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Review

MATRIX METALLOPROTEINASES IN TUMOR BIOLOGY - A SPECIAL ATTENTION ON THEIR ROLE IN HNSCC

A. Anastasov, T. Vlaykova*

Department of Chemistry and Biochemistry, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

ABSTRACT

Contemporary fundamental investigations in the field of oncology are focused on understanding of molecule mechanisms of tumor development, progression and metastasizing. These processes involve controlled and directed proteolysis of the components of basal membrane (BM) and extracellular matrix (ECM) that is catalyzed by variety of proteolytic enzymes, including matrix metalloproteinases (MMPs). MMPs are a large family of Zn-dependent neutral endopeptidases and their basic activity is the degradation of matrix proteins. In addition they cleave diversity of non-matrix proteins, such as growth factors, signal and receptor molecules, thus modifying indirectly the cellular and tissue behavior.

Based on their substrate specificity and structural organization they are subgrouped into collagenases, stromelysins, gelatinases, matrilysins, and membrane-type matrix metalloproteinases.

The MMP activity is very strictly controlled at the level of gene transcription, latent zymogene activation, and inhibition by endogeneous inhibitors. MMPs are synthesized in non-active latent zymogene form (proMMPs) and are activated after secretion. The proteolytic activity of MMPs is controlled by the members of a family of anti-proteinases known as tissue inhibitors of metalloproteinases (TIMPs) and other non-specific inhibitors.

The expression and activity is strictly controlled by signal transduction on the level of transcription and maturation and is affected by variety of factors, including genetic. So far, several polymorphisms in the promoter regions of MMP genes have been identified that influence the transcription activity of MMP genes and protein levels of the enzymes.

The current review attempts to summarize the information about the role of MMPs and their inhibitors in development, growth, invasion, tumor angiogenesis and metastasizing of neoplastic diseases, particularly in squamous cell carcinoma of head and neck (HNSCC).

Key words: MMP, tumor biology, polymorphisms, HNSCC

INTRODUCTION

MMPs are family of highly homologous extracellular Zn^{2+} dependent neutral endopeptidases, which are also known as matrixins. Recent studies have indicated that some MMPs are found also intracellulary and may act on intracellular proteins (1, 2). Metal ions and mainly zinc ions play a crucial role in the catalytic process. MMPs basic activity is the degradation of the components of the basal lamina or extracellular matrix proteins. MMPs are naturally inhibited by specific inhibitors called tissue inhibitors of metalloproteinases, TIMPs (2-5). In addition, metal chelators can also inhibit MMPs. MMPs are active at physiological pH and they are secreted as zymogens, which require extracellular activation (2-6).

***Correspondence to:** *Tatyana Vlaykova*
Dept. Chemistry and Biochemistry, Medical Faculty, Trakia University, Stara Zagora, Bulgaria, e-mail: tvlaykov@mf.uni-sz.bg

1. Classification and action

The family of MMPs consists of more than 20 members (currently 23 in humans), which are differ in substrate specificity, regulation and potential interactions with additional MMP and TIMP family members (3, 4, 7). On the basis of their substrate specificity the MMPs can be sorted into five groups: collagenases (MMP-1,

-8 and -13), stromelysins (MMP-3, -10 and -11), gelatinases (MMP-2 and -9), matrilysins (MMP-7 and -26), and membrane-type matrix metalloproteinases (MT-MMPs)(1, 2, 5).

Other classification of MMPs is based on their structure; so those are formed new eight subgroups (5, 8). Five of these groups contain secreted MMPs and three groups contain membrane-bound MMPs. The group of secreted MMPs includes the following members: MMP-1, 2, 3, 8÷13, 18, 19, 20, 21, 23, 26, 27 and 28. The membrane-bound MMPs are: MMP-14, 15, 16, 24, 17 and 25.

Matrix metalloproteinases are able to degrade virtually all protein components of the extracellular matrix (ECM), basal lamina, clotting factors, cell-cell and cell-matrix adhesion molecules, cell-membrane precursor forms of growth factors, growth factor binding proteins, growth factor receptors, other proteinases and proteinase inhibitors, as well as their own inactive zymogene forms (3-5, 7, 8). That is why, they are considered to intervene in the metabolism of normal interstitium. In general MMPs have different substrate specificities and preferences: for an example, collagenases cleave native collagens into small fragments, which are spontaneously denatured into gelatin and are further degraded by other MMPs, *e.g.* gelatinases (3-5, 7, 8).

