

## Original Article

# Expression of matrix metalloproteinase-8 and matrix metalloproteinase-13 in mast cells of human periapical lesions

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**Abstract:** Objective: This study aimed to detect the expression of matrix metalloproteinase-8 (MMP-8) and MMP-13 in mast cells (MCs) of human periapical lesions and to discuss the pathogenic role of MCs in periapical lesions. Methods: Ninety samples were divided into three groups: (1) periapical granuloma group (n=30); (2) periapical cyst group (n=30); (3) normal periodontal membrane group (n=30). The samples were fixed in 10% neutral formalin for over 48 h and made into serial sections. After H&E staining, histological changes were observed under the optical microscope. Moreover, double immunofluorescence (DIF) staining was performed to detect expression of MMP-8 and MMP-13 in MCs of periapical lesions under the fluorescence microscope. Results: Compared with the normal control group, the number of MMP-8 and MMP-13 double positive MCs in the periapical lesions increased significantly ( $P<0.01$ ). There was no significant difference in the density of MMP-8 and MMP-13 double positive MCs in the periapical cyst group and periapical granuloma group ( $P>0.05$ ). Conclusion: The number of MCs increased significantly in periapical lesions and there was a considerable increase in the density of MMP-8 and MMP-13 double positive MCs. These results indicate that MCs positive for MMP-8 and MMP-13 might contribute to the pathogenesis of chronic apical periodontitis.

**Keywords:** Chronic periapical disease, mast cell, matrix metalloproteinase

## Introduction

Periapical periodontitis is an acute or chronic inflammatory lesion around the apex of a tooth root [1]. Inflammatory bone resorption is mediated by cytokines (CKs) secreted by the immunologically competent cells or inflammatory cells in the periapical tissues [2]. Mast cells (MCs) are important effector cells as well as regulatory immune cells secreting a variety of CKs that are involved in periapical diseases and bone resorption related to periodontal diseases [3-6].

Matrix metalloproteinases (MMPs) are Zn- and Ca-dependent endopeptidases that degrade extracellular matrix (ECM). MMPs are involved in the mobility of osteoclasts by degrading collagen and they also act as important proteolytic enzymes that degrade bone matrices [7].

Many studies have been published on the roles of MMPs in dental caries and pulp diseases. However, the roles of MMPs in periapical diseases are discussed less often, and no reports on the involvement of MMPs-positive MCs in human chronic periodontitis have been published. We performed double immunofluorescence (DIF) staining to detect expression of MMP-8 and MMP-13 in MCs in different types of periapical lesions. Based on the detection results, the pathogenic role of MCs expressing MMP-8 and MMP-13 in chronic apical periodontitis are discussed.

## Materials and methods

### Subjects

Case selection: Sixty cases that received periapical curettage for periapical cysts or periapi-

cal granulomas at the Oral Medical Center of the Second Clinical Medicine College of Jinan University from July 2010 to August 2014 were included. The cases were aged 15 to 62 years old, including 33 males aged  $37 \pm 19.52$  years and 27 females aged  $32 \pm 15.25$  years. There were no significant differences in age or gender of the subjects. Thirty cases had their healthy premolars extracted for orthodontic reasons, including 14 males and 16 females with an average age of  $19 \pm 5.06$  years. Systemic diseases were excluded, and none of the cases received antibiotics treatment within three months. Females who were pregnant or took oral contraceptives were excluded. All cases signed the informed consent.

The following groups were studied: (1) Periapical granuloma group (n=30): X-ray finding of circular or elliptical zones with reduced density in periapical region of the infected tooth, having <1 cm diameter and clear boundaries; (2) periapical cyst group (n=30): X-ray finding of circular or elliptical zones with reduced density in periapical region of the infected tooth, having >1 cm diameter and clear boundaries surrounded by the bone white line; (3) normal control group (n=30); (4) healthy premolars extracted for orthodontic reasons; (5) Developed tooth roots; (6) No X-ray findings of periapical lesions.

