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Increased expression of extracellular matrix metalloproteinase inducer is associated with matrix metalloproteinase-1 and -2 in gingival tissues from patients with periodontitis

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Background and Objective: Extracellular matrix metalloproteinase inducer (EMMPRIN), an immunoglobulin-like cell surface glycoprotein, could promote collagenolytic balance in favor of the expression and activation of matrix metalloproteinases (MMPs). This study was to investigate the expression of EMMPRIN in gingival tissues from different periodontal conditions and to correlate it with the production of MMP-1 and MMP-2.

Material and Methods: Gingival biopsies were collected from 15 patients with untreated advanced chronic periodontitis and 15 patients with aggressive periodontitis (AgP). The control group consisted of 12 subjects diagnosed either as periodontally healthy individuals or as individuals with a gingival index of one (H/G1). The peptides and mRNA of EMMPRIN, MMP-1 and MMP-2 were detected by immunohistochemistry and semi-quantitative reverse transcriptase-polymerase chain reaction, respectively.

Results: The expression of EMMPRIN, MMP-1 and MMP-2 peptides in periodontally healthy tissues was mainly confined to the gingival epithelium. The EMMPRIN was strongly expressed in the cell membrane of the basal layer. Immunoreactivity for EMMPRIN was more intensive and more widespread in periodontitis, extended from the epithelial layers to the underlying connective tissues, and was essential in both inflammatory and fibroblast-like cells. In addition, MMP-1 and MMP-2 showed the same localized expression. The chronic periodontitis group had a significantly higher mRNA expression of EMMPRIN and MMP-2 compared with the H/G1 subjects ($p < 0.05$). Production of MMP-1 and MMP-2 by gingival tissues was correlated with the mRNA level of EMMPRIN ($r = 0.463$, $p = 0.013$ for MMP-1 and $r = 0.404$, $p = 0.033$ for MMP-2).

Conclusion: The expression of EMMPRIN in human normal and diseased gingiva might contribute to periodontal physiological and pathological processes; moreover, its increased production might be associated with MMP-1 and MMP-2 expression.

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Periodontal disease is characterized by periodontal attachment loss and bone destruction. Although periodontitis is initiated by specific subgingival microbiota, matrix metalloproteinases (MMPs), mainly generated by the host, play a major role in connective tissue breakdown (1). The extracellular matrix (ECM) of periodontal tissues consists principally of collagens: type I is the major component, along with types III, IV, V and VI. Non-collagen proteins such as elastin, fibronectin, laminin, tenascin, thrombospondin, entactin and proteoglycans are also present (2). Most of these matrix constituents can be degraded by MMP-1, MMP-2, MMP-3, MMP-7 and MMP-11, which are expressed by resident cells that are mostly fibroblast cell lines (3). Several members of the MMP family have been proven to be involved in periodontal tissue destruction, including MMP-1, MMP-2, MMP-3, MMP-8, MMP-9 and MMP-13 (3–6). Clearly, expression of MMPs involves complex interactions between cell surface receptors and ECM, cytokines and growth factors. The activity of MMPs is under control of several regulatory mechanisms, including the upregulation of expression by inducers (3,5,7).

Extracellular matrix metalloproteinase inducer (EMMPRIN, also known as CD147 or basigin), a member of the immunoglobulin superfamily and formerly called tumor cell-derived collagenase stimulatory factor, is a 57 kDa transmembrane glycoprotein (8). It has a broad tissue distribution and has been shown to be expressed in numerous normal, inflammatory and neoplastic cells (8–13). It has been reported that EMMPRIN is enriched in a variety of carcinomas and contributes to tumor invasion and metastasis, not only stimulating nearby fibroblasts to secrete an increased amount of MMPs but also upregulating the expression of MMPs to degrade ECM (14–17). Enhancement of the production of MMPs by EMMPRIN might also be related to the etiology of rheumatoid arthritis (18,19).

It has been demonstrated that EMMPRIN could stimulate the production of MMP-1, MMP-2 and MMP-3, while it showed no effect on tissue inhibitor of matrix metalloproteinases (14,18–20). Therefore, EMMPRIN could promote the collagenolytic balance, contributing to both release and upregulation of MMPs. The ability of EMMPRIN to stimulate production of MMPs suggests that this molecule could be related to several normal and pathological tissue modulatory processes, as well as tissue remodeling (14). The presence of soluble forms of EMMPRIN in the gingival crevicular fluid (GCF) of patients with different periodontal diseases has previously been studied. Increased EMMPRIN levels in GCF are associated with enhanced severity of periodontal inflammation, indicating that these molecules can participate in the regulation of periodontal disease progression (21). However, the contribution of EMMPRIN and MMPs in the extracellular matrix destruction of periodontal disease is still unknown.

