Mouse의 치아 발육시 Runx2의 발현 양상

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국문초록

Runx2는 runt gene family에 속하는 전사조절 인자로써 뼈의 형성과 골아세포의 분화에 중요한 역할을 담당하고 있다. Runx2-haploinsufficency는 쇄골의 저형성 및 두개 봉합의 지연을 특징으로 하는 쇄골두개 이형성증을 일으키며, 치아에 있어서는 법랑질의 저형성, 영구치 맹출지연 등을 보인다. 이에, 치아의 발육 및 맹출에 미치는 Runx2의 영향을 알아보기 위해 in situ hybridization 방법으로 태생 1, 4, 7, 14, 21일 된 쥐의 하악 및 제1대구치를 사용하여 실험을 실시하였다. Runx2-full length는 태생 1일과 4일에 치낭 및 그 주위조직에 보이지만 Runx2-typeII는 보이지 않았다. Runx2-full length는 태생 7일에 치관 교합면 부위의 법랑모세포에 발현하였고, 1주일 후인 태생 14일에는 백악법랑경계 상방의 치관인접면 법랑모세포에서 발현되었다. 이에 반해 Runx2-typeII는 법랑모세포에서 발현하지 않았다. 또한 태생 21일에서는 두 가지 이성질체가 모두 하악골에서 발현을 보였다. 이런 결과를 종합해볼 때, Runx2-full length는 치아의 맹출과 연관이 있으며, 법랑모세포의 분화 및 이로 인한 법랑질형성에 영향을 주지만 Runx2-typeII는 하악골의 형성에만 영향을 미치는 것으로 사료된다.

주요어 : Runx2, 치낭, 쇄골두개 이형성증, 치아발육, 치아맹출

I. Introduction

Runx2 is a transcription factor in homologous with Drosophila runt gene. Previously it is also referred to as PEBP2αA (polyoma virus enhancer-binding protein), AML-3 (acute myeloid leukemia) and Cbfa1 (core-binding factor a1)[1,2]. Runx2 is essential for bone formation during embryogenesis and a critical gene for osteoblast differentiation and osteoblast function[3-5]. Runx2 has two major N-terminal isoforms, designated typeI/p56 (PEBP2αA, starting with the sequence MRIPVD) and typeII/p57 (Til-1 G1, starting with the sequence MASNSL)[6,7], which showed different expression patterns in mouse calvarial development. Runx2-typeI plays an important role in several steps of osteoblasts differentiation from early commitment to final differentiation. On the other hand Runx2-typeII is restricted to later events of the cell differentiation[8].

Analysis of Runx2-deficient mice revealed that osteoblasts differentiation is arrested and Runx2-haploinsufficency causes cleidocranial dysplasia (CCD)[9,10]. CCD is an autosomal-dominant inherited disorder characterized by hypoplastic clevicle and delayed ossification in fontanelles and wormian bones[11]. Dental defects are possibly shown to CCD patients : multiple supernumerary teeth, irregular and compressed permanent tooth crowns, hypoplastic and hypomineralized defects in enamel and dentin, an excess of ep-

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ithelial root remnants, the absence of cellular cementum, and abnormally shaped roots[12-17]. In addition, delayed eruption of the secondary dentition is a constant finding.

Tooth morphogenesis is achieved by reciprocal and inductive exchange of molecular signals between ectoderm and neural crest-derived ectomessenchyme[18]. After morphogenesis is achieved in the alveolar bone of the jaw some degree, tooth is ready to be prepared for eruption. Eruption periods are composed of two stages ; stage of intraosseous eruption and stage of mucosal penetration and preocclusal eruption[19]. Between these, stage of intraosseous eruption has a quite relation with dental follicle theory, one of the various eruption theories, which is that dental follicle has a ability of bone formation and bone resorption to adjacent alveolar bone[20-22]. This ability of dental follicle may be regarded as the ability of bone remodeling characterized by interaction of osteoclasts and osteoblasts. In fact, intraosseous tooth eruption is a good model of bone remodeling.

