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법랑질 형성과정에서 MMP-20의 조절을 통한 ODAM의 법랑질 석회화 조절

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치의생명공학과

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목 차

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ABSTRACT

ODAM modulates enamel mineralization through the regulation of MMP-20 expression during amelogenesis

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It was previously hypothesized that odontogenic ameloblast-associated protein (ODAM) plays a functional role in the maturation of enamel for proper mineralization. Therefore, it was examined whether ODAM modulates enamel mineralization through the regulation of MMP-20 expression in this study. We investigated the expression of ODAM and MMP-20 during amelogenesis in normal and MMP-20-deficient mice *in vivo* and *in vitro*. ODAM is weakly expressed in secretory ameloblasts and strongly expressed in maturation-stage ameloblasts. The expression level of ODAM does not differ between wild-type and MMP-20-deficient ameloblasts. In ameloblast-lineage cells (ALCs), the expression of ODAM correlates with MMP-20 expression between four and seven days in culture. This time frame overlaps with the secretory stage of amelogenesis. *ODAM* mRNA was found in various cancer cells including gastric cancer. However, *ODAM* mRNA was not expressed in normal oral

epithelial cells. The overexpression of ODAM in ameloblasts resulted in up-regulation of the *MMP-20* gene. In contrast, inactivation of ODAM by siRNA abrogated MMP-20 expression. Calcium binding property of rODAM revealed that rODAM clearly bound ^{45}Ca by a dot blot technique. Here, it was also demonstrated the entry of exogenous rODAM into the nucleus and cytoplasm of ALCs. These results suggest that ODAM serves an important regulatory function in the mineralization of enamel through the regulation of MMP-20.

Keywords Ameloblast, ODAM, MMP-20, Mineralization, Regulation

Introduction

Enamel development is a complex biological process consisting of three stages: a secretory, a transition, and a maturation stage. During the secretory stage, tall columnar ameloblasts secrete specialized proteins, including amelogenin (Gibson et al. 2001; Hu et al. 2001), ameloblastin (Fukumoto et al. 2004; Krebsbach et al. 1996), and enamelin (Hu et al. 2001) into the enamel matrix. Two novel molecules, odontogenic ameloblast-associated protein (ODAM) and amelotin (AMTN) have recently been described as members of the secretory calcium-binding phosphoprotein (SCPP) gene cluster (Kawasaki and Weiss 2003, 2008; Sire et al. 2007). The localization of ODAM and AMTN to the extracellular matrix has not yet been demonstrated. Matrix metalloproteinase-20 (MMP-20, also known as enamelysin) and Kallikrein-4 (KLK4) have also been shown to play roles in enamel formation (Lu et al. 2008; Hu et al. 2002). The production of enamel matrix by ameloblasts is essential for enamel formation, but little information is available on the differentiation, maturation, and mineralization of ameloblasts during amelogenesis.

The physiological significance of ODAM is still emerging. The cDNA transcript of ODAM (FLJ20513) was originally cloned from the human KATO III cell line (Sugano et al. 2000; Sekiguchi et al. 1978) and has been observed in calcifying epithelial odontogenic tumor (CEOT)-associated amyloids (Solomon et al. 2003). ODAM was reported to be a gastric cancer-specific gene based on serial analysis of gene expression data (Aung et al. 2006). Rat ODAM protein was identified from the secretome profile of rat enamel organ cells using the signal trap method (Moffatt et al. 2006). ODAM has been shown

to be specifically expressed in ameloblasts during the maturation stage of enamel development. Data also indicate that ODAM involved in ameloblast maturation and enamel mineralization. However, the precise function of ODAM remains largely unknown. In addition to ameloblasts, ODAM is also expressed in odontoblasts, lactating mammary glands, nasal and salivary glands, tongue, and gingiva (Dey et al. 2001; Rijnkels et al. 2003). ODAM expression has been observed in normal and malignant ameloblasts. In addition, ODAM is found in gastric, lung, and breast neoplasias (Kestler et al. 2008). Taken together, these data suggest a broader physiologic role for ODAM.

In ameloblasts and other tissues, the expression of MMP-20 correlates with ODAM expression. MMP-20 is primarily expressed in ameloblasts, although transient expression has also been detected in odontoblasts (Bourd-Boittin et al. 2004). MMP-20 expression has also been detected in pathological tissues, including ghost cells of calcifying odontogenic cysts (Takata et al. 2000a), odontogenic tumors (Takata et al. 2000b), and human tongue carcinomas (Väänänen et al. 2001).

