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Matrix Metalloproteinases As Novel Biomarkers and Potential Therapeutic Targets in Human Cancer

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ABSTRACT

The matrix metalloproteinase (MMP) family of enzymes is comprised of critically important extracellular matrix remodeling proteases whose activity has been implicated in a number of key normal and pathologic processes. The latter include tumor growth, progression, 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. In this review, we summarize the literature that establishes these enzymes as important clinical targets, discuss the complexity surrounding their choice as such, and chronicle the development strategies and outcomes of their clinical testing to date. The status of the MMP inhibitors currently in US Food and Drug Administration approved clinical trials is presented and reviewed. We also discuss the more recent and successful targeting of this enzyme family as diagnostic and prognostic predictors of human cancer, its status, and its stage. This analysis includes a wide variety of human cancers and a number of human sample types including tissue, plasma, serum, and urine.

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INTRODUCTION

Matrix metalloproteases (MMPs) are a multigene family of zinc-dependent endopeptidases that share a similar structure and which collectively, have the capacity to degrade virtually every component of the extracellular matrix (ECM). The basic domain structure of MMP family members is provided in Figure 1. MMP activity is inhibited specifically and reversibly by a group of structurally related, endogenous inhibitors known as tissue inhibitors of metalloproteases (TIMPs). To date, four TIMPs have been identified: TIMP-1, -2, -3, and -4.[1-3] The role of MMPs and TIMPs in tumor growth, metastasis, and angiogenesis has been widely investigated. We refer the reader to a number of comprehensive reviews on this topic[4-7] as well as for a review of the general biochemistry of the MMP family.[2,8,9] Based on their substrate specificity, MMPs have been divided into distinct subclasses: collagenases, gelatinases, stromelysins, and matrilysins. However, MMPs exhibit considerable promiscuity with respect to their substrates, leading to considerable redundancy in biologic functions as discussed below.

A related family of enzymes, the a disintegrin and metalloprotease (ADAMs), include integral membrane and secreted glycoproteins comprised of two subgroups: the membrane-anchored ADAMs[10-12] and the secreted ADAMTSs (Fig 1).[13] Like MMPs, some ADAM family members have a zinc binding consensus sequence at their catalytic site and display proteolytic activity. ADAMs are multifunctional enzymes involved in ectodomain shedding, regulation of growth factor availability, and in cell-cell/matrix interactions in both normal and pathologic states.[10-12] Unlike the MMPs, a role for ADAMs in tumorigenesis has only now begun to be explored.

FUNCTIONAL ROLES OF MMPS IN CANCER

Proteolysis of ECM

In most organs, the principle components of the ECM are collagens and numerous other proteins including laminin, entactin, and proteoglycans that make up the basement membrane. Tumor cells overexpress proteases and/or induce expression of these enzymes in neighboring stromal cells in order to degrade the basement membrane and invade the surrounding tissue. Several MMPs have been implicated in the ECM degradation associated with tumor growth and angiogenesis. This proteolytic activity is also required for a cancer cell to invade a nearby blood vessel (intravasation) and then extravasate at a distant location and invade the distant tissue in order to seed a new metastatic site (Fig 2).

