

Matrix metalloproteases: Underutilized targets for drug delivery

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Abstract

Pathophysiological molecules in the extracellular environment offer excellent targets that can be exploited for designing drug targeting systems. Matrix metalloproteases (MMPs) are a family of extracellular proteolytic enzymes that are characterized by their overexpression or overactivity in several pathologies. Over the last two decades, the MMP literature reveals heightened interest in the research involving MMP biology, pathology and targeting. This review describes various strategies that have been designed to utilize MMPs for targeting therapeutic entities. Key factors that need to be considered in the successful design of such systems have been identified based on the analyses of these strategies. Development of targeted drug delivery using MMPs has been steadily pursued; however, drug delivery efforts using these targets need to be intensified and focused to realize the clinical application of the fast developing fundamental MMP research.

Keywords: Drug delivery, drug targeting, matrix metalloprotease (MMP), prodrug, drug activation

Abbreviations

AP-1	activator protein-1	RECK	reversion-inducing cysteine-rich protein with Kazal motifs
DAF	decay accelerating factor	scFv	single chain antibody variable fragment
Dox	doxorubicin	SeV	Sendai virus
DTS	drug targeting system	SNPs	single nucleotide polymorphisms
ECM	extracellular matrix	TGF	tumor growth factor
EGF	epidermal growth factor	TIMPs	tissue inhibitors of metalloproteases
EPR	enhanced permeability and retention	TNF	tumor necrosis factor
ETS	erythroblastosis twenty-six	TNFR	tumor necrosis factor receptor
FGF	fibroblast growth factor	TSP-1	thrombospondin-1
FMG	fusogenic membrane glycoprotein	uPA	urokinase-type plasminogen activator
GALV	gibbon ape leukemia virus envelope glycoprotein	VEGF	vascular endothelial growth factor
HCC	hepatocellular carcinoma		L-amino acids in the peptide sequences are designated by one-letter abbreviations used under standard convention
IgG	immunoglobulin G		D-amino acids in the peptide sequences are designated by three-letter abbreviations used under standard convention.
IL	interleukin		MMP cleavage sites within peptides are denoted by “ ~ ”
LV	left ventricular		
MMP	matrix metalloprotease		
MMPIs	matrix metalloprotease inhibitors		
mPEG	poly(ethylene glycol) methyl ether		
PA	protective antigen		
PEG-PD	poly(ethylene glycol)-peptide		

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Introduction

The search for a “magic bullet” has been the major focus of drug delivery and drug discovery research for many years (Ehrlich and Bolduan 1906). If active agents or delivery systems can be devised that selectively treat a disease while having no effect on healthy cells, tissues and organs, that particular disease will no longer plague humankind. However, for most diseases, the search for a drug targeting system (DTS) has not been successful to date. The major reason that the DTS search has failed is that most biologic processes are active in many tissues at varying levels. Diseased tissues tend to exhibit normal biology that only exists at either an elevated level or decreased level compared to the equivalent normal tissue or other normal tissues in the body. This fact has driven researchers to identify targets that have the largest difference between normal and diseased tissue or to find novel ways of utilizing multiple properties of the disease state to amplify the difference between normal and diseased tissue. In this way, we continue to investigate novel targets for designing and delivering active chemotherapeutic agents that are similar to the proposed “magic bullet”. Matrix metalloproteases (MMP) are one of such promising targets being explored for designing an array of DTS.

The drug targeting field has attempted to classify the various methods used for DTS design. The classification schemes utilize the physical, chemical and biologic cues of the disease that are exploited to release or activate a drug to describe the DTS (Lubbe et al. 2001). A DTS that utilizes a specific biochemical signal at the cellular level to target delivery would easily be classified as site-directed, 2nd order, cellular and active delivery. This is because the targeting would deliver the agent to the disease location in response to a specific biochemical extracellular event at the cellular level. Such a targeting mechanism would easily and clearly describe the topic of current review, MMP targeting.

Matrix metalloproteases (MMPs)

It has been more than four decades since the first collagen degrading activity was observed during metamorphosis in tadpoles (Gross and Lapiere 1962). This observation marked the beginning of discovery of a family of enzymes recently categorized into the MMPs family. Much has been discovered about MMP structure, activation and inhibition, regulation of gene expression, and role in normal physiology and in diseases in this span and much is left to be determined (Brinckerhoff and Matrisian 2002).

MMPs, also referred to as matrixins, are a family of approximately 24 human zinc-containing endopeptidases that are together capable of degrading all components of the extracellular matrix (ECM) and

many other proteins. MMPs are produced as latent zymogens which must be proteolytically activated in the body to form the functional enzyme (Nagase and Woessner 1999). MMPs have been subdivided into at least five classes based on their structure (Borkakoti 1998; Rundhaug 2005), Table I. Matrilysins (MMP-7 and -26) consist of a propeptide domain and a catalytic domain with the zinc binding site. The collagenases (MMP-1, -8 and -13), in addition to the minimal matrilysin domain structure, contain a hemopexin-like domain connected to the catalytic domain via a proline-rich hinge region. The stromelysins (MMP-3, -10 and -11) have similar structural domains as the collagenases but broader substrate specificity. The gelatinases (MMP-2 and -9) contain an additional region of three fibronectin type II repeats within their catalytic domains. The fifth subclass of MMPs is the membrane-type MMPs (MT-MMPs) which are bound to the cell surface via a C-terminal transmembrane domain (MMP-14, -15, -16 and -24) or glycosylphosphatidylinositol anchor (MMP-17 and -25). Other MMPs not included in above classification are metalloelastase (MMP-12), enamelysin (MMP-20), epilysin (MMP-28), MMP-19, MMP-27 and MMP-23. These MMPs with an exception of MMP-23 have domain structure similar to collagenases but have not been categorized in that subclass for various reasons. MMP-23 is unique in that it lacks the hinge and hemopexin domains and instead contains a short carboxy-terminal domain containing cystein array (Parks et al. 2004).

Variations in the residues forming the catalytic domain play a major role in binding and catalysis, but discrete MMP substrate binding domains exist in non-catalytic regions that further increase the substrate specificity of different MMPs (Overall 2002). Various approaches, including proteomics, affinity based methods and genetic knockout, have been used to identify MMP substrates (McQuibban et al. 2000; Guo et al. 2002; Tam et al. 2004). In general, significant overlap is observed in specificity of different MMPs for natural physiological matrix and non-matrix substrates. For example, collagen I is degraded by MMP-1, -2, -8 and -13; fibronectin by MMP-2, -3, -7, -10 and -11; and ProMMP-2 by MMP-14 and -16 (Nagase and Fields 1996). Among the non-ECM substrates, MMP-2 and -9 can activate latent TGF β 1 and TGF β 2 while MMP-1, -3 and -7 release TNF α from the cell surface (McCawley and Matrisian 2001; Stamenkovic 2003). In addition to natural protein cleavage, cleavage site specificity of several MMPs, mainly MMP-1, -2, -3, -7, -8, -9, -13 and -14, have been studied using large series of synthetic peptides based on natural substrates, phage display libraries and mixture-based libraries (Netzelarnett et al. 1991; Nagase and Fields 1996; Deng et al. 2000; Turk et al. 2001). If the cleavage site of a protease is

Table I. Mammalian matrix metalloproteinases (MMP) family.

MMP subclass	MMP designation	Common name	Domain structure*
Matrilysins	MMP-7 MMP-26	Matrilysin	SP - Pro - Catalytic
Collagenases	MMP-1 MMP-8 MMP-13	Interstitial collagenase Neutrophil collagenase Collagenase-3	SP - Pro - Catalytic - ^{Hinge} - Hemopexin - like
Stromelysins	MMP-3 MMP-10 MMP-11	Stromelysin-1 Stromelysin-2 Stromelysin-3	SP - Pro - Catalytic - Hemopexin - like
Gelatinases	MMP-2 MMP-9	Gelatinase A Gelatinase B	SP - Pro - F - Catalytic - (Fn) - (Fn) - (Fn) - Hemopexin - like or C5 - Hemopexin - like
Membrane-type MMPs	MMP-14 MMP-15 MMP-16 MMP-24 MMP-17 MMP-25	MT1-MMP MT2-MMP MT3-MMP MT5-MMP MT4-MMP MT6-MMP	SP - Pro - F - Catalytic - Hemopexin - like - TM - Cs or GPI
Others	MMP-12 MMP-20 MMP-19 MMP-27 MMP-28	Metalloelastase Enamelysin Epilysin	SP - Pro - Catalytic - Hemopexin - like SP - Pro - F - Catalytic - Hemopexin - like
	MMP-23		SP - Pro - F - Catalytic - Cys - IgG - like

SP, signal peptide; Pro, pro-domain; F, furin-cleavage site; Fn, fibronectin repeat; C5, type-V-collagen-like domain, TM, transmembrane domain; Cs, cytosolic; GPI, glycosylphosphatidylinositol; Cys, cysteine array; Ig, immunoglobulin.

* Domain structures adapted from Parks et al. (2004).

defined as ...P3-P2-P1 ~ P1'-P2'-P3'... where P# is any specific amino acid, cleavage occurs between P1 and P1' amino acids. MMPs generally require hydrophobic amino acids at P1' and P2, prefer hydrophobic or basic amino acids at P2', proline at P3, and small residues particularly serine at P1. Although, a number of features have been identified for the cleavage site motif that are common to all MMPs examined, certain differences have also been observed that can distinguish MMPs. Whereas MMP-1, -2 and -9 prefer small residues, MMP-3, -7 and -14 select for methionine at P3'. MMPs are also different in their relative tolerance for aromatic amino acids at P1'. Although P2 is generally hydrophobic, the particular residues vary from enzyme to enzyme. Only, MMP-2, -3 and -7 select glutamic acid at P1. Thus, even though there is large similarity in the optimal sequences for cleavage by MMPs, it is possible to design substrate peptides that are selectively cleaved (>1000-fold difference in activity) by specific MMPs, an important aspect for utilizing MMPs as targets for drug delivery (Turk et al. 2001). The overlapping cleavage specificity is controlled *in vivo* by specific binding sequences to natural substrates and through exquisite regulation of the temporal and spatial control of MMP activity.

As part of the conserved structure, all MMPs contain a "cysteine switch" that maintains the latency of the enzyme prior to activation. The sulfhydryl cysteine in the highly conserved PRCGXPD sequence in the propeptide domain coordinates with the catalytic zinc ion to prevent activity (Van Wart and Birkedal-hansen 1990). MMPs are activated *in vivo* by proteolytic removal of the propeptide domain. MMP activation is regulated by one of several proteolytic cascades. One such cascade is initiated by plasmin generated from plasminogen through the action of urokinase-type plasminogen activator (uPA). Plasmin can activate proMMP-1 and proMMP-3. Activated MMP-3 can in turn activate proMMP-1 and -9 (He et al. 1989; DeClerck and Laug 1996; Ramos-DeSimone et al. 1999). Another cascade utilizes MT1-MMP to activate ProMMP-2 and -13 and focuses the activity at the cell surface (Sato et al. 1994; Knauper et al. 2002). Activated MMP-2 and -13 can both activate proMMP-9 (Fridman et al. 1995; Knauper et al. 1997). Thus, there are many cross-talk events between MMP activation cascades due to several MMPs having the capability to activate other MMPs and self-activate.

Regardless of the cascade utilized to activate a particular MMP, extracellular activation of MMPs results from a two-step process. The first step is the cleavage by an activator protease in the exposed loop in the propeptide region thus destabilizing its zinc coordination. This is followed by a second cleavage, usually by a MMP, releasing the mature enzyme (Nagase 1997). Specific MMP family members

(MMP-11, MT-MMPs, MMP-23 and -28) can also be activated by intracellular furin convertase enzymes (Pei and Weiss 1995; Sato et al. 1997). The multitude of activation mechanisms allows very precise control of MMP activity even when the expression levels of the proteases are constant. However, inhibition mechanisms exist that aid in further controlling MMP activity.

