〔Original〕

Gene expression of insulin-like growth factor family during tooth development of the mouse

Yuko SUZUKI[1)], Nobuko OBARA[2)], and Shunichi SHIBATA[3)]

1）Division of Biostatistics, Department of Clinical Psychology, School of Psychological Science, Health Sciences University of Hokkaido
2）Division of Histology, Department of Oral Growth and Development, School of Dentistry, Health Sciences University of Hokkaido
3）Maxillofacial Anatomy, Department of Maxillofacial Biology, Graduate School, Tokyo Medical and Dental University

Abstract

The organogenesis of tooth germs requires epithelial−mesenchymal interactions, which are mediated by cytokines and growth factors such as insulin−like growth factors (IGFs). The expression of IGFs and IGF action−regulating IGF−binding proteins (IGFBPs), in the developing mouse molar teeth was determined by *in situ* hybridization. *IGF−I* receptor (*IGF−IR*) was detected in the enamel organ and mesenchyme ; and *IGF−I*, *IGF−II*, in the enamel organ at the cap stage of E14 mouse embryos. Also, *IGFBP−2*, *−3*, *−4*, and *−5* were expressed at this stage : *IGFBP−2* was expressed in the whole enamel organ, whereas *IGFBP−3* and *−5* were expressed only in a part of it. In the mesenchyme surrounding the enamel organ, *IGFBP−3* and *−4* were expressed. At the late cap stage of E15 embryos, *IGFBP−2* was expressed in the inner and outer enamel epithelium ; and *IGFBP−5*, in the outer enamel epithelium. At the bell stage from E17 to P0, *IGF−II* was detected in the cervical loop and odontoblasts. *IGF−IR* was expressed weakly in the inner enamel epithelium. *IGFBP−2* expression was observed in the inner and outer enamel epithelium, and then became restricted to the inner enamel epithelium at P0. At this stage, *IGFBP−5* was expressed in the outer enamel epithelium. *IGFBP−3* was detected in the dental follicle, and *IGFBP−4* was found in both the dental pulp and dental follicle. At the crown formation stage from P0 to P13, *IGF−II* and *IGF−IR* were detected in the ameloblasts and odontoblasts. *IGFBP−2* and *−5* were expressed in the stellate reticulum. *IGFBP−5* was detected in the dental pulp from P9 to P13. At the root formation stage from P5 to P13, strong expression of *IGF−IR* was observed in Hertwig's epithelial root sheath (HERS). Also, *IGFBP−2* and *−5* were expressed in it. These data suggest a role for these molecules as local mediators of tooth growth and differentiation.

Key words：*IGF−I*, *IGF−II*, *IGF−IR*, *IGFBP*, *in situ* hybridization, molar tooth germ

Introduction

The insulin−like growth factor (IGF) system is known to be essential for normal growth and development. In the tooth morphogenesis, IGF−I appears to regulate the proliferation and differentiation : immunoreactivity for IGF−I is observed in the enamel organ and mesenchyme at the cap stage, and in the inner and outer enamel epithelium at the bell stage in the rat molar. Differentiating ameloblasts, and secretory ameloblasts and odontoblasts also express IGF−I in rat incisors at embryonic day (E) 13−E19 (Joseph et al., 1993 ; 1994a). As detected by *in situ* hybridization, IGF−I signals are expressed in the apical loop and secretory ameloblasts as well as in the odontoblasts in the rat incisor (Joseph et al., 1996). IGF−I also aids in crown formation and in the enlargement of the size of tooth germs (Young, 1995). In the root formation stages, immunoreactivity for IGF−I is observed in Hertwig's epithelial root sheath (HERS ; Symons et al., 1996). In *in vitro* organ cultures of postna-

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tal day (P) 5 mouse molars, exogenous IGF−I promotes active root growth (Fujiwara et al., 2005), suggesting that its receptor, IGF−IR, is expressed in HERS. Also, IGF−IR is expressed in the secretory ameloblasts, odontoblastic epithelium, and mesenchyme in rat incisors (Joseph et al., 1994b ; 1999). Moreover, Yamamoto et al. (2006) showed that IGF−II and IGF−IIR were expressed in the apical loop of the incisors, inner and outer enamel epithelium, and secretory and mature ameloblasts in the rat incisor. Treatment of cultured mouse first molar tooth germs with insulin, IGF−I, and IGF−II increased the synthesis of amelogenin and ameloblastin (Takahashi et al., 1998), which are enamel −specific gene products.

