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REVIEW

The Role of Matrix Metalloproteinases in Tumor Angiogenesis and Tumor Metastasis

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Although a considerable amount of effort has been placed on discovering the etiologies of cancer, the majority of the basic cancer research existing today has focused on understanding the molecular mechanism of tumor formation and metastasis. Metastatic spread of tumors continues to be a major obstacle to successful treatment of malignant tumors. Approximately 30% of those patients diagnosed with a solid tumor have a clinically detectable metastasis and for the remaining 70%, metastases are continually being formed throughout the life of the tumor. Even after the tumor is excised, the threat of death is attributable to the metastasis that may occur through the remaining tumor cells. In addition, treating the metastasis often proves futile since metastasis often vary in size, composition, and anatomical location. New treatments blocking the formation of metastasis will provide greater chances of survival for cancer patients. One family of enzymes that has been shown over the years to play a role in tumor progression is the matrix metalloproteinase (MMP) family. The main function of MMPs, also known as matrixins, is degradation of the extracellular matrix physiologic function involving MMPs include wound healing, bone resorption and mammary involution. MMPs, however, also contribute to pathological conditions including rheumatoid arthritis, coronary artery disease, and cancer. Tumor cells are believed to utilize the matrix degrading capability of these enzymes to spread to distant sites. In addition, MMPs also are thought to promote the growth of these tumor cells once they have metastasized. This review will discuss the role of MMPs and their inhibitors in tumor invasion, angiogenesis and metastasis with special emphasis on the gelatinases, MMP-2 and MMP-9. (Pathology Oncology Research Vol 7, No 1, 14–23, 2001)

Keywords: matrix metalloproteinases; metalloproteinase inhibitors; gelatinases; tumor angiogenesis; metastasis

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List of Abbreviations

Amino-terminal – NH2-terminal; Carbohydrate Antigen 19-9 – CA19-9; Carcinoembryonic Antigen – CEA; Cerebrospinal Fluid – CSF; Endothelial Cell – EC; Extracellular Matrix – ECM; Human Dermal Microvascular Endothelial Cell – HDMVEC; Human Umbilical Vein Endothelial Cell – HUVEC, Interleukin-1 Alpha – IL-1α; Interleukin-1 Beta – IL-1β; Interleukin-8 – IL-8; Intravital Videomicroscopy – IVVM; Matrix Metalloproteinase – MMP; Matrix Metalloproteinase Inhibitor – MMPI; Membrane-Type Metalloproteinase – MT-MMP; Phorbol 12-Myristate 13-Acetate – PMA; Polymorphonuclear Cells – PMNs; Thrombospondin-1 – TSP-1; Tissue Inhibitor of Metalloproteinase – TIMP; Transforming Growth Factor Beta – TGF-β; Tumor Necrosis Factor – TNF; Vascular Endothelial Cell Growth Factor – VEGF

Introduction to the MMP family

The first mammalian MMP, interstitial collagenase, was discovered over thirty years ago by Gross and Lapiere in amphibian tissue.[10] Since then, over 14 members have been added to the family. The MMP family can be subdivided into five groups: the collagenases, the stromelysins, the gelatinases, PUMP-I or matrilysin, and membrane-type (MT) MMPs. Although the classification system was developed on the basis of substrate specificity, it is now recognized that there is some overlap between some members of the family (see *Table 1*). For example, MMP-2 has been reported to have the ability to cleave fibrillar collagen similar to the collagenases.[11]

This multigene family of metal containing proteases share several common characteristics: (1) each degrade at least one component of the basement membrane; (2) they

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are active at physiological pH; (3) they require 2 Zn^{++} ions/molecule in order to be active; (4) they are inhibited by metal chelators and tissue inhibitors of metalloproteinases (TIMPs); and (5) they are secreted as zymogens and require activation extracellularly.[12,13]

Comparing the protein and cDNA sequences of cloned MMP molecules reveal a number of conserved regions within the family. All latent MMPs contain at least three domains: (1) a hydrophobic pre-peptide domain that is necessary to signal secretion, (2) an amino terminal propeptide domain which is removed upon activation, and (3) the Zn^{++} containing catalytic domain.[8,12,13] This basic 3-part structure is present in all of the MMPs. Within the family, however, further subdivisions exist based on distinct structural variations. Matrilysin (MMP-7), the smallest member of the MMP family is comprised of only the basic core structure. The remaining enzymes all contain a "hemopexin" domain connected to the catalytic domain by a hinge region. The hemopexin domain contains the TIMP binding site and may also be involved in receptor binding. The membrane-type metalloproteases (MT-MMPs) are a unique subdivision in that they contain a transmembrane domain at the carboxyl terminal anchoring the molecule to the cell surface. Another subdivision, the gelatinases (MMP-2 and MMP-9) contain a fibronectin-like domain within the catalytic domain responsible for collagen binding. MMP-9 has an additional collagen V-like sequence within the catalytic domain downstream of the Zn^{++} binding.

