression levels of tight junction proteins, such as Claudin-1, ZO-1 and ZONAB, assessed by immunohistochemistry, confocal microscopy and image analysis.

Results

Hematoxylin-Eosin stained incisor and molar sections revealed that functional ameloblasts fail to form in Epfn (-/-) mice, and as a result teeth from these animals completely lack enamel. Odontoblasts in Epfn (-/-) mice show a defective polarization, with nuclei often appearing at the apical side of the cell (Figure 1; top). We found that tight junction proteins Claudin-1, ZO-1 and ZONA-B where downregulated in teeth from Epiprofin deficient mice. ZO-1 protein is expressed in ameloblastic cells and the signal was accumulated at the basal and apical pole of the cell membrane. At a similar developmental stage, the inner enamel epithelium of *Epfn*-null incisor and molars showed largely reduced levels of ZO-1. In the mutant E17.5 incisor, ZO-1 labelling levels in cells of the inner enamel epithelium dropped to a mere 22%, relative to those present in normal labial-side ameloblasts ($p<0.001$; Tukey's *post-hoc* test. One-way ANOVA), and were comparable to intensity levels found in inner enamel epithelial cells of the lingual incisor side, where ameloblastic cells do not naturally differentiate. Similarly, ZO-1 labelling levels were also significantly decreased in odontoblasts of *Epiprofin/Sp6* (-/-) mice, being reduced by 40% in both incisor and molars ($p<0.001$). Claudin-1 was expressed in the inner enamel epithelium and stratum intermedium, but this expression was also clearly reduced in *Epiprofin/Sp6* deficient mice (Fig. 1; middle). Finally, the expression of ZONA-B was decreased in incisor and molar teeth of *Epiprofin*-null mice at all developmental stages tested (Fig. 1; bottom).

Discussion

Taken together, these results show alterations in ameloblastic and odontoblastic cells regarding their capability to form functional tight junctions. Cell signalling pathways regulated by these junctions also appear to be affected, which could contribute to explain why these dental cells fail to differentiate properly in the absence of *Epiprofin/Sp6*.

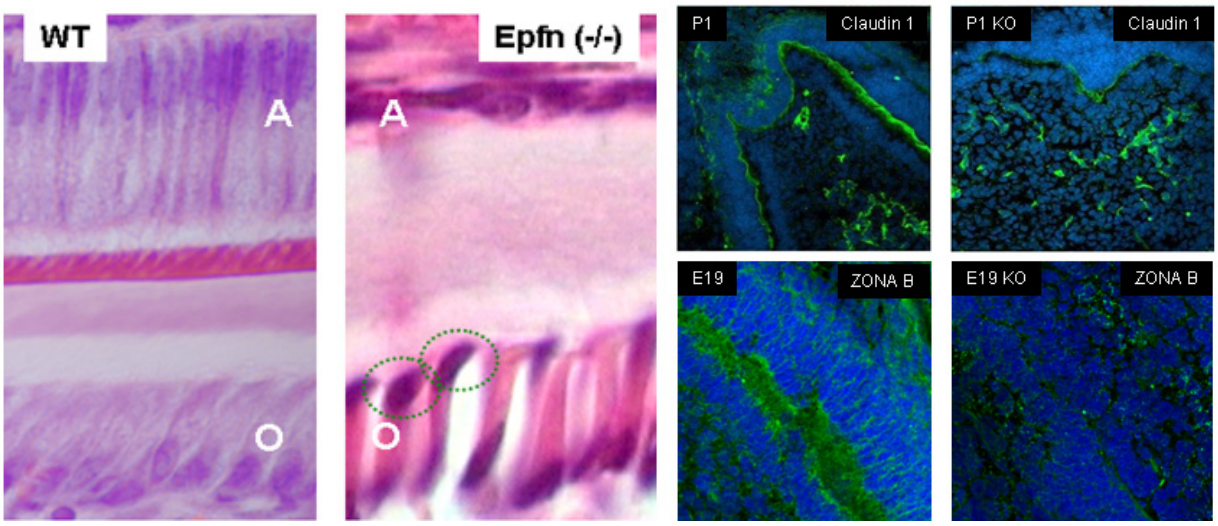

Figure I. Top: Defective polarization and differentiation of Ameloblast and Odontoblast cells in Epfn (-/-) mice. Note the absence of ameloblasts and the incorrectly polarized odontoblasts in the mutant incisor (dotted circles) Middle: Claudin-1 immunohistochemistry of WT and Epfn-null P1 molars. Note the decrease in labelling of cells in the inner enamel epithelium. Bottom: ZONA-B immunohistochemistry in E19 Epfn-deficient molars also reveals a decreased labelling in ameloblastic and odontoblastic lineage cells.

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