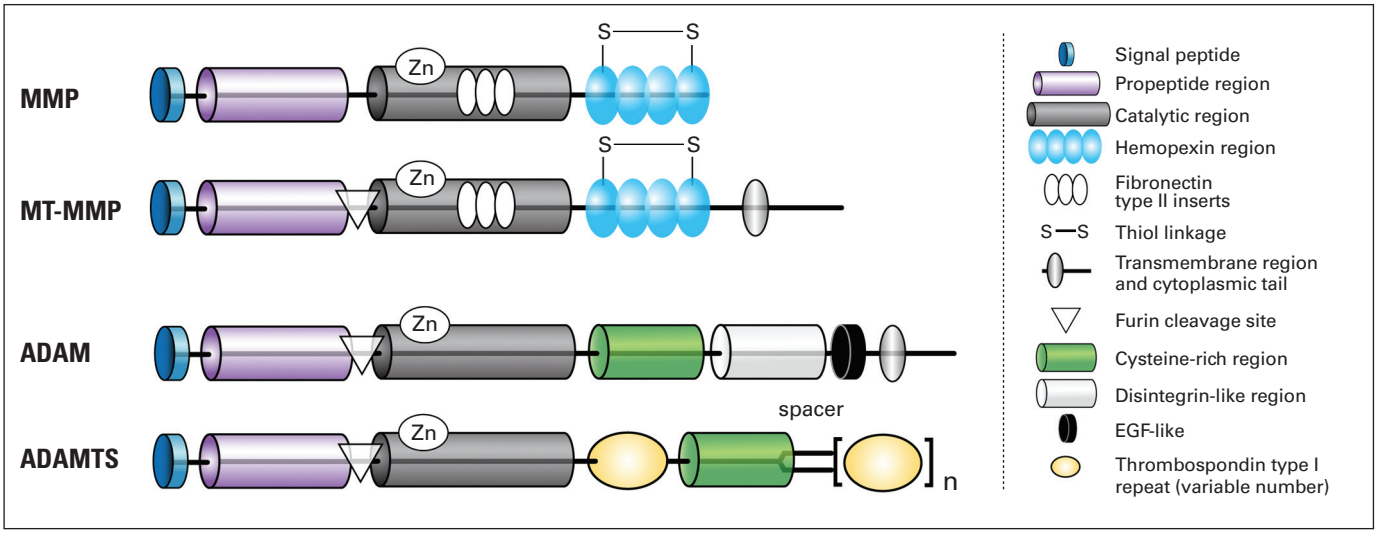

Fig 1. Basic domain structure of matrix metalloprotease (MMP) and a disintegrin and metalloprotease (ADAM) family members. The characteristic domain structure of MMPs includes the signal peptide domain, which guides the enzyme into the rough endoplasmic reticulum during synthesis, the propeptide domain, which sustains the latency of these enzymes until it is removed or disrupted, the catalytic domain, which houses the highly conserved Zn^{2+} binding region and is responsible for enzyme activity, the hemopexin domain, which determines the substrate specificity of MMPs, and a small hinge region, which enables the hemopexin region to present substrate to the active core of the catalytic domain. The subfamily of membrane-type MMPs (MT-MMPs) possesses an additional transmembrane domain and an intracellular domain. MMPs are produced in a latent form and most are activated by extracellular proteolytic cleavage of the propeptide. MT-MMPs also contain a cleavage site for furin proteases, providing the basis for furin-dependent activation of latent MT-MMPs before secretion. ADAMs are multidomain proteins composed of propeptide, metalloprotease, disintegrin-like, cysteine-rich, and epidermal growth factor–like domains. Membrane-anchored ADAMs contain a transmembrane and cytoplasmic domain. ADAMTSs have at least one thrombospondin type I sequence repeat motif. EGF, epidermal growth factor.

Modulation of Cell Adhesion, Migration, and Epithelial to Mesenchymal Transition

ECM degradation products display unique biologic properties that can trigger a variety of cellular signals. For example, cleavage of collagen IV and laminin-5 generates cryptic peptides that can in turn promote migration of tumor cells.[14,15] MMP substrates include non-ECM molecules, ranging from growth factor precursors and cell surface adhesion molecules to angiogenic inhibitor precursors (Fig 2). E-cadherin is cleaved by MMP-3, MMP-7, and ADAM10,[16,17] leading to the release of soluble E-cadherin and the disruption of cell-cell interactions leading to disruption of cell adhesion and increase in migration. In addition, several integrins can serve as substrates for MMPs (Fig 2). For example, MT1-MMP can process pro-α_v, -α_5, -α_3,[18] a process that can contribute to $\alpha_v\beta_5$-mediated signaling and migration in breast tumor cells.[19]

MMPs have also been implicated in the epithelial to mesenchymal transition (EMT), a hallmark of cancer progression to metastasis.[20] During EMT, tumor cells acquire migratory characteristics and more readily invade into surrounding tissues and metastasize to secondary sites. Activation of growth factors and cleavage of adhesion molecules are some of the proposed mechanisms underlying MMP-induced EMT. Proteolytic activation of latent transforming growth factor–β has been shown to be essential during MMP-28–induced EMT.[21] MMP-3–induced EMT has been shown to be the result of E-cadherin cleavage[22] and increased expression of an alternatively spliced form of Rac1b.[23]

Processing of Cytokines and Receptors

Recent studies point to an emerging role for MMPs in modulating aspects of immunity and inflammation during tumorigenesis.[24] Cytokine signaling is an integral aspect of inflammation. A variety of cytokines, cytokine receptors, and chemokines have been found to undergo MMP-mediated cleavage. For example, MMP-7 and/or ADAM17 activity is required for release of the proangiogenic inflammatory cytokine tumor necrosis factor (TNF) -α from its membrane-bound form.[25-27] In breast cancer, MMP-9 expression is upregulated in tumor-associated stromal cells including neutrophils, macrophages, and lymphocytes[28] and may play a role in tumor-associated inflammation.

Processing of Growth Factors and Receptors

Several members of the MMP and ADAM family can regulate cellular proliferation by modulating the bioavailability of growth factors or cell-surface receptors (Fig 2). For example, the bioavailability of insulin-like growth factors (IGFs) is mainly regulated by IGF binding proteins (IGFBP). MMP-1, -2, -3, and ADAM12 cleave IGFBP-3,[29-31] while IGFBP-1 is a substrate for MMP-11.[32] Ligands for several growth factor receptors are processed by MMP/ADAM family members as well. Chief among them are the epidermal growth factor (EGF) receptor ligands: heparin-binding EGF (HB-EGF), amphiregulin, betacellulin, heregulin, and epiregulin. Normally, signaling via the EGF receptor (EGFR) pathway is tightly controlled. In cancer, as a consequence of increased shedding of active EGFR ligands and induction of constitutively active EGFR kinases, signaling through these pathways is upregulated, resulting in uncontrolled proliferation, migration, and survival of cancer cells. MMP-3, -7, ADAM17, and ADAM12 can proteolytically release HB-EGF allowing it to transactivate the EGFR[33-35] and in turn promote tumor growth and angiogenesis.[36] ADAM17 overexpression in breast cancer cells can lead to increased proliferation, migratory, and invasive capacity of cancer cells,[37] properties that can be attributed in part, to the increased