2. Structure

All proMMPs contain at least three conserved domains. The first one is a hydrophobic signal peptide at the N-terminal end that targets the secretion of non-membrane bound proMMPs and is subsequently removed from the latent enzyme. The second domain is an amino terminal propeptide containing a conserved cysteine residue into "cysteine switch" motif. The cysteine residue forms a covalent bound with the catalytic zinc ion, thus maintaining the inactive latent state of the proMMPs and this domain is removed from the latent MMPs upon activation. The third domain is the catalytic one and contains a highly conserved zinc binding sequence. Glutamate- and aspartic acid-rich sequences at the flanking sides of this domain are thought to bind calcium ion. Besides the basic three domains, at the C-terminal end of most of the MMP molecules there is a hemopexin domain, which is connected to the catalytic domain via a proline-rich linker peptide, called "hinge region" (3-5, 7, 8).The hemopexin domain is the binding site for TIMPs and has an important role in regulation of substrate specificity and proteolytic activity (9). In particular, hemopexin domain is absolutely required in collagenases (MMP-1, MMP-8,

MMP-13) for cleavage of the triple helical interstitial collagens (3-5, 7, 8, 10).Exceptions of this structure are the molecules of matrilysins (MMP-7 and MMP-26) and MMP-23; they do not contain the linker peptide and the hemopexin domain (1, 2).

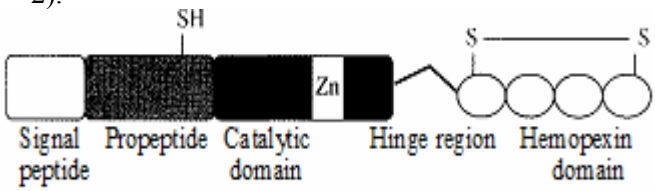

Figure 1. Schematic representation of the general structure of MMPs (5).

A majority of MMPs contain the above described five domains and belong to the subgroup of simple hemopexin-domain-containing MMPs (MMP-**1,** MMP-3, MMP-8, MMP-10, MMP-12, MMP-13, MMP-19, MMP-20, MMP-27)**.** Although, these five domains are fundamental, some MMPs have distinct structural variations; the gelatinases (MMP-2 and -9) contain in their catalytic domain three fibronectin type II inserts that are important for collagen binding; and MMP-9 has an extra collagen V-like sequence within the catalytic domain downstream of the Zn^{2+} binding (1-5, 7, 8, 11).

The furin-activated secreted MMPs (MMP-11, 28 and 21) as well as MMP- 23, and all membrane-type MMPs have an alternative proteolytic activation site – a recognition motif for furin-like enzymes, called a furin cleavage site, located between the propeptide and the catalytic domains (1, 2, 5, 11). Only one of the MMPs (MMP-21) contains an additional vitronectin-like insert next to the furin cleavage site (5, 12).

The membrane-type MMPs (MMP-14, -15, -16 and -24) are a unique subdivision in that they contain, in addition to the five domains and the furin cleavage site, a transmembrane domain accompanied by a short cytoplasmic domain anchoring the molecule to the cell surface (3, 5, 11, 13). Instead of the transmembrane and cytoplasmic domains the GPI-anchored MMPs contain a glycosylphosphatidylinositol (GPl)-

anchoring domain (MMP-17 and MMP-25) (3, 5, 11)..

MMP-23 is a type II transmembrane MMP, because it has a quite different structure from the other MMPs. At the N-terminus it has a cytoplasmic tail and signal anchor that targets it to the cell membrane, whereas in the C-terminus there is a unique cysteine- and proline-rich array and an Ig-like domain (4, 5, 8, 14, 15).

3. Regulation of MMP activity

The MMP activity is very strictly controlled at the level of gene transcription, latent zymogen activation, interaction with specific ECM components and inhibition by endogeneous inhibitors (1, 2, 11, 16, 17).

The latent zymogens are activated when the interaction of Zn^{2+} in the active site with cysteine residue in the propepride domain is cleaved. A variety of enzymes have the ability to activate the MMP proenzymes: trypsine 2, catepsines G, B and L, PMN elastase, plasminogen activators etc. Once MMPs are activated they also have capability of activating themselves and other proMMPs (3). An important role in activation of secreted proMMPs, particularly, proMMP-2 and proMMP-13, have the MT-MMPs (3, 15).

The proteolytic activity of MMPs is controlled by the members of a family of anti-proteinases known as tissue inhibitors of metalloproteinases (TIMPs). The TIMP gene family consists of 4 members: TIMPT-1, -2, -3 and -4. They can inhibit the activity of all members of MMP family by binding to the conserved Zn-binding sequence. They also can inhibit the activation of several zymogens (15). The local balance between MMPs and TIMPs is a major factor involved in regulation of ECM degradation in normal and pathological processes.

