POTENTIAL OF MATRIX METALLOPROTEINASES AND THEIR INHIBITORS IN COLORECTAL CARCINOMA PATIENTS

LJILJANA MAYER¹, MIHAELA GAĆE¹, SANJA DOBRIJEVIĆ¹ and ZVJEZDANA ŠPACIR PRSKALO¹

¹Department of Clinical Chemistry University Hospital for Tumors, Sestre milosrdnice University Hospital Center, Ilica 197, 10000 Zagreb, Croatia

Summary

Colorectal carcinoma (CRC) is the third most common type of cancer worldwide. Early stages of colorectal cancer are associated with good survival rates. However, most colorectal cancers are diagnosed at advanced stages when disease is no longer limited to bowel wall. Penetration through the bowel wall is partly attributed to disfunctionate extracellular matrix (ECM) remodeling.

Matrix metalloproteinases (MMPs) are enzymes that have an important role in ECM degradation. Activity of MMPs is controlled by tissue inhibitors of metalloproteinases or TIMPs. MMP-1, -2, -3, -7, -9, -13 blood levels correlate with the stage of disease. In fact, determining the concentrations of MMPs and TIMPs in plasma/serum may differentiate colorectal adenomas from colorectal carcinomas. Furthermore, a correlation between the anastomotic leakage in colorectal cancer surgery and levels of TIMP-1, MMP-8 and MMP-9 has been found. TIMP-1 was also considered in monitoring chemotherapy effects.

In this paper we review current applications of quantifying MMPs and TIMPs and their implications for clinical practice.

KEY WORDS: Extracellular matrix, matrix metalloproteinase, tissue inhibitors of metalloproteinases, colorectal carcinoma

Sažetak

Kolorektalni karcinom je treći oblik tumora prema učestalosti u svijetu. Rani stadiji kolorektalnog karcinoma povezuju se s višom stopom preživljenja. Međutim, kod značajnog broja bolesnika s kolorektalnim karcinomom bolest se dijagnosticira u već uznemiravajućoj stupnju bolesti, koji karakterizira prodor tumora u stijenku crijeva, što dijelom doprinosi poremećaju pregradnje izvanstaničnoga matriksa.

Matriks metaloproteinaze (MMP) su enzimi koji imaju značajnu ulogu u pregradnji izvanstaničnog matriksa. Njihova je aktivnost regulirana posredstvom tkivnih inhibitora metaloproteinaza (TIMP). Koncentracije MMP-1, -2, -3, -7, -9, -13 u krvi koreliraju sa stupnjem bolesti. Mjerenjem koncentracija MMP i TIMP u plazmi/serumu može se razlikovati kolorektalni adenom od karcinoma. Nadalje, kod bolesnika s kolorektalnim karcinomom je pronađena povezanost između pojavljivanja anastomozne i koncentracije TIMP-1, MMP-8 i MMP-9. Dodatna moguća primjena TIMP-1 jest i praćenje učinka terapije.

U ovome članku prikaz trenutno mogućih potencijala određivanja koncentracija MMP i TIMP te njihove primjene u kliničkoj praksi.

KLJUČNE RIJEČI: Izvanstanični matriks, matriks metaloproteinaza, tkivni inhibitori metaloproteinaza, kolorektalni karcinom.
INTRODUCTION

The extracellular matrix (ECM) is secreted by cells and surrounds the cells in tissues. It has long been believed to be merely the structural support to cells since its characteristics are specific to certain tissue morphology. Extracellular matrix was shown not to be simply a passive, mechanical support for cells, but in fact an extraordinarily complex scaffold composed of a variety of biologically active molecules that are highly regulated and critical for determining the action and fate of the cells that it surrounds.

Degradation of ECM macromolecules is an important feature of morphogenesis, development, tissue remodeling and repair. While, regulated in homeostasis, disregulation in degradation is observed many pathophysiological conditions (arthritis, nephritis, fibrosis, atherosclerosis, heart failure) as well as in carcinogenesis (1,2).

Heterogenous types of proteinases are involved in ECM degradation, but the key enzymes are considered to be matrixins or matrix metalloproteinases (MMPs), large family of calcium-requiring zinc endopeptidases. Activity of MMPs is specifically controlled by their inhibitors known as tissue inhibitors of metalloproteinases (TIMPs). In fact, balance between MMPs and TIMPs is essential in remodeling of ECM environment (3,4).

This review describes biological properties, structure, function of both, MMP and TIMP with the emphasis, their possible application as a diagnostic and prognostic tool in patients with colorectal cancer.

