



TARGETING MATRIX METALLOPROTEINASES: AN IMPORTANT STRATEGY IN CANCER THERAPEUTICS

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ABSTRACT

Matrix metalloproteinases (MMPs), also known as matrixins, are a family of zinc-dependent proteinases, which are thought to play a central role in the breakdown of extracellular matrix. Collagen, elastin, gelatin and casein are major components cleaved by MMPs. The breakdown of these components is essential for many physiological processes such as embryonic development, morphogenesis, reproduction, and tissue resorption and remodelling. MMPs have also been implicated in pathological processes of tumor growth, invasion and metastasis as well as the dysregulated angiogenesis that is associated with these events. As a result, these proteases have come to represent important therapeutic and diagnostic targets for the treatment and detection of human cancers. A number of MMP inhibitors are being developed for the treatment of cancer. The most extensively studied class of MMP inhibitors includes collagen peptidomimetics and nonpeptidomimetic inhibitors of the MMP active site, tetracycline derivatives and bisphosphonates. This review summarizes current knowledge regarding these proteins, their regulation and activation, their participation in cancer and also provides a comprehensive summary of the MMP inhibitors that are currently in clinical trials.

Keywords: Matrix metalloproteinases (MMPs), Extracellular matrix (ECM), Tissue inhibitors of matrix metalloproteinases (TIMPs), Cysteine switch.

1. INTRODUCTION

Cancer is a class of diseases characterized by uncontrolled cell division and the ability of these cells to invade other tissues, either by direct growth into adjacent tissue or by migration of cells to distant sites. This unregulated growth is caused by a series of acquired or inherited mutations to DNA within cells, damaging genetic information that define the cell functions and removing normal control of cell division. The major goal in anticancer drug discovery is to develop innovative therapies that exhibit a real improvement in effectiveness and/or tolerability. In cancer therapy, remarkable progress has been made in the identification of new targets. Cancer research is largely focused on prospective targets identified by basic science such as the oncogenic signal transduction pathway, oncogenes, tumor suppressor genes, and genes involved in the regulation of the cell cycle and apoptosis or programmed cell death¹. The importance of proteinases in tumor invasion was first recognized in 1925 when Fischer found that a lytic substance from sarcoma cells could degrade the fibrin stroma. Later it was found that the serine proteinase plasminogen activator (PA) played an important role in activating plasminogen to plasmin. Apart from PAs, proteinases such as serine, cysteine and metalloproteinases have been associated with cancer. It is important to realize that high levels of extracellular proteolytic activity are not restricted to the malignant phenotype, but are also seen in a number of physiological processes such as embryo implantation, wound healing, and angiogenesis. A common feature in these processes is the breaching of histological barriers with the degradation of the extracellular matrix (ECM)

composed of basement membrane and extracellular stroma².

2. MATRIX METALLOPROTEINASES

Matrix metalloproteinases (MMPs), also known as matrixins, are a large group of zinc-dependent proteases responsible for cleaving and rebuilding connective tissue components such as collagen, elastin, gelatin and casein. Found in invertebrates, vertebrates and plants, their phylogenetic origin emerged from lower organisms, namely *Bacteroides fragilis*³. Common properties of the MMPs include the requirement of zinc in their catalytic site for activity and their synthesis as inactive zymogens that generally need to be proteolytically cleaved to be active. Normally the MMPs are expressed only when and where needed for tissue remodeling that accompanies various processes such as during embryonic development, wound healing, cartilage-to-bone transition during ossification and trophoblast invasion into the endometrial stoma during placenta development. However, aberrant expression of various MMPs has been correlated with pathological conditions, such as periodontitis, rheumatoid arthritis and tumor cell invasion and metastasis⁴. Extracellular matrix assists with the organization and differentiation of cells, helps exchange information between cells and acts as a physical barrier against microorganisms. MMPs play an important role in the degradation of extracellular matrix, a process that takes place during developmental stages such as growth and morphogenesis. Due to their physiological functions, high levels of MMPs activity are observed in diseases and pathological processes involved in connective tissue degradation such as inflammation and cancer. These



enzymes play crucial roles within organisms and are present in several forms and modifications, which differ in location, substrate specificity and regulation.

2.1. CLASSIFICATION OF MMPs

MMPs family consists of 28 endopeptidases that share homologous protein sequences, with conserved domain structures and specific domains related to substrate specificity and recognition of other proteins. They have in common roughly 40% of their primary structures. MMPs are classified based on their pre-synthetic region on

chromosomes and their various substrate specificities into six subgroups: collagenases (MMP-1, MMP-8 and MMP-13), gelatinases or type IV collagenases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10 and MMP-11), matrilysins (MMP-7 and MMP-26), membrane-type MMPs (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24 and MMP-25) and others (MMP-12, MMP-18, MMP-19, MMP-20, MMP-23 and MMP-28)⁵. Number designations MMP-1 to MMP-28 are used for classification⁶, but some have still not been identified through this system (Table 1).

Table 1: Classification of Matrix Metalloproteinases

MMP	Metalloproteinase	Mol. Wt. (kDa)	EC classification	Substrates
MMP-1	Collagenase (type I, interstitial)	43	EC3.4.24.7	Collagens (I, II, III, VII, VIII and X), gelatin, entactin/nidogen, IL-1 β proteoglycans, fibrinogen, TNF precursor, MMP-2, MMP-9
MMP-2	Gelatinase A	72	EC3.4.24.24	Collagens (I, IV, V, VII, X, XI and XIV), gelatin, elastin, aggrecan, fibronectin, TNF precursor, α 1-PI, MMP-1, MMP-9, MMP-13
	Gelatinase type IV collagenase	66		
MMP-3	Stromelysin-1	46	EC3.4.24.17	Collagens (III, IV, V, VII and IX), gelatin, elastin, TNF precursor, α 1-PI, MMP-2/TIMP-2, MMP-7, MMP-8, MMP-9, MMP-13
	Proteoglykanase			
MMP-7	Matrilysin	20	EC3.4.24.23	Collagens (IV and X), gelatin, aggrecan, elastin, plasminogen, α 1-PI, MMP-1, MMP-2, MMP-9, MMP-9/TIMP-1
MMP-8	Neutrophil collagenase	58	EC3.4.24.34	Collagens (I, II, III, V, VII, VIII and X), gelatin, fibronectin, α 2M
MMP-9	Gelatinase B	92	EC3.4.24.35	Collagens (IV, V, VII, X and XIV), gelatin, entactin, aggrecan, elastin, fibronectin, plasminogen, TNF precursor, α 1-PI, α 2M
MMP-10	Stromelysin-2	46	EC3.4.2.22	Collagens (III-V), gelatin, casein, elastin, MMP-1, MMP-8
MMP-11	Stromelysin-3	44	-	Unknown (the most likely casein)
MMP-12	Macrophage metalloelastase	45	EC3.4.24.65	Collagen IV, gelatin, elastin, casein, fibronectin, plasminogen, TNF precursor, α 1-PI
MMP-13	Collagenase-3	55	-	Collagens (I, II, III, IV, IX, X and XIV), gelatin, plasminogen, fibronectin, osteonectin, MMP-9
MMP-14	MT1-MMP	54	-	Collagens (I-III), gelatin, casein, fibronectin, proteoglycans, α 1-PI, α 2M, MMP-2, MMP-13
MMP-15	MT2-MMP	61	-	Fibronectin, gelatin, entactin, laminin, perlecan, MMP-2
MMP-16	MT3-MMP	55	-	Collagen III, gelatin, laminin, fibronectin, α 1-PI, α 2M, MMP-2
MMP-17	MT4-MMP	54	-	Gelatin, TNF- α precursors, fibronectin
MMP-18	Collagenase-4	53	-	Collagens (I,II,III,VIII and X), gelatin
MMP-19	RASI-1	45	-	Gelatin, aggrecan, fibronectin, laminin
MMP-20	Enamelysin	22	-	Amelogenin; aggrecan
MMP-21	-	-	-	Unknown
MMP-22	-	-	-	Unknown
MMP-23	-	-	-	Unknown
MMP-24	MT5-MMP	62	-	Unknown
MMP-25	Leukolysin / MT6-MMP	-	-	Pro-gelatinase A, fibrin, fibronectin, collagen IV, gelatin
MMP-26	Endometase, matrilysin-2	19	-	Gelatin, fibrinogen, fibronectin, vitronectin
MMP-27	-	-	-	Unknown
MMP-28	Epilysin	-	-	Casein

