MATRIX METALLOPROTEINASES AND THEIR ROLE IN ORAL DISEASES: A REVIEW

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Abstract

Matrix metalloproteinases (MMPs) are zinc dependent endopeptidases that are capable of degrading extra cellular matrix proteins. The activity of MMPs is seen not only during normal organogenesis and wound healing, but also in pathological conditions like inflammatory diseases and tumor invasion. This review describes the structure, function and regulation of MMPs and also highlights their role in certain oral diseases like oral cancer, periodontitis and dental caries.

Key words: MMPs, extra cellular matrix, oral cancer, invasion

Introduction

The controlled and co-ordinated synthesis, breakdown and remodeling of the extracellular matrix (ECM) are critical events in normal physiological conditions like embryonic development, wound healing and angiogenesis. Remodeling of the ECM is carried out by different protease systems, which can be divided into four subgroups according to their amino acid residue required for catalytic activity: cysteine proteases, aspartic proteases, serine proteases and metalloproteinases. The metalloproteinases are further divided into several superfamilies, the important one being the metzincin superfamily. Matrix metalloproteinases (MMPs) belong to the metzincin superfamily (i.e) they bind zinc at the catalytic site and have a conserved 'Met-turn' motif. [1-3]

Classification of MMPs:

Based on the substrate specificity MMPs are classified into the following types [3]:

1. **Collagenases**
   MMP-1 (collagenase-1, interstitial collagenase)
   MMP-8 (collagenase-2, neutrophil collagenase)
   MMP-13 (collagenase-3)
2. **Gelatinases**

MMP-2 (gelatinase A, 72-kDa gelatinase)
MMP-9 (gelatinase B, 92-kDa gelatinase)

3. **Stromelysins**
   MMP-3 (stromelysin-1)
   MMP-10 (stromelysin-2)
   MMP-11 (stromelysin-3)
   MMP-12 (metalloelastase)
4. **Matrilysins**
   MMP-7 (matrilysin, PUMP-1)
   MMP-26 (matrilysin-2)
5. **MT-MMPs (Membrane type)**
   MMP-14 (MT1-MMP)
   MMP-15 (MT2-MMP)
   MMP-16 (MT3-MMP)
   MMP-17 (MT4-MMP)
   MMP-24 (MT5-MMP)
   MMP-25 (MT6-MMP)
6. **Other MMPs**
   MMP-18
   MMP-19
   MMP-20 (enamelysin)
   MMP-21
   MMP-23
   MMP-27
   MMP-28 (epilysin)

Collagenases:

MMPs 1, 8 and 13 are the major secreted collagenases that initiate degradation of fibrillar collagens (type I, II, III, V and IX). The fibrillar collagens are cleaved at a specific site to produce triple helical fragments, which denature spontaneously to gelatin at 37^0C. Matrix metalloproteinase-1 cleaves preferentially type

III collagen and is expressed in various normal cells, such as keratinocytes, fibroblasts, endothelial cells, macrophages and chondrocytes. It can be detected *in vivo not only* in physiological situations like embryonic development and wound healing, but also in chronic cutaneous ulcers and many cancers. MMP-8 cleaves type I and II collagens and is synthesized by maturing leukocytes in bone marrow, stored intracellularly in granules and released in response to extracellular stimuli. Matrix metalloproteinase-13 was originally cloned from a breast tumor and cleaves preferentially type II collagen and also gelatin more effectively than other collagenases. It is expressed during bone development and gingival wound repair as well as in pathological situations, such as in squamous cell carcinomas (SCC) of different organs, chondrosarcomas and melanoma. [4]

Gelatinases

Gelatinase A is expressed in a variety of normal cells, including fibroblasts, keratinocytes, endothelial cells and chondrocytes. Gelatinase B is produced by keratinocytes and macrophages. Gelatinases are able to degrade type IV, V, VII, X, XI, and XIV collagens, gelatin, elastin, proteoglycan and fibronectin and have a vital role in cancer invasion. [4]

Stromelysins

MMPs 3 and 10 are homologous in structure and substrate specificity. Matrix metalloproteinase-3 is expressed by keratinocytes, fibroblasts and chondrocytes. It can activate TGF-β, TNF-α and IL-1β. Matrix metalloproteinase-10 was initially identified in an adenocarcinoma cDNA library, but can also be detected in SCCs of the head, neck and lung. Additionally, MMP-10 is expressed by migrating keratinocytes in skin wounds and by migrating enterocytes in intestinal ulcers. MMP-11 is expressed in mesenchymal cells located close to epithelial cells during physiological and pathological tissue remodeling. It is expressed in most invasive human carcinomas and is associated with a poor clinical outcome in breast cancer. MMP-12 was initially found in alveolar macrophages of cigarette smokers and is the most effective MMP against elastin. [4,5]