### *Sample collection and treatment*

Periapical lesion samples (periapical granulomas and periapical cysts) were collected by periapical curettage. For the normal control group, periodontal membranes were harvested from the root surface of the extracted premolars. The samples were immediately fixed in 10% neutral formalin for over 48 h. The samples were dehydrated, made transparent, soaked in wax, paraffin embedded, and made into 5  $\mu$ m thick serial sections.

### *H&E staining and histological observation*

H&E staining was performed and histological changes were observed under the optical microscope.

### *DIF staining for MCs positive for MMP-8 and MMP-13*

Primary antibodies were anti-tryptase antibody (Abcam) at a 1:200 dilution and antibodies

against MMP-8 (Boster) and MMP-13 (Boster) at a 1:100 dilution. Secondary antibodies were goat anti-mouse IgG (H + L) Alex Fluor 555 and goat anti-rabbit (H + L) Alex Fluor 488 (Cell Signaling Technology), at a 1:200 dilution. Tissue sections were conventionally dewaxed and rehydrated with ethanol. Antigen retrieval was performed using microwave oven and citrate buffer, and the cells were blocked with goat serum. Next, the cells were incubated with primary antibodies and then with secondary antibodies in the dark. DAPI was added for nuclear staining in fluorescence microscopy. The slides were then sealed and observed under the fluorescence microscope. Green fluorescence indicated tryptase-positive cells, while red fluorescence indicated MMP-8-positive for MMP-13-positive cells. Positive expression was localized to nuclei or cytoplasm. In the same visual field, green fluorescence emitted by tryptase-positive cells overlapped with the red fluorescence emitted by the MMP-8- and MMP-13-positive cells to give an orange color. Tissue sections were observed by two pathologists under the fluorescence microscope who were blinded to the research. MMP-8 and MMP-13 double positive MCs were counted. The area of the tissue sections was measured, and the number of immunofluorescence-positive cells per unit area was calculated (cells/mm<sup>2</sup>). The average value was taken as the average density of MMP-8 and MMP-13 double positive MCs in the tissue section.

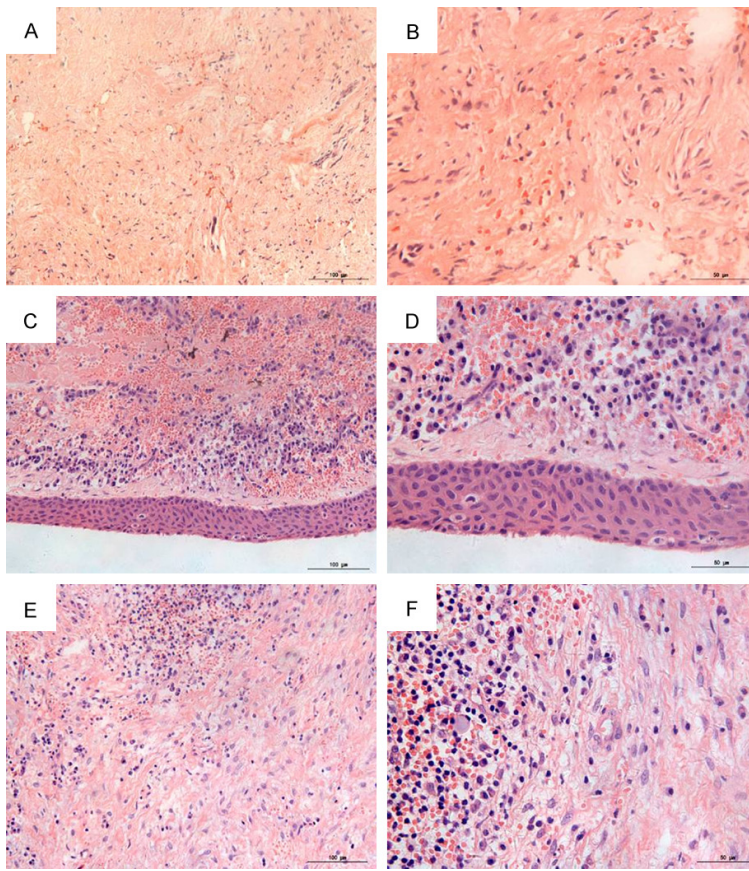
### *Statistical analysis*

Data are expressed as mean  $\pm$  SD. Statistical analyses were performed using SPSS 13.0 software. Average densities of MMP-8 and MMP-13 double positive MCs were compared between the groups. Kruskal-Wallis test for completely randomized design was performed. Nemenyi test was used for pairwise comparisons between three independent samples, and  $P < 0.05$  was taken to indicate statistical significance.

