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Expression of metalloproteinases and their tissue inhibitors in gingiva affected by hereditary gingival fibromatosis: analysis of three cases within a family

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Background and Objective: Hereditary gingival fibromatosis (HGF) is a benign disorder manifested by fibrous enlargement of keratinized gingiva. Evidence exists concerning the role of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in mediating normal and pathological processes, including HGF. Nevertheless, there are few and contradictory results on the analysis of MMPs and TIMPs transcripts in this pathology.

Material and Methods: We studied the expression of the transcripts encoding MMP-1, -2 and -9 and TIMP-1 and -2 in gingival biopsies, obtained from three cases of HGF within a family, by semi-quantitative reverse transcriptase-polymerase chain reaction analysis. Samples were also processed for gelatin zymography.

Results: Except for MMP-9, most transcripts presented a higher level of expression in biopsies from HGF patients compared with control subjects. Accordingly, MMP-9 gelatinase activity was detected at low and similar levels among samples. Moreover, MMP-2 enzymatic activity was not detected at all.

Conclusion: The mRNA expression of MMP-1 and -2 and TIMP-1 and -2 does not explain the gingival overgrowth presented in these cases. In addition, it is suggested that the gene expression of those molecules in the course of HGF is regulated at the translational or post-translational level.

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Hereditary gingival fibromatosis (HGF) is a benign disorder manifested by a slowly progressive, non-hemorrhagic, fibrous enlargement of keratinized gingiva (1). It is characterized by dense fibrous connective tissue, rich in collagen fibers and other extracellular matrix (ECM) molecules, covered by a hyperplasic epithelium (1). As an

isolated finding, it is mostly sporadic, and an autosomal dominant inheritance pattern is suggested (2). Evidence exists concerning the role of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in mediating normal and pathological processes, including embryogenesis, arthritis, periodontal disease, cancer and fibrotic diseases (3–6). However, the cellular and molecular mechanisms underlying the accumulation of dense fibrous connective tissue are poorly understood (1). There are few and contradictory results on the expression of MMPs and TIMPs transcripts *in vitro* (5–7), while there are no reports in gingiva biopsies from HGF patients. Thus, we aimed to study the expression of the transcripts encoding MMP-1, -2 and -9 and TIMP-1 and -2 by a semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR), along with the gelatinase activity, in gingival biopsies obtained from three cases of HGF within a family.

Material and methods

This study was conducted in accordance with the Declaration of Helsinki and written informed consent was obtained from all patients before sample collection.

Sample collection

Gingival biopsies were collected from three patients presenting HGF within the same family and from three other patients with healthy gingiva (used as control subjects) at the School of Dentistry of the Federal University of Rio de Janeiro, Brazil. None of them was receiving any medication known to cause gingival overgrowth. The HGF samples included only fibrous and non-inflamed gingival tissue.

RT-PCR conditions

Total RNA isolation and purification and cDNA synthesis were performed according to a previously published study (4). Briefly, after cDNA amplification, efficiency of synthesis was confirmed by PCR with primers (5′-ATCACCATCTTCGAGGAGCG-3′ and 5′-CCTGCTTCACCACCTTC-TTG-3′) for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Next, we analysed the expression patterns of the following genes: MMP-1, -2 and -9 and TIMP-1 and -2 (also see Ref. 4 for sequences of primers). The PCR reactions for each gene were carried out with 30 μL of the corresponding mix: 125 μL of 10× Taq Pol buffer (Ludwig Biotecnologia, São Paulo, Brazil); 10 μL of 25 mM dNTPs with equimolar amounts of dTTP, dATP, dCTP and dGTP (Invitrogen, Paisley, UK); 62.5 μL of 25 mM $MgCl_2$; 5 μL of primer sense and 5 μL of primer antisense (100 pmol/μL of each primer, Gene link, Hawthorne, NY, USA) and 795 μL of water. Furthermore, 0.3 μL of Taq polymerase (Ludwig Biotecnologia; 5 U/μL) was added to the PCR reaction followed by 2 μL of cDNA of each patient. Positive and negative controls were also assessed in each experiment. The PCR products were visualized in 2% agarose gels stained with ethidium bromide.

