Matrix Metalloproteinases: Pro- and Anti-Angiogenic Activities

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Matrix metalloproteinases (MMP) are a family of structurally related proteinases most widely recognized for their ability to degrade extracellular matrix, although recent investigations have demonstrated other biologic functions for these enzymes. MMP are typically not constitutively expressed, but are regulated by: (1) cytokines, growth factors, and cell–cell and cell–matrix interactions that control gene expression; (2) activation of their proenzyme form; and (3) the presence of MMP inhibitors [tissue inhibitors of metalloproteinases, (TIMP)]. MMP have important roles in normal processes including development, wound healing, mammary gland, and uterine involution, but are also involved in angiogenesis, tumor growth, and metastasis. Angiogenesis, characteristically defined as the establishment of new vessels from pre-existing vasculature, is required for biologic processes such as wound healing and pathologic processes such as arthritis, tumor growth, and metastasis. Blocking of MMP activity has been studied for potential therapeutic efficacy in controlling such pathologic processes. Synthetic MMP inhibitors, most notably the hydroxymates, have been engineered for this purpose and are presently in clinical trial. These inhibitors may have broad *versus* specific MMP inhibitory activity. As increased non-matrix degrading capabilities of MMP are recognized, however, i.e., cytokine activation, processing of proteins to molecules of distinct biologic function, it becomes less clear whether the nonselective inhibition of MMP activity for all pathologic processes involving MMP is appropriate. This review focuses upon the contribution of MMP to the process of tumor invasion and angiogenesis, and discusses the design and use of MMP inhibitors as therapeutic agents in these processes. ***Key words: angiostatin/endothelial cell/hydroxymates. Journal of Investigative Dermatology Symposium Proceedings 5:47–54, 2000***

Matrix metalloproteinases (MMP) are a structurally related family of proteinases (**Fig 1**) that are part of a larger superfamily of zinc-dependent endoproteinases called metsincins (Stocker *et al*, 1995). MMP are widely recognized for their ability to degrade extracellular matrix, and as a family are capable of degrading all extracellular matrix components. Presently, there are over 20 human MMP described, and although most MMP are secreted extracellularly, certain MMP are membrane associated [membrane-type (MT) MMP]. The collagenases (MMP-1, –3, and –13) cleave native fibrillar collagen types I, II, and III (Goldberg *et al*, 1986; Hasty *et al*, 1987; Welgus *et al*, 1990; Frieje *et al*, 1994). Stromelysin-1 and –2 (MMP-3, MMP-10) have broad substrate specificity. Stromelysin-3 (MMP-11) has a more restricted activity (Basset *et al*, 1990) and has been found to function in the release of matrix-bound growth factors (Manes *et al*, 1997). Gelatinase A (MMP-2, 72 kDa) and gelatinase B (MMP-9, 92 kDa) degrade denatured collagens and basement membrane components (Hibbs *et al*, 1987; Wilhelm *et al*, 1989). Gelatinase A is typically produced by mesenchymal cells, and gelatinase B is found in neutrophils, other inflammatory cells, and endothelial cells (Saarialho-Kere *et al*, 1993a, b; Stahle-Backdahl and Parks, 1993; Romanic and Madri, 1994; Zucker *et al*, 1995; Lee and McCulloch, 1997; Nguyen *et al*, 1998; Vu and Werb, 1998; Xie *et al*, 1998; Makela *et al*, 1999). Matrilysin (MMP-7) cleaves types I, III, IV, and V collagens, fibronectin, and procollagenase-1 (Quantin *et al*, 1989). More recently described members of the MMP family are five membrane-type matrix metalloproteinases (MT-MMP), membrane-associated proteases that function not only in matrix remodeling but also in pericellular activation of pro-MMP (Knauper *et al*, 1996; Knauper and Murphy, 1998). MMP-19 (Pendas *et al*, 1997) and enmelysin (MMP-20) (Llano *et al*, 1997) are the newest MMP described. MMP-19 has been demonstrated in synovial capillary endothelial cells during acute inflammation, findings that may suggest a role in angiogenesis (Kolb *et al*, 1999). MMP-20 presently has no known function in angiogenesis. In addition to their matrix degrading capabilities, investigations have also determined that MMP have important roles in other biologic processes; most notably, activation of other MMP (Knauper *et al*, 1996; Knauper and Murphy, 1998) and certain cytokines (TNFα) (Black *et al*, 1997), modulation of cell adhesion (Makela *et al*, 1999; Sarkissian and Lafyatis, 1999) and the proteolysis of parent molecules to biologic proteins with separate and specific activities (angiostatin from plasminogen) (Dong *et al*, 1997; Patterson and Sang, 1997; Cornelius *et al*, 1998).

