Received: 12 September 2021 | Revised: 5 April 2022 | Accepted: 17 April 2022

DOI: 10.1111/jre.12996

Journal of PERIODONTAL RESEARCH WILEY

ORIGINAL ARTICLE

Expression of matrix metalloproteinase-3 and -10 is up-regulated in the periodontal tissues of aged mice

Yoko Kageyama[1] | Megumi Nakamura[2] | Yohei Igari[1] | Satoshi Yamaguchi[1] | Akiko Oguchi[3] | Yasuhiro Murakawa[3] | Yoshinori Hattori[1] | Yasuyuki Sasano[2]

[1]Division of Aging and Geriatric Dentistry, Tohoku University Graduate School of Dentistry, Sendai, Japan

[2]Division of Craniofacial Development and Tissue Biology, Tohoku University Graduate School of Dentistry, Sendai, Japan

[3]RIKEN Center for Integrative Medical Sciences, Yokohama, Japan

Correspondence
Megumi Nakamura, Division of Craniofacial Development and Tissue Biology, Tohoku University Graduate School of Dentistry, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan.
Email: megumi.nakamura.a6@tohoku.ac.jp

Funding information
JSPS KAKENHI, Grant/Award Number: JP18K09517, JP18K09760 and JP18K17110

Abstract

Objective: The present study was designed to investigate the whole transcriptome of periodontal tissues of both young and aged mice to identify the characteristic up-regulation of protease genes with aging and to localize their translated protein products in the periodontal tissues.

Background: The metzincin protease superfamily is composed of matrix metalloproteinases (MMPs), a disintegrin and metalloproteinases, and a disintegrin and metalloproteinases with thrombospondin motifs. Up-regulation of these extracellular matrix-degrading proteases has been implicated in senescence of tissues and organs, including the skin. However, few studies have investigated the expression profiles of these proteases and potential involvement in aging of periodontal tissues.

Methods: Periodontal tissues with the surrounding mandibular bones were collected from 50- and 10-week-old mice. Total RNA was extracted from the periodontal tissue and analyzed by cap analysis of gene expression (CAGE) to identify differentially expressed genes encoding the metzincin proteases. Furthermore, quantitative real-time polymerase chain reaction (qRT-PCR) was performed to validate the CAGE results, and the phenotypic expression of proteases involved in aging was localized via immunohistochemical analysis.

Results: The CAGE results showed that the expression levels of MMP-3, -10, and -12 were up-regulated at 50 weeks. Subsequent qRT-PCR analysis showed that the gene expression levels of MMP-3 and -10 were significantly increased with age. MMP-10 immunoreactivity was localized exclusively in the cementum and alveolar bone adjacent to the periodontal ligament and was stronger and broader in aged mice than young mice. MMP-3 immunoreactivity was localized in the periodontal ligaments at both 10 and 50 weeks.

Conclusion: In the present study, we demonstrated that the expression of MMP-3 and -10 increased with aging and identified their characteristic localizations in aged periodontal tissues.

KEYWORDS
aging, matrix metalloproteinase, stromelysin, transcriptome analysis

1 | INTRODUCTION

Tissues are composed of cells and extracellular matrix (ECM), which provides an external environment for the cells to regulate their differentiation and function. Homeostasis of the extracellular environment is maintained by regulating proteolysis of the ECM.[1] Extracellular matrix remodeling contributes to developmental morphogenesis, wound healing, tumor growth, and invasion, as well as maintenance of ECM homeostasis.[1-4]

The metzincin protease superfamily is composed of three major families of ECM proteases: matrix metalloproteinases (MMPs), a disintegrin and metalloproteinases (ADAMs), and a disintegrin and metalloproteinases with thrombospondin motifs (ADAMTSs). Members of these enzyme families are endopeptidases that contain a zinc ion in the catalytic active site. MMPs are either secreted or membrane-bound. Currently, 24 MMP genes have been identified in mammals, including 23 identified in humans.[1,5] Thus far, 22 and 37 ADAMs—membrane-anchored proteases—have been identified in humans and mice, respectively, along with 19 ADAMTSs, which are secreted extracellular proteases.[1,6,7] Recently, elevated expression of ECM-degrading proteases of the metzincin protease superfamily has been suggested to be implicated in tissue senescence. MMPs are involved in the cleavage of collagens, which are the major ECM proteins in the skin. Previous studies have demonstrated that mRNA expression of MMP-1, -3, -9, -10, -11, -23, -24, and -27 increased and that of type I procollagen decreased in aged human skin.[8,9] Another study showed that expression of constitutively active MMP-1 mutants in cultured young human skin organs and fibroblasts caused fragmentation of collagen fibrils, similar to that observed in aged skin, which resulted in reduced production of procollagen and altered morphology and function of dermal fibroblasts.[10] However, few studies have focused on the expression profiles and roles of members of the metzincin protease superfamily in aged periodontal tissues and potential involvement in the aging of periodontal tissues.

Conventional quantitative real-time polymerase chain reaction (qRT-PCR) is a reliable technique for analysis of gene expression; however, it can simultaneously detect the expression of only a limited number of genes. Sequence-specific probes for target genes are required for hybridization-based techniques. In recent years, the development of next-generation sequencing technologies, such as RNA sequencing (RNA-seq) and cap analysis of gene expression (CAGE) has made it possible to simultaneously sequence millions of DNA fragments of both known and unknown genes. RNA-seq identifies random RNA fragments to sequence, whereas CAGE captures and sequences the 5′ ends of capped RNAs.[11-15]

In this study, the whole transcriptomes of the mandibles of young and aged mice were subjected to CAGE in order to identify differentially expressed MMP, ADAM, and ADAMTS genes involved in the aging of periodontal tissues. Furthermore, qRT-PCR was performed to validate the CAGE results and immunohistochemical analysis was used to localize the phenotypic expression of the proteases involved in aging.