2.1.1. Collagenases

This group of MMPs includes Collagenases-1, -2 and -3 (MMP-1, MMP-8, and MMP-13, respectively). These are the main secreted neutral proteinases, which can initiate the degradation of native helix of fibrillar collagens⁷. The hemopexin domains of these MMPs are essential for specific binding and cleavage of this substrate. All three collagenases can cleave a specific site (Gly⁷⁷⁵-Ile/Leu⁷⁷⁶) at each α -chain of the trimeric collagen molecule. The resulting N-terminal $\frac{3}{4}$ and C-terminal $\frac{1}{4}$ fragments are

spontaneously denatured at 37°C to gelatin, which can be further degraded by other proteinases. Three collagenases have overlapping activities on the fibrillar collagen types I, II and III, but their substrate preferences are different. MMP-1 displays highest catalytic efficiency against type III relative to type I collagen, whereas MMP-8 has reversed preference and MMP-13 preferentially cleaves collagen type II. MMP-13 also displays higher gelatinase activity and has broader substrate specificity than MMP-1 and -8.



MMP-1 was the first MMP discovered based on its activity in metamorphosing tadpole tail. It was also the first MMP purified to homogeneity and cloned as a cDNA. Fibroblasts of various origins, chondrocytes, osteoblasts, endothelial cells, hepatocytes, macrophages and monocytes and tumor cells secrete MMP-1 *in vitro*⁷. MMP-8 is synthesized by polymorphonuclear leukocytes during their maturation in bone marrow, stored in intracellular granules, and released in response to external stimuli⁸. MMP-13 is expressed during fetal bone development, postnatal bone remodeling, and gingival wound repair. In addition, MMP-13 expression has been associated with pathological conditions such as severe chronic inflammation in osteoarthritic cartilage and chronic wounds, as well as malignant tumor invasion⁹.

2.1.2. Gelatinases

Gelatinases include MMP-2 (gelatinase A, 72-kDa gelatinase) and MMP-9 (gelatinase B, 92-kDa gelatinase). These differ from the other MMPs by containing three head-to-tail repeats homologous to the type II repeat of the collagen-binding domain of fibronectin. These domains are required for gelatinases to bind and cleave collagen and elastin. The hemopexin domain does not affect MMP-2 binding to collagen, but similarly to collagenases it is critical for the initial cleavage of the triple helical type I collagen¹⁰. The hinge domain of MMP-9 contains an additional type V collagen-like insert. Unlike other MMPs, the MMP-2 and MMP-9 proenzymes can bind TIMP-2 and TIMP-1, respectively.

A wide range of normal and transformed cells of fibroblastic, endothelial and epithelial origin constitutively express MMP-2. Expression of MMP-9 is more restricted and is often low in normal tissues, but it can be induced when tissue remodeling occurs during development, wound healing and cancer invasion. MMP-9 is secreted by alveolar macrophages, polymorphonuclear leukocytes, keratinocytes, and invading trophoblasts and by several transformed cell lines, but not by fibroblastic cells. Gelatinases primarily cleave denatured collagen and intact collagen type IV in basal membranes. They are also able to cleave denatured collagen type V, VII, X, XIV, fibronectin, elastin and aggrecan. MMP-2 is known to cleave native collagen type I¹¹. Other studies have shown that MMP-2 binds to intact collagen to prevent autolytic inactivation. In addition to gelatin and other forms of denatured collagen, MMP-9 cleaves a number of other physiological substrates¹². MMP-9 has been suggested to affect angiogenesis by releasing ECM bound vascular endothelial growth factor (VEGF).

2.1.3. Stromelysins

Stromelysins include stromelysin-1, -2 and -3 (MMP-3, MMP-10, and MMP-11, respectively). The domain structures of stromelysins resemble those of collagenases. However, they are unable to cleave native fibrillar collagens. Stromelysins have relatively broad substrate specificity. The majority of stromelysins cleave

non-collagenous extracellular matrix proteins (proteoglycans, glycoproteins, fibronectin and laminin). Moreover, these enzymes can cleave other MMPs. Stromelysin 2 (MMP-10) can degrade the ends of propeptide domains of neutrophil collagenases (MMP-8), cleaving it at the Gly⁷⁸-Phe⁷⁹ site, leading to the activation of MMP-8 only¹³. Stromelysin-3 is inactive against many ECM components; instead it can cleave proteinase inhibitors, α_2 -macroglobulin (α_2 M) and α_1 -PI¹⁴, and insulin-like growth factor binding protein. It also differs from most secreted MMPs by having recognition sequence for proprotein convertases between the pro- and catalytic domains.

2.1.4. Matrilysins

Matrilysins-1 and -2 (MMP-7 and MMP-26, respectively) are the MMPs with broad substrate specificities. Matrilysins are the smallest MMPs that lack the C-terminal hemopexin-like domains present in other MMPs. Matrilysin consists of only a pro-peptide domain and a catalytic domain. MMP-7 is also known as PUMP and it cleaves a number of substrates including collagen types IV and X, elastin, fibronectin, gelatin, laminin, and proteoglycans. It is secreted as a 28 kDa proenzyme that can be activated *in vitro* by organomercurials (e.g., p-amino phenylmercuric acetate (APMA)) and trypsin and *in vivo* by MMP-3 to form a 19 kDa active MMP-7. The active MMP-7 can activate pro-MMP-1 and pro-MMP-9. Elevated levels of MMP-7 are reported in cycling endometrium as well as in colorectal cancers and adenomas, hepatocellular carcinomas, rectal carcinomas, and approximately 50% of gliomas¹⁵.