MMPs can be inhibited by general protease inhibitors, e.g. α 2-macroglobulin, angiogenesis inhibitor thrombospondin-1 (TSP-1) and reversion-inducing cysteine-rich protein with Kazal motifs (RECK) (Egeblad and Werb 2002), much as these inhibitors act on other proteases. More specifically, MMP activity is regulated by endogenous inhibitors, primarily the tissue inhibitors of metalloproteases (TIMPs). TIMPs act by forming 1:1 complexes between their N-terminal domain and the activated catalytic zinc in MMPs (Bode and Maskos 2003). The TIMPs exhibit differential inhibitory activity against different MMPs based primarily on binding efficiency to the MMPs. Since the discovery of TIMPs, contradicting functions have been identified, specifically association of ProMMP-2 with TIMP-2 is necessary for MMP-2 activation by MT1-MMP (Goldberg et al. 1989; Cao et al. 1998). TIMPs also have other biological functions that are not related to MMP inhibition and are not entirely understood (Brew et al. 2000). Thus, the MMP-TIMP inhibition system is quite complex and caution is warranted in interpreting observations due to these conflicting roles of TIMPs. Further, complicating the regulation of MMP activity, MMPs are not only regulated by activation and natural inhibition, but also at the transcriptional and post-transcriptional levels.

Most of the MMPs are not constitutively expressed but are induced by specific stimuli, such as cytokines, growth factors, activated oncogenes and cell-ECM interactions (Westermarck and Kahari 1999). Several MMP promoter regions contain multiple *cis*-acting elements including activator protein-1 (AP-1) and erythroblastosis twenty six (ETS) binding sites (Overall and Lopez-Otin 2002; Rivat et al. 2003). These sites in cooperation with several other upstream elements regulate MMP gene expression. These various promoters also confer the tissue specificity for MMP expression (Crawford and Matrisian 1996). Single nucleotide polymorphisms (SNPs) in these promoter regions alter the transcriptional regulation of the MMP gene and have been associated with specific diseases (Ye 2000). MMP levels are also controlled at the post-transcriptional stage. MMP-9 levels have been shown to be controlled by translational efficiency in murine prostate carcinoma cells (Jiang and Muschel 2002) and translational repression is responsible for the discordance observed between mRNA levels and protein expression of human MMP-13 following interleukin-1 β or transforming growth factor- β 1 stimulation (Yu et al. 2003). Beyond these

four levels of MMP regulation, further regulation is exerted by localization of MMP activity in the pericellular space.

Many MMPs are typically anchored to the cell membrane after secretion and hence act only on their specific substrates in the immediate environment of the cell. In addition to MT-MMPs which contain membrane anchoring regions, several other interactions of MMPs with cell surface proteins have been reported that facilitate localized cell migration and invasion. MMP-2 can bind to $\alpha_v\beta_3$ -integrin in a proteolytically active form on the surface of invasive cells (Brooks et al. 1996). Also, a ternary complex of $\alpha_2\beta_1$ -integrin, proMMP-1 and collagen function together to drive and regulate keratinocyte migration indicating that several MMPs are directly localized by cell-surface receptors (Dumin et al. 2001). In addition to integrins, other cell surface receptors are involved in MMP focalization. Localization of MMP-9 to the cell surface by hyaluronan receptor CD44 is required to promote tumor invasion and angiogenesis by activating latent transforming growth factor- β (Yu and Woessner 2000). Heparan sulfate proteoglycans on or around epithelial cells and in the underlying basement membrane have been found to be the docking molecules for MMP-7 (Yu and Woessner 2000). As suggested for MMP-1 and 9, these MMP anchors might serve dual function of proenzyme activation and assisting enzyme-substrate interaction (Parks et al. 2004). Through these specific docking mechanisms, MMPs are spatially regulated allowing precise control of their proteolytic activity.

As a final level of regulation, cellular catabolism of MMPs is mediated by the low density lipoprotein receptor-related protein (Hahn-Dantona et al. 2001). It is clear that MMPs are highly regulated by every known mechanism of protein regulation. This is due to the control needed in normal physiology to precisely activate specific molecules and degrade other molecules.

Normal physiologic MMP function

Though MMPs were discovered as ECM degrading enzymes, an emerging view is that matrix degradation is not the only (or even primary) function of these enzymes (Woessner 2002; Mott and Werb 2004). MMPs act on many non-matrix proteins including cytokines, chemokines, receptors, cell-cell adhesion molecules, latent growth factors and antimicrobial peptides often potentiating activity of these proteins. Through these non-conventional substrates, MMP activity has been extended to cell growth, signaling, migration, differentiation, and apoptosis (Vu and Werb 2000; McCawley and Matrisian 2001). MMPs are involved in normal physiological processes requiring ECM modification, such as ovulation (Zhang and Nothnack 2005), muscle homeostasis

(Carmeli et al. 2004), rapid modeling and remodeling of connective tissues (Hulboy et al. 1997) and wound healing (Parks 1999).

Specific MMP gene knockout mice have enabled researchers to aim at detecting functions of various MMPs in normal developmental physiology. Surprisingly, mice that lacked MMP-2, -3, -7, -11 and -12 showed little or no phenotypic alterations under normal conditions. Inflammatory and immune-related differences were observed in all *mmp*-null mice described (Parks et al. 2004). Redundancy in MMP function might have hindered the phenotype appearance in these cases (Lee and Murphy 2004), but this hypothesis needs further confirmation. Despite several MMPs having no visible role in development, the role of MMPs during development has been observed in MMP-9 and MT1-MMP deficient mice. Deletion of MMP-9 caused a slight delay in bone growth-plate development (Vu et al. 1998). More convincingly, MT1-MMP knock-out mice have severe phenotypic dwarfism, osteopaenia, and arthritis and the mice survive only a short time after birth (Holmbeck et al. 1999). Mice lacking MMP-2 and MT1-MMP die immediately after birth with respiratory failure, abnormal blood vessels, and immature muscle fibers reminiscent of central core disease (Oh et al. 2004). More studies with multiple MMP gene deletions are needed to elucidate the role of individual MMPs in normal physiology particularly due to the interdependence of many MMP activation events and activation cascades. The roles of MMPs in normal physiology and development are still poorly understood, but the presence and overactivity of MMPs in many diseases have become quite apparent despite the lack of clear understanding of normal function of these proteases.

MMPs in pathologic conditions

Considering the variety of effects of MMPs on cells and their microenvironment it is not surprising that MMPs have been linked with a number of diseases and the list has only been increasing in recent years. As such, a brief review of MMPs in various pathologic conditions will be highlighted with attention drawn to specific cases while citing recent reviews. The list below is by no means complete, but the listing is intended to exemplify the wide impact of various MMPs in several pathologies.

Arthritis

Arthritis was the first disease to be linked with MMPs after the observation that rheumatoid synovial tissue degraded collagen (Harris and Krane 1972). Studies over the past 40 years have confirmed that MMPs, especially the collagenases, and TIMPs play an important role in connective-tissue destruction

in rheumatoid arthritis and osteoarthritis (Martel-Pelletier et al. 2001; Celiker et al. 2002; Mengshol et al. 2002; Burrage et al. 2006). Specifically, transcription-region SNPs in tissue type plasminogen activator and MMP-2 have been implicated as being related to development of rheumatoid arthritis while transcription-region SNPs in MMP-1, -3, -7, -9, and -13 are not correlated with arthritis development (Rodriguez-Lopez et al. 2006). However, overactivity of these MMPs has been implicated in arthritis and as potential therapeutic targets in arthritis (Martel-Pelletier et al. 2001; Mengshol et al. 2002).

Inflammation and innate immunity

MMP activity in inflammation and immunity has recently been thoroughly reviewed and the many actions of MMPs in immunity clearly defined (Parks et al. 2004). Increased or misregulated levels of many MMPs are observed in many diseases that are associated with inflammation. Injury induces expression of several MMPs by epithelial cells, the first line of innate defense, e.g. MMP-1 for repair of skin wounds and MMP-7 for reepithelialization in lung and gut (Pilcher et al. 1997; Dunsmore et al. 1998). MMP-7 is also expressed by healthy mucosal epithelium in most adult tissues that are exposed to external environment. MMP-7 provides defense against microorganisms and enables apoptosis in these tissues (Saarialhokere et al. 1995).

Not only are MMPs defensive as proteolytic enzymes, but MMPs take part in the maturation of the inflammatory process. MMPs also help to establish chemokine gradients by acting on the macromolecules in ECM to which chemokines are usually bound. Thus, MMPs indirectly regulate the influx of inflammatory cells in the tissue showing an inflammatory response. Several studies have indicated that MMPs can affect the activity of various cytokines including interleukin-1 β , interferon- β , vascular endothelial growth factor, epidermal growth factors, fibroblast growth factors, transforming growth factor- β 1 and tumor necrosis factor (TNF). MMPs are involved in both activation and inactivation of these inflammatory mediators and thus can control or support inflammation. MMP-1, 2, 3, 13 and 14 cleave chemokines thus converting the chemokines into antagonistic derivatives, thereby disrupting further recruitment of immune effector cells (McQuibban et al. 2002). MMPs have been suggested to regulate the expression of chemokine receptors that may slow immune response (Khandaker et al. 1999).

Cancer

As can be seen in many excellent recent reviews (Zucker et al. 2000; Foda and Zucker 2001; McCawley and Matrisian 2001; Egeblad and Werb 2002; Lynch

and Matrisian 2002) more may be known about the association between MMPs and cancer than is known about the role of MMPs in normal physiology. MMPs are known to break down the ECM to allow tumor growth, microenvironment development, metastasis and invasion (Sternlicht et al. 1999). MMPs break down the matrix to simply allow growth, but they also are known to release angiogenic and proliferation promoting growth factors from the ECM (McCawley and Matrisian 2001; Overall et al. 2004).

Association of MMPs, primarily MMP-2 and 9, with tumor invasion and metastasis has now been well established through numerous *in vivo* studies (Liotta et al. 1980; Stetler-Stevenson 1990). There is ongoing debate concerning whether MMPs in certain cancers are expressed by the stromal cells surrounding the tumor rather than the tumor cells themselves (Hofmann et al. 2005), but there is little debate that these MMPs are associated with cancer. In addition to MMP-2 and 9, MT1-MMP (MMP-14) has been proposed as being present in nearly every form of cancer if not the cause of many of the malignant transformations (Golubkov et al. 2005a,b). MMP-1, -2, -3, -7, -9, -13 and -14 are overexpressed in colorectal cancers and degree of overexpression can be correlated with the stage of the disease and the prognosis. In contrast, overexpression of MMP-12 is associated with increased survival in colorectal cancer (Zucker and Vacirca 2004). MMPs and TIMPs are widely expressed in both small cell lung cancer and non-small cell lung cancer (Michael et al. 1999; Thomas et al. 2000). In some cases, circulating MMP levels have been greatly increased when lung cancer is present (Hrabec et al. 2001).

Due to these associations and many others, MMPs have attracted significant attention as therapeutic target for cancer. However, MMPs also have anti-metastatic and anti-proliferative actions (Overall et al. 2004). Since, several MMPs degrade ECM proteins that reveal hidden (cryptic) adhesion sequences that may deter migration or metastasis (Pirila et al. 2003; Schenk and Quaranta 2003) or release anti-angiogenic protein fragments (Bergers et al. 2000), MMPs are now known to be pro-, counter- and anti-cancer targets (Overall and Kleinfeld 2006).

Angiogenesis

In addition to cancer-related angiogenesis, a definite link between MMPs and angiogenesis has been observed in various diseases characterized by neo-vascularization (Kvanta et al. 2000; Rundhaug 2005). MMPs are localized to invasive blood vessels and vascular endothelial cells have been observed to make a variety of MMPs in culture. Many growth factors including vascular endothelial growth factor, fibroblast growth factor-2 and TNF- α that regulate angiogenesis also induce MMP expression

(Stetler-Stevenson 1999). MT1-MMP and MMP-2 and 9 seem to be predominant MMPs involved with angiogenesis (Bergers et al. 2000; Fang et al. 2000; Galvez et al. 2001; Oh et al. 2004), but many other MMPs may also be associated.

Cardiovascular diseases

As previously described for angiogenic diseases, MMPs play an important role in cardiovascular diseases (Newby et al. 2006). MMP-1, -2, -3, and -9 found in the atherosclerotic plaque might be responsible for plaque progression and rupture (Galis et al. 1994). MMPs may be present to degrade the plaque, as part of the inflammatory cascade, or in an attempt to remodel the disease.

MMP-2 overexpression and activity has been shown to induce lower contractility in cardiac tissue (Wang et al. 2006). Increased MMP levels have been demonstrated to cause adverse left ventricular (LV) remodeling after myocardial infarction. Inflammatory processes occurring during the infarction play a role in induction of MMPs. Several distinct signaling pathways interact in myocardial interstitium and have a combined effect on MMP induction and activation. Hence, it is proposed that pharmacological intervention at the level of these signaling molecules may provide potential therapeutic strategies for the adverse ventricular remodeling associated with the progression of heart failure (Janicki et al. 2004; Tsuruda et al. 2004; Ahmed et al. 2006).