Matrilysins

Matrix metalloproteinase-7 was originally identified as the small putative uterine metalloproteinase (PUMP). Unlike most other MMPs expressed only in response to injury, MMP-7 is expressed by non-injured, non-inflamed mucosal epithelium in many tissues. Matrix metalloproteinase-7 is up-regulated in many tumors, especially of epithelial origin, such as breast, lung and skin cancers. MMP-7 can also inhibit tumor angiogenesis by producing angiostatin. Matrix metalloproteinase-26 is detected in placenta and uterus and is also widely expressed in diverse tumor cell lines and in malignant tumors. [3,4]

Membrane type MMP

The structure of MT-MMP is similar to other MMPs, except for a transmembrane domain and a furin site between the propeptide and catalytic domain, cleaving of which leads to their activation. Localization of MT-MMPs at the cell surface implies that they have a significant role in regulation of cell–matrix interactions and activation of other MMPs. For example, MT1- and MT2-MMP activate MMP-13. The expression of MT-MMPs, especially MT1-MMP, has been detected in tumor cells or adjacent stromal cells in a variety of human cancers. [2,4]

Other MMPs

Matrix metalloproteinase-19 is widely expressed in tissues, including placenta, lung, pancreas, ovary, spleen and intestine. Matrix metalloproteinase-19 is capable of degrading many components of the ECM and basement membrane (BM), but cannot activate any pro-MMPs. MMP-20 or enamelysin is exclusively expressed in ameloblasts and odontoblasts of developing teeth and helps in degrading amelogenin, Matrix metalloproteinase-23, cloned from an ovary cDNA library, is expressed mainly in the ovary, testis and prostate and have a specialized role in reproductive processes. It has a unique structure with a short prodomain and C-terminal domain. Epilysin or MMP-28 was cloned from testis and keratinocyte cDNA libraries. It is expressed at high levels particularly in testis and in injured epidermis. [1,2]

Structure of MMPs (Figure 1)

MMPs are products of different genes dispersed in the genome, although there is an MMP gene cluster in chromosome 11. Matrix metalloproteinases are structurally similar, but differ in substrate specificity, in that each MMP has the ability to degrade a particular subset of matrix proteins. Most members of the MMP family have three basic domains namely: an amino terminal propeptide domain, a catalytic domain and a hemopexin-like domain at the carboxy terminal.[2]

The propeptide domain consists of 80–90 amino acids containing a cysteine residue, which interacts with the catalytic zinc atom via its side chain thiol group. The catalytic domain consists of two zinc ions and one calcium ion. One of the two zinc ions is present in the active site and is essential for the proteolytic activity of MMPs. The second zinc ion, which is also known as structural zinc and the calcium ion are present in the catalytic domain approximately 12 A° away from the catalytic zinc. The hemopexin-like domain of MMPs is highly conserved and shows sequence similarity to the plasma protein, hemopexin. The hemopexin-like domain plays a functional role in substrate binding and also in interactions with the tissue inhibitors of metalloproteinases (TIMPs), a family of specific MMP inhibitors.[2,5]

In addition to these basic domains, certain structural and functional domains are incorporated and/or deleted in some family of MMPs. For example, MMP-2 and MMP-9 have incorporated three repeats homologous to the type-II module of fibronectin into the catalytic domain. These repeats involved in binding to denatured collagen or gelatin are known as the gelatin binding domain or fibronectin type-II like domain. This domain is unique to the gelatinases, and so these enzymes are considered as a separate subgroup among members of the MMP family. Another example is incorporation of a hydrophobic stretch of 25 amino acids at the carboxy terminus and a recognition motif for furin-like convertases at the end of the propeptide domain in MT-MMPs. The hydrophobic stretch is a transmembrane domain which localizes the MT-MMPs to the cell membrane, while the recognition motif provides an alternate cleavage site for MMP activation.[4]

Regulation of MMPs

The activity of the MMPs is regulated at multiple levels including transcription, activation and inhibition.

