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RAPID COMMUNICATION

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Expression of osteopontin mRNA in odontoclasts revealed by in situ hybridization during experimental tooth movement in mice

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Introduction

Orthodontic tooth movement requires the resorption and apposition of bone adjacent to the teeth. When orthodontic forces are applied, resorption of the root and repair of the cementum occur just as they do on adjacent bone [1,2]. Root resorption in orthodontic tooth movement was shown to be caused by odontoclasts. Odontoclasts appeared at the root surface after the appearance of osteoclasts during experimental tooth movement [2,3]. However, studies of odontoclasts in bone resorption are few in comparison with those on osteoclasts [2–7]. In addition, there are only a few reports using molecular biological methods to investigate the characteristics of odontoclasts [5–7].

Osteopontin (OPN) was originally isolated from bone and identified as a major non-collagenous bone matrix protein. A sialic acid-rich phosphorylated glycoprotein, it contains a motif of glycine-arginine-glycine-aspartic acid-serine (GRGDS) in its amino-acid sequence that promotes cell attachment via cellular $\alpha_V \beta_3$ integrin [8,9]. OPN is considered to play important roles to promote or regulate the adhesion, attachment, and spreading of osteoclasts to the bone surface during bone resorption [10,11]. During physiological or experimental tooth movement in rats, OPN mRNA was expressed in osteocytes and osteoclasts in the resorption site of the interradicular septum [12,13]. These findings also suggest that OPN participates in bone formation and resorption.

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Osteoclasts and odontoclasts have morphological and functional similarities [2–7], and this suggests that OPN may play an important role in root resorption during orthodontic tooth movement. The present study was carried out to investigate the expression of OPN mRNA in periodontal tissue during experimental tooth movement in mice, and to examine the expression of OPN mRNA in odontoclasts, using in situ hybridization.

Materials and methods

Animals and tissue preparation

Six-week-old male ICR mice, weighing 25–30 g, were anesthetized, and an elastic module was inserted interproximally between the upper first and second molars on the right side. Control mice were treated in a similar manner, but elastic was not used. One, 3, 5, 7, and 14 days after the elastic insertion, the animals were anesthetized and perfused with 4% paraformaldehyde (PFA), pH 7.4. The maxillary bones and surrounding tissues were dissected and fixed in the above solution for approximately 24 h at 4°C. The specimens, decalcified in 20% ethylenediaminetetraacetate (EDTA), pH 6.3, for 14 days at 4°C, were dehydrated and embedded in paraffin. The tissue blocks were cut into mesio-distal serial sections, 5-μm-thick. Histological examination focused on the interradicular septum of the first molar. The experiment was approved by the Animal Committee of Okayama University.

Probe preparation

Digoxigenin (DIG)-11-uridine triphosphate (UTP)-labeled single-stranded RNA probes were prepared with a DIG RNA labeling kit (1175025; Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. A 0.47-kb fragment of mouse OPN and a 0.95-kb fragment of mouse osteocalcin (OSC) were used to generate sense and anti-sense probes [14,15].

In situ hybridization

In situ hybridization was performed by a previously reported method [12]. Sections were deparaffinized and incubated with proteinase K in Tris-HCl, 1 mM EDTA for 15 min at 37°C, and fixed with 4% PFA. Then the sections were treated with 0.2 N HCl for 20 min and incubated with 50% formamide for 3 h at 42°C. The sections were incubated with hybridization solution containing a DIG-UTP-labeled RNA probe, deionized formamide, dextran sulfate, Denhardt's solution, Tris-HCl, NaCl, sodium dodecylsulfate (SDS), EDTA, and yeast tRNA, in a moisture chamber for 16 h at 42°C. After hybridization, the slides were incubated with RNase A in TNE (10 mM Tris-HCl, 500 mM NaCl, 1 mM EDTA) for 30 min at 37°C. Then, the slides were blocked with 1.5% blocking reagent (1096176; Roche Diagnostics) in DIG buffer 1 (100 mM Tris-HCl [pH 7.5], 150 mM NaCl) for 60 min at room temperature. After being blocked, the slides were incubated for 40 min at room temperature with sheep anti-DIG alkaline phosphatase Fab fragment (1:2000; 1093274; Roche Diagnostics). For the color reaction, the sections were incubated in the dark for 6 h at room temperature with NBT/BCIP (368 μg/ml nitroblue tetrazolium salt and 184 μg/ml 5-bromo-4-chloro-3-indolyl-phosphate) in DIG buffer 3 (100 mM Tris-HCl [pH 9.5], 100 mM NaCl, 50 mM $MgCl_2$). The color reaction was stopped when the sections were washed in distilled water.