Recently, we reported that ODAM is primarily involved in the maturation of enamel, and is required for enamel mineralization during tooth development. Enamel mineralization is mediated by MMP-20 (Park et al. 2007). Therefore, in the present study, we want to determine whether ODAM regulates the expression of MMP-20 and consequently modulates enamel mineralization.

Materials and methods

1. Immunohistochemistry

All experiments involving animal were performed according to the Dental Research Institute guidelines of Seoul National University. The MMP-20-deficient mice were kindly provided by Dr. John D. Bartlett (Department of Developmental Biology, Harvard School of Dental Medicine). Mandibles and maxillae of mice (postnatal age of one and 16-days) were decalcified in a 10% ethylenediaminetetra - acetic acid (EDTA, pH 7.4) solution at 4°C and processed for immunohisto-chemistry. As described previously, ODAM protein expression was detected using an ABC kit (Vector Lab, Burlingame, CA, USA) with a rabbit anti-rat ODAM antibody (0.2 μg/ml) as the primary antibody and a biotin-labeled goat anti-rabbit IgG (1:200) (Park et al. 2007).

2. Cell culture

Immortalized ameloblast-lineage cells (ALCs) were kindly provided by Dr. T Sugiyama (Akita University School of Medicine, Akita, Japan) and cultured as previously described (Nakata et al. 2003). MDPC-23 odontoblast-like cells (Hanks et al. 1998) were provided by Dr. Hanks and Nör (University of Michigan, MI, USA). Osteoblast-like MG-63 cells (Franceschi et al. 1985) were obtained from ATCC (Rockville, MD, USA). Periodontal ligament (PDL) fibroblasts were obtained from an explant culture of healthy human PDL tissue (Kim et al. 2007). NIH3T3 fibroblast cells (Eschenfeldt and Berger 1986) were also used. In order to observe the expression of ODAM in various cancer cells and normal oral epithelial cells, we have used several

cancer cell lines (Fadu, Head & neck cancer cell line; AGS, Gastric cancer cell line; T24, Kidney cancer cell line; KB, Oral cancer cell line; SH-SY5Y, Neural cancer cell line) and INOK cells (mouse normal oral epithelial cell). Cells were grown and maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS; Gibco BRL), 10 ng/ml recombinant human epithelial growth factor (EGF; Sigma-Aldrich, St. Louis, MO, USA), and antibiotics. Cells were cultured at 5% CO_2 in a 37°C incubator. To induce cell differentiation and mineralized nodule formation, confluent cells were treated with 50 μg/ml ascorbic acid, 10 mM ß-glycerophosphate, and 5 μM dexamethasone for up to two weeks.

3. Transient transfection, RNA extraction, RT-PCR, and real-time PCR analysis

ALCs were plated (2×10^5 cells/ 60-mm dish) and cultured for 24 h until approximate confluency (50%-60%). Cells were transiently transfected with 2 μg of one plasmid; a pCMV-driven ODAM plasmid, a U6-driven ODAM siRNA plasmid, or an empty vector control (mock) (Park et al. 2007). Transfections were performed using the Lipofectamine Plus™ reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Each sample was run in duplicate and each experiment was repeated three times. Forty-eight hours post-transfection, total RNA was isolated using TRIzol (Invitrogen). Total RNA was used for cDNA synthesis and PCR amplification. The primer sequences that were used are as follows: *ODAM,* 5'-ccagcaggctagtcctatgtcctatgtgg-3'/ 5'-cgcgtcgacatgagatcagtg-3'; *MMP-20,* 5'-agctgtgagcaactgatgactgga-3'/5' -acagctagagccaagaacacacct-3'; and *GAPDH,* 5'-accacagtccatgccatcac-3'/5'-

tccaccaccctgttgctgt-3' (forward/reverse). Real-time PCR was performed on an ABI PRISM 7500 sequence detection system with SYBR GREEN PCR Master Mix (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The PCR protocol was as follows: 94°C for 1 min, followed by 40 cycles of 95°C for 15 s and 62°C for 34 s. All reactions were performed in triplicate and normalized to the housekeeping gene, *GAPDH*. Relative differences were calculated using the comparative cycle threshold (C_T) method.

