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THE BIOLOGY OF MATRIX METALLOPROTEINASES

A BIOLOGIA DAS METALOPROTEASES DA MATRIZ

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Matrix metalloproteinases (MMPs) are an important family of zinc-dependent endopeptidases that mediate the extracellular matrix (ECM) degradation. These enzymes have been implicated in pathologic oral processes such as periodontal tissue destruction, root caries, tumor invasion and temporomadibular joint disorders. In the present work we review some general aspects of matrix metalloproteinases, and discuss the role of theses enzymes in normal physiology and pathology with emphasis on the oral environment.

UNITERMS: Matrix metalloproteinases (MMPs); Oral mucosa.

MATRIX METALLOPROTEINASES: GENERAL ASPECTS

Matrix Metalloproteinases (MMPs) form an important family of metal-dependent endopeptidases that represent the major class of enzymes responsible for degradation of extracellular matrix (ECM) components. Collectively, MMPs are capable of degrading all ECM proteins[2]. All family members are secreted as inactive proenzymes (zymogens) and are thought to be activated in the tissue by cleavage of the propeptide. All MMPs contain Zn^{2+} at the catalytic site and, in addition, require Ca^{2+} for stability and activity.[2]

The first report about a matrix metalloproteinase was published in 1962 by Jerome Gross and Charles Lapière[5]. They found an active enzyme in the culture media of tissue fragments of tail fin skin that degraded the triple helix of native type I collagen. Since then, at least sixteen human MMPs have been characterized (Table 1). MMPs are classified into five main classes (collagenases, gelatinases, stromelysins, membrane-type and others, including the matrilysin) on the basis of their putative substrate specificity and internal homologies.

The members of the MMPs family are organized into three basic, distinctive, and well-conserved domains based on structural considerations: amino-terminal propeptide, catalytic domain, hemopexin-like domain at the carboxy-terminal (Figure 1). The amino acid sequence homology between the MMPs members is highest at the amino-terminal pro-fragment region and the zinc atom catalytic site. Some additional domains or short inserts can be found attached to the common structure in several MMPs, like MMP-2 and –9 that contain a gelatin-binding domain insert between the catalytic and active site domain.[28]

ROLE OF MATRIX METALLOPROTEINASES IN VIVO

Matrix Metalloproteinases are expressed in response to specific stimuli by resident connective tissue cells as well as the major inflammatory cell types that invade the tissue during remodeling events in vivo.[2] Evidences for the role of any particular metalloproteinase in a pathological process is provided by findings as the presence of metalloproteinase mRNA in lesional cells and activity of MMPs in lesions.[9] Such evidences suggest that collagenases could have a fundamental role during ECM degradation since these enzymes have the unique ability to cleave type I collagen that will be further degraded by others proteinases.

The MMPs activity has been related to a number of important diseases such as joint destruction in

TABLE 1- The matrix metalloproteinase family.

Enzyme	Number	kDa	Preferred Substrate
Interstitial collagenase	MMP-1	57/52	Helical collagen, proMMP-2, proMMP-9
Neutrophil Collagenase	MMP-8	85-64	Helical collagen
Collagenase-3	MMP-13	52-42	Helical collagen
Gelatinase A	MMP-2	72/66	ProMMP-9, gelatin, fibronectin
Gelatinase B	MMP-9	92/80	Gelatin, fibronectin, elastin, collagen IV, V, VII, X, and denatured type I collagen
Stromelysin-1	MMP-3	60/55	Fibronectin, laminin, elastin, proteoglican, collagen IV, V, IX, X proMMP-1, -7, -8, -9, -13
Stro melysin-2	MMP-10	60/55	Fibronectin, laminin, elastin, proteoglican, collagen IV, V,IX,X
MT1-MMP	MMP-14	66/54	ProMMP-2, -13, helical collagen
MT2-MMP	MMP-15	72/60	----------
MT3-MMP	MMP-16	64/53	ProMMP-2
MT4-MMP	MMP-17	57/53	----------
Matrilysin	MMP-7	28/19	Fibronectin, elastin, collagen IV
Metalloelastase	MMP-12	54/22	Elastin
Enamelysin	MMP-20	54/22	Dental enamel matrix

