Published in final edited form as:

Arch Oral Biol. 2017 June ; 78: 6–12. doi:10.1016/j.archoralbio.2017.02.001.

Expression of odontogenic ameloblast-associated protein in the dental follicle and its role in osteogenic differentiation of dental follicle stem cells

Shaomian Yao¹, Chunhong Li¹, Michael Beckley², and Dawen Liu¹

¹Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana

²Department of Oral and Maxillofacial Surgery, University of the Pacific, Arthur A. Dugoni School of Dentistry, San Francisco, California

Abstract

Objective—Odontogenic Ameloblast-Associated Protein (ODAM) is encoded by a secretory calcium-binding phosphoprotein cluster gene, which generally plays an important role for mineralization. Dental follicle (DF) is essential in regulating bone formation for tooth eruption. This study aims to reveal ODA expression in the DFs of developing and erupting molars, and to determine the possible role of ODA.

Design—DFs were collected from human third molars and rat mandibular molars for gene expression assessment and for establishment of cell cultures. RT-PCR and western blot were conducted to determine ODA expression. Over- or silencing expression of ODA in the dental follicle stem cells (DFSCs) was done by transfecting the cells with ODA plasmid or siRNA to evaluate ODA effects on osteogenesis.

Results—Rat DFs weakly expressed ODA at early-postnatal days, but a chronological increment of ODA expression from days 1 to 11 was observed. Differences in expression of ODA were seen in the human DFs of different individuals. In vitro, ODA was expressed in DFSCs, but almost no expression in DF-derived fibroblast-like cells. Forcing the DFSCs to overexpress ODA accelerated osteogenesis, whereas continuously silencing the ODA in the DFSCs reduced osteogenesis only at 2 weeks of osteogenic induction.

Conclusions—ODA is differentially expressed in the DFs of different age molars. Its expression is coincident with the increased bone formation of tooth crypt during tooth eruption in rat DFs. Increase of ODA expression may accelerate osteogenic differentiation of DFSCs. Thus, ODA expression in the DF may regulate bone formation for timely tooth eruption.

*Corresponding Author: Dr. Shaomian Yao, Department of Comparative Biomedical Sciences, School of Veterinary Medicine, Louisiana State University, Baton Rouge, Louisiana 70803, USA; Tel: +1-225-578-3324, Fax: +1-225-578-9895; shaomia@lsu.edu.

No Conflict of Interests

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Dental Follicle (DF); Osteogenic differentiation; Stem cells; Odontogenic Ameloblast-Associated Protein (ODAM); Gene expression

Introduction

Tooth development involves a series of complicated mineralization processes. Teeth and other hard tissues can be viewed as the results of mineralization in the extracellular matrix. Secretory calcium-binding phosphoproteins (SCPPs) are macromolecules involved in mineralization in bone development. Odontogenic Ameloblast-Associated Protein (ODAM, also known as Apin) is encoded by a gene in the SCPP cluster (Kawasaki and Weiss 2003), and ODA M has been reported to be primarily expressed in the cells and tissues related to mineralization, such as in ameloblasts (Moffatt et al., 2008) and in the enamel organ (Park et al., 2007; Moffatt et al., 2008). It is believed that ODA M plays an essential role in tooth maturation (Kestler et al., 2008). In previous studies, we observed a rapid bone growth in the bony crypt of rat erupting molars, and we proposed that the bone growth in the tooth crypt is essential for the tooth to erupt (Wise et al., 2007; Wise et al., 2011).

The dental follicle (DF) is a loose connective tissue sac surrounding the unerupted tooth. A major function of DF is to regulate tooth eruption via timely and spatially controlling osteogenesis and osteoclastogenesis (Wise and King 2008). In a preliminary study, we found that the rat DF expresses ODA M. It is logical to ask if expression of the ODA M in the DF varies during tooth eruption, and if the human DF also expresses ODA M. Given that the function of ODA M is related to mineralization, it was the objective of this study to determine if the ODA M produced by the DF participates in osteogenesis for the alveolar bone growth seen in the erupting teeth. To address these questions, we collected human DFs from impacted third molars of human patients to determine ODA M expression and rat DFs from pups to determine the chronological expression of ODA M in the first mandibular molar at different postnatal days prior to the onset of the tooth eruption. Because studies have proved the existence of stem cells in the DF (Yao et al., 2008; Honda et al., 2011), we have proposed that the dental follicle stem cells (DFSCs) may contribute to bone formation needed for tooth eruption. Thus, another objective of this study was to explore ODA M expression in the DFSCs and to determine if ODA M can promote osteogenic differentiation of DFSCs.

