

## Glucose-Regulated Protein 78: A Novel Therapeutic Target for Amelogenin-Induced Periodontal Tissue Regeneration

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### Abstract

Amelogenin, the major component of a commercial enamel matrix derivative (Straumann® Emdogain), is commonly employed in periodontology. It is mainly used in periodontal surgery to stimulate the regeneration of periodontal tissues, including the cementum, periodontal ligament, and alveolar bone. However, the precise molecular mechanisms underlying amelogenin-induced regeneration have not yet been elucidated. Thus, to gain further insight into how amelogenin induces periodontal tissue regeneration, we performed a protein interaction screen using recombinant full-length amelogenin (rM180) as bait. Coupling affinity chromatography with proteomic analysis identified glucose-regulated protein 78 (Grp78) as a new amelogenin-binding protein. We further demonstrated that the interaction between amelogenin and Grp78 not only contributes to cell proliferation in osteoblastic cells but also enhances cell migration in periodontal ligament stem/progenitor cells. Although the potentiation effects of Grp78 should be further examined *in vivo*, our findings indicate the significant therapeutic potential for amelogenin-induced periodontal tissue regeneration.

**Keywords:** Glucose-regulated protein 78 (Grp78); Amelogenin; Extracellular matrix (ECM)

### Commentary

The periodontium is mainly composed of two hard tissues and two soft tissues; the former include the alveolar bone and cementum covering the tooth root, and the latter include the periodontal ligament (PDL) tissue and gingival tissue. Periodontal disease is characterized by irretrievable damage of the tooth-supporting periodontium and is the major cause of tooth loss in adults. However, the conventional subgingival root debridement or periodontal flap surgery can only help to prevent the progression of periodontal disease and do not provide a cure. Various types of periodontal regeneration strategies have been developed to date, and application of the extracellular matrix (ECM) has been proposed as one of the most successful therapeutic strategies [1]. The ECM is well known to serve as a biological scaffold for tissue regeneration by modulating cells through the transduction of a variety of signaling pathways [2]. Furthermore, the ECM contributes to angiogenesis, recruitment of stem/progenitor cells, and remodeling of damaged tissues [3].

Enamel matrix derivative (EMD) (Straumann® Emdogain) was developed according to a concept of mimicking the tooth developmental environment [4]. EMD has been widely applied for periodontal tissue regeneration, and long-term successful clinical results have been obtained [5]. Many *in vitro* studies have supported the notion that EMD not only contributes to osteoconductive activity [6] but also enhances the multi-lineage differentiation of human PDL cells [7]. Amelogenins, the major (>95%) component of EMD, belong to the ECM protein family [8]. During tooth development, ameloblasts secrete amelogenins onto the dentin root surface [9]. Consequently, amelogenin induces the formation of the PDL, thereby promoting alveolar bone formation [10]. As an ECM protein, amelogenin (Xelma®, Molnlycke Health Care, and Gothenburg, Sweden) has also been used as an effective and standard treatment for hard-to-heal wounds [11]. Moreover, recent studies have demonstrated that recombinant amelogenin alone could induce *in vivo* periodontal tissue regeneration [12]. Although amelogenins have been suggested to serve as bioactive candidates for periodontal

regeneration [4], their downstream signaling molecules have not been clearly elucidated.

To gain further insight into the molecular basis of amelogenin-induced periodontal tissue regeneration, we performed an affinity chromatography-based proteomic analysis to identify amelogenin-specific binding proteins in osteoblastic cells [13]. After confirming the endocytosis of recombinant amelogenin as a precondition for physiological interaction, we found that glucose-regulated protein 78 (Grp78) was the only protein present in both the cytosolic and membrane fractions of the osteoblastic cell lysate. Grp78 is a member of the heat shock protein 70 family and is well known as an endoplasmic reticulum (ER) chaperone, which regulates the ER stress signaling pathway [14]. Recent studies have demonstrated that ER stress signaling mediates bone formation [15], and we found that the biological interaction between amelogenin and Grp78 contributed to osteoblastic cell proliferation.

PDLSCs play a central role in regenerating the periodontium, possess a self-renewal property, and differentiate into fibroblasts, osteoblasts, or cementoblasts in response to stimuli in their microenvironment [16].

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As successful periodontal regeneration requires the participation of mesenchymal stem cell (MSC)-derived PDL stem cells (PDLSCs) [17], we also evaluated the interaction using the PDLSC [18]. Although Grp78 is located mainly in the ER, in stem/progenitor cells and undifferentiated tumor cells, it translocates to the cell surface, where it acts as a receptor for cell-surface signalling [19-21]. Consistent with these reports of the cell surface localization of Grp78, our confocal analysis confirmed the cell surface co-localization of amelogenin with Grp78, and showed that Grp78 specifically mediates the endocytosis of amelogenin. Considering the cell surface localization of Grp78 in PDLSCs, Grp78 appears to be an amelogenin-specific receptor in PDLSCs. We also found that the biological interaction between Grp78 and amelogenin contributes to cell migration without affecting cell proliferation. Cell migration involves cytoskeletal reorganization and induce the formation of membrane protrusions in the forms of lamellipodia and filopodia [22]. Small GTPases of the Rho family, Rac1, Cdc42, and Rho, are key molecules for cytoskeletal dynamics and control the formation of lamellipodia, filopodia, and stress fibers, respectively [23]. We further demonstrated that Rac1 activation and lamellipodia formation are critical in amelogenin-induced cell migration in PDLSCs.

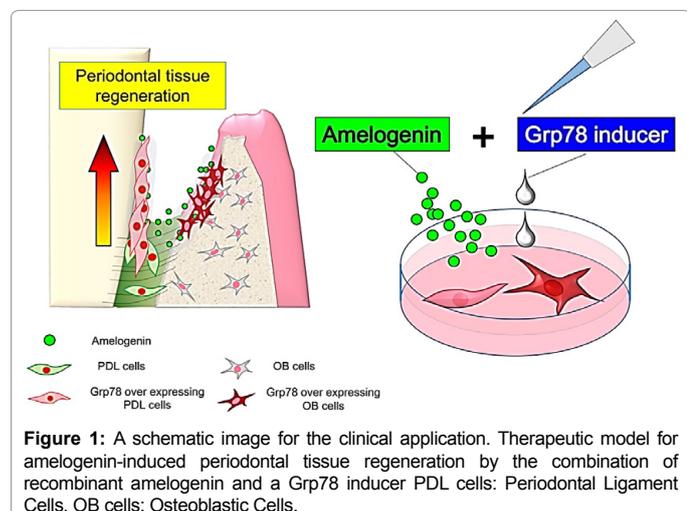
In conclusion, the biological interaction of amelogenin with Grp78 shows significant therapeutic potential for amelogenin-induced periodontal tissue regeneration. The results of our *in vitro* studies suggest the possibility that this interaction in osteoblasts can lead to bone regeneration, and the resulting enhanced migration of PDLSCs is an essential component for the initiation of periodontal tissue regeneration. Considering that the commercial product Straumann® Emdogain is derived from the porcine EMD, development of a new procedure through the combination of recombinant amelogenin and a Grp78 inducer could be a safe and reliable therapeutic strategy based on the clinical achievement of Straumann® Emdogain and the molecular basis of amelogenin. Although these effects should be further examined *in vivo*, our findings indicate the significant therapeutic potential for amelogenin-induced periodontal tissue regeneration (Figure 1).

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## Author Disclosure Statement

The authors have declared that no conflicts of interest exist.

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