MMP are expressed in an inactive, or zymogen form, and activity is dependent upon extracellular activation of the enzyme that requires cleavage of the cysteine-containing pro-enzyme

Manuscript received May 25, 2000; revised July 17, 2000; accepted for publication August 2, 2000.

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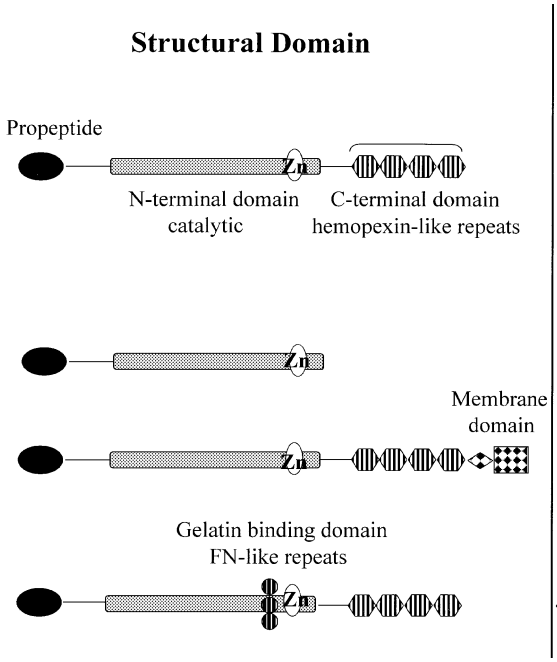

Figure 1. MMP family, structural domains, and substrates.

region that contacts the zinc atom in the active catalytic site (**Fig 1**) (Woessner, 1998). X-ray crystalographic studies have contributed greatly to our understanding of MMP structure and concomitant activity. Studies of full-length collagenase-1 (MMP-1) (Li *et al*, 1995) reveal that the N-terminal catalytic domain and the C-terminal hemopexin domain are connected by a flexible proline-rich linker, and that the hemopexin domain contains four units of a four-stranded antiparallel beta sheet resulting in a four-bladed propeller-like structure. Physiologic MMP inhibitors, known as tissue inhibitors of metalloproteinases (TIMP 1–4), inhibit MMP by forming noncovalent bimolecular complexes with them to block activation or block the active enzyme site itself (Gomis-Ruth *et al*, 1997b). Synthetic MMP inhibitors have also been engineered to bind irreversibly to the Zn^{++} atom of the enzyme active site (Brown, 1998). The C-terminal hemopexin domain is responsible for MMP substrate and inhibitor specificity, but the actual inhibition of MMP activity occurs due to binding of both physiologic (TIMP) and synthetic inhibitors (hydroxamates) to the catalytic domain. Based upon their 3-dimensional structures, specificity pocket subsites (designated S) within the MMP are formed that determine binding of the specific inhibitor amino acid segments, and result in inactivity of the enzyme. More specifically, determination of the crystal structure of the TIMP-1-MMP-3 complex reveals that the critical TIMP binding residues surround the TIMP disulfide bond between Cys^1 and Cys^{70}, with Cys^1 located on top of the MMP-3 catalytic site coordinating the catalytic Zn (Gomis-Ruth *et al*, 1997a).

Regulation of MMP activity is dependent upon gene expression, enzyme activation, and the presence of inhibitors. With few exceptions (Saarialho-Kere *et al*, 1995), MMP are not constitutively expressed and investigations into gene regulation have found that growth factors, cytokines and cell–matrix interactions are important regulators of MMP gene expression (reviewed by Birkedal-Hansen *et al*, 1993). In fact, the regulatory region of most MMP genes contain an AP-1 binding site and TRE-element, which are classically involved in this regulation (Angel *et al*, 1987a, b; Gaire *et al*, 1994; Pierce *et al*, 1996). Transcription factors that have been implicated in MMP gene regulation via these, and other sites (PEA3/ets) (Gum *et al*, 1996), and include members of the jun family (Mauviel *et al*, 1996; Solis-Herruzo *et al*, 1999), AP-2 and YB-1 (Mertens *et al*, 1998), NF-κB (Bond *et al*, 1998), and Egr-1 (Haas *et al*, 1999). Tyrosine kinase and protein kinase C signaling pathways have been implicated in the control of MMP expression (Sudbeck *et al*, 1994; Vincenti *et al*, 1999).