2.1.5. Membrane-type matrix metalloproteinases

Membrane-type matrix metalloproteinases (Membrane-associated MMPs, MT-MMPs) are a unique subclass of MMPs which include MMP-14 (MT1-MMP), MMP-15 (MT2-MMP), MMP-16 (MT3-MMP), MMP-17 (MT4-MMP), MMP-24 (MT5-MMP) and MMP-25 (MT6-MMP). Four of these i.e. MT1-MMP, MT2-MMP, MT3-MMP and MT5-MMP have transmembrane and cytosolic domains at the C-terminus. The other two MT-MMPs (MT4-MMP and MT6-MMP) do not have a cytosolic domain and are thought to be GPI (glycophosphatidyl inositol) anchored to the cell surface. MT-MMPs exhibit similar substrate specificity analogous to free MMPs. They degrade mainly collagen but also other substrates as well. The main difference to other MMPs is their association with the cell membrane. In addition to its location, MT-MMPs also differ in its activity from other types of MMPs. MT1-MMP is five to seven times less effective in cleaving hydrolysed collagen type I than its analogue MMP-1. It is however eight times more effective in cleaving gelatin in comparison to MMP-1¹⁶. MT4-MMP can cleave gelatin and synthetic substrates, but cannot cleave collagen type I and IV, fibronectin and laminin¹⁷. MT2-MMP, MT3-MMP and MT4-MMP can cleave proMMPs prior to activation.



2.1.6. Macrophage Elastase and other MMPs

Macrophage elastase (MMP-12) shares its ability to cleave elastin with other MMPs (gelatinases and matrilysins). It is also able to cleave fibronectin, laminin, collagen, basal membrane, chondroitin sulphate etc¹⁸. This enzyme enables macrophages to penetrate basal membrane and, thus, rebuild the inflammatory tissue. MMP-19 also degrades basal membrane. In addition, MMP-20 can degrade tooth enamel, specifically emalogenin¹⁹. MMP-18 is also known as Xenopus collagenase-4 or xCol4. It is an interstitial collagenase and is known only as a Xenopus enzyme. Sequence comparison of MMP-18 with other MMPs suggests that MMP-18 is not a homolog of any known collagenase. MMP-23 differs from all other characterized MMPs by having unique cysteine-rich, proline-rich, and IL-1 type II receptor-like domains instead of the C-terminal hemopexin-like domain. It has also an atypical N-terminal prodomain that lacks the conserved "cysteine switch" sequence, but contains a potential membrane spanning region.

2.2. STRUCTURE OF MMPs

X-ray crystallography and nuclear magnetic resonance (NMR) studies have made it possible to determine the structures of many MMPs²⁰. MMPs are zinc and calcium-dependent endopeptidases, which are synthesized from inactive proMMPs. Commonly secreted from cells in its inactive form, with the exception of membrane-associated MMPs (MT-MMPs), this prevents MMPs from cleaving essential components in cells. The enzyme is divided into three domains: N-terminal propeptide, C-terminal domain (or hemopexin-like domain) and catalytic domain (Fig. 1)²¹.



Figure 1: Structure of Matrix Metalloproteinase

2.2.1. N-Terminal Propeptide

N-terminal propeptide ensures enzyme latency by containing approximately 80 amino acids. The most important functional amino acid within the N-terminal propeptide is cysteine, which interacts with catalytic zinc ions through the thiol group and constitutes the cysteine switch (Fig. 2). In the propeptide a highly conserved sequence (Pro-Arg-Gly-Cys-X-Pro-Asp, where X represents any amino acid) is present. Cleavage of the propeptide triggers proMMP activation²².

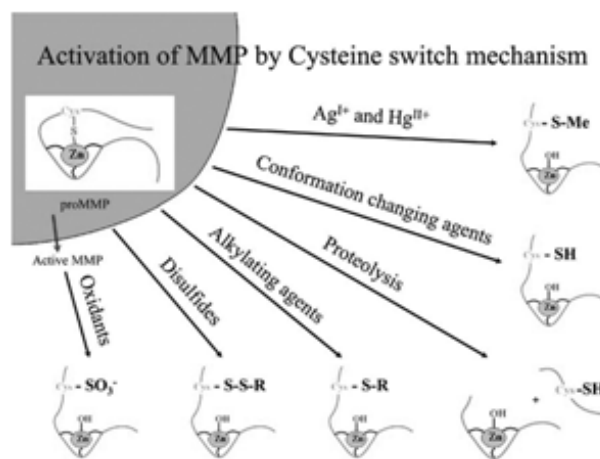


Figure 2: Activation of MMPs by cysteine switch mechanism²³. Activation of MMPs through cysteine switch can be achieved by several ways i) treatment with oxidants, ii) disulfides, iii) alkylating agents, iv) proteolytic cleavage, v) usage of agents changing conformation, and vi) silver(I) and mercury(II) ions.

2.2.2. C-Terminal Domain

The C-terminal domain (or hemopexin-like domain) is structurally similar to proteins of the hemopexin family. The domain has a relatively large surface area for protein-protein interactions e.g. cell membrane receptors. It is ellipsoid shaped with propeller-like subdomain, where each leaf of the "propeller" is composed of 4 antiparallel β -sheets and one α -helix. The first and fourth leaf are linked by a disulfide bridge²⁴. For collagenase-1, the catalytic and C-terminal domains²⁴, are freely attached by a flexible proline-rich peptide linker (hinge). The length of the hinge is extremely variable 16 amino acid residues in collagenase to 65 amino acid residues in MMP-15. The specific function of the hinge is not fully understood.

2.2.3. Catalytic Domain

The catalytic domain consists of five β -sheets, three α -helices and connecting loops. It is composed of 170 amino acids and contains zinc-binding motif (His-Glu-His-XX-XXXX-Gly-His, where X represents any amino acid) associated with methionine, which forms a unique structure known as the methionine loop. The catalytic domain contains two zinc(II) ions and two or three calcium(II) ions. The first Zn^{2+} ion present in the active site directly participates in catalytic processes. The second Zn^{2+} ion (also called structural) and Ca^{2+} ions are approximately 12 nm far from the Zn^{2+} ion in the catalytic site. Calcium ions are necessary to stabilize the domain structure²⁵.