Root formation has a relation with tooth eruption ether. While the demonstrations that rootless teeth erupt mean that root formation does not cause eruption[23], formation of the root during the intraosseous stage is an energetically benefit effect of eruption. Especially, eruption speed of intraosseous stage is only limited in speed by the rate of bone remodeling carried out by dental follicle, but during stage of mucosal penetration and preocclusal eruption, eruption speed is much faster because of the rate of root elongation and bone apposition in apical region[19].

In CCD patients who have the defect of osteoblasts activity originated by mutation of Runx2 gene, they show commonly delayed eruption of the permanent teeth. And the most part of them have an experience of dental disability in late youth. Evenly, surgical procedures such as the extraction of all deciduous teeth and the removal of bone overlying the crypts of the unerupted teeth may be needed to promote eruption. Also long term orthodontic treatment is usually necessary for actively erupting and aligning of the impacted permanent teeth[24].

Thus, the aim of this study is to evaluate the role of Runx2 in the tooth development and eruption through analyzing the expression pattern of Runx2 by in situ hybridization during crown (late bell stage) and root formation of tooth.

Ⅱ. Materials and Methods

1. preparation of tissues

Mandibular first molars of ICR mouse were used for the experiment. Mandible of newborn mice at postnatal day 1, 4, 7, 14, and 21 were dissected in dulbecco's phosphate-buffered saline (pH 7.3), and were divided into two parts in the median line. Each side of mandible including first molar was fixed overnight at 4℃ in 4% paraformaldehyde (PFA) in PBS.

Tissues were demineralized in 12.5% ethylenediamintetracetic acid (EDTA) with 2.5% PFA in PBS for about 4 weeks and were dehydrated in ethanol series and embedded in paraffin. Sections of 5㎛ were mounted on silanized slides, dried overnight and stored at 4℃.

2. Preparation of probes

The Runx2 probe was prepared as follows. A 1.6 Kb fragment of mouse Runx2-full length coding sequence was digested with BamHI, XbaI and subcloned into pBluescript SK (Stratagene, La Jolla, CA). As Cbfa1 isoform gene, 400bp Runx2-typeII specific sequence including 5' UTR and coding sequence was digested with BamHI and subcloned into pBluescript SK. To generate antisense and sense transcripts, the plasmids were linearized and transcribed.

Runx2-full length coding sequence was linearized with BamH1 and transcribed with T3 polymerase for a antisense strand, and linearized with Xba I and transcribed with T7 RNA polymerase for sense probe. Runx2-typeII specific sequence was linearized with Xho I and transcribed with T7 polymerase of a antisense strand, and linearized with Sac I and transcribed T3 polymerase for a sense probe.

3. In situ hybridization on tissue sections

In situ hybridization was performed using ^{35}S-UTP-labelled riboprobes as described (Vainio et al., 1991). Briefly, radiolabeled RNA antisense and sense probes

synthesized by in vitro transcription were purified by ethanol precipitation. For hybridization, paraffin-embedded section were deparaffinized and digested with 7㎍/㎖ proteinase K (Sigma)for 15min before the probe was applied. Hybridization was done with the riboprobes of 50000-60000 cpm/㎕ in hybridization mixture including hybridization buffer composed of 50% deionized formamide, 10% dextran sulfate, 1× Denhardt's solution, 20mM Tris-HCL, 0.3 M Nacl, 20mM DTT, 0.5 ㎎/㎖ tRNA, 5 mM EDTA and 10 mM NaH_2PO_4, pH 8.0. After overnight incubation at 50℃, sections were washed using high stringency solution (50% formamide), treated with 20 ㎍/㎖ RNase A (Sigma), and dehydrated. Then, sections were dipped into NTB-2 autoradiographic emulsion (Kodak, Rochester, NY) for 2 sec, dried, and exposed at 4℃ for 2-3 weeks in a dark room. The sections were developed with developer (Kodak D19), fixer (Kodak 300). Tissue sections were conterstained with hematoxylin and stored at 4℃ until examined.

