Matrix Metalloproteinases in Cancer and in Periodontal diseases.

BMS Essay For Master's

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MMPs in Cancer and in Periodontal Diseases.

The Matrix Metalloproteinases (MMPs) are family of highly homologous zinc dependent proteinases that collectively cleave most, if not all components of the extracelluler matrix (ECM). These proteinases are involved in serious pathogenesis processes such as periodontal diseases, arthritis, cancer, atherosclerosis, pulmonary emphysema and osteoporosis. In this essay I will mostly emphasize the role of MMPs in periodontal diseases. In periodontal diseases, gingival sulcular epithelium expresses several, rather than single, collagenolytic MMPs. Similarly, the MMPs are overexpressed in a variety of malignant tumor types, and their overexpression is associated with tumor aggressiveness and metastatic potential. The specific alteration of the MMPs observed in malignant tissues and their participation in some of the major oncogenic mechanisms have fueled interest in the design and evaluation of MMP inhibitors as anticancer agents. It will be most interesting if we can use these inhibitors in periodontal diseases. This expectation is supported by the inhibitory effect of tetracycline analogues as MMP inhibitors, which are being used in periodontal treatment as well in the treatment of colon cancer.

The human MMP gene family consists of at least 18 structurally related members. These MPPs are grouped into five subclasses: Collagenases (MMP-1, MMP-8 & MMP-13), gelatinases (MMP-2 & MMP-9), stromelysins (MMP-3, MMP-7, MMP-10, and MMP-11), membrane type (MT) - MMPs and non-classified MMPs (Table1). This scheme is loosely based on substrate specificities. This classification is satisfactory for the collagens of type 1, 2 and 3, but it is not satisfactory for the gelatinases, referred to also as type 4 collagenases, because stromelysin digests type 4 collagen better than the gelatinases.

Table 1. Matrix matalloproteinase (MMP) family

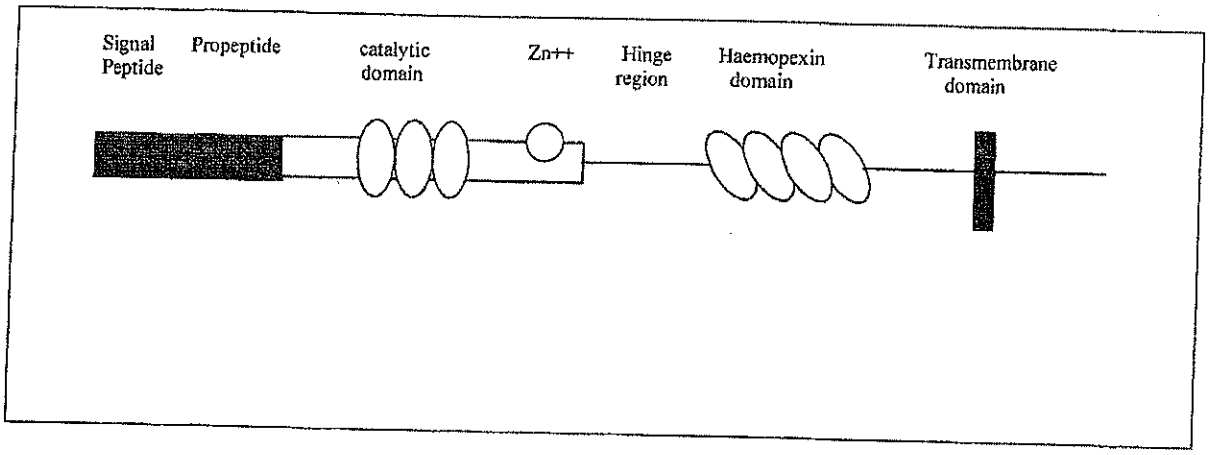
Group	MMP	Substrate(s)
Interstitial collagenase	MMP-1	Collagen types 1, 2, 3, 7,& 10
Neutropil collagenase	MMP-8	Collagen types 1, 2, 3, 7,& 10
Collagenase-3	MMP-13	Collagen types 1, 2, 3, 7,& 10
Gelatinase A	MMP-2	Gelatinase types 1, 4, 5 & 10; Laminin 5
Gelatinase B	MMP-9	Gelatinase types 1, 4, 5 & 10; Laminin 5
Matrilysin	MMP-7	Gelatin, fibronectin & pro-MMP-1
Stromelysin-1	MMP-3	Collagen types 3, 4, 9, & 10; gelatin; pro-MMP-1; laminin; & proteoglycan
Stromelysin-2	MMP-10	Collagen types 3, 4, 9, & 10; gelatin; pro-MMP-1; laminin; & proteoglycan
Stromelysin-3	MMP-11	α-1 antiprotease
Metalloelastase	MMP-12	Elastin

MT1-MMP	MMP-14	Pro-MMP-2, gelatin & collagens
MT2-MMP	MMP-15	Pro-MMP-2
MT3-MMP	MMP-16	Pro-MMP-2
MT4-MMP	MMP-17	Not Known
RASI-1	Collagenase 4	Not Known
Enamelysin	MMP-20	Not Known
	MT5-MMP	Not Known

The characteristics of the matrix metalloproteinases are:

1. The catalytic mechanism depends on zinc at the active center.
2. The proteinases are secreted in zymogen form.
3. The zymogen can be activated by proteinases or by organomercurials.
4. The cDNA sequences have homology with that of collagenase.
5. The enzymes cleave one or more components of the extracellular matrix (ECM).
6. Activity of the activated MMP is also inhibited by tissue inhibitors of metalloproteinases (TIMP). Fig1 illustrates its domain structure.