2 | MATERIALS AND METHODS

2.1 | Experimental animals

Male C57BL/6 mice, aged 10 and 50 weeks, were housed under specific-pathogen-free conditions with a 12-h light–dark cycle, a constant temperature of 23 ± 3°C, and *ad libitum* access to a stock (solid) diet (Nosan Corporation, Yokohama, Japan) and tap water. All procedures were approved by the Animal Research Committee of Tohoku University.

2.2 | RNA extraction

Six mice at 10 and 50 weeks of age were euthanized by inhalation of isoflurane (FUJIFILM Wako Pure Chemical Corporation). Then, the mandible in the region containing all molars was resected, and the skin and crowns of the molars were removed. The sample containing the tooth roots with the surrounding periodontal tissues was immersed in QIAzol Lysis Reagent (QIAGEN), homogenized mechanically as finely as possible with an ultrasonic homogenizer, and stored at −80°C until total RNA extraction with an RNeasy Lipid Tissue Mini Kit (QIAGEN) and an RNase free DNase set (QIAGEN).

2.3 | Identification of differentially expressed genes (DEGs) by CAGE

Tissues from two 10-week-old mice and two 50-week-old mice (two biological replicates) were used for identification of DEGs. The quality of the total RNA extracted from the mandible samples was checked. A CAGE library was prepared and sequenced by Kabushiki Kaisha DNAFORM with an Illumina NextSeq 500 system (Illumina). The trimmed clean reads were mapped to the mouse (mm9) reference genome with the STAR ultrafast universal RNA-seq aligner (v 2.5.2b).[16] The read count data were normalized and converted to log2 counts per million. The R package edgeR v 3.24.3[17] was used to identify up-regulated DEGs in aged mice. DEGs were defined as genes with a false discovery rate (FDR) of <0.01.

2.4 | Gene ontology enrichment analysis

Gene ontology (GO) enrichment analysis of DEGs was performed using the Database for Annotation, Visualization and Integrated Discovery Bioinformatics Resources 6.8 (https://david.ncifcrf.gov/). A probability (*p*) value of <.05 was considered statistically significant.

2.5 | qRT-PCR analysis of mRNA expression

cDNA was synthesized from 5 μl of total RNA isolated from the tissues of six 10-week-old mice and six 50-week-old mice using SuperScript III Reverse Transcriptase (Thermo Fisher Scientific) and random primers (Thermo Fisher Scientific). Specific primers were designed for the mRNA of MMP-3, -10, and -12 because the expression of each was up-regulated at 50 weeks rather than at 10 weeks, as determined by CAGE. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control gene. The primer sequences were as follows: Mmp3 forward, 5′-GTA TTC AGT CCC TCT ATG GAA CTC-3′ and reverse, 5′-CAG GTT CCA GAG AGT TAG ACT TG-3′; Mmp10 forward, 5′-TCC AGG AAT TGA GCC ACA AG-3′ and reverse, 5′- GGG TCA AAC TCG AAC TGT GAT-3′; Mmp12 forward, 5′-GCA GTC CTC TAT TTC AAA AGA CAC-3′ and reverse, 5′-CCA AAC CAG CTT GTA CTT TTC AAT G-3′; and Gapdh forward, 5′-CTC GTC CCG TAG ACA AAA TGG-3′ and reverse, 5′-AGG TCA ATG AAG GGG TCG T-3′. mRNA expression was quantified by qRT-PCR using FastStart Essential DNA Green Master hot start reaction mix (Roche Diagnostics) and a LightCycler Nano system (Roche Diagnostics) with the following cycling conditions: an initial 10-min enzyme activation step at 95°C followed by 40 cycles of denaturing at 95°C for10 s, annealing at 60°C for10 s, and elongation at 72°C for 15 s. Melting curve analysis was carried out by heating from 60°C to 95°C at a temperature transition rate of 0.1°C per s.

2.6 | Statistical analysis

Statistical analysis was performed with IBM SPSS Statistics for Windows, version 22.0. (IBM Corp.). The qRT-PCR data obtained from the 10- and 50-week-old mice were compared with the Shapiro–Wilk normality test followed by the unpaired *t*-test. A probability (*p*) value <.05 was considered statistically significant.

2.7 | Tissue preparation and histology

Mice aged 10 and 50 weeks were euthanized at by inhalation of isoflurane (FUJIFILM Wako Pure Chemical Corporation), and the mandibles were resected and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C overnight. The fixed specimens were dehydrated with a graded series of ethanol solutions and embedded in paraffin after decalcification in 10% ethylenediaminetetraacetic acid in 0.01 M phosphate buffer at 4°C for 1.5 months for the mandibles of 10-week-old mice or 2 months for those of 50-week-old mice. Afterward, the paraffin-embedded specimens were cut into serial 5-μm-thick sections, which were stained with hematoxylin and eosin (H&E).

2.8 | Immunohistochemical analysis

Paraffin-embedded sections adjacent to those stained with H&E were deparaffinized, immersed in 3.0% hydrogen peroxide in absolute methanol to block endogenous peroxidase, treated with 0.5% normal goat serum, and then incubated with rabbit anti-human MMP-3 monoclonal antibody (ab52915; Abcam) at a dilution of 1:50 or rabbit anti-human MMP-10 polyclonal antibody (ab199688; Abcam) at a dilution of 1:500 at room temperature for 2 h. After washing with phosphate-buffered saline (PBS), the sections were incubated in Histofine® Simple Stain MAX PO detection reagent (Nichirei Co.) for 30 min at room temperature, and washed again with PBS. Afterward, immunoreactivity was visualized with 3,3′-diaminobenzidine solution and the sections were counterstained with methyl green. Control sections were processed routinely except that 5% normal goat serum was used as a substitute for the antibodies.