MMP regulation

Regulation of the MMPs occurs on three levels: alteration of gene expression, activation of latent zymogens, and inhibition by tissue inhibitors of metalloproteinases. The cooperative effects of these three factors provides a tightly controlled regulation of MMPs in normal physiological states. Alteration of all three levels of control have been associated with tumor cell progression.[14]

Gene Regulation

MMPs and TIMPs are thought to be regulated by a variety of cytokines, growth factors, steroid hormones and phorbol esters.[8] Despite a vast array of research in this area of MMP regulation, transcriptional activation is not fully understood. These factors cause variable patterns of expression in different tissues and have variable effects on the different MMP family members, complicating the understanding of gene regulation of MMPs in both physiological and pathological states. One of the most potent family of inducers are phorbol esters such as PMA.[8,15] Other inducers include interleukin (IL)-1α, IL-1β, IL-8, transforming growth factor (TGF)β-1, and tumor necrosis factor (TNF).[8,9,13] Other factors implicated in stimulating MMP regulation include basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), and vascular endothelial cell growth factor (VEGF).[16-18] Important to remember is that there is often a balancing effect within the corresponding physiological inhibitors of MMPs, the tissue inhibitor of metalloproteinases (TIMP). To create a favorable state for invasion, there must be a balance of protease to

Table 1. **Matrix Metalloproteinase Family***

MMP Name and No	*Molecular Weight (kDa)* *Latent*	*Active*	*ECM Substrate*
Interstitial Collagenase (MMP-1)	55	45	Fibrillar Collagens
Neutrophil Collagenase (MMP-8)	75	58	Fibrillar Collagens
Collagenase-3 (MMP-13)	60	48	Fibrillar Collagens
MMP-18	?	?	Fibrillar Collagens
Stromelysin-1 (MMP-3)	57	45	Laminin, fibronectin, non-fibrillar collagens
Stromelysin- (MMP-10	57	44	Laminin, fibronectin, non-fibrilar collagens
Stromelysin-3 (MMP-11	51	44	Laminin, fibronectin, non-fibrillar collagens
Matrilysin (MMP-7)	28	19	Laminin, fibronectin, non-fibrillar collagens
Metalloelastase	54	45/22	Elastin
MT1-MMP (MMP-14	66	60	Gelatinase A, fibrillar Collagens, Proteoglycans, ECM glycoproteins
MT2-MMP (MMP-15)	68	62	Gelatinase A
MT3-MMP (MMP-16)			Gelatinase A
MT4-MMP (MMP-17)			
Gelatinase A (MMP-2)	72	66	Type I, IV, V and fibrillar collagens; gelatin
Gelatinase B (MMP-9)	92	86	Type IV, V collagen, gelatin
MMP-19	61	?	Gelatin

*The MMP family are listed here in addition with their molecular weights and their substrates.
The subdivisions correspond to a combination of structural and substrate homology. The newest members including MMP-18, MMP-19, and the MT-MMPs have not yet been fully characterized.

inhibitor in order for the cell to migrate and invade. Although the exact role of cytokines and growth factors is not clear, it appears that they act in conjunction to regulate both MMPs and their inhibitors to create the environment necessary for either physiological or pathological processes.

In addition to cytokines and growth factors, MMP production can be regulated by environmental factors. For example, extracellular matrix (ECM) component peptides have been observed to have an effect on MMP production. The laminin-1 peptide, AG-73 has been shown to enhance gelatinase production and increase in vivo B16-F10 melanoma cell lung and liver metastasis.[19] Vitronectin, collagen, and elastin have also been shown to induce collagenases and gelatinases expression in either tumor cells or fibroblasts.[20-22] Our laboratory has done extensive work in identifying thrombospondin-1 (TSP-1), a 450 kDa extracellular matrix protein, as a stimulator of MMP-9 production in endothelial cells, pancreatic cancer cells, and breast cancer cells.[23,24] The capacity of TSP-1 to upregulate MMP-9 is mediated in part by the CSVTCG peptide sequence present in the type one properdin repeats of TSP-1. Synthetic peptides containing the CSVTCG sequence probably bind and block a specific TSP-1 receptor.[24] These peptides represent a potential therapeutic approach for the treatment of metastasis. These data all suggest that the cell environment plays a key role in regulating MMP production.