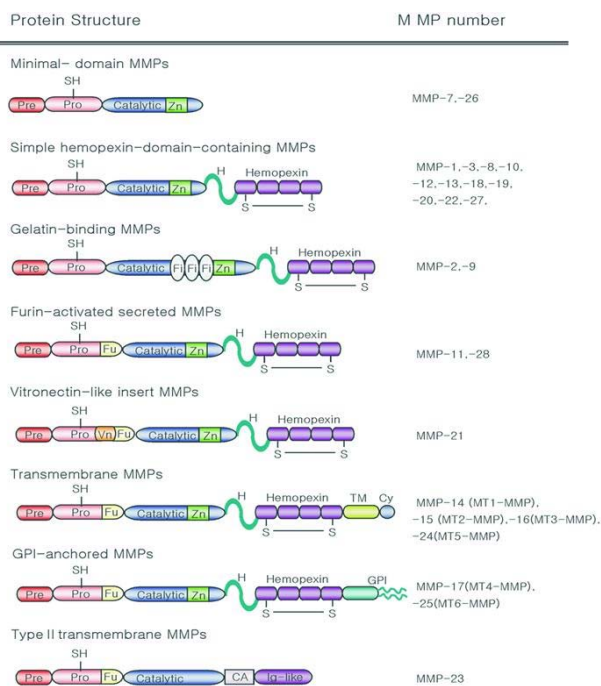


Figure 3: Different domains of MMPs²⁶

[**Pre:** signal sequence, **Pro:** propeptide with a free zinc-ligating thiol (SH) group, **Hemopexin:** hemopexin-like domain, **Fu:** furin-susceptible site, **Zn:** zinc-binding site, **Fi:** collagen-binding fibronectin type II inserts, **H:** hinge region, **TM:** trans-membrane domain, **Cy:** cytoplasmic tail, **GPI:** glycosylphosphatidylinositol-anchoring domain, **CA:** cysteine array domain, **Ig-like:** immunoglobulin-like domain].

The hemopexin-like domain is characteristic for collagenase and it is necessary for the degradation of specific amino acid sequences in interstitial collagen. The catalytic domain of MMPs has proteolytic activity²⁷. Matrilysin MMPs, however, do not contain this domain. MT-MMPs possess this catalytic domain. However, deletion of the hemopexin domains in MT1-, MT2-, MT3-, MT5-, and MT6-MMP does not impair their abilities to activate proMMP2²⁸. A transmembrane domain, this domain contains one hydrophobic chain composed of approximately 25 amino acids with a protease specific recognition motif (Arg-X-Lys-Arg, where X represents any amino acid), except MT4-MMP and MT6-MMP, which are connected to the cell surface by a glycosylphosphatidylinositol (GPI) transmembrane anchor²⁵. Gelatinases contain domains showing structural similarities with matrix proteins, three tandem copies of the domain with a sequence similarity to fibronectin type II (58 amino acid moieties long) are present in all gelatinases.

Matrilysins (MMP-7 and MMP-26) belong to the smallest members of MMPs and do not contain the hemopexin-like domain. For MMP-23 the hydrophobic N-terminal signal anchor is specific²⁹. Structures of other domains of matrilysins are similar to other MMPs, i.e. a signal sequence, the latent domain, catalytic domain and C-terminal propeptide-like hemopexin, but lack structures

specific for other groups. Some MMPs, such as MT-MMPs and stromelysin-3, contain protein convertase specific recognition motifs (Arg-X-Arg-X-Lys-Arg, where X represents any amino acid).

3. REGULATION AND ACTIVATION OF MMPs

The role of MMPs in ECM degradation can be regulated at many stages, including gene activation and transcription, messenger ribonucleic acid (mRNA) stability, translation and secretion of latent proenzymes, binding of proenzymes to cell membranes and/or ECM components, proenzyme activation, inactivation by endogenous inhibitors and degradation or removal of active or inactive enzyme species. At the level of transcription, many of the MMPs appear to be regulated by similar mechanisms. MMPs are responsive to cytokines, growth factors and hormones. In general, most of the MMPs are induced by interleukin-1 β (IL-1 β), tumour necrosis factor- α (TNF- α), platelet derived-growth factor (PDGF), transforming growth factor- α (TGF- α), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF) and nerve growth factor (NGF) and are repressed by transforming growth factor- β (TGF- β)³⁰. Interleukin-1 α (IL-1 α) appears to be acting as an obligatory intermediate regulator for stimulation of interstitial collagenase expression³¹. Sensitivity of individual MMPs to these factors varies from enzyme to enzyme, and is tissue-specific. Specificity is maintained by induction and repression of distinct MMP family members. MMP production has also been found to be regulated by the pericellular environment, cell matrix interactions, and components of the ECM. IL-1 α and other cytokines have also been shown to stimulate interstitial collagenase release from fibroblasts in response to interaction between extracellular matrix and cell surface receptors³². Interestingly, gelatinase A exhibits only a slight response to cytokine and growth factors, and calcium influx suppresses gelatinase A mRNA and protein synthesis.

Under some conditions, transcriptional activation of the MMP genes may be a requirement for ECM turnover. Current evidence also suggests that transcriptional activation alone may not be sufficient. Activation of proenzyme forms of these proteases is required for initiation of matrix degradation and acquisition of the invasive phenotype. The balance of activated proteases and endogenous inhibitors is crucial for determining the extent of ECM turnover. The mechanism for mammalian MMP activation is referred to as the "cysteine switch" (Fig 2). In this mechanism, an unpaired cysteine residue in the profragment co-ordinates with the active site zinc atom and maintains the latency of the enzyme³³. When this cysteine-zinc atom interaction is interrupted by chemical or physical means a conformational change occurs and it results in subsequent proteolytic cleavage of the amino-terminal profragment³⁰.

So far, two possible pathways for *in vivo* activation of MMPs have been proposed. Latent interstitial collagenase, stromelysin-1 and gelatinase B may be



activated by the plasmin cascade. These events occur either at the cell surface via urokinase plasmin activator (uPA) receptor or distant from the site of secretion of the enzyme³⁴. Briefly, the zymogen plasminogen is cleaved by uPA or tissue plasmin activator (tPA) into plasmin. PA may be inhibited by an endogenous PA inhibitor (PAI). Plasmin cleaves 84 amino-terminal amino acids from latent fibroblast stromelysin to form activated stromelysin and cleaves 81 amino-terminal amino acids from latent interstitial collagenase to form partially activated interstitial collagenase. Activated stromelysin may subsequently increase the activity of the partially activated interstitial collagenase 5-8 fold by clipping an additional 15 amino acids from its carboxy-terminus³⁴. In contrast, activation of gelatinase A appears to occur in a cell-mediated fashion, although the exact mechanism of this activation is controversial. Plasmin/PA mediated activation of insoluble gelatinase A has been reported, while others have reported that purified progelatinase A and progelatinase A/tissue inhibitor of metalloproteinase-2 (TIMP-2) complex are degraded, not activated, by plasmin. It has also been demonstrated that serine proteases, such as plasmin and uPA, are not responsible

for cell-mediated activation of gelatinase A, because it is seen that serine protease inhibition failed to block cell-mediated activation of progelatinase A. Carboxy-terminally truncated gelatinase A is not activated by a similar mechanism, and therefore the carboxy terminus appears to be necessary for activation³⁵. The cell-surface associated activator has recently been isolated and shown to be an integral membrane matrix metalloprotease, referred to as MT-MMP³⁶.

