

## SONIC HEDGEHOG AND FGF SIGNALING ARE IMPORTANT FOR TOOTH ROOT DEVELOPMENT

Masato S.Ota<sup>1</sup>, Mitsushiro Nakatomi<sup>1</sup>, Sachiko Iseki<sup>1</sup>, Taka Nakahara<sup>1,2</sup>, Kazuhiro Eto<sup>1</sup>

<sup>1</sup>Section of Molecular Craniofacial Embryology, Graduate School, Tokyo Medical and Dental University, Tokyo, Japan. <sup>2</sup>Section of Developmental and Regenerative Dentistry, School of Dentistry at Tokyo. The Nippon Dental University. Tokyo, Japan.

**INTRODUCTION:** The cell-therapy for the tooth root tissue regeneration combined with tissue-stem cells and tissue-engineering technology would be a very powerful tool in the future, but there is not enough accumulation of basic knowledge about tooth root development. In fact, several signalling molecules in the Shh, FGF, BMP and Wnt families appear to regulate the developmental steps of tooth morphogenesis. Here, we examined the biological effects for the root development of typical signaling pathway, SHH<sup>1</sup> and FGF<sup>2</sup>.

**METHODS:** Mice C57BL/6 wild-type and heterozygous *Ptc<sup>mes</sup>* mutant (Makino *et al.*, 2001) mice were used for the experiments..

**Immunohistochemistry.** Immunohistochemistry was carried out essentially according to the manufacturer's methods(Roche). **In situ hybridization and Realtime RT-PCR** In situ hybridization and realtime RT-PCR were performed according to the standard protocol. **Recombinant protein, Protein-beads , and Kidney capsule grafting** P5 mandibular molars were dissected just before root formation and recombinant proteins were added with beads in attempts to examine the functions in tooth root formation. We examined SHH (50 µg/ml) protein and FGF2 and FGF18 (250µg/ml).

**RESULTS: Disturbance of Tooth Eruption and Root Formation in Homozygous *Ptc<sup>mes</sup>* Mutants.** All lower molars had finished eruption in control littermates by P28 (Fig. 1A; n = 11/11), whereas in homozygous mutants, all molars showed delayed eruption (Fig. 1B; n = 7/7). In addition, all tooth roots of mutants (right tooth in Figs. 1C-1E) were shorter than controls (left tooth in Figs. 1C-1E), especially the third molars (white arrow in Fig. 1E). The length of all tooth roots (asterisks in Fig. 1F;  $P < 0.001$ ). We examined gene expressions of several molecules involved in SHH pathway and FGF family by realtime RT-PCR (data not shown). Interestingly, *Fgf18* was significantly repressed in the mutants. Therefore, we examined a functional assay of signaling molecules in developing tooth root using recombinant proteins (Fig.2) and observed that FGF2 and FGF18, but not SHH, significantly stimulated tooth root elongation (data not shown).

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Fig. 1: Disturbance of tooth eruption and root formation in homozygous *Ptc<sup>mes</sup>* mutants at P28. Wild type (left) vs. *Ptc<sup>mes</sup>* (right).

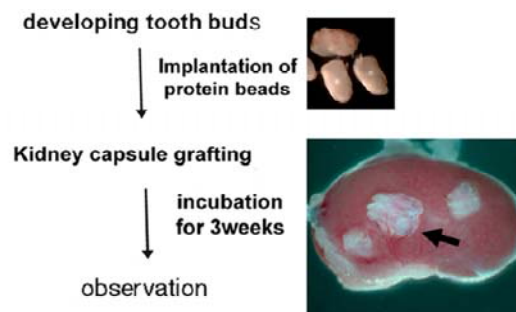


Fig. 2: functional assay of signaling molecule in developing tooth root.

### DISCUSSION & CONCLUSIONS:

Our findings indicated that SHH signaling pathway is important for tooth root development and FGFs effectively promote the tooth root elongation and periodontal tissue formation.

**REFERENCES:** <sup>1</sup>Nakatomi M. et al. (2006) *J. Dent Res.* 85:427-31. <sup>2</sup>Ota M. et al. (2007) *J. Oral Tissue Engn* 4:137-142.

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