Comparative Morphological Study on the Embryonic and Neonatal Development of the Filiform Papillae and Teeth in Mice

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Introduction

During development, the mouse tongue is divided into oral and pharyngeal sections by the terminal sulcus and its dorsal surface is covered with lingual papillae1). The lingual papillae perform various functions during mastication, transportation and swallowing of food2), as well as have a barrier function that prevents the penetration of harmful substances such as bacteria3). On the dorsal surface of the tongue, four types of lingual papillae are found: circumvallate, fungiform, foliate, and filiform, each of which is distributed on the surface of the tongue according to specific patterns (Fig. 1)1,4,5). Based on the histological analysis of the presence or absence of taste buds, the lingual papillae are divided into gustatory papillae, which plays an important role in the gustatory system, and mechanical papillae, which plays a role in mastication3). Circumvallate, fungiform and foliate papillae with taste buds belong to the gustatory papillae and filiform papillae without taste buds belong to the mechanical papillae3).
mice, one dome-shaped circumvallate papilla with keratinized epithelium is located in the center of the terminal sulcus\(^6,7\), the fungiform papillae have a single taste bud in the apical portion of the thinly keratinized or non-keratinized epithelium\(^8\), and the foliate papillae with red leaf-like ridges are variable in size and shape and have many taste buds in the non-keratinized epithelium\(^9\). Filiform papillae, the most common and cone-shaped of the lingual papillae are divided into two types according to the shape of the tip and the location of distribution\(^3\). The filiform papillae located in the intermolar eminence have a pointed end while those located in other regions have a rounded end\(^3\). It is not yet known which of the developmental processes determines the morphological difference between filiform papillae. Recently, many studies of tongue papillae have been conducted and reported in various ways using protein expression, signaling pathways, molecular mechanisms, and tissue interactions but have mainly been about the gustatory papillae and taste buds\(^1,10-12\). To date, filiform papillae development remains unclear\(^13-15\).

In the early stages of development, teeth and lingual papillae are induced and developed through special and complex epithelial-mesenchymal interactions\(^16,17\). Organs that are induced and formed by epithelial-mesenchymal interactions include the salivary and mammary glands, hairs, and feathers, and these organs have similarities in their initiation and formation processes, although their morphological, anatomical, and physiological properties are significantly different\(^1,18\). The process of tooth development is well known and classified into the bud, cap, bell, crown, and functional stages according to the morphological characteristics\(^19,20\), and is completed through prenatal and postnatal development\(^20\). The completion of the teeth marks the beginning of the weaning phase, and accordingly, many oral tissues and organs are completed, and it is believed that their developmental completion time will be related to each other.

The purpose of this study was to clarify the embryonic and neonatal development of the filiform papillae and mandibular molar tooth, and discuss the developmental relationship between these organs by comparing the developmental completion times.

**Materials and Methods**

Animal maintenance and sacrifice for experiments were conducted in accordance with the guidelines of Youngsan University for the care and use of animals in research.

1. **Animals**

   Embryos and mice were obtained from ICR outbred mice. Embryos at embryonic day 15 (EM15), 17 (EM17) and 21 (EM21) and mice at neonatal day 1 (NE1), 5 (NE5), 10 (NE10) and 21 (NE21) were used for the experiments. Tissues dissected from embryos and mice were washed with cold phosphate-buffered saline (PBS), then fixed with cold 4% paraformaldehyde in PBS, and processed for histological analysis.

2. **Histological analysis**

   The fixed tissues were decalcified with 10% ethylenedi-
aminetetraacetic acid for 4 weeks. After washing the decalcifying solution from the tissues, dehydration was performed with a series of graded ethyl alcohols. The dehydrated tissues were treated with xylene and then embedded in paraffin wax. The tissues were cut to a thickness of 6∼7 μm and sections were stained with hematoxylin and eosin for observation with a light microscope.