4. TISSUES INHIBITORS OF MATRIX METALLOPROTEINASES

Tissue inhibitors of matrix metalloproteinases (TIMPs) are major endogenous regulators of MMP in tissue. These proteins' molecular masses range from 21 to 30 kDa. The TIMP family consists of: TIMP-1, TIMP-2, TIMP-3 and TIMP-4. Binding of the TIMPs to their specific MMPs results in the inhibition of MMP activity. MMPs bind with TIMPs in a 1:1 ratio forming binary noncovalent complexes with high dissociation constant K_d (10^{-9} – 10^{-10}). MMP-TIMP formations block substrate cleavage binding sites³⁷. Classification and characterization of the data associated with TIMP are shown in the Table 2³⁸.

Table 2: Classification of Tissue Inhibitors of Metalloproteinases (TIMPs)

TIMP	Inhibited MMP	Mol. Wt. (kDa)	Expression	Tissues	Localization
TIMP-1	All except MMP-14	28.5	inducible	bones, ovary	diffusible
TIMP-2	All	21.5	constitutive	placenta	diffusible
TIMP-3	MMP-1,-2,-3,-9 and -13	21	inducible	kidney, brain	ECM associated
TIMP-4	MMP-1,-2,-3,-7 and -9	22	-	heart	diffusible

TIMP-1, a 184 amino acid glycoprotein of 28.5 kDa, exhibits a 41% sequence homology with the non-glycosylated 21.5 kDa TIMP-2. It is more widely distributed than the other TIMPs and inhibits the activity of all the active MMPs. Its binding to MMP-9 and MMP-1 occurs via a reversible non-covalent binding to the catalytic domain of the MMP protein. TIMP-1 is not cleaved by this binding and can be recovered with full activity from complexes with MMP-3. The activity of MMPs can also be inhibited by α 2-macroglobulin. However, it has been shown that transfer of MMP-1 to α 2-macroglobulin does not occur if MMP is already complexed with TIMP-1. TIMP-2, a 194 amino acid unglycosylated protein of 21.5 kDa, has 41% and 44% sequence homology to TIMP-1 and TIMP-3, respectively. It inhibits the activity of all active MMPs and regulates the activation of pro-MMP-2 by binding to its C-terminal region. In addition to their inhibitory function, both TIMP-1 and TIMP-2 have been shown to have erythroid potentiating activity and cell growth-promoting activities.

TIMPs catalysis of disulfide bond formation between cysteines prevents autocatalytic MMPs activation through the cysteine switch mechanism. In addition, TIMPs in complexes with other molecules involve in the inhibition of already active MMP as it was known in MT-MMP. Activation of proMMP-2 by MT1-MMP and TIMP-2 has widely been studied. MT-MMP can form dimers and

multimers on the cell surface via hemopexin domains (Fig. 4). ProMMP-2 binds its hemopexin domain to the C-terminal domain of TIMP-2, which is linked via the N-terminal domain to MT1-MMP (Fig. 4), while simultaneously inhibited by TIMP. Complex (MT1-MMP)-(TIMP-2)-(ProMMP-2), subsequently leads to splitting the conservative sequence of the propeptide located in the catalytic site of proMMP-2 by MT1-MMP (Fig. 4).

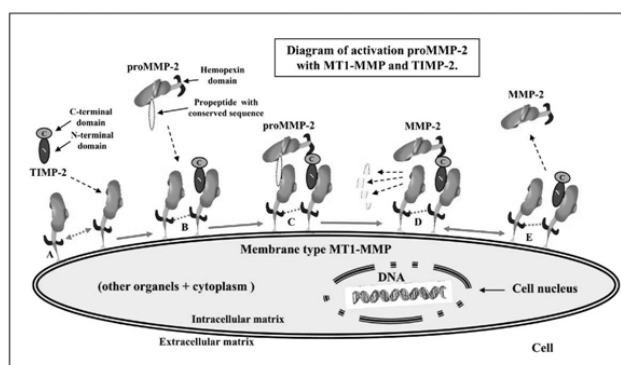


Figure 4: Mechanisms of pro-MMP-2 activation by trimer formation with TIMP-2 and MT1-MMP on a cell membrane³⁹. (A) Two membrane type MT1-MMPs are anchored on the cell membrane. (B) One molecule TIMP-2 selectively interacts with one MT1-MMP and forms MT1-MMP-TIMP2 complex. (C) This complex binds proMMP-2. (D) The second MT1-MMP anchored on the

cell membrane free of TIMP-2 cleaves propeptide from proMMP2. (E) This process leads to activation of MMP-2, which is released into extracellular matrix.

The expression of TIMP-1 and TIMP-2 is regulated differently by cytokines (e.g. tumour necrosis factor- α), which may modify the expression of TIMP-1 through the induction of nuclear transcription factors NF- κ B. TIMP-3, a 21 kDa glycoprotein, is expressed by a variety of cells. It has an affinity for components of the ECM and as a consequence it is largely sequestered there. It forms a non-covalent, stoichiometric complex with both latent and active MMPs. TIMP-3 prevents the activation of pro-MMP-2 by MT2-MMP. It differs from other TIMPs in that it is directly linked to components of the extracellular matrix (TIMP-1 and TIMP-2 is free in the interstitial space). Therefore, TIMP-3 modulates the activity of MMP more effectively than other TIMPs⁴⁰. Expression of TIMP-3 is quite high in eye tissue. TIMP-4 was detected in low concentrations in the kidney and colon and is absent in the lungs, liver and brain. By contrast, high expressions of TIMP-4 were detected in the heart⁴¹. Overproduction of TIMP inhibits the growth of various types of tumour cells. Decreasing the concentration of TIMP in the remodelling process during repair of damaged tissue increases the collagenolytic activity and allows tumour cells to disrupt the extracellular surroundings and migrate to neighboring tissues. Strict regulation and maintaining specific levels of collagenase and its inhibitors is necessary for tissue remodelling. Its disruption can significantly alter the functional characteristics of tissue⁴².