The IGF family is associated with high−affinity IGF−binding proteins (IGFBPs). The actions of the IGFs appear to be regulated and coordinated by these IGFBPs, which are thought to have 4 major functions that are essential to the regulation and coordination of the biological activities of IGFs. That is, IGFBPs are considered 1) to act as transport proteins in the bloodstream and to control the efflux of IGFs from the vascular space ; 2) to prevent IGFs from being degraded and to prolong their half−lives ; 3) to provide a means of tissue and cell type−specific localization, and 4) to directly modulate the interaction of the IGFs with their receptors and thereby indirectly control their biological actions (Swisshelm et al., 1995 ; Burger et al., 1998 ; Duan, 2002). Recent evidence suggests that IGFBPs can also have direct IGF−independent actions on various cellular functions : IGFBP−4 caused a marked inhibition of ceramide −induced apoptosis of human breast cancer cells, which lack IGF−IR (Zhou et al., 2003).

Little is yet known about the expression and localization of IGFBPs during tooth development. In previous studies, we showed by *in situ* hybridization that *IGFBPs* were expressed in developing submandibular and von Ebner's glands, in developing lingual papillae, in the olfactory epithelium and in the taste buds, suggesting that they regulate the morphogenesis of these tissues (Suzuki, 2005 ; Suzuki et al., 2005 ; Suzuki, 2006 ; Suzuki, 2007). Moreover, the expressions of mRNAs of *IGF−I*, *IGF−II*, and *IGF−IR* in developing mouse molars remained unknown. Therefore, the goal of this study was to examine the temporal and spatial pattern of *IGFs* and *IGFBPs* during the development of the murine molar tooth.

Materials and methods

Animals and tissues

Timed pregnant ddY mice, whose embryos were considered as being at day 0 on the morning of discovery of the vaginal plug, were obtained from Sankyo Laboratories (Tokyo, Japan). They were maintained in a heat− and humidity−controlled vivarium with food and water provided *ad libitum*. Experimental protocols concerning animal handling were reviewed and approved by the Animal Ethics and Research Committee of the Health Sciences University of Hokkaido.

To obtain embryos, pregnant females were killed by cervical dislocation and carefully dissected out their uteri bearing fetuses (E14−E17). Postnatal mice at P0, P5, P9, and P13 were killed by an overdose injection of Nembutal (Dainippon Pharmaceutical, Osaka, Japan). For *in situ* hybridization, the mandibles were fixed with 4% paraformaldehyde in 0.1M phosphate buffer (pH7.4) overnight at 4℃. Each specimen was washed in phosphate−buffered saline (PBS) solution, cryoprotected with 25% sucrose, and embedded in OCT compound (Oken, Tokyo, Japan). The tissues were sectioned coronally at a thickness of 8−10 μm, and the sections were then collected and placed on silane−coated slides.

RNA probes and *in situ* hybridization

cDNA fragments of IGF−I, IGF−II, IGF−IR, IGFBP−2, IGFBP−3, IGFBP−4, IGFBP−5, IGFBP−6, and IGFBP−7 genes were generated by the reverse transcription polymerase chain reaction (RT−PCR) by using total RNA extracted from the tongue and then used for the synthesis of cRNA probes. The sequences of the primers were as follows : 5'−TCTCTCTCTGGCCGACGAGT−3' and 5'−GAGCAGAAGTCACCGAATCG−3' (977 bp, AF056187) for IGF−IR, 5'−GTGGATGCTCTTCAG TTCGT−3' and 5'−ACACTCCTAAAGACGATGTT−3' (520 bp ; NM010512) for IGF−I, 5'−ATTGGGTTCCCC TCGTTAGC−3' and 5'−GTATCTGGGGAAGTCGTCC G−3' (950 bp, NM010514) for IGF−II ; 5'−GACGCTA CGCTGCTATCCCA−3' and 5'−GTCTCCTGCTGCTC GTTGTA−3' (614 bp ; NM008342) for IGFBP−2 ; 5'−G GAAACATCAGTGAGTCCGA−3' and 5'−GCTGAGG

