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치관, 치근 상아질에서 우식의 진행에 따른 Matrix Metalloproteinase- 8과 13의 서로 다른 발현 양상에 관한 연구

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<Abstract>

Differential Expressions of Matrix Metalloproteinase- 8 and 13 in Human Coronal and Radicular Dentin with Progression of Caries

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Background: Matrix metalloproteinases (MMPs) are proteolytic enzymes involved in degradation of the extracellular matrix. MMP-8 has been reported to be involved in the degradation of collagen in progression of dental caries. MMP-13 was found to be expressed in both normal and caries pulp, implying its involvement in the pathogenesis of dental caries. Methods: Four extracted teeth were used. They were categorized into four grades according to caries progression. Three sections were prepared from each separated crown and root. Immunofluorescence of the FITC of the MMP-8 and 13 in coronal and radicular dentin was analyzed by confocal microscopy. Results: Immunofluorescence signals that were indicative of MMP-8 were observed both in radicular and coronal dentin in the sound, C1 and C3 carious teeth. In C2 carious teeth, immunofluorescence signals that were indicative of MMP-8 were observed only in radicular dentin. Immunofluorescence signals that were indicative of MMP-13 were observed both in radicular and coronal dentin in the sound teeth. In C1, C2 and C3 carious teeth, immunofluorescence signals that were indicative of MMP-13 were not observed both in radicular and coronal dentin. Conclusion: Immunofluorescence signals revealed that MMP-8 and 13 were present in coronal and radicular dentin, and was differently expressed as caries progressed.

Key words : Coronal Dentin, Dental Caries, MMP-8, MMP-13, Root Dentin.

Ⅰ. INTRODUCTION

Matrix metalloproteinases (MMPs) is the proteolytic enzyme that is involved in the inflammatory diseases in the body through degradation of extracellular matrix[1]. The MMPs promote tissue remodeling and cell migration by degrading all kinds of proteinaceous components in the ECM extensively. There have been a lot of studies about the role of MMPs in the oral tissues. The MMPs are known to play an fundamental role in invasion and growth of oral tumor as well as in development and remodeling of oral tissue[23]. Their expression were observed in pulps and periapical lesions and are found to be closely related to periodontal disease especially[45]. In periodontal disease, MMPs that are classified as collagenase, such as MMP-1, 8 are known to cause absorption as well as destruction

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of alveolar bone and gingiva[6]. It is thought that the reason is because the major component of alveolar bone or periodontal tissue is the type I collagen . Recent studies has reported that MMP-2, 9 that are classified as gelatinases are involved in dental caries[8]. It has been reported that they are synthesized and secreted in the form of proenzyme from odontoblast and then embedded in the dentin matrix . After being embedded in the dentin matrix[9], they are closely related to autodegenerative processes, such as the degradation of the exposed dentin matrix during the progression of caries[10], the inflammatory process of pulps[11] and the process of dentin adhesion[2].

It has been found that MMP-8 exists in carious dentin through western blot analysis and gelatine zymography [810,], indicating that it is the main collagenase present in dentin[12]. MMP-13, as well as MMP-8 is the MMPs that are classified as collagenases. And according to previous studies about the role of MMPs in dental caries, it has been reported that MMP-13 was expressed in pulps of sound and carious dentin[13]. Therefore, it is known that it is a collagenase that exists in the pulps, but it is not known if it exists in dentin. In addition, studies have shown that MMP-8 also existed in dentin of sound tissues and caries. But the studies were performed using Western blot analysis. There were no studies that observed the expressions using immunofluorescence staining. Also there are no studies that classified teeth according to the progression of caries and observed changes in expression patterns by dividing them into the crown and root. In this study, we classified teeth according to the progression of caries and divided teeth into crown and root. And we observed the expressions of MMP-8 and-13 that are classified as representative collagenases through immunofluorescence staining. Identifying the MMP-8 and MMP-13 expression patterns according to the progression of caries will have important implications for understanding the role of the endogenous collagenase in relation to the progression of caries.