Tartrate-resistant acid phosphatase (TRAP)

Tartrate-resistant acid phosphatase (TRAP) staining was performed with an acid phosphatase leukocyte kit (Sigma Chemical, St. Louis, MO, USA) according to the manufacturer's instructions. Subsequently, the sections were counterstained with hematoxylin.

Results

One day and 3 days after the start of tooth movement, no odontoclasts were observed around the teeth (data not shown). Five days after the start of tooth movement, odontoclasts expressing OPN mRNA appeared on the surface of the root, not only on the compression side but also on the tension side (Fig. 1B,C). Seven days after the start of the tooth movement, odontoclasts were still observed on both sides (Fig. 1D,F,G). The cells were multinuclear and positive for TRAP staining (Fig. 1D). Most expressed OPN mRNA strongly; however, some TRAP-positive odontoclasts did not express OPN mRNA (Fig. 1D,F).

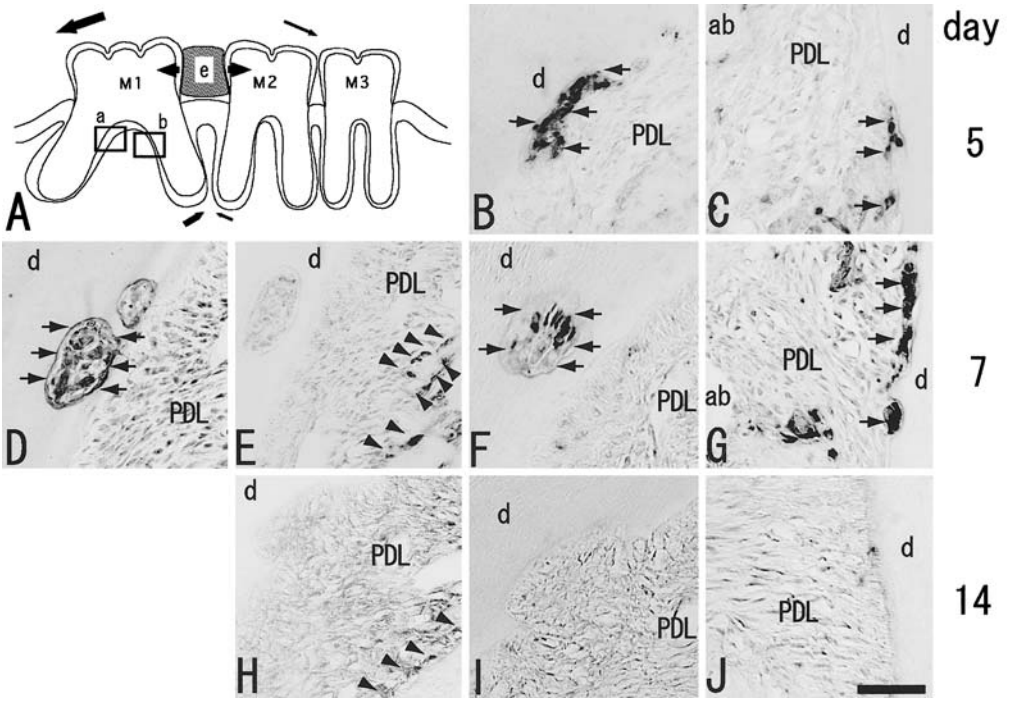

Fig. 1. Expression of osteopontin (OPN) mRNA in odontoclasts. **A** Schematic drawing of experimental tooth movement. An elastic module (*e*) was inserted interproximally between the upper first (*M1*) and the second (*M2*) molars. The *arrows* indicate the direction of the force applied. Small rectangles (*a* and *b*) indicate the tension area enlarged in **B**, **D**, **E**, **F**, **H**, and **I**; and the compression area enlarged in **C**, **G**, and **J**. In situ hybridization for osteopontin is shown in **B**, **C**, **F**, **G**, **I**, and **J**. **D** Tartrate-resistant acid phosphatase (TRAP) staining; **E** and **H** in situ hybridization for osteocalcin; **B** and **C**, 5 days; **D**, **E**, **F**, and **G** 7 days; **H**, **I**, and **J**, 14 days after the beginning of tooth movement. The *arrows* indicate odontoclasts. The *arrowheads* indicate osteoblasts and lining cells. *ab*, alveolar bone; *d*, dentin; *PDL*, periodontal ligament. *Bar*, 50 μm

Fourteen days after the start of tooth movement, odontoclasts were not observed. However, resorption lacunae remained (Fig. 1I).