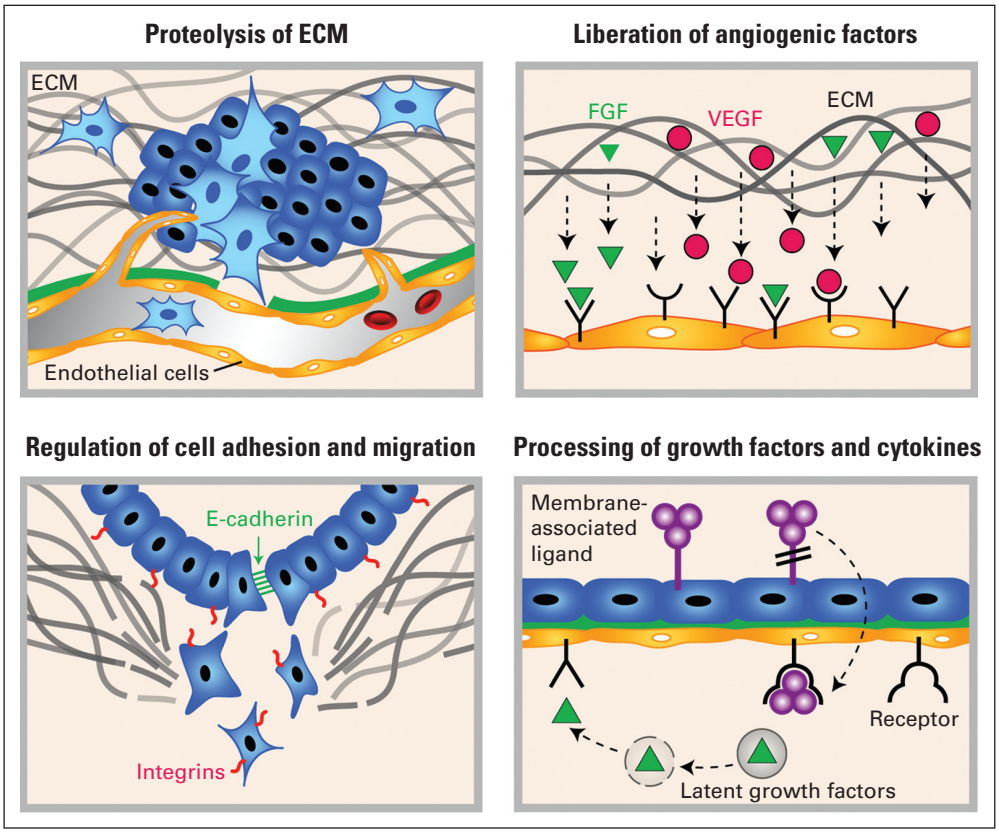

Fig 2. Multiple functions of matrix metalloproteases (MMPs) in cancer progression. (counterclockwise) MMPs degrade components of extracellular matrix (ECM), facilitating angiogenesis, tumor cell invasion, and metastasis. MMPs modulate the interactions between tumor cells by cleaving E-cadherin, and between tumor cells and ECM by processing integrins, which also enhances the invasiveness of tumor cells. MMPs also process and activate signaling molecules, including growth factors and cytokines, making these factors more accessible to target cells by either liberating them from the ECM (eg, vascular endothelial growth factor [VEGF] and basic fibroblast growth factor [FGF]) and inhibitory complexes (eg, transforming growth factor-β), or by shedding them from cell surface (eg, heparin-binding epidermal growth factor).

shedding of basement membrane transforming growth factor–α and amphiregulin in these cells.[38,39]

MMPs have been shown to promote angiogenesis through their release of angiogenic factors stored in the ECM such as vascular endothelial growth factor (VEGF)[40,41] and basic fibroblast growth factor (bFGF; Fig 2).[42] Stroma-derived MMP-9 can facilitate the liberation of ECM-sequestered VEGF during tumor angiogenesis.[41] Similarly, cleavage of perlecan by MMP-1 and MMP-3 releases active bFGF.[42]

Role in Tumor-Associated Angiogenesis

Angiogenesis is the formation of new blood vessels from a preexisting one. It is widely believed that a tightly controlled balance of pro- and antiangiogenic molecules regulates angiogenesis in tumors. MMPs play complex and sometimes conflicting roles in regulating angiogenesis. Remodeling of the ECM during angiogenesis is accomplished largely through the activity of MMPs.[4,8,43] Angiogenic mitogens, such as bFGF and VEGF, can stimulate the production of MMPs by capillary endothelial cells.[44,45] Studies have also demonstrated that MMPs are involved in the angiogenic switch, one of the earliest stages of tumor growth and progression. In a model of tumor progression which reliably recapitulates this switch, MMP-2 was shown to play an important role in the development of the angiogenic phenotype.[46] It has also been shown that MMP-9 can be a regulator of the angiogenic switch in a pancreatic tumor model,[41] further confirming the pro-angiogenic role of MMPs. These findings strongly suggest that MMP activity is critical, not only to the initiation of angiogenesis, but to the maintenance of the growing vascular bed, which in turn supports tumor growth and metastasis. MMP activity can, however, result in the production of negative regulators of angiogenesis as well. For example, MMPs have now been shown to cleave the parent molecules plaminogen and collagen XVIII into the cryptic, endogenous angiogenesis inhibitors, angiostatin[47,48] and endostatin,[49] respectively. These data underline the importance of MMPs as both positive and negative regulators of angiogenesis and cancer.

