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Aldh1a2 is a maker of dental follicle in tooth germs at cap stage

Jinsun Kim and Sung-Won Cho*

Division of Anatomy and Developmental Biology, Department of Oral Biology, Yonsei University College of Dentistry

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Retinoic acid is an important signaling molecule in animal development. Deficiency of retinoic acid causes loss of teeth and skull deformity. In the early stages of tooth development, retinoic acid has been reported to regulate tooth morphology. The major enzymes of retinoic acid formation, *Aldh1a2* and *Aldh1a3*, are expressed during tooth development. However, no expression pattern of the two genes was reported for each tooth development stage.

In this study, we examined the expression patterns of *Aldh1a2* and *Aldh1a3* genes in the tooth germ at cap stage. *Aldh1a2* showed strong expression only in the dental follicle of the tooth germ, but *Aldh1a3* expression was weakly observed throughout the tooth germ. *Aldh1a2* could be used as a new marker of dental follicle.

Keywords: retinoic acid, *Aldh1a2*, *Aldh1a3*, tooth, development, dental follicle

INTRODUCTION

Retinoic acid, an oxidized form of vitamin A, is a signaling molecule with crucial and numerous functions in development of organs in vertebrate embryos. When female rodents were fed with a vitamin A-deficient diet during pregnancy, the offspring showed congenital defects in many organs[1].

The active all-*trans* form of retinoic acid acts as a ligand for nuclear retinoic acid receptors, which function as transcription factors[2]. Disruptions in retinoic acid signaling cause malformations of cranial neural crest cells which form the facial and cranial structures, including cartilage, bone, and teeth[3-6].

Retinoic acid signaling has been studied previously on tooth development. Mouse mutants lacking retinoic acid receptors develop deformed skulls without teeth[7]. On the other hand, exposure to the excess retinoic acid alters dental epithelial morphology, and possibly switches tooth identity between molars and incisors[8]. In zebrafish, the chemically blocking retinoic acid synthesis results in the complete absence of teeth[9], and exogenous retinoic acid induces the formation of an extensive symmetrical pattern of supernumerary teeth. An excess of retinoic acid appeared

* Corresponding author: Sung-Won Cho
Department of Oral Biology, Yonsei University College of Dentistry Yonsei-ro 50, Seodaemun-gu, Seoul 03722, KOREA
Tel: 82-2-2228-3068, E-mail: chosome1@yuhs.ac

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to reduce alkaline phosphatase activity and retard growth and differentiation of molar in mouse[10].

Retinoic acid is a metabolic product of *Aldh1a2*, which is one of the aldehyde dehydrogenase family[11]. *Aldh1a2* has been reported to play an important role during early development of limb, heart, and tooth[11-13]. *Aldh1a2* is expressed in part of the dental papillae and dental follicles at E18.5[13,14]. It has also been reported that *Aldh1a2* is expressed in dental papilla and involved in regular growth and differentiation from dental papilla cells to odontoblasts between E16 and E18[15].

Aldh1a3 is a cytosolic homodimer that participates in the synthesis of retinoic acid and plays an important role in development[16]. Aldh1a3 oxidizes both all-trans-retinal and 9-cis-retinal to retinoic acid. *Aldh1a3* is expressed in various late-stage embryonic and adult rodent tissues including tooth, intestine, kidney, brain, retina, prostate, skeletal muscle, lung, liver and pancreas[14].

Others have studied the effects of exogenous retinoic acid on the development of teeth after bud stage at E12 and E13[17]. However, the morphological changes in teeth were not significant after the treatment. Not only the function but also the expression pattern of *Aldh1a2* and *Aldh1a3* is not well known. In the present study, we examined the expression patterns of *Aldh1a2* and *Aldh1a3*, two major enzymes in retinoic acid synthesis, at the developmental stage, which have not yet been reported, in tooth.

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 ICR mice (Koatech Co, Korea) were used in this study.

Whole mount *in situ* hybridisation

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, Japan), and sectioned at a thickness of 8 μm.