3 | RESULTS

3.1 | Identification of metzincin proteases whose expressions increase with aging of mouse mandibles

Whole transcriptome analysis with CAGE was performed to identify DEGs in specimens collected from the 10- and 50-week-old mice. A total of 23,290 genes were detected, of which 381 were identified as DEGs (FDR >0.01). Of these, 362 were up-regulated and 19 were down-regulated at 50 weeks (Figure 1). The genes with significantly

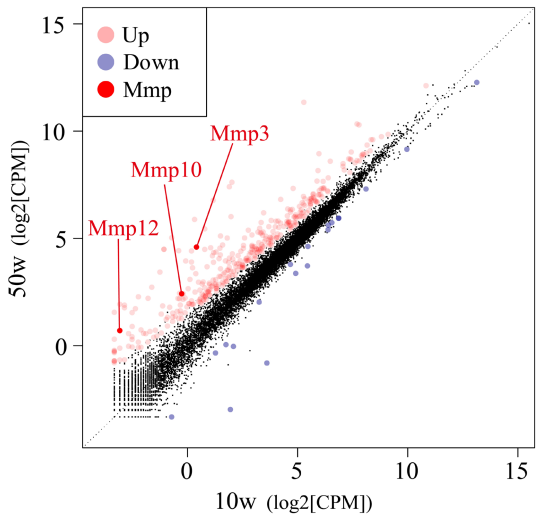

FIGURE 1 Scatter plot of DEGs. Among the 23 290 genes detected by CAGE, 362 were up-regulated (indicated by red dots) and 19 were down-regulated (indicated by blue dots) at 50 weeks (FDR >0.01). The gene expression values are presented as $\log_2$ counts per million

TABLE 1 Up-regulated DEGs at 50 weeks with logFC >2.5 and FDR <0.01

Up-regulated genes					
Gene symbol	**logFC**	**FDR**	**Gene symbol**	**logFC**	**FDR**
Defb6	8.81	1.69E-15	Psca	3.58	4.55E-12
Il13ra2	7.48	4.82E-05	ENSMUST00000103365	3.57	6.36E-06
Atp12a	6.92	3.09E-05	uc009bpu.1	3.57	1.37E-06
Cldn7	6.89	1.65E-18	Klk6	3.46	7.69E-04
Csn1s1	6.88	4.92E-05	Saa3	3.30	1.53E-09
Il23a	6.86	5.62E-05	Slco1a5	3.27	6.10E-03
Prl2c2	6.79	2.43E-04	ENSMUST00000103549	3.25	9.38E-05
uc009eln.1	6.35	1.75E-03	Tspan1	3.24	5.77E-04
1700024P16Rik	6.35	1.49E-03	ENSMUST00000103364	3.19	5.65E-04
Cd80	6.34	1.34E-03	Sprr2h	3.18	3.73E-05
Prl2c4	6.27	2.11E-03	Ptprn2	3.15	3.56E-05
ENSMUST00000103326	6.07	1.75E-05	ENSMUST00000103316	3.11	4.78E-10
uc008xzf.1	6.06	3.87E-24	ENSMUST00000103481	3.10	4.41E-03
Csf3	5.88	2.23E-14	ENSMUST00000103356	3.09	2.39E-07
Cxcl3	5.87	2.09E-29	Slurp1	3.08	3.12E-13
ENSMUST00000103369	5.84	8.23E-06	Glycam1	3.08	5.46E-03
ENSMUST00000103359	5.69	2.33E-15	Wfdc2	3.07	1.06E-18
ENSMUST00000103403	5.67	1.16E-06	Il1f5	3.03	1.88E-12
Cxcl5	5.62	4.21E-52	Vnn1	3.00	4.35E-11
Mmp10	5.62	4.58E-09	Icos	2.99	4.04E-03
U90926	5.59	7.03E-23	Krt16	2.98	2.20E-31
Sprr2d	5.59	9.92E-103	ENSMUST00000103377	2.93	1.54E-04
Prss22	5.10	1.88E-12	Mmp12	2.89	5.10E-08
ENSMUST00000103401	5.02	1.38E-05	Ggt1	2.86	2.65E-15
ENSMUST00000103475	5.01	9.30E-04	Sprr2f	2.76	2.42E-06
4732456N10Rik	4.96	5.24E-16	Nov	2.76	1.50E-17
Sprr2e	4.95	9.14E-18	Casp14	2.75	1.45E-06
Cxcl2	4.92	1.26E-08	ENSMUST00000103498	2.74	3.23E-03
Tmprss11g	4.80	8.37E-58	Lce1k	2.71	1.10E-07
ENSMUST00000133772	4.74	8.80E-05	Cxcl13	2.71	4.51E-04
Rprl1	4.60	2.35E-04	2300005B03Rik	2.71	6.08E-11
Spink12	4.40	4.81E-05	Gprc5a	2.70	8.83E-09
Cxcl1	4.35	7.18E-15	Bmpr1b	2.70	7.12E-03
Abp1	4.28	5.89E-05	ENSMUST00000103496	2.70	5.24E-03
Mmp3	4.25	8.68E-09	Krt6b	2.64	1.05E-34
ENSMUST00000103370	4.18	2.40E-15	Il1rn	2.63	6.39E-27
ENSMUST00000103445	4.17	1.01E-08	ENSMUST00000103367	2.63	9.27E-13
Prl2c5	4.11	8.80E-05	Atp13a4	2.60	2.09E-06
Nr1h4	4.10	5.69E-03	Tmprss11d	2.60	2.27E-31
Gm1045	4.10	1.27E-52	Cxcl9	2.56	1.37E-10
ENSMUST00000103325	3.83	5.69E-05	Krt10	2.53	1.62E-04
Slc6a14	3.75	2.63E-29	Krt6a	2.51	2.20E-31
ENSMUST00000103547	3.65	1.07E-04	Mfsd4	2.50	8.30E-07

increased expression at 50 weeks with log fold-change (logFC) >2.5 and FDR <0.01 are shown in Table 1. Genes for MMP-3, −10, and −12 were up-regulated, whereas none of the genes for ADAMs and ADAMTSs were differentially expressed (Table 1, Figure 1). The most significantly up-regulated gene was Mmp10 (logFC = 5.62, FDR = 4.58E-09), followed by Mmp3 (logFC = 4.25, FDR = 8.68E-09) and Mmp12 (logFC = 2.89, FDR = 5.10E-08). GO enrichment analysis was performed to characterize the biological functions of DEGs, and GO biological process terms with more than ten DEGs as the result of the analysis are listed in Table 2. Genes for MMP-3, -10, and -12 were included in the third most enriched category, "proteolytic."

