Comparison of expression patterns of cathepsin K and MMP-9 in odontoclasts and osteoclasts in physiological root resorption in the rat molar

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Introduction

Dental root resorption in human permanent teeth occurs under pathologic (e.g. excessive force from traumatic occlusion or orthodontic treatment, periodontal inflammation and tumorigenesis), and non-pathologic conditions (Ne et al., 1999). Either form of root resorption is thought to be followed by trauma in the periodontal ligament (Henry and Weinmann, 1951; Furseth, 1968; Bosshardt and Schroeder, 1994) and therefore might increase the risk of tooth loss due to fracture or extraction. Root resorption lacunae are principally formed by odontoclasts, which develop from the mature monocyte/macrophage lineage and have cytologic features similar to osteoclasts, including multiple nuclei, ruffled borders, clear zones, and tartrate-resistant acid phosphate (TRAP) activity (Domon et al., 2006). It has been proposed that osteoclasts undergoing even more differentiation have a greater number of nuclei with a higher bone resorption activity (Teti et al., 1991; Lees et al., 2001; Manolson et al., 2003). Since odontoclasts normally have fewer nuclei and less clear zones compared with osteoclasts (Tanaka et al., 1990; Ne et al., 1999), we hypothesized that odontoclasts possess less cell differentiation in...
their matrix resorption characteristics than osteoclasts. This may be a fundamental difference that explains why the dental root is less susceptible to resorption by multinucleated cells as compared with alveolar bone.

Osteoclasts and odontoclasts resorb mineralized tissues by lowering the pH in the resorption lacuna, followed by tissue degradation through the secretion of proteolytic enzymes classified into two families: cysteine proteinases such as the cathepsin family, and matrix metalloproteinases (MMPs). In particular, cathepsin K and MMP-9 are characteristic proteinases expressed in osteoclasts and odontoclasts (Wucherpfennig et al., 1994; Oshiro et al., 2001). Cathepsin K is a cysteine proteinase that is specific for osteoclastic bone matrix solubilization (Garnero et al., 1998), as illustrated by the reduced bone resorption in patients with the osteopetrosis-like disease pycnodysostosis, which is caused by a genetic mutation of the cathepsin K gene (Gelb et al., 1996). MMP-9 (also called type IV collagenase or gelatinase B) is strongly expressed in osteoclasts (Wucherpfennig et al., 1994) but is more important for cell migration rather than degradation of the bone matrix (Vu et al., 1998; Colnot et al., 2003). MMP-9 deficiency impairs osteoclast precursor movement from blood vessels to the bone surface (Engsig et al., 2000). Thus, the expression patterns of cathepsin K and MMP-9 in odontoclasts and osteoclasts are of interest regarding bone resorption and cell differentiation.

There is no in vitro method for the examination of odontoclasts, and in vivo comparison of odontoclasts and osteoclasts is difficult. For the purposes of the current study, physiological root resorption in the rat molar is a useful model because the histological features are similar to the odontoclasts and osteoclasts of human permanent teeth (Sicher and Weinmann, 1944; Hardt, 1988; Nagaoka et al., 2002; Kashiwazaki et al., 2003; Kimura et al., 2003). Since moderate mechanical stress causes a physiological drift of molars in a distal direction with aging (Sicher and Weinmann, 1944; Roberts and Morey, 1985), root resorption possesses spatial and temporal characteristics (Kimura et al., 2003). First, the tartrate-resistant acid phosphatase (TRAP)-positive precursor cells for odontoclasts or osteoclasts start appearing in periodontal tissues at 3 weeks of age, and then odontoclasts actively resorb the distal surface on the distal roots of rat maxillary second molars from 4 to 6 weeks of age (Sicher and Weinmann, 1944; Hardt, 1988). After the decrease followed by the peak of root resorption at 5 weeks, newly formed resorption lacunae on the distal roots of second molars are mostly undetectable by 8 weeks—even though the osteoclasts keep resorbing the alveolar bone facing the distal roots of the second molar (Nagaoka et al., 2002). These observations indicate that the differentiation of odontoclasts varies with aging in physiological root resorption in the rat molar.