In the present study, we investigated the expression of EMMPRIN in gingival tissues from different periodontal conditions and analyzed the correlation between the mRNA level of EMMPRIN and that of MMP-1 and MMP-2.

Material and methods

Study population

Thirty Chinese patients (21–51 years old) were selected for the study. Fifteen patients (mean age 44.8 ± 5.1 years; 36–51 years old) had untreated advanced chronic periodontitis and 15 patients (mean age 33.3 ± 5.3 years; 21–38 years old) had been diagnosed with aggressive periodontitis (AgP) according to criteria established in 1999 (22).

The control group consisted of 12 subjects with a mean age of 25.9 ± 8.9 years (12–56 years old) clinically diagnosed either as periodontally healthy individuals or as individuals with a gingival index of one (H/G1).

Exclusion criteria included diabetes, hypertension, history of vascular diseases or other systemic diseases, drugs taken during the 3 months before tooth extraction, smoking, and oral diseases other than chronic periodontitis.

The purposes and procedures of the study were explained, and written informed consent obtained from all subjects according to the Declaration of Helsinki. In addition, the protocols were reviewed and approved by the Ethics and Investigation Committee of the School & Hospital of Stomatology, Wuhan University.

Collection of samples

In the experimental group, biopsies were harvested during extraction of teeth with a poor periodontal prognosis. All biopsies were taken from sites with bleeding on probing and probing depths ≥ 6 mm. The control group biopsies were collected during crown lengthening procedures or during extractions for orthodontic purposes. The biopsies contained oral epithelium, gingival connective tissue and sulcular/junctional epithelium.

Adjacent to the selected sites, the following clinical parameters were measured at the time of gingival biopsy: plaque index (Silness & Loe, 1964), gingival index (Loe & Silness, 1963), sulcus bleeding index (Mazza, 1981) and probing depth (23–25). All measurements were performed at six sites per tooth and were carried out by the same investigator to minimize variability.

Tissue samples in each group were divided into two parts: one-third of the specimens each group were immediately fixed in 4% paraformaldehyde solution for 24 h, dehydrated, embedded in paraffin and used for immunohistochemistry, while two-thirds of the specimens each group were stored at −80°C until used for total RNA extraction.

Immunohistochemistry

Serial paraffin sections of biopsies were cut 4 μm thick, and immunohistochemistry was performed using a three-step immunoperoxidase procedure. First, the tissue section was deparaffinized, rehydrated and incubated with normal horse serum diluted to 1:50 in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (Zhongshan, Beijing, China) for 20 min at room temperature to block non-specific binding. Subsequently, the

sections were incubated overnight at 4°C with rabbit anti-human EMMPRIN polyclonal antibody (3 μg/mL, prediluted; Zymed, San Francisco, CA, USA), MMP-1 or MMP-2 antibody (5 μg/mL, Lab Vision Corp., Fremont, CA, USA). Sections were then incubated with biotinylated mouse anti-rabbit immunoglobulin G (Zhongshan) for 30 min at 37°C, followed by incubation with avidin–biotin–peroxidase complex (Zhongshan) for 30 min at 37°C with the addition of 3,3′-diaminobenzidine tetrahydrochloride (Zhongshan) for 5 min at room temperature. Between the reaction steps, the sections were each washed with PBS three times for 5 min. Finally, the slides were counterstained with Mayer's hematoxylin, dehydrated in a graded ethanol series, cleared in xylene and coverslipped. As a negative control, normal rabbit immunoglobulin G with irrelevant specificity was used instead of the primary antibody at the same concentration.

Semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was extracted from gingival tissues using TRIzol™ reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Purified RNA was resuspended in autoclaved diethyl pyrocarbonate-treated water, and the concentration was measured by ultraviolet spectrophotometry (UV-2100PC; Shimadzu Corporation, Kyoto, Japan) with an absorbance wavelength of 260 and 280 nm. The RNA concentrations were determined from absorbance at 260 nm, and purity was assessed by the ratio of optical density at 260 and 280 nm.