Expression of OSC mRNA was observed in osteoblasts and lining cells, but was not observed in odontoclasts 7 days after the start of tooth movement (Fig. 1E). Fourteen days after the start of tooth movement, the cells observed in the resorption lacunae on the root surface did not express OSC mRNA (Fig. 1H).

Discussion

Terai et al. [13] reported that OPN mRNA was expressed in osteoclasts and osteocytes during experimental tooth movement in rats, and the numbers and populations of osteocytes and osteoclasts expressing OPN mRNA changed during the tooth movement. The expression patterns of OPN mRNA in osteocytes and osteoclasts in mice in the present study were similar to those in the rat model. One day after the start of tooth movement, OPN mRNA was expressed in osteocytes located on the compression side of alveolar bone. After 3 days, the expression of OPN mRNA in osteocytes became more intense and extended to osteocytes situated on the tension side, while the number of osteoclasts increased and active bone resorption occurred (data not shown). Therefore, these findings also suggested that OPN may play an important role in bone resorption triggered by mechanical stress, and that this mouse model of experimental tooth movement is useful.

There are several reports about odontoclasts in root resorption, and the characteristics of these cells were considered to be similar to those of osteoclasts, they were multinuclear, had a well-developed ruffled border, and were located in resorption lacunae [2–6]. In the present study, cells located in the resorption lacunae on the root surface at 5 and 7 days after the beginning tooth movement were multinuclear, and positive for TRAP staining. Therefore, these cells were identified as odontoclasts. Most odontoclasts in this study strongly expressed OPN mRNA. A recent study showed that the phosphatase activity of the TRAP enzyme is closely related to OPN bioactivity by dephosphorylation [16]. OPN is, possibly, a multifunctional protein that acts in the coupling of bone resorption and formation in the process of bone remodeling under mechanical strain [13,17]. Therefore, OPN may play an important role in root resorption similar to the role it plays in bone resorption. However, in the present study, some TRAP-positive odontoclasts did not express OPN mRNA. Terai et al. [13] reported that OPN mRNA was expressed in osteoclasts in contact with the bone surface, but was not expressed in osteoclasts away from the bone surface during experimental tooth movement. In addition, OPN mRNA was detected strongly in osteoclasts attached to the bone at sites of active bone resorption in a murine experimental arthritis model [18]. These findings suggest that odontoclasts expressing OPN mRNA actively resorbed the root surface.

In the present study, some odontoclasts that strongly expressed OPN mRNA were accumulated on the root surface. OPN is considered to play important roles to promote or regulate the adhesion and attachment of osteoclasts to the bone surface during bone resorption [10,11]. Therefore, OPN seems to play a role in anchoring odontoclasts to the root surface during root resorption. However, Sasakura et al. [19] reported that odontoclasts were found near cementocytes which expressed OPN mRNA, suggesting that OPN secreted by cementocytes has a role in odontoclast migration to the root surface. An in vitro migration assay demonstrated the chemotactic activity of OPN on osteoclast precursors [13]. The increase in OPN mRNA expression in osteocytes adjacent to resorption sites suggests that OPN exerts a paracrine effect on osteoclasts on the bone surface [13,16]. Therefore, further study is required to define the role of OPN expression in odontoclasts during root resorption.

We previously reported that OSC mRNA was expressed in osteogenic cells, e.g., osteoblasts, lining cells, and cementoblasts, during physiological tooth movement in rats [12]. It is suggested that OSC plays an important role in bone formation and cementogenesis. In the present study, OSC mRNA was expressed in osteoblasts and lining cells at 7 and 14 days after the start of tooth movement. However, it was not expressed in any cells in the resorption lacunae on the root surface. Brudvik and Rygh [2] reported, in their ultrastructural study, that newly mineralized cementum was not observed on the resorbed root surface by 10 or 14 days after the start of experimental tooth movement in rats, but was observed by 21 days. In the present study, none of the cells in the resorption lacunae on the root surfaces expressed OPN or OSC mRNA at 14 days. These findings suggest that active root resorption had finished within 14 days after the beginning of experimental tooth movement, but that cementogenesis on the resorbed root surface had not yet started. Fourteen days after the beginning of experimental tooth movement might be the time of a reversal phase between resorption and formation in the turnover of cementum.

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