Differences between odontoclasts and osteoclasts are not well understood because there has been no experimental model for a comparison of these cells. Physiological root resorption in rat molars may be suitable to draw distinctions between osteoclasts and odontoclasts. We therefore, examined the expression patterns of cathepsin K and MMP-9 in odontoclasts and osteoclasts using in situ hybridization (ISH) in this model.

**Materials and Methods**

**Tissue preparation**

All experimental protocols followed the guidelines for animal use and care issued by the Tohoku University Animal Use and Care Committee. Male Wistar rats at 3, 4, 5, and 6 weeks of age were used in the study. Seven to 10 rats from each age group were anesthetized with sodium pentobarbital (Dainihon Seiyaku, Osaka) and fixed with 4% paraformaldehyde and 0.5% glutaraldehyde in a 0.1M phosphate buffer, pH 7.4, by perfusion through the aorta for 30 min at a perfusion rate of 3 ml/min. Maxillae were dissected out from the rats and divided into right and left halves. After fixation in the same fixative overnight at 4°C, the samples were washed in ribonuclease (RNAse)-free phosphate-buffered saline (PBS). All experimental procedures following this step were performed under RNase-free conditions. Specimens were decalcified in 10% ethylenediaminetetraacetic acid (EDTA) in a 0.01M PBS, pH 7.4, for 2–8 weeks at 4°C. The samples were then dehydrated in a graded series of ethanol, infiltrated with xylene, and embedded in paraffin. Horizontal serial sections of 5 μm thickness were cut and prepared for hematoxylin-eosin (H-E) staining, TRAP staining, or ISH analysis.

**TRAP staining and histomorphometric analysis of TRAP-positive cells**

To determine the morphometric characteristics of three kinds of TRAP-positive cells (odontoclasts, osteoclasts, and their precursor cells), we measured the number of cellular nuclei and the TRAP-positive area, which both correlate to their resorption activities (Teti et al., 1991; Lees et al., 2001; Manolson et al., 2003; Trebec et al., 2007; Hu et al., 2008). Briefly, the serial sections from maxillae were stained with TRAP after deparaffinization and dehydration. The TRAP solution was a mixture of 9.6 mg of naphthol AS-BI phosphate substrate (Sigma-Aldrich, St. Louis, MO, USA) dissolved in 0.6 ml of N,
N-dimethylformamide and 84 mg of fast red-violet LB diazonium salt (Sigma-Aldrich), 58.2 mg of L(+)-tartaric acid (Wako, Tokyo), 240 ml of 10% MgCl\textsubscript{2} and 4 ml of 3 M sodium acetate buffer (pH 5.0) dissolved in 56 ml of distilled water. The mixture was passed through a 0.22mm filter before use. The sections were incubated for 30 min in the TRAP solution at 37°C in the dark and then washed with distilled water for 10 min, followed by DAPI staining (Roche Diagnostics, Indianapolis, IN, USA) to count the number of nuclei. For quantification of TRAP-positive cells in the periodontal tissue, three sections from five maxillae of 3-, 4- and 5-week-old animals (i.e. 45 sections in total) were stained with TRAP and the representative images were captured with confocal (CLSM, Leica, Heidelberg, Germany) and fluorescence (AX-80, Olympus, Tokyo) microscopes. The images were analyzed using NIH image and Adobe Photoshop. The results are expressed as the average number of nuclei and the mean of the TRAP-positive area in osteoclasts, odontoclasts, and precursor cells. Statistical analysis of the data was performed by one-way ANOVA followed by the Bonferroni procedure for multiple comparisons, with the level of significance set at p < 0.01.

