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New Therapeutic Strategy for Regenerating Periodontal Tissue Based on the Combination of Amelogenin and Reapplications of Existing Grp78 Inducer

Takao Fukuda[1*], Terukazu Sanui[1*], Kyosuke Toyoda[1], Urara Tanaka[1], Kensuke Yamamichi[1], Takaharu Taketomi[2], and Fusanori Nishimura[1]

[1]*Department of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, Japan*

[2]*Dental and Oral Medical Center, Kurume University School of Medicine, Kurume, Japan*

[*]***Corresponding authors:*** *Takao Fukuda, Department of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, Japan*

Terukazu Sanui, Department of Periodontology, Division of Oral Rehabilitation, Faculty of Dental Science, Kyushu University, Fukuoka, Japan, Tel: 81926426358; E-mail: tfukuda@dent.kyushu-u.ac.jp

Terukazu Sanui and Takao Fukuda contributed equally to this work

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Abstract

The present study aims to develop a highly safe regenerative therapy that effectively induces periodontal tissue regeneration by targeting amelogenin and its newly associated molecule, glucose-regulated protein 78 (Grp78). The enamel matrix derivative (Emdogain® Gel, Straumann) is currently the only bioregeneration tool in use that is approved by the Japanese Ministry of Health, Labour and Welfare. However, in addition to the safety issues that exist owing to it being a foreign protein, a unified opinion on its mechanism of action at the signal transduction level remains unclear. Previously, our laboratory was the first to report the identification of a new amelogenin-associated molecule, Grp78, in osteoblasts. This association was later shown to promote the migration of periodontal ligament stem cells (PDLSCs), which play critical role in periodontal regeneration. Based on these results, we aim to establish the molecular basis for periodontal tissue regeneration via the combined use of recombinant amelogenin and inducers of Grp78 which could be applied by reapplication of existing drugs (drug repositioning).

Keywords: Enamel matrix derivative (EMD); Amelogenin; Glucose-regulated protein 78 (Grp78)

Introduction

Many of the recent periodontal treatments have focused on tissue regeneration, and some are producing positive results. Of these, an enamel substrate protein extracted from porcine fetal teeth germ, called enamel matrix derivative (EMD), is frequently used in periodontal regeneration surgery [1]. EMD has already been commercialized (known commercially as Emdogain® Gel), and is currently the only substance that uses biologically active proteins for the regeneration of periodontal tissue to have been approved by the Japanese Ministry of Health, Labour and Welfare. However, despite much effort to elucidate the molecular mechanism of EMD action, no unified opinion has been reached regarding its effects on the regeneration of periodontal tissue [1], its inhibition of downgrowth of the gingival epithelium [2], or its anti-inflammatory action [3]. Additionally, since the product is a foreign protein derived from porcine tooth germ, some health issues such as the possibility of allergic reactions and contamination with unknown viruses cannot be completely excluded, although no harmful effects have been reported [4].

The major (>95%) component of EMD, amelogenin, is an extracellular matrix (ECM) protein that is central to the regeneration of periodontal tissue [5]. The application of ECM proteins in regenerative medicine is not novel, with these proteins being implemented in wound recovery and other regenerative treatments in the medical field. For instance, amelogenin (Xelma®, Molnlycke Health Care, Gothenburg, Sweden) is also used in the treatment of hard to heal ulcers [6]. The application of amelogenin to periodontal tissue regeneration is based on an attempt to mimic the tooth developmental environment [7]. Amelogenin is secreted by ameloblasts during germ tooth formation and, through its deposition into the dentin, it participates in the formation of periodontal tissues such as the cementum [8]. In the oral cavity of amelogenin knockout (KO) mice, the cementum is malformed and the osteoclasts exhibit abnormal differentiation [9]. Moreover, administration of recombinant amelogenin alone has been reported to induce periodontal regeneration in experimental animals [10]. To date, amelogenin-associated molecules such as LAMP-1, Noggin, cytokeratin, and N-acetylglucosamine have been reported, however these molecules target ameloblasts during the formation of enamel [11]. Presently, there is little progress in research into the molecular interactions between amelogenin and the cell group that migrates during periodontal tissue regeneration and coordinates osteoinduction related to repair. Therefore, identification of amelogenin-binding molecules in cells, such as osteoblasts and mesenchymal stem cell (MSC)-derived PDL stem cells (PDLSCs), which are important in the regeneration of periodontal tissue, was an indispensable topic of study. By conducting a proteome analysis focused on amelogenin, the applicants were able to succeed, for the first time worldwide, in identifying a new amelogenin-binding proteins in osteoblasts that includes Grp78 [12]. Furthermore, we also found that the interaction between amelogenin and Grp78 not only enhances cell proliferation in osteoblasts [12] but also contribute to cell migration in PDLSCs [13].