A RT-PCR assay was performed to measure the mRNA levels of EMMPRIN, MMP-1 and MMP-2. One microgram of total RNA was used in 20 μL volume reverse transcription (RT) reactions using TOYOBO ReverTra Ace-α (TOYOBO, Osaka, Japan) following the manufacturer's instructions. Synthesized cDNA was then used for PCR analysis. The PCR analyses were performed in 25 μL volume, containing 1 μL of cDNA, 2.5 μL of 10× PCR buffer, 50 nM of primers and 1 unit of Taq polymerase (Takara Biotechnology Co. Ltd, Dalian, China). β-actin gene expression served as an endogenous control. Primer sequence, annealing temperature and thermal cycles are listed in Table 1. All RT-PCR reactions were performed in duplicate.

The PCR products were visualized on 1.5% agarose gel with ethidium bromide staining and analyzed densitometrically with a GeneGenius Gel Imaging System (Syngene, Cambridge, UK). The intensity of the bands was quantified under ultraviolet light and normalized with respect to those for β-actin mRNA.

Statistical analysis

The mRNA level differences between EMMPRIN, MMP-1 and MMP-2 were compared among the three groups using the Kruskal–Wallis test. The subject was used as the unit of measurement. All values are expressed as the means ± SD. *Post hoc* two-group comparisons were assessed with Bonferroni-corrected Mann–Whitney *U*-tests if there were significant differences ($p < 0.05$). Analysis of statistical correlation was performed using Pearson's correlation coefficient. All data analysis was performed using SPSS 13.0 statistical package (SPSS Inc., Chicago, IL, USA).

Results

Clinical assessments

The mean clinical data for the sampling areas are listed in Table 2. As expected, significantly higher plaque index, gingival index and bleeding on probing index scores, as well as probing depth measurement, were noted in chronic periodontitis and AgP groups compared with the H/G1 group ($p < 0.001$). In contrast, chronic periodontitis and AgP groups had similar clinical data ($p > 0.05$).

Immunohistochemistry

Both EMMPRIN and MMP-2 peptides were detected in all gingival specimens. The MMP-1 peptide was detected in all chronic periodontitis and AgP patients and in two of four H/G1 patients (Table 3).

The expression of EMMPRIN peptides in periodontal healthy tissues was mainly confined to the gingival epithelium and strongly expressed in the basal layer (Fig. 1A). Moderate or weak immunoreactivity was detected in the spinous layers and spottily weak expression in the papillary layers subjacent to the epithelium was also apparent. Two types of expressing cells were observed: gingival epithelial cells and fibroblast cells (Fig. 1B), with the former exhibiting strong expression in the cell membrane and the latter weak expression in the cytoplasm. Some

Table 1. Primers used for RT-PCR

Gene	Primer	Denaturing temperature (°C) /duration (s)	Annealing temperature (°C) /duration (s)	Lengthening temperature (°C) /duration (s)	Number of Cycles	Product length (bp)	GenBank accession number
EMMPRIN	F: GGC CAG AAA ACG GAG TTC AA R: GCG CTT CTC GTA GAA GA	94/60	57.5/60	72/60	32	492	NM_001728.2
MMP-1	F: CAT CCA AGC CAT ATA TGG ACG TTC R: TCT GGA GAG TCA AAA TTC TCT TCG T	94/60	59.5/60	72/90	32	611	NM_002421.2
MMP-2	F: ATG ACA GCT GCA CCA CTG AG R: CTC CTG AAT GCC CTT GAT GT	94/45	59/50	72/60	34	425	NM_004530.2
β-actin	F: GCG AGA AGA TGA CCC AGA TCA TGT T R: GCT TCT CCT TAA TGT CAC GCA AGA T	94/30	62/30	72/30	30	300	NM_001101.2

variations in the expression of MMP-1 and MMP-2 were observed among subjects, but the expression areas were the same as for EMMPRIN, and mainly confined to the epithelium (Fig. 1D,G). Expression of MMP-1 in the spinous and granular layers as well as papillary layer nearby was moderate (Fig. 1E), while in the sequential section, MMP-2 was weakly expressed and spotty in the basal and papillary layers (Fig. 1H). Both MMP-1 and MMP-2 were sometimes weakly expressed in all layers of the epithelium in other samples. However, all three peptides were not observed in the reticular layer of connective tissues (Fig. 1C,F,I).