## **Results**

### *Histological observation*

No inflammatory cell infiltration was observed in the normal control group (**Figure 1A, 1B**). In contrast, extensive inflammatory cell infiltration was observed in the epithelial layer of



**Figure 1.** Histological observation of samples in different groups (HE staining). A, B: Normal control group; C, D: Periapical cyst group; E, F: Periapical granuloma group; A, C, E:  $\times 200$ ; B, D, F:  $\times 400$ .

the periapical cyst, presenting as intercellular edema and predominance of neutrophil granulocytes (**Figure 1C, 1D**). Capillary and fibroblast proliferation was seen in periapical granulomas, with dispersed infiltration by neutrophil granulocytes, lymphocytes, plasmocytes, and macrophages. There was fibrous tissue hypertrophy surrounding the inflammatory granulation tissues as well as focal distribution of foam cells inside the granulation tissues (**Figure 1E, 1F**).

#### *DIF staining for MMP-8-positive MCs*

Results of DIF staining for MMP-8-positive MCs in different groups are shown in **Figure 2**. MCs were positive for MMP-8 expression in each group and there were very few MMP-8-positive MCs with a scattered distribution in the periodontal membrane in the normal control group (**Figure 2A-D**). In contrast, the number of MMP-8-positive MCs in the periapical gra-

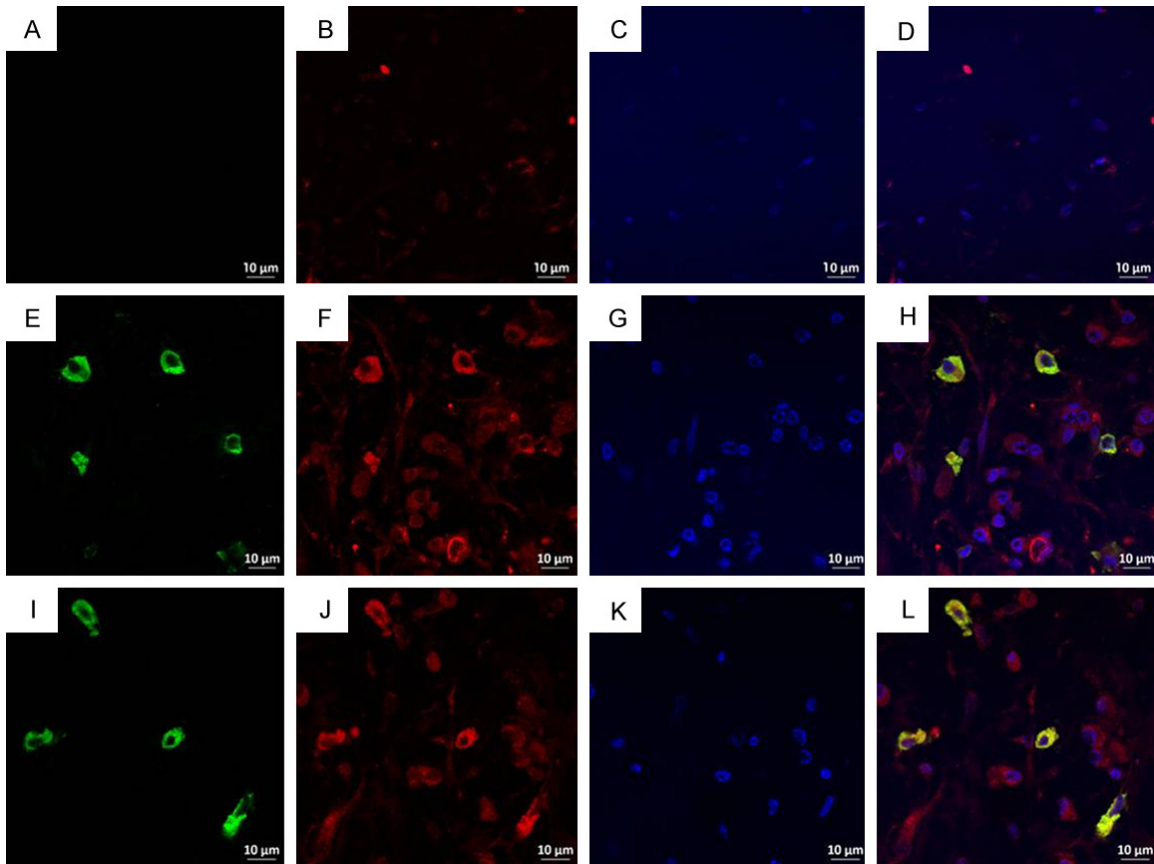
nulomas increased significantly with degranulation of MCs (**Figure 2E-H**). A large amount of MMP-8-positive MCs were observed in periapical cysts with apparent degranulation of MCs (**Figure 2I-L**).