Extracellular matrix

Extracellular matrix is a complex mixture of negatively charged polysaccharides (glycosaminoglycans), adhesive glycoproteins (such fibronectin and lamin) and fibrous proteins (elastin, collagens), which is secreted by cells and surrounds them in tissues (5,6). ECM represents both structural support for cells (which is tissue specific mechanical support (through collagen, laminin, proteoglycan and fibronectin) and a signaling platform composed of diverse biologically active molecules which are strictly regulated and are critical in determining the function of these cells (proliferation vs. cell cycle arrest, motility vs. immobility, survival vs. apoptosis) (7).

In most carcinomas, ECM proteins are over-expressed: increasing crosslinked collagen type I is a marker of metastatic potential of tumor and poor prognosis in women with breast cancer; laminin expression positively correlates with invasiveness of tumors; proteoglycans are involved in cell adhesion and they are over-expressed in epithelial tumors; increased stroma fibronectin is an indicator of tumor progression in prostate cancer (8,9).

The communication between cells and between cells and their ECM is established through ligand-receptor interactions, which are the triggers of communication cascades which finally result in changes in gene expression and consequently changes in ECM. One mechanism would be fibronectins binding to the integrins and transmitting the signal for cytoskeletal remodeling; growth factors bind to their receptors and initiate the cell growth. In fact, some proteins, like collagen and fibronectin, can imitate transforming growth factors (i.e. TGF-β) and activate its signaling pathway even in the absence of TGF-β receptors (10). The other mechanism would be that matrix proteins themselves apart from providing mechanical support, contain growth factor-like domains, which can bind to growth factor receptors, and thus stimulate the cell growth.

Apart from extracellular growth stimulation tumours require new blood vessels for further growth. In normal tissue pro-angiogenic factors and endogenous inhibitors of angiogenesis are in balance. Tumor cells produce pro-angiogenic factors, such as angiogenic growth factors VEGF-A and FGF-2 which control matrix-degrading proteinases (11). When tumor cells secrete VEGF-A and FGF-2 in their microenvironment, they increase the activity of matrix-degrading proteinases and results in remodeling of ECM. Therefore, the inhibition of matrix-degrading proteinase activity may also stop or slow down neoangiogenesis and thereby slow down the tumor progression. This might be a new therapeutic strategy for antineoplastic treatment (12,13).

Matrix metalloproteinases

The matrix metalloproteinase family includes 24 proteinases. MMPs remodel tissue via degradation of ECM. MMPs are synthesized as proenzymes (in inactive form, proMMP). Proenzyme contains propeptide (part of about 80 amino ac-
ids), catalytic metalloproteinase domain (170 amino acids), a linker peptide variable length (hinge region) and hemopexin domain (200 amino acids) (14,15). MMPs secondary structure elements are three alpha-helices and connecting loops. Position of alpha-helix and loops form MMP substrate binding catalytic domain. In accordance with their substrate specificity and structure MMPs are divided into 5 groups: collagenases, gelatinases, membrane type, stromelysins, matrilysins (16). Members of MMP family are described in detail in Table 1.

MMPs identify a hydrophobic amino acids (Leu, Ile, Met, Phe, Tyr) in target proteins and cleave a peptide bond before that part. MMP-12 is an exception, it cleaves in X-Lys bound (17). The hydrophobic amino acid residues fit into the enzyme active site S1′ pocket with variable depth depending on particular member of MMPs family. Other, noncatalitic domains also participate in the enzyme activity and specificity. For example, fibronectin domains of MMP-2 and MMP-9 are important for enzyme activity on type IV collagen, gelatin, and elastin. The loop region in MMP-1 just before the catalytic site helix is neccesery for collagenolytic activity (18).

MMPs are zinc-dependent endopeptidases. The essential zinc atom in the catalytic domain

