건전 상아질과 우식 상아질에서 matrix metalloproteinase-13의 발현 양상 분석
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<Abstract>
Differential Expressions of Matrix Metalloproteinase-13 in Sound and Carious Dentin

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Matrix metalloproteinases (MMPs) are proteolytic enzymes involved in degradation of the extracellular matrix. In a previous study, MMP-13 was found to be expressed in pulp implying its involvement in the pathogenesis of dental caries. Two extracted teeth were used. A sound tooth and a tooth with wide range of dental caries were used. Two sections were obtained each from isolated crown and root. Immunofluorescence of the FITC of the MMP-13 in coronal and radicular dentin was analyzed by confocal microscopy. Immunofluorescence signals that were indicative of MMP-13 were observed in coronal dentin of sound teeth and in carious teeth with a wide range of caries. Marked immunofluorescent reaction was observed in the border line of caries infected and affected coronal dentin. MMP-13 expression was not detected in the root dentin. The expressions of MMP-13 in carious dentin imply the roles of MMP-13 in caries progression.

Key words: Coronal Dentin, Dental Caries, Matrix Metalloproteinase-13, Radicular Dentin.

I. INTRODUCTION

Matrix metalloproteinases (MMPs) are proteolytic enzymes involved in inflammatory conditions through degradation of the extracellular matrix. MMPs take fundamental roles in oral tissue development and remodeling, and also play crucial role in invasion and growth of oral tumor. They are often expressed in the pulp and periapical lesions, and profoundly associated with periodontal diseases, in particular. MMPs classified as collagenases such as MMP-1, and 8 are commonly activated in periodontal diseases, and are known to generate destruction and absorption of alveolar bone and gingiva. This is thought to be attributable to type I collagen which is the major component of alveolar bone and periodontal tissue. Recent studies proposed that MMPs classified as gelatinases such as MMP-2, -9, and others are involved in dental caries. Those are reported to be synthesized and released in the form of proenzyme from odontoblasts, embedded in the dentin matrix, and
II. MATERIALS AND METHODS

1. Preparation of teeth

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 26–36 years old. One sound tooth without caries and one tooth with a wide range of caries that invaded dentin were used.

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 tooth 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 cemento–enamel junction using a high-speed diamond burr under douching. And it was placed in tubes and stored at −40°C.

2. Specimen preparation

The prepared dentin was fixed in 10% formaldehyde for 24–36 h. The prepared dentin was completely demineralized with 10% EDTA for 6–8 weeks. The demineralized dentin was 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. Two sections were prepared each from the crown and root obtained from a single tooth and used for MMP–13 and negative control staining. Each section was cut parallel to the major 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 deparaffinized 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–13 expression in carious and sound dentin. The deparaffinized and rehydrated sections were blocked in blocking buffer with horse serum for 40 min and incubated with anti–mouse MMP–13 primary monoclonal antibody (1:25 dilution, Abcam, Cambridge, UK) overnight at room temperature. The sections were rinsed with phosphate–buffered saline and incubated for 30 min in specific anti–mouse FITC conjugated secondary antibody (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

The expression of MMP–13 was observed using immunofluorescent staining in coronal and radicular dentin of sound tooth and carious tooth with caries invaded to dentin. In figure 1 and 2, MMP–13 expression was only observed in the coronal dentin of sound tooth, and was not seen in the radicular dentin of sound tooth. The expression of MMP–13 in carious coronal dentin was differently distributed depending on caries involved areas. In figure 3, Immunofluorescence signal intensity was lowest in the sound dentin, and strong immunofluorescent reaction was detected in caries–affected dentin. Intense immunofluorescent reaction was observed around the dentinal tubule, and distinctive expression was seen in the border of infected and affected dentins. Decomposition of minerals was found according to the progress of caries in the wide dentinal tubule of affected dentin. In figure 4,

Fig. 1. shows the images of MMP–13 expression in the normal coronal dentin. MMP–13 expression generally showed the tendency of scattered distribution

Fig. 2. shows the image of MMP–13 expression in the normal radicular dentin. MMP–13 was not expressed.

Fig. 3. shows the image of MMP–13 expression in the caries–affected coronal dentin. MMP–13 was distinctively expressed in the front part of affected dentin and was not expressed in the deep coronal dentin.

Fig. 4. shows the image of MMP–13 expression in the caries–affected radicular dentin. but no expression was found.
expression of MMP-13 was not detectable in the root of carious tooth. Control specimens revealed no immunofluorescent 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