CAATGTACGTCGT−3' (458 bp ; X8158.1) for IGFBP −3 ; 5'−GGAGAAGCCCCTGCGTACAT−3' and 5'−AC CCCTGTCTTCCGATCCAC−3' (434 bp ; X76066) for IGFBP−4 ; 5'−AGTAACGTTGAGTGACGCGT−3'and 5'−CAGTGTTGGGGGTGCGTACT−3' (750 bp ; L 12447) for IGFBP−5 ; 5'−TAATGCTGTTGTTCGCTG CG−3' and 5'−CACTGCTGCTTGCGGTAGAA−3' (552 bp ; NM008344) for IGFBP−6 ; and 5'−AAGGTC CTTCCATAGTGACG−3' and 5'−CAGGGTTATAGCT GTCGGCT−3' (439 bp ; NM008048) for IGFBP−7. The specificity of cDNA of these riboprobes was confirmed by the BLAST analysis. The PCR was carried out for 35 cycles. Each resulting fragment was cloned into *Hin*d III/*Eco*RI sites of pT7/T3 DH5α−FT™ (Invitrogen, Tokyo, Japan) and sequenced. Digoxigenin (DIG) −labeled antisense and sense probes were produced by use of a RNA transcription kit (Roche Diagnostics, Mannheim, Germany).Sections were washed in PBS and then treated for 20 minutes with 0.2 N HCl and thereafter for15−20 minutes with proteinase K (1μg/ml in PBS, Takara, Kyoto, Japan) at 37℃. Next, the sections were washed in PBS, and refixed with 4% paraformaldehyde in 0.1M phosphate buffer for 20 minutes. After having been washed twice in PBS, the sections were air−dried and hybridized. Hybridization was performed at 47℃ for 16 hours with RNA probes in a hybridization solution containing 50% formamide, 0.3M NaCl, 0.02M Tris −HCl, 1mM EDTA, 10% dextran sulfate, 1X Denhardt's solution, 1mg/ml yeast tRNA and 0.02% SDS. Hybridized sections were washed for 1 hour at 47℃ in a solution containing 50% formamide and 2XSSC, and thereafter twice in 2XSSC for 5 minutes each time. Then, they were treated with 20μg/ml of RNase (TypeII−A, Sigma) at 37℃ for 30 minutes, and washed at 47℃ in 50% formamide/2XSSC followed by 50% formamide/1 XSSC, each for 1 hour. After having been washed 3 times in PBS, the sections were incubated with 1% blocking reagent (Boeringer Manheim GmbH, Manheim, Germany) in maleic acid buffer (pH7.5) for 1 hour at room temperature. Subsequently, they were incubated overnight at 4℃ with alkaline phosphatase−conjugated anti−DIG Fab fragments (Boeringer) diluted 1 : 500 in PBS. After 3 washes in PBS, chromogenic reactions were carried out by using NBT/BCIP (Boeringer).

Results

Prior to detection of *IGFBPs*, we examined the expressions of *IGF−I*, *−II* and *IGF−IR* in developing mouse lower first molars thoroughly. In the cap stage at E14, *IGF−IR* was expressed in the enamel organ and mesenchyme (Fig.1A). At this stage, *IGF−I* expression was weak and only observed in the enamel organ (Fig.1 B). Also, *IGF−II* expression was weakly observed in the enamel organ. Strong expression of *IGF−II* was observed in mesenchyme cells surrounding Meckel's cartilage (Fig.1C). From late cap stage at E15 to the bell stage at E17, the enamel organ differentiated into the inner and outer enamel epithelium, the stellate reticulum, and the stratum intermedium. The inner enamel epithelium proceeded down in the cervical direction to form the cervical loop ; and, in particular, there was intense expression of *IGF−II* in it (Fig.1D). At P0, the tooth germs increased in size and the inner enamel epithelial cells differentiated into ameloblasts. Also, the odontoblasts became differentiated ; and dentin was formed near the cusp tips. At this stage, *IGF−IR* was expressed weakly in the inner enamel epithelium (Fig.1E). Also, *IGF−I* was expressed weakly in the inner enamel epithelium (Fig.1F). *IGF−II* expression was observed in the cervical loop, in the odontoblasts, and in the mesenchymal cells of the dental follicle (Fig.1G). At P5−P9, enamel and dentin were formed in the tooth crown. In the tooth root, the inner and outer enamel epithelium formed the double−layered HERS, which proliferated apically and directed root morphogenesis. *IGF−II* was expressed in the odontoblasts and weakly in the ameloblasts at P5 (Fig.1H). At that time weak *IGF−IR* expression was also observed in the ameloblasts and in the odontoblasts. Strong expression was observed in the HERS at P9 (Fig.1I). *IGF−I* signals disappeared during postnatal development.

Among *IGFBP* mRNAs, *IGFBP−1* mRNA is detectable only in adult liver tissue (Suzuki et al. 2005), and so it was not examined in the present study. *IGFBP−6* and *−7* were not detected in tooth specimens by the *in situ* hybridization technique. Therefore, expression levels of *IGFBP−2*, *−3*, *−4*, and *−5* mRNAs were examined in the developing teeth. *IGFBP−2* was expressed in the enamel organ in the cap stage at E14 (Fig.2A). At

