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Original Article

Overexpressing the NH_2-terminal fragment of dentin sialophosphoprotein (DSPP) aggravates the periodontal defects in *Dspp* knockout mice

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ABSTRACT

Objective: Previous studies have shown that dentin sialophosphoprotein (DSPP) is not only essential to the formation and mineralization of dentin but also plays an important role in forming and maintaining a healthy periodontium. Under physiological conditions, DSPP is proteolytically processed into the NH_2-terminal and COOH-terminal fragments, and these fragments are believed to perform different functions in the mineralized tissues. Previous studies in our group have demonstrated that the NH_2-terminal fragment of DSPP inhibits the formation and mineralization of dentin, while the role of this fragment in periodontium is unclear.

Methods: We analyzed the periodontal tissues of the transgenic mice overexpressing the NH_2-terminal fragment of DSPP in the *Dspp* knockout background (referred to as "*Dspp* KO/DSP Tg" mice), in comparison with wild type mice and *Dspp* knockout mice. The approaches used in this study included histology, micro-computed tomography, back scattered scanning electron microscopy and resin-casted scanning electron microscopy.

Results: *Dspp* KO/DSP Tg mice exhibited a greater reduction of the alveolar bone, more remarkably altered canalicular systems around the osteocytes, less cementum, more radical migration of the epithelial attachment towards the apical direction, and more severe inflammation in molar furcation region, than in the *Dspp* knockout mice.

Conclusion: Overexpressing the NH_2-terminal fragment of DSPP worsened the periodontal defects in *Dspp* knockout mice, indicating that the NH_2-terminal fragment of DSPP may exert an inhibitory role in the formation and mineralization of hard tissues in the periodontium.

1. Introduction

Dentin sialophosphoprotein (DSPP) discovered by cDNA cloning using an odontoblast library was intitailly thought to be dentin-specific [1,2]. Later on, its expression was also found in the long bone, alveolar bone and cementum [3,4]. Genetic studies have shown an association of DSPP mutations with dentinogenesis imperfecta (DGI) in humans [5–8]. Gene ablation studies revealed that *Dspp* knockout (*Dspp* KO) mice have severe dental defects characterized by dentin hypomineralization, widened predentin zone and enlarged pulp chamber, resembling the tooth defects observed in human DGI [9]. These in vivo studies have confirmed the critical role of DSPP in dentinogenesis. A recent study by our group has shown that *Dspp* KO mice develop periodontal diseases manifesting a reduction of alveolar bone, decreased deposition of cementum and altered morphology of osteocytes in the alveolar bone [10]. These recent findings indicate that in addition to its critical role in dentin formation, DSPP is also essential for the formation and maintenance of a healthy periodontium.

Physiologically, DSPP is proteolytically cleaved by astacin proteases into the NH_2-terminal and COOH-terminal fragments [2,11–13]. The NH_2-terminal fragments of DSPP exist in the extracellular matrix (ECM) of rat/mouse dentin as two forms, dentin sialoprotein (DSP) and the proteoglycan form of this fragment (DSP-PG); the former has no glycosaminoglycan (GAG) chains while the latter has two GAG chains made of chondroitin-4-sulfate [14,15]. The COOH-terminal fragment of DSPP is referred to as dentin phosphoprotein (DPP) [14]. Previous studies in our group have established that the posttranslational processing (cleavage) of mouse DSPP at the NH_2-terminus of Asp^{452}

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is an activation step essential to the formation and mineralization of dentin and alveolar bone [16,17]. We have shown that the substitution of Asp^{452} by Ala^{452} prevents the cleavage of DSPP into fragments, which subsequently leads to defects in the mouse dentin and periodontium [16,17].

The exact mechanisms of how the cleaved fragments of DSPP function in the dentin and periodontal tissues remain largely unknown. in vitro analyses have shown that DPP is a strong initiator and regulator for the formation and growth of hydroxyapatite crystals [18,19], while DSP (the NH_2-terminal fragment of DSPP without the GAG chains) has no significant effects on the formation and growth of hydroxyapatite crystals [20]. Recent in vivo studies have demonstrated that overexpressing the NH_2-terminal fragment of DSPP (including both DSP and DSP-PG) worsened the dentin defects in *Dspp* KO mice [21], indicating that DSP and/or DSP-PG may inhibit the formation and mineralization of dentin. This investigation focuses on the specific roles of the NH_2-terminal fragments of DSPP in periodontium, by analyzing the alveolar bone, cementum and periodontal ligaments in the mutant mice overexpressing the NH_2-terminal fragment of DSPP but lacking the endogenous *Dspp* gene. Our results showed that overexpressing the NH_2-terminal fragment of DSPP worsened the periodontal defects in *Dspp* KO mice.