3.2 | mRNA expression of MMP-3, -10, and -12 with aging of mice

The expression levels of MMP-3, -10, and -12, the ECM-degrading metzincin proteases in the mandibles of aged mice, as identified by CAGE, were further examined using qRT-PCR. The mRNA expression levels of MMP-3, -10, and -12 were compared between the specimens collected from the 10- and 50-week-old mice with correction based on the expression of GAPDH (Figure 2). The mRNA expression levels of MMP-3 and -10 were significantly increased at 50 weeks ($p = .035$ and $p < .001$, respectively). There was no significant difference in MMP-12 expression between the specimens collected from the 10- and 50-week-old mice ($p = .054$), whereas MMP-12 expression tended to increase at 50 weeks.

3.3 | Localization of MMP-10 in mouse mandibular periodontal tissues

Immunohistochemical analysis was performed to localize the MMP-10 protein. MMP-10 immunoreactivity could be observed in the cementum and along the interface between the cementum and dentin of the 10- and 50-week-old mice (Figures 3, 4 and 6). In particular, the immunoreactivity of the interface at 50 weeks was intense (Figures 3C,D and 4C,D). The surface layer of alveolar bone at the apical region of the teeth facing the periodontal ligaments of the 10- and 50-week-old mice was strongly immunoreactive for MMP-10 (Figure 3A,B,E,F). Moreover, the cementum and alveolar bone facing the periodontal ligaments showed intense immunoreactivity for MMP-10 (Figure 4A–D). Scattered immunoreactivity for MMP-10 was also observed in the periodontal ligaments (Figure 4C,D). Sparse spotted immunoreactivity could be observed in the dentin, which tended to be more intense at 50 weeks than at 10 weeks, and no specific MMP-10 immunoreactivity could be detected in the pulp at either 10 or 50 weeks (Figures 3C,D and 4C,D).

TABLE 2 GO biological process terms for DEGs with $p < .05$ and count >10

Term	Count	*p*-value
Keratinocyte differentiation	28	1.20E-28
Cell adhesion	26	1.20E-07
Proteolysis	25	1.10E-05
Peptide cross-linking	23	6.20E-26
Inflammatory response	23	1.70E-08
Immune response	19	2.10E-07
Epidermis development	18	3.10E-17
Immune system process	16	8.40E-04
Innate immune response	16	1.30E-03
Regulation of cell proliferation	15	1.10E-05
Keratinization	14	1.10E-14
Cellular response to lipopolysaccharide	14	2.00E-05
Response to lipopolysaccharide	11	9.20E-04

3.4 | Localization of MMP-3 in mouse mandibular periodontal tissues

MMP-3 immunoreactivity was observed in the cells and fibers in the periodontal ligament near the alveolar crest but not in the dentin, dental pulp, or cementum either at 10 or 50 weeks (Figures 5A–D and 6). Scattered strong immunoreactivity was also identified in the periodontal ligaments around the apical region of the teeth at both 10 and 50 weeks (Figure 5A,B,D,F).

4 | DISCUSSION

Whole transcriptome analysis of the mandible in young and aged mice showed that 362 DEGs were up-regulated and 19 were down-regulated in the aged mice. The number of up-regulated

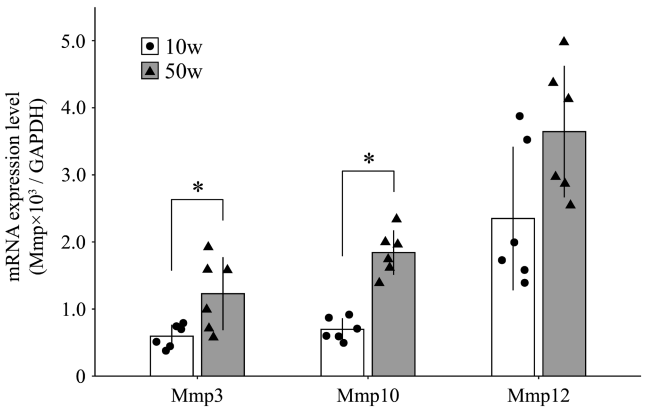

FIGURE 2 mRNA expression levels of MMP-3, -10, and -12 as determined by qRT-PCR and corrected based on GAPDH expression. Six mice aged 10 and 50 weeks were used for qRT-PCR, and RNA samples were extracted from the mandible of each mouse ($n = 6$). The reactions for each sample were performed in triplicates. The mRNA expression levels of MMP-3 and MMP-10 were significantly increased at 50 weeks ($p = .035$ and $p < .001$, respectively). MMP-12 expression tended to increase at 50 weeks

