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The Effects of LAMP1 and LAMP3 on M180 Amelogenin Uptake, Localization and Amelogenin mRNA Induction by Amelogenin Protein

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We previously demonstrated that the uptake of M180 amelogenin protein in dental epithelial cells (HAT-7) results in increased levels of amelogenin mRNA through enhanced mRNA stabilization. To determine the processes involved in the uptake of extracellular M180 amelogenin by cells and in amelogenin intracellular trafficking in the amelogenin protein-mediated amelogenin mRNA expression pathway, we investigated the effects of LAMP1 and LAMP3, which are candidate M180 amelogenin receptors, on M180 amelogenin uptake, localization and amelogenin mRNA induction by amelogenin protein, using anti-LAMP-1 and anti-LAMP-3 antibodies and siRNA analysis. The results indicate that LAMP3 blocking by anti-LAMP-3 decreases M180 amelogenin uptake, but does not affect amelogenin mRNA induction by amelogenin protein, suggesting that LAMP3 is related to amelogenin degradation. Down-regulation by siRNA of LAMP1, which is the receptor for small amelogenin protein (LRAP), does not affect M180 amelogenin uptake, localization or amelogenin mRNA induction by amelogenin protein. Thus, while LAMP1 is the specific receptor for LRAP, it is not a receptor for M180 amelogenin. These findings will aid further research into the understanding of M180 amelogenin function and expression.

Key words: amelogenin, LAMP1, LAMP3, ameloblast, tooth development.

Abbreviations: FITC, fluorescein isothiocyanate; LAMP, lysosomal associated membrane protein; LRAP, leucine-rich amelogenin peptides.

Amelogenin is the major protein component of enamel matrix. The expression of amelogenin is spatially temporally regulated at both the transcription– and post-transcription levels in tooth development (*1–5*). We demonstrated in a previous study that the uptake of amelogenin protein in dental epithelial cells (HAT-7) results in increased levels of amelogenin mRNA through enhanced mRNA stabilization (*5*, *6*). This finding is important for understanding amelogenin overexpression in tooth development. However, little is known about the processes involved in the uptake of extracellular amelogenin by cells and amelogenin intracellular trafficking in the amelogenin protein-mediated amelogenin mRNA induction pathway.

Alternative splicing of amelogenin pre-mRNA leads to the production of many isoforms (*7–13*). The smaller splice products, produced upon the deletion of exon 6ABC are known as the leucine-rich amelogenin peptides, LRAP or [A-4]/M59 and have been shown to act differently, as signalling molecules affecting odontogenic and other cell types (*14–17*). The larger forms, such as M180 amelogenin protein, contain the intact proline-rich, hydrophobic exon 6 domains, and are important for enamel mineralization (*18*). Thus, LRAP and M180 amelogenin have different functions, and the regulation of M180 amelogenin protein expression appears to differ from the regulation of LRAP expression.

The mammalian endocytic system plays a number of important roles, including the processing of extracellularly derived nutrients, regulation of activated surface receptors, maintenance of membrane homeostasis and defence against external pathogens. In these endocytic processes, internalized ligands are first delivered to endosomes. Subsequently, ligands, or ligand–receptor complexes, are either delivered to lysosomes for degradation or recycled to the plasma membrane or Golgi (*19*). Several highly glycosylated lysosomal membrane proteins have been identified (*20*), and are known to be related to cell endocytosis (*21*, *22*). Lysosomal-associated membrane protein-1 (LAMP-1) and LAMP-2 are major protein components of the lysosomal membrane (*23*). At steady state, LAMP1 is highly expressed in late endosomes and lysosomes. However, in many cell types, LAMP1 can also be observed in early endocytic compartments (*19*). Moreover, LAMP1 immunoreactivity is also observed at the plasma membrane of most cell types (*23–25*). Recently, LAMP1 was identified as a cell surface receptor for a mouse small amelogenin protein, [A-4]/M59 (LRAP) (*26*). A previous study reported that LAMP1 is expressed by ameloblasts at all stages of amelogenesis, and extracellular Emdogain (the major component of

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which is amelogenin) is transferred into the cell cytoplasm via direct passage of amelogenin into LAMP1-positive vesicles (*27*). Amino acid sequence of M180 amelogenin almost overlaps to LRAP, without exon 6 ABC. This suggests that LAMP1 could bind to M180 amelgenin, so LAMP1 is the candidate for the cell surface receptor for M180 amelogenin.

A member of the tetraspanin superfamily, LAMP-3 (also known as CD63) is a well-understood lysosomal integral membrane glycoprotein (*28–30*). Tetraspanins mediate signal transduction events that play a role in the regulation of cell development, growth, mobility and activation (*30*, *31*). LAMP3 localizes not only in late endosomes, lysosomes and secretary vesicles, but also expresses in the plasma membrane of most cell types (*32–34*). LAMP3 has been shown to internalize anti-LAMP3 antibodies in HUVECs, and then transport these antibodies to late endosomes and Weibel–Palade bodies (*32*), suggesting that LAMP3 plays a role in trafficking LAMP3-binding molecules between different parts of the cytoplasm. LAMP3 has also been shown to interact with amelogenin by yeast two-hybrid analysis (*35*), and endocytosed amelogenin co-localizes in CD63-positive cells (*27*). These findings suggest that LAMP1 and LAMP3 are candidate cell surface receptors for M180 amelogenin.

To elucidate the uptake pathway of M180 amelogenin, we examined the effects of LAMP1 and LAMP3 on M180 amelogenin uptake, localization and amelogenin mRNA induction by amelogenin protein. Our results suggest that LAMP3 is important for M180 amelogenin uptake and localization, but not for amelogenin mRNA induction by amelogenin protein, and that LAMP1 is not involved with M180 amelogenin uptake, localization or amelogenin mRNA induction by amelogenin protein.