4. Implication of MMPs in health and disease

MMPs participate directly in a number of physiological processes where degradation of components of the ECM is needed. Such processes are wound heeling, regeneration, endometrial cycling, neovascularization and the embryonic stages of organ development. They also can modify indirectly the cellular and tissue behavior by modulation the structure and activity of various extracellular proteins, growth factors, signal and receptor molecules. MMPs cleave different proteins into fragments, which change their biological activity. As an example, fragmentation of laminin-5 and collagen type IV exposes the cryptic sites of these proteins that promote migration (5). MMPs are also involved in the releasing of growth factors from their binding proteins and cell-membrane-bound precursor forms. The cleavage by MMPs of cell adhesion receptors or for the ligands of cell surface receptors can affect the multiple intracellular signaling cascades and thus may change cell responses. Soluble MMPs may participate in integrin-mediated signaling cascades being ligands for the integrins (18).

The expression and activity of MMPs is very strictly controlled in order to maintain the normal functions of tissues. This is done trough the balance between MMPs and their inhibitors (1, 7, 17). Violation of this balance is observed in different pathological conditions such as rheumatoid arthritis, osteoarthritis, atherosclerotic plague rupture, peridontitis, dermal photoaging, chronic ulceration, COPD, Bronchial asthma and cancer (2, 4, 19-21). MMPs synthesized by cancer cells and adjacent stromal cells are currently known to contribute not only to the tumor growth, invasion and metastasis, but almost to every step of cancer development; penetration the basal lamina, infiltration of lymphatic or blood vessel, and cancer cell survival (4, 7, 8, 15, 20). It is well known that neovascularization is critical for the continuous growth and invasion of cancers (3, 4, 15, 22). Angiogenesis occurs through series of steps, as some of them require ECM proteolysis (23, 24). It has been well documented that some MMPs participate in tumor angiogenesis, and in cancer invasion and metastasis and poor prognosis (3, 25-27).

The degradation of the components of ECM and particularly basal membrane (proteoglycan, fibronectin, laminin, gelatin, collagen type III and IV) is provided by MMP-3 due to its broad substrate specificity. Increased expression of MMP-3 has been associated with raised tumor invasiveness, lymph node metastasis, the degree of invasion into the blood vessels and shorter disease-free survival (26, 28, 29). The destruction of basal lamina is provided by gelatinases (MMP-2 and -9) which digest type IV collagen and promote invasion and metastasis of the tumor cells (29-32). Normally, MMP-2 is found in serum and it is regularly expressed by most of the cells,

whereas, MMP-9 is expressed only by polymorphonuclear leukocytes and its expression is induced in normal keratinized cells by several factors (12-O-tetradecanoylphorbol-13-acetate [TPA], growth factor, cytokines and so forth) (4, 33). MMP-9 was associated with microvessel density (MVD) and VEGF expression, and was shown to act as a controller of the tumor neovascularization. It was suggested that MMP-2 have more important role rather than MMP-9 (29).

The expression of MMP-7 and -13 was associated with poor survival (34), whereas MMP-13 was reported to be often expressed in tumor cells of head and neck carcinoma, but rarely in normal tissue, because of its association with rapid matrix turnover during local invasion and growth of the malignant tumour (35). In addition, MMP-13 was associated with higher proliferative activity of malignant melanoma, but not with the prognosis of the patients (25).

5. Functional polymorphysms of *MMPs*

Most of the genes of MMPs are highly polymorphic. So far a variety of single nucleotide polymorphisms (SNPs) have been found in the promoter regions of *MMP* and *TIMP* genes. These polymorpisms were associated with altered gene expression and enzyme activity of MMPs, which might eventually affect the individual susceptibility to neoplasms. Some of these SNPs identified in the promoter regions of *MMP2* and *TIMP2* have been shown to abolish the Sp1-binding site (CCACC box), which in turn diminishes promoter activity of the genes and down-regulates the transcriptional activity of theses genes. As a consequence an imbalance between the activities of TIMP-2 and MMP-2 may occur. The C to T transition located at position -1306 in the promoter region of *MMP2* (-1306 C>T) has displayed a lower promoter activity of the variant T allele and the high transcriptional and enzyme activity of MMP-2 has been proposed to be a result of CC genotype (36). A transversion G to C at position -418 within the consensus sequence for the Sp1-binding site (GAGGCTGGG) in the promoter region of *TIMP2* has also been identified and suggested to down-regulate the transcriptional activity of *TIPM2* (7, 37). A promoter polymorphism has been reported in the promoter region of gelatinase-B gene (*MMP9*). The SNP at position -1562 is due to a C to T substitution (*MMP9* -1562 C>T). In vitro studies have shown that the C to T substitution results in the loss of binding of a nuclear protein to this region of MMP9, and further to an increase in transcriptional activity in macrophages (38).