Fonte: Woessner, 1998

rheumatoid arthritis, osteoarthritis, abdominal aortic aneurysm, acute myocardial infarction and cancer.[29] For tumor cells to metastasize, it produces MMPs in order to break away from its neighbors, force its way into the surrounding stroma, and penetrate the basement membrane until the blood vase. MMPs also participate in normal remodeling processes such as embryonic development, post-partum involution of the uterus, bone remodeling, ovulation and wound healing.[27]

The function of MMPs has been studied using transgenic mice: knockouts for MMP-3, -7, -9 and –12 and over expressions of MMP-1 and –3.[21] The problem of using this kind of analysis appears to be the high functional redundancy of MMPs. The ablation of a particular MMP will induce the expression of other MMPs, in order to compensate for the loss. In view of this fact, it is believed that the multiplicity of MMP forms underlines the extreme importance of these enzymes for the maintenance and repair of the ECM.[28]

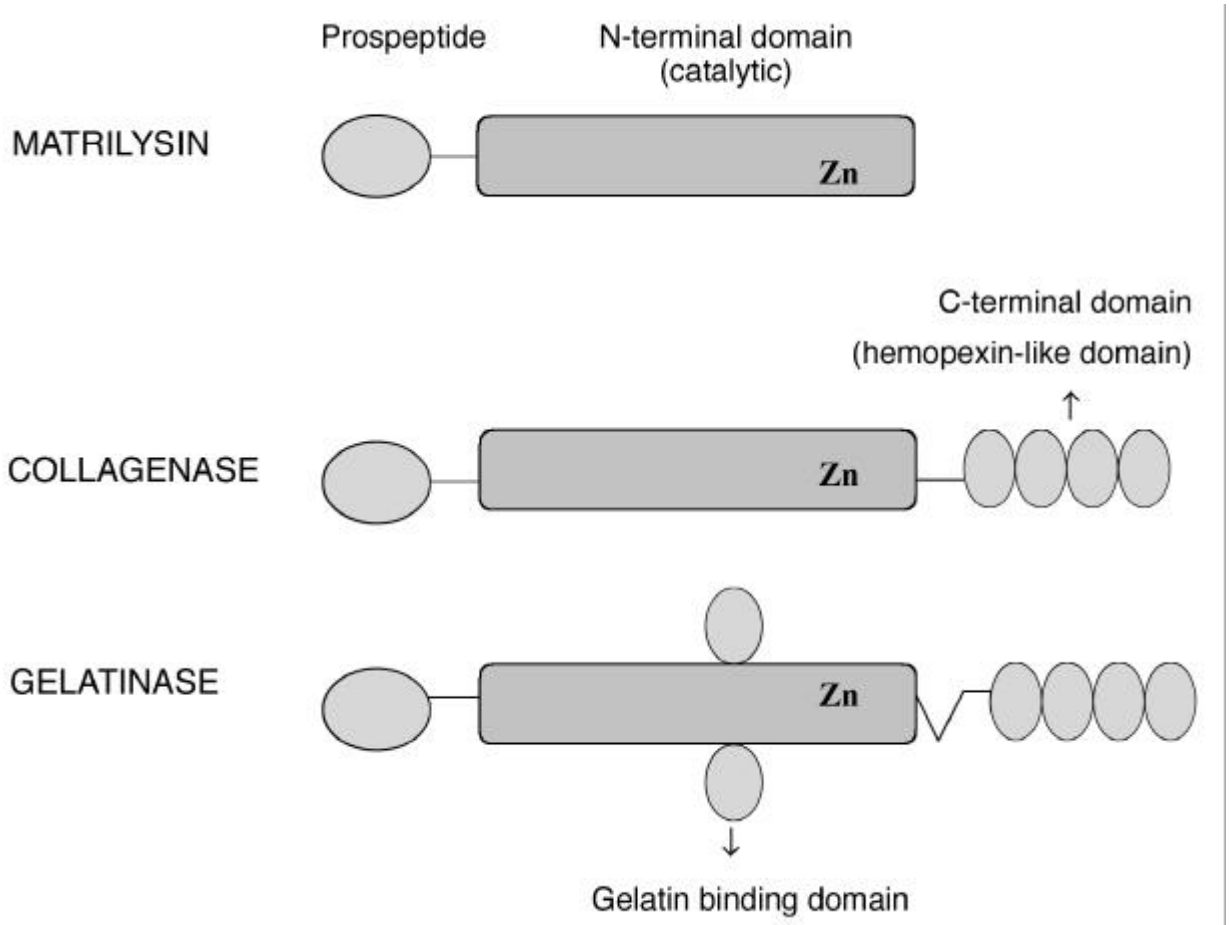

FIGURE 1- Domain Structure Of MMPs.

POLYMORPHISM IN MATRIX METALLOPROTEINASE GENE PROMOTER

The activity of MMPs is regulated at multiple levels, including conversion of proenzyme to the activated form, inhibition by tissue inhibitors of MMPs (TIMPs) in tissues and regulation of transcription. However, the synthesis of MMP appears to represent the key step, since most MMPs genes are expressed only when active physiological or pathological tissues remodeling takes place[29].

Gene promoters are regions that control gene transcription. Recently, DNA polymorphisms have been found in the promoter region of several MMPs. Polymorphism represents natural sequence variants (alleles), which may occur with more than one form, having a frequency greater than 1% in a human population. Approximately, ninety percent of DNA polymorphisms are single nucleotide polymorphisms (SNP) due to single base exchange.