4. SDS PAGE and western blot

Total protein (30 μg) was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Membranes were blocked for 1 h with 5% nonfat dry milk in PBS containing 0.1% Tween-20 (PBS-T) and incubated overnight with primary antibody (ODAM, 1:1500) diluted in PBS-T buffer at 4°C. After washing, membranes were incubated for 1 h with anti-rabbit-igG and goat anti-rabbit-IgG conjugated with horseradish peroxidase (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA).

5. ^{45}Ca binding assay

For the ^{45}Ca binding assay, 1 μg and 10 μg of rODAM was dot-blotted onto a polyvinylidene difluoride membrane (Bio-Rad Lab, Tokyo, Japan). The membranes were washed four times with the solution containing 60 mM KCl and 10 mM imidazole-HCl, pH 7.4, for 15 min, and incubated in the same buffer containing 1 mCi/liter of $^{45}CaCl_2$. After 24 h incubation, the membrane

was rinsed with 50% ethanol for 10 min and air-dried. Autoradiographs of the ^{45}Ca-labeled protein were obtained by exposing the dried membrane overnight to Kodak XAR-5X-ray film. The experiments were carried out twice in duplicate.

6. Expression and purification of recombinant rat ODAM (rODAM)

The coding region of ODAM was amplified by PCR using the following primers: 5'-caggctgctagcatgtcctatgtggttcc-3' and 5'-gtaaactgcagcttatggttctcttaggctatc-3'. The PCR product was cloned into the NheI and PstI sites of pRSET-A (Invitrogen) to generate pRSET-ODAM. The *E. coli* strain, BL21 (DE3) pLysS, was transformed with pRSET-ODAM and cultured at 37°C in Luria-Bertani (LB) medium. At an OD_{600} of 0.6 to 0.8, isopropyl-β-D-thiogalactopyranoside (IPTG; Elpis-Biotech., Inc., Taejeon, Korea) was added to a final concentration of 1 mM and incubated for 3 h. Cells were sonicated in a phosphate lysis buffer with lysozyme. The lysate was purified on Ni-NTA agarose (Qiagen, Inc., Hilden, Germany), washed with 50 mM imidazole and eluted by 250 mM imidazole. All buffers were made according to the QIAexpressionist manual (Qiagen) and contained protease inhibitors (phenylmethanesulphonylfluoride (PMSF), Roche Applied Science, Mannheim, Germany) and β-mercaptoethanol (5 mM, Fluka and Riedel, Buchs, Switzerland) in the native buffers. Fractions were analyzed by SDS-PAGE and western blot using an anti-His tag monoclonal antibody

7. Localization of rODAM in ALCs by immunofluorescence

Recombinant rat ODAM was dissolved in PBS (pH 7.4) to generate a stock solution of 3 mg/ml stock. ALCS were treated with 10 μg of exogenous

ODAM for 4 h. Cells were then fixed with 4% paraformaldehyde in PBS for 10 min, followed by permeabilization with Triton X-100 in PBS, and quenched with NH_4Cl (50 mM) in PBS. Cells were incubated for 1 h with an anti-ODAM antibody, washed with PBS, and incubated for 1 h with a fluorescein-labeled secondary antibody (Vector Lab). The cells were counterstained with DAPI to visualize the nucleus. After rinsing, the cells were examined by confocal microscopy (Olympus, Tokyo, Japan).

8. Statistical analysis

The data were analyzed for statistical significance using a non-parametric Mann-Whitney test.

Results

1. ODAM expression in ameloblasts from wild-type and MMP-20-deficient mice

Previous data have indicated that ODAM is involved in enamel maturation and is required for enamel mineralization during tooth development (Park et al. 2007). Therefore, we sought to determine the protein expression level of ODAM during different stages of ameloblast differentiation. During the presecretory stage, differentiating ameloblasts show no ODAM protein expression (Fig. 1a). However, expression of ODAM in ameloblasts was detected in the cytoplasm and the enamel matrix during the secretory stage. ODAM was deposited adjacent to the distal region of the cell (Fig. 1b). ODAM expression was clearly visible in the cytoplasm of ameloblasts in the postsecretory transition stage, as well as in cells in the underlying papillary layer (Fig. 1c). In maturation-stage ameloblasts, ODAM was highly visible in the supranuclear region and the distal end (Fig. 1d).