Immunoreactivity for EMMPRIN was more intense and more widespread in the periodontitis samples (Fig. 2A). There were no significant differences in the peptide expression pattern and extent between the chronic periodontitis patients and the AgP patients. The EMMPRIN was still strongly expressed in the basal layer, extending to all layers of the epithelium and subepithelial connective tissues (Fig. 2B), moderately expressed in the spinous and granular layers, and weakly expressed in the keratinized layer. The underlying connective tissues displayed strong and intense EMMPRIN-stained inflammatory cells (prominent lymphocyte infiltration) as well as spindle-shaped fibroblast-like cells in the areas infiltrated by inflammatory cells (Figs 2C and 3A). The fibroblast-like cells in these areas also weakly expressed EMMPRIN (Fig. 3B). EMMPRIN was detected within the cytoplasm of the inflammatory and fibroblast-like cells (Fig. 3C). Significant expression also appeared in intercellular spaces and blood vessels (Fig. 3C,D). The sequential sections labeled for MMP-1 (Fig. 2E) and MMP-2 (Fig. 2H) showed sparse and moderate expression in all layers of the epithelium, and a common distribution with EMMPRIN throughout the subepithelial connective tissues. In addition, immunoreactivity was strong and intense in the areas infiltrated by inflammatory cells (Fig. 2F,I).

Semi-quantitative RT-PCR

The expression of EMMPRIN, MMP-1, MMP-2 and β-actin mRNA in the diseased and healthy groups of gingival tissues is presented in Fig. 4. mRNA for both EMMPRIN and MMP-2 was detected in all the samples, whereas MMP-1 mRNA was detected in only two of 8 H/G1 patients, seven of 10 chronic periodontitis patients and eight of 10 AgP patients. The frequencies of expression for EMMPEIN, MMP-1 and MMP-2 mRNA are shown in Table 3.

The relative values of EMMPRIN, MMP-1 and MMP-2 mRNA in the diseased and healthy gingival tissues are shown in Table 4. There was a significant difference in the mRNA levels of EMMPRIN and MMP-2 among study groups ($p = 0.042$ for EMMPRIN and $p = 0.016$ for MMP-2). The chronic periodontitis group had significantly higher mRNA levels of EMMPRIN and MMP-2 compared with that of the H/G1 group ($p = 0.037$ for EMMPRIN and $p = 0.016$ for MMP-2).

Regarding MMP-1 mRNA expression, there was no significant difference among study groups ($p = 0.213$). The chronic periodontitis group tended to have higher MMP-1 mRNA levels compared with the H/G1 group, although this difference did not achieve statistical significance. The mRNA expression frequency for the AgP group was higher compared with that for H/G1 group (Table 3).

Correlation between EMMPRIN and expression of MMP-1 and MMP-2

The relationship between the mRNA expression of EMMPRIN and the production of MMP-1 and MMP-2 was analyzed by means of Pearson's correlation coefficient. The increased EMMPRIN mRNA level was significantly correlated with MMP-1 and MMP-2 in periodontal disease ($r = 0.463$, $p = 0.013$ for MMP-1 and $r = 0.404$, $p = 0.033$ for MMP-2; Fig. 5).

Table 2. Clinical parameters of the sampling sites in study groups

	H/G1	Chronic periodontitis	AgP
Number of patients	12	15	15
Age (years)	25.9 ± 8.9	44.8 ± 5.1	33.3 ± 5.3
Plaque index	0.52 ± 0.18	2.15 ± 0.67*	1.40 ± 0.97*
Gingival index	0.75 ± 0.46	2.35 ± 0.58*	2.20 ± 0.79*
Sulcus bleeding index	0	3.40 ± 0.52*	3.00 ± 1.56*
Probing depth (mm)	1.25 ± 1.04	6.87 ± 1.41*	7.29 ± 1.84*

All values are expressed as means ± SD. Significant difference among groups was assessed by Kruskal–Wallis test ($p < 0.01$). * Significant difference chronic periodontitis and AgP groups from H/G1 group. (Mann–Whitney *U*-test, $p < 0.01$).