MMP are expressed by various cell types during processes of development, as well as during certain physiologic and pathologic processes. In cancer, MMP activity has been implicated in tumor invasion and metastasis. The matrix degradative activity of tumor cells themselves, the interstitial cells of the surrounding matrix, tumor-associated inflammatory cells, and endothelial cells of the tumor vasculature has been studied. Work investigating the MMP expression of tumors is based upon the premise that increased protease activity leads to the removal of physical barriers to invasion (Kleiner and Stetler-Stevenson, 1999) and correlates with tumor growth, tumor cell intravasation into the vasculature, extravasation, and metastasis (Sloane *et al*, 1993). In support of this, increased MMP expression has been found in many malignant tumors (Basset *et al*, 1990, 1997; Sato *et al*, 1994; Tolivia and Lopez-Otin, 1994). Correspondingly, inhibition of MMP activity through inhibition of multiple regulatory and activating pathways has been investigated (**Fig 2**). Increased expression of the physiologic inhibitor TIMP has been found to reduce the invasive and metastatic capacity of transformed cells in certain murine tumor models (Montgomery *et al*, 1994). Regulators of MMP expression that suppress MMP synthesis in certain cancer cell types, such as the retinoids (Li *et al*, 1999), have been investigated for their potential antitumor activity (Schoenermark *et al*, 1999). In seeming contradistinction to these findings, there is some evidence that MMP may function as regulators of cellular apoptosis (Vu *et al*, 1998).

Tumors are heterogeneous in their expression of MMP and the MMP expressing cell type (tumor *versus* stromal) varies. In certain cancers, MMP, such as matrilysin, characteristically localise to tumor cells themselves (Powell and Matrisian, 1996), whereas induction or activation of stromelysin-1 expression is typically found within the stromal cells (Nagase, 1998). The gelatinases, however, may be expressed by both tumor cells and cells of the surrounding stroma (Birkedal-Hansen *et al*, 1993). In both basal cell and squamous cell carcinomas, altered MMP-2 and TIMP expression has been demonstrated (Wagner *et al*, 1996). Conflicting results have been reported with respect to the relationship of MMP expression and the invasiveness of melanoma cells both *in vitro* and in an *in vivo* murine model (Montgomery *et al*, 1994; Huijzer *et al*, 1995). Nonetheless, with the increasing recognition of the multiple biologic functions performed by MMP, the simplistic assumption that increased tumor MMP expression correlates with increased tumor growth and metastasis cannot always be supported. In this paper, we will specifically address the role of MMP expression as it contributes to one area important to

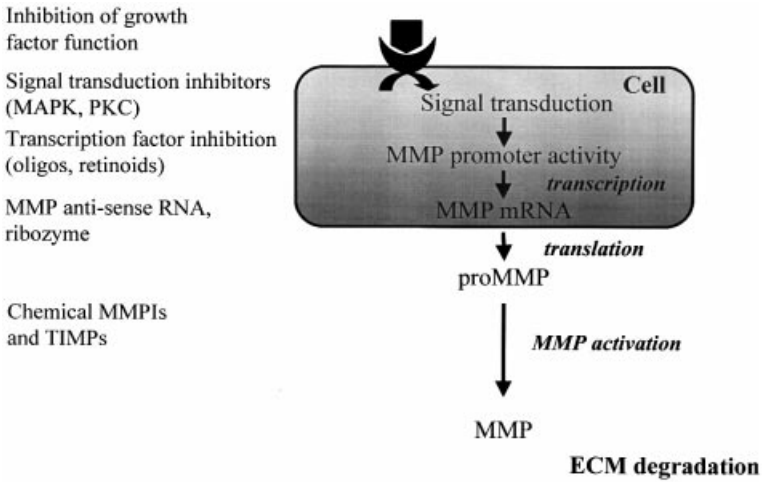

Figure 2. Targets for inhibiting MMP expression and activity. Reproduced with permission from Westermerck and Veli-Matti (1999).

tumor growth and metastasis – tumor angiogenesis – and the potential implications that inhibiting these enzymes may have on tumor behavior.