MMPS AS BIOMARKERS OF CANCER

One of the more promising and exciting applications of MMPs in human cancers is as potential cancer biomarkers, both diagnostic and prognostic. Below we review their exploitation as tools for early detection, disease progression, and metastasis. We have specifically included those biomarkers for which validation studies have accompanied the preliminary discovery analyses (Table 1).[50-96]

Breast Cancer

Evidence is emerging that members of the MMP and/or ADAM family can serve not only as potential markers for diagnosis and prognosis, early detection, and risk assessment, but also as indicators of tumor recurrence, metastatic spread, and response to primary and adjuvant therapy for breast cancer. MMP-9 levels in tumor tissue as well as serum, plasma, and urine are significantly elevated in patients with breast cancer.[51-53,71]

We have previously reported that the detection of urinary ADAM12 in patients with breast cancer is predictive of disease status

Table 1. Candidate MMP and ADAM Biomarkers of Cancer

Type of Cancer and MMPs/ ADAMs	Detected in Tissue/ Body Fluid	Method of Analysis
Breast		
MMP-13[50]	Tissue	IHC
MMP-9, TIMP-1[51]	Serum, tissue	ELISA, IHC
MMP-9[52-54]	Urine, serum, plasma, tissue	Gelatin zymography, IHC
ADAM12[55]	Urine	Immunoblot
ADAM17[56]	Tissue	ELISA, immunoblot
MMP-1[57]	Tissue, nipple aspirates	Gene analysis
Pancreas		
MMP-9[58]	Pancreatic juice, serum	ELISA, immunoblot
MMP-2[59]	Pancreatic juice, tissue	Gelatin zymography
MMP-7[60,61]	Tissue, plasma	IHC, RT-PCR, ELISA
ADAM9[62]	Tissue	IHC
Lung		
MMP-9, TIMP-1[63,64]	Serum, bronchial lavage	ELISA
MMP-7[65]	Tissue	IHC
MMP-1[66,67]	Tissue	Gene analysis
Bladder		
MMP-9[68]	Tissue	IHC
MMP-9, MMP-2[69,70]	Urine	Gelatin zymography
MMP-9[71,72]	Urine	Gelatin zymography, immunoblot, ELISA
MMP-9, telomerase[73]	Urine	Gelatin zymography, cytology
Colorectal		
MMP-2[74,75]	Tissue, plasma	IHC, ELISA
MMP-9[76]	Tissue	IHC
MMP-2, MMP-9[77]	Plasma	ELISA
MMP-7[78]	Serum	ELISA
MMP-1[79]	Tissue	ELISA
MMP-13[80]	Tissue	Gelatin zymography
Ovarian		
MMP-9[81]	Tissue	Gelatin zymography
MMP-9, MMP-14[82]	Tissue	IHC
MMP-2[82,83]	Tissue	IHC
MMP-2, MMP-9, MMP-14[84]	Tissue	IHC
ADAM17[85]	Tissue	RT-PCR, IHC
Prostate		
MMP-2, MMP-9[86,87]	Plasma, tissue	ELISA, IHC
MMP-2[88]	Tissue	IHC
MMP-9[70]	Urine	Gelatin zymography
ADAM8[89]	Tissue	IHC
ADAM9[90]	Tissue	IHC
Brain		
MMP-2[91,92]	Tissue	IHC, ELISA, gelatin zymography
MMP-9[93,94]	Tissue	IHC, ELISA, gelatin zymography
MMP-2, MMP-9[95,96]	Tissue, cerebrospinal fluid, urine	IHC, ELISA, gelatin zymography

Note: For each of the studies listed above, N ≥ 40, with the exception of brain tumor biomarker studies where larger sample sizes were not available.
Abbreviations: MMP, matrix metalloproteinase; ADAM, a disintegrin and metalloprotease; IHC, immunohistochemistry; ELISA, enzyme-linked immunosorbent assay; RT-PCR, reverse transcription polymerase chain reaction.

and stage and that ADAM12 protein levels in urine increase with progression of disease.[55] Expression of a related enzyme, ADAM17, is significantly elevated in high-grade breast tumor tissue and correlates with shorter overall patient survival.[56] Immunohistochemical (IHC) analysis of human breast biopsy materials have indicated that MMP-13 may be a useful prognostic indicator for invasive breast cancer. In this study, tumor-derived MMP-13, correlated with expression of Her2/neu and TIMP-1 and with aggressive tumor phenotypes and inversely correlated with overall patient survival.[50]

Recently, efforts have focused on the use of MMPs and ADAMs as potential biomarkers of early breast cancer. Studies from our laboratory indicate that urinary MMP-9 and ADAM12, in addition to being predictive markers for breast cancer, may also prove useful as noninvasive breast cancer risk assessment tools.[97] Differential global gene expression analysis of tissue samples from patients with atypical ductal hyperplasia and a history of cancer versus patients with no history or who did not subsequently develop breast cancer indicated that MMP-1 mRNA and protein levels were upregulated in precancerous lesions.[57] MMP-1 mRNA could also be detected in cells collected from nipple aspirates suggesting that MMP-1 might be useful as a diagnostic marker for screening atypical ductal hyperplasia to identify women with lesions that may eventually develop into cancer.[57]