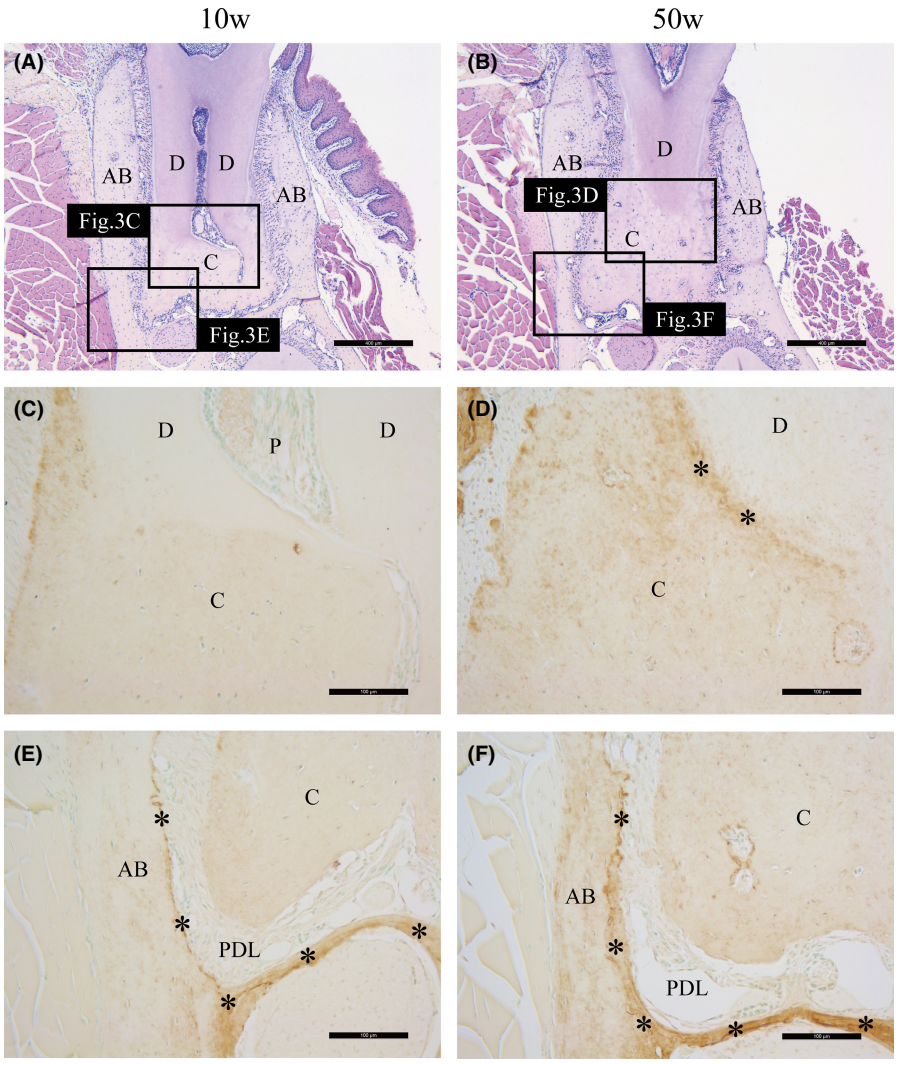

FIGURE 3 Immunohistochemical results of MMP-10 at 10 and 50 weeks are shown in (C, E) and (D, F), and the corresponding H&E-stained histological regions are indicated in (A) and (B). These H&E-stained sections are identical to (A) and (B) in Figure 5, respectively. Cementum and the interface region with dentin showed intense immunoreactivity (*) for MMP-10 at 50 weeks (D) as compared with the corresponding region at 10 weeks (C). The surface layer of alveolar bone facing the periodontal ligaments was strongly immunoreactive (*) at 10 weeks (E) and 50 weeks (F). AB, alveolar bone; C, cementum; D, dentin; P, pulp; PDL, periodontal ligaments. Scale bars = 400 μm in (A, B) and 100 μm in (C–F)

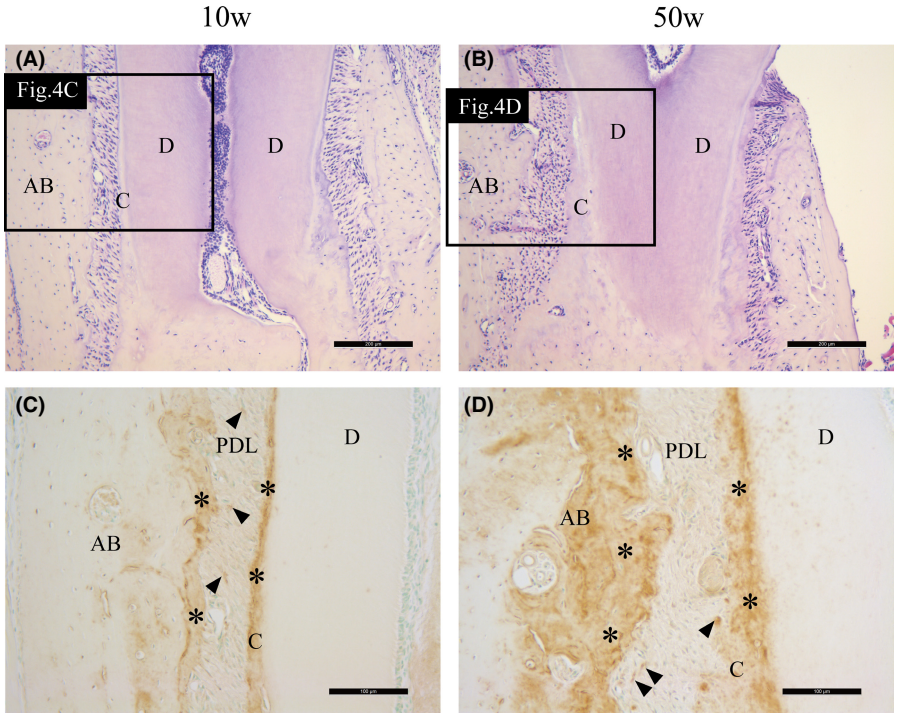

FIGURE 4 Immunohistochemical results of MMP-10 at 10 and 50 weeks are shown in (C) and (D), and the corresponding H&E-stained histological regions are indicated in (A) and (B). Cementum and alveolar bone facing the periodontal ligaments showed strong immunoreactivity (*) and the intensity was more widespread at 50 weeks (D) than at 10 weeks (C). Scattered immunoreactivity of MMP-10 (arrowheads) in the periodontal ligaments. AB, alveolar bone; C, cementum; D, dentin; PDL, periodontal ligaments. Scale bars = 200 μm in (A, B) and 100 μm in (C, D)