4. Histological examination

Tissue sections were prepared with mouse mandibular firstmolar from postnatal day 1, 4, 7, 14, 21, and stained with hematoxyline and eosin for general histological examination.

Ⅲ. Results

1. Morphologic change in each stage

To examine the development of murine mandibular first molar on each stage, we performed hematoxylin and eosin staining. At the postnatal day 1, dental follicle surrounded the dental organ and dental papila. Also formation of enamel and dentin was observed in the dental organ (Fig. 1A). At the postnatal day 4, the morphology of developing tooth was similar that of postnatal day 1, but thickness of enamel and dentin was much thicker than that of previous stage

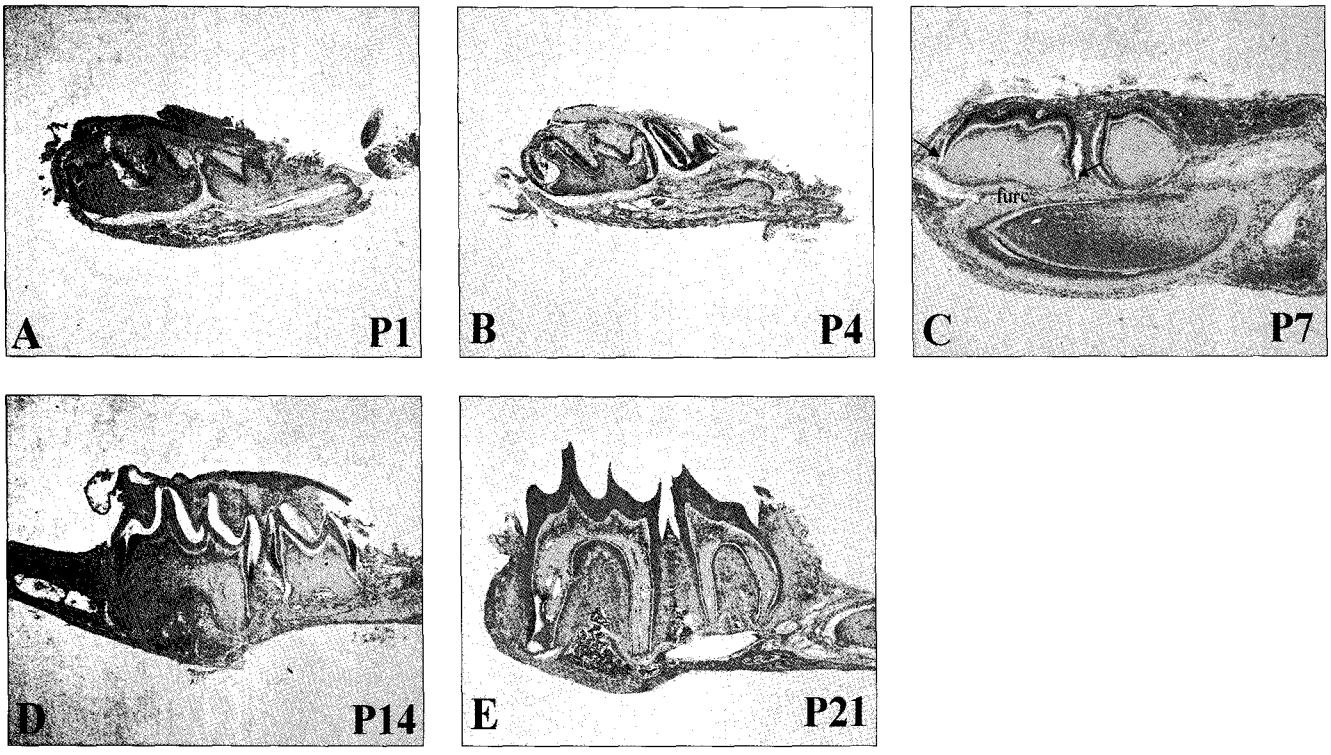

Fig. 1. HE staining of mandibular first molar and surrounding tissue. Sagittal sections of murine mandibular first molar and surrounding tissue, P1, P4, P7, P14, P21, were stained with hematoxylin and eosin. A. At P1, crown was surrounded with dental follicle and formation of enamel and dentin was more or less. B. At P4, the morphology of developing tooth was similar that of postnatal day 1. C, At P7, development of roots was initiated and formation of furcation area (furc) was observed and dental follicle disappeared. Also, according as root formation CEJ (arrows) could be clearly identified. D, At P14, root development was progressed and furcation area was easily detected. E, At P21, tooth was erupted in oral cavity, but root formation was not completed. arrows, CEJ; furc, furcation.

(Fig. 1B). At postnatal day 7, development of roots was initiated and formation of furcation area was observed and dental follicle disappeared. Also, according as root formation CEJ could be clearly identified (Fig. 1C). At postnatal day 14, root development was progressed and furcation area was easily detected (Fig. 1D). At postnatal day 21, tooth was erupted in oral cavity, but root formation was not completed (Fig. 1E).

2. Expression of Runx2-full length

mRNA of Runx2-full length is expressed in dental follicle and surrounding tissue at postnatal day1 and 4 (Fig. 2A, B, C, D). At postnatal day 7, it is expressed in ameloblasts of occlusal surface of enamel and bone area surrounding the tooth (Fig. 2E, F). In comparison with previous stage, at postnatal day 14, it is expressed in ameloblasts of proximal surface of