MATERIALS AND METHODS

Cell Culture—A rat dental epithelial cell line, HAT-7, which is an ameloblast-like cell line originating from the apical bud of rat incisor (*36*), was used in this study and was cultured as previously described (*5*, *6*, *37*, *38*).

FITC Labelling of Recombinant Amelogenin Protein—The recombinant mouse amelogenin (M180) used in this study was expressed using the baculovirus insect cell protein expression system as previously described (*5*). The FluoReporter FITC Protein Labeling Kit (F6434) was purchased from Molecular Probes (USA). FITC-amelogenin labelling was performed as previously described (*5*).

LAMP1 or LAMP3 Blocking and Uptake of FITC-amelogenin—Rabbit anti-LAMP1 polyclonal antibody (ab24170, Abcam, MA, USA) and rabbit anti-LAMP3 polyclonal antibody (sc-15363, Santa Cruz, CA, USA) were used to block LAMP1 and LAMP3, respectively. Rabbit polyclonal IgG-isotype control (ab27478, Abcam) was used as the control. Briefly, HAT-7 cells were seeded on coverglass slips the day before the experiment and cultured to 40–60% confluence. The cells were washed with serum-free culture medium and then incubated in anti-LAMP3 or anti-LAMP1 antibody at 40 μg/ml in serum-free medium for 16 h at 37°C. After antibody blocking, 10 μg/ml of FITC-amelogenin was added and the cells incubated for 4 h at 37°C. Subsequently, the cells were washed three times with phosphate-buffered saline (PBS), and then fixed in 4% paraformaldehyde for 20 min at room temperature. The nuclei were stained with Hoechst dye. After PBS washes, the coverslips were mounted on slides and the cells were observed under a confocal laser scanning microscope (Zeiss, LSM 510).

LAMP1 and LAMP3 Immunofluorescent Staining—Rabbit polyclonal anti-LAMP3 (FL-238) antibody (Santa Cruz) was used for LAMP3 immunostaining and rabbit polyclonal anti-LAMP1 antibody (ab24170, Abcam) was used for LAMP1 immunostaining. Alexa Fluor 594, goat anti-rabbit IgG (H+L) (A11037, Molecular probes, OR, USA) was used as the secondary antibody. Briefly, cells cultured on coverglass slips were washed three times with PBS and fixed in 4% paraformaldehyde for 20 min at room temperature. After PBS washes, the cells were blocked in 1% BSA–PBS for 1 h at room temperature and then incubated for 1 h at 37°C with the primary antibody diluted 1:50 in 0.1% BSA–PBS. The cells were then washed and incubated for 1 h at 37°C with the secondary antibody diluted 1:200 in 0.1% BSA–PBS. The nuclei were stained with Hoechst dye. The coverslips were mounted and the cells were observed under a laser confocal microscope (Zeiss, LSM 510).

LAMP1 siRNA Knockdown—The LAMP1 ds-siRNA oligonucleotide sequence was GGA GAA GGA UAU UUA CUG ATT (Rat LAMP1, accession No: M34959, 371–389 nt, designed and constructed by Takara, Ohtsu, Japan). The LAMP1 siRNA control sequence was AGG UAA UCA AGG UGG UAU ATT. The RNA interference was performed by transient transfection of the ds-siRNA into the cells using Lipofectamine RNAiMAX transfection reagent (Invitrogen, Carlsbad, CA, USA). Briefly, HAT-7 cells were incubated in a 6-well plate the day before the experiment and cultured to 50–70% confluence. A dilution of 5 μl of Lipofectamine RNAiMAX in 250 μl of Opti-MEM was mixed with a dilution of 4 μl of LAMP1 ds-siRNA in 250 μl of Opti-MEM and then incubated at room temperature for 20 min. After changing the medium to antibiotic-free medium, the Lipofectamine RNAi MAX/LAMP1 ds-siRNA mixture (ds-siRNA final concentration: 32 nM) was applied to the cells. The knockdown efficiencies were confirmed by determining the decreases in the levels of LAMP1 mRNA and protein expression. LAMP1 mRNA expression was determined using the real-time RT–PCR SYBR method. The decreased levels of LAMP1 protein expression were confirmed using immunofluorescent (IF) staining, as described above.

Real-Time RT–PCR—Amelogenin and LAMP1 mRNA expression levels in rat HAT-7 cells were determined using the real-time RT–PCR SYBR method described previously (*5*). The house-keeping gene GAPDH was used as the endogenous control. The primers used in this study were as follows: for amelogenin, forward 5′-TGG GAG CCC TGG TTA TAT CAA-3′, and reverse 5′-GCG GCT GCC TTA TCA TGC T-3′; for GAPDH, forward 5′-GCC CCC AAC ACT GAG CAT-3′, and reverse 5′-CCA GGC CCC TCC TGT TGT-3′; for LAMP1, forward 5′-AGT GTC CAG CAC ATG TAT TTC ACA TAT-3′, and reverse 5′-AGT GTC GGG CCC TTT GG-3′.