Densities of MMP-8-positive MCs in tissues samples in different groups are shown in **Figure 3**. The densities of MMP-8-positive MCs varied between the three groups ( $P < 0.01$ ). The highest density was observed in the periapical cyst (mean rank 69.53). Nemenyi test indicated significant difference in the density of MCs positive for MMP-8 between the normal control group and periapical cyst group ( $P < 0.01$ ) and also between the normal control group and periapical granuloma group ( $P < 0.01$ ). However, no such difference was found between the periapical cyst group and periapical granuloma group ( $P > 0.05$ ).

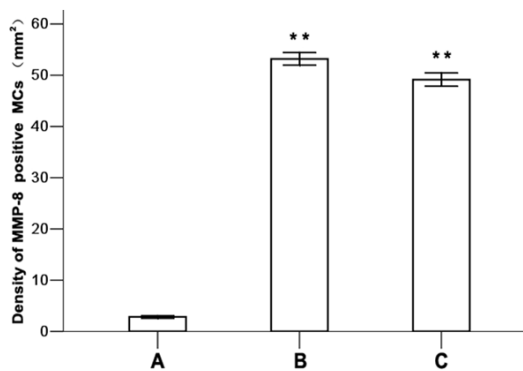
#### *Results of DIF staining for MMP-13-positive MCs*

Results of DIF staining for MMP-13-positive MCs in different groups are shown in **Figure 4**. As indicated by the figure, MCs were positive for MMP-13 expression in each group. There were very few MMP-13-positive MCs that were scattered in the periodontal membrane in the normal control group (**Figure 4A-D**). In contrast, the number of MMP-13-positive MCs in the periapical granulomas increased significantly with degranulation of MCs (**Figure 4E-H**). A large amount of MMP-13-positive MCs were observed in periapical cysts with apparent degranulation of MCs (**Figure 4I-L**).

Densities of MMP-13-positive MCs in tissues samples in different groups are shown in **Figure 5**. The densities of MMP-13-positive MCs varied between the three groups ( $P < 0.01$ ). The highest density was observed in the periapical cyst (mean rank 73.60). Nemenyi test indicated significant difference in the density of MCs positive for MMP-13 between the nor-



**Figure 2.** MMP-8-positive MCs by DIF staining in different groups. A-D: Normal control group ( $\times 630$ ); E-H: Periapical granuloma group ( $\times 630$ ); I-L: Periapical cyst group ( $\times 630$ ). A, E, I: tryptase; B, F, J: MMP-8; C, G, K: DAPI; D, H, L: Merged.



**Figure 3.** Densities of MMP-8-positive MCs in different groups. A: Normal control group (n=30); B: Periapical cyst group (n=30); C: Periapical granuloma group (n=30). Mean  $\pm$  SD. \*\*P<0.01 vs A.

mal control group and periapical cyst group (P<0.01) and also between the normal control group and periapical granuloma group (P<0.01). However, no such difference was found