In sections from wild-type mice, the enamel matrix was mostly removed in maturation-stage enamel (Fig. 1e). However, in MMP-20-deficient mice, the enamel matrix persisted as an abnormally thick layer of protein along the normal dentin surface (Fig. 1F). ODAM protein strongly localized to the supranuclear region of the ameloblasts as well as to the distal end of the ameloblasts in both wild-type and MMP-20-deficient mice (Fig. 1g, h).

2. ODAM expression in various cancer cells and normal oral epithelial cells

ODAM mRNA was found in various cancer cells, Fadu (Head & neck cancer cell line), AGS (Gastric cancer cell line), T24 (Kidney cancer cell line), KB (Oral cancer cell line), and SH-SY5Y cells (Neural cancer cell line). However, *ODAM* mRNA was not expressed in normal oral epithelial cells (Fig. 2).

3. ODAM is expressed by ameloblasts *in vitro* and regulates MMP-20

Since we observed differences in the enamel matrix between wild-type and MMP-20-deficient mice, we want to examine the correlation between ODAM and MMP-20 *in vitro*. In ALCS undergoing differentiation, a time-dependent expression of both ODAM and MMP-20 was observed. ODAM expression was seen on day 0 and gradually increased with time throughout the culture period (Fig. 3a). By western analysis, ODAM appeared as a doublet at around 35 kDa, which corresponds to previously described reports of two forms of ODAM (Park et al. 2007). MMP-20 expression was also detected on day 0, increased through day 7 of culture, and gradually decreased thereafter.

Next, we wanted to determine the effect of alterations in ODAM expression on MMP-20 expression. Over-expression of ODAM in ALCs resulted in an increase in the expression of *MMP-20* mRNA (Fig. 3b, c). In contrast, siRNA-mediated loss of ODAM expression decreased *MMP-20* expression (Fig. 3b, c). *MMP-20* expression was higher in cells overexpressing ODAM than in normal ALCs (Fig. 3c).

3. Calcium binding property of rODAM

Calcium binding ability of rODAM was demonstrated using a dot blot technique. The results show rODAM clearly bound ^{45}Ca (Fig. 4).

4. Localization of rODAM in ALCs

Next, we wanted to determine the subcellular localization of ODAM. In control ALCs, faint ODAM staining was seen in the nucleus and cytoplasm (Figs. 5a, b, c). After the addition of exogenous rODAM, ODAM protein was clearly visible in the nucleus and cytoplasm (Figs. 5d, e, f). MMP-20 protein expression was increased by exogenous rODAM treatment (Fig. 5g).

Disussion

Data supporting the expression of ODAM in secretory ameloblasts are controversial. Our lab has previously shown that ODAM is weakly expressed in secretory ameloblasts (Park et al. 2007). In contrast, ODAM is strongly expressed in maturation-stage ameloblasts. These data agree with results presented here. However, other reports indicated that ODAM is not expressed during the secretory stage of amelogenesis (Moffatt et al. 2006, 2008). Therefore, an aim of the present study was to clarify the expression of ODAM during early amelogenesis. ALCs are regarded as relatively undifferentiated. ALCS undergo differentiation under certain culture conditions (Park et al. 2007; Nakata et al. 2003). When ALCs were cultured in differentiation media, we observed the expression of ODAM on day 4, which overlaps with the secretory stage of amelogenesis (Park et al. 2007). In addition, we show that ODAM protein was also expressed by ALCs prior to culturing these cells in differentiation medium. We also observed the presence of MMP-20 protein in ameloblasts. MMP-20 was expressed during the same stage of amelogenesis as ODAM. ODAM localizes to the same regions as MMP-20 in tumors (Murphy et al. 2008). Considering the coinciding expression patterns of ODAM and MMP-20 observed here and the effect of altering ODAM on MMP-20 levels, ODAM expression could alter MMP-20 expression during amelogenesis.

MMP-20 is expressed during the secretory and transition stages of amelogenesis but not during enamel maturation (Bourd-Boittin et al. 2005). KLK4 is believed to be the prominent degradative enzyme that clears enamel protein from the

matrix during maturation (Lu et al. 2008). However, there was no significant change in *KLK4* mRNA levels following increase or loss of ODAM expression (Park et al. 2007). Therefore, we suggest that ODAM modulates the early stage of mineralization by the regulation of MMP-20.