MATRIX METALLOPROTEINASES AND ANGIOGENESIS

The contribution of MMP to angiogenesis has been studied through both *in vitro* and *in vivo* investigations, and involves cell–cell and cell–matrix interactions together with proteolysis. Growth factors and cytokines have been identified that promote angiogenesis, and include vascular endothelial cell growth factor (VEGF) (Cao *et al*, 1998; Shweiki *et al*, 1993; Yamagishi *et al*, 1997), basic fibroblast growth factor (bFGF, FGF-2) (Kandel *et al*, 1991) (reviewed by Friesel and Maciag, 1995), hepatocyte growth factor (HGF) (Rosen and Goldberg, 1997), tumor necrosis factor alpha (TNFα) (Koolwijk *et al*, 1996; Leibovich *et al*, 1987) and platelet-derived growth factor-beta (PDGF-β) (Battegay *et al*, 1994). Interestingly, many of these same factors are regulators of MMP gene expression (VEGF, TNFα, bFGF; Cornelius *et al*, 1995; Qin *et al*, 1998) in endothelial cells and other cell types, although this regulation may not be *directly* related to their effect on angiogenesis.

In vitro work has demonstrated the role of endothelial cell MMP in the degradation of basement membrane matrix proteins (collagen type IV and laminin), endothelial cell migration on proteins of the interstitial and provisional matrix (collagen type I and fibrin), and endothelial cell–matrix interactions that promote endothelial cell ''differentiation'' *in vitro* (the formation of endothelial cell ''tubes'' or ''chords'') (**Tables I** and **II**). Collagenase is induced in microvascular endothelial cells migrating upon type I collagen in the presence of angiogenic cytokines (Cornelius *et al*, 1995), and is required for endothelial cells to invade a collagen type I gel matrix (Fisher *et al*, 1994). Plating endothelial cells on the EHS-derived basement membrane matrix Matrigel (Collaborative Biomedical, Twin Oak Park, Bedford, MA), containing types IV collagen, proteoglycans and laminin, induces the formation of endothelial cell ''tubes'' within 18–24 h (Kubota *et al*, 1988), and provides one *in vitro* model of endothelial cell morphogenesis. In this model, both type IV collagenases (MMP-2, –9) (Schnaper *et al*, 1993) and serine proteases (urokinase plasminogen activator, uPA) (Schnaper *et al*, 1995) are induced, and inhibition of either protease type decreases tube formation, although at distinct stages. Microvascular endothelial cells cultured within three-dimensional collagen gels express the membrane-type MMP, MT1-MMP, the inhibition of which delays their differentiation into tube-like structures (Chan *et al*, 1998). Work by other investigators demonstrated that, in a similar system, MT1-MMP was coordinately expressed with, and involved in, the activation of MMP-2 (Haas *et al*, 1998). Additionally, endothelial cells plated in a fibrin gel utilize MT1-MMP for fibrinolysis in this system (Hiraoka *et al*, 1998), an activity that is characteristically ascribed to the serine proteinases and is required for provisional matrix migration. In some studies, the expression of endothelial cell MMP, specifically MMP-9 (gelatinase B, 92 kDa gelatinase) by microvascular (small vessel) cells, the prototypic cell involved in angiogenesis, and macrovascular (large vessel) cells, differs according to cell type (Cornelius *et al*, 1995; Nguyen *et al*, 1998).

Table I. Endothelial cell MMP and TIMP

collagenase (MMP-1)	TIMP-1
gelatinase A (MMP-2)	TIMP-2
gelatinase B (MMP-9)	
stomelysin (MMP-3)	
MT1-MMP (MMP-14)	
MP-19	

Table II. Endothelial cell MMP in angiogenesis

In vitro assays	*In vivo* assays	Knock outs
migration assays	corneal pocket	MMP-2
collagen I gels	dorsal air sac	MMP-9
Matrigel gels firbrin gels	Chick chorioallantoic membrane	

In separate investigations, Brooks *et al* demonstrated that MMP-2 (gelatinase A, 72 kDa gelatinase) is expressed on the surface of invasive cells and endothelial cells involved in active angiogenesis, bound to the cell surface integrin αvβ3 (Brooks *et al*, 1996). These investigators had previously shown that αvβ3 is an endothelial cell surface integrin that is required for angiogenesis both *in vitro* and *in vivo* (Brooks *et al*, 1994). This group then demonstrated that the noncatalytic hemopexin fragment of the MMP-2 domain (**Fig 1**), termed PEX, mediates the MMP-2-αvβ3 binding, and that recombinant PEX could inhibit angiogenesis by competing for this binding (Brooks *et al*, 1998). Recently, a family of angio-inhibitory proteins, called METH-1 and METH-2, which also contain a metalloproteinase domain, has been described (Vazquez *et al*, 1999). These proteins also share a disintegrin and thrombospondin domain. Thrombospondin has well-recognized antiangiogenic activity (Good *et al*, 1990; Iruela-Arispe *et al*, 1991; Tolsma *et al*, 1993; Dameron *et al*, 1994; Volpert *et al*, 1995; Grant *et al*, 1998) with some investigators also demonstrating angiogenic properties, dependent upon the specific protein domain expressed (Qian *et al*, 1997).