RESULTS

Effects of Anti-LAMP1 and Anti-LAMP3 Antibody on M180 Amelogenin Uptake and Localization—We showed in previous studies that extracellular M180 amelogenin is taken up by HAT-7 cells and localizes in the perinuclear region near the ER, where amelogenin protein increases its own mRNA expression level through enhanced mRNA stabilization (*5*, *6*). Recently, amelogenin-binding proteins have been cloned using the yeast two-hybrid assay (*35*). In that study, an integral membrane protein, LAMP3, was observed to interact with amelogenin. Moreover, LAMP1 was identified as a cell surface receptor for a mouse small amelogenin protein, LRAP (*26*). In the present study, to analyse the M180 amelogenin uptake pathway, LAMP1 or LAMP3 expression was blocked by anti-LAMP1 or anti-LAMP3 antibody and FITC-labelled M180 amelogenin was subsequently added. After 4 h, the uptake and localization of FITC-amelogenin was observed by laser scanning microscopy. The signals of FITC-amelogenin in the cytoplasm and perinuclear were significantly decreased by anti-LAMP3 antibody treatment when compared to the IgG control (Fig. 1A). Prior to antibody treatment FITC-amelogenin was localized in the perinuclear region near the ER; however, upon antibody treatment the localization of FITC-amelogenin changed to the cytoplasm (Fig. 1B). No significant differences in the uptake and localization of FITC-amelogenin were observed by anti-LAMP1 antibody treatment when compared with the IgG control (Fig. 1A). These results indicate that LAMP3 is involved in the uptake and localization of M180 amelogenin in HAT-7 cells, but LAMP1 is not.

Effect of LAMP3 Overexpression on M180 Amelogenin Uptake and Localization—Next, we found that LAMP3 overexpression resulted in significantly greater uptake of FITC-amelogenin (Fig. 2, left panel) compared with transfection of the control vector (Fig. 2, right panel). These results also suggest that LAMP3 is important for M180 amelogenin uptake in HAT-7 cells.

FITC-labelled M180 Amelogenin Co-localizes with LAMP3 in the Perinuclear Region in HAT-7 Cells—To elucidate the uptake pathway, we determined the localization of LAMP3 and amelogenin in HAT-7 cells. Exogenously added FITC-labelled M180 amelogenin

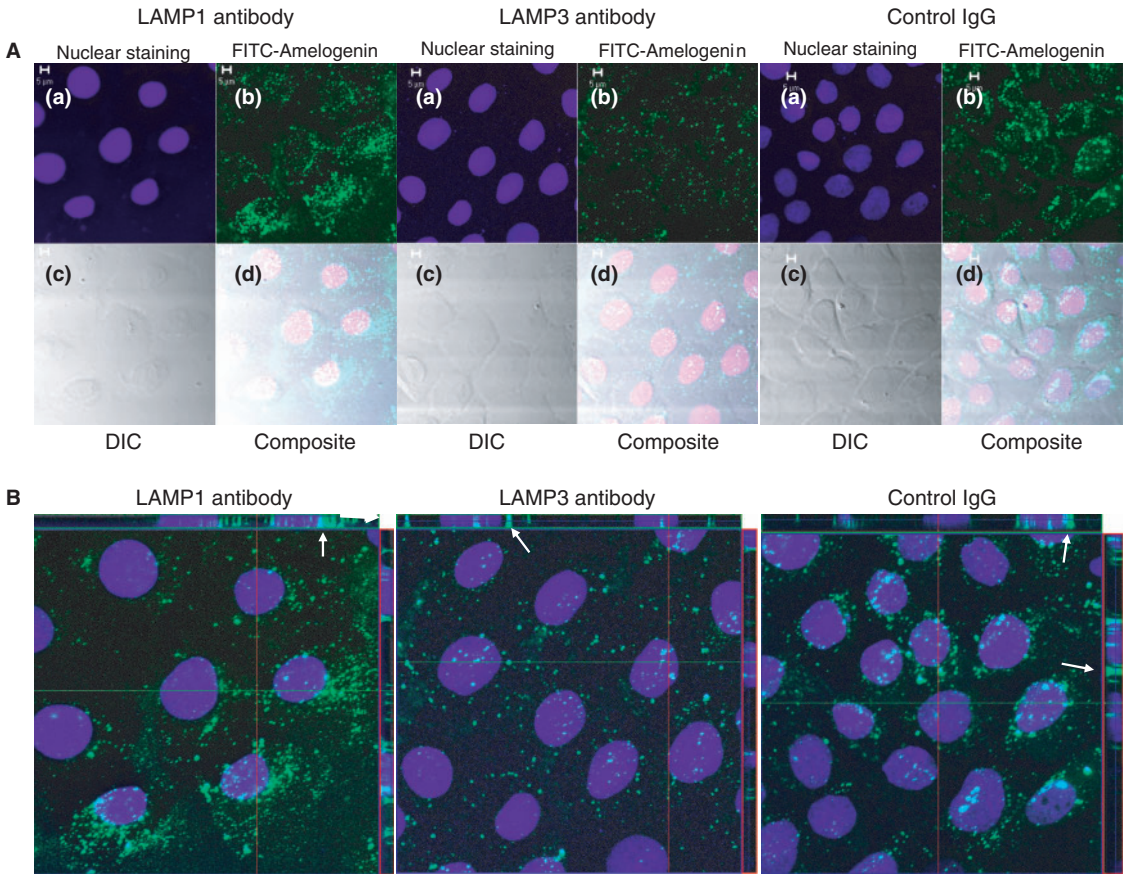

Fig. 1. **Effects of anti-LAMP1 and anti-LAMP3 antibody on amelogenin uptake and localization.** (A) Confocal images of uptake and localization of exogenously added FITC-amelogenin in HAT-7 cells. Left panel: anti-LAMP-1 antibody; Center panel: anti-LAMP3 antibody addition; right panel: rabbit IgG as control. HAT-7 cells were treated with 40 μg/ml of antibody for 16 h and then 10 μg/ml of FITC-labelled amelogenin protein was added exogenously to the cells for 4 h, after which they were observed under a laser scanning confocal microscope (Zeiss, LSM 510). (a) Split images of nuclear staining, (b) FITC-amelogenin signals, (c) differential interference contrast image, (d) composite of a, b and c. (B) The Z-section images of FITC-labelled amelogenin protein localization. The arrows indicate the localization of endocytosed FITC-amelogenin in the perinuclear region.

