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Gene expression patterns of *Etv5* and *Etv6* in molar tooth germs at the cap stage

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The Fgf signaling pathway plays an important role in the sequential process of tooth development and morphogenesis. Ets (E-twenty-six) is a family of transcription factors regulated by the Fgf signaling pathway and is involved in the development of various organs. Although most Ets proteins act as transcriptional activators, Etv5 (Ets version 5) and Etv6 (Ets version 6) reportedly act as transcriptional repressors. Expression of *Etv5* mRNA has been reported in developing teeth, whereas *Etv6* expression has not been reported yet. In this study, we examined the expression pattern of *Etv6* in tooth development and compared the expression patterns of *Etv5* and *Etv6*. *Etv5* was strongly expressed in epithelium, except in the enamel knot, while *Etv6* was strongly expressed in dental lamina and stellate reticulum. Although their expression patterns overlapped in the stellate reticulum, there were many differences overall. This result suggests that *Etv5* and *Etv6* regulate different genes

Keywords: Etv5, Etv6, mRNA expression, tooth, development

Introduction

Tooth is one of craniofacial organs which are regulated by Fgf signaling pathway during development[1,2,3,4,5]. Fgf signaling is required for tooth bud formation as shown in mutant mice lacking Fgfr2 IIIb[6]. FGF signaling plays a pivotal role in the folding of dental epithelia during tooth development by inducing cell proliferation in areas surrounding the non-dividing enamel knot[7]. It has also been reported that cells in enamel knot do not have FGF receptors[8].

The Ets (E-twenty six) is a large family of transcription factors that are downstream targets of Fgf signaling, and the family is involved in the development of various organs[9,10,11].

The *Ets version 5 (Etv5)* is one of Ets family and is mainly expressed in the epithelium during development of many organs[12]. Complete absence of Etv5 results in embryonic lethality. *Etv5* expression has been reported in the developing kidney, lung,

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heart, limb, salivary gland and tooth[12,13,14]. Etv5 have been shown to have a role in kidney branching morphogenesis and in limb pattern formation[15,16,17]. It has been reported that *Etv5* showed a restricted expression pattern in epithelial and mesenchymal cells surrounding the primary enamel knot[13]. While most Ets proteins act as transcriptional activators, a small number of members act as transcriptional repressors[18,19]. The role of Etv5 was reported as a repressor at least in limb pattern formation[20].

Etv6 (Ets version 6) is also a transcriptional repressor that binds DNA during embryonic development[21]. Homozygous disruption of *Etv6* in mice results in embryonic lethality[22]. Expression of *Etv6* has not been reported in developing teeth yet.

The aim of this study is to investigate the expression pattern of *Etv6* in developing teeth and to compare the expression patterns of *Etv5* and *Etv6*, which are two transcriptional repressors.

Materials and Methods

All experiments were performed according to the guidelines of the Yonsei University, College of Dentistry, Intramural Animal Use and Care Committee.

Animals

Embryos at E14.5 ICR mice (Koatech Co, *Pyeongtaek*, Korea) were used in this study.

Whole mount *in situ* hybridization

Maxillae and mandibles were isolated from the mouse embryos and fixed overnight in 4% paraformaldehyde and dehydrated in methanol. Hybridizations were performed on these embryos with digoxigenin-labelled cRNA probes in hybridization buffer for 20 hours at 69°C. Hybridization signals were detected by alkaline-phosphatase-conjugated anti-digoxigenin antibodies plus nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate, toluidine salt substrate (Roche, Mannheim, Germany).

Histology

Tissue samples were embedded in OCT compound (Tissue Tek®, Sakura Finetek, Tokyo, Japan), and sectioned at a thickness of 8 μm.

Results

Expression of *Etv5* was observed in developing mandibular molars and hair follicles in mouse embryos at E14.5 (Fig. 1A). *Etv6* was observed in molars but not in hair follicles (Fig. 1B). The expression of *Etv5* encircled the center of first molar tooth germ, while the expression of *Etv6* was a line across the first molar tooth germ in the anteroposterior direction. Both *Etv5* and *Etv6* were expressed weakly in the anterior region of tongue and the circumvallate papilla. Foliate papillae weakly expressed *Etv5*, while neither *Etv5* nor *Etv6* was expressed in fungiform papilla.

On the frontal section of the mandibular first molar, *Etv5* was strongly expressed in the epithelium of the dental organ except the enamel knot and lightly expressed in the oral epithelium and dental mesenchyme. Specifically, the lingual dental epithelium rather than the buccal dental epithelium showed the strongest expression of *Etv5* (Fig. 2A). *Etv6* was

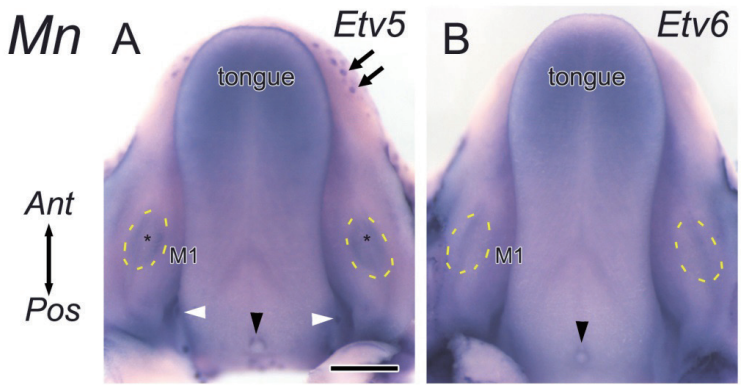

Figure 1. Expression pattern of *Etv5* and *Etv6* mRNA in mandible at E14.5. (A) In mandible (*Mn*), *Etv5* is strongly expressed in developing first molar (M1, yellow dotted line) and hair follicles (arrows). The *Etv5* expression surrounds the center (asterisk) of first molar. *Etv5* is also expressed in the anterior region of tongue, the foliate papillae (white arrowheads) and the circumvallate papilla (black arrowhead). **(B)** *Etv6* is expressed in a straight line across the first molar tooth germ in the anterior (Ant)-posterior (Pos) direction. *Etv6* is expressed in the anterior region of tongue and the circumvallate papilla. Scale bar size: 500㎛