It should be noted that for many years, physiologic and pathologic angiogenesis in the postnatal period has been defined as the "sprouting" of new vessels from differentiated, pre-existing vessels and has been distinguished from vasculogenisis, or the development of vessels during embryogenesis from progenitor cells. The *in vivo* role of MMP in blood vessel development has been studied in fetal angiogenesis, and MMP-1 was demonstrated in early microvessels developing from undifferentiated mesoderm (Karelina *et al*, 1995). Recent investigations have determined that endothelial progenitor cells may also play a role in postnatal neovascularization (Asahara *et al*, 1999). Studies of myocardial and hindlimb ischemia demonstrate vascular endothelial cell growth factor (VEGF)-dependent collateral artery growth (Takeshita, 1994). Further work by these investigators has determined that circulating endothelial cell progenitor cells are mobilized from the bone marrow following ischemia and VEGF induction, and may be incorporated into the neovasculature (Takahashi, 1999). To this author's knowledge, the contribution of MMP to this process has not yet been determined.

TUMOR ANGIOGENESIS

The requirement of a tumor and its metastasis to develop a functional vasculature for its survival and growth has been well established, and specific morphometric parameters have been determined *in vitro* and *in vivo* (Folkman, 1990, 1992). Tumor angiogenesis studies are driven by the need to understand the role of blood vessel development in tumor growth, with the ultimate goal of inhibiting growth and metastasis. Quantitative evidence has shown that in certain nonsmall cell lung carcinoma (Macchiarini *et al*, 1992), prostate (Wakui *et al*, 1992), and breast cancers (Weich *et al*, 1991), intratumoral microvessel density correlates with the development of metastasis, and may be an independent and significant prognostic indicator in certain tumors (Weidner, 1998). Folkman has proposed that tumor progression is associated with a switch to an "angiogenic phenotype" and develops after abrogation of the normal proliferative controls and tumor suppressor mechanisms (Folkman, 1992).

The clinical observation that the removal of the primary tumor in certain cancers led to the apparent increased growth of previously dormant metastasis instigated the search for a circulating tumor "factor" that inhibited metastatic growth. The first of these "factors" to be identified was from a murine model of Lewis lung carcinoma (LLC) and was a protease-generated product of plasminogen called angiostatin (O'Reilly *et al*, 1994). This protein was found to have endothelial cell antiproliferative properties *in vitro* (O'Reilly *et al*, 1994) and to inhibit angiogenesis of certain murine tumors *in vivo* (O'Reilly *et al*, 1996). In this model, it was later determined that a tumor-generated growth factor, GM-CSF, induces the protease that cleaves plasminogen to angiostatin (Dong *et al*, 1997), and that this protease is a macrophage MMP, MMP-12, or macrophage elastase (Dong *et al*, 1997; Cornelius *et al*, 1998) (**Fig 3**). Further studies have found that both serine proteases (plasmin) (Gately *et al*, 1996; Gately *et al*, 1997; Stathakis *et al*, 1997) and other MMP (MMP-2, –3, –7, –9) (Patterson and Sang, 1997; Cornelius *et al*, 1998) are also capable of generating angiostatin from plasminogen. In the LLC model, it was postulated that the angiogenesis inhibitor angiostatin blocks the development of a functional vasculature in the micrometastasis and consequently inhibits their growth. Other endogenous angiogenesis inhibitors have recently been described, most notably endostatin, a cleavage product of collagen XVIII (O'Reilly *et al*, 1997), although the specific protease(s) responsible for the cleavage have not been determined.

Another intriguing development in the area of tumor metastasis is the finding that, in certain *in vivo* models of lung metastasis, tumor cells remain *within* the microvasculature, and form distinct tumor cell colonies (Al-Mehdi *et al* 2000). Although not yet investigated, further study of the expansion, perhaps proliferation, and ultimately vascularization of such intravascular tumor colonies could reveal novel mechanisms of tumor angiogenesis, possibly involving MMP.