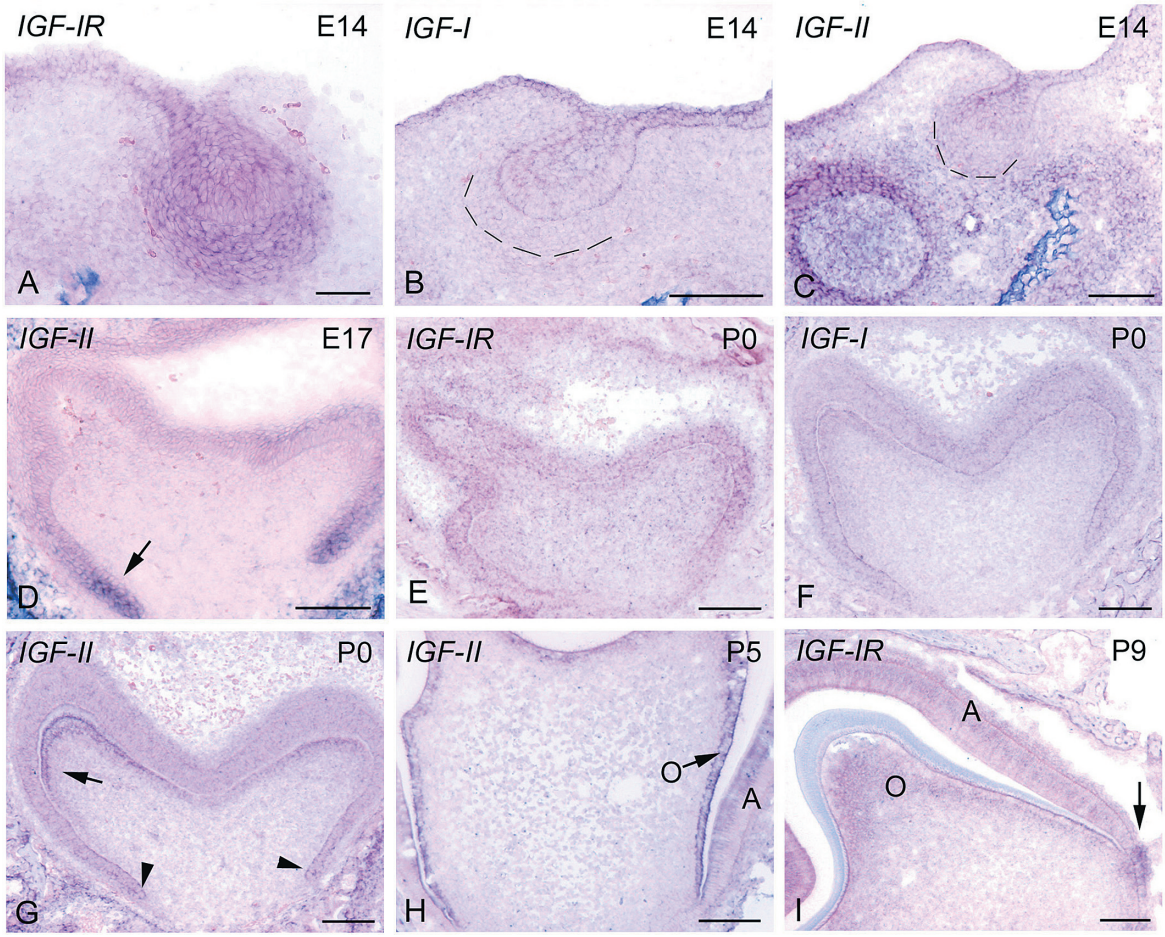

Fig.1 Expression of IGF−I, IGF−II, and IGF−I receptor mRNAs in the developing mouse lower first molars as detected by *in situ* hybridization with RNA probes. **(A)**−**(C)** E14, cap stage showing expression of *IGF−IR* **(A)**, *IGF−I* **(B)**, and *IGF−II* **(C)**. The dashed lines in B and C indicate the boundary of the tooth germ. **(D)** E17, bell stage showing expression of *IGF−II* in the cervical loop (*arrow*). **(E)**−**(G)** P0. bell stage. Weak expression of *IGF−IR* and *IGF−I* is seen in the inner enamel epithelium **(E, F)**. *IGF−II* expression **(G)** is seen in the cervical loop (*arrowheads*) and in the odontoblasts (*arrow*). **(H)** P5. *IGF−II* expression is observed in the odontoblasts (O) and ameloblasts (A). **(I)** P9. Weak *IGF−IR* expression is observed in ameloblasts (A) and odontoblasts (O). Hertwig's epithelial root sheath (HERS, *arrow*) expresses *IGF−IR*. Scale bars=100μm.