5. MMPs AND CANCER

MMPs are overexpressed in multiple tumor types when compared to normal tissues. The upregulation of the MMPs may be a secondary effect of the remodeling of the matrix and/or growth characteristics of the tumor. However, in cases in which increased MMP levels have been shown to be strong indicators of a negative prognosis, it is more likely that targeting those enzymes will impact tumor progression. Several studies have shown the possible use of MMPs in the future to augment treatment strategies in specific cancers⁴³. MMPs were classically thought to contribute to tumor metastasis via their matrix degrading activity, but in recent years, studies have implicated MMPs at virtually all stages of tumor progression from initial development of the tumor, growth, angiogenesis, invasion and metastasis and growth at the secondary site⁴⁴. There are several ways in which MMPs can increase tumor cell proliferation: first of all, MMPs can release the cell-membrane-bound precursors of some growth factors; they can render bioavailable peptide growth factors that are sequestered by ECM proteins and moreover they can control proliferation signals through integrins. MMPs also confer anti-apoptotic characteristics to cancer cells as can be seen by the release of FasL. For tumor cells to continue growing and to migrate to distant sites, the formation of new blood vessels is a fundamental step and many

studies with endogenous and synthetic inhibitors indicate the central role MMPs occupy in this process. MMPs can favor new blood vessel sprouting by simply eliminating physical barriers through the degradation of ECM structural components, or by the generation of pro-angiogenic factors. MMPs can also down regulate new vessel formation through the generation of angiogenesis inhibitors. MMPs take part in all events that lead to tumor cell detachment, invasion of the basement membrane and surrounding stroma and colonization of new sites.

5.1. Tumor Growth, Invasion and Metastasis

Tumor growth, invasion, and metastasis are a multistep and complex process that includes cell division and proliferation, proteolytic digestion of the extracellular matrix, cell migration through basement membranes to reach the circulatory system, and remigration and growth of tumors at the metastatic sites⁴⁵. MMPs degrade the basement membrane and extracellular matrix, thus facilitating the invasion of malignant cells through connective tissues and blood vessel walls and resulting in the establishment of metastases⁴⁶. The degradation of the extracellular matrix by MMPs not only facilitates metastasis but also promotes tumor growth by increasing the bioavailability of growth factors that reside in the extracellular matrix and are released during extracellular matrix degradation. MMP expression, although low or undetectable in most normal tissues, is substantially increased in the majority of malignant tumors. Numerous studies in a variety of tumor types, including lung, colon, breast, and pancreatic carcinomas, demonstrate over expression of MMPs in malignant tissues in comparison to adjacent normal tissues.

The MMPs in tumor tissues are produced not only by malignant tumors but also by stromal fibroblasts and inflammatory cells⁴⁷. These cells may produce cytokines and proteins that induce the production of MMPs by surrounding cells, creating extracellular networks of MMP secretion and activation. Furthermore, analyses of cellular components derived from primary tumor tissues or their corresponding lymph node metastases demonstrated increased expression of MMPs in the metastatic tissue, indicating that MMP expression is a component of the metastatic process. In addition to the well-documented 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 malignancies. In general, the gelatinases (MMP-2 and MMP-9) have been most consistently detected in malignant tissues and associated with tumor aggressiveness, metastatic potential and a poor prognosis. More recently, matrilysin (MMP-7) has been the focus of attention because its preferential expression in early-stage tumors and premalignant lesions may make it a suitable target for chemopreventative strategies.



5.2. Tumor Protection

MMP activities have also been traditionally associated with a variety of escaping mechanisms that cancer cells develop to avoid host immune responses. Some MMPs, such as MMP-9, can suppress the proliferation of T lymphocytes through IL-2R α signalling disruption⁴⁸. Likewise, MMP-11 decreases the sensitivity of tumor cells to natural killer cells by generating a bioactive fragment from α 1-proteinase inhibitor. MMPs can also modulate antitumor immune reactions by efficiently cleaving several chemokines or regulating their mobilization. MMPs, additionally, protects it host by stimulating protective and adaptive immune responses.

5.3. Apoptosis

MMPs have the ability to act on substrates that influence apoptosis. MMP-3 has pro-apoptotic activity on epithelial cells⁴⁹. By shedding the ectodomain of proHB-EGF, mature HB-EGF is formed, promoting cellular proliferation by activating ErbB4 receptor and inhibiting apoptosis. MMP-7 sheds the ectodomain of membrane-bound mFasL from cell membranes to generate soluble FasL (sFasL) that increases apoptosis through activation of Fas, excepting cancer cells that are refractory to this proapoptotic signal, because of proteic abnormalities in the signal transduction cascade⁵⁰. Concomitantly FasL protects cancer cells from chemotherapeutic drug toxicity. It was shown that MMP-1, MMP-9 and MT1-MMP are involved in autophagic cell death and apoptosis of breast tumor cells. Other MMPs, such as MMP-11, suppress tumor cell apoptosis inhibiting cancer cell death.

5.4. Angiogenesis

Angiogenesis is the formation of new blood vessels and in solid tumors it has been demonstrated that it is required for tumor growth. Angiogenesis is important for vascular remodeling and wound healing and its anomalies occur in numerous pathological conditions, such as rheumatoid arthritis, diabetic retinopathy, psoriasis, hemangiomas, and cancer⁵¹. Several pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) or transforming growth factor- β (TGF- β) are activated by MMPs, triggering the angiogenic switch during carcinogenesis and facilitating vascular remodelling and neovascularization at distant sites from a tumour⁵².

MMPs have been shown to have direct effects on endothelial cells, being necessary for cell migration and tube formation. MT1-MMP is mainly involved in endothelial cell migration and invasion of fibrin barriers, due to its fibrinolytic activity⁵¹. MMP-7 enhances endothelial cell proliferation, up-regulates endothelial expression of MMP-1 and MMP-2, and induces angiogenesis *in vivo*. Membrane vesicles containing MMP-2, MMP-9, and MT1-MMP are contained in endothelial cells, sometimes located near pseudopodia, and angiogenic stimulation of cells with bFGF and/or VEGF results in shedding of vesicles. bFGF stimulation by

MMP-9 enhances endothelial cell growth *in vitro*. MMP-2, MMP-9, and MMP-7 are expressed in vascular endothelial cells of tumors. Supplementary, MMP-7 stimulates vascular proliferation, as shown by several experiments. Oppositely, matrilysin-specific antisense oligonucleotides inhibit tumoral angiogenesis.

Recent findings have opened new connections between MMPs and angiogenesis generated by the induction of MMP-9 in tumor macrophages and endothelial cells. Contrary to these proangiogenic roles of MMPs, these enzymes also negatively regulate angiogenesis. MMP-2, MMP-7, MMP-9 and MMP-12 generate antiangiogenic polypeptides, by cleavage of plasminogen into angiostatin. Angiostatin inhibits endothelial cell proliferation and promotes endothelial apoptosis. MMP-3, MMP-7, MMP-9, MMP-13 and MMP-20 generate endostatin (another endogenous inhibitor of angiogenesis); by cleavage of collagen type XVIII⁵³. Also, MMPs are able to cleave the precursor of endostatin.