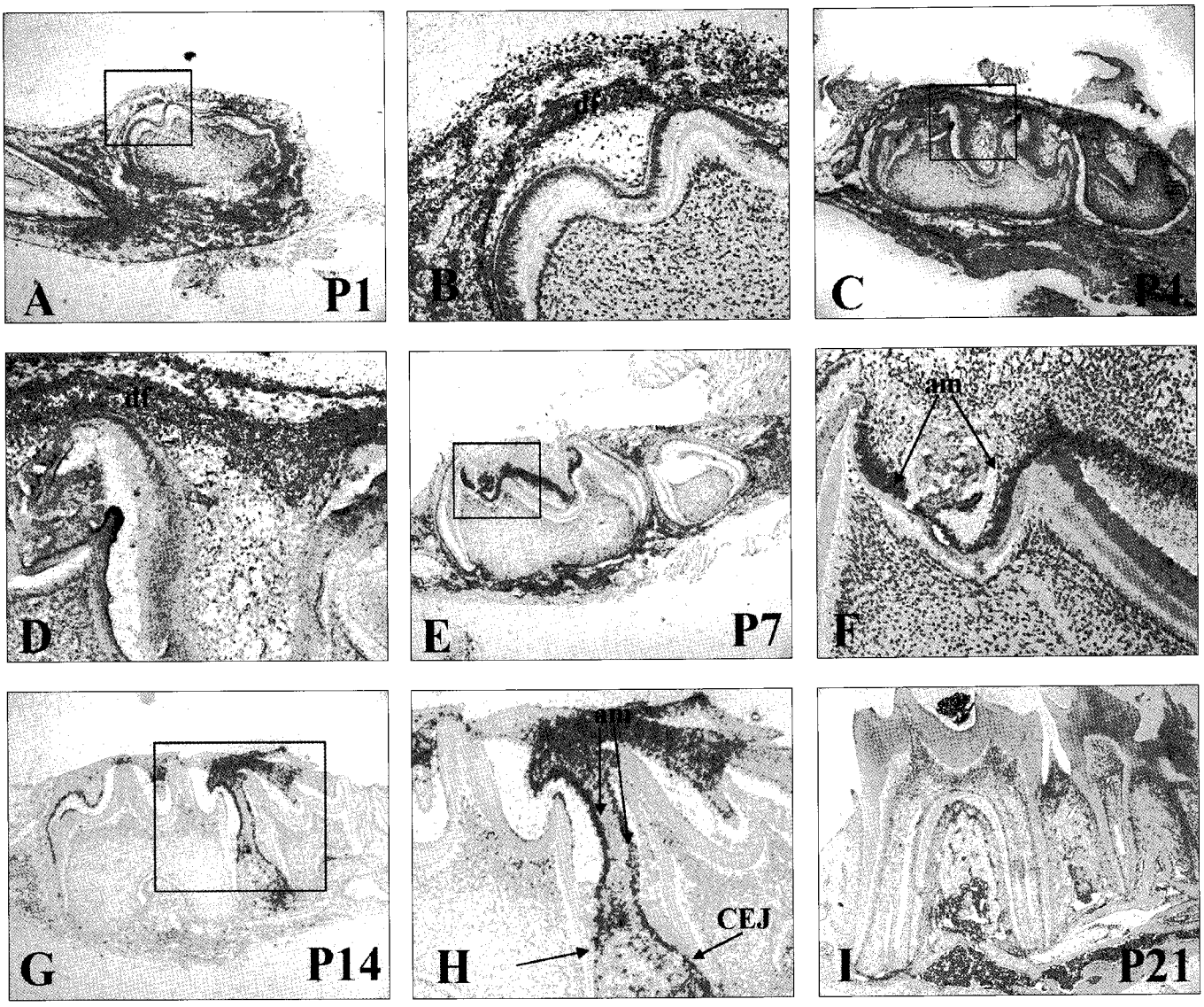

Fig. 2. Runx2-full length expression in mouse first molar and surrounding tissue during postnatal development.
A and C, At P1 and P4, mRNA of Runx2-full length is expressed in dental follicle and surrounding tissue (boxed area in A and C shown in higher magnification in B and D). E, At P7, mRNA of Runx2-full length is expressed in ameloblasts of occlusal surface of enamel and bone area under the tooth (boxed area in E shown in higher magnification in F). G, At P14, in comparison with previous stage, mRNA of Runx2-full length is expression ameloblasts of proximal surface of enamel (boxed area in G shown in higher magnification in H). I, At P21, mRNA of Runx2-full length is observed in bone area. ab, ameloblasts; CEJ, cementoenamel junction.