A SNP in the promoter region of human MMP-1 gene has been described.[18] An insertion/deletion of a guanine at position –1607 creates two different alleles, one having a single guanine (1G) and the other having two guanines (2G). The 2G allele together with an adjacent adenosine create a core-binding site (5‘-GGA-3‘) for Ets family of transcription factors that increases the transcriptional activity significantly.[18] Tumors bearing the 2G allele can secrete higher levels of MMP-1, and the presence of this allele was associated with the development of ovarian cancer[8].

Two functionally important genetic polymorphism have been detected in the gene promoter of MMP-9. One of them represents a SNP at position -1562 and the other a (CA)n microsatellite repeat at position -90. The SNP is a C to T substitution that increases the transcriptional activity. Zhang et al.[30] have found an association of the C-1562T polymorphism with the severity of coronary arteriosclerosis. The (CA)n is a multi-allelic microsatellite polymorphism where the most common form has a peak at the $(CA)_{14}$ allele and the second peak at the $(CA)_{21,}$ $(CA)_{22}$ and $(CA)_{23}$ alleles.[22] The 14CA repeats has only 50%

of the transcriptional activity of MMP-9 promoter comparing with the 21CA repeats. The relationship between the (CA)n repeats and abdominal aortic aneurysm and intracranial aneurysm[14] has been analyzed, but the data are contradictory.

A 5A/6A polymorphism has been reported in the MMP-3 (stromelysin-3) gene promoter. This SNP has been associated to arteriosclerosis in a number of genetic epidemiological studies. The frequency of 5A allele is significantly higher in affected individuals than in control subjects and the risk of acute myocardial infarction in individuals carrying one or two copies of the 5A allele was estimated to be 2.25 fold. MMP-3 is capable to degrade a wide range of extracellular matrix proteins, promoting the cleavage of atherosclerotic plaque.

The evidences presented suggest that genetic polymorphism in the MMPs genes are likely to be related to a wide range of diseases that are characterized by extracellular matrix degradation.