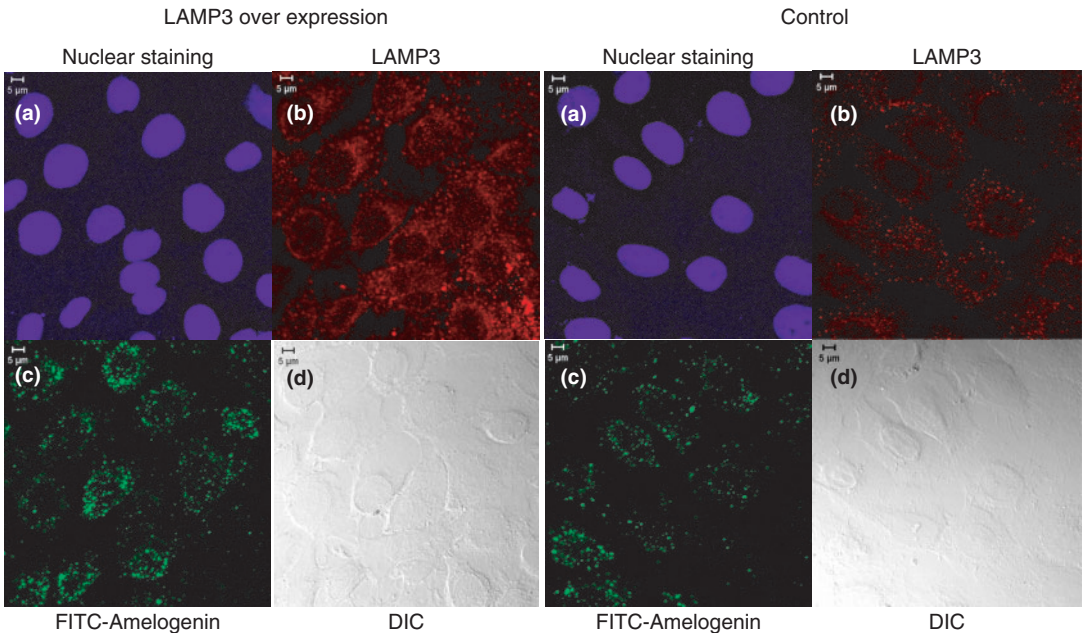

Fig. 2. **Effect of LAMP3 overexpression on amelogenin uptake and localization.** Confocal images of uptake of exogenously added FITC-amelogenin and LAMP3 immunofluorescent staining of HAT-7 cells transfected with LAMP3 expression vector (left panel) or control vector (right panel). (a) Split images of nuclear staining, (b) LAMP3 signals, (c) FITC-amelogenin signals and (d) differential interference contrast image.

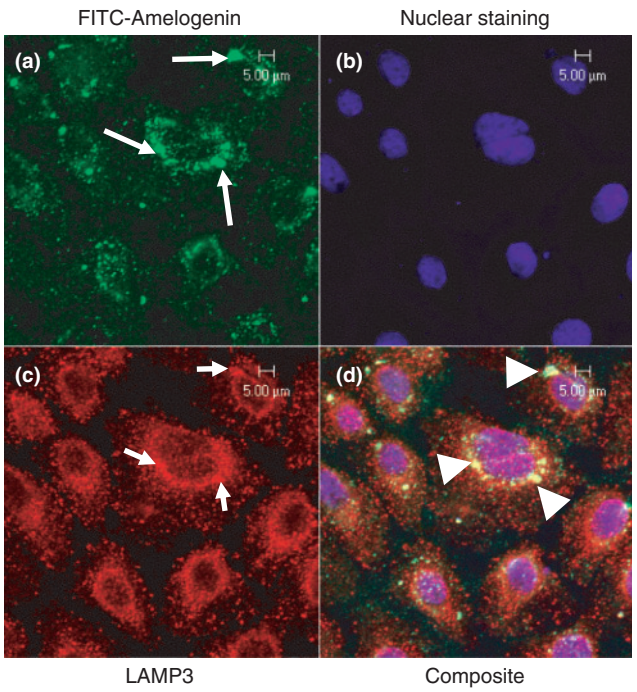

Fig. 3. **Co-localization of endocytosed FITC-amelogenin and LAMP3 expression.** (a) FITC-amelogenin signals, (b) split images of nuclear staining, (c) LAMP3 signals, (d) composite of a, b and c.

co-localized with endogenous LAMP3 at the perinuclear region, and LAMP3 was expressed throughout the cytoplasm as well as at the plasma membrane in HAT-7 cells (Fig. 3). These results suggest that LAMP3 is closely involved in extracellular M180 amelogenin uptake and M180 amelogenin intracellular trafficking in HAT-7 cells.

LAMP1 Down-regulation does not Affect the Uptake and Localization of M180 Amelogenin—Anti-LAMP1 antibody treatment did not inhibit the uptake of extracellular amelogenin. Because it was possible that the epitope specificity of the LAMP1 antigen was different from the amelogenin binding site, LAMP1 was down-regulated using siRNA to further clarify the involvement of LAMP1 in exogenous amelogenin uptake. LAMP1 siRNA efficiency was confirmed by mRNA expression level analysis, as shown in Fig. 4A. LAMP1 siRNA did not affect the uptake of extracellular amelogenin or its localization in the cytoplasm (Fig. 4B, left panel) compared to control siRNA transfection (Fig. 4B, right panel). These results indicate that LAMP1 is not involved in the uptake of extracellular M180 amelogenin or in its intracellular trafficking in HAT-7 cells.