6. INHIBITION OF MMPs AS ANTICANCER THERAPY

Although MMP involvement in pathology is more than simple excessive matrix degradation, MMP inhibition may be of therapeutic benefit⁵⁴. Given the important roles that MMPs play in tumor growth, metastasis and the dysregulated angiogenesis that drives them, there has been significant attention paid to the development of clinically useful antagonists of this enzyme family⁵⁵. MMP expression and activation involve multiple steps-transcription of MMP genes, secretion of the zymogen into the extracellular matrix, and activation of the zymogen; several of which are amenable to pharmacologic intervention. For example, inhibition of signal transduction transmitted through the MAP kinases such as PD 98059 and SB 203560, markedly inhibits the expression of MMPs and the invasive potential of cancer cell lines. A second approach to the inhibition of MMP expression is via the use of specific antisense oligonucleotides. A potential advantage of antisense strategies is their selectivity for a specific MMP subtype, thereby potentially resulting in fewer systemic toxic effects.

At present, inhibition of the function of MMPs in the extracellular matrix is being most actively pursued for anticancer therapy. The naturally occurring inhibitors of MMP activity (TIMPs) were the first compounds to be considered for clinical development. Theoretically, the ability of TIMPs to potently and specifically inhibit the activity of several MMPs could result in a beneficial therapeutic effect. However, the lack of effective methods of systemic gene delivery has limited the clinical utility of this approach, whereas the development of synthetic inhibitors of MMPs has been actively pursued and widely tested in clinical trials (Table 3)⁵⁶. Inhibitors of MMPs fall into three pharmacologic categories: 1) collagen peptidomimetics and nonpeptidomimetics, 2) tetracycline derivatives and 3) bisphosphonates.



Table 3: Matrix metalloproteinase inhibitors (MMPi) in clinical development

Compound	Class	Specificity	Company	Status
AG3340	Nonpeptidomimetic inhibitor	MMP-2 and -3	Agouron Pharmaceuticals (San Diego, CA)	Phase II/III
BAY 12-9566	Nonpeptidomimetic inhibitor	MMP-2 and -3	Bayer Corporation (Pittsburgh, PA)	Development halted
CGS 27023A	Nonpeptidomimetic inhibitor	Broad spectrum	Novartis Pharmaceuticals (East Hanover, NJ)	Phase I
Batimastat	Peptidomimetic inhibitor	MMP-1, -2, -3, -7, and -9	British Biotech (Oxford, U.K.)	No further development
Marimastat	Peptidomimetic inhibitor	MMP-1, -2, -7, and -9	British Biotech (Oxford, U.K.)	Phase II/III
Col-3 (metastat)	Chemically modified tetracycline	MMP-2 and -9	Collagenex (Newtown, PA)	Phase I

6.1. PEPTIDOMIMETIC MMP INHIBITORS

These compounds are pseudopeptide derivatives that have been synthesized to mimic the structure of collagen at the site where MMP binds to cleave it. The inhibitor binds reversibly at the active site of the MMP in a stereospecific manner and chelates the zinc atom on the enzyme activation site⁵⁷. Several zinc-binding groups have been tested for their ability to competitively inhibit MMP by binding at the active site; these groups include carboxylates, aminocarboxylates, sulfhydryls, derivatives of phosphoric acid, and hydroxamates. Most MMP inhibitors in clinical development are hydroxamate derivatives.

6.1.1. Batimastat

Batimastat, the first MMP inhibitor evaluated in cancer patients, is a nonorally bioavailable low-molecular weight hydroxamate. This compound is potent but relatively nonselective, with IC_{50} (concentration that causes 50% enzyme inhibition) values of less than 10 ng/mL for MMP-1, -2, -3, -7 and -9 inhibition. *In vitro*, batimastat had cytostatic effects against a variety of cancer cell lines and was not cytotoxic⁵⁸.

6.1.2. Marimastat

Marimastat is a synthetic low-molecular-weight MMP inhibitor that, in contrast to batimastat, is orally bioavailable, with an absolute bioavailability of 20%–50% in preclinical studies. The drug contains a collagen-mimicking hydroxamate structure that chelates the zinc ion at the active site of MMPs. Like batimastat, marimastat is relatively nonspecific, inhibiting the activity of MMP-1, -2, -3, -7, and -9 with IC_{50} s of 2.5, 3, 115, 8, and 1.5 ng/mL, respectively⁵⁹. Preclinical studies⁵⁹ of marimastat against lung and breast cancer experimental metastases models demonstrated a reduction in the number and size of metastatic foci in treated versus control animals. Marimastat has subsequently been evaluated in several phase I-II studies. The principal toxic effect of marimastat in these studies was the appearance of a dose-limiting inflammatory polyarthritis⁶⁰. The occurrence of this musculoskeletal toxic effect was related to marimastat dose, substantially limiting administration of the agent at doses more than 10 mg twice a day⁶¹. Marimastat is currently undergoing phase III clinical trials, both alone and in combination with

classic chemotherapy, in patients with various solid tumor types⁶². Overall survival of patients with advanced pancreatic cancer who were treated with marimastat was not better than that of patients treated with gemcitabine. However, patients with advanced gastric cancer who either had not received prior therapy or were stable after initial chemotherapy demonstrated a statistically significant improvement in disease-free and overall survival after treatment with marimastat than after treatment with placebo.

6.2. NONPEPTIDIC MMP INHIBITORS

Problems with the peptidic MMP inhibitors include poor oral bioavailability (except for marimastat) and a relative lack of specificity for the MMPs thought to contribute to cancer progression⁶³. In an effort to avoid these problems, several nonpeptidic MMP inhibitors have been rationally synthesized on the basis of the three-dimensional x-ray crystallographic conformation of the MMP active site. The rational chemical design of MMP inhibitors made possible the synthesis of compounds with specific inhibitory activity against the MMP subtypes that predominate in certain diseases, such as cancer and arthritis. For example, AG3340, BAY 12–9566 and BMS-275291 were designed to be relatively selective inhibitors of MMP-2, whereas Ro 32–3555 was designed to be specific for MMP-1, which is frequently associated with osteoarticular diseases, and is thus being developed for arthritis⁵⁸. AG3340, BAY 12–9566, BMS-275291, and CGS 27023A are currently undergoing clinical evaluation in cancer patients.

6.2.1. BAY 12–9566

BAY 12–9566 is an orally bioavailable biphenyl compound that is a potent inhibitor of MMP-2, -3, and -9, with an IC_{50} below 0.13 μ g/mL⁵⁸. BAY 12–9566 has completed phase I evaluations in patients with malignant disease, both alone and in combination with chemotherapy⁶⁴. BAY 12–9566 has subsequently been evaluated in phase III clinical trials in patients with pancreatic, ovarian and lung cancers. Administration of BAY 12–9566 to patients with advanced pancreatic cancer resulted in statistically significantly inferior survival time and time to treatment failure when compared with gemcitabine, necessitating early termination of the study. In addition, an interim analysis of a phase III study that compared continuous administration of BAY 12–9566 with placebo in patients



with small-cell lung cancer following response to conventional therapy has also been prematurely closed because of the inferior survival of patients treated with BAY 12–9566. On the basis of these results, clinical development of BAY 12–9566 has been suspended.