INHIBITORS AND INHIBITION OF MMP ACTIVITY

In the extracellular matrix (ECM), the activity of MMP is controlled by specific inhibitors known as tissue inhibitors of MMPs (TIMPs). TIMPs are small (21-28 kDa), multi-functional proteins that regulate MMP function both at the level of their activation and in their ability to hydrolyze a particular substrate. The MMPs inhibition by TIMPs occurs in a 1:1 stoichiometry and non-covalent fashion.

Four members of TIMP family have been so far described. TIMP-1 is more effective than TIMP-2 at inhibiting MMP-1 and MMP-3. In most cells, MMP-9 is secreted as a complex with TIMP-1, whereas TIMP 2 is associated with MMP-9. Experimental evidences suggest that TIMP-2 is 10 times more effective than TIMP-1 in inhibiting the activity of MMP-2.

The balance between production of MMPs and TIMPs represents a critical point to maintain the homeostasis of the ECM. It is recognized that a pathological breakdown of the ECM can be installed if there is excess of MMP activity in the tissue. For this reason, there is a great interest in the development of synthetic inhibitors of MMP, which could be used in medical therapy. Most attention has been given to zinc chelating agents. Tetracycline was shown to inhibit collagenase in gingival fluid and tissue, independent of their antibacterial activity. Gold salts are used to treat arthritis; these have been shown to work by the binding of a gold atom to a heavy metal site in the MMP distinct from that occupied by the catalytic zinc atom of the MMP[10].

MMPS AND ORAL ENVIRONMENT

Several evidences have supported the fundamental role of MMPs during development and remodeling of oral tissues. MMPs are required to remove the enamel matrix proteins during enamel maturation, resulting in a highly mineralized tissue.[7] MMPs are the major players in collagen breakdown during periodontal tissue destruction.[26] Gingival fibroblasts, keratinocytes, resident macrophages and polymorphonuclear leukocytes (PMN) are capable of expressing MMP-1, MMP-2, MMP-3, MMP-8, MMP-9,[2] inflammatory cytokines and growth factors that up regulate MMPs transcription. High levels of MMPs on the periodontal tissues provoke an imbalance between production and degradation of collagen, causing tooth attachment loss. Periodontitis patients have significantly higher levels of MMP-2 and MMP-9 than health subjects, and the amount of gelatinases decreases after periodontal treatment. The activation of MMP-2 and MMP-9 was also shown to have a crucial role in the destruction of dentin by caries. Additionally, these enzymes can potentate the degradation of extracellular matrix by activating collagenase-3 (MMP-13) and neutrophil collagenase.

Recently, we have demonstrated that divalent metal salts, as Zn, Cu, Hg and Sn, are capable to inhibit the activity of MMP-2 and MMP-9 at low concentration.[24] We have also observed that lead, cadmium and zinc inhibit the activity of enamel matrix proteinases in vitro.[3] Among the metals studied, zinc is extensively used in clinical dentistry. Besides being an important component of restorative materials, it is also used as an active component in toothpaste and mouthrinses. Clinical studies have shown that mouthrinses and dentifrices containing zinc salts can reduce plaque accumulation and calculus formation.[4,6,13, 15] Using an experimental gingivitis model, Saxton and Cummins[20] have also shown that zinc can effectively improve gingival health. These authors showed that dentifrices containing zinc citrate could reduce the development of gingival inflammation by 25%. Therefore, it is proposed that zinc can directly improve gingival health by directly inhibiting MMPs present in inflamed gingival tissue.

Large amounts of zinc and copper are constantly

released from amalgam[11]. The continuous salivary clearance would rapidly remove these metals from the mouth minimizing the interference with the MMPs present in saliva. However, in some cases such as amalgam tattoo and root-end filling, amalgam remains in direct contact with connective tissue for prolonged periods of time. The inhibition of MMP activity could have a local effect on the connective tissue around this material. There are many different formulations of amalgam with physical properties that may make them behave differently in regard to MMPs inhibition. Therefore, different formulations of amalgam may elicit different biological responses from connective tissue[23].