Cerebrovascular diseases

As MMPs allow breakdown of basement membrane, MMPs are expected to be present in many ischemic conditions. Not surprisingly, levels of active MMP-2 and 9 are markedly increased during cerebral ischemia (Clark et al. 1997). It has been hypothesized that activation of MMP-2 causes blood-brain barrier breakdown following focal cerebral stroke (Mun-Bryce and Rosenberg 1998) and MT-1 MMP, MMP-2 and 9 have been found to be focally present in cerebral aneurysms (Bruno et al. 1998). MMP upregulation, especially of MMP-2, -3 and -9, has been observed in intracranial hemorrhage and MMP-9 is correlated with cerebral edema (Fatar et al. 2005). MMP-2 and 9 seem to be involved in different types of dementia (Rosenberg et al. 2001; Adair et al. 2004) although the mechanism of involvement is unclear. Increased levels of MMP-9 and TIMPs have been found in arterio-venous malformations of the brain (Hashimoto et al. 2003).

Pulmonary diseases

Increased MMP-9 mRNA and protein levels have been detected in bronchial tissues of patients with

bronchial asthma. MMP-9 has been thought to be expressed by inflammatory cells in this case (Ohno et al. 1997). MMP-1 and 2 have been suggested to play an important role in airway smooth muscle hyperplasia and airway obstruction in asthma (Johnson and Knox 1999; Rajah et al. 1999). Elevated levels of various MMPs have been reported in chronic obstructive pulmonary diseases including pulmonary emphysema and chronic bronchitis (Segura-Valdez et al. 2000; Zheng et al. 2000). MMP-2 and 9 have been implicated in acute respiratory distress syndrome based on experimental animal studies (Torii et al. 1997). Increased MMPs have also been linked with pulmonary hypertension, acute lung injury and interstitial lung diseases (Ohbayashi 2002). The increased MMP levels in many of these diseases may be due to the increased turnover of lung epithelium, inflammatory response, or attempts to regulate the ECM mechanics, but as with many other pathologies, the presence and activity of MMPs is clear in many pulmonary diseases.

Ocular diseases

MMP-2 and -9 are expressed during corneal wound healing as long- and short-term response, respectively (Fini et al. 1992). MMPs are also involved in corneal neovascularization that occurs after extensive corneal damage (Mohan et al. 2000). MMP-2 and -9 have been found to be constitutively expressed in normal human vitreous and retina, respectively. Various MMPs have been found to be upregulated in pathologies of posterior segment of the eye, including age-related macular degeneration, proliferative diabetic retinopathy, glaucomatous optic nerve head damage, vitreal liquefaction and vitreoretinopathy (Sivak and Fini 2002). Also, a degenerative genetic eye disease, Sorsby's fundus dystrophy has been linked to a mutation in the *timp-3* gene (Weber et al. 1994).

Gastrointestinal diseases

High expression of MMPs has been recently implicated in the pathogenesis of many diseases of the gut characterized by ulceration. High expression of MMP-1 and 3 has been shown in Crohn's disease (Kirkegaard et al. 2004). MMP-1, -3, -7, -9, -10, -12 and -14 are overexpressed by different cell types in inflammatory bowel disease (Pender and MacDonald 2004). MMP-3 is overexpressed in necrotizing enterocolitis, a disease characterized by extensive hemorrhagic inflammatory necrosis of the terminal ileum and ascending colon of premature infants (Pender et al. 2003). MMP-1 and -3 are increased in peptic and duodenal ulcers (Saarialho-Kere et al. 1996) while MMP-7 is overexpressed in gastric epithelial cells exposed to *Helicobacter pylori* (Wroblewski et al. 2003). Certain gastrointestinal

diseases, e.g. collagenase colitis and diverticular disease, are characterized by increased synthesis of ECM and by the absence of certain MMPs and higher levels of TIMPs (Gunther et al. 1999; Mimura et al. 2004).

Oral diseases

As a final example of MMP-pathology relation, oral diseases are an excellent example of MMP as a drug target. Increased presence and activity of collagenases, especially MMP-8, has been known to cause substantial connective tissue destruction leading to periodontitis (Sorsa et al. 2004). The general MMP inhibitor, doxycycline, has been FDA approved for the treatment of periodontitis (Ingman et al. 1996). MMPs may also be involved in other oral diseases, such as dental caries and oral cancer (Chaussain-Miller et al. 2006). This one case where MMP targeting has yielded clinical acceptance should be heralded and promote further MMP targeting research in other areas.

This extensive list of MMP-related diseases indicates that MMPs are involved in various conditions and pathologies. Specific MMPs have been shown to be over or under active in the diseases. If appropriate methods can be identified, targeting MMPs for therapy can clearly be advantageous for disease treatment.

Targeting MMPs with small molecules

After MMPs were linked to tumor invasion and metastasis, they were considered as prime targets for anti-cancer drug discovery programs by almost all major pharmaceutical companies. Matrix metalloprotease inhibitors (MMPIs) developed under these programs included psuedopeptides mimicking MMP substrates as well as small molecules that bind the catalytic zinc. Non-peptidic small molecules were mostly hydroxamate derivatives (Whittaker et al. 1999). Following the intense competition in developing hydroxamate compounds and their failure in clinical trials, non-hydroxamate small molecules binding zinc are being developed as MMPIs (Breuer et al. 2005). Many MMPIs had reached clinical trials by the early 2000s with the majority of MMPIs being broad spectrum because of their non-selective binding mechanism and structural similarity in active sites of MMPs. The first MMPI to enter clinical development was British Biotech's batimastat, a broad-spectrum hydroxamate derivative. It was soon replaced by the orally bioavailable analog, marimastat, in clinical trials. Marimastat failed due to patients experiencing joint pain after long-term administration and lack of efficacy at the tolerated doses (Bramhall et al. 2001; Evans et al. 2001). The lack of efficacy was measured by survival of end-stage patients, but this may not have

been the appropriate patient population or therapeutic endpoint. A relatively selective inhibitor of MMP-2, -3 and -9, Bayer's BAY12-9566 (Tanomastat), proved to be worse than the standard treatment in a similar trial to marimastat. Several other phase III clinical trials failed to reach their end points of increased survival (Zucker et al. 2000; Fingleton 2003). In spite of failure as anti-cancer drugs so far, MMPIs have been considered as therapies for various other diseases. Animal models have shown their effectiveness in inflammatory diseases including multiple sclerosis, emphysema, aortic aneurism and atherosclerosis (Whittaker et al. 1999).

Several factors, such as lack of selectivity, mechanism of MMPI activity, trial design, and side effects, may have caused the failure of MMPI clinical trials. Redundancy and overlap of different MMPs in substrate specificities, expression patterns and functions along with complex interplay of various factors that regulate their induction, activation and inhibition also pose significant challenge in development of safe and effective MMP inhibitors. It is very important to determine which MMPs are potential drug targets (pro-tumorigenic) and which are clearly anti-targets (anti-tumorigenic) (Overall and Kleifeld 2006). Blocking MMP anti-targets counterbalances the beneficial effects of target inhibition thus leading to the failure of MMPIs. It is possible that the main cause of clinical failure of broad spectrum MMPIs is their non-selectivity and hence loss of protective actions of MMP anti-targets in cancer (Overall and Kleifeld 2006).

Therefore, identification and validation of specific MMPs as targets and anti-targets in different types and stages of cancer should be the first step in the drug development of MMPIs followed by discovery of new chemical leads for the specific inhibition of target MMPs that spare anti-targets. To satisfy the later requirement, new MMPIs need to be designed that bind specific features of individual MMP targets instead of relying on Zn^{2+} binding which is common to all MMPs. Efforts are now underway to explore such new generation of selective MMPIs. Novel Zn^{2+} binding groups that also interact with active site side groups have been designed (Puerta and Cohen 2003). Specific dynamic features offered by MMP active site flexibility can also be exploited to obtain selectivity (Morales et al. 2004; Bertini et al. 2005; Cuniasse et al. 2005). It is also important to optimize design of such MMPIs to avoid MMP anti-targets specifically MMP-3 and -8 (Overall and Kleifeld 2006).

In addition to careful MMPIs design, *in vivo* assays such as imaging with surrogate markers are required to prove the inhibition of MMPs by these inhibitors during clinical trials (Coussens et al. 2002; Overall and Lopez-Otin 2002) to fully understand the mechanisms and locations of action of these agents. As research in basic biology, molecular biology and

physiological and pathological functions of MMPs continues, careful clinical trial design may lead to clinical use of MMPIs for treatment of MMP-linked diseases in the future. Despite the disappointment of MMPIs as therapeutic molecules, other approaches that utilize MMPs to activate drugs have become heavily investigated in recent years.

Matrix metalloprotease activated drug delivery

As is clear from the action of MMPs in natural and pathologic states, MMPs cleave active agents from the ECM by degrading proteins. When specific MMPs are implicated as pharmacologic targets for a particular disease, MMPs can either be inhibited or be used to cleave prodrugs and thus release the active drug selectively in the diseased tissue overexpressing MMPs. Drug activation would lead to increased local concentration of the active drug at the disease site versus the surrounding tissue and other parts of the body where MMP activity is very low. Attempts to exploit MMP activity for targeting purposes do not readily appear in the literature until the late 1990s, making this a recently exploited target for DTS. This attractive idea of drug delivery has been pursued steadily since. Here, recent DTS research exploiting the activity of MMPs for targeting therapeutic entities has been grouped and briefly described. The major factors and limitations are identified.

MMP activated peptide prodrugs

The first class of MMP-activated DTS includes simple enzyme prodrugs, Figure 1, activated by MMPs. In the prodrugs, therapeutic drugs are attached to a MMP substrate peptide. When the peptide is cleaved, the drug becomes active. The prodrug can be orally available or parenterally administered and targeting depends only on the peptide sequence specificity. Many such prodrugs have been designed for various anti-cancer agents (Glazier 1997; Timar et al. 1998; Mincher et al. 2002; Copeland et al. 2005; Firestone and Telan 2005). MMP specific conjugates of doxorubicin (Dox) were

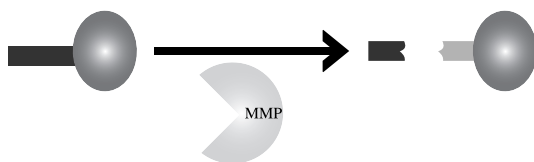


Figure 1. Schematic depiction of MMP-activation of a peptide prodrug. To utilize MMP targeting, an inactive peptide (blue) is attached to a drug (magenta). When MMP is present, the MMP (yellow) cleaves the peptide (blue) releasing an inactive peptide (blue) and a peptide-modified (aqua) drug (magenta). The peptide-fragment that is retained as part of the chemotherapeutic agent must be appropriate that the agent is active following MMP-cleavage.

shown to preferentially release leucine-modified Dox and Dox in a targeted fashion in MMP-expressing xenografts. It was found that peptide substrates with three or four prime side (—COOH terminal side of the cleavage site) residues like PLG ~ LYL and PLG ~ LYAL were cleaved efficiently by MMP-2 and -9. These conjugates also showed much higher therapeutic index than Dox in this preclinical model (Albright et al. 2005).

Peptidomimetic analogues of such peptide conjugates were designed for doxorubicin, auristatins and CBI-TMI. These peptidomimetic analogues were designed to release the free drug after MMP cleavage. However, these peptidomimetics were found to be non-substrates of MMPs. The peptide conjugates of Dox (PLG ~ LDox) and auristatin that were substrates of MMPs did not show selective activity against MMP-2 and -9 expressing cells versus non-expressing cells during a 4 h exposure. This study indicates the need for careful evaluation of substrate selectivity for these types of prodrugs (Kline et al. 2004).

Similarly, a topoisomerase inhibitor anthracene-9, 10-dione was attached to the C-terminus of MMP-9 substrate heptapeptide (ala-AALG ~ NVA-P) to produce a prodrug, EV1. Fluorescein isothiocyanate (FITC) was conjugated to EV1 at the N-terminus to form EV1-FITC. Intrinsic fluorescence of the FITC label was quenched internally by the anthraquinone chromophore when the heptapeptide was intact and fluorescence could be detected when the prodrug was cleaved by MMP-9. Selective activation of EV1-FITC by MMP-9 in diseased bone marrow and spleen cells was identified in the 5T33 multiple myeloma (MM) mouse model (Van Valckenborgh et al. 2005). Incorporating such fluorescent markers aids in following the fate of the conjugate and directly correlates the activation with the observed pharmacological effect without any ambiguity.