**Preparations of riboprobes**

Digoxigenin (Dig)- or fluorescein isothiocyanate (FITC)-labeled cRNA probes for rat cathepsin K, MMP-9, rat type I collagen (Col1\textsubscript{a1}), and bone sialoprotein (BSP) were prepared for ISH or double-labeling ISH, as partly described previously (Shimizu \textit{et al.}, 2001; Sasano \textit{et al.}, 2002). cRNA probes for each molecule were selected and transcribed from at least two different cDNA fragments. The specificities of the probes for each molecule were verified using ISH and by searching the GenBank database to avoid cross-reaction with other homologs. Reverse transcription-polymerase chain reaction (PCR)-based cloning of the genes of interest was performed using cDNA derived from embryonic rat limb buds. The sequence of amplimers used, PCR conditions, and GenBank accession numbers are summarized in Table 1. PCR amplification using Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA) involved initial denaturation at 95°C for 4.5 min, followed by 40 cycles of denaturation for 45 sec at 95°C, annealing at a primer set-specific temperature, cathepsin K at 60°C and MMP-9 at 56°C for 45 sec, and extension for 90 sec at 72°C, ending with an extension step of 8.5 min at 72°C. The PCR fragments were subcloned into the pCR\textsubscript{II}-TOPO vector (Invitrogen), analyzed to confirm the nucleic acid sequence (Hokkaido System Science, Sapporo), and transcribed into Dig-labeled or FITC-labeled sense or antisense cRNA probes using T7 or Sp6 RNA polymerase (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions. The information regarding \textit{in vitro} transcription is summarized in Table 1. The probes were electrophoresed on a 1% agarose gel to confirm purity.

**In situ hybridization analysis**

The protocol for ISH was based on that used in our previous work (Sasano \textit{et al.}, 2002). In brief, sections were deparaffinized in xylene, rehydrated in a graded series of ethanol, treated with 0.2 N hydrochloric acid for 20 min at room temperature, washed thoroughly in PBS, and then treated with 20 µg/ml proteinase K (Roche Diagnostics) for 30 min at 37°C prior to dehydration and drying. The dried sections were hybridized with riboprobes for cathepsin K, MMP-9, Col1\textsubscript{a1}, or BSP for 16 h at 45°C. After rinsing in 2× standard sodium citrate (SSC)/50% formamide, the sections were treated with 20 µg/ml RNase before further stringent washes with 2× SSC/50% formamide for 1 h at 45°C and 1× SSC/50%

| Molecule | Sequences for primers | Product length (bp) | Anti-sense RNA polymerase | Sense RNA polymerase | Restriction Enzyme |
|----------|-----------------------|--------------------|--------------------------|---------------------|------------------|
| Cathepsin-K | Upstream: GGGGCAGGATGAAAAGTTGTATGTA | 519 | T7 | Sp6 |
| (NM-031560) | Downstream: TCTCAGAAAAATGCCAGTTGTGTC | | BamHI | EcoRV |
| MMP-9 | Upstream: GGGGCAGGATGAAAAGTTGTATGTA | 465 | T7 | Sp6 |
| (NM-031055) | Downstream: TCTCAGAAAAATGCCAGTTGTGTC | | | | |
formamide for 1 h at 45°C. The sections were then rinsed thoroughly in PBS and incubated with alkaline phosphatase (AP)-conjugated anti-Dig antibody (Roche Diagnostics). After visualization, the sections were counterstained with methyl green and mounted.