Activation of Latent MMPs

Another level of MMP regulation is the activation of the zymogen/proenzyme secreted form of MMPs. All MMPs are secreted as zymogens with the exception of the MT-MMPs, the membrane sequestered subdivision. These latent zymogens must be activated in order to degrade matrix components. As mentioned previously, these proenzymes remain in an inactive form through an interaction between a cysteine in the proregion and the Zn^{++} ion in the active site. This interaction blocks access to the active site and cleavage of this site results in enzyme activation. Trypsin 2 has the ability to activate both free proenzyme and proenzyme:TIMP-1 complexes, the major form of MMP ***in vivo.***[25] Other agents which have been shown to activate MMPs include cathepsins G, B, and L, PMN elastase, and hypochlorous acid.[26] In addition, the plasmin/plasminogen system has been implicated in tumor cell invasion in part through its ability to activate MMPs.[27]

MMPs, once activated, are also capable of activating themselves. MMP-3 can activate MMP-2 and MMP-9 as can MMP-7. The gelatinases, MMP-2 and MMP-9, can activate each other. In contrast to other members of the family, MMP-2 does not seem to be activated by plasmin or stromelysin. There seems to be a tighter regulation of MMP-2 than the other MMPs perhaps in part to the fact that MMP-2 is the most commonly expressed MMP in normal tissues.[28] The newest subclass of MMPs, the MT-MMPs, have the capacity to activate pro-MMP-2.[29] The transmembrane domain is essential to MT-MMP's capacity to activate MMP-2. Understanding this level of regulation of MMP will provide yet another target for development of anti-cancer therapeutics.

Inhibitors of Metalloproteases

The third level of control of MMPs occurs through inhibition of enzymatic activity. Various physiological agents can have an inhibitory effect on MMPs including α2-macroglobulin and TIMPs, tissue inhibitors of metalloproteinases.[30] The α2-macroglobulin molecule, a large molecular weight (780 kDa) serum protein, can inhibit proteinases, but its size prevents the molecule from entering into tissue spaces. The TIMPs are much smaller molecules and are expressed in various tissues and fluids. There are four members of the mammalian TIMP family, TIMP-1, TIMP-2, TIMP-3, and TIMP-4.[31] The amino-terminal domain present in all TIMP molecules is responsible for the MMP inhibitory activity. The TIMPs form high affinity, non-covalent complexes with all active MMPs in a 1:1 stoichiometric ratio. In addition, TIMP-1 and TIMP-2 can block the pro forms of MMP-9 and MMP-2, respectively. The balance between protease and inhibitor is critical in determining net proteolytic activity.

TIMP-1 is a 28.5 kDa glycoprotein that has a wide variety of functions including growth factor activity, stimulating cell morphology changes, and inhibiting angiogenesis.[31] Increased TIMP-1 levels have traditionally been associated with reduced invasion and metastasis. Some controversy has arisen on the role of TIMP-1, however. Recent studies have shown that TIMP-1 may increase the invasive capacity of tumor cells due to its growth factor like activity.[32] TIMP-2, similar to TIMP-1, is associated with decreased metastatic potential. TIMP-2, a non-glycosylated 21 kDa protein, suppresses tumor growth and invasion.[31] In contrast to TIMP1 and TIMP-2, which are secreted, TIMP-3 is associated with the extracellular matrix.[31] A recent report describes melanoma cells transfected with the TIMP-3 gene reduced invasion and induced cell death in these cells ***in vitro.***[33] The most recently identified molecule is TIMP-4, a 24 kDa protein. Some controversy exists on the roles of TIMP-2 and TIMP-3 as well since several studies report increased expression of these compounds results in increased metastatic potential. Again, it is important to consider the ratio of protease to inhibitor which is critical to invasive capacity. A small change in the balance could have a profound effect on proteolysis.

Tumor progression: the angiogenic and metastatic cascade

Metastasis, a hallmark of cancer, is the colonization of distant sites with cells from the primary tumor.[3] The process of metastasis involves the survival of the primary tumor cell through a series of steps including: tumor cell invasion enabling it to break free from the primary tumor, intravasation, survival and extravasation into and from the blood or lymphatic circulation, and finally tumor cell colonization and angiogenesis to form the metastatic lesion.[3] Although the majority of those cells that escape from the primary tumor will not survive, those capable of invading into and out of the circulatory system will each give rise to a metastatic tumor.

