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Full length amelogenin binds to cell surface LAMP-1 on tooth root/periodontium associated cells

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ABSTRACT

Objectives: Lysosome-associated membrane protein-1 (LAMP-1) has been suggested to be a cell surface receptor for a specific amelogenin isoform, leucine-rich amelogenin peptide or LRAP. However, it is unclear if LAMP-1 is an amelogenin receptor for dental mesenchymal cells. The goal of this study was to determine if LAMP-1 serves as a cell surface binding site for full length amelogenin on tooth root/periodontium associated mesenchymal cells.
Design: Murine dental follicle cells and cementoblasts (OCCM-30) were cultured for 2 days followed by addition of full length recombinant mouse amelogenin, rp(H)M180. Dose–response (0–100 μg/ml) and time course (0–120 min) assays were performed to determine the optimal conditions for live cell surface binding using immunofluorescent microscopy. A competitive binding assay was performed to determine binding specificity by adding Emdogain® (1 mg/ml) to the media. An antibody against LAMP-1 was used to detect the location of LAMP-1 on the cell surface and the pattern was compared to cell surface bound amelogenin. Both amelogenin and cell surface LAMP-1 were immuno-co-localized to compare the amount and distribution pattern.
Results: Maximum surface binding was achieved with 50 μg/ml of rp(H)M180 for 120 min. This binding was specific as demonstrated by competitive inhibition (79% lower) with the addition of Emdogain®. The binding pattern for rp(H)M180 was similar to the distribution of surface LAMP-1 on dental follicle cells and cementoblasts. The high co-localization coefficient (0.92) for rp(H)M180 and LAMP-1 supports rp(H)M180 binding to cell surface LAMP-1.
Conclusions: The data from this study suggest that LAMP-1 can serve as a cell surface binding site for amelogenin on dental follicle cells and cementoblasts.

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Abbreviations: rp(H)M180, 180 amino acid recombinant mouse amelogenin; LAMP-1, lysosome-associated membrane protein-1; LRAP, leucine-rich amelogenin peptide; TRAP, tyrosine-rich amelogenin peptide; PDL, periodontal ligament; E–M, epithelial–mesenchymal; HERS, Hertwig's epithelial root sheath; NF1C, nuclear factor 1C; BSP (Bsp), bone sialoprotein; OCN (Ocn), osteocalcin; OPG (Opg), osteoprotegerin; Runx2, runt-related transcription factor 2; OPN (Opn), osteopontin; NGS, normal goat serum; FBS, fetal bovine serum; HRP, horseradish peroxidase; TSA, tyramide signal amplification; OCCM-30, murine immortalized cementoblasts; DMEM, Dulbecco's Modified Eagle's Medium; PBS, phosphate buffered saline; IDPs, intrinsically disordered proteins.

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1. Introduction

Periodontal disease is marked by destruction of periodontal tissues, which can lead to tooth loss if left untreated. Recognition that periodontal regeneration can be achieved, i.e., formation of new bone, new cementum and supportive periodontal ligament (PDL), has resulted in increased attempts to understand the cellular and molecular mechanisms and factors that regulate formation of these tissues. It is well established that epithelial–mesenchymal (E–M) interactions are required for formation of the tooth crown (enamel and dentin).[1–3] Recent data suggest tooth root development begins with Sonic Hedgehog signalling emanating from Hertwig's epithelial root sheath (HERS) cells (stimulated by a currently unknown source) inducing abutting dental papilla cells to express the transcription factor nuclear factor 1C (NF1C). Without NF1C expression, root odontoblast driven root development does not occur.[4,5] This confirms that E–M signalling initiates root dentin formation, a requirement for full root development including cementum formation and a functional periodontium.

Amelogenin expression has been detected by some investigators in HERS cells and root tissue during this developmental time point.[6–9] Given the developmental history of these tissues, the use of an epithelial signalling protein to regenerate periodontal tissues is conceptually appealing and thus research directed at understanding the role of epithelium derived molecules in promoting tooth root formation should assist efforts to improve upon existing regenerative therapies. In fact, Emdogain® (EMD, Straumann, Switzerland) a predominately amelogenin containing medicament (derived from porcine enamel matrix) has been marketed for use in regenerating periodontal tissues based on the concept that E–M interactions are required for root formation.[8,10] Whilst not completely predictable, meaningful regenerative results have been reported when EMD was used to treat bone and periodontal defects in both animal models and human patients.[11–18] An early event in periodontium formation/regeneration is the development of acellular cementum and EMD is reported to alter the activity of dental follicle cells[19] and cementoblasts[20] *in vitro*. The mechanism of these effects remains to be defined, although amelogenin has been considered as the major factor responsible for these activities.[21] However, as discussed below, additional factors may also be playing a role.