Materials and Methods

Dental follicle and cell cultures

Human DFs were collected from the impacted wisdom teeth removed at the Pacific Oral and Facial Surgery Center (Livermore, CA, USA). Human tissue collection has been approved by Institutional Review Board (IRB) of Louisiana State University. DFs of Sprague Dawley rats were surgically collected from the first mandibular molars of rat pups at postnatal days 1, 3, 5, 7, 9 and 11 in 3 independent litters for RNA extraction to determine chronological gene expression. The protocol for using rats has been approved by Institutional Animal Care

and Use Committee (IACUC) of Louisiana State University. For establishment of cell cultures, the DFs collected from rat pups or from the human patients were minced into small pieces and then digested with trypsin to obtain cell suspension. The cell suspension was centrifuged at 3000 RPM to collect cell pellets. For DFSC cultures, cells were cultured in the medium consisting of α -Minimum Essential Medium plus 20% fetal bovine serum (Atlanta Biologicals, Inc., Flowery Branch, GA, USA) and proper antibiotics. Our previous studies showed that cell populations derived from dental tissue under this culture condition contain stem cells (Yao et al. 2008). For non-stem dental follicle cell (DFC) cultures, cells were cultured in Minimum Essential Medium and 10% Gibco newborn calf serum (Thermo Fisher Scientific Inc., USA) with antibiotics. Studies indicated that DFC cultures derived from this condition are fibroblast-like cells (Wise et al., 1992), and do not possess capability of differentiation (Yao et al., 2008). For establishing both DFSC and DFC cultures, the primary cells were cultured in T-25 polystyrene flasks at 37°C, 5% CO₂. Non-adherent cells were removed by changing the medium after overnight incubation. The adherent cells remaining in the flasks were grown to 80–90% confluency before passaging into a T-75 flask. For subsequent passages, each T-75 flask was passaged into 3 flasks. The established cultures were subjected to osteogenic differentiation assay to evaluate their osteogenesis capability at passage 3, as described below in the section of “Induction of osteogenic differentiation”. The cells of passages 3 to 7 were used for the experiments in this study.

Determination of ODAM expression

DFs collected from first mandibular molars of rat pups of different postnatal days and from different human impacted molars were used for RNA isolation to determine ODAM expression. Total RNA was also extracted from the established human and rat dental follicle cell cultures. Total RNA were isolated using RNeasy kit (Promega, Madison, WI, USA) or Direct-zol™ RNA Kit (Zymo Research, Irvine, CA, USA) according to the manufacturers' instructions. The total RNA was further treated with Ambion Turbo DNase I kit (Thermo Fisher Scientific Inc. USA) to remove possible contamination of DNA. Next, the RNA was quantitated and analyzed with a Nanodrop spectrophotometer. RNA samples with OD260/OD280 greater than 1.8 were processed for RT-PCR to acquire gene expression data. Briefly, equal amounts of the RNA were reverse-transcribed into cDNA with MLV reverse transcriptase. Conventional PCR or real-time PCR was conducted using the cDNA and ODAM specific primers, and actin was used as the internal control in all RT-PCR analyses. For conventional PCR, the PCR products were electrophoresed in an agarose gel to determine the gene expression. For real-time PCR, threshold cycle (C_T) values were obtained by running the reactions in ABI 7300 real-time PCR system and relative gene expression (RGE) was calculated using the delta C_T method.

To determine if ODAM protein was indeed exist in the DF, rat pups of postnatal day 8 was sacrificed and DF of 1st and 2nd molars was isolated and lysed in CytoBuster™ Protein Extraction Reagent (EMD Millipore Corporation) containing protease inhibitors. 20µg of the protein was used for western blotting analysis for hybridization with anti-ODAM antibody (Biorbyt, Cambridge, UK). HRP conjugated secondary antibody was used for detection ODAM signal with SuperSignal West Dura Extended Duration Substrate (Thermo Fisher, Rockford, IL, USA).