Table 3. Frequencies of peptides and mRNA expression determined by immunohistochemistry and RT-PCR

	Immunohistochemistry						RT-PCR					
	H/G1 (n = 4)		Chronic periodontitis (n = 5)		AgP (n = 5)		H/G1 (n = 8)		Chronic Periodontitis (n = 10)		AgP (n = 10)	
EMMPRIN	4/4	100%	5/5	100%	5/5	100%	8/8	100%	10/10	100%	10/10	100%
MMP-1	2/4	50%	5/5	100%	5/5	100%	2/8	25%	7/10	70%	8/10	80%
MMP-2	4/4	100%	5/5	100%	5/5	100%	8/8	100%	10/10	100%	10/10	100%

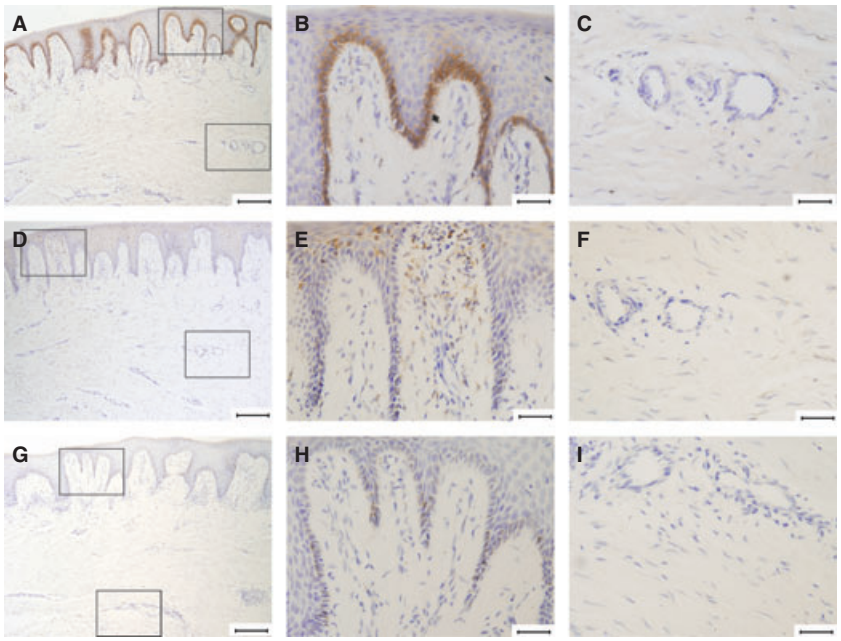

Fig. 1. The expression of EMMPRIN, MMP-1 and MMP-2 peptides in a human healthy gingival biopsy. (A) The expression of EMMPRIN is very strong in the basal layer. (B) EMMPRIN is mainly expressed in gingival epithelial cell membrane. There is weak expression in the spinous layer and sparse expression in the papillary layer subjacent to the epithelium. (D and G) The expression areas of MMP-1 and MMP-2 are mainly confined to epithelium. (E) Expression of MMP-1 in the spinous and granular layers as well as papillary layer is moderate. (H) MMP-2 is expressed weakly and spottily in the basal layer and papillary layer. EMMPRIN (C), MMP-1 (F) and MMP-2 peptides (I) show no immunoreactivity in the reticular layer of connective tissues. The boxed areas in (A) are expanded in (B) and (C), the boxed areas in (D) are expanded in (E) and (F), and the boxed areas in (G) are expanded in (H) and (I). (Original magnification 100 in A, D and G, bars = 100 μm; and 400 in B, C, E, F, H and I, bars = 25 μm).

Discussion

EMMPRIN is expressed in very low levels in most normal human tissues, including epidermal keratinocytes, retinal pigment epithelium, breast lobules and ductules, ovarian surface epithelium and colorectal epithelia, which suggests that EMMPRIN might play a physiological role in tissue remodeling by inducing stromal MMPs (8,14,26–29). In this study, we clearly showed the presence of EMMPRIN in human gingival tissues from both healthy and periodontitis subjects at the mRNA and protein levels for the first time. We found the EMMPRIN peptide mainly located in the basal layer of clinically healthy gingival tissues. In the gingival epithelium, basal cells are the most metabolically active cells and are responsible for epithelial renewal and exchange of materials with the subepithelial tissues. The integrity of the ECM and basement membrane is important in maintaining the stability of periodontal tissues (30–32). Tissue integrity is maintained by a balance between matrix degradation and production, which is regulated to a large extent through the action of MMPs in both normal tissue remodeling and pathological states (33,34). The unique expression patterns of EMMPRIN in gingival tissues indicate that EMMPRIN might play an important role in the turnover of connective tissues in both physiological and pathological conditions.