Figure1: The domain structure of human MMP



The general structure contains a signal peptide, a propeptide domain, a catalytic domain with a highly conserved zinc-binding site, and a hemopexin-like domain linked to the catalytic domain by a hinge region. Then finally a transmembrane domain at the c-terminal end of the haemopexin domain. And it represents a characteristic sequence of the membrane type MMPs (MT-MMPs). This transmembrane domain is absent in the matrilysin (MMP-7), and MMP-2 and MMP-9 contain fibronectin type 2 inserts within the catalytic domain.

Propeptide domain: The function of the propeptide is to maintain latency of the MMPs until a signal for activation is given. Evidence shows that the propeptide functions by virtue of the cysteine switch mechanism, involving the coordination of cys [73]
in the conserved sequence to the active center zinc atom.

Hemopexin/Vitronectin domain: There are four repeats in this region that have weak homology to hemopexin and vironectin. The amino end contains a hinge region and a disulfide bridge connects the extreme ends of this domain. The function of the domain is not completely clear, and, indeed, matrilysin and macrophage elastase function perfectly without this domain. Interstitial collagenase (MMP-1) has good catalytic activity without

this domain (enzymatically or mutagenically removed), but it loses its ability to digest the triple helix of collagen. This is also true for neutrophil collagenase (MMP-8). Various chimeras have been produced by manipulation of the cDNA whereby the C-terminal domains of various matrixins are interchanged. The C-domain of interstitial collagenase can cause the catalytic domain of stromelysin-1 to bind to collagen. C-domain also fails to digest collagen. However, collagenase activity depends not only on the C-domain but on specific sequences within the zinc-binding domain[1].

These MPPs are not stored in cells (except for neutrophils), but are secreted as inactive or latent proenzymes (also known as zymogens). In the activation process, the zymogen loses its signal peptide. The cysteine in the propeptide domain contacts zinc to maintain MMP latency. During activation, the propeptide domain is cleaved in several steps. Conversion from latency to active form can be mediated by physical means (chaotrpic agents and detergents), or chemical means (oxidants and organomeravials) or enzymatic means (trypsin, plasmin and other proteinases). Physical agents can unfold the structure to expose zinc, the chemical agents may react with sulfhydryl groups to inactivate cysteine or proteolytic enzymes can cleave the propeptide, even ahead of the cysteine. Sorsa et al [2] proposed that the neutrophil MMPs are primarily activated by oxidative pathways. PMN activation generates hydrogen peroxide (H_2O_2), which in the presence of Cl^-, is converted by myeloperoxidase to hypchlorous acid. This reactive oxygen species activates the pro-MMP. In contrast, the fibroblast MMP is primarily activated by proteinases such as stromelysin and plasmin. Pathology results when activation of excessive amounts of enzyme occurs and the level of active enzymes

exceeds that of endogenous inhibitors (e.g. TIMPs). It is clear that the control of extracellular proteolysis is critically important.

Regulation:

MMPs are highly regulated at the levels of both gene expression and protein activation. In general these genes are not expressed constitutively in vivo and the basal production of MMP in cell cultures is low. MMP gene transcription is induced by a variety of extracellular stimuli, such as cytokines (IL-4, IL-10), growth factors (EGF, TGF-2), basic fibroblastic growth factor and cell-cell or cell-matrix interactions.

Most MMPs are secreted as latent precursors then they are activated in the extracellular space. The proteolytic activity of these MMPs can be inhibited either by nonspecific protease inhibitors (e.g. α_2-macroglobulin) or by the specific tissue inhibitors of the metalloproteinase (TIMPs)[3]. The TIMPs are family of four structurally related proteins (TIMP-1, -2, -3and-4) which exert a dual control on the MMPs by inhibiting both the active form of the MMPs and their activation process[3]. The TIMP and α-macroglobulins bind in a noncovalent fashion to inhibit members of the MMP family. TIMPs control MMP activities pericellularly, whereas the α_2-macroglobulin functions as a regulator of MMPs in body fluids. During inflammation, however, this large molecular weight protein may escape the vasculature and function in the ECM. Other than the natural inhibitors there is a synthetic inhibitors such as Zn^{2+} and Ca^{2+} chelating agents. Zn^{2+} and Ca^{2+} chelating agents are potent inhibitors of enzyme activity in vivo, but they are toxic and not used in vivo as therapeutic agents[4]. The only proteinase inhibitors that have been tested for efficacy in periodontal therapy is tetracycline analogous.