this stage, *IGFBP−3* was expressed in a part of the enamel organ and in mesenchymal cells in the dental follicle (Fig.2B). *IGFBP−4* was expressed in the mesenchyme of the dental papillae and dental follicle (Fig.2 C). *IGFBP−5* was expressed in the outer enamel epithelium, but not inside of the enamel organ (Fig.2D). At E 15, *IGFBP−2* was expressed in the inner and outer enamel epithelium (Fig.2E), *IGFBP−5* was expressed intensely in the outer enamel epithelium on the lingual side. *IGFBP−2* and *−5* were also expressed in the cervical loop (Fig.2F). At E17, in the bell stage, *IGFBP−2* was expressed in the inner and outer enamel epithelium (Fig.2G). *IGFBP−3* was detected in the outer enamel epithelium and surrounding mesenchymal cells in the dental follicle (Fig.2H) ; and *IGFBP−4* in the mesenchymal cells of the dental papillae and dental follicle. Strong expression was observed in the pulp horn (Fig.2 I). *IGFBP−5* was expressed in the outer enamel epithelium (Fig.2J). At P0, when crown formation begins, *IGFBP−2* was found in the ameloblasts, stellate reticulum and the outer enamel epithelium (Fig.2K). *IGFBP−3* was expressed in the dental follicle and in blood vessels of the dental pulp (Fig.2L). Also at P0, *IGFBP−4* was seen in the dental pulp and in the dental follicle. In the dental pulp, the expression became weak in the pulp horn, but was observed in the central to basal region (Fig.3A) ; *IGFBP−5* in the outer dental epithelium and surrounding connective tissue (Fig.3B). At P5, *IGFBP−2* expression was similar to that at P0 (not shown). *IGFBP−3* expression became weak and remained only in the mesenchyme of the dental follicle near developing HERS (Fig.3C). Similarly, *IGFBP−4* was observed in

