MATRIX METALLOPROTEINASE AND THEIR INHIBITORS: MOLECULAR ASPECTS OF THEIR ROLES IN THE TUMOR METASTASIS

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ABSTRACT

The matrix metalloproteinases (MMPs) are a family of proteolytic enzymes, whose physiological functions include tissue remo-delling and embryogenesis. The importance of this group of proteins in the processes of tumor invasion and metastasis is now widely acknowledged, and has led to the search for MMP inhibitors for use as anticancer treatments in a clinical setting. The review aims to introduce current research relating to MMPs as well as their native and synthetic inhibitor, with particular emphasis on the molecular aspects of their roles in tumor metastasis.

Key words: Matrix metalloproteinase; Tissue inhibitor of matrix metalloproteinase; Tumor; Gene regulation

Tumor cell invasion and metastasis are now regarded as multi-step phenomena, involving proteolytic degradation of extracellular matrix (ECM), altered cell adhesion, physical movement of tumor cells and angiogenesis.

Extracellular proteinases are essential for tumor cells to be able to penetrate the basement memberane. Proteolytic degradation of ECM is also necessary when invasive tumor cells penetrate tissue, gain access to blood vessels and colonize distant sites (metastasis). In addition, angiogenesis involves proteolytic degradation of ECM by invasive endothelial cells.

There are four classes of proteinases that play roles in ECM degradation: serine, cysteine, aspartyl and matrix metalloproteinases(MMPs). MMPs are one of the four major classes of proteinases and thought to play a central role in the evolution of cancer. The expression of most MMPs is transcriptionally regulated by growth factors, hormones, cytokines[1, 2]. The proteolytic activities of MMPs are precisely controlled during activation from their precursors and inhibition by endogenous inhibitors -- tissue inhibitors of metalloproteinases (TIMPs). This review focuses on recent progress for the characters of MMPs and their inhibitors in the tumor metastasis, with emphasis on recent findings regarding the regulation of MMPs and the new drug design based on the MMPs and TIMPs.

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The Family of MMPs

Up to now, 26 members of MMPs family have been identified, the common features of this family include following: 1) structurally contain a zinc atom at their active site. 2) Often produced in an inactive form. 3) Primary structure typically contains two highly conserved regions, one is the N-terminal propetide domain and the other is the catalytic domain. 4) Inhibited by specific inhibitors known as tissue inhibitors of metalloproteinases(TIMP).

The MMPs family can be classified into five groups on the basic of sequence homology and substrate specificity. Table I lists currently known[3] vertebrate MMPs.

The first group consists of four collagenases : interstitial collagenase, neutrophil collegenase, collegenase 3 and 4, they can specially degrade type Ⅰ, Ⅱ, Ⅲ and fibrillar collagens . The second group consist of the 72 k Da and 92 k Da gelatinases , also referred to as type Ⅳ collegenase A and B, and their substrate specificity is very broad. The third group of MMPs consists of two closely related enzymes, stromelysin-1,2 and a more than distantly related enzyme matrilysin (also called putative uterine metalloproteinase, PUMP).They degrade a wide

variety of proteoglycans, fibronectin, laminin, as well as type Ⅳ collagen and type X collagen.

The fourth group is the group whose gene structures and functions have not been clearly studied. Stromelysin 3 despite its name, dose not fit the third group. mRNA in situ hybridization studies suggests that stromelysin-3 is important in malignant disease, and the substrate of stromelysin 3 is also different to the third group. Some newly discovered MMPs[4,5] such as MMP 19, 20, 21, 22, 23, 26 are not clearly studied, so they were classified to the fourth group. The fifth group is membrane-bound metallop-proteinase (MT-MMP) with recognized transmem brane domain, and they appear to specifically activate progelatinase A(MMP-2)[6,7]. Up to date, there are six newly discovered members of membrane-type (MT-MMP)[8,9,10,11]. MT-MMPs are the only MMPs so far identified that are not secreted products.

Table 1. Matrix Metalloproteinase Family

Matrix Metalloproeinase	MMP number	substrate or peculiarity
Class I		
Interstitial collegenase	1	Fibrillar collegens, type Ⅰ, Ⅱ, Ⅲ
Neutrophil (PMN) collegenase	8	Fibrillar collegens, type Ⅰ, Ⅱ, Ⅲ
Collegenase 3	13	Fibrillar collegens, type Ⅰ, Ⅱ, Ⅲ
Collagenase 4	18	some similar to Stromelysins
Class Ⅱ		
Gelatinase A (72 kDa)	2	collagen types Ⅳ, Ⅴ, gelatin
Gelatinase B (92 kDa)	9	collagen types Ⅳ, Ⅴ, gelatin
Metalloelatase	12	Elastin
Class Ⅲ		
Stromelysin-1	3	Laminin, fibronectin, proteoglycans
Stromelysin-2	10	Laminin, fibronectin, proteoglycans
Matrilysin (pump)	7	Laminin, fibronectin, proteoglycans
Class Ⅳ		
Stromelysin-3	11	1-antritrypsin
(No trivial name)	19	Rheumatoid arthritis-associated MMP(not clear)
Enamelysin	20	(not clear)
XMMP	21	Recently cloned MMP
CMMP (chicken)	22	Recently cloned MMP
(No trivial name)	23	new MMP, special in reproductive organs
Endometase	26	type I gelatin, related to Endometrial Tumor
Class Ⅴ		
Memberane-type		
MT1 2 3 4 5 6-MMP	14, 15, 16, 17, 24, 25	pro-gelatinase A and other unknown