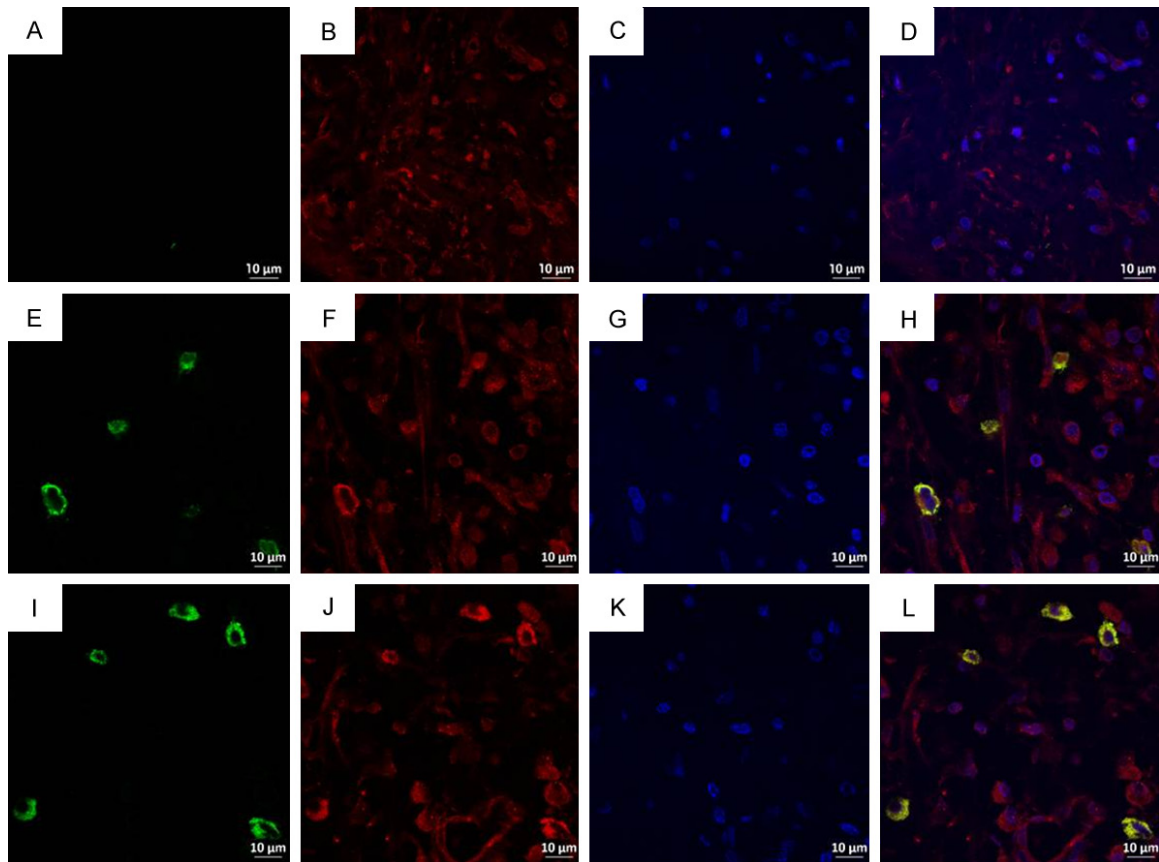
between the periapical cyst group and periapical granuloma group (P>0.05).

## Discussion

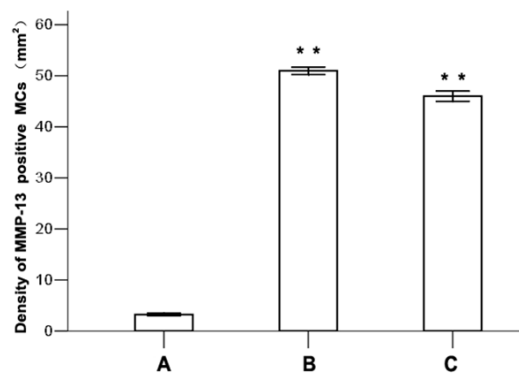
Periapical periodontitis is an acute or chronic inflammatory lesion around the apex of a tooth root [1]. Inflammatory bone resorption is mediated by cytokines (CKs) secreted by the immunologically competent cells or inflammatory cells in the periapical tissues [2].