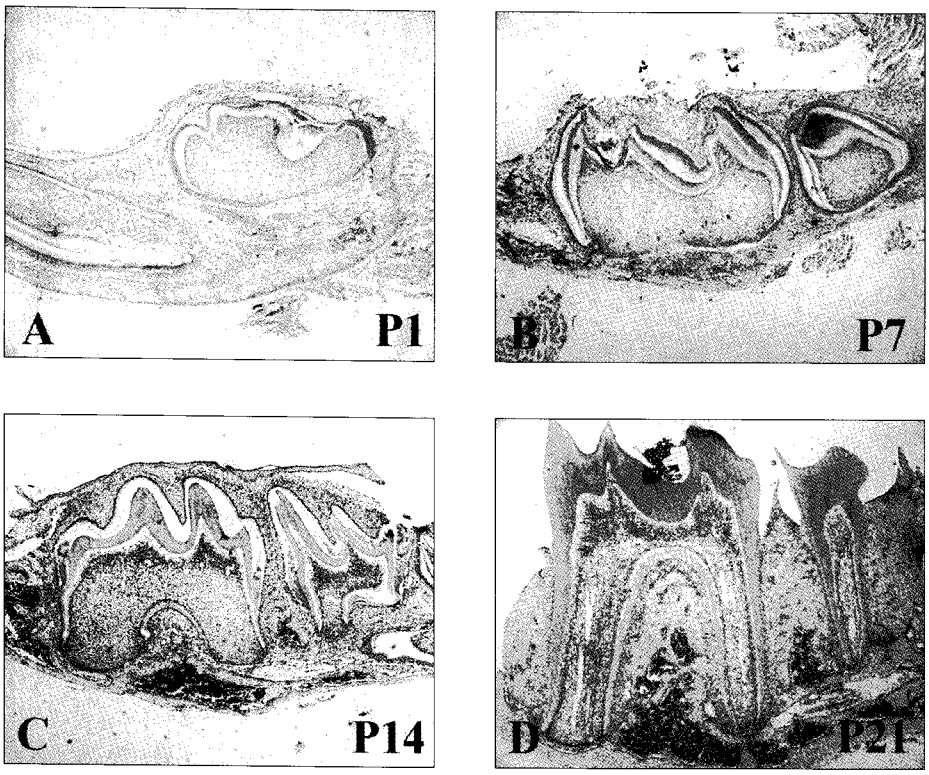

Fig. 3. Runx2-typeII expression in mouse first molar and surrounding tissue during postnatal development
A and B, At P1 and P7, mRNA of Runx2-typeII is not expressed. C and D, At p14 and P21, mRNA of Runx2-typeII is expressed in the bone area.

enamel (Fig. 2G, H). At postnatal day 21 it's expression is observed only in bone area (Fig. 2I).

3. Expression of Runx2-typeII

At postnatal day 1 and 7, mRNA of Runx2-typeII is not expressed (Fig. 3A, B). At postnatal day 14 and 21, it's expression is observed in the bone area (Fig. 3C, D).

Ⅳ. Discussion

Runx2 is a transcription factor which is essential for bone formation and osteoblasts differentiation and it is reported that haploinsufficency of Runx2 gene causes cleidocranial dysplasia (CCD). Clinical features of CCD patients are hypoplasia of clevicle and delayed ossification of cranial sutures. In dentition, deformation of permanent tooth of enamel surface, supernumerary teeth and delayed eruption of permanent teeth are observed. The aim of this study is to know the role of Runx2 by the analysis of the expression of Runx2 (full length and typeII) mRNA during tooth development and eruption periods by in situ hybridization. In result of ^{35}S-UTP labeled in situ hybridization, there are different expression pattern in each period.

In our study, HE staining revealed that root formation was achieved and at postnatal day 7 and mRNA of Runx2-full length is expressed in dental follicle at post natal day 1 and 4. The fact that Runx2-full length is expressed in dental follicle prior to root formation suggests that Runx2 takes part in tooth eruption.

Dental follicle is originated cranial neural crest mesenchyme and composed of a loose connective tissue surrounding the enamel organ of each tooth. Its main function to tooth eruption is the coordinated enlargement of the eruption pathway and formation of bone in the base of the bony crypt[25]. In other words, bone remodeling is achieved by dental follicle during tooth eruption. The experiment of dog premo-