MMP activated carrier-peptide drug conjugates

Since, peptide-prodrugs suffer from clearance and non-specific accumulation in non-target tissues, systems that would protect the drug from clearance, protect the drug from non-specific activation, and allow increased accumulation in the diseased tissue were developed, carrier-peptide drug conjugates, Figure 2. To allow biologic acceptance, the carrier molecule can be of natural origin, such as albumin which is the most abundant protein in the circulation. Using this scheme, a maleimide doxorubicin derivative incorporating a MMP-2 specific peptide sequence (GPLG ~ IAGQ) was synthesized that binds rapidly and selectively to cysteine of albumin. Thus, circulating or purified albumin were used as a macromolecular carrier that had the potential to accumulate in tumor tissue by the EPR effect. In addition, the

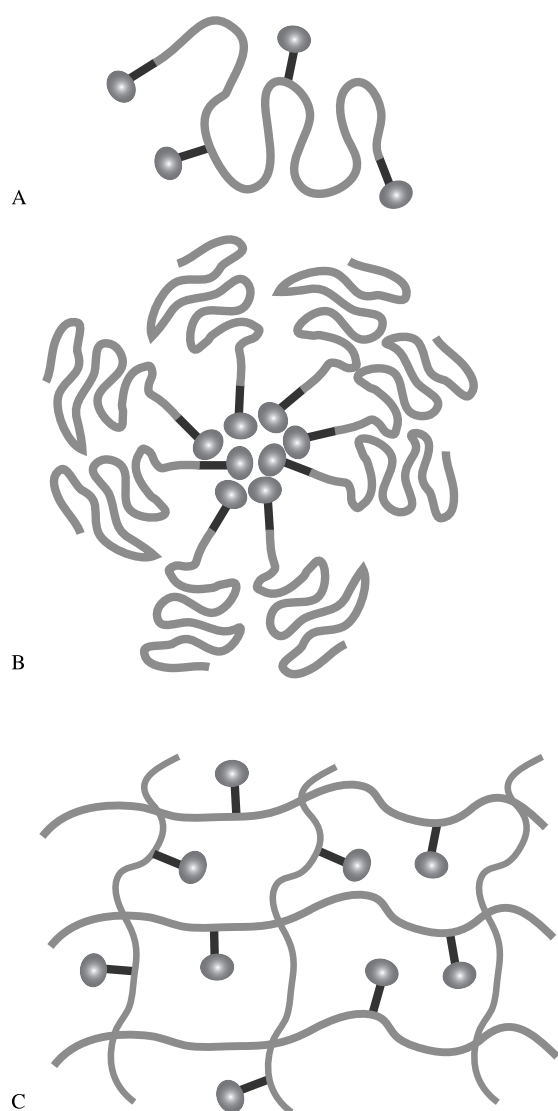


Figure 2. Schematic depiction of MMP-activation of carrier-peptide prodrugs. Three general subtypes of this category exist. In the first (A), a macromolecule is modified to bear one or more peptide-prodrugs on the macromolecule chain. The peptide-prodrug may be randomly placed on the chain, specifically placed on the chain (as in a protein), or at the end(s) of the macromolecular carrier. In the second, (B), macromolecular carriers may assemble into higher order structures such as micelles. In the final carrier-peptide conjugate (C), the drug is held within a three-dimensional hydrogel network. In each, the macromolecular carrier (green), MMP-sensitive peptide (blue), and therapeutic agent (magenta) are presented. The activation mechanism is similar to the prodrug (Figure 1) except the therapeutic agent (typically with an inactive portion of the MMP-sensitive peptide) is released from the inactive macromolecule.

peptide was used for further targeting the delivery of Dox to tumor cells expressing MMP-2. Efficient and specific cleavage of the conjugate by MMP-2 to produce peptidyl-Dox was observed in the presence of purified MMP-2, A375 melanoma cell culture supernatants and A375 melanoma tissue homogenates. When 100 μ M albumin conjugate was treated with 2 mU activated MMP-2 for 1.5 h, the initial peak

of the conjugate completely disappeared during reverse-phase HPLC analysis and a new peak for Dox-tetrapeptide was observed. In the presence of A375 melanoma tissue homogenates Dox-tetrapeptide was cleaved to an additional Dox-peptide and then to free Dox within minutes. The potency of the conjugate to inhibit A375 cell proliferation was lower than that of doxorubicin; however, this was expected due to remaining amino acids attached to the doxorubicin molecule after MMP activation. In A375 melanoma xenograft-bearing mice, equivalent intravenous doses of doxorubicin and the conjugate did not show significant difference in anti-tumor response, but the maximum tolerated dose of the conjugate was substantially increased thus allowing administration of high doses (Kratz et al. 2001; Mansour et al. 2003).

In addition to natural polymers, the strategy of MMP cleavable peptide prodrug has been combined with polymer therapeutics concept to design polymer-peptide-drug conjugates (Suarato et al. 2002; Benjamin et al. 2004). The targeting ability of simple prodrugs was further improved by conjugating peptide prodrug to a polymeric carrier that exploited passive targeting mechanism (Duncan 2003). MMP activated methotrexate-peptide-dextran conjugates were studied *in vitro* and *in vivo*. Dextran was modified to contain carboxymethyl groups used for conjugation to the peptide (PVG ~ LIG) as well as changing the polymer backbone to a negative overall charge. For comparison, MMP-insensitive conjugate containing scrambled peptide linker, GIVGPL, was prepared. The methotrexate-PVG ~ LIG-dextran conjugate released 89 and 61% peptidyl-methotrexate after 24 h in the presence of MMP-2 and -9, respectively, whereas the scrambled control peptide did not show any significant release. In initial experiments, MMP-2 did not exhibit the expected activation of the conjugate, but the activation of the conjugate was improved by blocking the negative charge on the dextran backbone, thus confirming the authors' previous suspicion that there was electrostatic repulsion between MMP-2 and the conjugate (Chau and Langer 2003; Chau et al. 2004). The conjugate was relatively stable in presence of serum, an important prerequisite for systemic delivery. Peptidyl-drug was released by MMP-2 and -9 positive cells while no cleavage was detected in the MMP-2 and -9 negative cells. Released peptidyl-methotrexate was effective in inhibiting the proliferation of tumor cells but with a lower potency than the free drug (Chau et al. 2004). The conjugate demonstrated enhanced anti-tumor efficacy compared to free drug and saline control in human tumor xenograft models expressing MMP-2 and -9 versus an alternate (RT-112) model with significantly lower MMP expression. However, the role of MMP-targeting mechanism in improving anti-tumor efficacy over the free drug could not be

confirmed as proliferation rate of RT-112 was also very slow and conjugate containing scrambled peptide, though acutely toxic, was also effective in controlling tumor size (Chau et al. 2006a,b). By comparing the plasma pharmacokinetics of the conjugates with free methotrexate, passive targeting was confirmed and may account for activity. Slightly more accumulation was found in the tumor tissue after treatment with MMP-sensitive conjugate than the MMP insensitive conjugate. However, since this difference in the tumor accumulation was not statistically significant, it was speculated that high dosage may have saturated the MMP mediated drug release and caused the endocytotic uptake to dominate. Also, most of the methotrexate in the tumor tissue was uncleaved thus downplaying the role of MMPs in tumor-specific delivery of the macromolecular peptidyl-methotrexate *in vivo*. Also, the cleavage of the conjugates in the small intestine and subsequent toxicity was surprising and did not correlate with MMP activation. The MMP-sensitive conjugates exhibited higher intestinal cleavage than the MMP-insensitive conjugate, but the MMP-insensitive conjugate was significantly more toxic. This confirmed that enzymes other than MMP were involved in the activity of the conjugates in normal tissues and particular attention must be paid to peptide selection (Chau et al. 2006a,b). This study showcased the complexity of factors that should be considered in interpreting *in vivo* data obtained with the drug-peptide conjugates designed to be cleaved specifically by MMPs.

Others have developed higher order structures utilizing the same carrier system type, Figure 2(B). The polymer-peptide-drug conjugates can be formulated as micelles if the drug is hydrophobic and a proper architecture is maintained. When micelles of poly(ethylene glycol) methyl ether (mPEG)-GPLG ~ V-Dox conjugates were injected intravenously in Lewis lung carcinoma xenograft bearing mice, the life span increased significantly compared to the free doxorubicin group. However, a higher dose of the conjugate than doxorubicin was required to show similar therapeutic effect (Bae et al. 2003). It was proposed that these systems took advantage of both the active targeting by the MMP-activation as well as the passive accumulation at the tumors based upon the EPR effect (Maeda et al. 2003).

In an attempt to circumvent systemic drug activation, locally implanted hydrogel-based devices have also been designed. Such implants placed at the site of the tumor after surgical resection can help prevent recurrence of the disease. A cytotoxic drug, cisplatin, was attached to MMP cleavable peptide (CG ~ LDD) pendant on the polymeric hydrogel network, Figure 2(C). The drug was released in the presence of MMPs, and MMPs expressed by the tumor cells. In the case of the hydrogel devices, drug release rate depends on the penetration of MMPs into

the network of hydrogel in addition to peptide cleavage kinetics (Tauro and Gemeinhart 2004, 2005). Each of these carrier-peptide drug conjugates has only begun to be examined. Further, research is ongoing to understand the properties that are necessary to control the drug activation process. These systems also can be expanded from small molecule delivery to biomacromolecule delivery.

MMP activated peptide and protein delivery

Much as small molecule therapeutics can be attached to carrier, biomacromolecules can be attached to carrier molecules for MMP activation. Decay accelerating factor (DAF), an anti-complement protein, is effective in reducing activity of complement in inflammatory diseases, including rheumatoid arthritis, but would have systemic effect unless inactivated as a prodrug. A MMP-3 and -8 sensitive polypeptide sequence derived from interglobular domain of aggrecan, a major proteoglycan in cartilage, was incorporated as a linker in a fusion protein of DAF with human immunoglobulin G (IgG). Fusion with IgG increased the circulation half-life of DAF while the MMP-sensitive linkers helped to target DAF to sites of inflammation and prevent systemic inhibition of the complement system. Specific cleavage was shown using purified MMPs, MMPs secreted from cytokine-stimulated chondrocytes and in the complex milieu of synovial fluid *ex vivo* in humans and rats and *in vivo* in rats. Initial *in vivo* studies demonstrating distribution of prodrug and efficacy in reducing inflammation are warranted (Harris et al. 2003). This system allowed a natural inhibitor to be delivered in a targeted manner.

MMP can also be used to activate cytolytic protein toxins. Two mutated anthrax toxin protective antigen (PA) proteins were constructed in which furin recognition site was replaced by the MMP-sensitive sequences GPLG ~ MLSQ and GPLG ~ LWAQ. The mutated proteins were completely resistant to furin but were efficiently processed by MMP-2 and 9. When combined with cytotoxin FP59, MMP activated PA proteins caused internalization of this cytotoxin leading to cell death. Thus, MMP-sensitive PA proteins selectively killed MMP-2 and 9-overexpressing human tumor cells in coculture model with gelatinase non-expressing cells (Vero cells). These proteins were also shown to be cleaved by MT1-MMP. This study indicated that PA activation occurred on the tumor cell surface and not in the supernatant and that the agents were selectively internalized within the activating cells (Liu et al. 2000).