We found more intensive and more widespread EMMPRIN immunoreactivity in periodintitis, which extended from the basal layer of the gingival epithelium to all layers and the underlying connective tissues. The serial sections of biopsies showed a common distribution of EMMPRIN with MMP-1, MMP-2 in the connective tissues, where EMMPRIN was expressed in the cytoplasm of inflammatory cells and fibroblast-like cells, as well as in the intercellular spaces. Previous studies have shown that EMMPRIN in activated lymphocytes and monocytes could contribute to the elevated levels of MMPs found in rheumatoid arthritis, which shows a strong association with periodontal disease as well as similarities in the pathogenic mechanisms of tissue destruction (18,19,31). It is now recognized that during active periodontitis, degradation of gingival connective tissues, mainly collagen, is due in part to the expression of MMPs *in situ* by inflammatory cells (monocytes, macrophages, lymphocytes and polymorphonuclear cells) and resident cells (fibroblasts, epithelial cells and endothelial cells; 6,35,36). Therefore, we suggest that EMMPRIN, through stimulating MMP production, could upregulate the proteolytic activity released by the recruited inflammatory and resident cells, thereby participating in periodontal disease progression.

The local expression of EMMPRIN peptides in human gingival tissues was confirmed by detection of EMMPRIN mRNA. We found higher mRNA levels of EMMPRIN and MMP-2 in the chronic periodontitis group compared with H/G1 subjects. These results are consistent with previous reports, in which increased EMMPRIN levels in human GCF were associated with an enhanced severity of periodontal tissue inflammation (21). EMMPRIN was initially isolated by Biswas *et al.* from the surface of tumor cells (8). In tumor cells, EMMPRIN has two forms: membrane-associated EMMPRIN and soluble EMMPRIN. The former may stimulate the production of MMPs by cells adjacent to the tumor cells; however, the latter may stimulate and activate the expression of MMPs by cells in the absence of physical contact with EMMPRIN (37,38). A previous study has demonstrated the presence of soluble forms of EMMPRIN in the GCF of patients

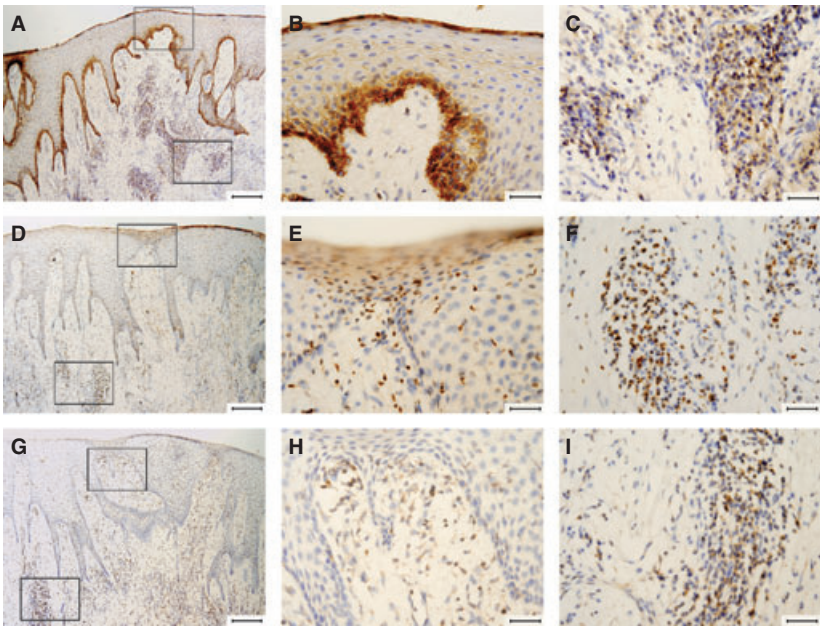

Fig. 2. The sequential sections show peptide expression of EMMPRIN, MMP-1 and MMP-2 in gingival tissue from an AgP patient. (A) EMMPRIN immunoreactivity is more intense and more widespread throughout the gingival tissue than in the H/G1 group. (B) EMMPRIN expression can be seen in all layers of the epithelium: very strong in the basal layer, and moderate within the spinous and granular layers in the epithelial membranes. (C) The underlying connective tissues exhibit intensely EMMPRIN-staining inflammatory cells (prominent lymphocyte infiltration) and spindle-shaped fibroblast-like cells in the areas infiltrated by inflammatory cells. Both MMP-1 (E) and MMP-2 (H) show sparse and moderate expression in all layers of the epithelium. The sequential sections labeled for MMP-1 (F) and MMP-2 (I) show the same distribution in connective tissues as EMMPRIN. The immunoreactivity is strong and intense in areas infiltrated by inflammatory cells. The boxed areas in (A) are expanded in (B) and (C), the boxed areas in (D) are expanded in (E) and (F), and the boxed areas in (G) are expanded in (H) and (I). (Original magnification 100 in A, D and G, bars = 100 μm; and 400 in B, C, E, F, H and I, bars = 25 μm).