**Double labeling fluorescence in situ hybridization (double-FISH)**

Double-labeling ISH experiments were performed using fluorescent-labeled probes to confirm the expression patterns of cathepsin K, MMP-9, Col1α1, and BSP in the periodontal tissue. In the double-labeling analysis, a signal amplification method using two tyramide fluorogenic substrates was used after posthybridization washes (Sasano et al., 2002). The tissue sections were hybridized simultaneously with FITC-labeled and Dig-labeled cRNA probes (1 μg/ml). After high-stringency washing, the sections were reacted with horseradish peroxidase (HRP)-anti-Dig (1:100) and AP-anti-FITC (1:1000) (Roche Diagnostics) antibodies followed by incubation in tyramide-biotin (1:50) (PerkinElmer, Boston, MA, USA) to amplify the peroxidase signal. The signals were visualized by Streptavidin-Alexa 488 (1:200) (Molecular Probes, Eugene, OR, USA) and Fast Red (2-hydroxy-3-naphthoic acid-2′-phenylanilide phosphate fluorescent detection set) (Roche Diagnostics), respectively. The sections were mounted in an aqueous mounting medium (Immunon Lipshaw, Pittsburgh, PA, USA) after DAPI nuclear staining (0.5 μg/ml) (Roche Diagnostics). Images were obtained with a confocal or fluorescence microscope.

**Fig. 1.** Histological features of physiological root resorption in rat molars (a–c). Hematoxylin-eosin staining of physiological root resorption at 3 (a), 4 (b) and 6 (c) weeks of age. Root resorption lacunae are not seen on the distal surface of the distal root of rat second molars until 3 weeks of age, but several precursors (white arrowhead) to resorbing cells with several nuclei are present in the middle of the periodontal ligament (a). A resorption lacuna (arrowhead) with odontoclasts appears on the root surface of the upper second molar at 4 weeks (b). No multinucleated cells are observed in the root resorption lacunae (arrowhead) at 6 weeks, and restoration of the cementum on the root surface has occurred (c). The alveolar bone facing the resorption lacunae has many multinucleated cells, regardless of age (a–c). White arrowheads: precursor cells, Arrowheads: resorption lacunae. Bars=100 μm
Cathepsin K and MMP-9 expression in odontoclasts

using a cooled CCD camera system (Cool SNAP, Roper Co., Chiba). To clarify the expression patterns of proteolytic enzymes in resorbing cells, we analyzed the MMP-9 expression in cathepsin K-positive cells. Following previous methods (Lees et al., 2001; Manolson et al., 2003), cathepsin K-positive cells were categorized into four types: small osteoclasts (≤ 5 nuclei), large osteoclasts (≥ 6 nuclei), odontoclasts, and precursor cells in periodontal tissues. Five experiments for Double-FISH were conducted with at least 8 sections obtained from a rat maxilla at 3 weeks of age. With respect to evaluations for the positivity/negativity of MMP-9 transcripts, the detection thresholds of fluorescence intensity were established by the laser power and exposure time in CLSM. The population of MMP-9 expression in cathepsin K-positive resorbing cells was analyzed using the Pearson's chi-square test in SPSS (ver. 10), with the level of significance set at p < 0.05.

Localization of mRNA in cathepsin K-positive cells in periodontal tissue in 3-week-old rat molars

Cell polarity involves the localization of transcribed mRNA, which is maintained in specific cellular sites for protein synthesis. Osteoclasts have a distinct cell polarity that causes them to move in the direction of the bone surface upon differentiation (Laitala-Leinonen et al., 1996; Adamo et al., 1999; Lopez de Heredia and Jansen, 2004). To examine whether the precursor cells appearing in periodontal tissues on the distal side of rat second molars at 3 weeks of age are moving to the molar root or alveolar bone, we investigated the intracellular localization of mRNA signals in double-FISH. Since MMP-9 expression was detected by ISH and double-FISH, in other kinds of cells, such as osteoblasts and periodontal cells, we examined results for cathepsin K. Three positions (alveolar bone side, dental root side, and unidentifiable) were categorized by hybridization positive signals against cell nuclei, and these were compared among odontoclasts, osteoclasts, and precursor cells at 3 weeks of age.

Results

Histological appearance of root resorption in H-E staining

As reported previously (Nagaoka et al., 2002; Kashiwazaki et al., 2003), root resorption lacunae were not observed on the distal surface of the distal root of rat second molars until 3 weeks of age, but several multinucleated cells were present in the middle of the periodontal ligament (Fig. 1a). Serial sections showed that these cells were detached from the alveolar bone or molar root and were TRAP-positive. The cells were defined as precursors of odontoclasts and/or osteoclasts.