II. MATERIALS AND METHODS

1. Preparation of teeth

Four freshly extracted teeth with closed apices were used. Teeth extracted as treatment were collected after obtaining consent from patients, with the approval of the Institutional Review Board for Human Studies of the Catholic University of Korea, Yeouido St. Mary's Hospital. Patients were aged from 26 to 66 years. In total, four teeth were used; they were categorized into four grades, based on caries progression:

Normal : Without caries

Grade 1 (C1 grade caries) : Caries limited to enamel.

Grade 2 (C2 grade caries) : A wide range of caries that invaded dentin.

Grade 3 (C3 grade caries) : A wide range of caries that invaded pulp.

The dental caries was classified in advance using clinical observation and radiographs, and caries invasion was confirmed visually during enamel elimination and crown separation procedures after tooth extraction.

Blood and periodontal ligament (PDL) were wiped off the extracted teeth with gauze immediately after extraction. The enamel of the crown was removed using a high-speed diamond burr under douching, and then the crown and root were separated from the cement-enamel junction using a high-speed diamond burr under douching. After pulp had been eliminated from the crown and root, they were placed in tubes and stored at -40℃.

2. Specimen preparation

The prepared dentin was fixed in 10% formaldehyde for 24-36 h. The prepared dentin was completely demineralised with 10% EDTA for 6-8 weeks. The demineralised dentin were washed four times with distilled water, dehydrated in an ascending ethanol series (70%, 80%, 90%, 95% and

100%), rinsed twice with xylene and embedded in paraffin. We used three sections obtained from crown and root apart in staining of MMP-8, MMP-13 and negative control and each section was cut in a direction parallel to the long axis of the tooth. Five micrometer-thick sections were obtained using a microtome (Automatic Rotary microtome, Leica, RM2255, Germany) and mounted on adhesive microscope glass slides. Sections were deparaffinised in xylene, rehydrated in a descending ethanol series (100%, 90%, 80% and 70%) and finally in distilled water.

3. MMP-13 immunofluorescence

The sections were used for identification of MMP-8, MMP-13 expression in sound and carious dentine. The deparaffinised and rehydrated sections were blocked in blocking buffer with horse serum for 40 min and incubated with anti-mouse MMP-8, MMP-13 primary monoclonal antibody (1:25 dilution, Abcam, Cambridge, UK) overnight at room temperature. The antibody specifically recognizes MMP-8 in both its 65 kDa latent and 52 kDa active forms and MMP-13 in both its 60 kDa latent and 48, 34 kDa active forms. The sections were rinsed with phosphate-buffered saline and incubated for 30 min in specific anti-mouse FITC conjugated secondary antibody (anti-MMP-8 and anti MMP-13, Abcam, Cambridge, UK). Finally, the sections were rinsed twice in phosphate-buffered saline, mounted with DAPI mounting medium (Vector Laboratories, Peterborough, United Kingdom) and examined using a Confocal microscope (LSM 510 Meta; Carl Zeiss Co. Ltd. Germany). Negative controls were performed by omitting primary antibodies.

Ⅲ. RESULTS

We divided dentins of sound and carious teeth into crowns and roots and then observed MMP-8 and MMP-13 expressions using immunofluorescence staining. Different expression patterns in sound dentin and carious dentin were shown. And the changes in the expression patterns depending on the progress of caries could be observed. Also different expression patterns in crown and root depending on the progression of caries could be observed.

Figure 1 is the picture obtained by observing the expression of MMP-8 in sound and caries dentin using immunofluorescence staining. Through observation of MMP-8 expression, we could observe positive stainings scattered across both crowns and roots in sound dentins. In the case of sound dentins, we could observe the greater number of positive spots in the crowns than in the roots. In the case of C1 whose caries were limited only to enamel, we could observe positive spots in crown and root, despite a small number of the positive spots compared to sound dentin. In the case of C2 whose caries were involved in dentin, the expression was observed in the dentin of root, not in the dentin of crown. In the case of C3 whose caries involved in pulp, positive spots in crown were rarely observed and a large number of positive spots in root were observed compared to the roots of C1 and C2.