Induction of osteogenic differentiation

Cells (DFSCs or DFC) were seeded in the flasks or plates at a cell density around 8.0k-10.0k cells/cm² in α -MEM plus 20% FBS (V/V). After the cells reached about 90% confluency, the cell growth medium was replaced with osteogenic induction medium consisting of DMEM-low glucose (1.0g/L), 10% FBS, 50 μ g/mL ascorbate-2 phosphate, 10⁻⁸ M dexamethasone and 10 mM β -glycerophosphate (Sigma-Aldrich, St. Louis, MO, USA) with medium changes every 4 days for designated times. The cells then were fixed with 10% Neutral Buffered Formalin for 5 minutes and then stained with 1% Alizarin red solution for 5 minutes. The Alizarin red solution was removed and the cells were rinsed with deionized water. For further assessment of osteogenesis, alkaline phosphatase (ALP) assay was conducted in some experiments as detailed below.

Alkaline phosphatase (ALP) assay

ALP activity was measured with the QuantiFluo™ Alkaline Phosphatase Assay Kit (BioAssay Systems, Hayward, CA, USA). Specifically, 150 μ l CytoBuster Protein Extraction Reagent (Millipore Novagen) containing protease inhibitor cocktail was added to each well and the cells were scraped off from the culture surface. The cell suspension was collected into a 1.5ml- microtube and placed in ice for 30 minutes to allow lysis of the cells. Next, the lysate was centrifuged at 15,000 rpm for 5 minutes. Then, 10 μ l of cell lysate supernatant of each sample was pipetted into each well of black 96-well plate, and 90 μ l of assay buffer was added to each well. ALP standard curve was established in the same manner by mixing 10 μ l of ALP standard with 90 μ l of assay buffer in each well. After 15 minutes of incubation at room temperature, the plate was read at excitation of 360nm and emission of 450nm with a SpectraMax-M2 plate reader (Molecular Devices, LLC, Sunnyvale, CA). The ALP activity was calculated using the equation given in the manufacturer's protocol (BioAssay Systems).

Effect of ODAM overexpression and knockdown on osteogenesis of DFSCs

To study the effect of ODAM on osteogenesis, ODAM overexpression experiments were conducted by transfecting plasmid vector, namely, pCMVrODAM containing rat ODAM (rODAM) coding sequence driven by a CMV promoter into human and rat DFSCs. The plasmid, pCMV3tag3 (Agilent Technologies, Santa Clara, CA, USA) without insertion of the ODAM coding sequence, was also transfected into the cells to serve as the control. Briefly, cells were seeded in 12-well plates or T25 flasks. When they reached about 80–90% confluency, the plasmids (ODAM or control plasmids) were mixed with PolyJet™ transfection reagent (SignaGen Laboratories, Gaithersburg, MD, USA) and then added to the cultures according to the manufacturer's instruction. After overnight incubation, the medium along with the transfection reagent was removed, and cells were collected at designated time points for RNA isolation to determine the overexpression of the transgene rODAM by RT-PCR. For induction of differentiation, osteogenic differentiation medium was added to the transfected cells, and incubated for 5, 7 and 14 days, followed by Alizarin red staining as described earlier and by ALP analysis. For ALP analysis, 150 μ l of CytoBuster Protein Extraction Reagent (Millipore Novagen) containing protease inhibitor cocktail was added to

each well to lyse the cells for collecting total protein. ALP activity was determined as described earlier.

To study if knockdown ODAM affects osteogenesis, DFSCs were transfected with ODAM-siRNA to knock down ODAM expression. Specifically, cells were seeded in 12-well plates, and ODAM-target siRNA [5'-GAAUGGUAUCUGUCCUCGUAUAGCUG-3', which was synthesized by Integrated DNA Technologies, Inc. (ITD, Coralville, Iowa, USA) or a scrambled siRNA (control) were mixed with RNAiMax (Invitrogen, Waltham, MA, USA) according to the manufacturer's protocol. The siRNA mixture was added to the cultures at a final siRNA concentration of 20nM. Transfection was terminated by medium change after 24 hours of incubation. Cells were collected for RNA isolation as described above and then real-time RT-PCR was used to determine knockdown efficiency. The transfected cells were subjected to osteogenic induction for up to 4 weeks with transfection repeated weekly. The experimental scheme is illustrated in Fig 1. Cell lysates were collected at 2, 3 and 4 weeks for ALP activity analysis (BioAssay Systems) to evaluate osteogenic differentiation.