![Graph showing comparison of the number of nuclei and the area of TRAP-positive area per cell](image)

**Fig. 2.** Comparison of the number of nuclei (a) and the TRAP-positive area (b) in osteoclasts (Oc), odontoclasts (Od), and precursor cells (PC). Based on measurements of the number of nuclei (a) and the TRAP-positive area (b) per cell, the mean values of the nuclear number and TRAP-positive area in osteoclasts are significantly higher than those in odontoclasts or in precursor cells in periodontal ligaments, while there is no significant difference between odontoclasts and precursor cells. Error bars represent the standard error of the mean. * P < 0.01 (Bonferroni test).
Odontoclasts began to resorb the root surface of the rat second molar and to form resorption lacunae at 4 weeks (Fig. 1b). TRAP-positive odontoclasts were apparent in root resorption lacunae until 5 weeks, but not after 6 weeks, when restoration of the cementum began to occur (Fig. 1c). The alveolar bone facing the resorption lacunae in rat second molars had many multinucleated osteoclasts, regardless of age (Fig. 1a–c).

Nuclei count and Measurement of TRAP-positive area in osteoclasts, odontoclasts, precursor cells

The mean of the nucleus number in osteoclasts (n=580) was $2.7 \pm 0.1$ (the standard of the mean) whereas those in odontoclasts and that in precursor cells were $2.1 \pm 0.1$ and $2.1 \pm 0.1$, respectively. The nucleus number in osteoclasts was significantly larger than that in odontoclasts (n=255) and in precursor cells (n=149) of periodontal ligaments (Fig. 2a). With respect to the TRAP-positive area, that for osteoclasts appeared approximately three times larger than the others (Fig. 2b).

Expression of cathepsin K and MMP-9 in bone tissue

Strong positive hybridization signals for cathepsin K and MMP-9 were observed in osteoclasts, which were defined as multinucleated cells on the surface of the alveolar bone (Fig. 3a, c). No hybridization signal was identified in adjacent sections processed with control sense RNA probes (Fig. 3b, d). Osteoblasts also had some...
hybridization signals for MMP-9 (Fig. 3c). To examine which type of cells expressed MMP-9, we carried out double-FISH experiments using cRNA probes for MMP-9 and BSP. As shown in figure 4, osteoblasts with the positive signals for BSP (red) also showed distinct MMP-9 expression (green).