Metastasis can be described clinically as four different patterns: No metastasis is present at the initial diagnosis but appears within (1) months or (2) years after the primary tumor is removed; (3) metastasis is present at the time of diagnosis of the primary tumor; and (4) only the metastasis is detectable at the time of diagnosis.[34] Metastasis that appears after several years have been termed "dormant" metastasis. Considerable research has been done to understand the molecular changes a normal cell undergoes to become malignant. However, no single gene has been implicated as a metastasis-specific gene. There is likely to be a cascade of events that occur to create a malignant cell.

Angiogenesis or neovascularization is a tightly regulated system occurring rarely in a normal physiologic environment.[34] Reproduction, embryogenesis, and wound healing are among the few situations requiring angiogenesis. The formation of these new blood vessels is one of the major factors that stimulates tumor growth. Tumors without a blood supply are only able to grow 1-2 mm^3, and formation of a blood supply is critical to forming metastases.[35] A tumor cell cannot escape from the primary tumor until the tumor has been vascularized and once the metastatic cell has reached its target organ, angiogenesis is required for the metastases to grow.[34] Angiogenesis occurs through a series of steps including (1) the release of angiogenic factors (2) the release of proteolytic enzymes to degrade the basement membrane of the postcapillary venule (3) EC migration toward the tumor (4) EC proliferation and (5) microvessel formation and differentiation.[36] Tumors which bear a higher percentage of angiogenic cells have been shown to have increased metastatic potential and are more aggressive tumors.[37] Similarly, agents which are capable of inhibiting angiogenesis, such as anti-bFGF, subsequently reduce growth of malignant cell lines ***in vivo***.[37]

Proteases are required by the malignant cell to invade the ECM. The metastatic cell uses proteases to invade through the basement membrane and its underlying connective tissue and then subsequently through the basement membrane of the small blood vessels and lymphatics. These processes are then repeated upon extravasation of tumor cells from the vessel.[38] ***In vitro*** and ***in vivo*** studies show a definite correlation between gelatinase expression and metastasis. In addition, MMPs are essential factors in tumor angiogenesis (see ***Figure 1***).[39] Proteolytic activity is required during the formation of the capillary bud in order for the endothelial cell to migrate out through the peri-capillary membrane and through the ECM. In addition, capillary elongation, lumen formation, and ECM remodeling all require proteolytic activity. Recent studies have implicated MMPs as an important protease component in angiogenesis perhaps as a downstream modulator to known angiogenesis-related molecules such as VEGF and thrombospondin-1.[24,40]

The role of MMPs in tumor angiogenesis

Many studies have shown that endothelial cells are capable of differentially expressing and activating MMPs and TIMPs. Type I collagen is the predominant constituent of the perivascular ECM, and as mentioned previously, a variety of MMPs are capable of degrading collagen type I including interstitial collagenase and neutrophil collagenase.[39] Several studies have shown that MMP-1 is a critical protease in the angiogenic cascade. Immunofluorescent staining revealed that more aggressive skin tumors displayed a higher number of collagenase-containing blood vessels.[41] This collagen-degrading activity has been shown to be specific through a number of specific MMP inhibitors such as TIMP, Batimastat, and Marimastat which are capable of suppressing MMPs and therefore capillary tube formation ***in vitro*** and ***in vivo***.[42]

A number of angiogenic factors have been shown to regulate the production of MMPs in endothelial cells. Our

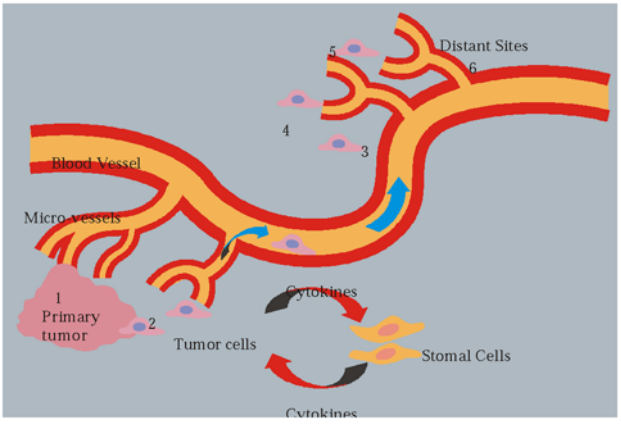

Figure 1. *Schematic of tumor growth dependent on angiogenesis. Tumor cells multiply and grow, eventually requiring a blood supply to sustain further growth. Cytokines secreted by the tumor cells and adjacent stromal elements stimulate endothelial cells to produce MMPs needed for endothelial cell migration and tube formation.*

laboratory has show that TSP-1 can also stimulate BAECs to secrete MMP-9.[24] The role of TSP-1 in angiogenesis has been controversial. We have shown that TSP-1 is a bifunctional modulator of angiogenesis acting in part through MMP-9 regulation. In a collagen gel tube formation assay, TSP-1 stimulated endothelial tube formation at low concentration (5 μg/ml) while at higher concentrations (15 μg/ml), TSP-1 inhibited tube formation. The ability of TSP-1 to either induce or inhibit tube formation was attributable to the amount of MMP-9 produced. Optimal MMP-9 levels were induced by lower concentrations of TSP-1. Increasing amounts of MMP-9 caused excessive proteolysis and inhibited tube formation.