Two functional insertion/deletion polymorphisms have been identified in the promoter regions of *MMP1* (-1607insG, rs1799750) and *MMP3* (-1171insA, rs3025058) (11, 39). The 2G allele of -1607insG polymorphism of *MMP1* has been associated with augment transcription of MMP-1 because the guanine insertion created a core-binding site (5'-GGA-3') for the Ets transcriptional factor family, leading to a higher expression of MMP-1 (40). On opposite, the 6A allele of -1171insA polymorphism in *MMP3* has been associated with reduced gene expression in vitro compared to 5A allele (38).

There are a great amout of papers suggesting that genetically determined matrix-degrading capacity due to functional polymorphisms in *MMP/TIPM* genes is associated with susceptibility to cancers of the lung (41), gastric cardia (42), breast (43), colorectal cancer (44), head and neck cancers(11, 39) or may influence invasiveness, metastasis and poor prognosis of some types of cancer such as melanoma (45), colorectal cancer (46), breast cancer (47)and gastric cancer (48).

6. MMPs in HNSCC

Head and neck malignancies consist of a heterogeneous group of neoplasia as the squamous cell carcinoma (HNSCC) is the most common malignant lesion of the head and neck and represents 5% of newly diagnosed cancers in the Western world and up to 40% of all malignancies in Thailand and South East Asia (7, 11).

Risk factors are tobacco smoke, alcohol use and nutritional deficiency, but only a fraction of exposed individuals develop the disease, which may refer to a genetic susceptibility of the individuals (7, 11).

HNSCCs often present difficult problems in clinical situation (49), which make its treatment a difficult process. HNSCC is characterised by its capacity to invade adjacent tissues and metastasise loco-regionally. In 30-40% of cases local invasion, lymph node metastasis, and distant metastases are

observed. In this respect a better understanding of the molecular mechanism of carcingenesis, and progression, and the identification of potential biological markers of this disease are needed to provide accurate prognostic markers and useful information for appropriate and effective therapy of the patients.

Carcinogenesis of the head and neck and the tumor growth are multi-step processes, which are influenced by the balance between cell proliferation and apoptosis, angiogenesis, cell-cell and cell-ECM interactions and ECM degradation. In this respect, MMP have been largely studied as enzymes involved in the degradation of matrix and in development and progression of head and neck malignancies. (50).

It has been demonstrated that expression of multiple MMPs and TIMPs is a characteristic of HNSCC and that no specific member of the MMP family is solely responsible for HNSCC progression (51). Nevertheless, it has been suggested the potential role of MMP-2, MMP-7, MMP-9 and MMP-11 in progression and metastasis of human HNSCC (11, 52), however, the mechanism(s) which lead to their overexpression *in vivo* are largely unknown.

It was shown that in cases of HNSCC, MMP-2 and MMP-9 are not related to the tumor size, but MMP-2 and MMP-9 correlated with the risk of metastasis in the lymph nodes. MMP-2 was reported as a useful marker for evaluation of tumor invasion and metastasis, while the expression of MMP-9 was associated with tumor neovascularization and poor survival rate of HNSCC patients (52, 53).

Genetic polymorphisms in promoters of *MMP2* and *TIMP2* and genetically determined balance of MMP-2 and TIMP-2 might be associated with the development, progression and aggressiveness of HNSCC. Thus, the homozygous CC genotype of *MMP2* -1306C>T SNP might be a risk factor for development of HNSCC, while a moderately increased risk of the cancer was associated with the genotypes containing the variant C allele of *TIMP2* -418G>C SNP (7).

The investigations of *MMP1* -1607insG SNPS in HNSCC have reported controversial results: in a Caucasian and Indian populations individuals homozygous for 2G/2G were at lower risk of developing malignancy than the 1G/1G carriers (39, 54), while in a Japanese population the 2G/2G genotype was associated with a higher risk for HNSCC (55). Similarly, diverse results were reported for the role of *MMP3* -1171insA as predisposing factor of HNSCC: the 6A allele was associated with decreased risk in Caucasians, whereas no significant difference in the genotype distribution between patients and controls was defined in Japaneses (11, 39, 54, 55).

Since MMPs seem to have a prominent role in cancer development, the possibility remains that MMP inhibitors given in combination with chemotherapy at an early stage of cancer development may have a positive clinical outcome. However, so far the outcome of these trials has been disappointing, principally due to the lack of efficacy and the presence of unwanted side effects (3, 4, 20).

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