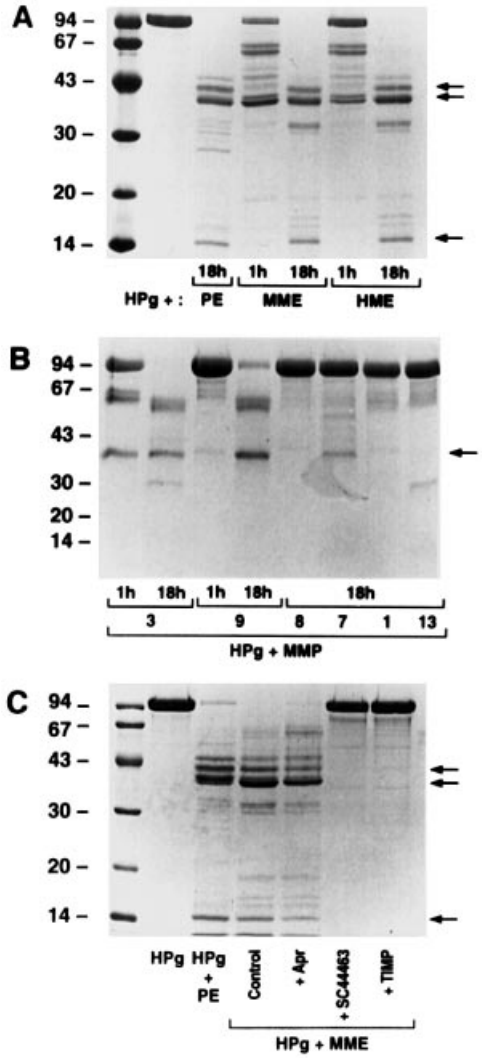

Figure 3. Cleavage of plasminogen by matrix metalloproteinases. (*A*) Pancreatic elastase (PE) [39 mM] and the MMP [final concentration 5×10^{-7} M] mouse and human macrophage elastase (MMP-12, MME and HME, respectively) were incubated with 4 μM plasminogen (HPg) [final concentration] for 1 or 18 h at 37°C. The reaction mixtures were stopped with SDS sample buffer containing DTT and subjected to electrophoresis on a 10% SDS-polyacrylamide gel. (*B*) The MMP [final concentration 5×10^{-7} M] stromelysin (MMP-3), 92 kDa gelatinase (MMP-9), collagenase-2 (MMP-8), matrilysin (MMP-7), collagenase-1 (MMP-1) and collagenase-3 (MMP-13) were incubated with 4 μM HPg [final concentration] for the indicated times. (*C*) MMP-12 was preincubated with either aprotinin (Apr) [100 KIU/ml], a hydroxymate MMP-inhibitor (SC 44463) [25 μM], or TIMP [25 μM] for 1 h prior to the addition of HPg. For all parts, the *arrows* denote 38 kDa, 35 kDa, and 14 kDa cleavage products. The 38 k Da product was subsequently sequenced and is consistent with angiostatin (kringle regions 1–4). Reproduced with permission from Cornelius *et al* (1998), Copyright 1998, American Association of Immunologists.

Nonetheless, it is indeed interesting that the MMP have been recently implicated in the *inhibition of* angiogenesis, as suggested by their role in the generation of angiogenesis inhibitors (Dong *et al*, 1997; Patterson and Sang, 1997; Cornelius *et al*, 1998) and the antiangiogenic activity of certain specific MMP domains (Brooks *et al*, 1998; Vazquez *et al*, 1999). As reviewed above, investigations have previously demonstrated that both endothelial cell serine proteases [urokinase plasminogen activator (uPA), tissue-type plasminogen activator (tPA) and plasmin] (Pepper *et al*, 1987,

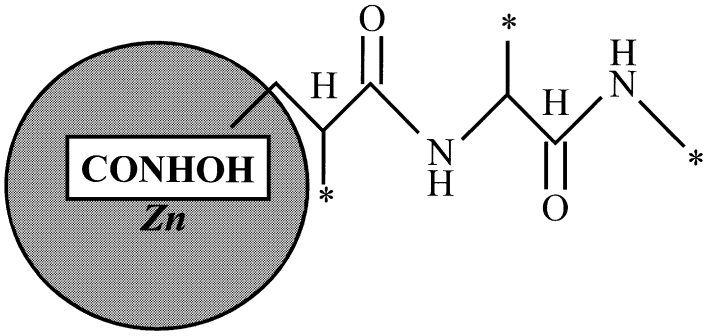

Figure 4. Synthetic MMP inhibitors. Hydroxymate group (CONHOH) binds to active site Zn ion of the MMP. Asterisks denote molecule sites that affect recognition of specific MMP.