RESULSTS

In maxilla, *Aldh1a2* mRNA was strongly expressed in the whisker region, but was not expressed in developing incisor and molar. Expression of *Aldh1a2* was strongly observed in the surrounding tissues of the developing incisor and molar. A Linear expression of *Aldh1a2* was also observed in the diastema region between the developing incisor and molar. *Aldh1a2* expression surrounding tooth germs was more prominent on the palatal side than on the buccal side of the developing molar (Fig. 1A).

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Aldh1a3 was widely expressed in whiskers and the central part of the primary palate (Fig. 1B). *Aldh1a3* was expressed weakly in the tissue surrounding the posterior half of the developing incisor, but not in the developing molar.

In mandible, *Aldh1a2* was strongly expressed in the anterior region of the tongue. *Aldh1a2* was not expressed in the tooth germ, but was strongly expressed in the surrounding tissue of the developing molar (Fig. 1C). *Aldh1a3* was expressed weakly in tongue and the surrounding tissues of developing molar (Fig. 1D).

On the section of the mandibular first molar, *Aldh1a2* was strongly expressed in the dental follicle surrounding the tooth germ. *Aldh1a2* was strongly expressed on the outside of the dental follicle than on the inside. *Aldh1a2* expression was weakly observed in dental papilla and dental organ, but not in the oral

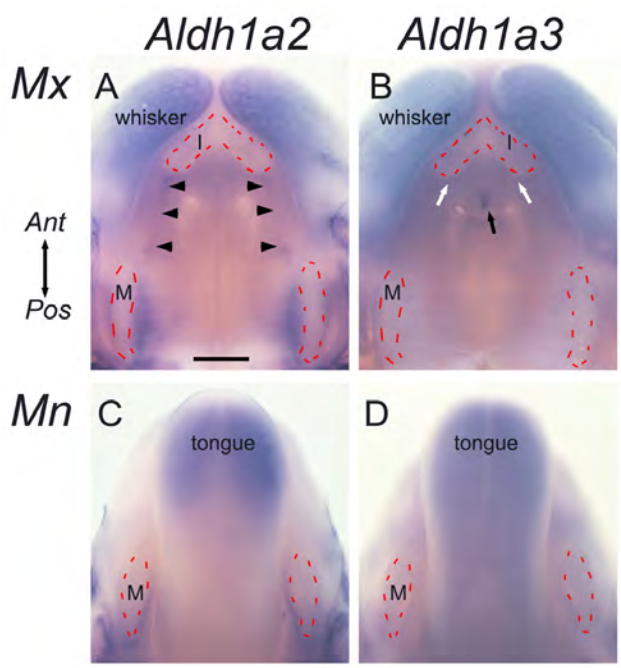

Figure 1. Expression pattern of *Aldh1a2* mRNA and *Aldh1a3* mRNA in maxilla and mandible at E14.5. **(A)** In maxilla (*Mx*)**,** *Aldh1a2* is strongly in whisker region and surrounding tissues of the developing incisor (I) and molar (M). *Aldh1a2* is also expressed in a straight line in the diastema region between the developing incisor and molar (arrowheads). **(B)** *Aldh1a3* is widely expressed in whisker region and the central part of the primary palate (black arrow). Aldh1a3 is expressed weakly in the posterior of the developing incisor (white arrows), but not in the developing molar. **(C)** In mandible, *Aldh1a2* is expressed in the anterior region of the tongue and the surrounding tissue of the developing molar, but is not expressed in the developing molar. **(D)** *Aldh1a3* is expressed weakly on the tongue and the surrounding tissue of the developing molar. Scale bar size: 500 ㎛