Amelogenins are the most abundant proteins of the enamel matrix and belong to a family of proteins formed as a result of alternative splicing of a single primary transcript.[22,23] One of these alternatively spliced products is known as LRAP, or M59/[A-4] to emphasize the absence of the polar hydrophilic amino acid sequence translated from amelogenin exon 4.[24–26] In addition to their structural role in enamel formation, amelogenins have been shown to be involved in a range of activities, including mineral nodule formation and intercellular signalling.[25,27,28] Amelogenin and associated peptides are secreted primarily by dental epithelial cells known as ameloblasts.[29] Although there are conflicting reports,[30] amelogenins were found to have low level expression in other cells, including odontoblasts[31] and HERS cells that line the root during early phases of cementogenesis.[32,33] HERS cells are thought to either undergo apoptosis and/or transform into cementoblast-like cells, as well as remain as remnant epithelial rest cells within the mature PDL.[34–36]

Mice null for the amelogenin gene exhibited a defect in crown enamel formation[37] and also expressed low levels of transcripts and proteins for bone sialoprotein (BSP) and osteocalcin (OCN),[28] two markers of the mature cementoblast and osteoblast phenotype. A root phenotype (cementum defects) was also reported in these amelogenin-null mice, although this defect seems to relate to changes in osteoclast behaviour after the root is fully formed so it is not developmental in nature, per se.[10] These data suggest that epithelial cells or products are required for proper development of periodontal tissues, including cementum and a functional PDL.[36] A large body of work suggests a signalling function for amelogenins. Viswanathan et al. demonstrated that expression of both *Ocn* and *Bsp* were decreased when immortalized cementoblasts were treated with high dose of amelogenin.[28] Interestingly, when the same cell type was treated with LRAP or TRAP (tyrosine-rich amelogenin peptide, a degradation product of full length amelogenin), similar effects were observed: *Ocn* was down-regulated whilst osteopontin (*Opn*) was up-regulated in a dose–response fashion.[27,38] These effects were seen as early as 6 h post-treatment for *Opn*. Amelogenin spliced product LRAP/M59/[A-4] has been shown to enhance the expression of runt-related transcription factor 2 (*Runx2*), a master switch for defining the osteoblastic phenotype.[25] LRAP treatment of either wild-type or amelogenin-null mouse embryonic stem cells induced a significant increase in mineral matrix formation, and significant increases in *Bsp* and osterix gene expression.[39,40] These *in vitro* data complement *in vivo* data in support of a role for amelogenins in modulating the expression of mesenchymal mineralized tissue-associated genes.

Dental follicle cells constitute the dental follicle region (a loose connective tissue) surrounding the developing tooth. Dental follicle cells play a critical role in the process of root development and tooth eruption.[41] In addition, substantial evidence indicates that dental follicle cells are progenitors of periodontal mesenchymal cells including cementoblasts, PDL fibroblasts, and alveolar osteoblasts.[36,42,43] Dental follicle cells and/or cementoblasts are the proposed target cells for amelogenin signalling in the periodontal region. Addition of EMD to immortalized murine dental follicle cells resulted in increased *Opn* mRNA level and decreased *Ocn* mRNA expression. EMD also blocked the induced mineralization by dental follicle cells *in vitro*.[19] However, whether this was caused by amelogenin or other factors in EMD is not clear.[21] Nevertheless, how amelogenins and other factors regulate tooth root/periodontium development needs to be further elucidated.

Tompkins et al. demonstrated that LRAP/M59/[A-4] regulates mesenchymal cells (mouse myoblast cell line C2C12) at least partly through a 95 kDa cell surface receptor (LAMP-1).[44] They also showed another possible receptor appearing as a 75 kDa band in electrophoresis. In a simultaneous study, Wang et al. reported that enamel matrix proteins interacted with a number of secreted membrane proteins and integral proteins, including human CD63 antigen (LAMP-3),[45] using the yeast two-hybrid assay. However, in a more recent study using