Down-regulation of LAMP1 and LAMP3 does not Affect Up-regulation of Amelogenin mRNA Expression by M180 Amelogenin Protein Treatment—To determine the effect of LAMP1 and LAMP3 down-regulation on amelogenin mRNA expression, amelogenin mRNA induction by M180 amelogenin protein treatment was determined under LAMP1 siRNA or anti-LAMP3 antibody treatment. In the case of LAMP1 siRNA, after 6 h of LAMP1-siRNA transfection, amelogenin was added and the cells subsequently cultured for 24 h. The LAMP1 mRNA level was then determined to verify knockdown efficiency. Amelogenin mRNA induction was determined with or without LAMP1-siRNA treatment (Fig. 5A). No significant difference in amelogenin mRNA induction was observed between LAMP1 siRNA treatment and the controls. In the case of LAMP3, after 6 h of anti-LAMP3

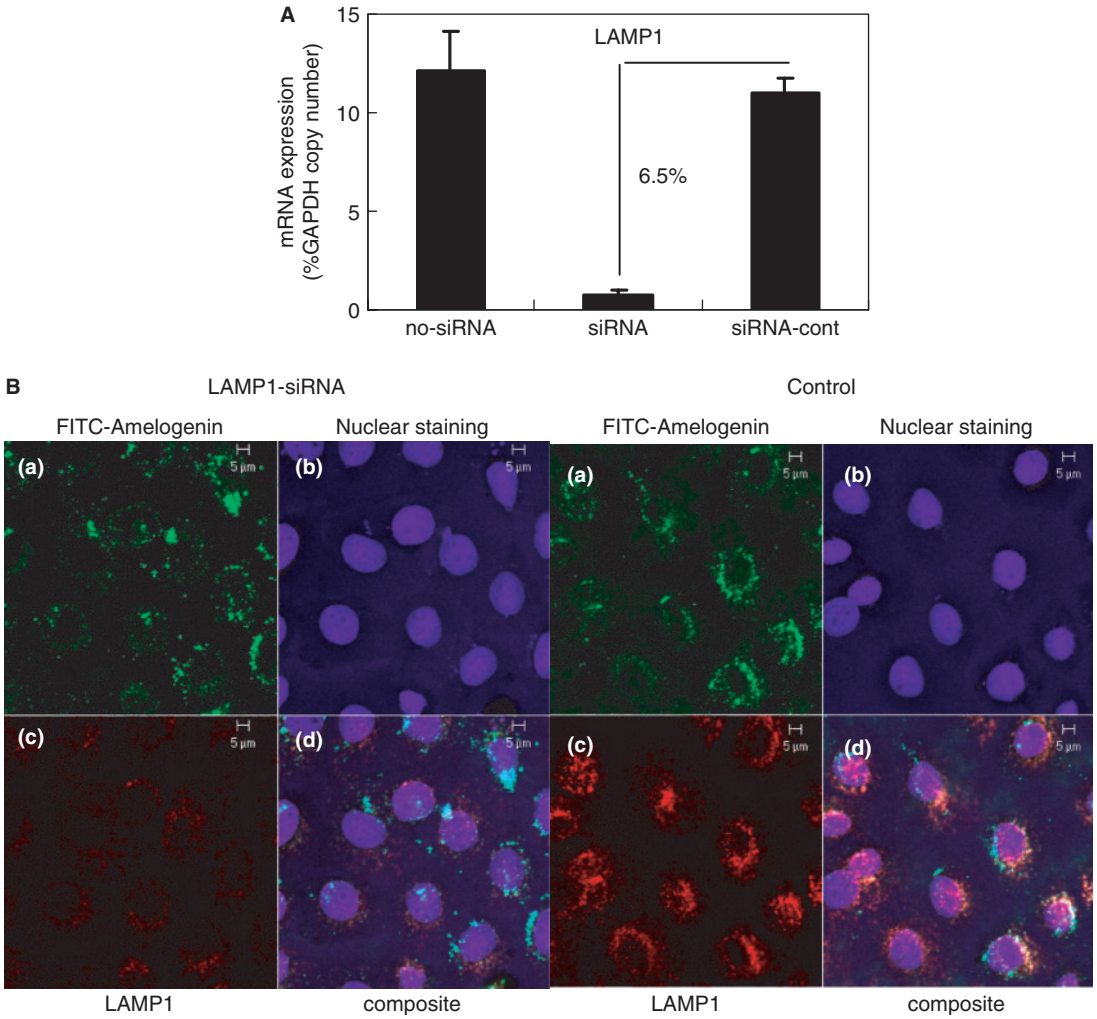

Fig. 4. **Effect of LAMP1 siRNA on amelogenin uptake and localization.** (A) Endogenous LAMP1 mRNA expression by LAMP1 siRNA and control-siRNA transfection. The siRNA knockdown efficiencies were confirmed by mRNA expression levels. Values from at least three independent experiments are represented in (A). Error bars indicate mean ± SD. (B) Confocal images of uptake of exogenously added FITC-amelogenin and LAMP1 immunofluorescent staining in HAT-7 cells transfected with LAMP1 siRNA (left panel) and control-siRNA (right panel) transfection. (a) FITC-amelogenin signals, (b) split images of nuclear staining, (c) LAMP1 signals and (d) composite of a, b and c.

antibody treatment, amelogenin was added and the cells subsequently cultured for 24 h. Amelogenin mRNA induction was then determined with or without anti-LAMP3 antibody treatment. No significant difference in amelogenin mRNA induction was observed between anti-LAMP3 antibody treatment and the control (Fig. 5B). These results suggest that although LAMP3 is important for M180 amelogenin uptake, neither LAMP1 nor LAMP3 are involved in amelogenin mRNA induction by M180 amelogenin protein.