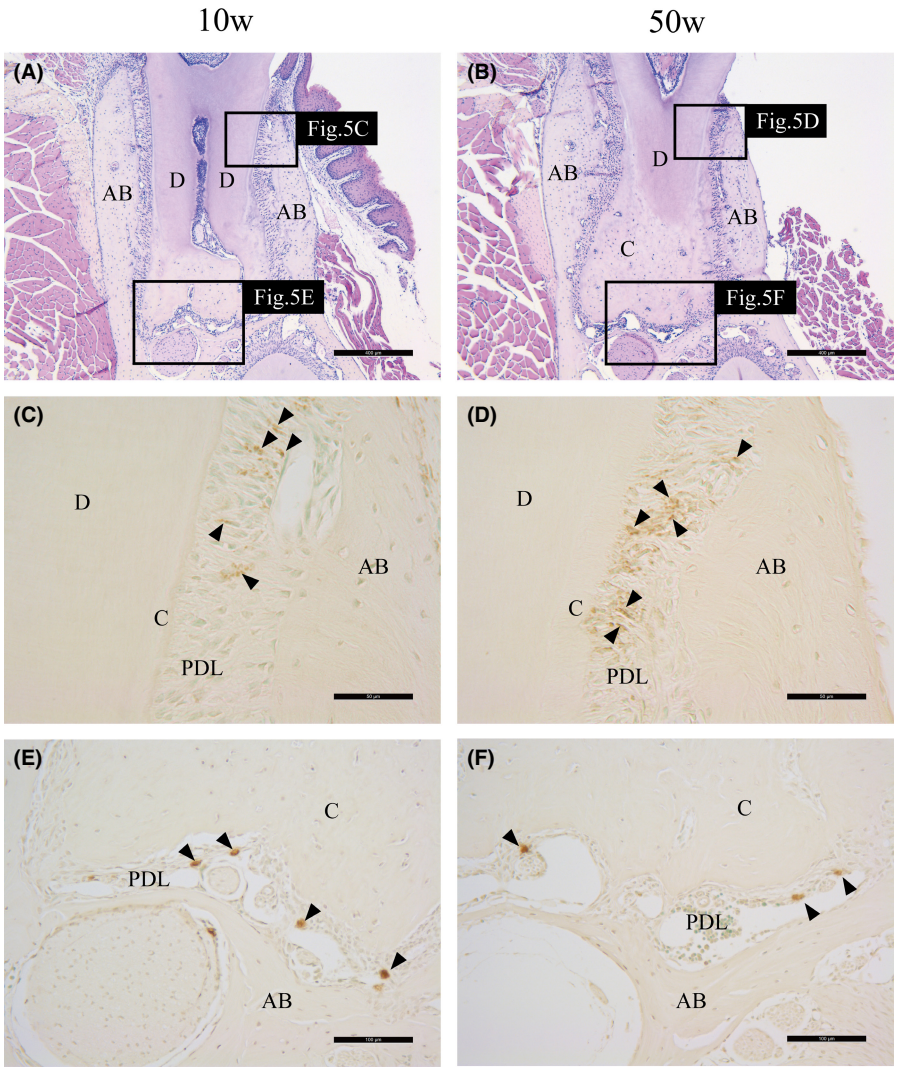

FIGURE 5 Immunohistochemical results of MMP-3 at 10 and 50 weeks are shown in (C, E) and (D, F), and the corresponding H&E-stained histological regions are indicated in (A) and (B). These H&E-stained sections were used for (A) and (B) in Figure 3. Periodontal ligaments showed positive immunoreactivity (arrowheads) near the alveolar crest (C, D) and scattered strong immunoreactivity (arrowheads) around the apical region of the teeth (E, F) at both 10 and 50 weeks. AB, alveolar bone; C, cementum; D, dentin; PDL, periodontal ligaments. Scale bars = 400 μm in (A, B); 50 μm in (C, D); and 100 μm in (E, F)

DEGs was expected to be lower that of down-regulated DEGs in the periodontal tissues of aged mice due to age-related decreases in physiological function, but the opposite was observed. Among the up-regulated DEGs, three (Mmp3, Mmp10, and Mmp12) encoded the major ECM proteases in the metzincin protease superfamily. A previous study demonstrated that mRNA expression of MMP-1, -3, -10, and -12 was increased in senescent human and mouse fibroblasts in vitro, which was in agreement with the results of the present study.[18] Furthermore, the potential candidates associated with aging in periodontal tissues were narrowed down to MMP-3 and -10 by qRT-PCR. MMP-3 and -10, also known as stromelysin-1 and -2, respectively, have wide substrate specificity and are capable of cleaving a variety of ECM proteins, including proteoglycans, Type IV collagen, laminin, and fibronectin.[4] Hence, these MMPs are defined as senescence-associated secretory phenotype factors[18] and it is likely that both are involved in aging of periodontal tissues.

Gene ontology enrichment analysis was performed to investigate the functional categories of DEGs (Table 2). The third most enriched GO term was proteolytic, where Mmp3, 10, and 12 were included, and the fifth one was inflammatory response, including proinflammatory cytokines such as IL1 family members (IL1B, IL1F6, IL1F8, IL1F9, and IL23A) and chemokines (CXCL1, CXCL2, CXCL3, CXCL5, CXCL9, and CXCL13). A previous study reported that the gingivae of aged mice, as opposed to young mice, were characterized by increased inflammation and elevated expression of proinflammatory cytokines, such as interleukin-1β and tumor necrosis factor-α,[19] suggesting that natural aging leads to elevated expression of inflammatory factors. Other studies reported that inflammatory stimulation up-regulated the expression levels of MMP-3 and -10 in cultured human periodontal ligament cells. Therefore, in the present study, natural aging was presumed to activate inflammatory mediators in periodontal tissues, which then induced the expression of MMP-3 and -10. The GO enrichment analysis also indicated that the DEGs were mainly enriched in GO terms related to epithelial keratinization and immune response. Considering that the skin was removed from the sample for CAGE analysis, keratinization might occur in the gingival mucosa. Based on these results, aging might promote gingival keratinization and immune responses to chronic inflammation. The results of this study showed that MMP-10 immunoreactivity was localized exclusively in the cementum and alveolar bone adjacent to the periodontal ligament and