The roles of the type IV collagenases, MMP-2 and MMP-9, in angiogenesis have been explored in a variety of studies. In a tube formation assay model, MMP-2 was produced by HUVECs as they formed tube like structures and this formation could be inhibited by adding TIMP-1 or TIMP-2 to the cultures.[43] A recent study examining the progression of mycosis fungoides, a hematological tumor of T-cell lineage, revealed that the more advanced the tumor, the microvessel density increases as does MMP-2 and MMP-9 expression.[44] The MMP-2 was expressed mostly by the microvascular cells of the blood vessels within and surrounding the tumor in addition to fibroblasts adjacent to the tumor stroma. MMP-9 was present in the tissue macrophages located close to the tumor nodules. In contrast, both MMP-2 and MMP-9 were only weakly expressed in normal tissue.

Perhaps the greatest evidence associating MMP-2 in the angiogenic process is the recent MMP-2 knock-out model examining angiogenesis and tumor progression.[45] Gelatinase A deficient mice displayed reduced tumor induced angiogenesis as measured by the dorsal air sac assay. In addition tumor volumes of B16-BL6 melanoma cells and Lewis lung cancer cells when injected intradermally decreased by 39% and 24%, respectively. The number of lung colonies also decreased by 54% for the B16-BL6 melanoma cells and 77% for the Lewis lung cancer. Therefore, host derived gelatinase A is necessary to promote tumor angiogenesis and tumor progression.

Similarly, the MMP-9 knock-out model also provides key evidence to the role of gelatinase B in angiogenesis. MMP-9 knock-out mice exhibit an abnormal pattern of skeletal growth plate vascularization and ossification. This aberrant pattern of vascularization returns to normal after transplanting wild-type bone marrow cells indicating that MMP-9 expression in cells of bone marrow origin is one factor that regulates normal vascularization in the skeletal growth plate. Important to note, however, is that vascularization, apoptosis, and ossification compensates to produce a normal growth plate after three weeks postnatal in these animals. The control on angiogenesis is believed to be mediated by a delayed release of an angiogenic activator mediated through the lack of MMP-9 expression. These data suggest that MMP-9 plays a regulatory role in angiogenesis not only through proteolytic activity but also through other downstream angiogenic factors.[46]

Evidence for MMPs in tumor metastasis

In order for a tumor cell to intravasate and extravasate, the collagen-rich ECM and basement membrane must be degraded. This degradative ability can be through either enzymatic capacities of the tumor cell or through enzymatic activity of cellular components of the matrix, such as fibroblasts. Likely there is a cooperation between the two components enabling the tumor cell to reach its target organ and survive. Although all five major classes (serine, aspartic, cysteine, threonine, and metalloproteinases) are involved in metastasis, a great deal of emphasis has been placed on the type IV collagenases, MMP-2 and MMP-9.[38] Type IV collagen is a major structural protein in the basement membrane and ECM. A number of studies have linked elevated MMP-2 and MMP-9 levels with an increased metastasis. The conclusions which can be drawn thus far are that the number and the relative levels of MMPs increase with tumor progression.

Several recent studies have been done to try and characterize the phenotypic and enzymatic profiles of more aggressive tumor cell lines. Selection of progressively more invasive human lung carcinoma cells from an established CL1cell line, revealed that the more invasive cells had a higher expression of MMP-9.[47] These cells had a 4 to 6 fold increase in invasive activity over the parentals and had an increased metastatic potential in vivo. MMP-9 has also been shown to be overexpressed in advanced stage melanoma cells.[48] Cell lines from early stage melanoma lesions revealed no MMP-9 expression while those cells from advanced lesions not only expressed MMP-9 but were also capable of being induced to secrete more MMP-9 by TGF, IL-1 and TPA.[48] Other tumor models involving MMP-9 in their invasive phenotype include human non-hodgkins lymphoma cells and human giant cell tumors.[49,50] Examining MMP-9 production in human giant cell tumors, tumors characterized by frequent vascular invasion, revealed that MMP-9 is highly expressed by the giant cells and the absence of collagen and laminin in the basement membrane correlated with those regions of high MMP-9 expression. MMP-9 through all of these data is certainly one of the key enzymes involved in invasion and metastasis.