Metastatic breast cancer involves the regional lymph nodes (LN) and liver or bone resulting in significant morbidity. Several independent studies have used circulating MMP-9 activity to predict metastatic spread of disease as well as to monitor patient response to primary and adjuvant therapy and to evaluate outcome.[54,98] High levels of serum MMP-9 and TIMP-1 are associated with increased incidence of LN metastasis and decreased relapse-free and overall survival rates.[51] MMPs may also be useful in predicting therapeutic efficacy. Plasma MMP-9 levels decrease after the surgical removal of primary breast tumors and a progressive decrease in plasma MMP-9 was observed in patients who responded well to adjuvant therapy.[54] Importantly, in all patients who suffered a relapse of disease there was a gradual increase of plasma MMP-9 activity 1 to 8 months before the clinical diagnosis of recurrence.[54] High MMP-14 mRNA expression ($>$ 10% cells) in primary breast tumor tissue can also predict shorter overall survival.[99]

Pancreatic Cancer

Nowhere is the need more urgent for sensitive and specific biomarkers for early diagnosis and to screen high-risk patients than in pancreatic cancer. This disease is extremely difficult to diagnose in its early stages due to a lack of specific symptoms and the limitations of current diagnostic methods. Several studies have evaluated differentially expressed biomarkers for pancreatic cancer using tissue, blood, or pancreatic juice. Serum and tissue levels of MMP-9 are significantly higher in patients with pancreatic ductal adenocarcinoma than in patients with chronic pancreatitis and healthy controls.[58] Active MMP-2 levels are upregulated in the pancreatic juice of patients with cancer (100%) as compared with patients with chronic pancreatitis (2%) or normal controls (0%).[59] Similarly, plasma as well as tumor tissues from patients with pancreatic ductal adenocarcinoma have significantly elevated MMP-7 levels[60] which may predict shortened survival of patients.[61]

Of the ADAMs studied to date, only ADAM9 has been analyzed in relation to pancreatic cancer. IHC analysis of ADAM9 in tumor tissue samples indicated that an increased expression of this protease correlates with poor tumor differentiation and shortened overall survival.[62]

Lung Cancer

Several studies have reported that plasma and/or serum levels of MMP-9 and TIMP-1 are elevated in patient with stage III or IV lung cancer when compared with those in patients with nonmalignant lung diseases.[63,64] Retrospective studies of non–small-cell lung cancer (NSCLC) tissue found that MMP-7 expression was higher in squamous cell carcinomas than in adenocarcinomas and correlated with significantly lower overall survival in patients.[65] In a similar study that included 159 patients with stage III and IV NSCLC, MMP-7 status correlated inversely with overall response to chemotherapy. These findings suggest that MMP-7 expression in NSCLC may be a significant prognostic factor and could be predictive of response to chemotherapy and outcome. MMP-1 overexpression has been reported in lung cancer cells[100] and DNA variants of *MMP-1* have been linked to lung cancer risk and susceptibility.[66] Several single nucleotide polymorphisms within the *MMP-1* gene have been shown to be significantly associated with the risk of early-onset lung cancer in particular for subgroups with high smoking intensity.[67]

Studies of ADAM-type proteases as biomarkers for lung cancer are rare. One exception is ADAM28, whose levels were found to be higher in lung carcinoma tissue compared with healthy lung tissue and correlated significantly with tumor size and LN metastasis.[101]

Bladder Cancer

As is expected, a majority of the biomarker studies in patients with bladder cancer have focused on urine. Studies from our group and others have shown that urinary MMP-2 and MMP-9 levels correlate with presence of bladder cancer as well as stage and grade of disease.[69-73] We have recently reported the identification of several MMP species in urine from patients with primary tumors in the bladder and prostate including MMP-2, MMP-9, MMP-9/neutrophil gelatinase-associated lipocalin complex and MMP-9 dimer.[70] Each urinary MMP species was detected at significantly higher rates in urine from patients with cancer as compared with controls. The difference in detection of MMP species in the urine of the two types of cancers studied may serve as a tumor-specific fingerprint that can indicate both the presence of a tumor as well as its location.[70] Increased levels of MMP-9 and MMP-2 in urine correlate with increased expression of these proteases in bladder tumor tissue as well.[68] Urinary MMP-9 levels when combined with telomerase analysis of exfoliated cells from voided urine could also increase the sensitivity of cytology, a commonly used method for bladder cancer detection and monitoring.[73] ADAM12 mRNA and protein were found to be upregulated in bladder cancer tissue and urinary ADAM12 levels were higher in patients with bladder cancer.[102]

Colorectal Cancer

MMP-2 and MMP-9 have been studied as potential prognostic biomarkers of colorectal cancer. A study of patients with Dukes' stages A to D colorectal cancer found that high levels of MMP-2, but not MMP-1, -7, and -13, in the malignant epithelium and stroma were associated with decreased survival.[74] Elevated plasma MMP-2 levels have also been shown to correlate with lymph node metastasis.[75] Enhanced MMP-9 staining in primary tumors was found to be an independent marker of poor prognosis in a study with T3-T4 node-negative patients.[76] Plasma MMP-2 and MMP-9 levels were significantly elevated in patients with colorectal cancer and those with adenomatous polyps, and significant reduction in both were observed after tumor resections, suggesting their potential as markers for therapeutic efficacy.[77] These MMPs may not be prognostic markers for tumor recurrence, however, since plasma proMMP-2 and -9 activities did not correlate with disease relapse after surgery.[103]