MMPs, as Zn- and Ca-dependent endopeptidase, were first found in the 1960s, and they are considered major mediators in the destruction of extracellular matrix (ECM). MMPs perform dual functions that include regulating cell differentiation and maintaining homeostasis and degrading ECM to induce tissue injury, occurrence of diseases, and tumor metastasis [8-10]. Based on the substrate and homology of MMPs, MMPs are divided into six types: col-

## Mast cells of human periapical lesions



**Figure 4.** MMP-13-positive MCs by DIF staining in different groups. A-D: Normal control group ( $\times 630$ ); E-H: Periapical granuloma group ( $\times 630$ ); I-L: Periapical cyst group ( $\times 630$ ). A, E, I: Tryptase; B, F, J: MMP-8; C, G, K: DAPI; D, H, L: Merged.



**Figure 5.** Densities of MMP-13-positive MCs in different groups. A: Normal control group (n=30); B: Periapical cyst group (n=30); C: Periapical granuloma group (n=30). Mean  $\pm$  SD. \*\*P<0.01, compared to A.

lagenase, gelatinase, stromelysin, membrane type matrix metalloprotease, matrilysin, and other MMPs [8, 11-15]. Many studies have been devoted to the role of MMPs in dental

caries and pulp diseases, and very few are concerned with MMPs in periapical diseases. Collagenases and gelatinases are the major MMPs related to periapical diseases. ECM in the periodontal membrane and alveolar bone are mainly composed of collagenous fibers. Basement membrane is mainly composed of type IV collagen/gelatin, which can be degraded into small fragments by collagenase. The fragments are further decomposed by gelatinases and other non-specific cathepsins, which leads to periapical tissue damage [16, 17].

MMP-8 and MMP-13 are both collagenases. MMP-8 was first found in neutrophil granulocytes, and it is also known as neutrophil collagenase. Recent studies have shown that MMP-8 is expressed in fibroblasts, endothelial cells, smooth muscle cells and macrophages. MMP-13 is cloned from breast cancer cells and expressed mainly in fibroblasts, endothelial cells, and plasmacytes [8, 15, 16]. It

was once believed that MCs were the main effector cells in allergic inflammatory responses. But recent studies have demonstrated that MCs secrete a variety of cytokines that are involved in periapical lesions and periodontal diseases. As effector cells and regulatory immune cells, MCs play an important role in both innate immunity and adaptive immune response and participate in immune defense [18]. MCs can secrete histamine and tumor necrosis factor- $\alpha$  that promote bone resorption in periapical lesions. However, no reports have been published on MCs secreting MMP-8 and MMP-13 in periapical lesions that are involved in periapical lesions. We found that MCs were positive for both MMP-8 and MMP-13 in periapical cysts and periapical granulomas by DIF staining. Moreover, the number of MMP-8 and MMP-13 double positive MCs in chronic periapical lesions increased significantly compared with the normal control group. This indicated a pathogenic role of MMP-8 and MMP-13 double positive MCs in chronic periapical periodontitis.

Marcal et al. [19] reported a higher density of MCs in periapical granuloma than in periapical cyst, while Rodini et al. [20] and Drazic et al. [18] described opposite results. We found no significant difference in the density of MMP-8 and MMP-13 double positive MCs in periapical granuloma and periapical cyst. Disagreement in the number of MCs between different studies may arise from differences in morphological analysis and sample size or from the aggravation of periapical lesions in acute phase [21].

MCs secrete tryptase that can activate and induce the production of collagen by fibroblasts [22], while promoting organization of periapical granulation tissues [20]. We observed the expression of MMP-8 and MMP-13 in MCs in chronic periapical lesions. ECM, especially collagen, is the substrate for MMP-8 and MMP-13. Therefore, MCs secrete a variety of mediators that are involved in the rebuilding of connective tissues. Changes in the mediators secreted by MCs may influence the chronic course of periapical lesions. When MCs are activated, the products of degranulation will aggravate periapical lesions and promote bone resorption. Some researchers have used ketotifen to inhibit secretion of neurotransmitters by MCs, thus reversing sclerotic changes

[23]. Therefore, controlling the synthesis of MMP-8 and MMP-13 by MCs or regulating the functions of MCs may become a new therapeutic target in periapical diseases.

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## Disclosure of conflict of interest

None.

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