Similarly, a natural cytolytic peptide, melittin, can be incorporated into MMP-activated systems. Melittin is highly active and targeting is necessary to prevent side-effects. Biotinylated melittin-MMP-2 cleavable peptide, Figure 3, has been interacted with avidin, as

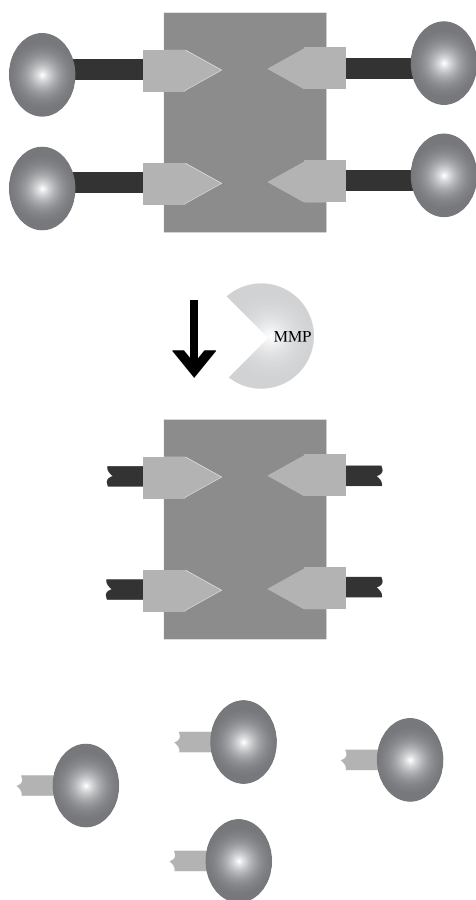


Figure 3. Schematic structure of the avidin-biotin-peptidyl-melittin MMP activated DTS. Cleavage of the MMP-sensitive peptide by MMP releases the cytolytic melittin. Avidin (green), biotin (light blue), MMP-sensitive peptide (dark blue), peptidyl (aqua)-melittin (magenta) are presented before and after MMP-cleavage.

a carrier, rendering melittin inactive until the peptide linker was cleaved by MMP-2. Unfortunately, the MMP-sensitive peptide sequence was not described by the authors. Specificity of the DTS was shown by strong cytolytic activity against cancer cells with high MMP-2 activity while being much less cytolytic against normal mouse L-cells. In mice, B16 tumors injected with the conjugate were significantly smaller compared to untreated tumors at the end of 25 days. Not only was there a difference in size, but the conjugate inhibited further growth of the tumor (Holle et al. 2003).

In an attempt to reduce tumor proliferation, TNF can be used as a prodrug. However, many TNF prodrugs have not exhibited adequate specificity. Multi-component prodrugs composed of a fusion protein of TNF with an N-terminal single chain antibody variable fragment (scFv) targeting abundant tumor markers and a C-terminal TNF receptor inhibitory fragment (TNFR1) were proposed for dual-level targeting. A MMP cleavable TNF prodrug (scFv-TNF-Linker-TNFR1) was designed

by introducing distinct MMP-2 recognition motifs between the TNF and TNFR1 fragments. Two MMP-2-sensitive linkers described in this study were comprised of three consecutive repeats of the motif GPLG ~ VRGK or HPV G ~ LLAR. Processing of these TNF prodrugs by recombinant active MMP-2 was confirmed by western blot analyses. The prodrugs were designed to be specifically activated upon antigen binding. These prodrugs were found to be activated by MMP-expressing HT1080 cells after specific membrane binding of the prodrug via the targeting scFv (Gerspach et al. 2006).

Overexpression of MMP in glioma has been used to target fusogenic membrane glycoprotein (FMG), potent therapeutic transgenes, which otherwise induce undesirable fusion in normal human astrocytes and fibroblasts. Expression constructs were made expressing the hyperfusogenic FMG, Gibbon Ape Leukemia Virus envelope glycoprotein (GALV), linked to a blocking ligand (the C-terminal extracellular domain of CD40 ligand) via a MMP cleavable linker (GALV M40). The MMP cleavable linker, GGPLG ~ LWAGG was based on the commercially available fluorogenic substrate of MMP-2 and -9 (Netzelarnett et al. 1991). A control construct containing the non-MMP specific linker GGGGS was used for comparison. When tested in malignant glioma and normal human astrocytes cell lines expressing varying levels of MMPs, fusion occurred after transfection with GALV M40 to an extent dependent on expression of MMP in the specific cell line. The fusion observed was completely blocked by broad-spectrum MMP inhibitors. Transfection with vectors expressing MMP-insensitive peptides exhibited little fusion and cell death. Fusion was also observed in MMP-poor cell lines transfected with GALV M40 constructs after mixing with MMP overexpressing, untransfected glioma cells as well as when stable transfectants expressing MT-1- and MT-2-MMP were established in these MMP-poor cell lines. When pre-infected U87 cells were inoculated in nude mice, the GALV M40 infected group showed significant suppression of tumor growth compared to control groups (Johnson et al. 2003). Therapeutic efficacy of this targeting approach was proved in glioma xenografts *in vivo* by using adenoviral vectors encoding the targeted constructs (Allen et al. 2004). These biomacromolecule delivery systems exemplified how specific proteins and peptides can be released, and similar strategies can be designed for MMP-targeted activation of almost any biomacromolecule.

MMP activated DNA delivery

MMPs have been used for targeting viral vectors for gene delivery. Formation of non-covalent complexes between homotrimeric viral envelope glycoproteins and a larger extraviral glycoprotein component (SU)

are required for the fusion of viral and cellular membranes. Inhibitory polypeptides have been fused with the N-terminus of the SU component of the viral membrane to block the infectivity of the retroviral vectors by inhibiting this complex formation. Protease-cleavable linkers were used for the attachment of inhibitory polypeptides such that exposure to relevant protease cleaves the polypeptide from the vector, thereby restoring the infectivity, Figure 4. Whether this protease targeting strategy can be used to target gene delivery *in vivo* using MMP was tested using retroviral vectors displaying two different inhibitory polypeptides, epidermal growth factor (EGF) residues 1–53 and CD40 ligand residues 116–261, fused through MMP cleavable linker (AAAPLG ~ LWA). The EGF domain was shown to be efficiently cleaved from the chimeric envelope by activated MMP-2, whereas control chimeric envelope containing MMP-insensitive linker (AAAGGGGS) was unaffected. MMP-activatable vectors could discriminate between MMP-rich and MMP-poor cells in a heterogeneous mixture of the two cell types whereas control vector containing non-cleavable linker did not show any significant selectivity. When the EGF-displaying vectors were administered by intratumoral inoculation of xenografts in nude mice,

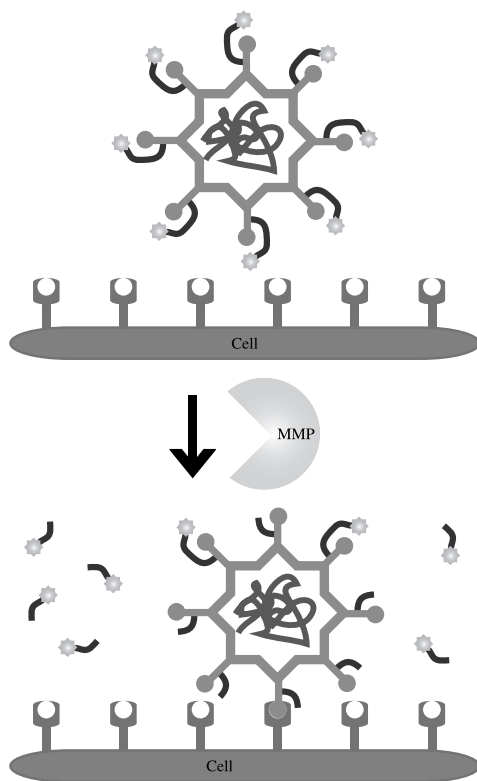


Figure 4. Schematic depiction of viral activation by MMPs. Blocking agents (orange star) are attached to the viral attachment and fusion proteins (green circles) via MMP-cleavable linkages (blue lines). The blocking agents prevent viral integration with the host cell (pink). Upon MMP cleavage, viral integration is possible as the blocking agents are freed and host integration is possible.

selective transduction of MMP-rich xenografts was apparent (Peng et al. 1997, 1999).

Similarly, a recombinant Sendai virus (SeV) that is selectively allowed to propagate and produce progeny in response to human tumor cells expressing MMPs has also been generated. This is a non-virulent oncolytic SeV in which the tryptic cleavage site of fusion glycoprotein (GVPQSR) is changed to a site susceptible to MMP cleavage (PLG ~ MTS). Cleavage is required for activation of fusion protein and thus for the infectivity of the virus. This is considered “gene delivery” due to the fact that the virus cannot form progeny in a tissue unless the viral protein is activated. The recombinant virus with the MMP cleavage motif spread efficiently in cell lines expressing MMPs, but not in cells that do not express MMP. The virus spread extensively in tumor xenografts in mice without disseminating to the surrounding normal cells (Kinoh et al. 2004). These two cases exemplify the creative ways that MMPs can be used to deliver specific agents to diseased tissue. MMPs have also been used to improve the targetability of drug-carrier systems to incorporate MMP-sensitivity.

MMP-deprotection of liposomes

Due to the tissue specific but not disease specific nature of some targeting ligands, combining tissue-specific ligand-targeting with disease-specific MMP-deprotection of the ligand can be used to allow selective disease targeting, Figure 5. Specifically, MMPs have been used to improve the targeting ability of galactosylated liposomes to hepatocellular carcinoma (HCC). These liposomes incorporated dioleoylphosphatidylethanolamine (DOPE) conjugated with poly(ethylene glycol) (PEG) bound to a MMP-2 substrate peptide (GPLG ~ IAGQ). The liposomes also contained a galactosylated cholesterol derivative (Gal-C4-Chol). These liposomes (Gal-PEG-PD-liposomes) were designed to target HCC by two mechanisms, MMP-deprotection and galactose-receptor binding. Due to galactose, the ligand, being buried within the PEG corona, liposomes would not be taken up by normal hepatocytes. In the local environment of HCC cells, the MMP-sensitive peptide can be cleaved by MMP-2, thus exposing the galactose moieties that can be selectively recognized by asialoglycoprotein receptors on HCC cells. Release of PEG coating from the liposomes due to specific cleavage by MMP-2 was confirmed by time-dependent appearance of $\text{NH}_2\text{-IAGQ-DOPE}$ after incubation of liposomes with purified hMMP-2. The PEG-PD cleavage was blocked by a known MMP inhibitor. The liposomes selectively delivered the drug load to HCC cells. The concept was confirmed by showing higher uptake of hMMP-2 preactivated Gal-PEG-PD-liposomes in HepG2 cells (Terada et al. 2006). This dual targeting mechanism approach can be extended to other types of drug carriers.

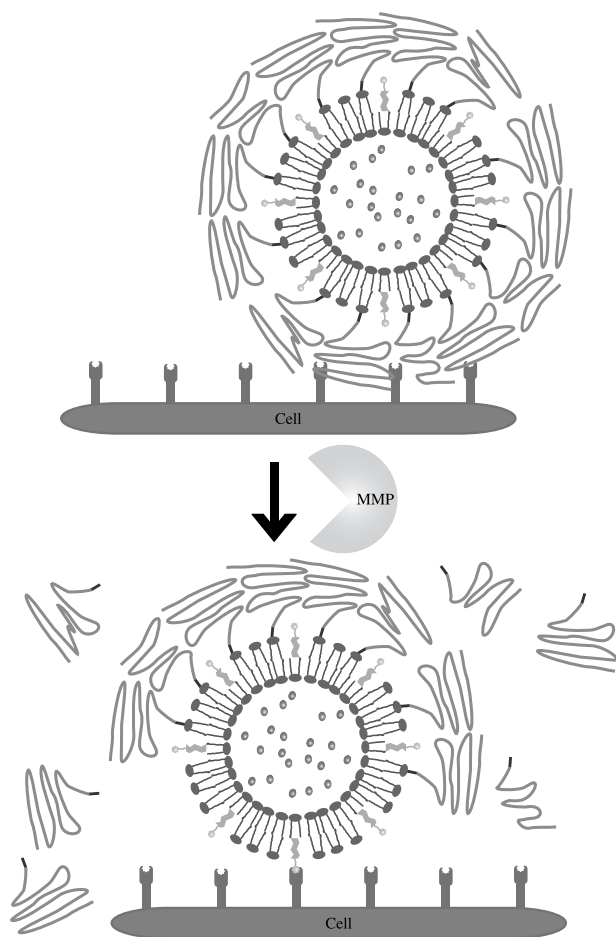


Figure 5. Schematic depiction of MMP-deprotection of liposomes. Poly(ethylene glycol) (light green) is released from MMP-sensitive peptide (dark blue line)-containing lipids (red) freeing cholesterol-bound galactose (orange star) to bind to liver cancer cells.