In addition to the gelatinases, serum MMP-7 levels were reported to predict decreased survival in patients with advanced colorectal cancer.[78] A study of paired colorectal tumor and normal mucosal tissues revealed the significant correlation between MMP-1 levels and pathology (ie, Dukes' stage, tumor depth, and lymphatic invasion).[79] MMP-13 activity was significantly elevated in tumor samples and the tumor to normal tissue ratio of MMP-13 activity significantly correlated with poor survival.[80]

Ovarian Cancer

MMP-2, -9, and -14 are among the most studied MMPs as biomarkers for ovarian cancer. MMP-9 activity in tissue extracts was significantly increased in advanced ovarian cancers (International Federation of Gynecology and Obstetrics stage III) compared with

benign tumors and was found to be an independent prognosticator of poor survival.[81] In another study of invasive epithelial ovarian cancer, high stromal expressions of MMP-9 and -14 were significantly correlated with cancer progression and were independent prognostic markers.[82] Correlation with ovarian cancer progression has also been reported for MMP-2[82,104] and elevated levels of MMP-2 in cancer cells of peritoneal implants were associated with a significant risk of death in stage III ovarian carcinomas.[83]

Tissue MMPs have also been shown to distinguish different histotypes of ovarian cancer, which is a significant finding given that different histotypes have different prognoses.[105] A recent study showed that more than 90% of clear-cell carcinomas expressed moderate to high levels of MMP-2 or MMP-14, compared with 30% to 55% of the other ovarian cancer histotypes (serous, endometroid, and mucinous), whereas MMP-9 was expressed more widely in other histotypes.[84] Importantly, the cellular source of MMPs must be considered when evaluating MMPs as ovarian cancer biomarkers. For example, strong MMP-9 levels in cancer cells were associated with longer survival whereas strong stromal MMP-9 was associated with shorter survival, suggesting a dual role for MMP-9 during ovarian cancer progression.[106]

Among the ADAM family members, ADAM17 expression was significantly increased in both early and advanced ovarian cancer tissues and correlated with the expression of HB-EGF.[85]

Prostate Cancer

MMP-2, -9, -15, and -26 expression in tissue or serum have been positively correlated with Gleason score in prostate cancer.[107-109] Among these MMPs, the activities of plasma MMP-2 and -9 increased significantly in metastatic prostate cancer.[86] Furthermore, overexpression of MMP-2 in cancer tissue was associated with shorter disease-free survival in a study with T3N0-2M0 patients.[88] Analysis of MMP-2 and -9 levels in radical prostatectomy specimens revealed these two as significant predictors of cancer recurrence.[87] These two enzymes may also be markers of therapeutic efficacy, since both the levels and activities of plasma MMP-2 and -9 decreased significantly in metastatic patients after therapy.[86] In addition, increased urinary MMP-9 activity has been shown to distinguish between prostate and other types of cancer (eg, bladder cancer).[70] MMPs can also be combined with other markers to increase their predictive capability. For example, the mRNA ratio of gelatinases (MMP-2 and MMP-9) to E-cadherin in biopsy samples independently predicted prostate cancer stage.[110]

ADAM8 levels in prostate cancer tissue have been significantly correlated with higher tumor status, positive nodal status, and higher Gleason scores.[89] Higher ADAM9 levels were associated with shortened prostate-specific antigen relapse-free survival.[90]

Brain Tumors

Elevated tissue levels of MMP-2 and MMP-9 have been reported in aggressive brain tumors.[91-94] Positive MMP-2 expression in tissue was also associated with shorter survival in patients with malignant brain tumors.[92] Both latent and activated forms of MMP-2 and MMP-9 have been detected in the cerebrospinal fluid of patients with brain tumors.[95] In studies of primary glial tumors and other CNS tumors, we have recently shown that detection of MMP-2, MMP-9, MMP-9/neutrophil gelatinase-associated lipocalin complex, and/or VEGF in the urine predicted disease status and therapeutic efficiency of patients with brain cancer.[96,111] Importantly, these studies showed that the upregulation of MMP-2 and -9 in the source tumor tissue was also reflected in CSF as well as in urine of these patients.

It is important to note that the measurement of MMPs in body fluids, in particular serum or plasma, can be influenced by the type of fluid and method of collection and storage. For example, basal MMP-9 levels in serum/plasma can be influenced by the use of EDTA or heparin,[112] a problem that can be alleviated by using sodium citrate instead.[113] Another issue to be considered is that of sample storage. For example, it has been reported that plasma MMP-9 is unstable and degrades rapidly even when stored at −80°C.[114,115] This problem can be alleviated by storing samples in liquid nitrogen and analyzing them shortly after collection.

The context in which these or any other biomarkers are utilized in the clinic is a critical consideration. It has been suggested that, rather than being used as screening tools, MMPs might best be used to provide useful clinical information as part of a longitudinal assessment of a patient's disease progression and therapeutic efficacy with the patient himself/herself serving as an internal control.

MMPS AS THERAPEUTIC TARGETS

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 family. There are a number of matrix metalloproteinase inhibitors (MMPIs) that are currently being tested in all three phases of clinical trials against a variety of human cancers (Table 2).[116-119] The promise of this therapeutic approach has yet to be realized and the academic, pharmaceutical, and biotechnology arenas continue to debate the potential issues underlying the lack of therapeutic success in cancer treatment. Although certainly not in the majority, there have been some promising results from some clinical trials. For example, Neovastat administration resulted in a significantly longer median survival time in patients with refractory renal cell carcinoma in a phase II trial.[120] However, in many cases, when there appeared to be positive results, the reports indicated that although biomarker levels decreased in the course of the treatment, positive clinical correlates were not necessarily observed.