MMP-2 has also been observed to be overexpressed in more aggressive tumor cells. Comparing a highly metastatic mouse mammary tumor cell line to its parental poorly metastatic cell line, show the highly metastatic cells express more MMP-2.[51] The level of pro-MMP-2 vs. active MMP-2 also plays a role in determining invasive

and metastatic capacity. The MMP-2 activation ratio in tumor tissue was also higher in pancreatic carcinoma patients with positive regional lymph nodes than those without metastasis.[52] The increase in MMP-2 activity levels correlates with the metastatic potential of the two carcinoma types. Elevated levels of MT-MMPs have been shown to increase activated levels of MMP-2 in breast, cervical, and lung carcinomas producing higher levels of invasiveness and metastasis. Transfection of several cell lines (HT-1080 fibrosarcoma, MCF-7 breast carcinoma, and U251.3 glioma cell lines) with the MT-MMP gene caused an increase in tumor cell invasion and migration.[53] Cell lines displaying an intermediate level of activation were the most invasive while those cells with a high level of activation were the least invasive. This is probably due to the balance required between MMPs and TIMP to create a controlled proteolytic system.

Although the major role of MMPs in metastasis has been inferred from the ***in vivo*** and ***in vitro*** data presented above to be breakdown of the ECM, recent studies have proposed additional roles for the MMP family. A recent review summarized these new roles for MMPs in invasion and metastasis.[38] Most of the ***in vivo*** and ***in vitro*** assays designed to examine the role of MMPs on tumor invasion measure the end results, as in the number of micrometastases formed. The mechanism, however, remains unknown. Intravital videomicroscopy (IVVM) allows for the observation of the metastatic cascade by following the tumor cell through the microcirculation. The results from these experiments suggest that the destruction of tumor cells in the circulation and during extravasation do not contribute as much as previously thought to the inefficiency of metastasis. Rather, the growth of the individual tumor cell once in the target organ appears to be the rate limiting step.[38] Tumor cells engineered to overexpress TIMP-1 were shown to extravasate at rates equal to wild-type cells but were unable to form proliferative colonies within the target organ.[54] Although these data suggest that MMPs may play a role in tumor cell growth, the studies of MMPs involvement in ECM degradation and basement membrane invasion still support the core role of MMPs in metastatic invasion. The steps in which MMPs play a role are summarized in ***Figure 2.***

Matrix metalloproteinase expression

MMP expression in various tissue types has been examined through immunohistochemical analysis and in situ hybridization. MMPs are expressed in a variety of tissues and are a necessary component of the normal tissue remodeling process that occurs during wound healing, pregnancy, and bone resorption. MMP-2 seems to be the most common enzyme expressed in normal tissues, with expression being the highest in stromal elements.[55] The localization of the MMPs in tumor tissue is similar to that of normal tissue. Although there are several studies showing MMP-2 and MMP-9 production to be localized to the tumor cells, the majority show MMP-2 and MMP-9 to be produced by the tumor stromal elements. Immunohistochemical studies of several types of carcinomas including ovarian, thyroid, hepatocellular, and gastrointestinal show MMP expression in stromal fibroblasts.[56-59] In both ovarian and hepatocellular cancers, MMP-2 was localized to the tumor cell membrane at both the invasive fronts and at sites of vascular invasion.[58,59] In breast carcinoma, tumor cells themselves were capable of modulating MMP-2 binding via MT1-MMPs as well as via TIMP-2 and Vβ3 integrins.[60] An explanation as to why both the tumor cells and the adjacent stromal cells express MT1-MMP, MMP-2, and MMP-9 may be that both cellular components contribute to a different part of the metastatic cascade. The tumor cell MMPs may contribute to the invasive growth of the tumor while the stromal elements contribute to the remodeling process and the desmoplastic reaction that occurs in the tissue adjacent to the tumor.