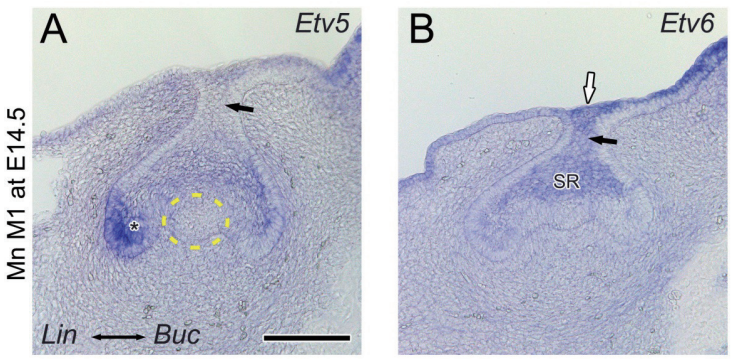

Figure 2. Expression pattern of *Etv5* and *Etv6* in molar tooth germs at cap stage. (A) In the frontal section of mandibular (Mn) first molar (M1) at E14.5, *Etv5* is strongly expressed in the dental epithelium except the enamel knot (yellow dotted line) and weakly expressed in the oral epithelium and dental mesenchyme. *Etv5* expression is stronger in the lingual (Lin) dental epithelium (asterisk) than the buccal (Buc) dental epithelium. *Etv5* expression is not observed in the dental lamina (black arrow), which connects the oral epithelium with the dental epithelium. **(B)** *Etv6* is strongly expressed in oral epithelium (white arrow), dental lamina (black arrow) and stellate reticulum (SR), but not in the inner dental epithelium or in the outer dental epithelium. Scale bar size: 100 ㎛

strongly expressed in the oral epithelium on tooth germ and the stellate reticulum. *Etv6* expression was not observed either in the inner dental epithelium or in the outer dental epithelium (Fig. 2B). Interestingly, there was no *Etv5* expression but strong *Etv6* expression in the dental lamina, which connects the oral epithelium with the dental epithelium.

Discussion

In previous studies it has been reported that Etv5 was strongly expressed in the dental mesenchyme and the dental epithelium except enamel knot in tooth germs at cap stage[13]. Our study confirmed that *Etv5* is not expressed in enamel knots. However, unlike previous studies, *Etv5* was not strongly expressed in the dental mesenchyme in our study. This result is consistent with other previous study reporting that *Etv5* is mainly expressed in the epithelium during development of many organs[12]. The strong *Etv5* expression in dental mesenchyme in the previous study might be due to the [^{35}S]UTP-labeled riboprobe, which is too sensitive to distinguish any difference in expression level between tissues[13]. In the present study, by using the digoxigenin-labelled riboprobe, we found a new fact that lingual dental epithelium expressed *Etv5* more strongly than buccal dental epithelium did.

Ets proteins activate or repress the transcription of target genes together with other transcription factors in order to promote the authenticity of promoter binding sites[18]. The aim of this study was to compare the expression patterns of *Etv5* and *Etv6*, since they have been to be transcriptional repressors in previous studies[16,17,20]. As results, their expression patterns overlapped in the stellate reticulum, but there were many differences in overall. It is not known whether Etv5 and Etv6 both act as repressors in tooth germ. Even assuming they both function as repressors, the expression result suggests that *Etv5* and *Etv6* play different kind of roles in regulating other genes.

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한글초록

모자시기 어금니 치배에서의 Etv5와 Etv6의 발현 양상

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Fgf 신호 전달 경로는 치아발달 단계적 진행과 치아 형태형성에 있어 매우 중요한 역할을 한다. Ets (E-twenty six)는 Fgf 신호 전달 경로에 의해 조절되는 전사 인자들의 집단을 말하며, 여기에 속한 전사 인자들은 다양한 기관의 발달에 관여한다. 대부분의 Ets의 단백질은 활성 전사인자로 작용하지만 Etv5와 Etv6는 억제 전사인자로 작용한다는 보고가 있었다. 발생중인 치아에서 *Etv5* mRNA의 발현은 보고된 적이 있으나, *Etv6*의 발현은 아직 보고된 적이 없다.

본 연구에서는 발생 중인 치아에서 *Etv6* mRNA의 발현 양상을 조사하였으며 그 발현양상을 *Etv5*의 발현양상과 비교하였다.

결과로서, *Etv5*는 enamel knot을 제외한 상피에서 강하게 발현되었으며, *Etv6*는 dental lamina와 stellate reticulum에서 강하게 발현되었다. Etv5와 Etv6의 발현 양상이 stellate reticulum에서는 겹치기도 하였지만, 전반적으로 많은 차이가 있었다. 이것으로 보아 Etv5와 Etv6의 역할은 동일하지 않은 것 같다.

주제어: Etv5, Etv6, mRNA발현, 치아, 발생