1991; Blei *et al*, 1993; van Hinsbergh *et al*, 1997) and MMP (Fisher *et al*, 1994; Moscatelli and Rifkin, 1988; Takigawa *et al*, 1990; Le Querrec *et al*, 1993; Ray and Stetler-Stevenson, 1994; Taraboletti *et al*, 1995; Stetler-Stevenson, 1999) can contribute to angiogenesis through subendothelial basement membrane degradation, endothelial cell migration, and ultimately, the formation of a newly formed vasculature. Substantiating these findings are studies in MMP-9 deficient mice that have demonstrated decreased ossification and growth plate vascularization (Vu *et al*, 1998). Tumor models in MMP-2 deficient mice have shown reduced angiogenesis and tumor progression (Itoh *et al*, 1998). Additionally, the expression of MMP-2 and MT1-MMP, which are the MMP most frequently implicated in angiogenesis, has been found to correlate with malignant progression in gliomas, a highly vascular tumor (Lampert *et al*, 1998). Work such as this has led to the experimental and current use of MMP inhibitors in clinical trials in certain types of cancer (Breattie and Smyth, 1998; Davies *et al*, 1993; DeClerck *et al*, 1992, 1997; Tarboletti *et al*, 1995).

MMP AND ANTI-ANGIOGENIC AGENTS

Physiologic inhibitors of MMP include the TIMP as well as the more general inhibitor $\alpha 2$ macroglobulin. Proteolytic and biologic activity of MMP is partially regulated by the expression of TIMP. MMP often exist complexed with TIMP, and there is evidence to suggest that this complex forms after their secretion (Nguyen *et al*, 1998). *In vivo* murine tumor models have shown that invasiveness of certain tumors may be inversely related to tumor cell expression of TIMP-1 (Soloway *et al*, 1996) and that overexpression of TIMP-3 by tumor cells reduces tumor growth, possibly by its angiostatic activity (Anand-Apte *et al*, 1996). Other systems have used recombinant TIMP protein(s) to inhibit MMP and *in vitro* angiogenesis (Takigawa, 1990; Schnaper *et al*, 1993), murine tumor invasiveness (Bao *et al*, 1996), and blood vessel development (Valente *et al*, 1998). In seeming contradiction, however, studies have shown that TIMP may promote development of certain tumors (Koop *et al*, 1994) and may perform separate biologic functions other than MMP inhibition, such as being effectors of cell proliferation (Murphy *et al*, 1993). As with the MMP, evidence exists that, in certain tumors**,** TIMP also have tumor promoting activity (summarized in Blavier *et al*, 1999). The clinical use of recombinant TIMP proteins as antitumor or antiangiogenic agents may be limited by this potential dual function together with the low plasma half-life of the recombinant protein that necessitates unrealistic dosage regimens and protein concentrations (Blavier *et al*, 1999).

Synthetic MMP inhibitors (MMPI) have been engineered based upon knowledge of the MMP structure and subsites, combined with the capability to chelate Zn^{++} at the active catalytic site (Skotnicki *et al*, 1999). The most widely used Zn^{++}-chelating compounds contain a hydroxamic acid group (**Fig 4**) (Brown, 1998; Skotnicki *et al*, 1999)**.** Structural modifications of regions within the inhibitor backbone alter their recognition of MMP enabling development of inhibitors that are more specific to certain MMP (Skotnicki *et al*, 1999). As previously described, this work has been greatly aided by determination of the MMP catalytic site and MMP/inhibitor complex structures via X-ray crystallography (see *Introduction*). Available X-ray structures of MMP-3 and MMP-8 have been used to design synthetic compounds with complimentary composition to their respective MMP specificity pocket(s) (Matter *et al*, 1999).