Results

Conventional RT-PCR results showed no detectable transcript of ODAM in neonatal rat DF at day 1 and only a weak expression of ODAM at postnatal day 3. A notable increase of expression was seen at day 5 and a huge increase seen at day 7. The incremental trend continued until postnatal day 11 (Fig. 2a). The real-time RT-PCR analysis clearly revealed a continuous incremental pattern of ODAM expression from postnatal day 1 until day 11 (Table 1). A huge increase was seen at postnatal day 7 at which the average expression was increased about 300-fold compared to postnatal day 1. At postnatal day 11, ODAM expression was over 1000 times higher than day 1 (Table 1). Conventional RT-PCR revealed that the DFs of human third impacted molars also expressed ODAM. Differential expression was seen in different individuals or between mandibular molar and maxillary molar in the same patient (Fig. 2b). Western blot analysis showed the presence of ODAM protein in the DF (Fig. 2c).

Two populations of cells, dental follicle cells (DFC) and dental follicle stem cells (DFSCs) were established from the human and rat DF, respectively. The DFC are fibroblast-like cells that have no capability of differentiation, whereas the DFSCs can be induced for multiple lineage differentiations (Wise et al., 1992; Yao et al., 2008). When the DFSCs or DFC (derived from human or rat) were cultured in the osteogenic differentiation induction medium for 2 weeks, strong osteogenesis was observed in the DFSCs, but no osteogenesis was seen in the DFC as revealed by Alizarin red staining (Fig. 3a). RT-PCR revealed expression of ODAM in the cultured rat and human DFSCs, but no or weak ODAM expression in the DFC (Fig. 3b).

Because rapid increase of ODAM expression in the rat DFs was coincident with the bone growth in the tooth crypt during the tooth eruption, we conducted ODAM overexpression experiments by transfection of rODAM expression plasmids into human or rat DFSCs to mimic the in vivo rapid increase of ODAM expression seen in the DFs. High levels of rODAM transgenic expression appeared to last at least 9 days post-transfection as

determined by RT-PCR (Fig. 4a, b). In particular, although some reduction of the rODAM transgene expression was seen at days 5 and 9 in the transfected human DFSCs as compared to day 2 (Fig. 4a), the increased expression of the transgene in the rODAM transfected cells was still remarkable when comparing to the control (Fig. 4a). For the rat DFSCs, the transgene expression remained almost constant until day 9 post-transfection (Fig. 4b). To ensure the detection of only transcription (mRNA) of the rODAM transgene, but not the contaminated plasmid DNA in the RT-PCR assay, RNA samples (without reverse transcription) were directly used in PCR for detecting rODAM. The results were all negative (Fig. 4a, b, bottom panel), indicating that the RNA samples were not contaminated by plasmid DNA and the amplification of the rODAM seen in RT-PCR was really from transcription of the rODAM transgene.

The transfected DFSCs were incubated in osteo-induction medium for assessment of their osteogenesis. Alizarin red staining showed that more calcium deposition was seen in the rODAM transfection treatment than the control transfected with 3T3 plasmid at days 5 and 7 of induction, but no notable differences were observed at 14 days of induction for both human and rat DFSCs (Fig. 4c). ALP assay revealed a similar pattern, i.e., rODAM transfected cells had a significantly higher ALP activity than the control transfection at days 5 and 7 of induction, and no significant difference was seen at day 14 (Fig. 4d, e). ALP has long been known to play important roles in osteogenesis (Siffert 1951), and increase of ALP activity often has been used to measure the osteogenic differentiation of stem cells and active bone formation (Birmingham et al., 2012; Eastell et al., 2012). Significantly higher ALP activity in ODAM transfected DFSCs versus the control DFSCs indicates greater osteogenic differentiation in ODAM transfected cells when they are subjected to osteo-induction.