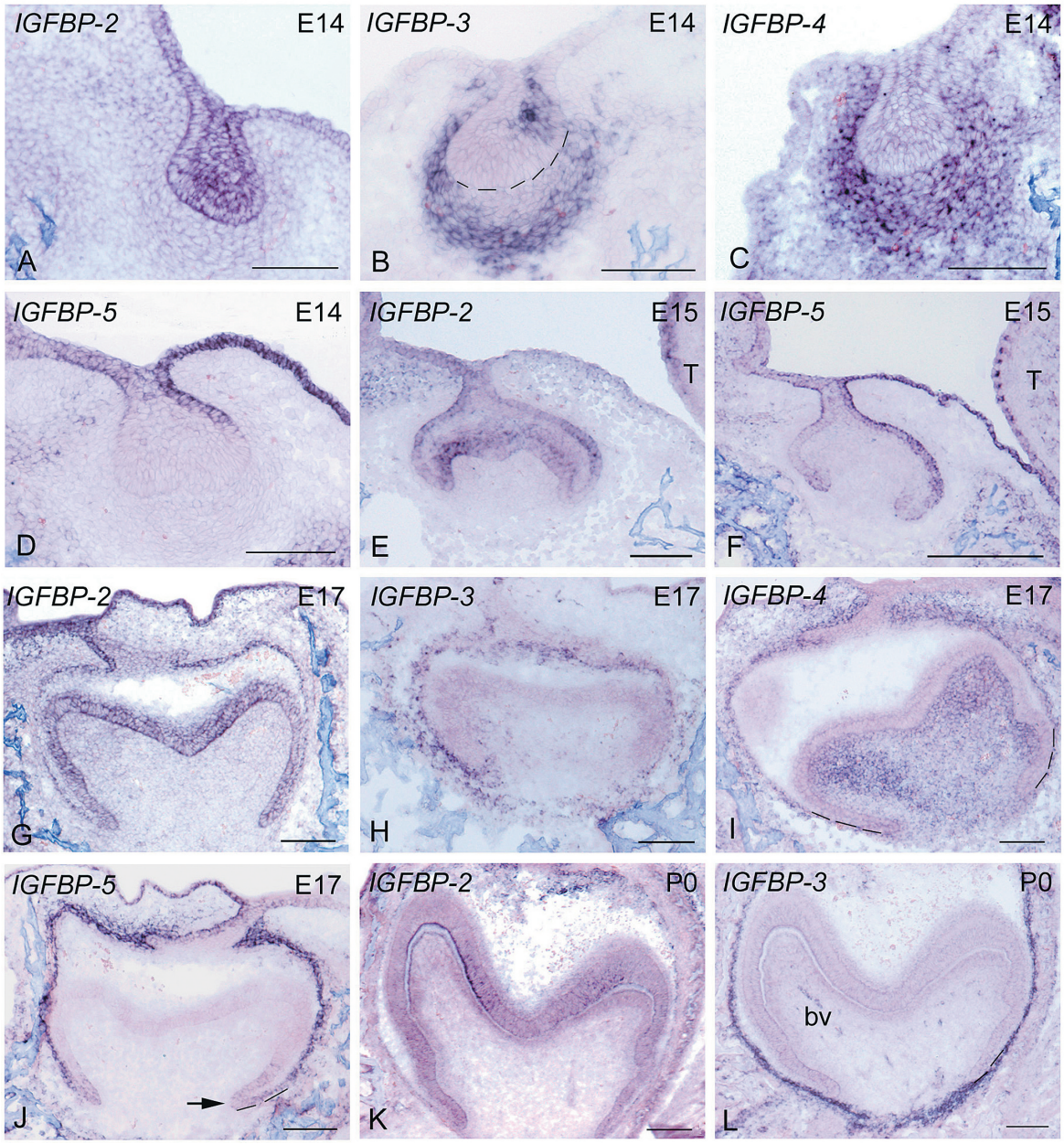

Fig.2 Expression of IGFBP mRNAs from E14 to P0 in mouse lower first molars as detected by *in situ* hybridization with RNA probes. **(A)**−**(D)** E14. cap stage. *IGFBP−2* expression appears in the enamel organ **(A)**. *IGFBP−3* expression is seen in a part of the enamel organ and in the mesenchyme **(B)**. The dashed lines indicate the boundary of the enamel organ. *IGFBP−4* expression is observed in the mesenchymal cells in the dental papillae and dental follicle **(C)**. *IGFBP−5* expression is seen in a part of the enamel organ **(D)**. **(E)**, **(F)** E15. *IGFBP−2* expression is observed in both outer and inner enamel epithelium **(E)**. *IGFBP−5* is expressed in the outer enamel epithelium on the lingual side and in the cervical loop **(F)**. **(G)**−**(J)** E17. bell stage. *IGFBP−2* expression is seen in the inner and outer enamel epithelia **(G)**. *IGFBP−3* is expressed in the outer enamel epithelium and in the dental follicle **(H)**. *IGFBP−4* expression is seen in the dental papillae and dental follicle. The dashed lines indicate the border between the cervical loop and mesenchyme **(I)**. *IGFBP−5* is found in the outer enamel epithelium and cervical loop **(J**, *arrow*). The dashed line indicates the border between cervical loop and mesenchyme **(J)**. **(K)**, **(L)** P0. *IGFBP−2* is expressed in the ameloblasts and stellate reticulum **(K)**. *IGFBP−3* is detectable in the surrounding mesenchyme and in blood vessels (bv). The dashed lines indicate the border between the cervical loop and mesenchyme **(L)**. T tongue. Scale bars=100μm.

the dental follicle and weakly in the dental pulp (Fig.3 D). *IGFBP−5* expression was similar to that at P0 (not shown). At P9, *IGFBP−2* was strongly expressed in the stellate reticulum and weakly in the odontoblasts and ameloblasts (Fig.3E). At this stage, *IGFBP−5* was expressed at the cell−rich zone of the dental pulp and in the stellate reticulum (Fig.3F). *IGFBP−3* and *−4* expression levels had become undetectable at this stage. In the root formation stage, which is from P5 to P13, *IGFBP−5* was expressed in the developing HERS and

