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# The Tooth as a Model for Organ Development

**Han-Sung Jung, Jae-Young Kim, Eun-Jeung Kim, Soo-Jin Song and Mi-Jin Park**

*Division in Histology, Dept. of Oral Biology & Oral Science Research Center, College of Dentistry, Institute of Life Science and Biotechnology, Brain Korea 21 Project of Medical Sciences, Yonsei University, 134 Shinchon-Dong, Seodaemoon-Gu, Seoul 120-752, Korea*

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**To investigate tooth organ development, it is very important to understand how the epithelial and mesenchymal cells accommodate the growing embryos. Embryonic induction is a process by which the fate of the responsive tissues is altered following tissue interactions. It is a gradual process composed of many steps. Recently, many studies have shown the mutual interactions, as well as antagonistic systems, of several genes expressed in the process of tooth bud development. Also, craniofacial abnormalities resulting from defective genes also have been reported in many cases. We would like to revisit how much we have learned by studying tooth bud development in embryogenesis and propose further possible studies in which we may move forward in our knowledge and understanding of embryogenesis.**

**Keywords:** mouse embryogenesis, tooth, developmental biology

## Introduction

An important question in embryonic development is how cells and tissues become precisely arranged to form the body. Recently, developmental biology has indeed become one of the more stimulating areas of study because of the combination of embryology and molecular biology and announcements from the human genome project concerning the importance of stem cells in many developing organs.

To study embryonic development, it is useful to begin with a simple organ with well-defined morphological patterns as a model system. One of the simplest patterns observed is the maintenance of a minimum distance between neighboring elements, which is often called a "spacing pattern". In a spacing pattern, some cells from a group of originally equivalent precursor cells become different from their neighbors, thus forming a spaced array of determined cells. Examples of periodic patterns are found in the development of many vertebrate organs, such as in teeth (cusps) formation, feather bud formation, somite formation, sweat gland formation and scale formation. In this review, we will provide an overview of recent results in the study of tooth morphogenesis, then offer suggestions as to what questions remain to be solved in the near future.

### Overview of tooth bud development

The mammalian tooth begins a process of growth between the dental epithelium and neural crest-derived mesenchymal cells. As the growth occurs, the differentiating inner enamel epithelium facing the mesenchyme gives rise to enamel-forming ameloblasts, while the mesenchyme below gives rise to dentine-forming odontoblasts (Jernvall and Thesleff, 2000). Tooth bud formation can be observed as an outgrowth of the dental lamina in the embryonic mandible. This morphological phenomenon of tooth development shares features with other epithelial appendages, such as the mammary gland, hair and scales, in which development is also regulated by interactions between the epithelial and mesenchymal tissues (Chuong, 1998). Development of the molar shape begins at the distal end of a cap-resembling structure surrounding the mesenchymal cells, i.e., the dental papilla.

The primary enamel knot is a region of an epithelial structure that is histologically visible as a cluster of densely packed cells (Ohshima *et al.*, 1999). These cells one non-dividing and express several signaling molecules, with the co-expression of several signaling pathways (Jernvall and Thesleff, 2000). Those results suggest that the primary enamel knot is an embryonic signaling center (Vaahtokari *et al.*, 1996; Nieminen *et al.*, 1998). Signaling centers communicate with surrounding epithelial and mesenchymal tissues by secreting molecular signals, which are essential for tooth morphogenesis and development of other epithelial appendages

†Correspondence to: Dr. Han-Sung Jung, Dept. of Oral Biology, Division in Histology, College of Dentistry, Yonsei University, 134 Shinchon-Dong, Seodaemoon-Gu, Seoul 120-752, Korea

(Tickle *et al.*, 1975; Smith, 1993; Liem *et al.*, 1995). While the developmental context and outcome differs among different signaling centers, the genes used in molecular signaling are usually the same. For example, anterior-posterior limb patterning requires the zone of polarizing activity (ZPA), which can be mimicked by either implanting into limbs a bead releasing sonic hedgehog (Shh)-protein (Riddle *et al.*, 1993) or a tooth germ (Koyama *et al.*, 1996). These discoveries have made it obvious that one gene does not produce one structure.

At least four fibroblast growth factors (*Fgf*s) are expressed in a developing tooth and all promote cell division in cultured dental tissues. Moreover, two of the *Fgf*s, *Fgf4* and *Fgf9*, are only expressed in the enamel knot cells. *Fgf3* is expressed in the enamel knot and the underlying mesenchyme. *Fgf10*, the fourth *Fgf* transcribed in teeth, is expressed during the cap stage only in the mesenchyme (Kettunen and Thesleff, 1998). Moreover, the different FGF proteins do not stimulate all dental tissues equally. FGF4 and FGF9 stimulate cell proliferation of both dental epithelium and mesenchyme, whereas FGF10 stimulates cell division only in dental epithelium (Kettunen *et al.*, 1999, 2000).