**Results**

1. Embryonic development

1) Embryonic day 15 (EM15)

The lingual epithelium covering the dorsal surface of the tongue, which is thought to occur as filiform papillae at EM15, is observed as striated cuboidal epithelium cells (Fig. 2A). Microvilli were observed on the surface of cuboidal epithelial cells and showed characteristics before development began (Fig. 2B). Under the lingual epithelium,
Fig. 3. Light micrographs showing neonatal development of filiform papillae and tooth in coronal section of mice head. (A~D) NE1: (E~H) NE5: (I~L) NE10: (M~P) NE21. (A) LE with distinct FP in NE1. (B) Higher magnified micrograph of pointed end FP. (C) Higher magnified micrograph of rounded end FP with KG on surface of epithelium. (D) Molar tooth of the mandibles of NE1 showing a more advanced bell stage characteristic. (E) LE of NE5 showing a form similar to that of NE1. Higher magnified micrograph of pointed end (F) and rounded end (G) FP with numerous KG of NE5. (H) Mandibular molar tooth showing the characteristics of late advanced bell stage. (I) Matured FP with keratinized stratified squamous epithelium in NE10. Higher magnified micrograph of matured pointed end (J) and rounded ends (K) FP of NE10 with numerous KG and a keratinized layer that looks like a thick, bundle of threads on the surface and a well-developed CTC at the bottom. (L) Mandibular molar tooth of NE10 showing typical crown stage characteristics. (M) FP of NE21 showing a form similar to that of NE10. Higher magnified micrograph of mature pointed end (N) and rounded ends (O) FP of NE21 showing a form similar to that of NE10. (P) Molar tooth of functional stage erupted into the oral cavity in NE21. AB: ameloblast, CT: connective tissue, CTC: connective tissue core, D: dentin, E: enamel, FP: filiform papillae, G: gingiva, KE: keratinized epithelium, KG: keratohyalin granules, LE: lingual epithelium, OC: oral cavity, OB: odontoblasts, P: pulp, PS: palatal shelf, REE: reduced enamel epithelium, T: tongue.
a single layer of mesenchymal cells was observed (Fig. 2B).

The developing mandibular molar teeth at EM15 were characterized by the late bud stage, with the dental lamina formed from the primary epithelial band toward the mesenchyme with the dental organ and dental papilla differentiated from the tooth bud (Fig. 2C).

2) Embryonic day 17 (EM17)

On EM 17, the microvilli disappeared and the elongated and thickened lingual epithelium was observed on the dorsal surface of the tongue, confirming that the development of the filiform papillae was initiated and showed a typical striated squamous epithelium composed of cuboidal cells in the basal layer and squamous cells in the apical cell layers, but characteristic changes such as archlike structures were not confirmed (Fig. 2E).

The molar tissue at EM17 was divided into complete dental organ, dental papilla and dental follicle, and showed typical cap stage characteristics (Fig. 2F).

3) Embryonic day 21 (EM21)

The lingual epithelium at EM21 was more compact (Fig. 2G). The morphological changes of the surface and the development of filiform papillae with pointed and rounded ends began (Fig. 2H, 2I). Archlike structures that; migrated upward of the condensed mesenchymal cells located below the epithelium; were observed (Fig. 2H, 2I).

The developing mandibular molar tissue at EM21 was characterized by the early bell stage (Fig. 2J). That is, the cells constituting the dental organs differentiated into outer enamel epithelial cells, stellate reticulum cells, stratum intermedium cells, and inner enamel epithelial cells. Dental papilla cells in contact with the inner enamel epithelial cells differentiated from cuboidal cells to columnar cells and were observed as presecretory odontoblasts (Fig. 2J).

2. Neonatal development

1) Neonatal day 1 (NE1)

At NE1, a clearly distinguished striated squamous epithelium of filiform papillae with connective tissue located below it was observed (Fig. 3A). Moreover, keratohyaline granules responsible for morphogenesis of the keratinized layer were found on the surface of the filiform papillae with rounded ends (Fig. 3C), unlike the epithelium of the filiform papillae with pointed ends (Fig. 3B).

The molar tissues of the mandibles at NE1 exhibited a more advanced bell stage characterized by the dentine and predentin layers formed by odontoblasts along with well-differentiated ameloblasts and odontoblasts (Fig. 3D).

2) Neonatal day 5 (NE5)

At NE5, the filiform papillae and connective tissue located below it were similar to that of at NE1, but they were more developed (Fig. 3E). Numerous keratohyaline granules were observed in both pointed- (Fig. 3F) and rounded-end (Fig. 3G) filiform papillae.

The developing mandibular molar tissue observed at NE5 has long and forming thick enamel, predentin, and dentine layers, and well-developed secretory ameloblasts, showing the characteristics of a late advanced bell stage (Fig. 3H).

3) Neonatal day 10 (NE10)

Filiform papillae with a keratinized stratified squamous epithelium and irregular connective tissue thought to be mature structures, were observed at NE10 (Fig. 3I). Namely, filiform papillae with pointed and rounded ends had numerous keratohyaline granules on the superficial layers, a thick keratinized layer which looked like a bundle of threads, and a well-developed connective tissue core beneath the keratinized layer (Fig. 3J, 3K).