lars[23] and the fact that CSF-1 and MCP-1 which recruit the osteoclast precursors has been detected in dental follicle[26-28] emphasize its function to tooth eruption. Runx2 regulates receptor activator of NF-KB ligand (RANKL)-RANK which is essential for osteoclastogenesis and OPG which inhibits RANKL-RANK signaling[29]. Also it has reported that Runx2-/- mice showed a lack of osteoclasts. So we suggests that a lack of Runx2 in dental follicle induces a low activity of osteoclastogenesis and it leads a failure of formation of eruption pathway.

In root formation, both isoforms show no expression. In fact, when root length of the normal permanent teeth reaches about one third of its final length, if overlying bone and primary teeth should be removed, we can expect of normal eruption of permanent tooth in CCD patients[30].

At postnatal day 7, while mRNA of Runx2-full length is expressed in ameloblasts of occlusal surface of enamel, one week later it is expressed in ameloblasts of proximal surface of enamel just above CEJ. This result suggests that differentiation of ameloblasts does not occur at the same time in the whole area. It is reported that the differentiation of ameloblasts is induced by odontoblasts and the odontoblasts differentiation always starts from the tips of the cusps where the enamel knot, a signaling center, is located[31]. So ameloblasts differentiation occur in turns of occlusal surface and proximal surface. A histopathological and analytical study of a permanent tooth from a patient with CCD shows enamel hypoplasia[12]. And our data shows that mRNA of Runx2-full length is expressed at postnatal day 4 and 7 on ameloblasts. So, it is suggested that insufficiency of Runx2 gene can not induce normal ameloblasts differentiation in CCD patients.