In Figure 2 is the picture obtained by observing the expression of MMP-13 in sound dentin and carious dentin using immunofluorescence staining. The expression level of MMP-13 was low compared to the expression level of MMP-8 as a whole. In the case of sound dentin, positive spots were observed in both crown and root, but the expression level in root was lower than the expression level in crown. However, in dentin in teeth with C1, C2 or C3 caries, no expression pattern was observed in both crown and root.

Control specimens revealed no immunohistochemical staining, confirming that no cross-reactions had occurred between the secondary antibodies and the dentin organic

matrix or with the inorganic phase (data not shown).

Ⅳ. DISCUSSION

In this study, MMP-8 and MMP-13 in crown and root of sound dentin and carious dentin were observed using immunofluorescence staining method. With the result that MMP-8 was expressed in sound dentin and carious dentin, we could support the previous report that had observed MMP-8 in both the sound dentin and the carious dentin using Western blot analysis[8,10]. In the cases of caries confined to enamel (C1 caries) and caries involved in pulp (C3 caries), the expressions were observed in both crown and root. But in the cases of caries dentin involved in dentin (C2 caries), the expression was not observed in crown, rather than in root. And, in the case of root dentin, the expression of the C3 caries involved in pulp increased more than the C1 and C2 caries did. It is thought that MMP-8 is the collagen degrading enzyme and is present in dentin, closely related to the collagen. And it is thought that when the pH decreases due to caries progression, the MMP-8 is activated and then involved in the degradation of collagen. It was thought that when caries was involved in dentin (C2 caries), the MMP-8 was dissolved with the degradation of collagen and that the MMP-8 was observed only in root dentin, not in coronal dentin. However, in the case of extensive caries involved in pulp (C3 group), it was thought that the MMP-8, the main MMP in saliva[14] had infiltrated into the exposed dentin directly and that a large number of positive spots had been observed in coronal and radicular dentin in the C3 group compared to the cases of C1 and C2 caries due to effects of the MMP-8 infiltrated from the saliva. Thus, it was thought that in the case of extensive caries, MMPs present in saliva had more effect on the caries, resulting in promotion of the progress of caries. It was also thought that because MMP-8 was known as one of the major MMPs in gingival crevicular fluid (GCF) , the MMP-8 derived from the GCF in the case of extensive caries involved in root also had an effect on the presence of MMP-8 in root dentin.

Previous studies revealed that there was MMP-13 in pulp[13] and that there was no expression of the MMP-13 in dentin. In this study, we observed a clear expression in crown and root of sound dentin by immunofluorescence staining method for the first time. However, the expression was not observed in both crown and root for dentins with caries (C1, C2 and C3 caries). It was thought that MMP-13 acted on the caries as the collagen degrading enzyme and that because the MMP-13 expression level in sound dentin was also low compared to the MMP-8 expression level and the MMP-13 was dissolved more easily according to the progression of the caries, the expression was not observed in carious dentin. In addition, the previous study compared the expression levels of MMP-13 in pulp according to the progression of caries and then reported that the expression of MMP-13 in the pulp with caries was down-regulated (PCR) and that this could be one of the defense mechanisms against the progression of caries. Thus, it was thought that because when the caries was involved in dentin, the expression of MMP-13 was suppressed, no MMP-13 was observed in dentin with caries. On the other hand, previous studies reported that no MMP-13 was observed in both saliva and GCF[10]. Thus, unlike MMP-8, it was thought that MMP-13 that penetrated from saliva and GCF did not affect the results despite caries progression and subsequent dentin exposure.