The topical application of zinc oxide has been shown to stimulate the healing of both chronic and acute wounds. Zinc oxide can reduce inflammatory reaction in the granulation tissue and significantly increase re-epithelialization of wounds.[1] The precise functions of MMP-2 and MMP-9 in wound healing are still controversial. The presence of MMP-9 in the leading edge of migrating epithelium suggests that this enzyme may be involved in keratinocyte migration during re-epithelialization after wounding. However, the activities of MMP-2 and MMP-9 were shown to be increased in diabetic healing-impaired mice when compared to nondiabetic mice.[12] The topic application of CMT-2, an inhibitor of MMPs activity, can enhance wound healing in diabetic rats.[16] These data indicate that elevated levels of MMPs may impair wound healing. Zinc oxide is fairly insoluble, it is slowly but continuously dissolved when applied on open wounds.[1] Therefore, zinc oxide can exert a prolonged and constant effect on the healing tissue. The inhibition of MMP-2 and MMP-9 activities may be one mechanism by which topical zinc oxide enhances wound healing[19].

Inhibition of MMPs can be a mechanism to prevent other destructive events. The role of proteolytic enzymes in the root caries process is not totally understood, but there are several evidences indicating that MMPs are required to remove the organic matrix during root-surface caries. Ramamurthy et al.[17] have reported that the treatment with non-antimicrobial tetracycline prevents not only the destruction of periodontium by MMPs, but also avoids the exposure of roots to host tissue. Metalloproteases from peri-implant sulcus fluid have been collected from loosing and well-fixed dental implants. Similar to periodontitis sites, the peri-sulcus fluid of loosing dental implants contains high levels of MMPs.[25] Thus, MMPs inhibitory therapy has been used during initial phases of dental implant in order to obtain a better insertion of dental implant to alveolar bone.

RESUMO

As metaloproteases da matriz (MMPs) representam uma importante família de enzimas zinco dependentes que modulam a degradação da matriz extracelular. Estas enzimas têm sido associadas a diversos processos patológicos que afetam a cavidade bucal, como a destruição do tecido periodontal durante a periodontite, cárie de raiz, invasão de tecidos por tumor e desordens da articulação temporomandibular. Neste trabalho nós apresentamos uma revisão dos aspectos gerais das metaloproteases da matriz e discutimos sobre o papel destas enzimas em processos fisiológicos e patológicos, dando ênfase ao papel destas enzimas no meio ambiente bucal.

UINITERMOS: Metaloproteases da matriz (MMPs); Mucosa bucal.

REFERENCES

1- Agren MS. Studies on zinc in wound healing. Acta Derm Venereol 1990; 154:1-36.

2- Birkedal-Hansen H. Role of matrix metalloproteinases in human periodontal diseases. J Periodontal 1993; 64:474-84.

3- Gerlach RF, Souza AP, Cury JA, Line SRP. Effect of lead, cadmium and zinc on the activity of enamel matrix proteinases in vitro. Eur J Oral Sci 2000; 108:327-34.

4- Giertsen E, Scheie AA, Rolla G. In vivo effects of zinc and chlorhexidine on dental plaque ureolysis and glycolysis. J Dent Res 1989; 68:1132-4.

5- Gross J, Lapière CM. Collagenolytic activity in amphibian tissues: a tissue culture assay. Proc Natl Acad Sci 1962; 54:1197-1204.

6- Harrap GJ, Saxton CA, Best JS. Inhibition of plaque growth by zinc salts. J Periondontal Res 1983; 18:634-42.

7- Hayakawa T. Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in development and disease of oral tissues. Dent in Japan 1998; 34:167-77.

8- Kanamori Y, Matsushima M, Minaguchi T, et al. Correlation between expression of the matrix metalloproteinase-1 gene in ovarian cancers and an insertion/deletion polymorphism in its promoter region. Cancer Res 1999; 59:4225-7.

9- Krane SM. Clinical Importance of metalloproteinases and their inhibitors. Ann NY Acad Sci 1994; 732:1-10.