Transcriptional regulation

At the level of transcription, cytokines and growth factors like TNF-α, IL-1, EGF, bFGF and PDGF can induce the production of MMPs, depending on the situation and cell type. Certain hormones (parathormone, progesterone, glucocorticoids), chemical agents (phorbol esters) as well as cell–cell and cell–matrix interactions can induce or repress the expression of MMPs. All these extracellular stimuli regulate MMP activity through the activator protein-1 binding site, which is situated in the proximal promoter region in inducible MMP genes (MMPs-1, -3, -7, -9, -10, -12, -13 and -19).[4]

Proenzyme activation

MMPs are usually produced in latent, non-active form called zymogen. Activation is required for the enzyme function which involves removal of the prodomain. This can be achieved by several proteolytic enzymes, including serine proteinases together with other MMPs. MMP activation in vivo involves tissue and plasma proteinases and bacterial proteinases together with oxidative stress. MMPs are usually activated extracellularly or at the cell surface, for example, activation of MMP-2 by a MMP-2/TIMP-2/MT1-MMP complex. Several MMPs may also be activated intracellularly by furin or related proprotein convertases.[2,4]

Inhibitors of MMPs

MMP activity can be controlled by inhibition in two ways: non-specific endogenous inhibitors such as *a*2-macroglobulin, and by specific tissue inhibitors of MMPs, TIMPs. Currently, four TIMPs (TIMP 1–4) are known to be expressed in vertebrates. TIMP-1, -2 and -4 are secreted, while TIMP-3 is sequestred to the ECM. Binding of the TIMPs to the catalytic domain results in efficient inhibition of enzymatic

activity of MMPs. TIMPs inhibit MMPs by forming 1:1 stoichiometric enzyme-inhibitor complexes. In the case of gelatinases, the TIMPs have been shown to bind to the zymogen forms of the enzymes and this interaction provides an extra level of regulation by preventing activation. It has been shown that TIMP-2 forms a trimolecular complex on the surface of the cell with MT1-MMP and proMMP-2, and regulates the formation and levels of concentration of mature MMP-2.[4,5]

Role of MMP in oral diseases

1. Periodontitis

Periodontitis refers to an inflammation of the gingival tissues in association with loss of attachment of periodontal ligament and bony support. With progressive loss of attachment, significant destruction of the periodontal ligament and adjacent alveolar bone occurs.

Significant evidence exists that collagenases, along with other MMPs, play an important role in the periodontal destruction. It was initially assumed that collagenases in periodontal disease would originate from microbial sources, as pathogenic bacteria are always present in periodontitis. However, mammalian collagenases (MMP-1, -8 and -13) cleave native collagen at a single locus, resulting in formation of two distinct fragments, while the bacterial collagenolytic proteases attack collagen at multiple sites, producing many short peptide fragments. In periodontitis, two fragments of collagen were noted and hence, the collagenases are thought to be derived from the host cells rather than the microbes.[6]

Makela *et al* have shown that periodontitis patients had statistically higher levels of both MMP-9 and MMP-2 gelatinases than the healthy subjects. Previously it was thought that the expression of MMP-8 was limited to neutrophils, but at present it is clear that other cell types present in the normal and diseased human periodontium (sulcular epithelial cells, fibroblasts, endothelial cells, macrophages and plasma cells) can be induced to express distinct MMPs including MMP-8.[7]

The evaluation of disease activity before significant destruction and measurement of successful treatment emphasizes the need for a chair-side diagnostic test in periodontal diseases. A dipstick test for MMP-8, using monoclonal antibodies against MMP-8, is a sensitive, specific, rapid and practical immunological test in gingival crevicular fluid (GCF). The test can be performed by a dentist without specific equipment, and measures the GCF MMP-8 level in 5 minutes. It differentiates healthy and gingivitis sites from periodontitis sites and reduction of GCF MMP-8 levels can be observed after successful periodontal treatment. GCF MMP-8 level testing is a very useful tool to monitor the beneficial effects of adjunctive doxycycline-medication for periodontitis patients.[8]

2.Dental caries

Dental caries is defined as irreversible microbial disease characterized by demineralization of inorganic portion and destruction of organic portion of teeth, resulting in cavitation. Demineralization is caused by microbial acids, and progression of the lesion is accompanied by degradation of dentin organic matrix to the point beyond remineralization. Traditionally, microbial proteolytic enzymes have been held responsible for this matrix degradation, but researches have demonstrate the presence of both pro and active forms of MMP-8, 2 and 9 in human dentinal carious lesions. Since the active forms of MMPs are short-lived, the presence of active MMPs indicates activation in site, suggesting their active role in the dentin matrix degradation. This is further supported by the finding that the pH changes taking place in caries lesion are extremely powerful activators for MMPs. These findings formed a base to the theory of a sequencial demineralization-MMP activation and dentin matrix degradation taking place in the dentin lesion.[9]

There are two possible sources for the MMPs in caries lesions: saliva and GCF. MMPs are also produced by odontoblasts and they are present in mineralized human dentin. The dentin matrix protein-1, osteopontin and bone sialoprotein have a role in eliciting the functional activity of MMP proforms. In vitro and in vivo studies have demonstrated a hydrolytic loss of collagen fibers in and under the adhesive layers of composite restorations and MMPs present in

dentin have been implicated in the degradation.[6] Like in other inflamed tissues, MMPs are present in inflamed dental pulp tissue and periapical lesions. The level of MMP-8 in periapical exudates decreases after successful root canal treatment, while in cases with persistent inflammation the levels remain high, indicating that MMP-8 dip-stick analysis from periapical exudate could be used to monitor inflammatory activity and the success of treatment in teeth with periapical lesions.[10]