Gelatin zymography assay

The protein content of homogenized samples was measured using the D-C Assay protein reagent (Bio-Rad, Hercules, CA, USA). Twenty micrograms of protein sample were mixed with 2× non-reducing sample buffer [0.5 M Tris-HCl, pH 6.8, 20% glycerol, 0.2% sodium dodecyl sulphate (SDS), 0.0005% Bromophenol Blue] and electrophoresed on 1.5-mm-thick 12% SDS-polyacrylamide gel elecrophoresis (SDS-PAGE) gels containing 0.3% gelatin as a protease substrate. Following electrophoresis, gels were washed in 2.5% Triton X-100, 50 mM Tris-HCl, pH 7.5, for 1 h to remove SDS and then washed with 50 mM Tris-HCl, pH 7.5. Subsequently, gels were incubated for 20 h at 37°C in incubation buffer (50 mM Tris-HCl pH 8.0, 0.02% sodium azide) on a rotary shaker. Afterwards, gels were stained in 30% methanol, 10% acetic acid and 0.2% (w/v) Coomassie Brilliant Blue for 1 h, followed by destaining in 30%

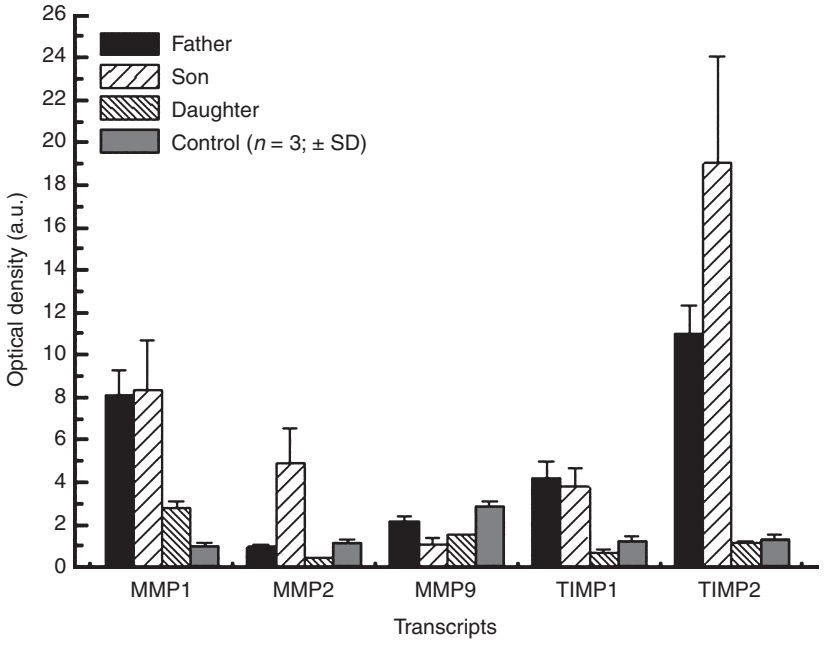

Fig. 1. Semi-quantitative analysis of mRNA transcripts of MMPs and TIMPs, in control and HGF-affected gingiva. Total RNA isolated from the experimental samples was subjected to reverse transcription followed by amplification by polymerase chain reaction. Products from those reactions were visualized in agarose gels and quantified by optical densitometry. All results were normalized for the GAPDH mRNA. The data presented here were obtained from each HFG patient and from a tissue pool of the three control patients. Results represent means ± SD of three experiments.

methanol and 10% acetic acid. Gelatinolytic activity was manifested as horizontal white bands on a dark blue background. Gels were scanned and bands were quantified by optical densitometry (ImageJ, 1.41J, NIH, USA; http://rsbweb.nih.gov/ij/).

Results

Semi-quantitative analysis of MMPs and TIMPs mRNA expression

The molecular analysis results are shown in Fig. 1. The mean transcript levels for MMP-1, MMP-2, TIMP-1 and TIMP-2 tended to be higher in HGF than in control tissues, whereas MMP-9 mRNA levels were slightly lower in the HGF samples.

Gelatin zymography assay

A representative zymogram and the comparison of data among samples are depicted in Fig. 2A,B, respectively. Gelatinase activity for MMP-2 was not detected at all. The levels of MMP-9 enzymatic activity were similar among HFG patients and control subjects. In addition, a discrepancy can be observed between proMMP-9 (92 kDa) and its active form (83 kDa), which occurs at very low levels.