6.2.2. AG3340

AG3340 is a nonpeptidic collagen-mimicking MMP inhibitor that was synthesized by use of a protein structure drug design program. The drug inhibits MMP -2, -9, -3 and -13, with IC_{50} s of below 0.13 ng/mL⁶⁵. AG3340 was evaluated in phase I clinical trials as well as in phase I–II feasibility studies in combination with chemotherapy and it is currently being evaluated in phase III randomized clinical trials in patients with non-small-cell lung and prostate cancers. At doses ranged from 2 to 100mg/day, AG3340 can be safely combined with mitoxantrone/prednisone and carboplatin/paclitaxel, and these combinations are currently being tested in randomized placebo-controlled phase III clinical trials as first-line therapy for patients with hormone-refractory prostate cancer and non-small-cell lung carcinoma, respectively.

6.2.3. CGS-27023A

CGS-27023A is a broad-spectrum inhibitor of MMPs. The agent does not inhibit the proliferation of cancer cell lines *in vitro*, but it does inhibit the invasion of tumor cells through matrigel as well as angiogenesis in various preclinical models. The combination of CGS-27023A with conventional cytotoxic agents resulted in additive or synergistic effects. CGS-27023A has been evaluated in a phase I clinical trial administered orally on a continuous dosing schedule at doses ranging from 150 to 600 mg in divided doses. The major toxic effects, which were encountered at doses exceeding 300 mg twice a day, consisted of cutaneous and musculoskeletal toxicity. Pharmacokinetic analysis revealed that administration of CGS-27023A at clinically tolerable doses yielded plasma concentrations that were several fold greater than the *in vitro* IC_{50} s for MMP-2, -3 and -9 and were sustained for longer than 10 hours after dosing.

6.3. TETRACYCLINE DERIVATIVES

The tetracycline derivatives inhibit not only the activity but also the production of MMPs and are thus being investigated for the treatment of disorders in which the MMP system becomes amplified, such as degenerative osteoarthritis, periodontitis and cancer^{66,67}. This family of agents comprises both the classic tetracycline antibiotics, such as tetracycline, doxycycline and minocycline, and as the newer tetracycline analogues that have been chemically modified to eliminate their antimicrobial activity (e.g., removal of the dimethylamino group from carbon-4 of the "A" ring). These agents inhibit the collagenases, MMP-1, -3, and -13, and the gelatinases, MMP-2 and -9, via multiple mechanisms⁶⁸, including 1) blocking the activity of mature MMPs by chelation of the zinc atom at the enzyme binding site, 2) interfering with

the proteolytic activation of pro-MMP into their active form, 3) reducing the expression of MMPs and 4) protecting MMPs from proteolytic and oxidative degradation⁶⁶. Some tetracycline derivatives have been evaluated in preclinical cancer models and have entered early clinical trials in patients with malignant diseases, including doxycycline and Col-3.

6.3.1. Doxycycline

Doxycycline is one of the classic antimicrobial tetracyclines that has been studied extensively in patients as an anticancer agent⁶⁹. Like related compounds, doxycycline exerts diverse inhibitory effects on MMP production and activity⁷⁰. The inhibitory effects of doxycycline on breast cancer tumor metastasis formation were potentiated by the addition of batimastat, suggesting that targeting MMPs through multiple distinct pathways may improve treatment efficacy.

6.3.2. Col-3 (metastat)

The chemically modified tetracyclines (CMTs) comprise a group of at least 10 analogues (CMT-1 to -10) that differ in their MMP specificity and potency. CMTs have several potential advantages over conventional tetracyclines: Their long-term systemic administration does not result in gastrointestinal toxicity, they reach greater plasma concentrations, and they have a longer elimination half-life, requiring less frequent drug administration. CMT-3, also known as Col-3 (metastat), is one of the most potent CMTs studied to date and has demonstrated antitumor activity both *in vitro* and *in vivo*. On the basis of these preclinical studies, Col-3 is currently being evaluated in phase I clinical trials in cancer patients treated by oral administration on a continuous dosing schedule. Preliminary pharmacologic analysis indicates that Col-3 has a prolonged elimination half-life and that, at clinically tolerable doses, steady-state plasma concentrations exceed the IC_{50} required for *in vitro* activity⁷¹.

6.4. BISPHOSPHONATES

The bisphosphonates are a class of drugs developed during the last three decades for use in disturbances of calcium homeostasis and, more recently, for the palliation and prevention of bone metastases in patients with breast cancer and multiple myeloma⁷². The agent exerts varied inhibitory effects on MMPs, including inhibition of their enzymatic activity. In addition, these agents inhibited TGF- β 1-induced MMP-2 secretion in PC-3 prostate cancer cell lines, resulting in inhibition of collagen degradation. Clodronate, one of the most frequently used bisphosphonates, also inhibited the expression of the MT1-MMP protein. The fact that these agents have been used clinically for a number of years and are well known both pharmacologically and toxicologically suggests that they should be investigated in properly designed clinical trials in patients with cancer or other diseases for which inhibition of MMPs may be beneficial⁷³.



7. CONCLUSION AND FUTURE PERSPECTIVES

Matrix metalloproteinases are considered promising targets for cancer therapy due to their strong involvement in malignant pathologies, as their expression is upregulated in such diseases and because they can degrade all components of the extracellular matrix. Preclinical studies analyzing MMP inhibition in tumor models brought positive results rising the idea that the development of strategies to inhibit MMPs may be a powerful tool to help defy cancer. Although clinical trials with MMP inhibitors have not been promising, MMPs are still interesting targets for therapy. More knowledge about the function of the specific MMPs is needed for the beginning of new clinical trials. In spite of the considerable progress made in the design of novel anti-cancer drugs in recent years, one clear lesson from the recent decades of research into cancer is that, although we can treat cancer and induce remission, survival rates have changed little in most cancers. Moreover, most anticancer drugs have several toxic side-effects that may produce a poor quality of life for cancer patients and considerable cost in supporting care.

From this point of view, one could conclude that the chances of developing effective anti-MMP therapies would greatly increase with improved knowledge of the contribution of MMPs to the progression of specific cancer types and stages with the appropriate tools for evaluating MMP inhibitory activity at both the molecular and clinical levels. Although, at the moment, the future of MMP inhibitors in the treatment of cancer appears to lie primarily in the "hands" of the pharmaceutical companies, what remains clear is that there is still much basic and translational groundwork to be done to develop and validate tools identifying tumor expressing target enzymes and, primarily, to assess the efficacy of specific compounds (and optimal doses) that significantly limit tumor-associated proteolytic activity. It is of concern, whether a balance between required activity and avoidance of toxicity, based on focused targeting of specific MMPs, can ever be achieved. The concept of MMPs is too intriguing to completely reject their development; however that at the current stage focus should again be on preclinical research.

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