Expression patterns of cathepsin K and MMP-9 in odontoclasts

Strong positive hybridization signals for cathepsin K (Fig. 5a) and MMP-9 (Fig. 5b) were observed in odontoclasts, which were defined as multinucleated cells in resorption lacunae on the surface of molar roots. The signals for cathepsin K and MMP-9 in resorption lacunae were strong from age 4 to 5 weeks of age, but were not observed after 6 weeks (Fig. 5c, d). ISH results for cathepsin K showed some cells with positive signals in the middle of periodontal ligaments at 3 weeks (Fig. 5e). On serial sections, these cells were confirmed to be distant from the surface of the bone and molar root. The cathepsin K-positive cells in periodontal tissues were characteristic
only of 3-week-old rats, and the intensities of the hybridization signals in odontoclasts did not differ from that in osteoclasts facing the alveolar bone. We therefore, compared the expression patterns in odontoclasts and osteoclasts using double-FISH, which is suitable for analysis of the expression of two genes in the same cell. Most odontoclasts and osteoclasts showed hybridization signals for cathepsin K and MMP-9 (Fig. 6a',b'). Precursor cells with a positive hybridization signal for cathepsin K in the periodontal ligament at age 3 weeks...
Fig. 6. Comparison of expression patterns for cathepsin K (red) and MMP-9 (green) with double-labeling fluorescence ISH. Images in (a–d) are obtained by phase-contrast microscopy and those in (a'–d') by confocal microscopy. Osteoclasts on the alveolar bone (a’) and odontoclasts on the dental root (b’) both show simultaneous expressions of cathepsin K and MMP-9. The intracellular localization of the cathepsin K and MMP-9 signals tend toward the alveolar bone side in osteoclasts (b’) and toward the dental root side in odontoclasts (b’). Precursor cells with cathepsin K signals in the periodontal ligament at 3 weeks of age (c’) also express MMP-9. In large osteoclasts with many nuclei (≥ 6 nuclei), the expression of MMP-9 is usually undetectable (68% of all large osteoclasts) (d’). Arrowheads: cathepsin K-positive osteoclast (a, d), odontoclast (b) and precursor cell (c). AB: alveolar bone, PDL: periodontal ligament, R: dental root. Bars = 50 μm (a, d), 10 μm (b) and 3 μm (c)
also had positive hybridization signals for MMP-9 (Fig. 6c'). However, some osteoclasts showed distinct cathepsin K signals but no MMP-9 signals, and these cells were large, strongly multinucleated (Fig. 6d') and expressly appeared at an age of 3 weeks. The number of cathepsin K-positive cells showing MMP-9 expression was counted, with classification of these cells into four types: small osteoclasts (≤ 5 nuclei), large osteoclasts (≥ 6 nuclei), odontoclasts, and precursor cells in periodontal tissues. MMP-9 signals were detected only in 31% of the large osteoclasts, but in 76% of the small osteoclasts (Table 2). Odontoclasts and precursor cells had similar expressions of cathepsin K and MMP-9 to those in small osteoclasts. Using Pearson’s chi-square test, there was a significant difference (P < 0.05) among the four groups.

**Intracellular localization of the cathepsin K hybridization signal**

To clarify the characteristics of the precursor cells appearing at age 3 weeks, we analyzed the intracellular localization of the fluorescence hybridization signal for cathepsin K, which is a molecule that is specific for resorbing cells, and compared this among odontoclasts, osteoclasts, and precursor cells at age 3 weeks. Three categories (dental root side, alveolar bone side, and un identifiable) were identified against cell nuclei. As shown in Figure 6, the intracellular localization of the cathepsin K signals tended toward the bone side in 74.7% of the osteoclasts (n=112) and toward the dental root side in 61.8% of the odontoclasts (n=34) (Bastani et al., 1996; Laitala-Leinonen et al., 1996; Nakamura et al., 2003). The precursor cells that appeared in the periodontal ligament at age 3 weeks were more frequent on the dental root side (40%, n=32) than on the bone side (11.3%, n=9) (Table 3).

**Discussion**

Physiological root resorption in rat molars is a useful model for studying the mechanism of dental root resorption and cementum regeneration. This process occurs spatially on the distal surface of distal roots of rat maxillary second molars and temporally at ages 4 to 6 weeks (Nagaoka et al., 2002; Kashiwazaki et al., 2003; Kimura et al., 2003). We have shown that odontoclasts involved in physiological root resorption express cathepsin K and MMP-9 mRNA, which are characteristic proteolytic enzymes in osteoclasts and also in odontoclasts of deciduous teeth (Oshiro et al., 2001). The increased expression levels of these enzymes at age 4 and 5 weeks are temporally coincident with the active period of physiological root resorption in the rat molar, and this observation may help to clarify the properties of odontoclasts compared with osteoclasts.