2. Materials and methods

2.1. Generation of transgenic mice overexpressing the NH_2-terminal fragment of DSPP in the Dspp-knockout background (Dspp KO/DSP Tg mice)

Detailed procedures for the generation of transgenic mice overexpressing the NH_2-terminal fragment of DSPP driven by a type I collagen promoter have been described in our previous publication [21]; these transgenic mice are named “DSP Tg mice”. We crossbred DSP Tg mice with *Dspp* KO mice obtained from the Mutant Mouse Regional Resource Center (MMRRC, UNC, Chapel Hill, NC), to generate a line of mutant mice that express the transgene encoding the NH_2-terminal fragment of DSPP but lack the endogenous *Dspp*; this line of mutant mice is referred to as “*Dspp* KO/DSP Tg” mice. Detailed information regarding the genotyping primers and the expression level of the transgene can be found in our precious report [21]. We employed histology, micro-computed tomography and scanning electron microscopy to assess the periodontal tissues of *Dspp* KO/DSP Tg mice, in comparison with those of the wild type (WT) and *Dspp* KO mice.

2.2. Histology (H&E staining)

For histology analyses, 3-month-old *Dspp* KO/DSP Tg, WT and *Dspp* KO mice were perfused from the ascending aorta with 4% paraformaldehyde (Sigma Aldrich Corporation, St. Louis, MO) in 0.1 M phosphate buffer. The heads from the sacrificed animals were then fixed in 4% paraformaldehyde for 48 h and then decalcified in 8% EDTA (pH 7.4) (Sigma Aldrich Corporation, St. Louis, MO) at 4 °C for approximately 2 weeks. The dissected mandibles were embedded in paraffin, and serial sections of 5 μm were prepared from these paraffin blocks. The sections were stained with hematoxylin & eosin (H&E) and observed under an Olympus microscope (Olympus BX51, Olympus Corporation, Center Valley, PA USA).

2.3. Micro-computed tomography (μ-CT)

We dissected the mandibles from the 3-month-old and 6-month-old WT, *Dspp* KO, and *Dspp* KO/DSP Tg mice (3 mice for each age group; i.e., totally, 6 animals for each type of mice). The mandibles were analyzed using the μ-CT (μ-CT 35 imaging system, Scanco Medical, Basserdorf, Switzerland), as we previously described [17,22]. We performed high-resolution scans (3.5 μm/slice) and three-dimensional reconstructions to assess the morphological characteristics of the mouse mandibles and to calculate the ratios of alveolar bone volume to total volume (BV/TV), among the three groups of mice at both ages. The BV/TV values were expressed as mean ± SD. Student's *t*-test was adopted in our statistical analyses to determine the significance levels of differences between two individual groups, using SPSS (SPSS v.17.0, IBM, Somers, NY).

2.4. Back scattered and resin-casted scanning electron microscopy (SEM)

For these experiments, the mandibles from 3-month-old mice ($n=3$ for each type of mice) were dissected and fixed in 2% paraformaldehyde with 2.5% glutaraldehyde (Sigma Aldrich Corporation, St. Louis, MO) in 0.1 M cacodylate buffer solution (pH 7.4) at room temperature. The detailed protocols used for processing, polishing and coating samples for back scattered and resin-casted SEM (Philips XL30 FEI scanning electron microscope, Philips, Hillsboro, OR, USA) analyses have been described in previous studies [10,17].

3. Results

3.1. Inflammatory infiltration and migration of epithelial attachment in the periodontium of Dspp KO/DSP Tg mice

Results from H&E staining demonstrated that when compared to the WT controls (Fig. 1A), the furcation region of the mandibular molar in *Dspp* KO mice (Fig. 1B) and *Dspp* KO/DSP Tg mice (Fig. 1C) had obvious inflammatory infiltration. The inflammatory reaction appeared more prevalent and severe in the *Dspp* KO/DSP Tg (Fig. 1C) than in the *Dspp* KO mice (Fig. 1B).

In comparison with the WT mice (Fig. 2A), both types of the mutant mice (Fig. 2B and C) showed the detachment and migration of the junctional epithelium, along with the disruption of periodontal ligaments in the interdental region between the mandibular first and second molars. The above defects in the *Dspp* KO/DSP Tg mice (Fig. 2C) were much more severe than in the *Dspp* KO mice (Fig. 2B).

3.2. Reduction of alveolar bone in Dspp KO/DSP Tg mice

Results from both H&E staining (Figs. 1 and 2) and μ-CT analyses (Fig. 3) showed that the reduction of alveolar bone in the *Dspp* KO/DSP Tg mice was greater than in *Dspp* KO mice. In the H&E-stained sections, *Dspp* KO mice (Fig. 1B) showed a moderate loss of alveolar bone in the furcation region of the first mandibular molar when compared to the WT control (Fig. 1A), while the same region in the *Dspp* KO/DSP Tg mice (Fig. 1C) was completely engulfed with the inflammatory cells and had only a few islands of bone left with the majority of the alveolar bone structures being destroyed.

When the alveolar bone volume fractions (BV/TV) were compared among the three types of mice (Fig. 3A and B), the *Dspp* KO/DSP Tg mice had the least values of BV/TV in either the 3- or 6-month-old age groups. The differences in BV/TV values were statistically significant between the *Dspp* KO and *Dspp* KO/DSP Tg mice at both ages.