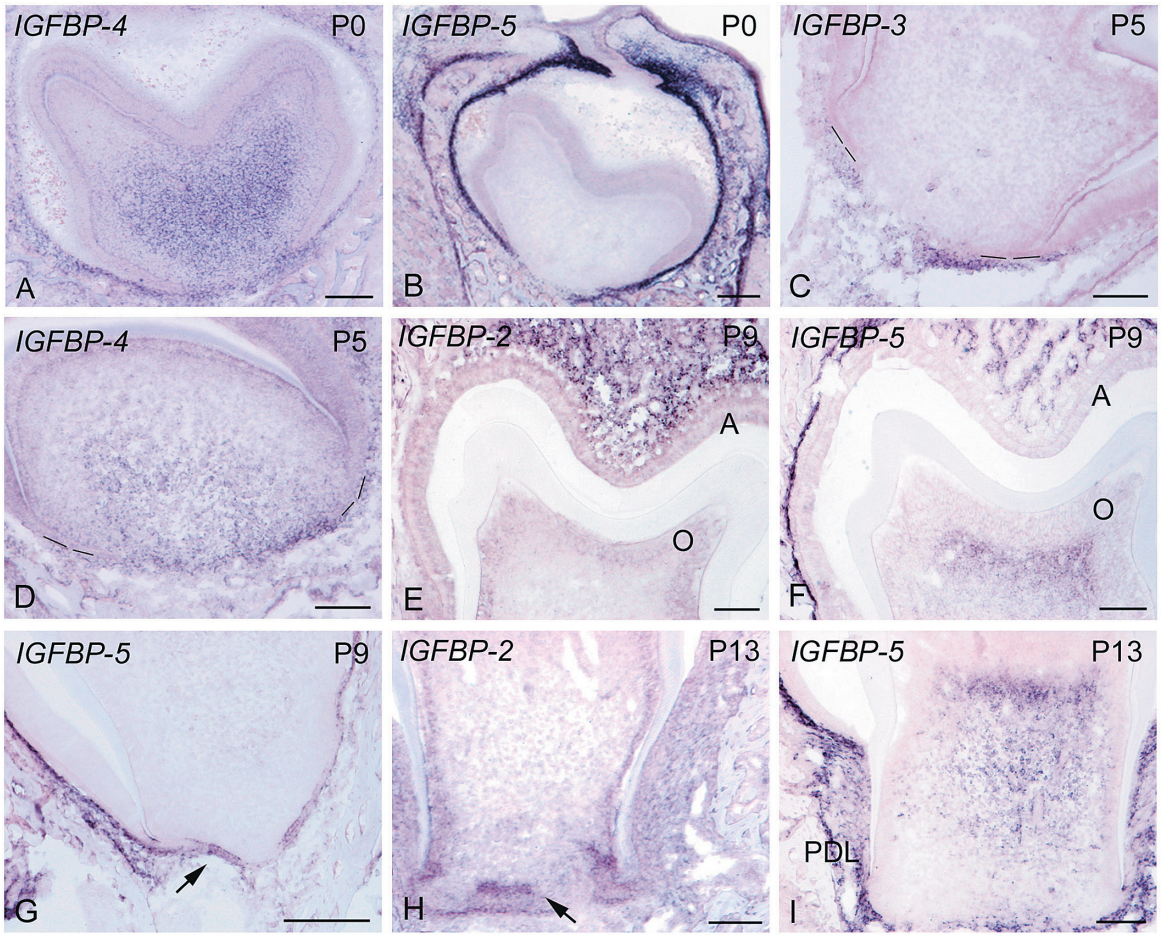

Fig.3 Expression of IGFBP mRNAs from P0 to P13 in mouse lower first molars as detected by *in situ* hybridization with RNA probes. **(A)**, **(B)** P0. *IGFBP−4* expression is seen in the dental pulp and dental follicle **(A)**. *IGFBP−5* expression is detectable in the outer enamel epithelium, and surrounding connective tissue **(B)**. **(C)**, **(D)** P5. *IGFBP−3* expression is seen in the basal region of the dental follicle, and in blood vessels. The dashed lines indicate the border between the cervical loop and mesenchyme **(C)**. *IGFBP−4* is expressed in the dental pulp and the dental follicle. The dashed lines indicate the border between cervical loop and mesenchyme **(D)**. **(E)**−**(G)** P9. *IGFBP−2* expression is evident in the stellate reticulum **(E)**. *IGFBP−5* is seen in the surface of dental pulp, the stellate reticulum **(F)**, and in HERS **(G**, *arrow***)** and the surrounding connective tissue. **(H)**, **(I)** P13. *IGFBP−2* expression is detectable in HERS **(H**, *arrow***)**; and *IGFBP−5* expression is seen in the dental pulp, HERS, and periodontal tissue (PDL , **I**). A ameloblast, O odonotoblast. Scale bars=100μm.

surrounding connective tissue (Fig.3G). At P13, *IGFBP−2* was found in HERS (Fig.3H). *IGFBP−5* expression remained detectable in the dental pulp (Fig.3I) and HERS (not shown). Also, *IGFBP−5* was expressed in the periodontal tissue (Fig.3I). Sections incubated with sense riboprobes of *IGFBP−2*, *−3*, *−4*, and *−5* displayed no reactivity (not shown). These results are summarized in Table1.

Discussion

In situ hybridization was used to examine the expression of *IGF−I*, *−II*, and *IGF−IR* in mouse mandibular first molars at various stages of tooth development. Although the intensity of the hybridization signal varied with the developmental stage, the distribution was consistent with that found in previous studies using immunohistochemistry or rat incisor specimens (Joseph et al., 1993, 1994a, b, 1996, 1999 ; Young, 1995 ; Symons et al., 1996 ; Yamamoto et al., 2006). However, *IGF−I* signal was weak in the present study, thus it was not detected in mesenchyme at the cap stage, in ameloblasts, odontoblasts, and HERS at the crown and root-formation stages. Since the expression of *IGF−IR* in HERS was suggested in *in vitro* organ cultures (Fujiwara et al., 2005), the signal could be detect for the first time in the present study.

The present study revealed that numerous IGFBPs were synthesized locally, suggesting that these molecules may modulate IGF action during tooth development. A specific pattern of IGFBP expression was ob-

Table 1. Summary of IGFBP expression pattern in the developing mouse teeth. Relative levels of expression (from +/− to ++) are based on hybridization intensity of sections, i.e., (++)=signal intense, (+)=signal moderate, (+/−)=signal weak. (−)= signal low to undetectable. A, ameloblast ; E, enamel organ ; DP, dental papilla (dental pulp) ; DF, dental follicle ; HERS, Hertwig's epithelial root sheath ; O, odontoblast ; PDL, periodontal tissue