### Table 1.

**FEATURES OF MATRIX METALLOPROTEINASES (1-17)**

<table>
<thead>
<tr>
<th>Designation</th>
<th>Common name</th>
<th>Chromosomal location</th>
<th>Structural class</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-1</td>
<td>Collagenase-1, interstitial collagenase, fibroblast collagenase, tissue collagenase</td>
<td>11q22-q23</td>
<td>Simple hemopexin domain</td>
<td>Type I and II fibrillar collagens</td>
</tr>
<tr>
<td>MMP-2</td>
<td>Gelatinase A, 72-kDa gelatinase, 72-kDa type IV collagenase, neutrophil gelatinase</td>
<td>16q13</td>
<td>Gelatin-binding</td>
<td>CCL2, CXCL 12</td>
</tr>
<tr>
<td>MMP-3</td>
<td>Stromelysin-1, transin-1, proteoglycanase, procollagenase-activating protein</td>
<td>11q23</td>
<td>Simple hemopexin domain</td>
<td>E-cadherin, Laminin, type IV collagen, Latent TGF-β1</td>
</tr>
<tr>
<td>MMP-7</td>
<td>Matrilysin, matrin; PUMP1, small uterine metalloproteinase</td>
<td>11q21-q22</td>
<td>Minimal domain</td>
<td>Pro-α-defensins, FAS ligand (CD95L), Latent TNF, Syndecan-1, E-cadherin, Elastin</td>
</tr>
<tr>
<td>MMP-8</td>
<td>Collagenase-2, neutrophil collagenase, granulocyte collagenase</td>
<td>11q21-q22</td>
<td>Simple hemopexin domain</td>
<td>Mouse CXCL5</td>
</tr>
<tr>
<td>MMP-9</td>
<td>Gelatinase B, 92-kDa gelatinase, 92-kDa type IV collagenase</td>
<td>20q11.2-q13.1</td>
<td>Gelatin-binding</td>
<td>Zona occludens 1, α1-Antiproteinase, Latent TGF-β1, Latent VEGF, Fibrin, NG2 proteoglycan</td>
</tr>
<tr>
<td>MMP-10</td>
<td>Stromelysin-2, transin-2</td>
<td>11q22.3-q23</td>
<td>Simple hemopexin domain</td>
<td>/</td>
</tr>
<tr>
<td>MMP-11</td>
<td>Stromelysin-3</td>
<td>22q11.2</td>
<td>Furin-activated and secreted</td>
<td>/</td>
</tr>
<tr>
<td>MMP-12</td>
<td>Metalloelastase, macrophage elastase, macrophage metalloelastase</td>
<td>11q22.2-q22.3</td>
<td>Simple hemopexin domain</td>
<td>Latent TNF</td>
</tr>
<tr>
<td>MMP-13</td>
<td>Collagenase-3</td>
<td>11q22.3</td>
<td>Simple hemopexin domain</td>
<td>Type I and II fibrillar collagens</td>
</tr>
<tr>
<td>MMP-14</td>
<td>MT1-MMP, MT-MMP1</td>
<td>14q11-q12</td>
<td>Transmembrane</td>
<td>ProMMP2, Fibillar collagens, Fibrin, Syndecan-1, γ2-Subunit of laminin-5</td>
</tr>
<tr>
<td>MMP-15</td>
<td>MT2-MMP, MT-MMP2</td>
<td>15q13-q21</td>
<td>Transmembrane</td>
<td>Fibrin</td>
</tr>
<tr>
<td>MMP-16</td>
<td>MT3-MMP, MT-MMP3</td>
<td>8q21</td>
<td>Transmembrane</td>
<td>Fibrin, Syndecan-1</td>
</tr>
<tr>
<td>MMP-17</td>
<td>MT4-MMP, MT-MMP4</td>
<td>12q24.3</td>
<td>GPI-linked</td>
<td>/</td>
</tr>
</tbody>
</table>
makes an intramolecular complex with the single cysteine residue in MMPs propeptide domain, a complex that blocks the activation of enzyme. After secretion, proMMPs can be activated with proteinases or nonproteolytic pathway (19,20). The most important event is the disassociation of the cysteine residue from the complex.

Activation of certain members of MMP family by plasmin is a part of fibrinolytic pathway in in vivo conditions. Plasmin is a product of plasminogen activation by tissue plasminogen activator and urokinase. It is known that plasmin can activate proMMP-1, proMMP-3, proMMP-7, proMMP-9, proMMP-10, and proMMP-13 (21,22). Activation of MMPs and interactions between fibrinolytic system and MMPs is shown in Figure 1.

![Figure 1. Interaction between fibrinolytic and metalloproteinase (MMP) systems](image)

Activation of proMMPs is gradual: α₂-macroglobulin and TIMPs are types of regulatory mechanisms that inhibit and regulate MMP activity. Plasma glycoprotein (α₂-macroglobulin) inhibits MMPs by entrapping them within the macroglobulin structure (23). The complex MMP- α₂-macroglobulin is rapidly cleared by endocytosis (by LDL receptor-related protein-1) (24,25). TIMPs interfere with MMPs activation by interacting with the intermediate MMP before it is completely activated.

Tissue inhibitors of metalloproteinases

Tissue inhibitors of metalloproteinases (TIMPs) are specific inhibitors of MMPs. Their expression influences development and tissue remodeling. In pathological conditions, such as carcinogenesis, changes of TIMP levels are important because of they effect on MMP activity.