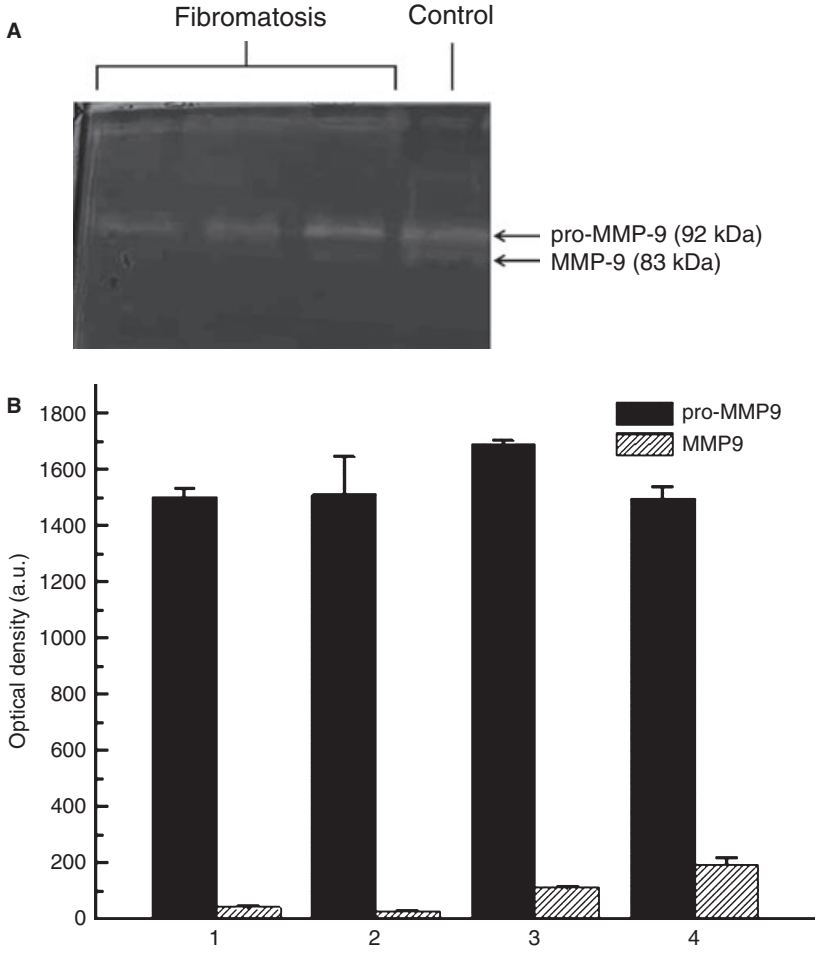

Fig. 2. Equal amounts of protein (20 μg) were loaded onto SDS-PAGE gels and separated by electrophoresis as described in the Material and methods section. (A) Representative zymogram. Bands correspond to 92 kDa (proMMP-9) and 83 kDa (MMP-9) in HGF (lanes 1–3) and control samples (lane 4). The presence of MMP-2 was not detected. (B) Zymogram gels were scanned, and bands were quantified by optical densitometry. The data presented here were obtained from each HFG patient and from a tissue pool of the three control patients. Results represent means ± SD of three experiments.

Discussion

Matrix metalloproteinase-mediated collagen degradation is an important mechanism for ECM turnover in wound healing and inflammation. Based on *in vitro* studies, diminished degradation of ECM has been proposed as a mechanism that contributes to the manifestation of HGF (8,9,10). Specifically, a decreased level of expression and activity of MMP-1 and MMP-2 have been shown in HGF-derived cells (9,10). Based on those studies, one can speculate that fibroblasts in HGF produce less MMPs and more ECM proteins, which result in ECM accumulation. Since TIMPs inhibit MMP activity, a high ratio of TIMPs to MMPs could also result in excess collagen accumulation (3). Colleta & Graner (2004) (10) suggested that gingival overgrowth develops through activation or selection of resident tissue fibroblasts, phenotypically characterized by increased proliferation, low levels of MMPs synthesis and abnormally high collagen production. In contrast, Gagliano *et al.* (2005) (9) did not found a lower level of expression of MMP-1 in HGF compared with control cells. Similarly, in our study, most transcripts presented a higher level of mRNA expression, with the exception of MMP-9. However, these results were observed in HGF biopsies and cannot be compared directly to the studies discussed. Moreover, the gene expression of these molecules could be controlled at a post-transcriptional level. In the present study, for instance, MMP-2 gelatinase activity was not detected at all, which is in disagreement with its mRNA expression.

In summary, it could be thought that the biological origin of gingival fibromatosis is complex and there is likely to be more than one molecular mechanism involved in the development of HGF. In conclusion, one could suggest that the mRNA expression of MMP-1 MMP-2, TIMP-1 and -2 does not explain the gingival overgrowth presented in these cases.

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