Current Design of MMPIs

Peptidomimetic MMPIs are pseudopeptide derivatives that mimic the structure of collagen at the MMP cleavage site.[121] These substrate-based MMPIs are usually broad spectrum and block MMP activity by occupying the substrate-binding site and chelating the zinc with, in most cases, a hydroxamic acid functional group. The earliest generation of these inhibitors, including batimastat (BB-94), had low water solubility and was therefore not orally available. The next generation of hydroxamate-based inhibitors, such as marimastat (BB-2516), was designed to be orally available, however, they were commonly associated with musculoskeletal syndrome, probably due to their off-target effects on non-MMP metalloproteinases.[122]

To improve specificity, the current knowledge of the three-dimentional conformation of the enzyme active site has been incorporated into the design of MMPIs. These structure-based inhibitors include tanomastat (BAY 12-9566; Bayer Corporation, West Haven, CT), prinomastat (AG3340; Agouron Pharmaceuticals, La Jolla, CA),

Table 2. MMP Inhibitors Currently in Clinical Trials for Cancer

Drug by Phase	Type of Cancer	Type of Drug	Target MMP
BMS-27591			
II	Prostate	Nonhydroxamate	MMP-1, 2, 8, 9, 14
III	Non–small-cell lung	Nonpeptidomimetic	
COL-3			
I	Advanced solid tumors	Chemically modified tetracycline	MMP-2, 9
II	Kaposi's sarcoma		
Dalteparin (trials completed)*			
II	Glioblastoma	Low molecular weight synthetic heparin	MMP-9
III	Advanced cancers (breast, lung, colon, prostate)		
Disulfiram			
I	Melanoma, solid tumors, non–small-cell lung	Tetraethyliuram disulfide	MMP-2, 9
II/III			
Genistein			
II	Breast, kidney, melanoma, prostate, bladder, pancreatic, breast cancer prevention	Soy isoflavone	MMP-2, 9
INCB7839			
I/II	Breast	Selective sheddase inhibitor	ADAM10, 17
Marimastat			
III	Breast	Hydroxamate peptidomimetic	MMP-1, 2, 3, 7, 9, 12
Neovastat (AE941; trials completed)†			
II	Multiple myeloma‡	Shark cartilage extract	MMP-2, 9, 12
III	Non–small-cell lung, kidney‡, breast, colorectal		
PCK 3145 (trials completed)			
I	Prostate‡	Prostate secretory protein 94-derived synthetic peptide	MMP-9
Prinomastat (AG3340)			
II	Glioblastoma, non–small-cell lung, prostate	Hydroxamate	MMP-2, 9, 13, 14
III		Nonpeptidomimetic	

Abbreviations: MMP, matrix metalloproteinase; ADAM, a disintegrin and metalloprotease.
*Completed trials in glioblastoma[116] and advanced cancers.[117]
†Completed trials in non-small cell lung cancer[118] and breast and colon cancer.[119]
‡Trials have been completed but results are unpublished.

and BMS-275291 Bristol-Myers-Squibb, New York, NY. Some of these inhibitors also contain hydroxamic acid group to chelate zinc, such as prinomastat. Musculoskeletal toxicity has also been reported in clinical trials with prinomastat and BMS-275291.[123,124] These MMPIs, while not as broad spectrum as the substrate-based MMPIs, still target several enzymes with similar potency. New structure-based inhibitors targeting a single MMP have been described for MMP-12 and -13.[125,126]

Another group of MMPIs are the chemically modified tetracyclines (CMTs), which do not possess antibiotic activities.[127,128] CMTs may inhibit MMPs by binding to the key metal ions, such as zinc and calcium, or by regulating MMP transcription.[129] CMTs used as MMPIs include metastat (COL-3), minocycline, and doxycycline.

Novel mechanism-based MMPIs have also been reported.[130] One of the first of such inhibitors, SB-3CT, was designed to be highly selective for gelatinases. It covalently binds to the active site of MMP-2 and restructures the enzyme back to its proenzyme state.[131] It reduced liver metastasis and increased survival in an aggressive mouse model of T-cell lymphoma.[132] A new variant of this inhibitor class further improves the specificity by exclusively targeting MMP-2.[133]

Several small molecule inhibitors targeted specifically to the ADAM family of enzymes have also been recently evaluated.[134-136] Of these, INCB7839 (Incyte Corporation, Wilmington, DE), a sheddase inhibitor, is currently in phase II clinical trials against breast carcinoma and several other solid tumors. Such inhibitors could prove to be particularly useful in targeting tumors dependant on EGFR signaling either as single agents or in a synergistic manner with currently approved tyrosine kinase inhibitors. Notably, unlike the first generation MMPIs, INCB7839 did not induce musculoskeletal adverse effects in initial animal studies.[136]

Far less attention has been given to the issues limiting the use of the TIMPs in the clinical setting, with the key limitations being the difficulty in the large scale production of biologically active protein and optimization of drug delivery. Just as is the case with their cognate enzymes, the TIMPs have also now been shown to be multifunctional proteins with activities independent of their MMP inhibitory ones.[4,137,138] Interestingly, there may be significant clinical potential in exploiting these non-MMP activities in that this strategy might permit circumvention of the limitations associated with the targeting of MMP inhibition alone.