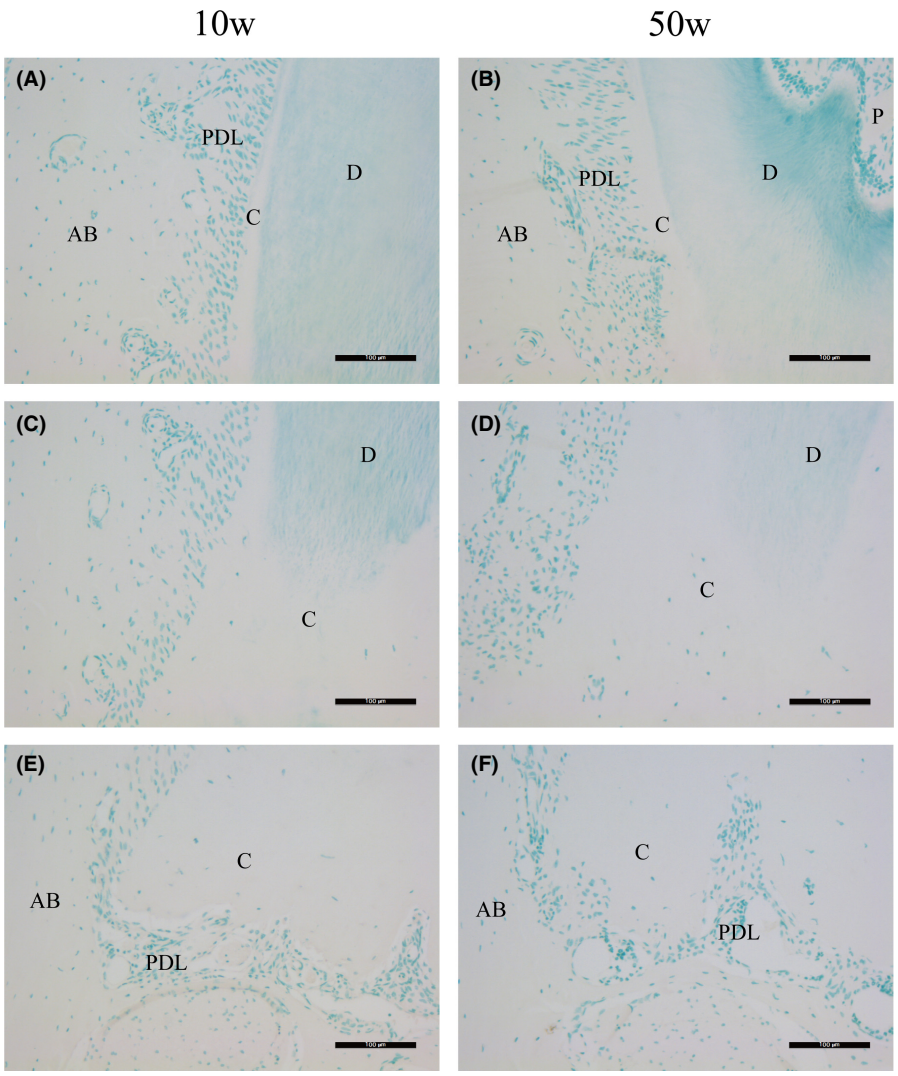

FIGURE 6 Negative controls for immunohistochemistry at 10 and 50 weeks are shown in (A, C, E) and (B, D, F), respectively. The identical section was used for (A) and (C). (A) shows the middle part of the tooth and (C) shows the area closer to the root apex. AB, alveolar bone; C, cementum; D, dentin; P, pulp; PDL, periodontal ligaments. Scale bars = 100 μm

was stronger and broader in aged mice than young mice. A previous study reported that MMP-10 was expressed in osteoblasts at sites of new bone formation in human neonatal ribs.[20] MMP-10 has also been shown to enhance BMP-2-promoted bone repair in a murine model of a calvarium bone defect.[21] These findings suggest that MMP-10 is expressed at sites where bone remodeling is active, such as bone development and wound healing. The immunoreactive areas of MMP-10 observed in this study, that is, alveolar bone and cementum that include Sharpey's fibers, may also be actively remodeled with aging. In fact, it has been reported that Sharpey's fibers are less ordered with decreases in number, length, and birefringence with aging.[22] In consideration of age-related changes, it is no wonder that the surrounding bone and cementum could be remodeled with aging.

The immunohistochemical analysis did not detect MMP-3 proteins in the dentin of either young or aged mice. However, previous studies reported the presence of MMP-3 in dentin, which had decreased after demineralization.[23–25] Thus, it is possible that some MMP-3 protein was lost in this study because the specimens were demineralized for more than a month prior to immunohistochemical analysis.

In a previous study, the levels of MMP-3 and MMP-10, as well as their tissue inhibitors, TIMP-1 and TIMP-2, in healthy human serum were compared among age groups and between genders.[26] The study reported that the serum levels of MMP-3 and TIMP-1 were positively correlated with age, while those of MMP-10 and TIMP-2 were negatively correlated with age, and that the serum levels of MMP-3, TIMP-1, and TIMP-2 were significantly higher in males than in females, whereas no significant difference was observed in the serum level of MMP-10. Interestingly, in our study, the expression of MMP-10 as well as MMP-3 increased with age in male mice. Due to the differences among animal species and analysis methods, it is difficult to compare the findings of the aforementioned study with ours. However, these findings indicate that an increase or decrease in the expression of circulating MMPs does not correlate with that of local MMPs, suggesting that each local MMP plays a site-specific role.

In this study, the qRT-PCR data indicated that MMP-12 mRNA expression tended to be higher in aged mice than young mice, although this difference was not statistically significant. A previous study reported that osteoclasts expressed MMP-12 in addition to MMP-9 and -14, and that MMP-12 was capable of cleaving the major bone matrix proteins, including osteopontin and bone sialoprotein.[27]

Although MMP-12 localization was not examined, it might be expressed in osteoclasts of the mandibular bone.

Our study demonstrated that the expression of MMP-3 and -10 increases with age and that these MMPs have different localization in the mandible. These results suggest that elevated expression of MMPs in a site-specific manner promotes degradation of the ECM, which contributes to aging of periodontal tissues. However, further investigations are required to clarify the roles of MMP-3 and -10 during the aging process and to provide more information to better understand the aging of periodontal tissue.