Matrix Metalloproteinases and Tumor Metastasis

The expression of matrix metalloproteinase and the cancer

Normally, the degradative activity of MMPs is tightly controlled both by the latency of the secreted enzymes as well as by the presence of naturally occurring inhibitors (TIMPs). However, in several disease such as arthritis and cancer. there appears to be a local and temporal imbalance between the levels of activated enzymes and their inhibitors. Based on our and other research group's results, in table 4, we show the expression changes of gelatinases A and B and their inhibitors in some kinds of tumor.

Several studies have shown that in cancer models the proportion of active MMPs overwhelms the local inhibitory activity surrounding the tumor. This MMP activity facilitates the direct expansion and local invasion of the primary tumor, facilitates the movement of tumor cells across the vascular basement membrane, make the local growth and invasion of any secondary tumor. MMP activity also contribute to the invasive growth of new blood vessels, which is a requisite for malignant tumor growth[13].

High levels of activated gelatinase A were

demonstrated in invasive cancer of breast, while the ratio of activated to latent gelatinase A was significantly higher in malignant versus benign breast cancer lesions and a higher proportion of activated enzyme was related to increasing tumor grade[14]. The expression of gelatinase A was correlated with the progression of colorectal and gastric cancer; gelatinase A was also found to be higher in invasive ovarian cancer than in benign ovary, and was particularly intense around micro-invasive cellular clusters.

The concentration of gelatinase A and B were significantly elevated in sera of lung cancer patients as compared to normal sera, and levels also were significantly higher in patients with distant metastases versus those without distant metastases. In non-small cell lung cancer tissue,there was also a highly significant correlation between the level of expression of gelatinase A and histopathological evidence of tumor spread.

Table. 2. The cancer related to imbalance of gelatinase

Cancer	Change of Enzyme
Breast cancer	Ratio of activated to altent enzyme ↑In malignant versus benign tumor
Colorectal, gastric cancer	The expression of enzyme ↑ , correlated with the tumor progression
Ovarian cancer	The enzyme activity is higher in the invasive ovarian cancer cells
Lung cancer	The concentration of MMP2, 9 in patients serum ↑ distant metastases versus un-metastases
Skin cancer	The expression of MMP 9 mRNA is detected in the squamous cancer cells ↑

*(↑ means "increase")

The Gene Regulation of MMPs Expressions

MMPs can be induced by phorbol esters, interleukin 1 (IL-1) and tumor necrosis factor (TNF-a) due to their ability to bind AP-1 elements localized in the MMP promoters. Recent studies not only emphasize on above mentioned soluble factors but also cell-matrix and cell-cell interactions as keys in gene expression of MMPs.

For examples : induction of MMP-1, -2, and -3 in fibroblasts by EMMPRIN (M6 antigen), a member of the immunoglobulin family expressed on tumor cell surface [15]; induction of MMP-9 in T lymphoma cells through leukocyte function-associated antigen-1 (LFA-1)-intercellular adhesion molecule-1 (ICAM-1)-mediated cell adhesion[16]; induction of MMP-2 in T cells through very late antigen 4 (VLA-4)-vascular cell adhesion molecule-1 (VCAM-1)-mediated adhesion to endothelial cells. In addition, our research indicated that MMP-2 and 9 can be induced by the surrounding matrix protein such as fibrinectin in the ovarian cell lines[17].

To sum up, some tumor cells-derived factor can stimulate normal cells to produce matrix metalloproteinase . This suggested tumor cells can trigger the production of proteinases by surrounding stromal cells. So, the ways for inhibiting tumor cell proteolysis are very potential therapeutic strategy to inhibit the tumor metastasis.

Native MMPs Inhibitor (TIMPs) and Synthetic MMPs Inhibitor (MMPIs)

Inhibition Mechanism of TIMPs and MMPIs:

The vertebrates TIMPs that have been characterized appear to define a family of four members: TIMP 1,2,3 and 4, named in order of their discovery. (see table 3). Their sequences have a highly conserved secondary structure imposed on the protein by six conserved disulfide bonds. There appeared to be two domains in the TIMP molecule: N-terminal domain possessing the metalloproteinase-inhibiting activity and C-terminal domain, perhaps important in protein location or complex formation with the progelatinases.