TIMPs (21 to 29 kDa) are group of 4 proteins: TIMP-1, TIMP-2, TIMP-3, and TIMP-4. Protein N-domain consist about 125 and C-terminal domain 65 amino acids. TIMP has a wedge like shape like a wedge and binds to the catalytic metalloproteinase domain similar like a supstrate. The N-terminal four amino acid residues and the CD-loop region next to them on TIMP-2 molecule are main points of interaction with MMP. Two of four amino acid residues are strictly conserved cysteines that make disulfide bonds. Cysteins is important in chelating the zinc in catalytic domain by expelling the water molecule bound to the zinc. They are connected to one another in the stoichiometric ratio 1:1.

It has been proved that TIMPs inhibit all members of metalloproteinase family, except TIMP-1 which is a poor inhibitor for MT1-MMP, MT3-MMP, MT5-MMP and MMP-19. TIMP-3 inhibits ADAM-17 (TACE), ADAM-10, ADAM-12, and the aggrecanases (ADAMTS-4 and ADAMTS-5) in contrast to other TIMP (26,27). Activation of MMPs and interactions between fibrinolytic system and MMPs is shown in Figure 1.
The role of TIMP-1 in tumorogenesis was linked to inhibition of apoptosis, cell growth stimulation and angiogenesis disregulation (32,33,34). Furthermore, it seems that TIMP-1 has a promising prognostic potential (higher values are connected with shorter survival) (35,36).

On the other hand, significant over expression of MMP-1, -2, -3, -7, -9, -13 in colorectal carcinoma patients was noted along with the correlation of their concentration with the stage of disease (37,38). Moreover, colorectal carcinoma cells themselves produce MMP-7. Mutation of APC gene causes nuclear beta-Catenin/TCF complex accumulation, which then acts as transcriptional factor for MMP over expression (39). Association between increased expression of MMP-3 in colorectal cancer and levels of microsatelite instability and poor prognosis was also described (40).

Furthermore, increased levels of MMP-9 were noted in early transition from colon adenoma to adenocarcinoma.

On the other hand, MMP-12 over-expression is associated with the better survival rates in colorectal carcinoma patients, probably through its inhibitory effect on angiogenesis.

Attempt of designing the drug with MMPs inhibitory properties were promising in animal model, but transition to humans proved disappointing, stressing once again multifactoriality and complexity of carcinogenesis. Recent research focus were matrix metalloproteinases MMPs-2 (gelatinase A) and -9 (gelatinase B) inhibitors due to their role in signal transduction via cell surface integral membrane proteins (CD44, αVβ/αβ1/αβ2 integrins and Ku protein) (41).

There are four types of TIMPs (1-4) in humans, with high degree of structural homology, but with quite different functions. While TIMP-1 inhibits angiogenesis directly through MMP-9 and VEGF, TIMP-2 selectively blocks the growth of microvascular endotel which is response to FGF-2 and VEGF-A. TIMP-3 promotes apoptosis, through caspase-8 pathway inhibits migration of endotel cells in vitro (42).

There are already several possible clinical applications of MMPs and TIMPs levels in prognosis and treatment reponse. Immunohistochemistry depicted higher MMP-9 expression in moderately and poorly differenitated tumors compared with well differenitated ones. Over-expression of MMP-2 and MMP-9 is associated with worse five-year survival. These two gelatinases are prognostically significant in both colon and rectal carcinoma (37). Gradual increase in immunostaining positivity for MMP-2, MMP-9, TIMP-1 and TIMP-2 was associated with tumor progression.

Plasma/serum concentration of MMPs and TIMPs may differentiate colorectal adenomas from colorectal carcinomas. Clinical trials are opened which explore their eligiblity for standard tumor stage markers. On the other hand, connection between elevated intraperitoneal concentration of MMP-8 and MMP-9 and the possibility of anastomotic leakage development after rectal cancer surgery has been researched for prediction of anastomotic leak risk (43). Another posible use of TIMP-1, due to its increased levels in apoptosis, could be the monitoring of the chemotherapy effect.

CONCLUSION

Cancer cells and normal human cells are surrounded by extracellular matrix (ECM). ECM used to be considered a passive cells environment, but now it is viewed as a dynamic communication matrix which may play a role in cancer development and progression. Heterogenous types of proteinases are involved in ECM degradation amongst which are matrix metalloproteinases (MMPs). Their inhibitors are TIMPs - tissue inhibitors of metalloproteinases. Changes in MMPs and TIMPs concentrations in serum/plazma correlate with patient’s survival. Intraperitoneal concentrations correlate with the risk of anastomotic leak in patient after rectal cancer surgery. Therefore, these parameters might be introduced in anastomotic leak assessment and monitoring chemotherapy effect.

LITERATURE

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Author address: Ljiljana Mayer, Department of Clinical Chemistry, University Hospital for Tumors, University Hospital Center Sestre milosrdnice, Ilica 197, 10000 Zagreb, Croatia. E-mail: ljiljana.mayer@kbscm.hr