Gene Expression of Matrix Proteins during Tooth Germ Development in Cbfa1 Knockout Mice

Kazuo Ichikawa[1)], Sathi Gul San Ara[1)], Hitoshi Nagatsuka[1)], Ying Hao Su[2)], Yoshiko Kitamura[1)],Junji Shimono[3)], Toshifumi Komori[4)] , Noriyuki Nagai[1)]

1)Department of Oral Pathology and Medicine. Graduate School of Medicine,Dentistry and Pharmaceutical Sciences, Okayama University.
2) Shanghai Medical University, China
3) Japan Institute for Advanced Dentistry, Osaka.
4) Nagasaki University, Dental School.

Abstract: Cbfa1(Runx2) is a well recognized factor for osteoblast differentiation. However its role during odontogenesis is not well known. We examined the morphogenesis of tooth and gene expression of matrix protein in Cbfa1-knockout mice at ed 17.5 and day 0 of birth and compared them with mandible bone development. Incisor tooth germ showed morphological and functional differentiation of odontoblasts with expression of osteopontin and osteocalcin, whereas the mandible bone-forming site displayed lack of osteoblastic differentiation, and absence of osteopontin and osteocalcin expression. Stage-specific and cytodifferentiation differences demonstrated: incisor tooth germ progressed to the bell stage, whereas molar tooth germ showed maturational arrest at bud to cap stage. Present findings suggest that 1. Cbfa1 is associated with morphogenesis of teeth and matrix protein gene expression, 2. Compared to the incisor tooth germ, the molar tooth germ is more strongly subjected to control by Cbfa1, and 3. In Cbfa1-knockout mice, the odontoblast-like cells in the incisor and the spindle cells in the mandible forming region showed different patterns of gene expression of matrix proteins which are common to both teeth and bones.

Key words: Gene Expression; Matrix Protein; Tooth germ; cbfa1; In situ hybridization.

Introduction

Odontogenesis and Osteogenesis are two different complex and highly regulated process two development of tooth and bone. Though these both process is distinct, are closely related in that their matrix producing cells share several molecular characteristics and also there matrix protein are common for bone and tooth. Recently a transcription factor called core binding factor alfa1 (cbfa1) has been identified. It belongs to the RUNX (runt related gene) (1). Cbfa1 determines the differentiation of mesenchymal stem cell to the osteoblastic lineage and directly regulates the bone matrix protein (2). Its key involvement in the signaling network regulating bone formation is further illustrated by the absence of osteoblast differentiation and complete lack of bone formation in cbfa1-knockout mice(3). Studies using cbfa-/- mice disclose that the tooth organs in these animals were severely misshapen and hypoplastic. This observation suggest that cbfa1 is also involved in regulating the morphogenesis of teeth. In the present study we examined the morphogenesis of the incisor and molar tooth germ in cbfa1 knockout mice, in order to elucidate more fully its involvement during odontogenesis.

Materials and Methods:

1.Cbfa1-KO mouse at ed 17.5 and day 0.
2.Hematoxylin and Eosin Staining (H&E)
3.In Situ Hybridization for Collagen type I,Osteonecttin, Osteopontin, Osteocalcin,Amelogenin and Dentin Sialophosphoprotein.

Results and Discussion

Our results showed that the incisor tooth germ at 0d was small and bell-shaped. There was differentiation to columnar ameloblast-like cells in the epithelium side and to odontoblast-like cells in the dental papilla also with predentin formation.Collagen type I, OSN, OPN and OSC signals were observed in the odontoblast-like cells. AG positive signal was seen in the ameloblast-like cells and DSPP signal was found in both ameloblast-like cells and odontoblast-like cells.

In the molar tooth germ at 0d we observed that the tooth germ had been arrested at the bud to cap stage and there was no differentiation to odontoblasts or ameloblasts.Collagen type I signal was observed in cells of the dental papilla and the surrounding mesenchymal cells.But no signals for OSN, OPN, OSC, AG and DSPP were expressed. At the presumptive bone forming region surrounding the Meckel's cartilage, although spindle cells had aggregated in a membrane form, no differentiation to oval shaped osteoblasts was observed. Collagen type I and OSN signals were detected.But signals for osteopontin and osteocalcin were absent.From the proteins common for bone and tooth OPN and OSC were detected neither in the molar nor in bone, but were found in the incisor in which also cellular differentiation had occurred. This suggests a mechanism of cellular differentiation or transcription regulation pathways different from Cbfa1 in the incisor odontoblast, determining the expression of OPN and OSC.From this results we can conclude that: The transcription factor Cbfa1 is associated with tooth morphogenesis, cellular differentiation and matrix protein gene expression. The difference in matrix protein expression suggests that this function is stronger in the molar tooth germ and that another transcription factor is involved in the incisor tooth germ.

References

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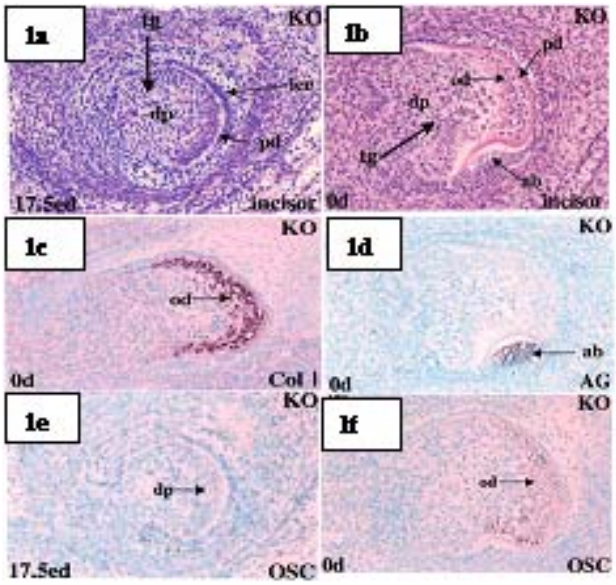

Fig.1a- shows at ed 17.5, the mandibular incisor tooth germ is bell shaped and small in cbfa1-knockout mice
Fig.1b- shows in 0days bell shaped tooth germ also present but large mRNA expression in mandibular incisor tooth germ in cbfa1 knockout mice.
Fig. 1c- at 0 days collagen type 1 in odontoblast like cell
Fig. 1d- Amelogenin detected at 0 day.
Fig. 1e & 1f- Signal for osteocalcin is detected at ed 17.5, and 0 days.