O5-DIFFERENTIAL EXPRESSION AND FUNCTIONAL SIGNIFICANCE OF GLUCOSE TRANSPORTERS DURING MURINE TOOTH DEVELOPMENT

Hiroko Ida-Yonemochi[1], Mitsushiro Nakatomi[1], Hidemitsu Harada[2], Hayato Ohshima[1]

[1]Division of Anatomy and Cell Biology of the Hard Tissue, Department of Tissue Regeneration and Reconstruction, Niigata University Graduate School of Medical and Dental Sciences, 2-5274 Gakkocho-dori, Chuoku, Niigata, Japan
[2]Division of Oral Histology and Developmental Biology, Department of Oral Structure and Function Biology, Iwate Medical University, 1-3-27 Chuo-dori, Morioka, Japan

Introduction

Glucose is an essential source of energy for mammalian cells and plays an important role for body metabolism, in which the transport of glucose across the cell membrane is accelerated by glucose transporters (GLUTs). To date, thirteen GLUTs have been identified and their tissue-specific expression patterns are suggestive to reflect the various glucose requirements of different tissues. Class I transporter comprises the well-characterized GLUT1-4, and GLUT1 is known to be the major glucose transporter expressed in many tissues and be responsible for fundamental glucose uptakes. GLUT2 is a low affinity isoform with glucose, and GLUT3 and GLUT4 are high affinity isoforms, which are mainly expressed in brain. However, there has been no available data on the expression and the role of GLUTs during tooth development. This study aims to clarify the functional significance of GLUTs during murine tooth development using immunohistochemistry and organ culture experiment.

Material and Methods

ICR mice from embryonic day 13 (E13) to postnatal day 10 (P10) were used in this study. All mice were administered with an intraperitoneal injection of BrdU 2 hrs before the fixation and perfused with 4% paraformaldehyde. Following decalcification in 10% EDTA when necessary, the heads were embedded in paraffin and the frontal or sagittal sections of teeth were cut at 4 μm. Immunohistochemistry was performed using antibodies against GLUT1-4, Ki67 and BrdU. For organ culture experiments, the mandibular molar germs were isolated from E13, E14 and E16 mice, and were cultured for 3, 7, 10 days with the inhibition of GLUTs by phloretin, a glucose transport inhibitor.

Results and Discussion

An intense GLUT1-immunoreaction was localized in the enamel organ of bud-stage tooth germ of mandibular first molars as well as the oral epithelium, where the active cell proliferation occurred. By the cap stage, the expression of GLUT1 in the dental epithelial cells was dramatically decreased in intensity, and subsequently began to appear in the stratum intermedium at the bell stage. On the contrary, GLUT2-immunoreactivity was observed in the stellate reticulum and dental papilla throughout all stages. Secretary ameloblasts and odontoblasts also showed immunopositive reaction for GLUT2. The expression of GLUT1 and GLUT2 was not overlapped in the dental epithelial and mesencymal cells during tooth development. With regard to GLUT3 and GLUT4, their immunoreactions were not observed in the tooth germs.

In vitro organ culture experiments, the inhibition of GLUT1/2 by phloretin in the bud-stage tooth germs (E13) induced the developmental arrest of explants at the bud stage and the squamous metaplasia of dental epithelial cells lacking the enamel organ. On the other hand, the development of tooth germs of E14 and E16 was not inhibited by phloretin treatment. Three days after phloretin treatment of E13 tooth germs, cell proliferation was inhibited in the dental epithelial cells and the apoptotic features were observed in the primary enamel knot areas. These results suggest that the disturbance of primary enamel knot formation by lack of glucose uptake into the enamel organ cells is attributed to arrested tooth formation.

Conclusions

The expression of GLUT1 and GLUT2 in the dental epithelial and mesencymal cells seems to be precisely controlled spatiotemporally, and the glucose uptake mediated by GLUT1/2 plays a crucial role in the early tooth morphogenesis.

References

1. Grobholz R *et al.* (1993) *Cancer Res* **53** (18): 4204-4211.
2. Kobori M *et al.* (1997) *Cancer Lett* **119** (2): 207-212.