The reason that expression patterns of MMP-8 and MMP-13 appeared different from each other, depending on the MMPs that are classified as collagen degrading enzymes and the progression of caries was thought to be because the mechanisms of action and the time in the progression of caries could be different and because the MMP-8 and the MMP-13 could have different effect on

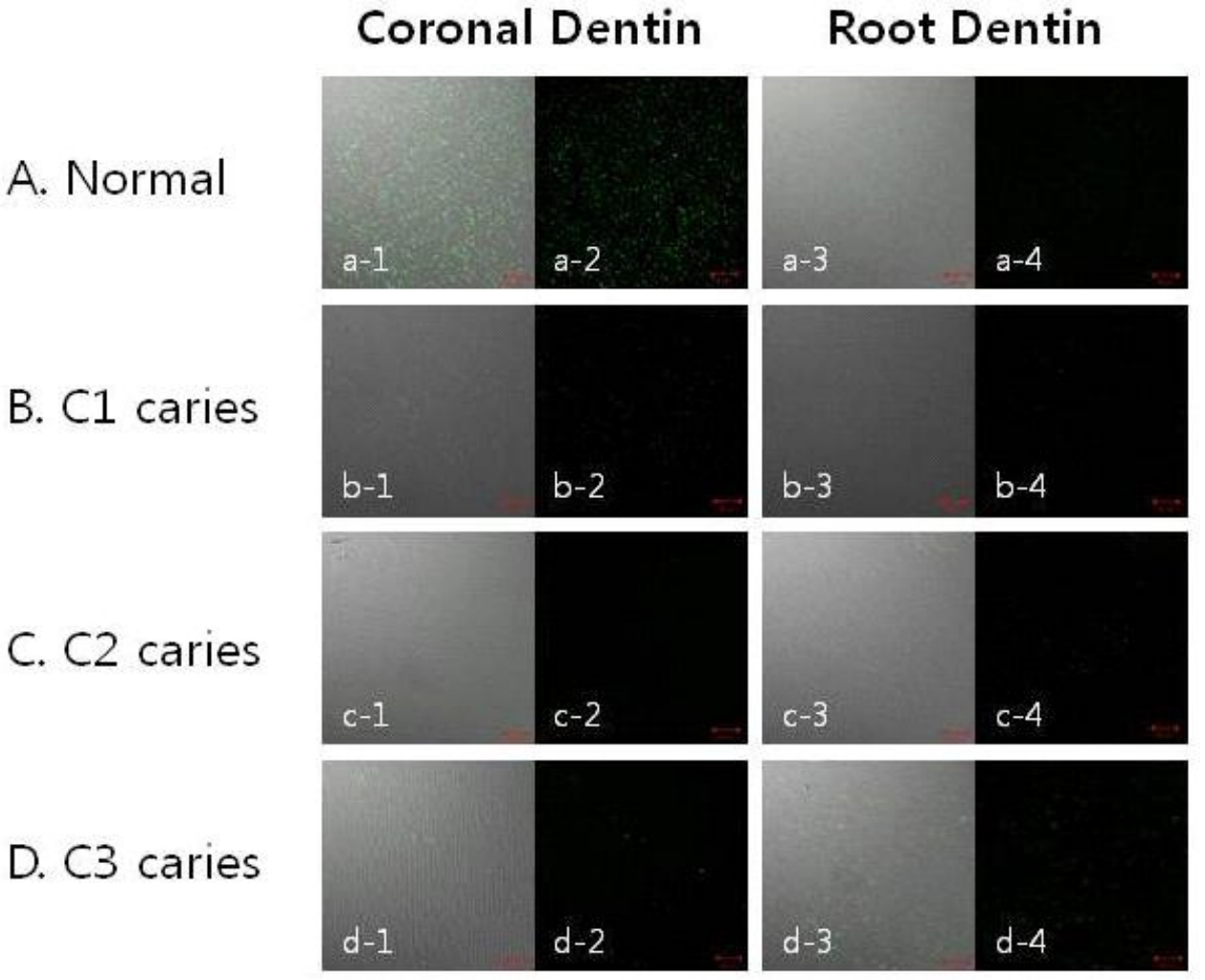

Fig. 1. Immunofluorescence images from coronal and radicular dentine revealing MMP-8 expression that is detectable as green fluorescence. Fluorescent images of MMP-8 positive staining labelled with FITC. The left column is a picture of the stained coronal dentin and the right column is a picture of the stained radicular dentin. A. This is a picture of the stained coronal and radicular dentin of sound teeth. The (a-1, 3) is a merged image and the (a-2, 4) is a confocal image. The (a-1, 2) is a picture of the stained coronal dentin of sound teeth. A lot of positive spots are observed. The (a-3, 4) is a picture of the stained radicular dentin of sound teeth. Positive spots that are scattered can be observed. B. This is a picture of the stained coronal and radicular dentin of teeth with C1 caries. The (b-1, 3) is a merged image and the (b-2, 4) is a confocal image. The (b-1, 2) is a picture of the stained coronal dentin of teeth with C1 caries. The frequency of positive spots is lower than the frequency of positive spots in coronal dentin of sound teeth. The (b-3, 4) is a picture of the stained radicular dentin of teeth with C1 caries. The frequency of positive spots is lower than the frequency of positive spots in radicular dentin of sound teeth. C. This is a picture of the stained coronal and radicular dentin of teeth with C2 caries. The (c-1, 3) is a merged image and the (c-2, 4) is a confocal image. The (c-1, 2) is a picture of the stained coronal dentin of teeth with C2 caries. The expression is not observed, but only cross-section of dentinal tubule is observed. The (c-3, 4) is a picture of the stained radicular dentin of teeth with C2 caries. The expression is sparsely observed. D. This is a picture of the stained coronal and radicular dentin of teeth with C3 caries. The (d-1, 3) is a merged image and the (d-2, 4) is a confocal image. The (d-1, 2) is a picture of the stained coronal dentin of teeth with C3 caries. The positive spot is sparsely observed. The (d-3, 4) is a picture of the stained radicular dentin of teeth with C3 caries. A large number of positive spots are observed compared to radicular dentins of teeth with C1 and C2 caries.