MMPs and clinical therapeutics

MMPs as diagnostic markers

Numerous studies have shown the higher the MMP expression in the tumor, the more aggressive the cancer. Measuring the MMP level in the serum, plasma, or CSF has

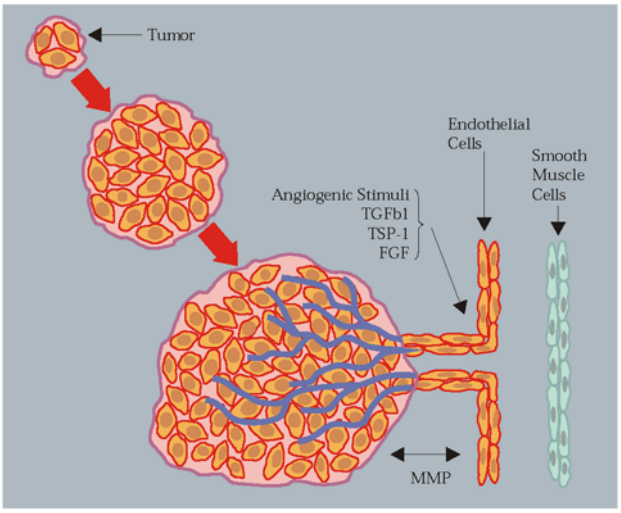

Figure 2. *Roles of MMPs in tumor cell metastasis. The roles of MMPs in metastasis are multifold. The steps where MMPs are involved are depicted in this schematic. 1) MMPs are involved in primary tumor growth and angiogenesis; 2) MMPs are secreted by both tumor cells and stromal cells upon cytokine stimuli thus enabling the tumor cell to invade and intravasate; 3) Extravasation of the tumor cell; 4) Tumor cell migration in ECM of distant site; 5) Growth and angiogenesis of distant metastasis*

received some attention as a possible predictor of tumor stage, metastasis, and recurrence. A study examining the serum levels of MMP-2 and MMP-9 in gastric carcinoma patients revealed a higher level of both pro-enzymes in the cancer patients vs. healthy volunteers.[61] In addition, the MMP level may be used in conjunction with other markers, such as CEA for colon carcinoma, to better determine the stage of the tumor. Other studies show that there is a correlation with MMP levels and tumor metastasis. Similar results were observed in prostate cancer where serum MMP-2 levels were higher in the carcinoma patients vs. healthy and benign prostatic hyperplasia patients, and among the cancer cases, there was a higher level in those patients with metastasis.[62] In addition to correlating with metastasis, MMP levels can also predict the recurrence of tumors. In a study examining the recurrence of urothelial cancer after resection showed that those patients with recurrence had a higher MMP-2:TIMP-2 serum level than those without recurrences.[62] Also, an independent study showed a higher MMP-2/TIMP-2/MT1-MMP level in bladder cancer patients is associated with decreased survival.[63]

Table 2. **Metalloproteinase Inhibitors and Their Clinical Uses***

Compound Class Name	*Action*	*Clinical Use*
Natural Inhibitors		
TIMP-1 (28.5 kDa)	Inhibits all activated MMPs; inhibits pro-MMP9	Possible target for gene therapy; basis for synthetic MMPI development
TIMP-2 (21 kDa)	Inhibits all activated MMPs; inhibits pro-MMP2	see TIMP-1
Synthetic Inhibitors		
Batimastat	Substrate analog to all MMPs	Used to generate preclinical data for marimastat
Marimastat	Substrate analog to all MMPs	Used in clinical trials in colon, pancreatic, ovarian cancer, etc.
Tetracycline	Weak MMP inhibitor	Used in rheumatoid arthritis and periodontal disease (clinical Trials)
Captopril	Chelates zinc, inhibiting MMPs	In vitro data suggests use in malignant gliomas

*A sample of MMPIs are listed here along with their potential clinical uses. MMPIs are still in clinical trials. They constitute a new class of emerging drugs targeting angiogenesis and metastasis formation.

Metalloproteinase inhibitors: chemotherapeutic agents

As explained previously, the TIMPs are one mechanism of control used physiologically to regulate MMP function. A number of studies have shown that overexpression of TIMPs produce a less metastatic phenotype presumably by reducing the amount of active metalloproteinases. Although the initial evidence for use as possible chemotherapeutic agents came from the studies involving natural TIMPs, the inability to mass produce and orally formulate natural TIMP cost-effectively have led to the development of synthetic MMPs. Some more common MMPIs are listed in *Table 2.*

Evidence for MMPIs as effective anti-cancer agents

Studies with the TIMP molecules provide the basis for developing synthetic MMPIs as anticancer agents. Experiments with recombinant TIMP-1 have shown that rTIMP-1 inhibits the invasion of tumor cells through amniotic membranes.[64] Administering rTIMP-1 to mice injected with metastatic B16 melanoma cells also inhibits the formation of lung metastasis.[64] Gastric cancer cells, B16F10 melanoma cells, and a human astrocytoma cell line transfected with the TIMP-1 gene showed metastasis with a reduced growth when injected into nude mice.[65-67] Additional evidence includes the ability of monoclonal antibodies against gelatinases to inhibit invasion in ***in vitro*** assays.[68,69] These data all suggest a role for MMPIs as a possible anti-cancer therapy. By altering the MMP-TIMP ratio, the invasive and metastatic ability of tumor cells can be altered.