with different periodontal diseases (21). The present study showed both membrane-associated EMMPRIN located in the epithelial cell membrane and soluble EMMPRIN located in the intercellular spaces of connective tissues in gingival tissues from periodontitis samples, whereas membrane-associated EMMPRIN was prevalent in clinically healthy gingival tissue, indicating that in periodontal pathological states, soluble forms of EMMPRIN derived from inflammatory cells and resident cells might play a major role by stimulating the release of MMPs.

Using semi-quantitative RT-PCR, we found that the mRNA levels of EMMPRIN, MMP-1 and MMP-2 for the AgP group were lower than that for the chronic periodontitis group and higher than that for H/G1 group, although there was no statistical significance. It is known that various categories of gingival tissues exist among different periodontal conditions. In this study, specimens in the chronic periodontitis group were derived from extracted hopeless teeth without periodontal treatments according to the clinical protocol, which ensured that the biopsies were taken from sites with active periodontitis. It has been observed that chronic periodontal destruction occurs as a result of the accumulation of local factors such as plaque and calculus. While AgP differs from chronic periodontitis primarily by the rapid rate of disease progression, it is characterized by specific pathogenic microorganisms as well as by microbial deposits disproportionately great for the disease severity, and has a familial (genetic) association (22). Further investigation is needed to elaborate the relevant mechanisms involved in the local expression of EMMPRIN and the significance of EMMPRIN in the pathogenesis of periodontal disease.

Although the precise function of EMMPRIN remains unknown, we showed a correlation between the expression of EMMPRIN and the production of MMP-1 and MMP-2 by semi-quantitive RT-PCR analysis. Stimulation of MMP-1 production by EMMPRIN is usually in the range of 1.5- to two fold in rheumatoid arthritis patients (19). Stimulation of MMP-2 production in tumor cells is more variable but rather modest (e.g. 1.5- to two fold), probably because fibroblasts could produce significant amounts of MMP-2 even without exogenous stimulation. The mechanism of stimulation of MMP-2 might be more complex than that for MMP-1. In addition to stimulating MMP-2 production, EMMPRIN can greatly enhance its activation (39–42). The present study showed a 2.5- to three times increase in MMP-1 production (0.165 ± 0.176 vs. 0.058 ± 0.113) and MMP-2 (0.505 ± 0.243 vs. 0.207 ± 0.177) in the chronic periodontitis group, compared with the H/G1 group, while the EMMPRIN expression increased. The results of this study suggest that EMMPRIN may be involved in the regulation of expression of MMPs in periodontal disease, and the increased expression of EMMPRIN was associated with an increase in MMP-1 and MMP-2.

In conclusion, we found, for the first time, expression of the EMMPRIN peptide and mRNA in both normal and periodontitis-affected human gingival tissues. Local expression of EMMPRIN in basal layers might contribute to both physiological and pathological processes in periodontium, and its increased production might be associated with the expression of MMP-1 and MMP-2, thereby contributing to the progression of periodontal disease.