ODAM expression in the DFSCs was knocked down by transfecting the cells with ODAM-siRNA using RNAiMax (Thermo Scientific). Maximal knockdown efficiency was seen at 72 hours post siRNA transfection at which time the ODAM expression was reduced about 90% (Fig 5a). The transfected cells were subjected to osteogenic differentiation induction for up to 4 weeks with siRNA transfection repeated weekly at weeks 1, 2 and 3. Cells were collected at weeks 2, 3 and 4 for ALP activity analysis for assessment of osteogenesis. The results showed that ALP activity was significantly reduced in the ODAM-siRNA transfected DFSCs only in two weeks of osteogenic induction, but no difference in three and four weeks of induction (Fig 5b). This result suggested that ODAM may only accelerate osteogenic differentiation at the early stage, but it is not essential as continuous knockdown of ODAM does not affect overall osteogenesis.

Discussion

Tooth eruption is a localized event which requires bone resorption and bone formation in the given tooth crypt. Bone resorption and bone formation are mainly regulated by the DF of the given erupting tooth. The DF exerts this regulatory role by timely and spatially expression of cytokines and growth factors to regulate osteoclastogenesis and osteogenesis (Wise and King 2008; Wise and Yao 2006). In previous studies, we examined rat first mandibular molars and found that bone growth starts in crypt of the rat first molar at the postnatal day 3, and rapid

bone growth starts at postnatal day 7. Such rapid bone growth continues during the course of eruption of rat first molar, and serves an eruption force to push the molar out of its crypt (Wise et al., 2007; Wise et al., 2011). Comparing this alveolar bone growth pattern to the ODAM expression profile in the DFs of the rat first mandibular molar determined in this study, we found that this bone growth pattern coincided well with the ODAM expression pattern. Because ODAM is involved in diverse activities relating to mineralization, such as ameloblast differentiation and enamel maturation (Lee et al., 2010), it is possible that the dramatic increase of ODAM expression seen after postnatal day 7 may contribute to the rapid alveolar bone growth needed for tooth eruption.

The dental follicle is a connective tissue sac consisting of majority of fibroblast-like cells. Various studies have reported the existence of stem cells in this tissue (i.e., DFSCs) in human, rat and other species (Honda et al., 2010; Yao et al., 2008). This study suggests that the fibroblast-like cells (non- stem cells) of the DF may not be the major contributor of the ODAM production because when cultured in vitro, the non-stem DFC do not express or only weakly express ODAM. However, because the DFSCs do express the ODAM and can differentiate toward osteogenic lineage, it is likely that they may contribute to the alveolar bone growth seen in the crypt during tooth eruption. Thus, we conducted in vitro studies with DFSCs to gain insight into the role of ODAM in osteogenesis.

DFSCs have been shown to possess capability of osteogenic differentiation in vitro when induced with a proper osteogenic medium (Yao et al., 2008; Mori et al., 2012) or in vivo when transplanted into bone defects (Rezai-Rad et al., 2015; Lucaciu et al., 2015). Culture of the DFSCs in osteogenic induction medium resulted in formation of calcium deposition (Yao et al., 2008; Li et al., 2012), and increased expression of the osteoblast master gene RUNX2 (Mori et al., 2012). Another study showed that ODAM cooperates with RUNX2 to modulate mineralization (Lee et al., 2010). Studies in our lab also revealed that rat DFSCs cultured in osteogenic medium dramatically and significantly increase the expression of osteogenic marker genes, osteocalcin and bone sialoprotein (Rezai Rad et al., 2015), suggesting that DFSCs can differentiate into osteoblasts under the proper conditions. In this study, we mimicked the increase of ODAM expression by transfecting the plasmid containing rat ODAM coding sequence into human and rat DFSCs, and found that increase of ODAM expression enhanced osteogenesis of the DFSCs at an early stage of osteo-induction when the transfected DFSCs were cultured in osteogenesis medium. However, overexpression of ODAM did not increase the overall osteogenesis at the end of the 14-day osteo-induction. Thus, it is likely that ODAM functions primarily to speed up the osteogenesis process at early stage of differentiation. The function of ODAM in accelerating osteogenesis was also supported by the ODAM loss-of-function experiment, in which knockdown of the ODAM expression in the rat DFSCs resulted in a significant reduction of osteogenesis at early stage as assessed by ALP activity. Thus the in vitro results might explain the in vivo observations of rapid alveolar bone growth in tooth crypt. The dramatic increased expression of ODAM in the DFSCs of the DF may accelerate the osteogenesis, and in turn at least partially contribute to the speedy alveolar bone growth seen after postnatal day 7 in the tooth crypt during tooth eruption of the rat first molar.