3. Oral cancer

Oral carcinogenesis is a complex, multistage process, where a normal cell undergoes genetic changes resulting in the ability to invade, and spread to, distant sites of the body. Interactions between the tumor and its microenvironment result in the production of proteolytic enzymes necessary for this process. In order for metastasis to occur, a cell must be able to detach from the primary tumor, and invade through BM and interstitial ECM. Furthermore, it has to respond to growth factors, proliferate as a secondary colony, induce angiogenesis and evade host defences.[4]

The initial observation of the importance of MMPs in cancer biology was that the ability of tumor cells to invade the surrounding tissue correlated with increased MMP levels. Subsequently, many MMP family members have been isolated from tumor cell lines or found to be over expressed in various tumor tissues. MMPs play a vital role in multiple stages of tumor progression, including growth, cell migration and angiogenesis.[6]

In SCC of the oral cavity, MMP-3 protein correlated with tumor size, invasion, and high incidence of lymph node metastases. The level of active MMP-2 may serve as a predictive marker of metastasis in oral SCCs. In addition, the high activity of MMP-2 and -9 correlated with the invasiveness of oral SCC and shorter disease-free survival after treatment. According to Sutinen et al, MMP-1 was detected in stromal fibroblasts and also in some neoplastic islands in oral SCCs.[11]

The metalloproteinases specifically degrading BM type IV collagen, MMP-2 and MMP-9 is up regulated in oral cancers. Since MMP-2 was secreted by numerous cultured malignant cell lines, it was originally speculated that it is the key enzyme in cancer growth and metastasis. Using immunohistochemistry and in situ hybridization, MMP-2 was, however, shown to be secreted not by the carcinoma cells but by the surrounding stromal fibroblasts. Unlike MMP-2, MMP-9 (gelatinase-B) is mainly synthesized by carcinoma and inflammatory cells of the carcinoma tissue.[12]

Matrix metalloproteinases enable tumor angiogenesis as they allow endothelial cells to invade through BMs to form new blood vessels. They also regulate endothelial cell attachment, proliferation, migration and growth, either directly or by the release of growth factors. Endothelial cells can produce at least MMP-1, -2, -9, -19 and MT1-MMP, while inflammatory cells and fibroblasts express many MMPs and also contribute to angiogenic phenotype. For instance, infiltration by mast cells and activation of MMP-9 coincides with the 'angiogenic switch' in premalignant lesions during squamous epithelial carcinogenesis.[13] Endostatin is an antiangiogenic factor derived proteolytically from hemidesmosomal type XVIII collagen. Endostatin inhibits in vivo invasive capacity of tongue carcinoma cells by inhibiting the activation of MMP-2, 9 and 13. This reveals that endostatin acts at two levels in oral cancer growth, by inhibiting endothelial cell growth and by reducing cancer cell capacity to modulate surrounding matrix by active MMPs.[14]

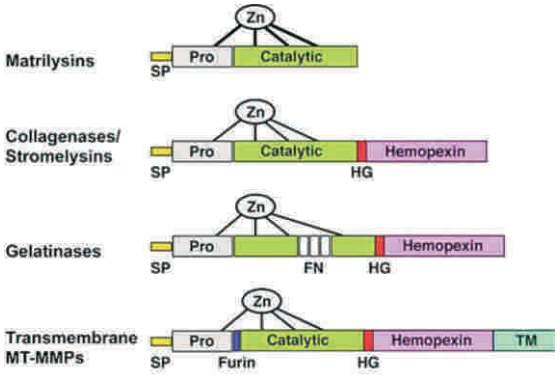

Fig 1: Structure of MMP

(SP-signal peptide, Pro-prodomain, Zn-zinc,

HG – hinge domain, FN – fibronectin like domain,

TM – transmembrane domain)

Conclusion

Besides the classic role of MMPs in the degradation of ECM in tumorigenesis, new roles are emerging that make the contribution of MMPs much more complex than initially thought. For instance, MMPs cleave receptors involved in cell adhesion, unmask cryptic sites of interaction, activate growth factors, and act on ECM components or other proteins to uncover hidden biologic activities that can affect cell proliferation, migration and angiogenesis. Therefore, the possible usefulness of specifically selected MMP inhibitors, like CTT-peptide in combination with other cytotoxic cancer drugs or bisphosponates would be worthy of investigation in treating oral cancer patients.

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