DISCUSSION

Amelogenin is a major component of the enamel matrix proteins, and plays an important role in enamel formation. In the process of amelogenesis, amelogenin dynamics include two pathways: amelogenin over expression in the pre-secretory/secretory ameloblasts and amelogenin degradation in the late stage of enamel formation. In this study, we examined the effects of LAMP1 and LAMP3 on M180 amelogenin uptake, localization and amelogenin mRNA induction by amelogenin protein. Our results suggest that LAMP3 is important for M180 amelogenin uptake and localization, but is not involved in amelogenin mRNA induction by amelogenin protein, and that LAMP1 is not involved in M180 amelogenin uptake, localization or amelogenin mRNA induction by amelogenin protein. Our findings increase our understanding of M180 amelogenin expression and degradation.

The re-uptake of extracellular amelogenin in ameloblasts is believed to play a role in both amelogenin expression in the pre-secretory/secretory stages and in amelogenin degradation in the post-secretory stage. In a previous study, immunohistochemistry of developing mouse mandibular incisor teeth showed higher levels of LAMP3 expression in late-stage amelogenesis compared to secretory or pre-secretory ameloblasts. We found in

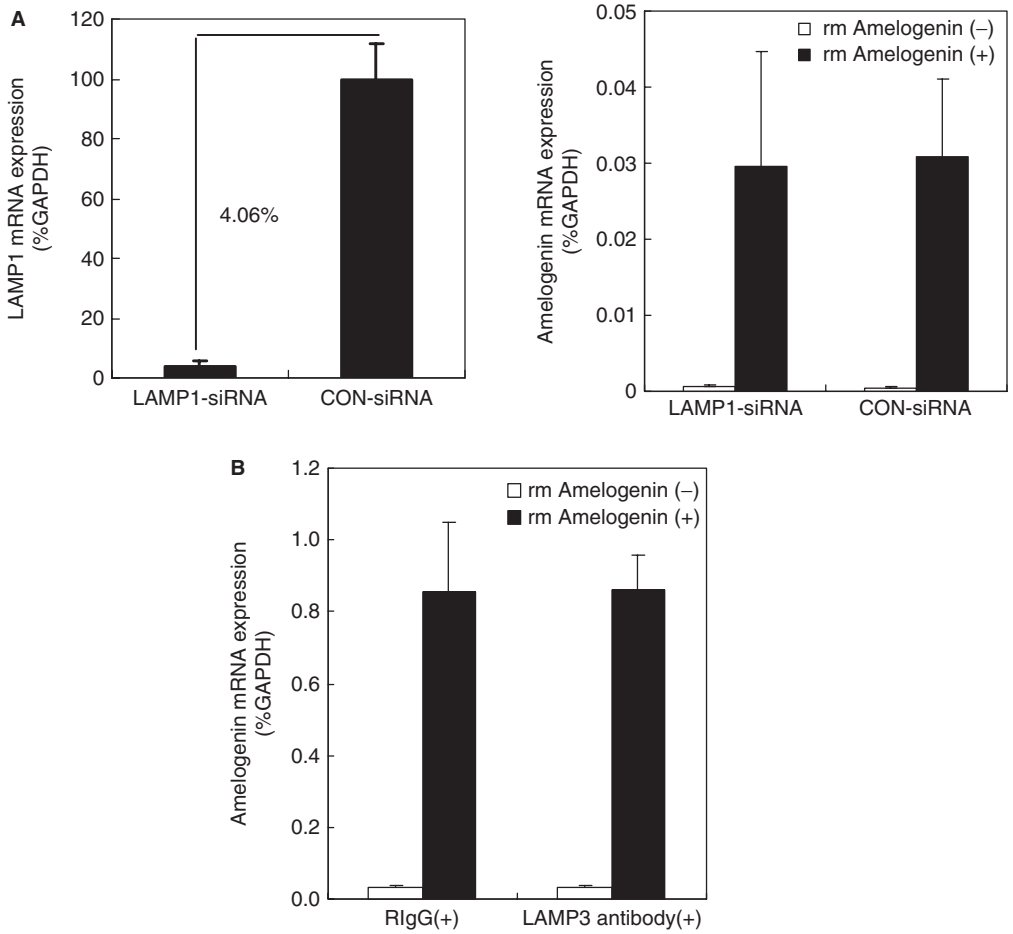

Fig. 5. **Down-regulation of LAMP1 and LAMP3 did not affect up-regulation of amelogenin mRNA expression by amelogenin protein treatment.** (A) Effect of LAMP1 siRNA on amelogenin mRNA expression by amelogenin protein treatment. Left: endogenous LAMP1 mRNA expression; right: amelogenin mRNA expression under same conditions. (B) Effect of LAMP3 antibody treatment on amelogenin mRNA expression by amelogenin protein treatment. Values from at least three independent experiments are represented. Error bars indicate mean ± SD.

the present study that LAMP3 is involved in uptake of exogenous M180 amelogenin but not with induction of amelogenin mRNA by amelogenin protein, suggesting that LAMP3 is related to amelogenin degradation. Thus, an as yet unknown receptor is involved with amelogenin protein-mediated amelogenin mRNA induction.