Enamel formation in the MMP-20-deficient mice is severely defective (Caterina et al. 2002), with enamel mineral content reduced by 50% and hardness decreased by 37% (Bartlett et al. 2006). In the present study, ODAM expression level was approximately the same in ameloblasts of MMP20-deficient mice and ameloblasts of wild-type mice. Therefore, we hypothesize that MMP-20 act downstream of ODAM in the ameloblast differentiation and mineralization pathway.

MMP-20 cleaves amelogenin to produce fragments commonly observed *in vivo* and is thought to regulate enamel mineralization (Simmer and Hu 2002). Using the broad-spectrum MMP inhibitor, marimastat, inhibition of mineralization was found to be associated with the inhibition of MMP-20 activation during amelogenesis (Bourd-Boittin et al. 2005). In the present study, we found that overexpression of ODAM in ameloblasts resulted in up-regulation of the *MMP-20* gene. In contrast, inactivation of ODAM by siRNA abrogated MMP-20 expression. These results suggest that while ODAM inactivation might inhibit mineralization by inhibiting activation of MMP-20, increased ODAM (by overexpression or treatment with rODAM) might enhance the onset of mineralization.

Recently, many proteins that show dual localization is found both in the nucleus and outside of the cell (Arnoys et al. 2007), and have physiological roles in the nucleus other than extracellular functions as a secreted protein

(Vuletic et al. 2009). Interestingly, dentin matrix protein 4, a novel secretory calcium-binding protein, modulates odontoblast differentiation (Hao et al. 2007). ODAM protein is found in both the extracellular compartment (extracellular medium) (Park et al. 2007) as well as the intracellular compartment (cytosol and nucleus) (Fig. 5). It was suggested that ODAM, a secreted protein, may mediate the adhesion of junctional epithelial cells to the tooth surface [Moffatt et al. 2008]. However, nothing is known about the possible intracellular functions of ODAM. It was also suggested that the short ODAM protein (153 amino acids) would be deficient in a secretory signal sequence and presumably would remain within the cell where it could have a more restrictive or differing functional role from that of larger ODAM product (279 amino acids) (Kestler et al. 2008). In the present study, we found that ODAM localizes to the nucleus and cytoplasm of normal ALCs. Furthermore, exogenous rODAM could be found localized to the nucleus and cytoplasm of ALCS. Based on these observations, we propose that, secreted ODAM protein re-enter the ameloblasts, escape the endocytotic pathway, and enter the nucleus, where they regulate MMP-20 gene expression either directly or indirectly through an interaction with other factors that control MMP-20 expression.

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Legend for figures

Fig. 1. ODAM immunoreactivity during amelogenesis in wild-type and MMP-20 -deficient mice.

a Presecretory-stage ameloblasts lack ODAM protein expression. **b** Secretory -stage ameloblasts express ODAM in their cytoplasm. **c** Transition-stage ameloblasts have obvious ODAM protein in the nucleus and entire cytoplasm, additionally ODAM protein in underlying papillary layer cells. **d** Maturation-stage **r**uffle-end ameloblasts have ODAM protein in the supranuclear region and their distal ends (hematoxylin counterstain). **e** Representative microphotograph of wild-type mice (hematoxylin & eosin (H&E) stain). **f** Representative microphotograph of MMP-20-deficient mouse showing an abnormally thick layer of enamel protein (*arrows*) along the normal dentin (De) surface (H&E stain). **g** Immunohistochemistry of ODAM in ameloblasts of wild-type mice. **h** MMP-20-deficient mice have a similar expression pattern of ODAM compared to normal ameloblasts. Am, ameloblast; En, enamel space; De, dentin. *Bar* 100 μm

Fig. 2. Expression of ODAM mRNA in various cancer cells and normal oral epithelial cells by RT-PCR.

Lane 1: Fadu, Head & neck cancer cell line. Lane 2: AGS, Gastric cancer cell line. Lane 3: T24, Kidney cancer cell line. Lane 4: KB, Oral cancer cell line. Lane 5: SH-SY5Y, Neural cancer cell line. Lane 6: INOK, mouse normal oral epithelial cell.