	E14			E17–P0			P5				P9–P13				
	E	DP	DF	E	DP	DF	A	O	DP	DF	A	O	HERS	DP	PDL
IGFBP–2	++	−	−	++	−	−	+	+	−	−	+/−	+/−	++	−	−
IGFBP–3	+	−	++	+	−	+	−	−	−	+	−	−	−	−	−
IGFBP–4	−	++	++	−	++	++	−	−	+	+	−	−	−	+	+
IGFBP–5	+	−	−	+	−	−	+/−	+/−	+	+	−	−	++	++	++

served during the embryonic stages ; i.e., *IGFBP–2* and *–5* mRNAs were localized in the developing enamel organ, whereas *IGFBP–4* mRNA was found exclusively in the mesenchyme. *IGFBP–2* and *–5* may facilitate receptor binding, resulting in IGF−induced protein and DNA synthesis (Werner & Katz, 2004). Their expression often overlaps in the same cells or tissue or are in adjacent tissues, e.g., taste buds, submandibular and von Ebner's glands (Suzuki et al., 2005 ; Suzuki, 2006), lung alveolae (Schuller et al., 1993), and ectoderm of limb buds and astrocytes (Green et al., 1994). In the present study, *IGFBP–2* mRNA was expressed in the inner and outer enamel epithelium in embryonic stages and became expressed in the ameloblasts, stellate reticulum and in HERS at postnatal stages. *IGFBP–5* was detected in the outer enamel epithelium at embryonic stages and in stellate reticulum and in HERS at postnatal stages. Therefore, in HERS, these binding proteins were expressed together with *IGF–IR*. This may promotes IGF action on the root growth. In contrast, *IGF–I*, *–II*, and the receptors were not expressed in the stellate reticulum in the present study and in the previous studies (Joseph et al., 1994a, b, 1999 ; Yamamoto et al., 2006), *IGFBP–2* and *–5* may have IGF−independent actions (Zhou et al., 2003) in the stellate reticulum. Moreover, the *IGFBP – 5* expression pattern was unique ; i.e., this binding protein was expressed not only in tissues that originated from the enamel organ, but also in the dental pulp and periodontal connective tissue in postnatal stages.

In adult serum, IGFBP−3 is the major IGF carrier protein (Clemmons, 1992) : and it is also expressed in developing peripheral tissues, such as the dermal papillae of lingual filiform papillae (Suzuki, 2005) and hair follicles (Batch et al., 1996). Most recent study revealed that porcine enamel organ−derived epithelial cells cultured on vitronectin−IGF−I−IGFBP−3 complex differentiated into ameloblasts−like cells that were able to secrete amelogenin proteins and form enamel−like tissues *in vivo* (Shinohara et al., 2012). In the present study, *IGFBP–3* was expressed not only in the dental follicle, but also in a part of the enamel organ at the cap stage, and the outer enamel epithelium at the bell stage. Also, *IGF–I* was expressed in the enamel organ. Therefore, *IGFBP–3* together with *IGF–I* may promote differentiation of the enamel organ.

IGFBP−4 is the member of the IGFBP family that appears to have an inhibitory effect on IGF action. The mechanism was shown to be the binding of IGFBP−4 to secreted IGFs, preventing their interaction with IGF receptors (Zhou et al., 2003). *IGFBP−4* together with other IGFBPs, such as *IGFBP–3* and *IGFBP–5*, is suggested to have a role in maintaining or causing the disappearance of mesenchyme and connective tissue of the tooth germs. In humans, other IGFBPs are suggested to act as anti−proliferative molecules suppressing the mitogenic effects of IGFs ; i.e., in deciduous teeth, the epithelial cells of Malassez are immunoreactive with antibodies against IGFBP−4 and −6, and the latter molecule inhibits IGF−II proliferative function (Götz et al., 2003). Human IGFBP−5 has no effect on proliferation but is anti−apoptotic and associated with the migration of gingival epithelial cells (Hung et al., 2008).

In conclusion, *IGFBP−2*, *−3*, *−4*, and *−5* locally expressed in the epithelium and mesenchyme of tooth germs, may play a role in tooth growth and differentiation.

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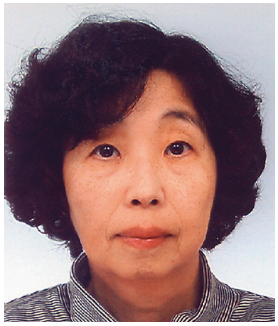

鈴木　裕子

昭和47年３月　弘前大学理学部生物学科卒
昭和49年３月　北海道大学大学院理学研究科動物学専攻修士課程修了
昭和54年９月　同上博士課程修了
昭和54年10月　北海道大学理学部研究生
昭和55年４月　北海道医療大学歯学部口腔解剖Ⅱ助手
昭和58年４月　同上講師
昭和60年４月　同上助教授
平成23年４月　北海道医療大学心理科学部臨床心理学科（大学教育開発センター）教授