In a recent publication, Wazen et al (2015) reported that teeth were erupted in ODAM KO mice. However, this is not contradictory to our observations because our results indicated that ODAM is not an essential gene for osteogenesis; i.e., without ODAM, osteogenesis can still occur. However, our results showed that increased ODAM could accelerate osteogenesis, thus without ODAM osteogenesis may occur at a slower speed, and the tooth eruption would not be inhibited and bone density would not be affected in the ODAM KO, but it may delay the tooth eruption. Further study may be conducted to determine if the tooth eruption is delayed in ODAM KO animals. Furthermore, one of the disadvantages in KO approach for gene functional study is that KO may activate some redundant gene(s), thus covers the real function of the gene of interest and lead to false conclusion. Thus, it is another possibility that the ODAM knockout does not affect tooth eruption because other redundant gene(s) was/were activated in the KO mice.

In conclusion, ODAM expression in the DF increases chronologically from postnatal days 1 to 11. This expression pattern correlates well with the increase of bone formation seen in the tooth crypt during tooth eruption. This study demonstrated that increased ODAM expression in vitro could function to accelerate the osteogenesis of the DFSCs. Thus, increased ODAM expression in the DF may promote osteogenic differentiation of DFSCs to contribute to alveolar bone formation seen in the tooth crypt for timely tooth eruption.

Acknowledgments

The authors thank Dr. Gary E Wise for editing the manuscript and his constructive suggestions and comments. This research was supported by a R01 grant (DE008911-21) from the National Institute of Dental and Craniofacial Research (NIDCR).

References

- Birmingham E, Niebur GL, McHugh PE, Shaw G, Barry FP, McNamara LM. Osteogenic differentiation of mesenchymal stem cells is regulated by osteocyte and osteoblast cells in a simplified bone niche. *Eur Cell Mater*. 2012; 23:13–27. [PubMed: 22241610]
- Eastell R, Garnero P, Audebert C, Cahall DL. Reference intervals of bone turnover markers in healthy premenopausal women: results from a cross-sectional European study. *Bone*. 2012; 50(5):1141–1147. [PubMed: 22348982]
- Honda MJ, Imaizumi M, Suzuki H, Ohshima S, Tsuchiya S, Satomura K. Stem cells isolated from human dental follicles have osteogenic potential. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2011; 111(6):700–708. [PubMed: 21147007]
- Honda MJ, Imaizumi M, Tsuchiya S, Morsczeck C. Dental follicle stem cells and tissue engineering. *J Oral Sci*. 2010; 52(4):541–552. Review. [PubMed: 21206155]
- Kawasaki K, Weiss KM. Mineralized tissue and vertebrate evolution: the secretory calcium-binding phosphoprotein gene cluster. *Proc Natl Acad Sci USA*. 2003; 100(7):4060–4065. [PubMed: 12646701]
- Kestler DP, Foster JS, Macy SD, Murphy CL, Weiss DT, Solomon A. Expression of odontogenic ameloblast-associated protein (ODAM) in dental and other epithelial neoplasms. *Mol Med*. 2008; 14(5–6):318–326. [PubMed: 18472969]
- Lee HK, Lee DS, Ryoo HM, Park JT, Park SJ, Bae HS, Cho MI, Park JC. The odontogenic ameloblast-associated protein (ODAM) cooperates with RUNX2 and modulates enamel mineralization via regulation of MMP-20. *J Cell Biochem*. 2010; 111(3):755–767. [PubMed: 20665536]
- Li C, Yang X, He Y, Ye G, Li X, Zhang X, Zhou L, Deng F. Bone morphogenetic protein-9 induces osteogenic differentiation of rat dental follicle stem cells in P38 and ERK1/2 MAPK dependent manner. *Int J Med Sci*. 2012; 9(10):862–871. [PubMed: 23155360]