Synthetic MMPIs

Two of the prototype MMPIs today are batimastat and marimastat. Batimastat, one of the first synthetic MMPIs, has a potent activity against most MMPs with an IC50 in the low nanomolar range. Batimastat acts by competitive, reversible inhibition by mimicing the substrate of MMPs. The long half-life and the route of administration of batimastat is convenient for animal models. Marimastat is another MMPI with similar inhibitory functions as batimastat. Marimastat is almost completely absorbed after oral administration and has a high bioavailability. The half life of marimastat is approximately 15 hours providing a

convenient twice a day dosing schedule. Marimastat is metabolized quickly in rodents, therefore, it has been used in clinical trials while batimastat has been used to generate preclinical data.

Preclinical data seem to strongly support the ability of MMPIs to reduce invasion and spontaneous metastases. Studies with the B16 murine melanoma model show a decrease in growth of subcutaneously implanted tumors and reduced spontaneous metastasis formation after surgical removal of the tumor. The number of lung colonies formed after IV injection of cells was also decreased by 68%.[70] More recently, in another colon cancer model, batimastat treated animals had decreased peritoneal carcinomatosis development and liver metastasis development. The treated animals also had significantly prolonged survival.[71] In addition, a hemangioma model using virus transformed endothelial cells measured the antiangiogenic ability of MMPIs through measuring new vessel formation. Batimastat treatment resulted in a decrease in vessel formation as assessed through hemoglobin content.[42]

Clinical trials examining the efficacy of marimastat also yielded favorable results. Clinical trials involving MMPIs must involve different parameters than just cytotoxic responses alone because MMPIs are not toxic to the tumor cell. The measure of effectiveness is the actual reduction of tumor growth along with the measure of survival time. The studies involving batimastat included the treatment of malignant ascites and malignant pleural effusion. These studies seemed to show favorable results; however, the poor bioavailability and the need to inject the drug directly into a body cavity led to the clinical trials with marimastat. In a recent review by Steward, an overview of the trials with marimastat are provided. Phase I studies revealed the severe joint and muscle pain that occurred with Marimastat at doses of 50 mg twice daily. Symptoms were decreased by decreasing the dose to 10 mg twice daily.[72]

Phase II studies have shown a decrease in tumor specific antigens, a measure of tumor activity, after treatment with marimastat. Studies done on pancreatic cancer patients show that there was a decrease in disease progression (as assessed through CT scan) and an increase in overall survival.[73] A more recent study with pancreatic cancer patients show optimal doses of batimastat to be 5, 10, and 25 mg twice daily.[74] Phase III trials are currently exploring the use of marimastat in conjunction with other chemotherapeutics.[75] Several impediments exist in the use of MMPIs, however. The side effect profile of marimastat and batimastat require reduced dosing of the drugs in order to reduce toxicity. In addition, study end points are difficult to establish as MMPIs are not cytotoxic to tumor cells, making some studies difficult to interpret.

Other therapeutic possibilities include blocking the signaling agents which can up-regulate MMP production. Our laboratory has discovered the ability of thrombospondin-1 to up-regulate MMP production in both endothelial cells and tumor cells.[24] Thrombospondin-1, a 450 kDa protein, has multiple domains with a variety of functions. TSP-1 has been shown to promote invasion in vitro and in vivo. One of the mechanisms involved is through the up-regulation of MMP-9. Specifically, the type-1 repeat peptide of TSP-1 has been shown to block the production of MMP-9 in endothelial cells by 86%. We interpret this result to mean that the peptide alone binds and blocks the receptor through which TSP-1 acts. The type I repeat peptides have been shown in mice to block the formation of B16 melanoma colonies in the lung. Peptides of matrix components such as the type I repeat of TSP-1 are possible anti-invasive therapies for the treatment of metastases formation. Other MMP inhibitors being studied in clinic trials include AG3340, COL-3, Neovastat, and BMS-275291.

Conclusions

The MMPs are a key family of enzymes used by tumor cells to invade and metastasize. The up-regulation of these enzymes during the invasive state can be caused by a variety of factors including increased production through cytokines and growth factors, increased activation through mechanisms such as uPa, and decreased inhibition by reduced levels of TIMP-1. Developing orally active synthetic inhibitors of MMPs is a possible treatment for controlling the metastatic potential of many tumors. While this therapy alone will not destroy the tumor, used in combination with other therapies, these MMPIs could halt the disease progression and slow the spread of the tumor. Research still continues in this field and exciting new therapeutic opportunities will ultimately emerge.

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