Previous studies have shown that LAMP1 co-localizes to internalized small amelogenin protein, LRAP, in the perinuclear region of cells (*26*, *39*, *40*). Exogenous LRAP transfer into the cytoplasm involves the direct passage of LRAP into LAMP1-positive vesicles, suggesting that LAMP1 may function as a specific, endocytosing ligand-binding receptor for LRAP. LAMP1 may also be involved in the trafficking of amelogenin to late endosomes or lysosomes (*26*, *27*). We found here that LAMP1 down-regulation by siRNA does not affect M180 amelogenin uptake in HAT-7 cells, which suggests that while LAMP1 may be the specific receptor for LRAP, it is not a receptor for M180 amelogenin.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Zhou, Y.L. and Snead, M.L. (2000) Identification of CCAAT/enhancer-binding protein alpha as a transactivator of the mouse amelogenin gene. *J. Biol. Chem.* **275**, 12273–12280
2. Zhou, Y.L., Lei, Y., and Snead, M.L. (2000) Functional antagonism between Msx2 and CCAAT/enhancer-binding protein alpha in regulating the mouse amelogenin gene expression is mediated by protein-protein interaction. *J. Biol. Chem.* **275**, 29066–29075
3. Xu, Y., Zhou, Y.L., Luo, W., Zhu, Q.S., Levy, D., MacDougald, O.A., and Snead, M.L. (2006) NF-Y and CCAAT/enhancer-binding protein alpha synergistically activate the mouse amelogenin gene. *J. Biol. Chem.* **281**, 16090–16098
4. Xu, Y., Zhou, Y.L., Ann, D.K., MacDougald, O.A., Shum, L., and Snead, M.L. (2006) Transcription factor sumoylation and factor YY1 serve to modulate mouse amelogenin gene expression. *Eur. J. Oral Sci.* **114**, 169–177
5. Xu, L., Harada, H., Tamaki, T.Y., Matsumoto, S., Tanaka, J., and Taniguchi, A. (2006) Reuptake of extracellular amelogenin by dental epithelial cells results in increased levels of amelogenin mRNA through enhanced mRNA stabilization. *J. Biol. Chem.* **281**, 2257–2262
6. Xu, L., Harada, H., and Taniguchi, A. (2006) The exon 6ABC region of amelogenin mRNA contribute to increased levels of