Fig. 3. ODAM and MMP-20 protein and mRNA expression.

a Western blot analysis of ODAM and MMP-20 protein expression in ALCs over a 14-day culture time course. Bands were measured by densitometric analysis of autoradiographic films. **b** Expression of *ODAM* mRNA after over-expression (over) or inactivation (inact) of ODAM as analyzed by real-time PCR. **c** Real-time PCR analysis of expression of *MMP-20* mRNA after over-expression and inactivation of ODAM. Graphs represent the relative amount of *ODAM* (**b**) and *MMP-20* (**c**) mRNA after normalization to *GAPDH* mRNA. The asterisk (*) indicate the values significantly different from the controls, according to the Mann-Whitney test ($P < 0.01$).

Fig. 4 Calcium binding property of ODAM.

Recombinant rat ODAM was dot-blotted onto a polyvinylidene difluoride membrane and detected by ^{45}Ca overlay. BSA was used as a negative control

Fig. 5 ODAM protein localizes to the nucleus and cytoplasm of ALCs.

Subconfluent ALCs were cultured in the absence (**a-c**) or presence (**d-f**) of rODAM (10 μg). ODAM localization was detected by immunostaining. The nuclei were visualized by staining with DAPI (**b**, **e**). The merged images are shown in the right panel (**c**, **f**). **g** Western analysis of ODAM and MMP-20 protein expression in ALCs after the addition of exogenous rODAM. *Bar* 50 μm

Figures

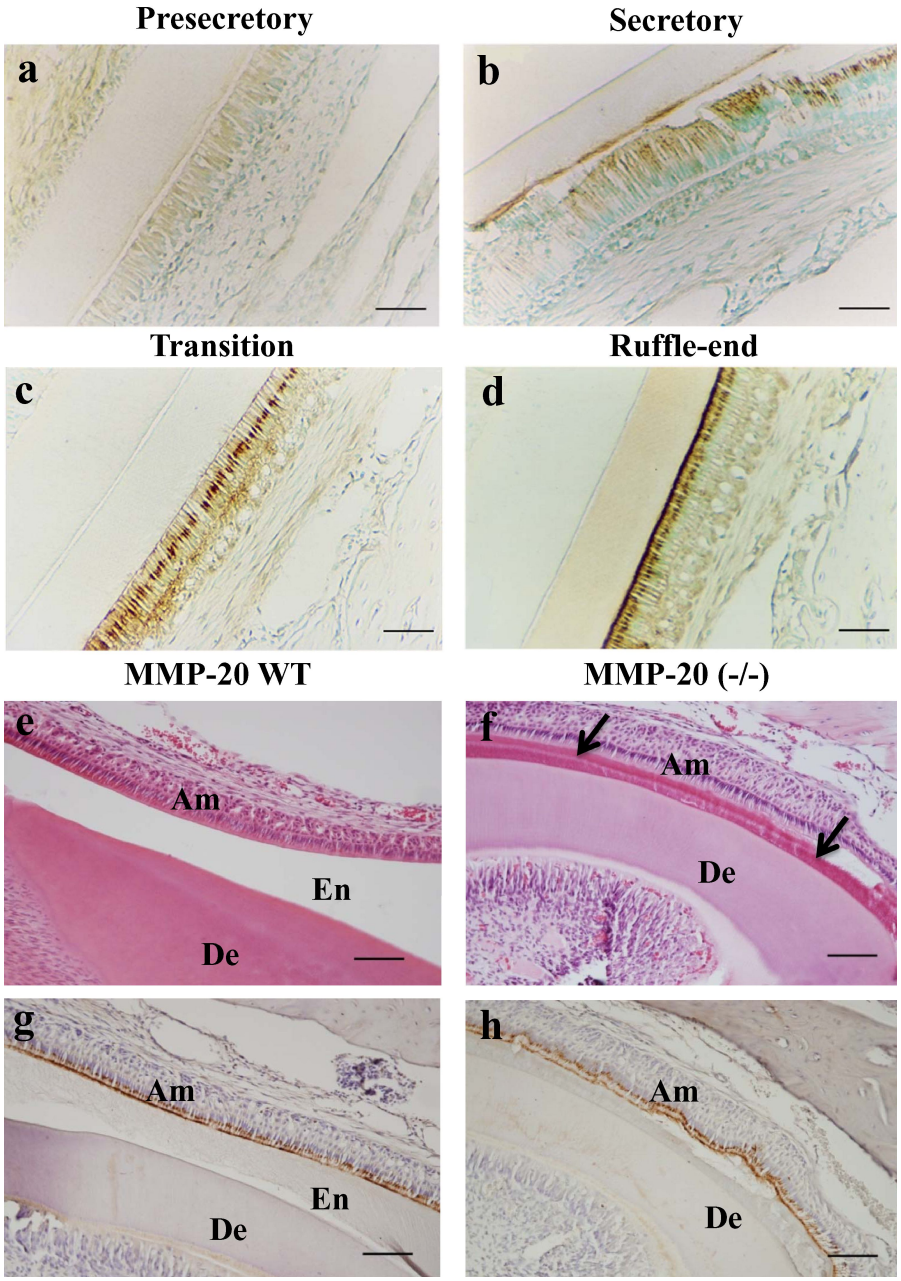
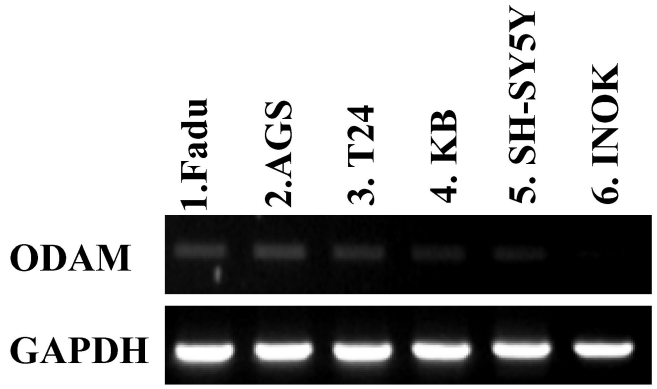