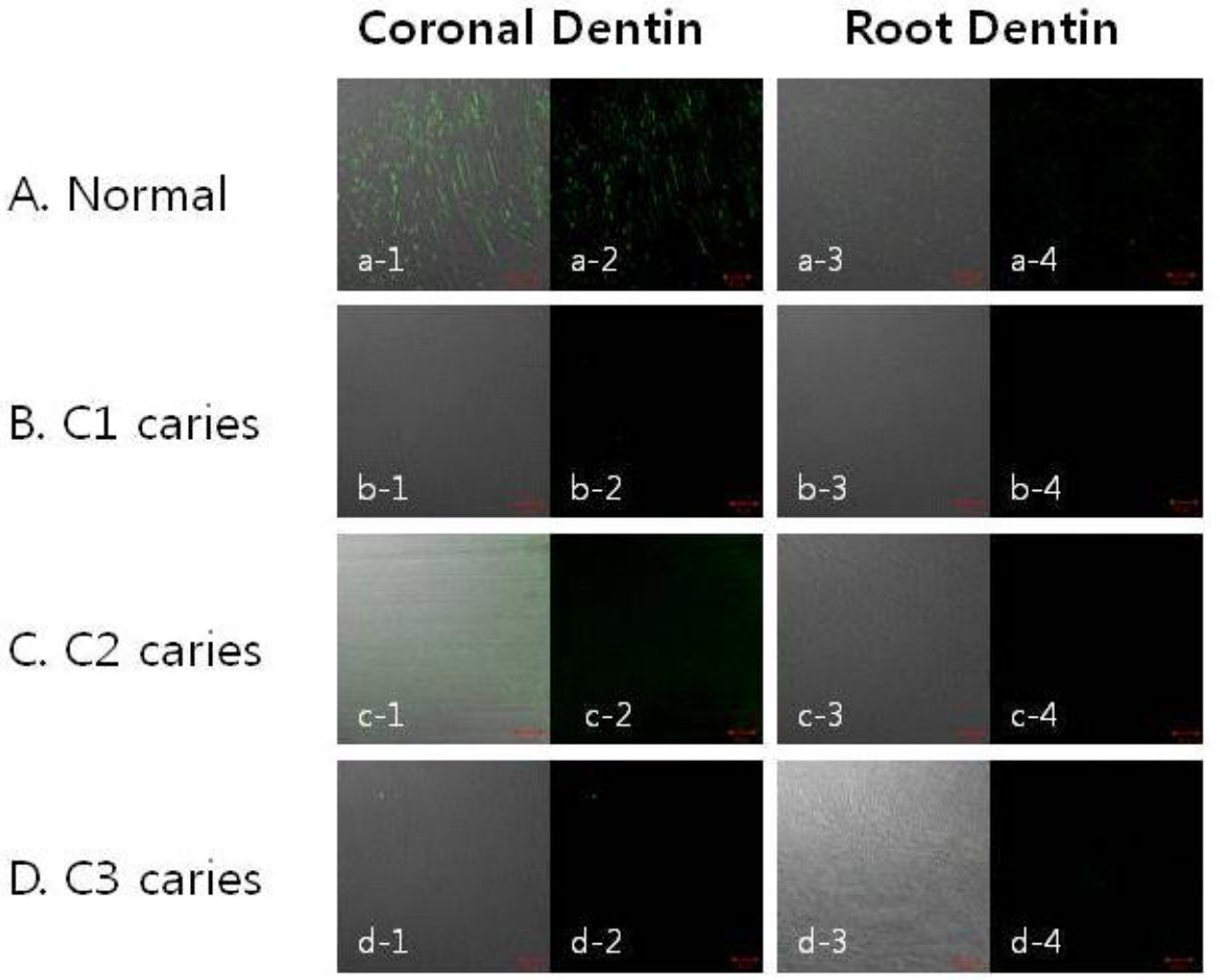

Fig. 2. Immunofluorescence images from coronal and radicular dentine revealing MMP−13 expression. Fluorescent images of MMP−13 positive labeled with FITC. The left column is a picture of the stained coronal dentin and the right column is a picture of the stained radicular dentin. A. This is a picture of the stained coronal and radicular dentin of sound teeth. The (a−1, 3) is a merged image and the (a−2, 4) is a confocal image. The (a−1, 2) is a picture of the stained coronal dentin of sound teeth. Significant positive spot is observed. The (a−3, 4) is a picture of the stained radicular dentin of sound teeth. Positive spots that are scattered can be observed. B. This is a picture of the stained coronal and radicular dentin of teeth with C1 caries. The (b−1, 3) is a merged image and the (b−2, 4) is a confocal image. The (b−1, 2) is a picture of the stained coronal dentin of teeth with C1 caries. The expression is not observed, but only cross−section of dentinal tubule is observed. The (b−3, 4) is a picture of the stained radicular dentin of teeth with C1 caries. No expression is observed. C. This is a picture of the stained coronal and radicular dentin of teeth with C2 caries. The (c−1, 3) is a merged image and the (c−2, 4) is a confocal image. The (c−1, 2) is a picture of the stained coronal dentin of teeth with C2 caries. No expression is observed. The (c−3, 4) is a picture of the stained radicular dentin of teeth with C2 caries. No expression is observed. D. This is a picture of the stained coronal and radicular dentin of teeth with C3 caries. The (d−1, 3) is a merged image and the (d−2, 4) is a confocal image. The (d−1, 2) is a picture of the stained coronal dentin of teeth with C3 caries. No expression is observed. The (d−3, 4) is a picture of the stained radicular dentin of teeth with C3 caries. No expression is observed.

caries. It was thought that MMP-8 still remained in the advanced root caries and played an important role in caries progression in root. It was also thought that because expression of MMP-13 was observed in sound dentin, the MMP-13 acted only in early caries in the progression of caries and then disappeared and that expression of MMP-13 was suppressed after early caries and played an auxiliary role in the activity of other enzymes. MMP-8 has been reported as the main MMPs present in saliva and GCF. But it has been reported that no MMP-13 was expressed both in the saliva and the GCF. Thus, it is thought that MMP-8 may penetrate directly into the exposed dentin and affect the progression of caries when dentin is exposed due to the progression of caries and that MMP-8 in dentin matrix of C3 caries is expressed more clearly for the same reason.