The primary enamel knot begins to disappear soon after the bud has branched out to form the cap. It is now known that the cells of the enamel knot disappear via apoptosis, or programmed cell death. This apoptosis is associated with the expression of another growth factor, bone morphogenetic protein-4 (Bmp4), in the enamel knot (Jernvall *et al.*, 1998). The Bmps have multiple roles in development. They appear to be generally involved in apoptosis in other body structures, such as rhombomeres and digits (Graham *et al.*, 1994; Pizette and Niswander, 1999). Biologically, apoptosis has been suggested to be a mechanism involved in controlling the duration of molecular signaling of the enamel knot (Vaahtokari *et al.*, 1996b; Jernvall *et al.*, 1998).

## Developmental regulatory genes

During tooth bud development, the process of events that forms a tooth involves crown morphogenesis, cementation, eruption and root formation. All these processes are exciting experimental subjects for developmental biologists.

Many transcription factors have master regulatory functions in the initiation and morphogenesis of individual organs. Probably the best example is *Pax6*, a transcription factor containing a paired box, which initiates eye development in different animals (Callaerts *et al.*, 1997). Tooth development depends on several key transcription factors. These include the homeobox genes, *Dlx1*, *Dlx2*, *Msx1* and *Msx2*, and the paired box genes, *Pax9* and *Lef1* (Peters *et al.*, 1998; Bei *et al.*, 1998). The function of these genes is to inhibit tooth development before or after at the bud stage. Also, tooth morphogenesis requires the function of the *runt*-domain containing transcription factor *cbfa1* (D'Souza *et al.*, 1999), which is the master control gene of osteoblast function and bone development.

The signaling molecules belong to several families: the hedgehog (HH), the bone morphogenetic proteins (BMPs), the fibroblast growth factors (FGFs) and the Wnt family signaling molecules. The tooth is the best example of an organ in which one or more members of these four families have been detected and described in detail. The conserved signaling networks regulate the development of bone and cartilage. Indian hedgehog (IHH), synthesized by prehypertrophic and hypertrophic chondrocytes, regulates the site of hypertrophic differentiation by signaling to the periarticular growth plate.

## Gene defects causing craniofacial malformations

Although defects in the craniofacial tissues result from defects in extracellular molecules, such as collagen and enamel proteins (Spranger *et al.*, 1994; Forsman *et al.*, 1994), most craniofacial abnormalities appear to be caused by defects in the developmental regulatory genes. Holoprosencephaly, which is caused by mutations in the *Shh* (Sonic hedgehog) gene, is one example. Craniosynostosis syndromes, which are caused by mutations in several different genes of the *FGF* signaling pathway, are also reported. Some gene defects that cause hypodontia have been identified. Mutations in the *Msx1* gene cause oligodontia (Vastardis *et al.*, 1996). Anhydrotic dysplasia (EDA) is caused by loss of function of ectodysplasin A, a cell surface molecule whose function is unknown and which is involved in mediating cell communication between the epithelium and mesenchyme (Kere *et al.*, 1996). Another example is the *cbfa1*-deficient transgenic mouse, which can be used to study various aspects of bone formation. The heterozygous mutant is considered as a model for human cleidocranial dysplasia (Huang *et al.*, 1997).

## Study of tooth development in the next 10 years

Remarkable knowledge has been gained from these studies of tooth morphogenesis and many laboratories are investigating various other aspects of tooth morphogenesis. We would like to suggest two fields of tooth development into which some of us have already stepped.

More detailed study of the periodontium is needed. Compared with root formation, crown morphogenesis might be easier to approach during tooth development since it can be investigated in a developing mandible. Indeed, many studies on crown morphogenesis have been reported and it is a good example for pattern formation study. Although recent dentistry techniques make it easy to replace tooth crowns with other materials, not much is known about the development and regeneration of the periodontium. Indeed, it is extremely difficult to approach from an experimental side, yet critical for dentists to know about it.

Secondly, producing a tooth from embryonic tissue is another area for study. Embryonic stem cells, as multipotent cells, can be transformed into three types of tissues: ectoderm, mesoderm and endoderm. Using modern techniques, we might

be able to produce a new tooth. Transfection of early marker genes for tooth bud initiation into stem cells and subsequent development of these stem cells within a special culture system, as in a molar shape or incisor shape scaffold, may produce calcified objects that can replace permanent teeth. The renal capsule of nude mice may be a candidate for incubation, but surely the other system works as an excellent culture system.

Information gained from these studies may form the basis of both new treatment and prevention methods, possibly affecting specific steps in tooth bud development. The use of BMPs in the stimulation of bone formation is already being tested. There are promising results from animal experiments in which the application of signal molecules has affected specific stages of morphogenesis and in some cases partially rescued development (Pispa *et al.*, 1999). The study of tooth development is now moving forward toward a new dimension in which all the applicable techniques can be used for producing new teeth.

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