Fig. 1. ODAM immunoreactivity during amelogenesis in wild-type and MMP -20-deficient mice.



1. **Fadu : Head & neck cancer cell line**
2. **AGS : Gastric cancer cell line**
3. **T24 : Kidney cancer cell line**
4. **KB : Oral cancer cell line**
5. **SH-SY5Y : Neural cancer cell line**
6. **INOK: Mouse normal oral epithelial cell**

Fig. 2. Expression of ODAM mRNA in various cancer cells and normal oral epithelial cells by RT-PCR.

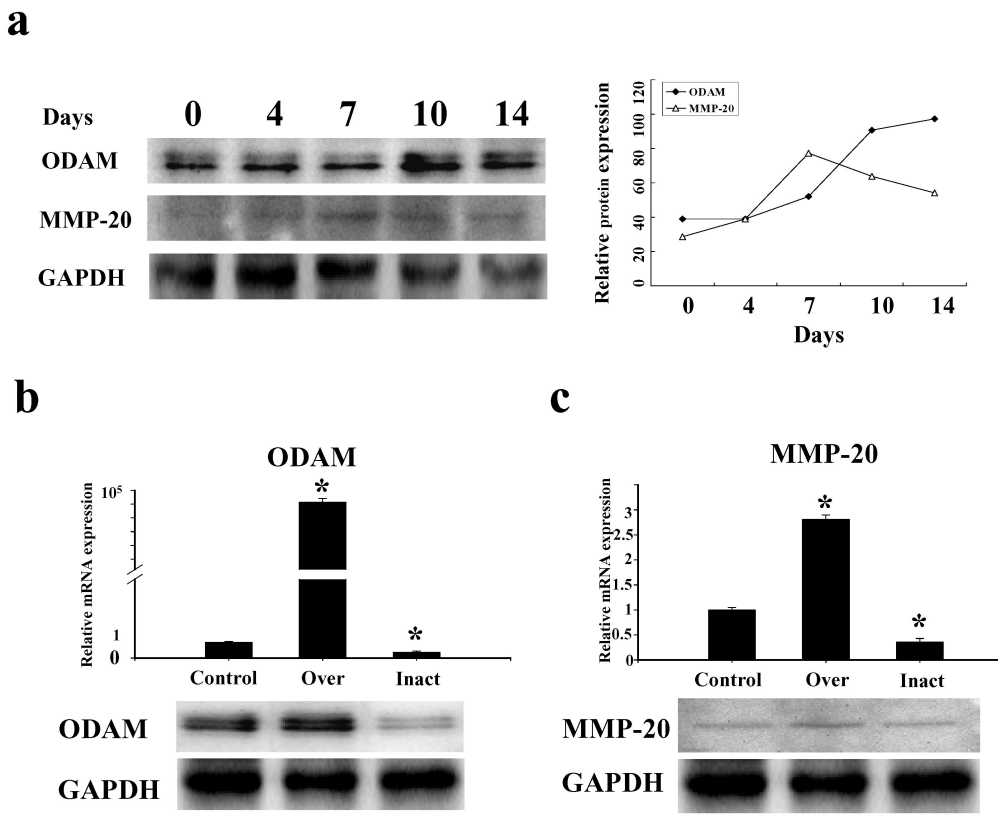


Fig. 3. ODAM and MMP-20 protein and mRNA expression.

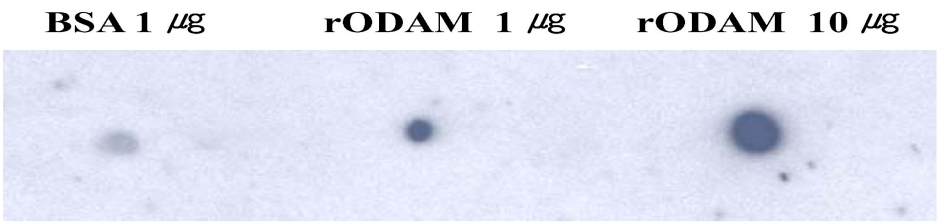


Fig. 4. Calcium binding property of ODAM.

