

A ROLE OF WNT5A IN CONTINUOUSLY GROWING MICE INCISORS

T Kagiya¹, N Fujiwara¹, K Ishizeki¹, J Xiao² & H Harada¹

¹*Department of Oral Anatomy II, School of Dentistry, Iwate Medical University, Morioka, Japan*

²*Department of Oral Biology, School of Stomatology, Dalian Medical University, Dalian, China*

INTRODUCTION: Tooth growth depends on precise control of basic cellular processes such as cell proliferation and differentiation. Mice incisors are regenerative tissues, which grow continuously throughout life. Some intercellular signalings including fibroblast growth factor (FGF) are essential for the maintenance of the incisor growth [1]. Wnt molecules have been implicated in the regulation of tooth development. *Wnt5a* gene expresses in the dental papilla and follicle [2]. However, little is known about the precise role of Wnt5a signaling during tooth development. To study the functions of *Wnt5a* in growing incisors, we examined the morphological features of incisors in *Wnt5a*-deficient mice.

METHODS: The wild-type (C57BL/6) and *Wnt5a*-deficient mice tooth germ (E-18) specimens which were embedded in paraffin were kindly provided by Dr. Changgong (University of Southern California, USA). To estimate the proliferative activity, they were sectioned and immunostained by Ki67. Rat dental epithelial cell line, HAT-7 and primary mouse dental papilla cells were cultured in the presence/absence of recombinant Wnt5a (R&D Systems, INC, Minneapolis, MN, USA) for 7days. Cell proliferation was measured by MTS assay (Cell Titer 96 Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA). Total RNA was extracted from primary mouse dental papilla cells at 5d, and the expression of *Fgf3* was analyzed by RT-PCR.

RESULTS: *Wnt5a*-deficient mice incisors were much shorter than wild type, the length of inner enamel epithelium as cell proliferation region was very short. On the other hand, morphological defect was not seen in the molars. The morphological features of mutant incisors were similar to those of *Fgf10*-deficient mice. But apical buds were observed clearly in *Wnt5a*-deficient mice incisors. Immunostaining analysis of Ki67 showed a decrease in the number of proliferating cells in *Wnt5a*-deficient mice incisors. To indicate the direct effect of cell proliferation in the dental epithelial cells and mesenchymal cells, we carried out MTS cell proliferation assay by HAT-7 and primary culture of mesenchymal cells. However,

recombinant Wnt5a did not have any effect on the proliferation of these cells. We investigated the relationship between *Wnt5a* and *Fgf3* expression in the incisors by the cell and the organ culture of mesenchyme. Recombinant Wnt5a prevented the *Fgf3* expression in the mesenchymal cells from disappearing.

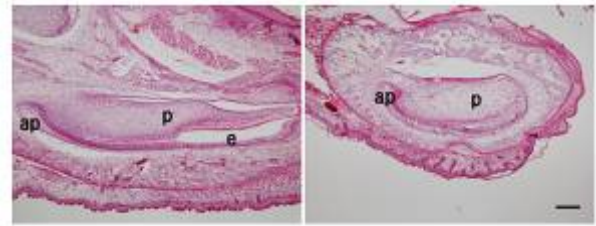


Fig. 1: Histology of *Wnt5a*-null mouse incisor: wild type (left) vs. *Wnt5a*-null (right). ap;apical bud, e;enamel, p;pulp, bar;200 μ m

DISCUSSION & CONCLUSIONS:

The morphological features of *Wnt5a*-deficient mice incisors were similar to those of *Fgf10*-deficient mice. It suggests that Wnt5a plays a role in proliferation of dental epithelium and/or mesenchyme in growing incisors. However, Wnt5a does not have a direct effect on cell proliferation in the cultured epithelial and mesenchymal cells of incisors. Furthermore, apical buds were seen in *Wnt5a*-deficient mice incisors. *Fgf3* expression was maintained by recombinant Wnt5a. These results suggest that Wnt5a plays a role in proliferation of inner enamel epithelial cells in continuously growing incisors through the induction and/or maintenance of *Fgf3* expression.

REFERENCES: ¹ H. Harada, T. Toyono, K. Toyoshima, et al (2002) *Development* **129** 1533-1541. ² L. Sarkar and P.T. Sharpe (1999) *Mech Dev* **85** 197-200.

ACKNOWLEDGEMENTS: This study was supported in part by a grant, KAKENHI (B) (No. 19390466 to H.H.) and the Open Research Project (2007-2011) (to N.F. and H.H. in Iwate Medical University) from MEXT.