In mRNA of Runx2-typeII, it is not expressed in dental follicle and ameloblasts but expressed in bone area. These results suggest that Runx2-typeII has a relation with bone formation rather than tooth formation and differentiation of ameloblasts.

In this study, we suggest that Runx2 have a relation of ameloblasts differentiation and an important role to tooth eruption made by dental follicle during intraosseous eruption stage. Also we can confirm that Runx2 has a role to bone formation.

Ⅴ. Summary

Runx2 gene is a transcription factor that belongs to the runt gene family and essential for bone formation and osteoblast differentiation. Runx2-haploinsufficency causes cleidocranial dysplasia (CCD), characterized by hypoplastic clevicle and delayed ossification in cranial sutures. Also in dentition, enamel hypoplasia and delayed eruption are observed in CCD patients. To evaluate the role of Runx2 in the tooth development and eruption, in situ hybridization was performed with mouse mandibles at postnatal stage 1, 4, 7, 14 and 21. mRNA of Runx2-full length is expressed in dental follicle and surrounding tissue at postnatal day 1 and 4, but mRNA of Runx2-typeII is not expressed.

While mRNA of Runx2-full length is expressed in ameloblasts of occlusal surface of tooth crown at post natal day 7, it is expressed in proximal surface just above CEJ at postnatal day 14. Otherwise, mRNA of Runx2-typeII is not expressed in any ameloblasts. At postnatal day 21, mRNA of both full length and typeII are expressed in the bone area. From these data, it is suggested that Runx2-full length has a quite relation with tooth eruption, ameloblasts differentiation and bone formation but Runx2-typeII has a relation with bone formation only.

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Abstract

EXPRESSION PATTERN OF RUNX2 IN MURINE TOOTH DEVELOPMENT

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Runx2 is a transcription factor in homologous with Drosophila runt gene and it is essential for bone formation during embryogenesis and a critical gene for osteoblast differentiation and osteoblast function. Runx2-haploinsufficency causes cleidocranial dysplasia (CCD). CCD is an autosomal-dominant inherited disorder characterized by hypoplastic clevicle and delayed ossification in fontanelles and wormian bones. Dental defects are possibly shown to CCD patients : multiple supernumerary teeth, irregular and compressed permanent tooth crowns, hypoplastic and hypomineralized defects in enamel and dentin, an excess of epithelial root remnants, the absence of cellular cementum, and abnormally shaped roots. In addition, delayed eruption of the secondary dentition is a constant finding.

The aim of this study is to evaluate the role of Runx2 in the tooth development and eruption through analyzing the expression pattern of Runx2 by in situ hybridization during crown (late bell stage) and root formation of tooth, using postnatal day 1, 4, 7, 14 and 21 mice mandibular molar teeth. mRNA of Runx2-full length is expressed in dental follicle and surrounding tissue at postnatal day1 and 4. At postnatal day 7, it is expressed in ameloblasts of occlusal surface of enamel and bone area surrounding the tooth. In comparison with previous stage, at postnatal day 14, it is expressed in ameloblasts of proximal surface of enamel. At postnatal day 21 it's expression is observed only in bone area. mRNA of Runx2-typeII is not expressed At postnatal day 1 and 7. At postnatal day 14 and 21, it's expression is observed in the bone area.

In this study, we suggest that Runx2 have a relation of ameloblasts differentiation and an important role to tooth eruption made by dental follicle during intraosseous eruption stage. Also we can confirm that Runx2 has a role to bone formation.

Key words : Runx2, Dental follicle, Cleidocranial Dysplasia, Tooth Development, Tooth Eruption