- Lucaciu O, Soriu O, Gheban D, Ciuca DR, Virtic O, Vulpoi A, Dirzu N, Câmpian R, Bociu G, Popa C, Simon S, Berce P, Bociu M, Crisan B. Dental follicle stem cells in bone regeneration on titanium implants. *BMC Biotechnol.* 2015; 15(1):114. doi: 10.1186/s12896-015-0229-6 [PubMed: 26718927]
- Moffatt P, Smith CE, St-Arnaud R, Nanci A. Characterization of Apin, a secreted protein highly expressed in tooth-associated epithelia. *J Cell Biochem.* 2008; 103(3):941–956. [PubMed: 17647262]
- Mori G, Ballini A, Carbone C, Oranger A, Brunetti G, Di Benedetto A, Rapone B, Cantore S, Di Comite M, Colucci S, Grano M, Grassi FR. Osteogenic differentiation of dental follicle stem cells. *Int J Med Sci.* 2012; 9(6):480–487. [PubMed: 22927773]
- Park JC, Park JT, Son HH, Kim HJ, Jeong MJ, Lee CS, Dey R, Cho MI. The amyloid protein APin is highly expressed during enamel mineralization and maturation in rat incisors. *Eur J Oral Sci.* 2007; 115(2):153–160. [PubMed: 17451507]
- Rezai Rad M, Liu D, He H, Brooks H, Xiao M, Wise GE, Yao S. The role of dentin matrix protein 1 (DMP1) in regulation of osteogenic differentiation of rat dental follicle stem cells (DFSCs). *Arch Oral Biol.* 2015; 60(4):546–556. [PubMed: 25596638]
- Rezai Rad M, Bova JF, Orooji M, Pepping J, Qureshi A, Del Piero F, Hayes D, Yao S. Evaluation of bone regeneration potential of dental follicle stem cells for treatment of craniofacial defects. *Cytotherapy.* 2015; 17(11):1572–1581. [PubMed: 26342992]
- Siffert RS. The role of alkaline phosphatase in osteogenesis. *J Exp Med.* 1951; 93(5):415–426. [PubMed: 14832392]
- Wazen RM, Moffatt P, Ponce KJ, Kuroda S, Nishio C, Nanci A. Inactivation of the Odontogenic ameloblast-associated gene affects the integrity of the junctional epithelium and gingival healing. *Eur Cell Mater.* 2015; 30:187–199. [PubMed: 26412389]
- Wise GE, He H, Gutierrez DL, Ring S, Yao S. Requirement of alveolar bone formation for eruption of rat molars. *Eur J Oral Sci.* 2011; 119:333–338. [PubMed: 21896048]
- Wise GE, King GJ. Mechanisms of tooth eruption and orthodontic tooth movement. *J Dent Res.* 2008; 87(5):414–434. [PubMed: 18434571]
- Wise GE, Lin F, Fan W. Culture and characterization of dental follicle cells from rat molars. *Cell Tissue Res.* 1992; 267(3):483–492. [PubMed: 1571962]
- Wise GE, Yao S. Regional differences of expression of bone morphogenetic protein-2 and RANKL in the rat dental follicle. *Eur J Oral Sci.* 2006; 114(6):512–516. [PubMed: 17184234]
- Wise GE, Yao S, Henk WG. Bone formation as a potential motive force of tooth eruption in the rat molar. *Clin Anat.* 2007; 20(6):632–639. [PubMed: 17415742]
- Yao S, Pan F, Prpic V, Wise GE. Differentiation of stem cells in the dental follicle. *J Dent Res.* 2008; 87(8):767–771. [PubMed: 18650550]

Highlights

- ODAM expression was detected in the human and rat dental follicle.
- ODAM expression is chronologically increased from postnatal day 1 to day 11 in the rat dental follicle, which is coincident with the increase of bone formation seen in the tooth crypt during tooth eruption.
- Continuous knockdown of ODAM expression in the dental follicle stem cells resulted in reduction of early stage osteogenic differentiation of the cells.
- Overexpression of ODAM in the Dental follicle stem cells appeared to accelerate osteogenesis of the cells, but had no effect on overall osteogenesis.