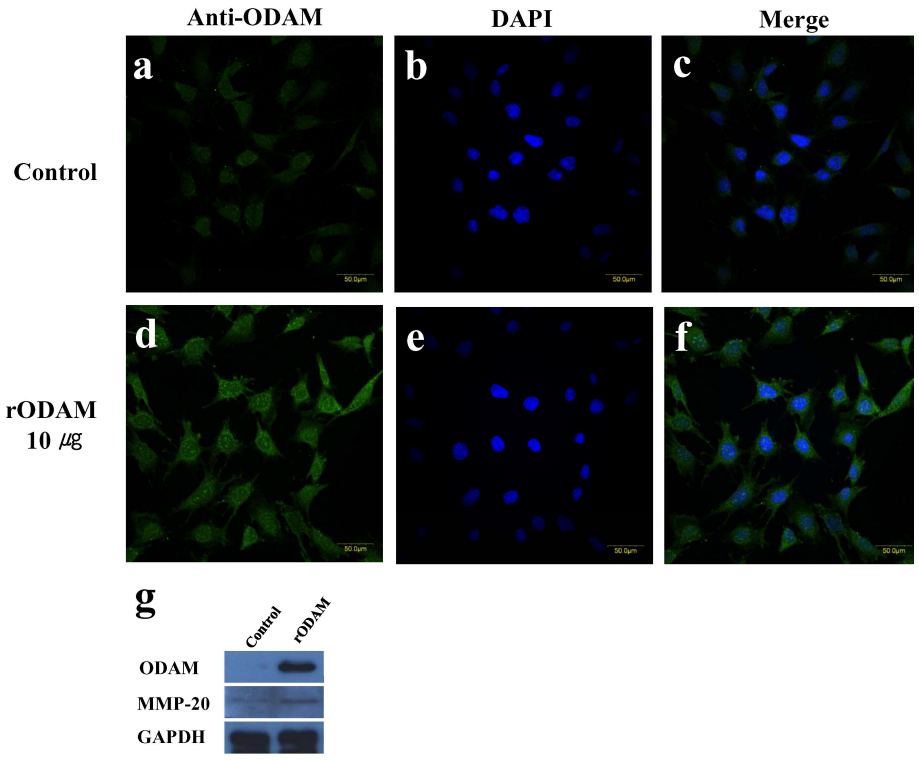


Fig. 5. ODAM protein localizes to the nucleus and cytoplasm of ALCs.

저작물 이용 허락서

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논문제목	한글 : 법랑질 형성과정에서 MMP-20의 조절을 통한 ODAM의 법랑질 석회화 조절 영어 : ODAM modulates enamel mineralization through the regulation of MMP-20 expression during amelogenesis				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집·형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포·전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송·출력을 허락함.

동의여부 : 동의(O) 반대( )

2010년 2월

저작자 : 한 평 호 (서명 또는 인)

조선대학교 총장 귀하