Challenges of Anti-MMP Therapy

The road to clinical use of MMPIs has not been straightforward. The reader is referred to a number of recent reviews for an extensive analysis of the design, development, and complexities that have slowed and limited the creation and mobilization of MMPIs into the clinic.[139,140] Several reasons might explain the unfavorable clinical outcomes in some of the MMPI clinical trials in various cancer types. Firstly, adverse effects, including musculoskeletal syndrome, have limited the maximum-tolerated dose of the early generation of MMPIs,

thereby limiting drug efficacy. Secondly, the best window for MMPI treatment may have been missed in patients recruited in the trials who are often at the most advanced, metastatic stage of cancer. In addition, the nonspecific nature of the inhibitors has negatively impacted the therapeutic efficacy of these inhibitors due, at least in part, to the wide range of MMPs and physiological events affected.

As is the case for many anticancer drugs, MMPIs may actually be more effective when administered at earlier stages of cancer progression rather than when a patient is suffering from end stage disease having experienced failure with all other conventional therapies. Therapy at an earlier stage in disease progression might also afford the opportunity to use lower doses of drug thereby perhaps limiting the toxicity that has so hampered these trials. Interestingly, Hanahan et al[141] demonstrated that MMPIs (in this case the broad spectrum, hydroxamate-based BB-94) would be most useful when tested in the very early stages of tumor progression, in the RIP1-Tag2 model of pancreatic carcinogenesis, such as at the time of the angiogenic switch.

As discussed earlier, MMPs can be multifaceted during cancer progression. Several MMPs have been shown to be protective against cancer. Tumor progression in various animal models has been inversely correlated with the expression status of MMP-3, -8, and -12.[142-144] In human studies, MMP-12 overexpression in the tumor has also been associated with more positive prognosis.[145] Importantly, the same MMP may play an opposite role at different stages of cancer progression. For example, a study with a transgenic mouse model of invasive squamous cell carcinoma suggested that MMP-9 stimulates proliferation during the early stages of cancer but restricts further malignant progression at later stages.[146] For many of these MMPs, it remains unclear exactly which effect, positive or negative, they are exerting and these effects can vary as a function of different cancer types and different stages of disease.[147]

The mechanisms underlying the putative protective activities of MMPs are not fully understood. One potential mechanism may be the processing of antiangiogenic factors (cryptic angiogenic inhibitors) from inactive parental proteins.[47-49,148,149] Another possible mechanism is the participation of MMPs in the host defense against a tumor[143,150] by modulating the activities of chemokines and cytokines.[151]

Another major challenge in developing MMP-targeted therapy is the accurate evaluation of MMPI efficacy in vivo. To overcome this hurdle, a MMP-2-sensitive imaging probe has been studied which contains a MMP-2 peptide substrate and quenched near-infrared fluorochromes.[152] Fluorescence is detected when the peptide is cleaved by active MMP-2. A significant reduction in probe fluorescence in implanted tumors was detected in live mice treated with MMPI prinomastat. These noninvasive imaging techniques make it possible to evaluate the therapeutic efficacy of MMPIs in patients and may also be used to monitor MMP activity at different stages of cancer progression. Taken together, such techniques provide insight into the functions of MMPs in cancer and may lead to effective MMP-targeting therapeutics.

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Glossary Terms

Angiogenesis: The process involved in the generation of new blood vessels. While this is a normal process that naturally occurs and is controlled by "on" and "off" switches, blocking tumor angiogenesis (antiangiogenesis) disrupts the blood supply to tumors, thereby preventing tumor growth.

Biomarker: A functional biochemical or molecular indicator of a biologic or disease process that has predictive, diagnostic, and/or prognostic utility.

MMP (matrix metalloprotease [metalloproteinases]): MMPs belong to a family of enzymes (zinc-dependent endoproteinases) that are involved in the degradation of the extracellular matrix. MMPs are involved in both normal and pathologic tissue remodeling, where their selective proteolysis is now appreciated to help regulate cell growth, angiogenesis, and invasiveness.

Prognostic (prognostic marker): A marker that predicts the prognosis of a patient (eg, the likelihood of relapse, progression, and/or death) independent of future treatment effects. A factor can be both prognostic and predictive.

ECM (extracellular matrix): Components that are extracellular and composed of secreted fibrous proteins (eg, collagen) and gel-like polysaccharides (eg, glycosaminoglycans) binding cells and tissues together. In addition, the ECM contains adhesion proteins (eg, fibronectin and laminin) that link components of the matrix both to one another and to attached cells. Depending on the tissue, the composition of the ECM differs. For example, collagen is the major component of ECM; elastin fibers contain cross-linked elastin; and integrins are cell surface receptors responsible for the attachment of cells to the ECM.

ADAMs (a disintegrin and metalloprotease): A family of integral membrane and secreted glycoproteins. ADAMs are multifunctional enzymes involved in cell-surface remodeling, ectodomain shedding, regulation of growth factor availability, ECM degradation, and mediation of cell-cell and cell-matrix interactions in both normal development as well as in pathological states.

MMPIs (matrix metalloproteinase inhibitor): Natural proteins or synthesized compounds that inhibit the activity of one or more MMPs.