10- Mallya SK, Van Wart HE. Mechanism of inhibition of human neutrophil collagenase by gold(I) chrysotherapeutic coumpouds. Interaction at a heavy metal binding site. J Biol Chem 1988; 264:1594-601.

11- Marek M. Interaction between dental amalgams and the oral environment. Adv Dent Res 1992; 6:100-9.

12- Neely AN, Clendering CE, Gardner J, Greenhalgh DG. Gelatinase activities in wounds of healing-impaired mice versus wounds of non-healing mice. J Burn Care Rehabil 2000; 21:395-402.

13- Opperman RV, Rölla G. Effect of divalent cations on the acidogenicity of dental plaque in vitro. Caries Res 1980; 14:422-7.

14- Peters DG, Kassam A, St Jean PL, Yonas H, Ferrel RE. Functional polymorphism in the matrix metalloproteinase-9 promoter as a potential risk factor for intracranial aneurysm. Stroke 1999; 30:2612-16.

15- Picozzi A, Fischmann SL, Pader M, Cancro LP. Calculus inhibition in humans. J Periodontol 1972; 43:692-5.

16- Ramamurthy MS, Kucine AJ, Mclean SA, McNamara TF, Golub LM. Topically applied CMT-2 enhances wound healing in Streptozocin diabetic rat skin. Adv Dent Res 1998; 12: 144-8.

17- Ramamurthy NS, Schroeder KL, McNamara TF et al. Root-surface caries in rats and humans: inhibition by non-antimicrobial property of tetracyclines. Adv Dent Res 1998; 12:43-50.

18- Rutter JL, Mitchell TI, Buttice G et al. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates na Ets binding site and augments transcription. Cancer Res 1998; 58:5321-5.

19- Santos MCLG, Souza AP, Gerlach RF, Tabchoury CM, Line SRP. Inibition of human gelatinase (matrix metalloproteinase-2 and matrix metalloproteinase-9) activity by zinc oxide: a possible mechanism to enhance wound healing. Brit J Dermatol 2001; 145:854-5.

20- Saxton CA, Cummins D. Effect of zinc citrate dentrifices on the development of gengivitis. J Dent Res 1989; 68:971.

21- Shapiro SD. Mighty mice: transgenic technology "knoch out" questions of matrix metalloproteinase function. Matrix Biol 1997; 15:527-33.

22- Shimajiri S, Arima N, Tanimoto A, et al. Shortened microsatellite d(CA)21 sequence down-regulates promoter activity of matrix metalloproteinase 9 gene. FEBS Lett 1999; 455:70-4.

23- Souza AP, Gerlach RF, Line SRP. Inihibition of human gelatinases by metals released from dental amalgam. Biomater 2001; 22:2025-30.

24- Souza AP, Gerlach RF and Line SRP. Inihibition of human gingival gelatinases (MMP-2 and MMP-9) by metal salts. Dent Mater 2000; 16:103-8.

25- Teronen O, Konttinen YT, Lindqvist C et al. Human neutrophil collagenase MMP-8 in peri-implant sulcus fluid and its inhibition by clodronate. J Dent Res 1997; 76:1529-37.

26- Van der Zee E, Everts V, Beertsen W. Cytokine-induced endogenous procollagenase stored in the extracellular matrix of soft connective tissue results in a burst of collagen breakdown following its activation. J Peridontol Res 1996; 3:483-488.

27- Woessner JrJF. Matrix metalloproteinases and their inhibitors in connective tissue remodeling. FASEB J 1991; 2145-54.

28- Woessner JrJF. The matrix metalloproteinase family. In: Parks WC, Mechan RP, editors. Matrix Metalloproteinase. California: Academic 1998. p.300-56.

29- Ye S. Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. Matrix Biol 2000; 19:623-9.

30- Zang B, Ye S, Herrmann SM, et al. Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. Circulation 1999; 99:1788-94.

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