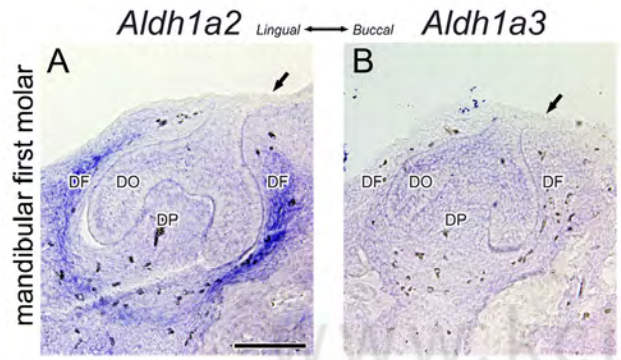

Figure 2. Expression pattern of *Aldh1a2* and *Aldh1a3* in the frontal section of mandibular first molar at E14.5. **(A)** *Aldh1a2* is strongly expressed in the dental follicle surrounding the tooth germ. *Aldh1a2* is weakly expressed in dental papilla (DP) and dental organ (DO), but is not expressed in oral epithelium (arrow). **(B)** *Aldh1a3* is weakly expressed in dental organ, dental papilla and dental follicle, but not in oral epithelium. Scale bar size: 100 ㎛

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epithelium (Fig. 2A). This result indicates *Aldh1a2* as a good marker of dental follicle. *Aldh1a3* was not expressed in the oral epithelium but was expressed weakly in the dental organ, dental papilla and dental follicle (Fig. 2B).

DISCUSSION

It has been reported that *Aldh1a2* was expressed in the dental papilla at E16.5 and E18.5[15]. In the present study, *Aldh1a2* expression was restricted in the dental follicle at E14.5. The difference in the expression pattern of *Aldh1a2* may result from the differences in stages of tooth development. Therefore, it is suggested that *Aldh1a2* is a good marker of dental follicle at E14.5.

Two genes have been proposed as markers for dental follicles. First, *ActivinβA* is expressed in the dental follicle during embryonic and postnatal tooth development, but strong *ActivinβA* expression is observed in the dental papilla, in the osteogenic mesenchyme and in ameloblasts[18]. Second, Golgi protein 49kDa (GoPro49) has been suggested as the more specific marker for the dental follicle during tooth development[18]. The *GoPro49*, which was shown to localise to the Golgi complex, surprisingly showed the mRNA expression restricted to a few mesenchymal tissues, such as cartilage and the dental follicle during development[19]. Currently *GoPro49* is the only Golgi protein with such a restricted expression pattern[18]. A specific function for *GoPro49* was proposed based on its expression pattern. During postnatal stages, *GoPro49* expression was observed mainly expressed in the root follicle, but not in the crown follicle. Therefore, the function of *GoPro49* in the root follicle was suggested to be related with the tooth eruption and movement[18].

Studies on *Aldh1a2* in addition to retinoic acid have been performed at the early stage of tooth development. In the subsequent stages of tooth development, the expression pattern and function of *Aldh1a2* has not been elucidated yet. It is necessary to confirm the expression pattern and function of *Aldh1a2* throughout embryonic and postnatal tooth development.

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한글초록

Aldh1a2, 모자시기 치아주머니의 새로운 표지자

김진선, 조성원

연세대학교 치과대학 구강생물학교실

레티노산은 동물의 장기 발생에 매우 중요한 신호분자이다. 레티노산의 결핍은 치아의 소실과 머리뼈의 변형을 초래한다. 치아발생 초기 단계에서 레티노산은 치아의 형태를 조절한다고 보고되었다. 레티노산 형성의 주요 효소인 *Aldh1a2*와 *Aldh1a3*가 치아 발생 과정 중에 발현된다. 하지만, 치아 발생 단계 별로 두 유전자의 발현 패턴이 보고되지 않았다.

이번 연구에서는 모자시기의 치배에서 *Aldh1a2*와 *Aldh1a3* 유전자의 발현 패턴을 살펴보았다. 결과로써, *Aldh1a2*는 치배의 치아주머니에서만 강한 발현을 나타냈지만, *Aldh1a3* 발현은 치배에서 전반에 걸쳐 약하게 관찰되었다. *Aldh1a2*는 치아주머니의 새로운 표지자로 사용될 수 있다.

주제어: 레티노산, *Aldh1a2*, *Aldh1a3*, 치아, 발생, 치아주머니

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