Many studies have shown that cathepsin K and MMP-9 are specific proteolytic enzymes in osteoclasts (Wucherpfennig et al., 1994; Oshiro et al., 2001), and it has also been reported that osteoblasts and osteocytes also express MMP-9 (McClelland et al., 1998). Our ISH results showed a moderate expression of MMP-9 in mononuclear cells in the periodontal ligament, and with double-FISH we confirmed that MMP-9-positive cells also expressed type I collagen or BSP. These results indicate that osteoblasts and periodontal fibroblasts express MMP-9, in addition to resorbing cells such as osteoclasts and odontoclasts. In contrast, cathepsin K expression was localized in multinucleated cells on the bone surface. Some mononuclear cells also showed cathepsin K expression, but these cells did not express type I collagen or BSP. Most large osteoclasts with many nuclei (≥ 6) only showed a signal for cathepsin K, and not for MMP-9. Cathepsin-K expression in osteoclasts may become dominant with an increased number of nuclei, i.e.

| Table 2. The synchronous expression of MMP-9 on cathepsin-K positive resorbing cells |
|-----------------------------------------------|
| Small osteoclasts | Large osteoclasts | Odontoclasts | Precursor cells |
| (n=118) | (n=32) | (n=55) | (n=80) |
| 76.2% | 31.2% | 80% | 88.8% |

Statistical significance among osteoclasts, odontoclasts, and precursor cells in Pearson’s chi-square test (P<0.05)

| Table 3. The intracellular localization of the cathepsin-K hybridization signal in bone resorbing cells. |
|---------------------------------------------------------------|
| Alveolar bone side | Dental root side | Un identifiable |
|-------------------|-----------------|----------------|
| Osteoclasts (n=150) | 74.7% | 1.3% | 24.0% |
| Odontoclasts (n=55) | 3.6% | 61.8% | 34.5% |
| Precursor cells (n=80) | 11.3% | 40.0% | 48.6% |
differentiation in vivo, whereas MMP-9 expression may decline. MMP-9 expressed by osteoclasts has been shown to be involved in cell migration, especially from blood vessels (Ishibashi et al., 2006; Muzylak et al., 2006). Our results suggest that cathepsin K, rather than MMP-9, is the characteristic proteolytic enzyme expressed in bone resorption.

The nuclei number and TRAP-positive area in osteoclasts is associated with their resorption activities (Teti et al., 1991; Lees et al., 2001; Manolson et al., 2003; Hu et al., 2008). Compared with osteoclasts, odontoclasts had fewer nuclei and less of a TRAP-positive area, both indicators for resorption activity in resorbing cells. These observations suggest that odontoclasts possess less cell differentiation in matrix resorption characteristics than osteoclasts. This finding corresponds to common knowledge in orthodontic treatment, in which the dental root suffers much less resorption by odontoclasts compared to alveolar bone resorption by osteoclasts (Avery, 2002). However, few findings explaining this observation have been available because of the lack of an in vitro model for the in vivo situation. Our data suggest that physiological root resorption in the rat molar provides a useful model to distinguish osteoclasts and odontoclasts in vivo.

Typical multinucleated cells with expressions of cathepsin K and MMP-9 were present in the middle of the distal periodontal ligament of the upper second molar in 3 week-old rats, and these cells were separate from mineralized tissues like the dental root or alveolar bone. With the expectation that the cells were precursors for osteoclasts and/or odontoclasts, we examined the cell polarity to determine whether the cells were moving to the dental root or alveolar bone. Osteoclasts have distinct cell polarity, and the localization of mRNA is thought to be coincident with polarized organelles, such as the ruffled border and actin ring (Laitala-Leinonen et al., 1996). In the physiological root resorption of the rat molar, the cell polarities of odontoclasts and osteoclasts indicated a dominant movement to the dental root and alveolar bone, respectively. More of the precursor cells appearing at 3 weeks were directed to the dental root (40%), compared with the alveolar bone (11.3%) and, moreover, we observed osteoclasts on the alveolar bone at age 3 weeks. This may indicate that more cathepsin K-positive cells at 3 weeks are precursor cells for differentiation into odontoclasts rather than osteoclasts.

In conclusion, our results show that odontoclasts involved in root resorption include cathepsin K and MMP-9 for proteolysis and possess less cell differentiation in matrix resorption characteristics than osteoclasts. We propose that physiological root resorption in the rat molar provides a good model for understanding the mechanism of root resorption and the cell differentiation of multinucleated cells.

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