The major component of dentin is mineralized collagen and type I collagen accounts for 90% of the extracellular matrix[16]. In addition, the dentin consists of proteoglycan (i.e., chondroitin-4/6-sulphate, decorin, biglycan, lumican and fibromodulin) and small integrin-bindingligand N-linked glycoproteins (SIBLINGs; i.e., bonesialoprotein, osteopontin, dentin matrix protein-1 and dentin sialophosphoprotein)[17]. It is known that there are MMPs in dentin and that MMP-2, 9 in gellatinase group and both MMP-8 and MMP-14, 20 in collagenase group are secreted from odontoblast during tooth development and buried in dentin.

It is found that MMP-8 exists in both caries dentin and sound dentin and play an important role in periodontal disease and peri-implantitis as collagen degrading enzymes[18] In addition, it has been reported that there are MMPs in both pulp and periapical tissues and that MMP-8 level in apical lesions could be a important criterion in determining whether the treatment was successful or not[19].

It is known that MMP-13 is the collagenase-3 that degrades most collagen fibers and acts as the most effective degrading enzyme, especially for type II collagen and that the MMP-13 exhibits high activity on gelatin unlike other collagen degrading enzymes[20]. It has been reported that MMP-13 was expressed in sound pulp and pulp with caries and that MMP-13 as well as MMP-8 is the major collagen degrading enzyme[13]. It has also been reported that MMP-13 was expressed in the GCF of teeth with apical lesions and that the researchers could estimate the transition from granuloma to cyst by analyzing the expression of MMP-13[12].

When the pH drops to less than 5.5 by lactic acid that is generated by cariogenic bacteria in the mouth, dental caries begins due to the decomposition of inorganic components of dentin[21]. It was thought that as a result, the organic matter in the dentin was exposed and that the dental caries progressed by the decomposition of organic matrix of dentin by bacterial enzymes and various decomposing enzymes. But according to the recent concept, it has been thought that only demineralization of inorganic components of dentin is caused by cariogenic bacteria and that the cariogenic bacteria cannot break down the organic matrix of dentin. And it has been thought that the concept that bacterial degrading enzymes decompose the dentin is not as important as we thought the concept previously[10]. It has been thought that the major factor in the pathogenesis of dental caries is the decomposition of dentin matrix by endogenous protease rather than the concept above[10]. It has been shown that MMP-2, 9 is an endogenous protease in dentin and is classified as gellatinase group and that the activity of the MMP-2, 9 plays an important role in the destruction of collagen[822,]. In this study, it has been thought that MMP-8 and MMP-13 that are classified as collagenase group are observed in both sound dentin and carious dentin and that the MMP-8 and MMP-13 play an important role in the degradation of collagen. MMPs present in mineralized dentin are activated by acidic pH due to lactate derived

from cariogenic bacteria. The endogenous MMPs are released and activated in the process of caries. And once the endogenous MMPs are activated, they can decompose organic matrix of dentin with caries. Thus, it is important to observe the expression of MMPs in carious dentin because organic matters of dentin themselves can be decomposed by endogenous MMPs.

This study has revealed the presence of MMP-8 and -13 in crown and root dentin of human teeth in the various distributions, patterns and amounts. Because the number of teeth used in this study was low, it was difficult to classify them more precisely according to the progression of dental caries and to conclude the resulting changes. However, the previous studies had reported that MMP-13 was expressed only in pulp of teeth. But this study revealed that MMP-13 was present in the dentin of teeth for the first time. In this study, we also observed MMP-8 that was expressed in various forms, depending on the progression of caries using immunofluorescence staining for the first time. These results suggest that MMP-8,-13 plays an important role in the degradation of collagen during the progression of caries as a collagen degrading enzyme. However, in this study, even though we revealed that both MMP-8 and MMP-13 were present in dentin, a lot remains to be done to assess the physiological and pathological importance and to identify the signaling pathways. It is thought that more studies are needed about these.

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