MMPs in cancer:

MMP expression is almost undetectable in most normal tissue, but is considerably increased in the malignant tumors. In addition to the overproduction and activation of MMPs in malignant tissue, there is now ample clinical evidence that overproduction of these molecules confers a poor prognosis in patients with a variety of cancer [5,7].

Whether specific member (s) of the MMP family are associated with oncogenesis is still unknown. In general, the gelatinases (MMP-2 and MMP-9) have been consistently detected in malignant tissues and seem to be indicator of tumor aggressiveness, metastatic potential, and a poor prognosis. Recently, matrilysin (MMP-7) seems to be a target for chemoprventive strategies [8,9].

The Regulation of Cytokines and Growth Factors:

First we have to explain the meaning of cytokines and growth factors. Cytokines are non-antibody proteins secreted by inflammatory leukocytes and some non-leukocytic cells, that act as intracellular mediators. They differ from classical hormones in that they are produced by a number of tissue or cell types rather than by specialized glands. They generally act locally in a paracrine or autocrine rather than endocrine manner.

Growth factors are complex family of polypeptides hormones or biological factors that are produced by the body to control growth division and maturation of blood cells by the bone marrow. They regulate the division and proliferation of cells and influence the growth rate of some cancers. The factors occur naturally but some can be synthesized using molecular biology techniques and are used clinically to stimulate normal white cell

production following chemotherapy or bone marrow transplantation. Cytokines and growth factors are endogenous transcriptional regulators secreted by inflammatory and mesenchymal cells. Generally they stimulate enzyme production. Each of the major cell types in the periodontal tissue is capable of producing a battery of MMPs when activated by a number of cytokines and growth factors. It was suggested that MMP-9 (gelatinase) is one of the major proteins produced by osteoclasts that mediate collagen degradation during bone resorption. The signals transmitted by the various cells in the periodontal lesion tend to amplify the production of MMPs.

Matrix metalloproteinase Inhibition:

The role of MMPs inhibitors is important because it is the imbalance between activated MMPs and their inhibitors that leads to pathologic breakdown of ECM in diseases such as periodontitis, arthritis, and cancer invasion. The proteolytic activity of MMPs is inhibited by non specific protease inhibitors, such as α2-macroglobulin and α-1 antiprotease, and by the specific tissue inhibitors of the metalloproteinases (TIMP). Tissue inhibitors of MMPs are widely distributed in fluids and tissues and are expressed by fibroblasts, Keratinocytes, endothelial cells, monocytes, and macrophages. The TIMPs are capable of inhibiting all of the active MMPs, binding strongly to their active site domains, and they may also prevent activation of latent forms of some of the MMPs. TIMP-1 is a glycoprotein, whereas TIMP-2 is its unglycosylated counterpart[10]. TIMP-3 has only recently been isolated. TIMPs can be activated by reduction and alkylation[11] and by serine proteinase proteolysis[12]. TIMP-1 is highly associated with the 92-KD gelatinase

(MMP-9) and fibroblast collagenase (MMP-1), whereas TIMP-2 is associated with the 72-KD form (MMP-2) [13].

The activity of matrix Metalloproteinase in periodontal disease:

The role of MMPs in periodontal destruction is strong and has been supported by a number of findings, including the production of elevated levels of collgenase by diseased gingival tissues in culture[14]. The detection of elevated levels of active rather than latent collagenase in the fluid of the periodontal pocket and in extracts of the adjacent gingival tissue[14, 15], inflammatory cells, particularly neutrophils, are also thought to play a major role in the MMP-mediated destructive lesion[16,17]. Additional evidence for this pathogenic pathway is the presence of elevated MMP protein in periodontal lesions supported by immunohistochemical studies[18, 19]. Moreover, the ability of MMP inhibitors such as doxycycline to retard periodontal breakdown further supports the pathologic role of these proteinases. Currently, some controversy exists concerning the origin of this excess collagenase in the periodontal pocket: Does it arise primarily from infiltrating PMNs? In this case, factors that regulate chemotaxis and degranulation would be of paramount interest. Or does it also involve excess expression of fibroblast-type MMPs (MMP-1 and MMP-2), which are produced by resident (fibroblast and epithelial) and infiltrating (macrophage) cells? Intracellular regulation by cytokines and growth factors would then be utmost importance. Moreover, the PMN-type and fibroblast-type MMPs may also respond differently in the ECM to factors that activate their respective zymogens (eg, proteolytic activation for fibroblast-type and oxidation activation for PMN-type procollagenase) and to inhibitors as well[20].

Conclusion:

It is an important coincidence that MMP inhibitors are now promising drugs in the treatment of patients with cancer as well as in periodontal treatment. This understanding has stimulated the new therapeutic approaches to a variety of medical disorders characterized by excess MMP activity. MMP inhibitors like batimastat and matimastat are currently being used in clinical trials for inhibition of cancer metastasis and other diseases. The nonantimicrobial low doses of the chemically modified tetracyclines are also being used as MMP inhibitors in the gingival tissue and gingival crevicular fluid with a resultant reduction in tooth eruption and periodontitis.

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