ACKNOWLEDGEMENTS

We thank Mr. Yasuto Mikami, Division of Craniofacial Development and Tissue Biology, Tohoku University Graduate School of Dentistry, for his assistance. We also thank K. K. DNAFORM for their technical support with CAGE. This work was financially supported by JSPS KAKENHI (Grant nos. JP18K09517, JP18K09760, and JP18K17110).

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

REFERENCES

1. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. *Nat Rev Mol Cell Biol*. 2014;15(12):786-801.
2. Hosseini F, Hassannia H, Mahdian-Shakib A, et al. Targeting of crosstalk between tumor and tumor microenvironment by beta-D mannuronic acid (M2000) in murine breast cancer model. *Cancer Med*. 2017;6(3):640-650.
3. Gill SE, Parks WC. Metalloproteinases and their inhibitors: regulators of wound healing. *Int J Biochem Cell Biol*. 2008;40(6-7):1334-1347.
4. Duffy MJ, Maguire TM, Hill A, McDermott E, O'Higgins N. Metalloproteinases: role in breast carcinogenesis, invasion and metastasis. *Breast Cancer Res*. 2000;2(4):252-257.
5. Ra HJ, Parks WC. Control of matrix metalloproteinase catalytic activity. *Matrix Biol*. 2007;26(8):587-596.
6. Kelwick R, Desanlis I, Wheeler GN, Edwards DR. The ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) family. *Genome Biol*. 2015;16:113.
7. Weber S, Saftig P. Ectodomain shedding and ADAMs in development. *Development*. 2012;139(20):3693-3709.
8. Qin Z, Balimunkwe RM, Quan T. Age-related reduction of dermal fibroblast size upregulates multiple matrix metalloproteinases as observed in aged human skin in vivo. *Br J Dermatol*. 2017;177(5):1337-1348.
9. Quan T, Shao Y, He T, Voorhees JJ, Fisher GJ. Reduced expression of connective tissue growth factor (CTGF/CCN2) mediates collagen loss in chronologically aged human skin. *J Invest Dermatol*. 2010;130(2):415-424.
10. Xia W, Hammerberg C, Li Y, et al. Expression of catalytically active matrix metalloproteinase-1 in dermal fibroblasts induces collagen fragmentation and functional alterations that resemble aged human skin. *Aging Cell*. 2013;12(4):661-671.
11. Murata M, Nishiyori-Sueki H, Kojima-Ishiyama M, Carninci P, Hayashizaki Y, Itoh M. Detecting expressed genes using CAGE. *Methods Mol Biol*. 2014;1164:67-85.
12. Kawaji H, Lizio M, Itoh M, et al. Comparison of CAGE and RNA-seq transcriptome profiling using clonally amplified and single-molecule next-generation sequencing. *Genome Res*. 2014;24(4):708-717.
13. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. *Nat Methods*. 2008;5(7):621-628.
14. Cloonan N, Forrest AR, Kolle G, et al. Stem cell transcriptome profiling via massive-scale mRNA sequencing. *Nat Methods*. 2008;5(7):613-619.
15. Carninci P, Kvam C, Kitamura A, et al. High-efficiency full-length cDNA cloning by biotinylated CAP trapper. *Genomics*. 1996;37(3):327-336.
16. Dobin A, Davis CA, Schlesinger F, et al. STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*. 2013;29(1):15-21.
17. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*. 2010;26(1):139-140.
18. Coppe JP, Patil CK, Rodier F, et al. A human-like senescence-associated secretory phenotype is conserved in mouse cells dependent on physiological oxygen. *PLoS One*. 2010;5(2):e9188.
19. Liang S, Hosur KB, Domon H, Hajishengallis G. Periodontal inflammation and bone loss in aged mice. *J Periodontal Res*. 2010;45(4):574-578.
20. Bord S, Horner A, Hembry RM, Compston JE. Stromelysin-1 (MMP-3) and stromelysin-2 (MMP-10) expression in developing human bone: potential roles in skeletal development. *Bone*. 1998;23(1):7-12.
21. Reyes R, Rodriguez JA, Orbe J, Arnau MR, Evora C, Delgado A. Combined sustained release of BMP2 and MMP10 accelerates bone formation and mineralization of calvaria critical size defect in mice. *Drug Deliv*. 2018;25(1):750-756.
22. Aaron JE. Periosteal Sharpey's fibers: a novel bone matrix regulatory system? *Front Endocrinol (Lausanne)*. 2012;3:98.
23. Hall R, Septier D, Embery G, Goldberg M. Stromelysin-1 (MMP-3) in forming enamel and predentine in rat incisor-coordinated distribution with proteoglycans suggests a functional role. *Histochem J*. 1999;31(12):761-770.
24. Boukpessi T, Menashi S, Camoin L, Tencate JM, Goldberg M, Chaussain-Miller C. The effect of stromelysin-1 (MMP-3) on non-collagenous extracellular matrix proteins of demineralized dentin and the adhesive properties of restorative resins. *Biomaterials*. 2008;29(33):4367-4373.
25. Mazzoni A, Papa V, Nato F, et al. Immunohistochemical and biochemical assay of MMP-3 in human dentine. *J Dent*. 2011;39(3):231-237.
26. Komosinska-Vassev K, Olczyk P, Winsz-Szczotka K, Kuznik-Trocha K, Klimek K, Olczyk K. Age- and gender-dependent changes in connective tissue remodeling: physiological differences in circulating MMP-3, MMP-10, TIMP-1 and TIMP-2 level. *Gerontology*. 2011;57(1):44-52.
27. Hou P, Troen T, Ovejero MC, et al. Matrix metalloproteinase-12 (MMP-12) in osteoclasts: new lesson on the involvement of MMPs in bone resorption. *Bone*. 2004;34(1):37-47.

How to cite this article: Kageyama Y, Nakamura M, Igari Y, et al. Expression of matrix metalloproteinase-3 and -10 is up-regulated in the periodontal tissues of aged mice. *J Periodont Res*. 2022;57:733–741. doi:10.1111/jre.12996