MMPs used in ligand targeting

An indirect, but more traditional, way of using MMPs to target drug delivery is through use of specific MMP inhibitors, Figure 6. A novel cyclic peptide inhibitor (CTT) specific for gelatinases (MMP-2 and -9) was complexed to liposomes to enhance their uptake by tumor cells expressing these MMPs. Thus, MMP-2 and -9 served as receptors of CTT ligand for liposomal targeting of invasive cells. CTT enhanced cellular uptake of liposome encapsulated, water-soluble fluorescent marker, rhodamine B, by 3–4-fold by gelatinase expressing cells. Augmented cytotoxicity in leukemia and sarcoma cells was obtained by enhanced delivery of adriamycin-containing CTT-liposomes compared to the liposomes without the peptide (Medina et al. 2001). The same concept was applied to tumor imaging using liposomes coated with Technetium-99m labeled CTT. These labeled liposomes showed tumor homing ability that could be detected by gamma imaging in live mice bearing KS1767 Kaposi's sarcoma xenografts (Medina et al. 2005). As such,

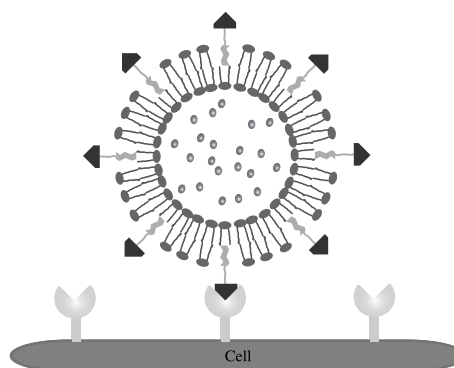


Figure 6. Schematic depiction of MMP-inhibitor as a ligand for MMP targeting. In this case, an MMP-inhibitor or MMP-binding molecule (blue triangle) act as a ligand for surface-bound MMP (yellow). The MMP may be a MT-MMP, an integrin-bound MMP, or any MMP bound to the ECM or cell.

MMP proteolytic activity can be used to target different diseases by releasing an active agent, or simple ligand-binding can be used to bind an agent to or near the surface of a cell using MMPs.

Key considerations for MMP activated drug targeting

As is apparent from the multitude of diseases in which MMPs are implicated, MMPs are viewed by many as therapeutic targets. MMPs have become a major area of DTS research in the last several years, but much is still to be discovered about the potential of MMPs as targets for drug delivery. Several issues are recurring in the literature and need to be addressed by MMP-based DTS researchers.

1. *Validation of specific MMPs as targets:* It is important to realize that MMP-targeted delivery systems are bound to face many of the same difficulties as MMPIs in the clinic. Hence, the clinical implication for the system must be clear so that specific MMPs overexpressed and overactive in the particular disease can be targeted by choosing specific and selective substrates as linkers. Similar MMPs may be targets in one disease and counter- or anti-targets in another, so choosing the appropriate MMPs for targeting is quite important. Also, choice of single MMP or multi-MMP targeting should be validated. MMP-2 and -9 have very similar substrate specificity and are difficult to differentially target. These two MMPs have been identified as targets and anti-targets in cancer (Overall and Kleinfeld 2006). Identifying MMP-2 (validated target) but not MMP-9 (suggested anti-target) specific substrates or DTS would be beneficial. Similarly, substrates that specifically target MMP-1, -2, and -7 but not others would be beneficial since they have all been identified as targets (Overall and Kleinfeld 2006).

2. *Specificity of the peptide linker:* Peptides used as MMP sensitive or MMP-inhibitory linkers should be specific substrates of the MMP intended to be exploited for targeting. It is desirable that the linker is rapidly cleaved once it encounters MMP in the diseased tissue. Enzyme kinetics studies should be conducted to ensure high catalytic efficiency of MMP towards the selected linkers. These peptide linkers can be rationally designed based on the literature available for MMP substrate specificities (Nagase and Fields 1996; Massova et al. 1997; Turk et al. 2001). Since, much is already known about the specificity of many MMPs, growth in this area may be in the non-natural peptide sequences or peptidomimetic chemistries.
3. *Selectivity of the peptide linker:* Peptide selectivity for the specific MMP for which it is designed is very important for the success of this targeting approach. Cleavage of the linker by other enzymes, including serum proteases or other MMPs expressed in non-disease tissues, would lead to non-specific release of drug causing systemic side effects (Chau et al. 2006a,b). Despite the depth of knowledge concerning substrate specificity, significantly less is known about MMP selectivity. Several reports have shown that most peptide substrates are cleaved by multiple MMPs with only 2–10-fold selectivity. In certain circumstances this may not be important, but when a target and counter-target both cleave a particular sequence, the MMP-based DTS will fail.
4. *Chemistry of the attachment of the peptide with the drug:* It is necessary that the bond introduced between the drug molecule and the peptide be stable so that non-specific release of the drug is avoided. The exact chemistry used to create the MMP-sensitive DTS will be based upon the carrier (if present) and the active molecule. In the case of peptide and protein molecules, using fusion technologies to create pro-drugs is a wise choice. When the active agent is a small molecule, covalent attachment is typically preferred as non-covalent bonds can cause increased non-specific release (Tauro and Gemeinhart 2005). Caution should also be exercised in using ester linkages which may be hydrolyzed faster than the MMP cleavage. The final delivery platform may dictate the design of the linkage as well. For example, links between the drug and pendant peptide in a hydrogel device intended to be implanted for long-term delivery should be very stable, whereas, such a link in a peptide–drug conjugate administered more frequently can be allowed to be degraded after the peptide is cleaved by the MMP. Finally, the chemistry of the attachment of drug to the peptide should be designed carefully such that the prodrug remains inactive until the peptide is cleaved while the peptidyl–drug moiety generated after cleavage regains its activity. To achieve this, it may be necessary to avoid involvement of functional groups of the drug molecule that are required for activity and exhibit less bulk tolerance during conjugation.
5. *Choice of the carrier:* In case a carrier macromolecule, e.g. polymer, protein or lipid, is used to prolong circulation of the peptide–drug conjugate, the carrier molecule should not hinder access of the enzyme to the linker. Such hindrance may be caused by the bulk of the carrier and/or electrostatic repulsion of negatively charged MMP catalytic site by the negative charge on the carrier (Chau et al. 2004). These difficulties can be overcome by selecting carriers that are not or less negatively charged and using a flexible linker between the carrier and the peptide. Non-specific or deleterious interactions are also possible between the carrier and the drug. It is possible that aggregation or misfolding could occur with either natural or synthetic macromolecular carriers. As such, basic characterization of the system is needed to confirm that higher-order structures are not formed, and if they are formed that the higher-order structures maintain functionality.
6. *Activity of peptidyl–drug moiety:* The currently described MMP-activated systems, and most foreseeable systems, typically retain approximately 1–4 amino acid attached to the drug after MMP cleavage of the prodrug. It is necessary to test the activity of this peptidyl–drug compared to the free drug. This is due to the endopeptidase nature of the MMP family (Nagase and Fields 1996; Turk et al. 2001). It is likely that the potency of the drug will be reduced due to attachment of amino acids, however, the decrease in potency should not compromise the advantage of increase in dosage allowed by reduced side effects. The peptidyl–drug moiety may be designed to be further cleaved to amino acid derivative of the drug and/or the active drug molecule by other enzymes present at the disease site. Chemistries that allow the original parent drug to be released would be advantageous in most circumstances (Kline et al. 2004).
7. *Cleavage profile in native disease tissue:* Biology of any disease tissue, especially tumor tissue, is very complex. Regulation of MMP activity at the disease site is further complicated by interplay of various induction, activation and inhibition mechanisms. Hence, it is very important to extend the proof of concept of MMP cleavable drug targeting to *in vivo* models. For example, cells in culture may not express TIMPs at the levels observed in tumor tissue and hence results obtained in the cell culture studies may not be reflected in animal models. Furthermore, it is also

important to take into consideration inter-species differences in MMP expression while making any extrapolation to human clinical application.

8. *Combination with other targeting strategies*: Delivery approaches can be designed that synergistically combine the MMP targeting mechanism with other active targeting strategies. For example, a cytotoxic protein that is targeted to a tumor cell surface protein and depends on MMPs for activation can be expected to have higher therapeutic index (Liu et al. 2000).

Conclusions

The abundance of MMPs in different diseases makes them attractive targets to be exploited for drug delivery. Development of targeted drug delivery using MMPs has been steadily pursued since the birth of the concept in late 1990s, however, not at a pace expected based on the emergence of new knowledge about MMPs and their association with various diseases. As research in molecular biology and pathophysiology of MMPs continues, more information is being made available to decide which specific MMPs should be targeted in particular diseases. Research experience has helped us to understand the important considerations for the design of drug targeting strategies utilizing MMPs. Based on this groundwork, drug delivery efforts using this ubiquitous target need to be intensified to realize the clinical application of the fundamental MMP research.

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References

- Adair JC, Charlie J, Dencoff JE, Kaye JA, Quinn JF, Camicioli RM, Stetler-Stevenson WG, Rosenberg GA. 2004. Measurement of gelatinase b (MMP-9) in the cerebrospinal fluid of patients with vascular dementia and Alzheimer disease. *Stroke* 35: E159–E162.
- Ahmed SH, Clark LL, Pennington WR, Webb CS, Bonnema DD, Leonardi AH, McClure CD, Spinale FG, Zile MR. 2006. Matrix metalloproteinases/tissue inhibitors of metalloproteinases—relationship between changes in proteolytic determinants of matrix composition and structural, functional, and clinical manifestations of hypertensive heart disease. *Circulation* 113:2089–2096.
- Albright CF, Graciani N, Han W, Yue E, Stein R, Lai ZH, Diamond M, Dowling R, Grimminger L, Zhang SY, Behrens D, Musselman A, Bruckner R, Zhang MZ, Jiang X, Hu D, Higley A, DiMeo S, Rafalski M, Mandlekar S, Car B, Yeleswaram S, Stern A, Copeland RA, Combs A, Seitz SP, Trainor GL, Taub R, Huang P, Oliff A. 2005. Matrix metalloproteinase-activated doxorubicin prodrugs inhibit ht1080 xenograft growth doxorubicin with less toxicity. *Mol Cancer Ther* 4:751–760.
- Allen C, McDonald C, Giannini C, Peng KW, Rosales G, Russell SJ, Galanis E. 2004. Adenoviral vectors expressing fusogenic membrane glycoproteins activated via matrix metalloproteinase cleavable linkers have significant antitumor potential in the gene therapy of gliomas. *J Gene Med* 6:1216–1227.
- Bae M, Cho S, Song J, Lee GY, Kim K, Yang J, Cho K, Kim SY, Byun Y. 2003. Metalloprotease-specific poly(ethylene glycol) methyl ether-peptide-doxorubicin conjugate for targeting anticancer drug delivery based on angiogenesis. *Drugs Exp Clin Res* 29:15–23.
- Benjamin AB, Pachence JJ, Rosa JG, Simon PM. 2004. Polymeric conjugates for tissue activated drug delivery. US patent Appl, 20040228831.
- Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, Hanahan D. 2000. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat Cell Biol* 2:737–744.
- Bertini I, Calderone V, Cosenza M, Fragai M, Lee Y, Claudio L, Mangani S, Terni B, Turano P. 2005. Conformational variability of matrix metalloproteinases: Beyond a single 3D structure. *Proc Natl Acad Sci USA* 102:5334–5339.
- Bode W, Maskos K. 2003. Structural basis of the matrix metalloproteinases and their physiological inhibitors, the tissue inhibitors of metalloproteinases. *Biol Chem* 384:863–872.
- Borkakoti N. 1998. Matrix metalloproteinases: Variations on a theme. *Prog Biophys Mol Biol* 70:73–94.
- Bramhall SR, Rosemurgy A, Brown PD, Bowry C, Buckels JAC. 2001. Marimastat as first-line therapy for patients with unresectable pancreatic cancer: A randomized trial. *J Clin Oncol* 19:3447–3455.
- Breuer E, Frant J, Reich R. 2005. Recent non-hydroxamate matrix metalloproteinase inhibitors. *Expert Opin Ther Pat* 15:253–269.
- Brew K, Dinakarandian D, Nagase H. 2000. Tissue inhibitors of metalloproteinases: Evolution, structure and function. *Biochim Biophys Acta, Protein Struct Mol Enzym* 1477:267–283.
- Brinckerhoff CE, Matrisian LM. 2002. Matrix metalloproteinases: A tail of a frog that became a prince. *Nat Rev Mol Cell Biol* 3:207–214.
- Brooks PC, Stromblad S, Sanders LC, vonSchalscha TL, Aimes RT, Stetler-Stevenson WG, Quigley JP, Cheresch DA. 1996. Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. *Cell* 85:683–693.
- Bruno G, Todor R, Lewis I, Chyatte D. 1998. Vascular extracellular matrix remodeling in cerebral aneurysms. *J Neurosurg* 89:431–440.
- Burrage PS, Mix KS, Brinckerhoff CE. 2006. Matrix metalloproteinases: Role in arthritis. *Front Biosci* 11:529–543.
- Cao J, Drews M, Lee HM, Conner C, Bahou WF, Zucker S. 1998. The propeptide domain of membrane type 1 matrix metalloproteinase is required for binding of tissue inhibitor of metalloproteinases and for activation of pro-gelatinase a. *J Biol Chem* 273:34745–34752.
- Carmeli E, Moas M, Reznick AZ, Coleman R. 2004. Matrix metalloproteinases and skeletal muscle: A brief review. *Muscle Nerve* 29:191–197.
- Celiker MY, Ramamurthy N, Xu JW, Wang MS, Jiang YF, Greenwald R, Shi YE. 2002. Inhibition of adjuvant-induced arthritis by systemic tissue inhibitor of metalloproteinases 4 gene delivery. *Arthritis Rheum* 46:3361–3368.