The molar tissue at NE10 had thick, well-developed enamel and dentine layers, but ameloblasts that shrank and lost function to become reduced enamel epithelium were observed, showing typical crown stage characteristics (Fig. 3L).

4) Neonatal day 21 (NE21)

The filiform papillae at NE21 were a matured form with no morphological differences from NE10 (Fig. 3M ∼ 3O). At NE21, the mandibular molar tooth erupted into the
Table 1. Comparison of Embryonic and Neonatal Development of Filiform Papillae and Tooth

| Developmental timing | Lingual papillae | Tooth | Phase |
|----------------------|------------------|-------|-------|
| Embryonic development |                  |       |       |
| EM13, EM14           | Developmental Initiation of fungiform papillae$^{26}$ | Embryonic phase |
| EM15                 | Developmental Initiation of filiform papillae$^{28}$ | Bud stage |
| EM17                 | Initiation stage of filiform papillae | Cap stage |
| EM21                 | Morphogenesis stage of filiform papillae | Early bell stage |
| Neonatal development |                  |       |       |
| NE1                  | Morphogenesis of filiform papillae | Bell stage | Sucking phase |
| NE5                  | Morphogenesis of filiform papillae | Late advanced bell stage |
| NE10                 | Functional stage of filiform papillae | Crown stage |
| NE21                 | Completion of taste bud in fungiform papillae$^{19,27}$ | Functional stage | Weaning phase$^{19,20}$ |

oral cavity and was at a functional stage (Fig. 3P).

Discussion

The development of oral tissues and organs, including the lingual papillae and teeth, begins very early in embryonic development, which is sensitive to genetic and epigenetic effects$^8$. The lingual papillae and teeth begin to develop, and the tissues are formed through epithelial-mesenchymal interactions$^{16,18}$. Despite their structural, anatomical, physiological and functional differences, it is known that their development processes have many similarities$^1,16$. Based on histological analysis results, the developmental process of the lingual epithelium for filiform papillae covering the dorsal surface of the tongue was classified into three stages: initiation, morphogenesis, and functional (Table 1). In the early stages of embryonic development, a characteristic of undifferentiated epithelial cells is the presence of microvilli on the surface of the epithelial cells, and when differentiation of the epithelial cells begins, the microvilli disappear$^{2,21,23}$. Based on this, EM17 was identified as the initiation stage of the development of filiform papillae. And then, rapid morphological changes in epithelial cells have been observed and this period is divided into morphogenesis stages. That is, the surface of the epithelium at EM21, PN1 and PN5 were clearly distinguished from the prominent papillae with pointed and rounded ends, and keratin granules were observed. The filiform papillae at PN10 reached the functional stage with well-developed keratohyalin granules and keratinized layers. Namely, pointed- and rounded-end filiform papillae were completed at PN10. Recently, it has been reported that the expression of Shh, Ptc, Gli-1, Bmp-4, and Fgf-8 signaling molecules had been confirmed in the embryonic developmental process of mouse lingual papillae, and they play an important role for morphogenesis in the initiation stage of embryonic development$^{24,25}$. Although there are no direct results on inducing and determining morphological differences between pointed- and rounded-end filiform papillae, Bmp-4 and Shh expression are thought to be particularly relevant$^1$. In contrast, there are reports that the developmental initiation of fungiform papillae occurs at EM13 or EM14$^{26}$, and taste buds in the fungiform papillae and teeth are completed at NE21 which is the weaning phase$^{19,27}$. The development of the filiform papillae initiates at EM15$^{28}$ and is completed before birth$^{29,30}$. However, in this study, the development of the filiform papillae initiated at EM17 and reached the functional stage at PN10 which is the sucking phase. The development of the filiform papillae initiated late and completed quickly in comparison with the fungiform papillae and teeth, and is completed through embryonic and neonatal development such as the tooth and salivary gland formed as a result of epithelial-mesenchymal interactions (Table 1). Although filiform papillae are known to maintain food in the oral cavity, increase the contact and friction surfaces between the tongue and food, mastication, swallowing, sucking$^{2,9,31,32}$, they are considered to play an important role in sucking rather than mastication as it is completed in the sucking phase.
Notes

Conflict of interest
No potential conflict of interest relevant to this article was reported.

Ethical approval
This article is not necessary for IRB screening because it is an experimental paper using animal.

Author contributions
Conceptualization, Data acquisition, Formal analysis, Funding, Supervision, Writing-original draft, Writing-review & editing: Soon-Jeong Jeong.

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