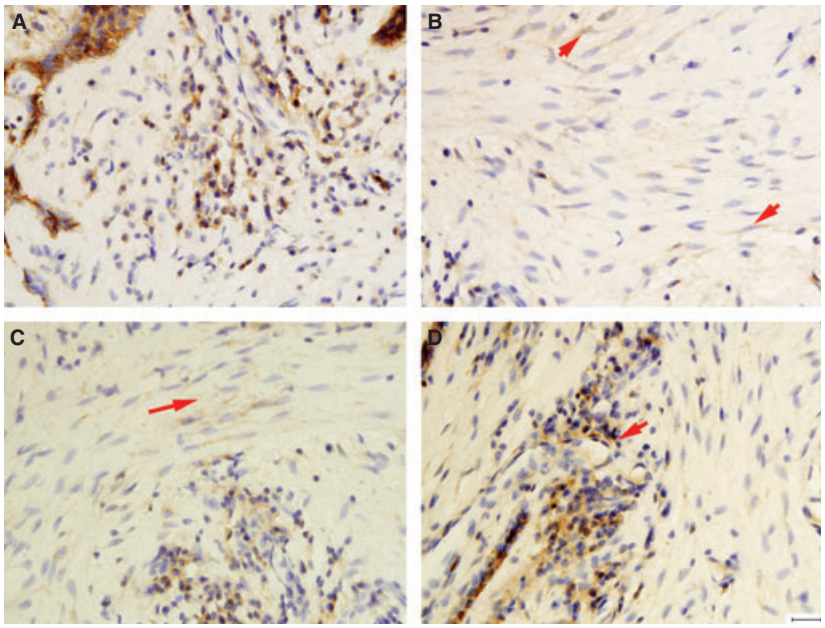

Fig. 3. Immunohistochemical demonstration of human EMMPRIN in gingival connective tissues of periodontitis. (A) The underlying connective tissues exhibit intensely EMMPRIN-staining inflammatory cells (prominent lymphocyte infiltration) and spindle-shaped cells in areas infiltrated by inflammatory cells. (B) The fibroblast-like cells weakly express EMMPRIN (red arrows) in areas not infiltrated by inflammatory cells. (C) EMMPRIN in both cytoplasm and intercellular spaces (red arrow). (D) EMMPRIN expression in a blood vessel (red arrow). (Original magnification 400 in A– D, bar = 25 μm).

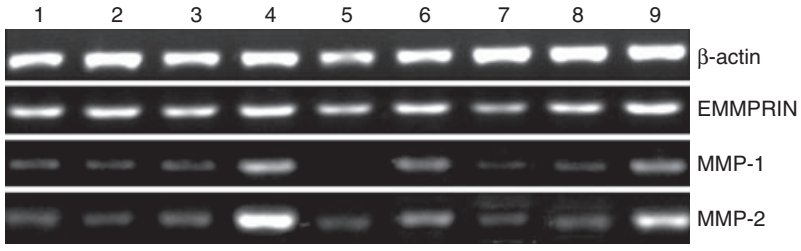

Fig. 4. EMMPRIN, MMP-1 and MMP-2 mRNA expression in the diseased and healthy gingival tissues. Representative gel showing bands with amplified cDNA. Lanes 1–4, chronic periodontitis samples; lanes 5–7, H/G1 samples; and lanes 8 and 9, AgP samples. There is no MMP-1 mRNA expression in lane 5.

Table 4. mRNA levels encoding EMMPRIN, MMP-1 and MMP-2 determined by RT-PCR in H/G1, chronic periodontitis and AgP groups

mRNA	H/G1 (n = 8)	Chronic periodontitis (n = 10)	AgP (n = 10)
EMMPRIN	0.670 ± 0.146	0.943 ± 0.263*	0.775 ± 0.203
MMP-1	0.058 ± 0.113	0.165 ± 0.176	0.117 ± 0.09
MMP-2	0.207 ± 0.177	0.505 ± 0.243*	0.331 ± 0.185

All values are expressed as the mean ± SD percentage of transcript levels relative to β-actin. The EMMPRIN and MMP-2 are significantly difference among groups by Kruskal–Wallis test. *Significant difference between chronic periodontitis and H/G1 groups (Bonferroni test) for EMMPRIN (p = 0.037) and for MMP-2 (p = 0.016).

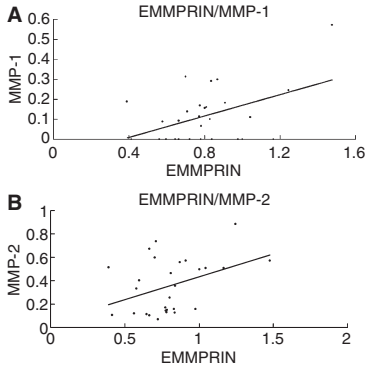

Fig. 5. Relationship between the expression of EMMPRIN mRNA and the production of MMP-1 (A) and MMP-2 (B) by gingival tissue from 28 subjects by Pearson's correlation coefficient (r = 0.463, p = 0.013 for MMP-1 and r = 0.404, p = 0.033 for MMP-2).

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