This study first examined the expression of MMP-13 in sound and carious dentin of the crown and root using immunofluorescent staining technique. Although previous studies identified the presence of MMP-13 in the pulp of tooth\(^{11}\), they could not observe MMP-13 expression in the dentin. Dental caries stimulate MMP-13 expression, and different aspects of expression are shown in the sound and carious dentin. Marked expression of MMP-13 was observed in the border line of caries infected and affected coronal dentin in C2 caries. However, MMP-13 was not expressed in deep coronal dentin. The expression was not observed in the radicular dentin of both sound and carious teeth. MMP-13 was mainly expressed in affected dentin, and this result was caused by inorganic components decomposition as carious progressed. In contrast, MMP-13 was not observed in infected dentin with collagen deformity since it was assumed to be dissolved. Although demineralization occurred in affected dentin, intrinsic MMPs have been suggested to have direct influence in the decomposition of adhesive layer in resin bonding compare with healthy dentin without collagen deformity. Thus, the intense expression of MMP-13 in caries-affected dentin implies that the faster decomposition of collagen fiber in hybrid layer could occur with the treatment of adhesion of restorative resin. In addition, it is partially involved in the failure of restoration\(^{12}\). A previous study identified a high level of MMP-2 in the affected dentin\(^{13}\). In a comparative study on the degrees of MMP-13 expression according to caries progression in the pulp, down-regulation (PCR) was reported in the expression of MMP-13 in the pulp of progressing dental caries, suggested as one of the defense mechanisms toward caries progression\(^{11}\). Therefore, no MMP-13 expression in the deep coronal dentin could be thought to be attributable to the suppression of expression caused by caries that invaded dentin. The previous studies have suggested that MMP-13 was not expressed in both saliva and GCF\(^9\). Despite the exposure of dentin due to caries progression, there was no influence of MMP-13 penetrated from saliva or GCF. Dentin is primarily composed of mineralized collagen, and type I collagen accounts for 90% of all extracellular matrix\(^{14}\). In addition, dentin comprises proteoglycan (i.e., chondroitin-4/6-sulphate, decorin, biglycan, lumican,fibromodulin)\(^{15}\) and small integrin-binding ligand N-linked glycoproteins (SIBLINGs; i.e., bonesialoprotein, osteopontin, dentin matrix protein-1, dentin sialophosphoprotein)\(^{16}\). MMPs that are detected in the dentin are embedded in dentin that odontoblasts secrete MMP-2, and -9 of gellatinases\(^7\) and MMP-8, -4, and -20 of collagenases during dentinogenesis\(^7\). MMP-13, also known as collagenase-3, decomposes most collagen fibers and is recognized as the most effective lyase in type II

![Fig. 5.](image) Fig. 5. shows the image of MMP-13 expression in the coronal pulp of carious dentin. Intense expression of MMP-13 was detected.
collagen, in particular. Unlike other collagenases, it shows high activity on gelatin, by 44 times higher than MMP−13 and 8 times higher than MMP−8. This high activity on gelatin is anticipated to decompose collagen fibers of MMP−13 and be involved in the decomposition of degradation product\textsuperscript{17}. The expression of MMP−13 was reported in sound and caries−affected pulp, and MMP−13 was found to be an important collagenase in the pulp along with MMP−8\textsuperscript{11}. Moreover, the expression of MMP−13 was seen in GCF of the teeth with periapical lesion, and the transition from apical granuloma to cyst has been suggested\textsuperscript{18}. When the pH in the mouth drops to less than 5.5 due to lactic acid produced by oral cariogenic bacteria, caries develop as the mineral substances in the dentin begin to decompose\textsuperscript{19}. Consequently, the organic substances of dentin were predicted to be exposed, and dental caries were thought to occur by the decomposition of dentin organic matrix due to bacterial enzyme, various lyases, and others. However, recent studies have suggested that cariogenic bacteria incur only dentin demineralization and are unable to decompose dentin organic matrix. The progression of demineralization generates the deformation of dentin matrix, and the dentin structures are damaged under this condition. Apatite crystallites are broken down first, and then the decomposition of collagen matrix occurs as non−collagenous proteins escape under acidic conditions and host−derived proteases are activated. The major cause of dental caries is assumed to be the decomposition of dentin matrix induced by intrinsic proteolytic enzymes\textsuperscript{9}. The activation of MMP−2 and 9 which are intrinsic collagenases of the dentin and belonging to gelatinase category is known to do fundamental role in the collagen destruction of the dentin\textsuperscript{7}. This study found out that MMP−13 plays crucial role in collagen decomposition taking place in caries progression by identifying the expression of MMP−13 belonging to collagenase group in sound and carious dentin. MMPs present in mineralized dentin are activated by an acidic pH brought about by lactate released from cariogenic bacteria. Once MMPs are released and activated during caries progression, activated dentin MMPs can decompose dentin organic matrix in carious tooth. Native triple helical collagen molecules are broken down by MMP−1 and −8, and hydrolyzed by gelatinases such as MMP−2 and −9\textsuperscript{20}. This interaction between intrinsic collagenase and gelatinases is considered the most important inflammatory response in collagen decomposition.

Although previous studies reported MMP−13 expression in the pulp, this is the first study to determine the presence of MMP−13 in the dentin using immunofluorescence analysis. This study found out that MMP−13 is a collagenase that plays a crucial role in collagen decomposition in the progression of caries along with MMP−8. Although the study has identified the presence of MMP−13 in the dentin, future studies are thought to be crucial to clearly elucidate the assessment and signaling process on physiological and pathological significance of MMP−13 expression in progression of caries.

This paper was translated in English at www.harrisco.net

V. REFERENCES

4. Sternlicht MD, Werb Z: How matrix metalloproteinases