- Chau Y, Langer RS. 2003. Important factors in designing targeted delivery of cancer therapeutics via MMP-2 mediation. *J Control Release* 91:239–240.
- Chaussain-Miller C, Fioretti F, Goldberg M, Menashi S. 2006. The role of matrix metalloproteinases (MMPs) in human caries. *J Dent Res* 85:22–32.
- Chau Y, Tan FE, Langer R. 2004. Synthesis and characterization of dextran-peptide-methotrexate conjugates for tumor targeting via mediation by matrix metalloproteinase ii and matrix metalloproteinase ix. *Bioconjugate Chem* 15:931–941.
- Chau Y, Dang NM, Tan FE, Langer R. 2006a. Investigation of targeting mechanism of new dextran-peptide-methotrexate conjugates using biodistribution study in matrix-metalloproteinase-overexpressing tumor xenograft model. *J Pharm Sci* 95:542–551.
- Chau Y, Padera RF, Dang NM, Langer R. 2006b. Antitumor efficacy of a novel polymer-peptide-drug conjugate in human tumor xenograft models. *Int J Cancer* 118:1519–1526.
- Clark AW, Krekoski CA, Bou SS, Chapman KR, Edwards DR. 1997. Increased gelatinase a (MMP-2) and gelatinase b (MMP-9) activities in human brain after focal ischemia. *Neurosci Lett* 238:53–56.
- Copeland RA, Albright CF, Combs AP, Dowling RL, Graciani NR, Han W, Higley CA, Huang PS, Yue EW, DiMeo SV. 2005. Peptidase-cleavable, targeted antineoplastic drugs and their therapeutic use. US patent 6844318.
- Coussens LM, Fingleton B, Matrisian LM. 2002. Matrix metalloproteinase inhibitors and cancer: Trials and tribulations. *Science* 295:2387–2392.
- Crawford HC, Matrisian LM. 1996. Mechanisms controlling the transcription of matrix metalloproteinase genes in normal and neoplastic cells. *Enzyme Protein* 49:20–37.
- Cuniasse P, Devel L, Makaritis A, Beau F, Georgiadis D, Matziari A, Yiotakis A, Dive V. 2005. Future challenges facing the development of specific active-site-directed synthetic inhibitors of MMPs. *Biochimie* 87:393–402.
- DeClerck YA, Laug WE. 1996. Cooperation between matrix metalloproteinases and the plasminogen activator-plasmin system in tumor progression. *Enzyme Protein* 49:72–84.
- Deng SJ, Bickett DM, Mitchell JL, Lambert MH, Blackburn RK, Carter HL, Neugebauer J, Pahel G, Weiner MP, Moss ML. 2000. Substrate specificity of human collagenase 3 assessed using a phage-displayed peptide library. *J Biol Chem* 275:31422–31427.
- Dumin JA, Dickeson SK, Stricker TP, Bhattacharyya-Pakrasi M, Roby JD, Santoro SA, Parks WC. 2001. Pro-collagenase-1 (matrix metalloproteinase-1) binds the alpha(2)beta(1) integrin upon release from keratinocytes migrating on type i collagen. *J Biol Chem* 276:29368–29374.
- Duncan R. 2003. The dawning era of polymer therapeutics. *Nat Rev Drug Discov* 2:347–360.
- Dunsmore SE, Saarialho-Kere UK, Roby JD, Wilson CL, Matrisian LM, Welgus HG, Parks WC. 1998. Matrilysin expression and function in airway epithelium. *J Clin Invest* 102:1321–1331.
- Egeblad M, Werb Z. 2002. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2:161–174.
- Ehrlich P, Bolduan C. 1906. Collected studies on immunity. New York, NY: John Wiley & sons. etc. etc. 2 l., iii–xi, p 586.
- Evans JD, Stark A, Johnson CD, Daniel F, Carmichael J, Buckels J, Imrie CW, Brown P, Neoptolemos JP. 2001. A phase ii trial of marimastat in advanced pancreatic cancer. *Br J Cancer* 85:1865–1870.
- Fang JM, Shing Y, Wiederschain D, Yan L, Butterfield C, Jackson G, Harper J, Tamvakopoulos G, Moses MA. 2000. Matrix metalloproteinase-2 is required for the switch to the angiogenic phenotype in a tumor model. *Proc Natl Acad Sci USA* 97:3884–3889.
- Fatar M, Stroick M, Griebel M, Hennerici M. 2005. Matrix metalloproteinases in cerebrovascular diseases. *Cerebrovasc Dis* 20:141–151.
- Fingleton B. 2003. Matrix metalloproteinase inhibitors for cancer therapy: The current situation and future prospects. *Expert Opin Ther Targets* 7:385–397.
- Fini ME, Girard MT, Matsubara M. 1992. Collagenolytic/gelatinolytic enzymes in corneal wound healing. *Acta Ophthal Suppl* 202:26–33.
- Firestone RA, Telan LA. 2005. Enzyme-activated anti-tumor prodrug compounds. US patent 6855689.
- Foda HD, Zucker S. 2001. Matrix metalloproteinases in cancer invasion, metastasis and angiogenesis. *Drug Discov Today* 6:478–482.
- Fridman R, Toth M, Pena D, Mobashery S. 1995. Activation of progelatinase-b (MMP-9) by gelatinase-a (MMP-2). *Cancer Res* 55:2548–2555.
- Galis ZS, Sukhova GK, Lark MW, Libby P. 1994. Increased expression of matrix metalloproteinases and matrix-degrading activity in vulnerable regions of human atherosclerotic plaques. *J Clin Invest* 94:2493–2503.
- Galvez BG, Matias-Roman S, Albar JP, Sanchez-Madrid F, Arroyo AG. 2001. Membrane type 1-matrix metalloproteinase is activated during migration of human endothelial cells and modulates endothelial motility and matrix remodeling. *J Biol Chem* 276:37491–37500.
- Gerspach J, Muller D, Munkel S, Selchow O, Nemeth J, Noack M, Petrus H, Menrad A, Wajant H, Pfizenmaier K. 2006. Restoration of membrane tnfr-like activity by cell surface targeting and matrix metalloproteinase-mediated processing of a tnfr prodrug. *Cell Death Differ* 13:273–284.
- Glazier A. 1997. Tumor protease activated prodrugs of phosphoramidate analogs with toxicification and detoxification functionalities. US patent 5659061.
- Goldberg GI, Marmer BL, Grant GA, Eisen AZ, Wilhelm S, He CS. 1989. Human 72-kilodalton type-iv collagenase forms a complex with a tissue inhibitor of metalloproteinases designated timp-2. *Proc Natl Acad Sci USA* 86:8207–8211.
- Golubkov VS, Boyd S, Savinov AY, Chekanov AV, Osterman AL, Remacle A, Rozanov DV, Doxsey SJ, Strongin AY. 2005a. Membrane type-1 matrix metalloproteinase (MT1-MMP) exhibits an important intracellular cleavage function and causes chromosome instability. *J Biol Chem* 280:25079–25086.
- Golubkov VS, Chekanov AV, Doxsey SJ, Strongin AY. 2005b. Centrosomal pericentriol is a direct cleavage target of membrane type-1 matrix metalloproteinase in humans but not in mice—potential implications for tumorigenesis. *J Biol Chem* 280:42237–42241.
- Gross J, Lapiere CM. 1962. Collagenolytic activity in amphibian tissues: A tissue culture assay. *Proc Natl Acad Sci USA* 48:1014–1022.
- Gunther U, Schuppan D, Bauer M, Matthes H, Stallmach A, Schmitt-Graff A, Riecken EO, Herbst H. 1999. Fibrogenesis and fibrolysis in collagenous colitis—patterns of procollagen types i and iv, matrix-metalloproteinase-1 and-13, and timp-i gene expression. *Am J Pathol* 155:493–503.
- Guo L, Eisenman JR, Mahimkar RM, Peschon JJ, Paxton RJ, Black RA, Johnson RS. 2002. A proteomic approach for the identification of cell-surface proteins shed by metalloproteinases. *Mol Cell Proteomics* 1:30–36.
- Hahn-Dantona E, Ruiz JF, Bornstein P, Strickland DK. 2001. The low density lipoprotein receptor-related protein modulates levels of matrix metalloproteinase 9 (MMP-9) by mediating its cellular catabolism. *J Biol Chem* 276:15498–15503.
- Harris ED Jr, Krane SM. 1972. An endopeptidase from rheumatoid synovial tissue culture. *Biochim Biophys Acta* 258:566–576.
- Harris CL, Hughes CE, Williams AS, Goodfellow I, Evans DJ, Catterson B, Morgan BP. 2003. Generation of anti-complement “prodrugs”—cleavable reagents for specific delivery of