Fig. 1.

Schematic illustration of experiment of continuously silencing ODAM expression by weekly-repeat transfection of the dental follicle stem cells (DFSCs) with ODAM siRNA or scrambled siRNA (control) during the weeks of osteogenic induction. Note that cells were repeatedly transfected at weeks 1, 2 and 3, and cell lysates were collected for ALP assay to evaluate osteogenic differentiation.

Fig. 2.

(a) Chronological expression of ODAM in the DF of rat first mandibular molar at different postnatal days determined by conventional RT-PCR. Note the chronologically increased expression of ODAM from postnatal day 1 to day 11. (b) ODAM expression in the human DF of impacted third molars collected from two individuals (Mad=mandibular molar; Max=Maxillary molar). Note the differential expression in different individuals and in mandibular and maxillary molars within the same individual. (c) western blot analysis showed the existence of the ODAM protein in the dental follicle of rat first and second molars (M1 and M2).

Fig. 3. Determination of osteogenic capability and ODAM expression in human and rat DF derived cells. (a) Alizarin red staining revealed that established human dental follicle stem cells (hDFSCs) and rat dental follicle stem cells (rDFSCs) showed strong osteogenesis vs. no osteogenesis in non-stem dental follicle cells (hDFC and rDFC) after osteogenic induction. (b) RT-PCR determined that the hDFSCs and rDFSCs substantially expressed ODAM whereas hDFC and rDFC either weakly expressed or did not express ODAM at all

Fig. 4.

Effect of overexpression of rat ODAM (rODAM) on osteogenesis in the human and rat dental follicle stem cells. Rat ODAM plasmids, pCMVrODAM (prODAM) and the control plasmid, pCMV-3tag3 (3T3), were transfected into the human or rat DFSCs by Polyjet method. (a) RT-PCR to determine overexpression of rODAM transgene at the indicated days post transfection (Days post-Tranf.) of the plasmids into the human DFSCs (hDFSCs). (b) RT-PCR to determine overexpression of rODAM transgene at the indicated days post-transfection of the plasmids into the rat DFSCs (rDFSCs). (c) Osteogenesis of the transfected hDFSCs and rDFSCs after osteogenic induction as revealed by Alizarin red staining. (d, e) Osteogenesis of the transfected hDFSCs and rDFSCs, respectively after osteogenic induction as revealed by alkaline phosphatase (ALP) activity assay. Note that overexpression of rODAM transgene increased and expedited the osteogenesis after 5 and 7 days of induction for both hDFSCs and rDFSCs; however, no significant difference was seen at 14 days of induction. Rat Actin (rActin) or human Actin (hActin) was used as endogenous controls in the RT-PCR analyses. ** indicates highly significant ($P < 0.01$) with Student T-test. NS= not significant

Fig. 5.

Effect of continuous knockdown of ODAM expression in rat DFSCs on osteogenic differentiation. (a) Real-time RT-PCR determined that ODAM expression was knocked down in the DFSCs by transfection of ODAM-siRNA. (b) Continuous knockdown of ODAM expression by siRNA significantly reduced ALP activity in DFSCs during 2 weeks of osteogenic induction as compared to the scrambled siRNA treatment; however, no significant difference of ALP activity in the DFSCs was seen after 3 and 4 weeks of osteogenic induction in the ODAM continuous knockdown treatment as compared to the scrambled siRNA control.

Table 1

Relative gene expression (RGE) of ODAM in the DF of rat first mandibular molars at different postnatal days as determined by real-time RT-PCR

Postnatal days	CT	CT	RGE
1	23.5±0.6	0.0±0	1±0 a
3	20.3±0.3	-3.6±0.8	17.4±8.0a
5	18.6±0.6	-5.3±0.3	39.9±7.0a
7	16.3±0.3	-7.7±0.2	217.0±33.9b
9	15.0±0.2	-8.7±0.5	486.7±145.6b
11	14.1±0.0	-9.7±0.6	1031.9±360.1c