The crystal structure of the complex formed between TIMP-1 and the catalytic domain of MMP-3 was determined by Gomis-Rüth et al. [18]. The critical residues involved in MMP inhibition are located around the disulfide bond between Cys1 and Cys70 (Fig. 1). The N-terminal -amino and carbonyl groups of Cys1 bidentately coordinate the catalytic Zn2+. The N-terminal segment Cys1-Thr-Cys-Val-Pro5 binds to the Ser68 and Val69 active site cleft of the MMP-3 enzyme. The side chain of Thr2 extends into the large S1' specificity pocket of MMP-3.

The native inhibitors TIMP-1,2,3,4 are potent[19] broad spectrum inhibitors (such as inhibit collagenases, stromelysins and gelatinases) and have been in development as therapeutic agents since their identification[20] and sequencing. However, the therapeutic use of these proteins is likely to de limited by their low oral bio-availability, and their size may also limit tissue penetration. Presently, efforts are underway to characterize the functional domains of

the TIMP molecules hopefully yielding new synthetic or recombinant approaches.

Synthetic matrix metalloproteinase inhibitors (MMPIs) have been developed since the early 1980s. The common MMPIs include: Bastimatat (also called BB94), SC44463, Marimastat and CGS 27023. The majority of these inhibitors are substituted peptide derivatives, analogues of the cleavage site in the collagen molecule with a metal-binding group in the position of the cleaved peptide bond. The metal binding group, commonly a thiol, sulphydryl, carboxyl or hydroxamate group, binds to the zinc atom in the activity site of the matrix metallopoteinase. Potencies have been reported in the low nano-molar range. However , this agents have too broad spectrum inhibition , the low selective ability make their serious side-effects. In the future, the selective inhibitors are likely to be pursued.

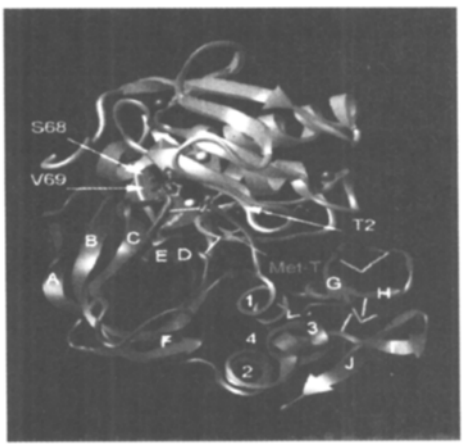

Fig 1: Ribbon diagram of the complex of TIMP-1 and the catalytic domain of MMP-3.

The image was prepared from the Brookhaven Protein Data Bank entry (1UEA), MMP-3 is shown in silver at the above, TIMP-1 is in red at the down. The purple spheres at the center indicates the zinc ions of the catalytic domain.

The Pre-clinical Studies of the Biological Activity of the Inhibitors of MMPs

Both native and synthetic inhibitors have been considered for therapeutic aims. Intra-peritoneal administration of recombinant TIMP-1 and TIMP-2 inhibited the colonization of lungs by both B16-F10 murine melanoma cells and ras-transfected rat embryo 4R cells [21]. Similar results were obtained with the synthetic MMPI SC44463 and BB94, which yielded more than 80% inhibition of lung colonization and tumor growth by B16-F10 cells[22] . These agents inhibit both the number and median weights of lung colonies of rat carcinoma cells. This implied the effect not only on seeding efficiency but also on the subsequent tumor growth.

By preventing remolding of the extracellular matrix, both native TIMPs and syntheses MMPIs can act as anti-tumor agents. In addition, the anti-tumor action appears to underlie another mechanism by which the inhibitors can exert an anti-angiogenic effect. In vitro studies have shown that TIMP-1 can inhibit the invasion of extra-cellular matrix by capillary endothelial cells. TIMP-1 has also been shown to inhibit tumor-induced vascularization of the rabbit cornea, presumably by blocking the remodeling of the extra-cellular matrix that precedes new capillary growth. These more recent studies broaden the ranges of the inhibitors' action [23].

From the above review, we could conclude that the ways to inhibit the MMPs activity of tumor cell have significant value. Native TIMPs and synthesis MMPIs not only inhibit the invasive tumor growth and angiogenesis, but also inhibit steps in the whole metastatic cascade.

Table 3. TIMP family

Name	TIMP-1	TIMP-2	TIMP-3	TIMP-4
Molecular mass	28 kd	21 kd	24 kd	22 kd
Messenger RNA	0.9 kb	1.1/3.5 kb	4.5-5.0 kb	1.4 kb
Associated proteins	pro-MMP9	pro-MMP2	ECM	not determined
Major sites of ovary expression	bone	placenta	kidney	heart

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