One of the first hydroxymates developed was Batimistat (BB-94, British Biotech). This MMP inhibitor was found to have poor solubility and was not suitable for oral or intravenous administration. It has shown efficacy, however, in early clinical studies when administered intraperitoneally for malignant pleural effusions and ascites (Ngo and Castaner, 1996). A second generation hydroxymate MMP inhibitor, Marimistat, another broad spectrum MMPI, has increased bioavailability and is in clinical trial as an antiangiogenic agent in certain cancers, including nonsmall cell lung cancer, metastatic breast cancer, small cell lung cancer and the highly vascular glioblastoma (NCI). Recent reports of completed clinical trials employing marimistat in advanced pancreatic carcinoma have demonstrated no survival advantage over chemotherapy, however (Yip *et al*, 1999). AG3340 (Agouron Pharmaceuticals, San Diego, CA), also a hydroxymate inhibitor, has selective inhibitory activity for the gelatinases, MT1-MMP and collagenase-3. In murine human tumor models, this agent has demonstrated dose-dependant growth inhibition and decreased tumor angiogenesis in certain colon, lung, and breast tumors (Shalinsky *et al*, 1999).

Other Zn^{++}-chelating groups that have been specifically synthesized to inhibit MMP include the mercaptoalcohols and mercaptoketones (Campbell *et al*, 1998; Levin *et al*, 1998). The bisphosphonates, a separate class of drugs that are currently in use for diseases of bone-resorption, have recently been recognized to have MMP inhibitor activity *in vitro*, possibly due to their cation-chelating ability (teronen *et al*, 1999). Another known class of drugs, the tetracyclines, have been recognized for their ability to inhibit MMP activity, originally in periodontal disease (Golub, 1983) in doses less than that exibiting antimicrobial activity. Such findings have provided the basis for the development of a class of tetracycline-based MMP inhibitors without antimicrobial activity, the chemically modified tetracyclines. A separate method used to identify MMP inhibitors is the screening of phage display peptide libraries. One group has screened for gelatinase-specific (MMP-2,

–9) inhibitors and identified cyclic peptides containing the sequence HWGF (Koivunen *et al*, 1999), prompting the synthesis of the synthetic peptide CTTHWGFTLV that has been shown to inhibit tumors in murine models that target angiogenic blood vessels (Koivunen *et al*, 1999).

Musculoskeletal pain and inflammation are a commonly reported side-effect of the hydroxymate MMPI that is characteristically dose-related (Nemunaitis *et al*, 1998). Along these lines, the ability of both the broad spectrum and selective MMPI to exhibit anticancer effects (in a mouse melanoma model) and to induce tendinitis (in a rat tendinitis model) (Drummond *et al*, 1999) has been investigated. Controlling for systemic dose and inhibitor potency, the MMP-selective (collagense-, gelatinase-) inhibitors caused less tendinitis but were also less effective as anticancer agents. Interestingly, at equivalent dosages, one of the broad spectrum MMPI tested that had less ability to inhibit certain MMP-like enzymes known as membrane protein ''sheddases'' also exhibited less capability to induce tendinitis. ''Sheddases'' are metalloenzymes that release membrane-associated proteins, some of which are growth factors and cytokines such as transforming growth factor (TGF-α) (Arribas *et al*, 1996) and tumor necrosis factor (TNF-α), and have previously been shown to be inhibited by certain MMPI (Gearing *et al*, 1994). In related work, MMPI have also been found to inhibit the release of L-selectin from the T lymphocyte surface, and consequently affect lymphocyte transgression through the vasculature and into the lymph node (Preece *et al*, 1996). As studies with MMPI progress, it is increasingly evident that these agents, like the MMP and sheddases that they inhibit, affect varied biologic processes other than tumor cell invasion and angiogenesis. Additionally, they are not cytotoxic but rather cytostatic, and if their efficacy in human studies is proven, their use as anticancer agents may ultimately be as selective adjuvants to chemotherapeutic agents in certain tumors.

CONCLUSIONS

The contribution of MMP to tumor growth, invasion, and angiogenesis has been established by multiple *in vitro* and *in vivo* investigations. In light of such findings, the inhibition of MMP activity has been investigated as a mechanism of inhibiting tumor growth and metastasis. Although the MMP profile of tumors is not homogenous, the expression of certain MMP, i.e., MMP-2, –9 and MT1-MMP, are implicated in both tumor invasion and angiogenesis. There is disagreement as to whether the application of broad-spectrum inhibitors, such as the early hydroxymate inhibitors, in distinction to the use of inhibitors that target certain MMP (i.e., the gelatinases) is most appropriate. Investigations aimed at improving our knowledge of the heterogeneity of specific tumor types and the host defense mechanism(s) involved will potentially allow more tumor-specific intervention of these therapeutic agents.

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