Grp78 is a member of heat shock protein (HSP) family that both participates in the cell defense [14] and controls Ca^{2+} homeostasis [15], which is important for hard tissue formation. While it is mainly located in the endoplasmic reticulum (ER), it also translocate to the

cell surface via its transmembrane domain [16]. Several studies of Grp78 are particularly relevant to the known actions of amelogenin, such as those shown below:

(1) Grp78 participation in osteoblast differentiation as a DMP-1-specific receptor [17]

(2) Cell surface expression of Grp78 influences cell proliferation [18], migration [19] and stem sell behavior [20]

(3) Grp78 promotes resolution of inflammation by inducing regulatory T cell [21,22]

These studies suggest the possibility that Grp78 exerts an influence on the cell-specific actions of Emdogain® Gel, namely, cellular adhesion, control of mesenchymal stem cell differentiation, suppression of epithelial cell proliferation, and anti-inflammatory action. Indeed, we have found that Grp78 participates in the promotion of osteoblast proliferation [12] and periodontal ligament stem cell migration [13] via an amelogenin stimulus. Grp78 is reported to have two anti-inflammatory pathways. First, Grp78 induces IL-10 secretion and attenuates TNF-a production in monocytes [21]. Second, while macrophage have also been known to play central roles in tissue regeneration, Grp78 stimulated monocytes change deactivated macrophage like phenotype to induce cytotoxic T lymphocyte antigen-4 $(CTLA\text{-}4)^{+}$ regulatory T cell [22]. Considering these findings, induction of Grp78 could have advantages for the treatment of periodontal disease by inducing periodontal tissue regeneration and resolution of inflammation.

In recent years, it has become clear that heat shock proteins (HSP) including Grp78 participate in tissue regeneration, and progress is being made in their use in retinal detachment [23] and myocardial protection [24]. Heat shock protein inducers are frequently used clinically as anti-gastric ulcer drugs (e.g. Teprenone) [25], and since its safety has been confirmed, they are expected to find a practical use in drug repositioning. Furthermore, previous study has shown that the level of histone H3 lysine 9 methylation (H3K9me), the heterochromatin marker, is decreased at the promoter of Grp78 in the ethanol-treated mice. As the expression level of histone H3K9 methyltransferase EHMT2 (G9a) is also selectively decreased [26], it is very likely the histone methyltransferases G9a plays a role in the expression of Grp78 in mammals. As G9a/GLP complex has also been shown to interact with DNA methyltansferases [27] and plays a role in the maintenance of DNA methylation at imprinted loci [28], the expression of Grp78 might be controlled by two different epigenetic mechanisms: one is associated with heterochromatic histone modification H3K9me and the other is DNA methylation. Therefore, by inhibition of G9a and its partner protein GLP clinically, it might also facilitate the regenerating of periodontal tissue as expression level of Grp78 might be increased.

Recent efforts in the treatment of periodontal disease have focused on cell-based endogenous regenerative technology by recruitment of stem cell-based therapeutics [29,30]. Due to their multipotency and paracrine effect, stem cells appear to have a promising therapeutic potential in regenerative medicine, thereby providing a cellular source for the regeneration of the different missing periodontal tissues. Although remarkable results have been produced by regenerative treatments using cell transplants, especially using stem cells, issues with their clinical application, such as the required facilities and costs, are currently imposing many hurdles. In contrast, the application of the clinically effective Emdogain® Gel and Teprenone will likely lead to the development of safe, molecularly targeted, regenerative treatments that target amelogenin, Grp78, and perhaps other HSPs.

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Author Disclosure Statement

The authors have declared that no conflicts of interest exist.

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