amelogenin mRNA through amelogenin protein-enhanced mRNA stabilization. *J. Biol. Chem.* **281**, 32439–32444
7. Lau, E.C., Simmer, J.P., Bringas, P., Hsu, D.D., Hu, C.-C., Zeichner-David, M., Thiemann, F., Snead, M.L., Slavkin, H.C., and Fincham, A.G. (1992) Alternative splicing of the mouse amelogenin primary RNA transcript contributes to amelogenin heterogeneity. *Biochem. Biophys. Res. Commun.* **188**, 1253–1260
8. Simmer, J.P., Hu, C.-C., Lau, E.C., Sarte, P., Slavkin, H.C., and Fincham, A.G. (1994) Alternative splicing of the mouse amelogenin primary RNA transcript. *Calcif. Tissue Int.* **55**, 302–310
9. Brookes, S.J., Robinson, C., Kirkham, J., and Bonass, W.A. (1995) Biochemistry and molecular biology of amelogenin proteins of developing dental enamel. *Arch. Oral Biol.* **40**, 1–14
10. Simmer, J.P. (1995) Alternative splicing of amelogenins. *Connect. Tissue Res.* **32**, 131–136
11. Bonass, W.A., Kirkham, J., Brookes, S.J., Shore, R.C., and Robinson, C. (1994) Isolation and characterisation of an alternatively-spliced rat amelogenin cDNA: LRAP–a highly conserved, functional alternatively-spliced amelogenin? *Biochim. Biophys. Acta* **1219**, 690–692
12. Li, R., Li, W., and DenBesten, P.K. (1995) Alternative splicing of amelogenin mRNA from rat incisor ameloblasts. *J. Dent. Res.* **74**, 1880–1885
13. Hu, C.-C., Bartlett, J.D., Zhang, C.H., Qian, Q., Ryu, O.H., and Simmer, J.P. (1996) Cloning, cDNA sequence, and alternative splicing of porcine amelogenin mRNAs. *J. Dent. Res.* **75**, 1735–1741
14. Boabaid, F., Gibson, C.W., Kuehl, M.A., Berry, J.E., Sneed, M.L., Nociti, F.H. Jr, Katchburian, E., and Somerman, M.J. (2004) Leucine-rich amelogenin peptide: a candidate signaling molecule during cementogenesis. *J. Periodontol.* **75**, 1126–1136
15. Veis, A., Tompkins, K., Alvares, K., Wei, K., Wang, L., Wang, X.S., Brownell, A.G., Jengh, S.M., and Healy, K.E. (2000) Specific amelogenin gene splice products have signaling effects on cells in culture and in implants in vivo. *J. Biol. Chem.* **275**, 41263–41272
16. Veis, A. (2003) Amelogenin gene splice products: potential signaling molecules. *Cell Mol. Life Sci.* **60**, 38–55
17. Tompkins, K., Alvares, K., George, A., and Veis, A. (2005) Two related low molecular mass polypeptide isoforms of amelogenin have distinct activities in mouse tooth germ differentiation in vitro. *J. Bone Miner. Res.* **20**, 341–349
18. Moradian –Oldak, J., Simmer, J.P., Lau, E.C., Diekwisch, T., Slavkin, H.C., and Fincham, AG. (1995) A review of the aggregation properties of a recombinant amelogenin. *Connect. Tissue Res.* **32**, 125–130
19. Cook, N. R., Row, P. E., and Davidson, H. W. (2004) Lysosome associated membrane protein 1 (Lamp1) traffics directly from the TGN to early endosomes. *Traffic* **5**, 685–699
20. Peters, C. and von Figura, K. (1994) Biogenesis of lysosomal membranes. *FFBS Lett.* **346**, 108–114
21. Duffield, A., Kamsteeg, E-J., Brown, A.N., Pagel, P., and Caplan, M.J. (2003) The tetraspanin CD63 enhances the internalization of the H,K-ATPase beta-subunit. *Proc. Natl Acad. Sci. USA* **100**, 15560–15565
22. Hunziker, W., Simmen, T., and Honing, S. (1996) Trafficking of lysosomal membrane proteins in polarized kidney cells. *Nephrologie* **17**, 347–350
23. Kannan, K., Stewart, R. M., Bounds, W., Carlsson, S. R., Fukuda, M., Betzing, K. W., and Holcombe, R. F. (1996) Lysosome-associated membrane proteins h-LAMP1 (CD107a) and h-LAMP2 (CD107b) are activation-dependent cell surface glycoproteins in human peripheral blood mononuclear cells which mediate cell adhesion to vascular endothelium. *Cell Immunol.* **171**, 10–19
24. Silverstein, R.L. and Febbraio, M. (1992) Identification of lysosome-associated membrane protein-2 as an activation-dependent platelet surface glycoprotein. *Blood* **80**, 1470–1475
25. Holcombe, R.F., Baethge, B.A., Stewart, R.M., Betzing, K., Hall, V.C., Fukuda, M., and Wolf, R.E. (1993) Cell surface expression of lysosome-associated membrane proteins (LAMPs) in scleroderma: relationship of lamp2 to disease duration, anti-Sc170 antibodies, serum interleukin-8, and soluble interleukin-2 receptor levels. *Clin. Immunol. Immunopathol.* **67**, 31–39
26. Tompkins, K., George, A., and Veis, A. (2006) Characterization of a mouse amelogenin [A-4]/M59 cell surface receptor. *Bone* **38**, 172–180
27. Shapiro, J.L., Wen, X., Okamoto, C.T., Wang, H.J., Lyngstadaas, S.P., Goldberg, M., Snead, M.L., and Paine, M.L. (2007) Cellular uptake of amelogenin, and its localization to CD63, and Lamp1-positive vesicles. *Cell. Mol, Life Sci.* **64**, 244–256
28. Metzelaar, M.J., Wijngaard, P.L., Peters, P.J., Sixma, J.J., Nieuwenhuis, H.K., and Clevers, H.C. (1991) CD63 antigen. A novel lysosomal membrane glycoprotein, cloned by a screening procedure for intracellular antigens in eukaryotic cells. *J. Biol. Chem.* **266**, 3239–3245
29. Stipp, C.S., Kolesnikova, T.V., and Hemler, M.E. (2003) Functional domains in tetraspanin proteins. *Trends Biochem. Sci.* **28**, 106–112
30. Yunta, M. and Lazo, P. A. (2003) Tetraspanin proteins as organisers of membrane microdomains and signalling complexes. *Cell Signal* **15**, 559–564
31. Mantegazza, A.R., Barrio, M.M., Moutel, S., Bover, L., Weck, M., Brossart, P., Teillaud, J.L., and Mordoh, J. (2004) CD63 tetraspanin slows down cell migration and translocates to the endosomal-lysosomal-MIICs route after extracellular stimuli in human immature dendritic cells. *Blood* **104**, 1183–1190
32. Kobayashi, T., Vischer, U.M., Rosnoblet, C., Lebrand, C., Lindsay, M., Parton, R.G., Kruithof, E.K., and Gruenberg, J. (2000) The tetraspanin CD63/lamp3 cycles between endocytic and secretory compartments in human endothelial cells. *Mol. Biol. Cell* **11**, 1829–1843
33. Escola, J.M., Kleijmeer, M.J., Stoorvogel, W., Griffith, J.M., Yoshie, O., and Geuze, H.J. (1998) Selective enrichment of tetraspan proteins on the internal vesicles of multivesicular endosomes and on exosomes secreted by human B-lymphocytes. *J. Biol. Chem.* **273**, 20121–20127
34. Arribas, M. and Cutler, D.F. (2000) Weibel-Palade body membrane proteins exhibit differential trafficking after exocytosis in endothelial cells. *Traffic* **1**, 783–793
35. Wang, H.J., Tannukit, S., Zhu, D.H., Snead, M.L., and Paine, M.L. (2005) Enamel matrix protein interaction. *J. Bone Miner. Res.* **20**, 1032–1040
36. Kawano, S., Morotomi, T., Toyono, T., Nakamura, N., Uchida, T., Ohish, M., Toyoshima, K., and Harada, H. (2002) Establishment of dental epithelial cell line (HAT-7) and the cell differentiation dependent on Notch signaling pathway. *Connect. Tissue Res.* **43**, 409–412
37. Xu, L., Takahashi, R., Harada, H., and Taniguchi, A. (2007) Effect of BMP-2 on gene expression of enamel matrix proteins at the dental epithelial cell line. *Open Biotechnol. J.* **1**, 18–20
38. Xu, L., Harada, H., Ikoma, T., and Taniguchi, A. (2008) Hydroxyapatite- and amelogenin protein-induced expression of mineralization-related genes in a dental epithelial cell line. *Open Biotechnol. J.* **2**, 116–120
39. Zou, Y., Wang, H., Shapiro, J.L., Okamoto, C.T., Brookes, S.J., Lyngstadaas, S.P., Snead, M.L., and Paine, M.L. (2007) Determination of protein regions responsible for interactions of amelogenin with CD63 and LAMP1. *Biochem. J.* **408**, 347–354
40. Iacob, S. and Veis, A. (2008) Identification of the functional activity of the [A-4] amelogenin gene splice product in newborn mouse ameloblasts. *Bone.* **42**, 1072–1079