- complement regulators to disease sites. *J Biol Chem* 278:36068–36076.
- Hashimoto T, Wen G, Lawton MT, Boudreau NJ, Bollen AW, Yang GY, Barbaro NM, Higashida RT, Dowd CF, Halbach VV, Young WL. 2003. Abnormal expression of matrix metalloproteinases and tissue inhibitors of metalloproteinases in brain arteriovenous malformations. *Stroke* 34:925–930.
- He CS, Wilhelm SM, Pentland AP, Marmer BL, Grant GA, Eisen AZ, Goldberg GL. 1989. Tissue cooperation in a proteolytic cascade activating human interstitial collagenase. *Proc Natl Acad Sci USA* 86:2632–2636.
- Hofmann UB, Eggert AAO, Blass K, Brocker EB, Becker JC. 2005. Stromal cells as the major source for matrix metalloproteinase-2 in cutaneous melanoma. *Arch Dermatol Res* 297:154–160.
- Holle L, Song W, Holle E, Wei YZ, Wagner T, Yu XZ. 2003. A matrix metalloproteinase 2 cleavable melittin/avidin conjugate specifically targets tumor cells *in vitro* and *in vivo*. *Int J Oncol* 22:93–98.
- Holmbeck K, Bianco P, Caterina J, Yamada S, Kromer M, Kuznetsov SA, Mankani M, Robey PG, Poole AR, Pidoux I, Ward JM, Birkedal-Hansen H. 1999. MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. *Cell* 99:81–92.
- Hrabec E, Strek M, Nowak D, Hrabec Z. 2001. Elevated level of circulating matrix metalloproteinase-9 in patients with lung cancer. *Respir Med* 95:1–4.
- Hulboy DL, Rudolph LA, Matrisian LM. 1997. Matrix metalloproteinases as mediators of reproductive function. *Mol Hum Reprod* 3:27–45.
- Ingman T, Tervahartiala T, Ding YL, Tschesche H, Haerian A, Kinane DF, Kontinen YT, Sorsa T. 1996. Matrix metalloproteinases and their inhibitors in gingival crevicular fluid and saliva of periodontitis patients. *J Clin Periodontol* 23:1127–1132.
- Janicki JS, Brower GL, Gardner JD, Chancey AL, Stewart JA. 2004. The dynamic interaction between matrix metalloproteinase activity and adverse myocardial remodeling. *Heart Fail Rev* 9:33–42.
- Jiang Y, Muschel RJ. 2002. Regulation of matrix metalloproteinase-9 (MMP-9) by translational efficiency in murine prostate carcinoma cells. *Cancer Res* 62:1910–1914.
- Johnson S, Knox A. 1999. Autocrine production of matrix metalloproteinase-2 is required for human airway smooth muscle proliferation. *Am J Physiol, Lung Cell Mol Physiol* 277:L1109–L1117.
- Johnson KJ, Peng KW, Allen C, Russell SJ, Galanis E. 2003. Targeting the cytotoxicity of fusogenic membrane glycoproteins in gliomas through protease–substrate interaction. *Gene Ther* 10:725–732.
- Khandaker MH, Mitchell G, Xu LL, Andrews JD, Singh R, Leung H, Madrenas J, Ferguson SSG, Feldman RD, Kelvin DJ. 1999. Metalloproteinases are involved in lipopolysaccharide- and tumor necrosis factor- α -mediated regulation of cxcr1 and cxcr2 chemokine receptor expression. *Blood* 93:2173–2185.
- Kinoh H, Inoue M, Washizawa K, Yamamoto T, Fujikawa S, Tokusumi Y, Iida A, Nagai Y, Hasegawa M. 2004. Generation of a recombinant sendai virus that is selectively activated and lyses human tumor cells expressing matrix metalloproteinases. *Gene Ther* 11:1137–1145.
- Kirkegaard T, Hansen A, Bruun E, Brynskov J. 2004. Expression and localisation of matrix metalloproteinases and their natural inhibitors in fistulae of patients with crohn's disease. *Gut* 53:701–709.
- Kline T, Torgov MY, Mendelsohn BA, Cerveny CG, Senter PD. 2004. Novel antitumor prodrugs designed for activation by matrix metalloproteinases-2 and -9. *Mol Pharm* 1.
- Knauper V, Smith B, LopezOtin C, Murphy G. 1997. Activation of progelatinase b (proMMP-9) by active collagenase-3 (MMP-13). *Eur J Biochem* 248:369–373.
- Knauper V, Bailey L, Worley JR, Soloway P, Patterson ML, Murphy G. 2002. Cellular activation of proMMP-13 by MT1-MMP depends on the c-terminal domain of MMP-13. *FEBS Lett* 532:127–130.
- Kratz F, Dreves J, Bing G, Stockmar C, Scheuermann K, Lazar P, Unger C. 2001. Development and *in vitro* efficacy of novel MMP2 and MMP9 specific doxorubicin albumin conjugates. *Bioorg Med Chem Lett* 11:2001–2006.
- Kvanta A, Shen WY, Sarman S, Seregard S, Steen B, Rakoczy E. 2000. Matrix metalloproteinase (MMP) expression in experimental choroidal neovascularization. *Curr Eye Res* 21:684–690.
- Lee MH, Murphy G. 2004. Matrix metalloproteinases at a glance. *J Cell Sci* 117:4015–4016.
- Liotta LA, Tryggvason K, Garbisa S, Hart I, Foltz CM, Shafie S. 1980. Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature* 284:67–68.
- Liu SH, Netzel-Arnett S, Birkedal-Hansen H, Leppa SH. 2000. Tumor cell-selective cytotoxicity of matrix metalloproteinase-activated anthrax toxin. *Cancer Res* 60:6061–6067.
- Lubbe AS, Alexiou C, Bergemann C. 2001. Clinical applications of magnetic drug targeting. *J Surg Res* 95:200–206.
- Lynch CC, Matrisian LM. 2002. Matrix metalloproteinases in tumor–host cell communication. *Differentiation* 70:561–573.
- Maeda H, Fang J, Inutsuka T, Kitamoto Y. 2003. Vascular permeability enhancement in solid tumor: Various factors, mechanisms involved and its implications. *Int Immunopharmacol* 3:319–328.
- Mansour AM, Dreves J, Esser N, Hamada FM, Badary OA, Unger C, Fichtner I, Kratz F. 2003. A new approach for the treatment of malignant melanoma: Enhanced antitumor efficacy of an albumin-binding doxorubicin prodrug that is cleaved by matrix metalloproteinase 2. *Cancer Res* 63:4062–4066.
- Martel-Pelletier J, Welsch DJ, Pelletier JP. 2001. Metalloproteinases and inhibitors in arthritic diseases. *Best Pract Res Clin Rheumatol* 15:805–829.
- Massova I, Fridman R, Mobashery S. 1997. Structural insights into the catalytic domains of human matrix metalloproteinase-2 and human matrix metalloproteinase-9: Implications for substrate specificities. *J Mol Model* 3:17–30.
- McCawley LJ, Matrisian LM. 2001. Matrix metalloproteinases: They're not just for matrix anymore! *Curr Opin Cell Biol* 13:534–540.
- McQuibban GA, Gong JH, Tam EM, McCulloch CAG, Clark-Lewis I, Overall CM. 2000. Inflammation dampened by gelatinase a cleavage of monocyte chemoattractant protein-3. *Science* 289:1202–1206.
- McQuibban GA, Gong JH, Wong JR, Wallace JL, Clark-Lewis I, Overall CM. 2002. Matrix metalloproteinase processing of monocyte chemoattractant proteins generates cc chemokine receptor antagonists with anti-inflammatory properties *in vivo*. *Blood* 100:1160–1167.
- Medina OP, Soderlund T, Laakkonen LJ, Tuominen EKJ, Koivunen E, Kinnunen PKJ. 2001. Binding of novel peptide inhibitors of type iv collagenases to phospholipid membranes and use in liposome targeting to tumor cells *in vitro*. *Cancer Res* 61:3978–3985.
- Medina OP, Kairemo K, Valtanen H, Kangasniemi A, Kaukinen S, Ahonen I, Permi P, Annala A, Sneek M, Holopainen JM, Karonen SL, Kinnunen PKJ, Koivunen E. 2005. Radionuclide imaging of tumor xenografts in mice using a gelatinase-targeting peptide. *Anticancer Res* 25:33–42.
- Mengshol JA, Mix KS, Brinckerhoff CE. 2002. Matrix metalloproteinases as therapeutic targets in arthritic diseases—bull's-eye or missing the mark? *Arthritis Rheum* 46:13–20.
- Michael M, Babic B, Khokha R, Tsao M, Ho J, Pintilie M, Leco K, Chamberlain D, Shepherd FA. 1999. Expression and prognostic significance of metalloproteinases and their tissue inhibitors in

- patients with small-cell lung cancer. *J Clin Oncol* 17:1802–1808.
- Mimura T, Bateman AC, Lee RL, Johnson PA, McDonald PJ, Talbot IC, Kamm MA, MacDonald TT, Pender SLF. 2004. Up-regulation of collagen and tissue inhibitors of matrix metalloproteinase in colonic diverticular disease. *Dis Colon Rectum* 47:371–378.
- Mincher DJ, Turnbull A, Bibby MC, Loadman PM. 2002. Tumor targeting prodrugs activated by metallo matrixproteinases patent PCT WO 02/072620 A1.
- Mohan R, Sivak J, Ashton P, Russo LA, Pham BQ, Kasahara N, Raizman MB, Fini ME. 2000. Curcuminoids inhibit the angiogenic response stimulated by fibroblast growth factor-2, including expression of matrix metalloproteinase gelatinase b. *J Biol Chem* 275:10405–10412.
- Morales R, Perrier S, Florent JM, Beltra J, Dufour S, De Mendez I, Manceau P, Tertre A, Moreau F, Compere D, Dublanchet AC, O’Gara M. 2004. Crystal structures of novel non-peptidic, non-zinc chelating inhibitors bound to MMP-12. *J. Mol Biol* 341:1063–1076.
- Mott JD, Werb Z. 2004. Regulation of matrix biology by matrix metalloproteinases. *Curr Opin Cell Biol* 16:558–564.
- Mun-Bryce S, Rosenberg GA. 1998. Gelatinase b modulates selective opening of the blood–brain barrier during inflammation. *Am J Physiol Regul Integr Comp Physiol* 43:R1203–R1211.
- Nagase H. 1997. Activation mechanisms of matrix metalloproteinases. *Biol Chem* 378:151–160.
- Nagase H, Fields GB. 1996. Human matrix metalloproteinase specificity studies using collagen sequence-based synthetic peptides. *Biopolymers* 40:399–416.
- Nagase H, Woessner JF. 1999. Matrix metalloproteinases. *J Biol Chem* 274:21491–21494.
- Netzelarnett S, Fields G, Birkedalhansen H, Vanwart HE. 1991. Sequence specificities of human fibroblast and neutrophil collagenases. *J Biol Chem* 266:6747–6755.
- Newby AC, Pauschinger M, Spinale FG. 2006. From tadpole tails to transgenic mice: Metalloproteinases have brought about a metamorphosis in our understanding of cardiovascular disease. *Cardiovas Res* 69:559–561.
- Ohbayashi H. 2002. Matrix metalloproteinases in lung diseases. *Curr Protein Pept Sci* 3:409–421.
- Ohno I, Ohtani H, Nitta Y, Suzuki J, Hoshi H, Honma M, Isoyama S, Tanno Y, Tamura G, Yamauchi K, Nagura H, Shirato K. 1997. Eosinophils as a source of matrix metalloproteinase-9 in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* 16:212–219.
- Oh J, Takahashi R, Adachi E, Kondo S, Kuratomi S, Noma A, Alexander DB, Motoda H, Okada A, Seiki M, Itoh T, Itoharu S, Takahashi C, Noda M. 2004. Mutations in two matrix metalloproteinase genes, MMP-2 and MT1-MMP, are synthetic lethal in mice. *Oncogene* 23:5041–5048.
- Overall CM. 2002. Molecular determinants of metalloproteinase substrate specificity—matrix metalloproteinase substrate binding domains, modules, and exosites. *Mol Biotechnol* 22:51–86.
- Overall CM, Kleinfeld O. 2006. Tumour microenvironment—opinion—validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. *Nat Rev Cancer* 6:227–239.
- Overall CM, Lopez-Otin C. 2002. Strategies for MMP inhibition in cancer: Innovations for the post-trial era. *Nat Rev Cancer* 2:657–672.
- Overall CM, Tam EM, Kappelhoff R, Connor A, Ewart T, Morrison CJ, Puente X, Lopez-Otin C, Seth A. 2004. Protease degradomics: Mass spectrometry discovery of protease substrates and the clip-chip, a dedicated DNA microarray of all human proteases and inhibitors. *Biol Chem* 385:493–504.
- Parks WC. 1999. Matrix metalloproteinases in repair. *Wound Repair Regen* 7:423–432.
- Parks WC, Wilson CL, Lopez-Boado YS. 2004. Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nat Rev Immunol* 4:617–629.
- Pei DQ, Weiss SJ. 1995. Furin-dependent intracellular activation of the human stromelysin-3 zymogen. *Nature* 375:244–247.
- Pender SL, MacDonald TT. 2004. Matrix metalloproteinases and the gut—new roles for old enzymes. *Curr Opin Pharmacol* 4:546–550.
- Pender SLF, Braegger C, Gunther U, Monteleone G, Meuli M, Schuppan D, MacDonald TT. 2003. Matrix metalloproteinases in necrotising enterocolitis. *Pediatr Res* 54:160–164.
- Peng KW, Morling FJ, Cosset FL, Murphy G, Russell SJ. 1997. A gene delivery system activatable by disease-associated matrix metalloproteinases. *Hum Gene Ther* 8:729–738.
- Peng KW, Vile RG, Cosset FL, Russell SJ. 1999. Selective transduction of protease-rich tumors by matrix-metalloproteinase-targeted retroviral vectors. *Gene Ther* 6:1552–1557.
- Pilcher BK, Dumin JA, Sudbeck BD, Krane SM, Welgus HG, Parks WC. 1997. The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix. *J Cell Biol* 137:1445–1457.
- Pirila E, Sharabi A, Salo T, Quaranta V, Tu HM, Heljasvaara R, Koshikawa N, Sorsa T, Maisi P. 2003. Matrix metalloproteinases process the laminin-5 gamma 2-chain and regulate epithelial cell migration. *Biochem Biophys Res Commun* 303:1012–1017.
- Puerta DT, Cohen SM. 2003. Examination of novel zinc-binding groups for use in matrix metalloproteinase inhibitors. *Inorg Chem* 42:3423–3430.
- Rajah R, Nachajon RV, Collins MH, Hakonarson H, Grunstein MM, Cohen P. 1999. Elevated levels of the igf-binding protein protease MMP-1 in asthmatic airway smooth muscle. *Am J Respir Cell Mol Biol* 20:199–208.
- Ramos-DeSimone N, Hahn-Dantona E, Siple J, Nagase H, French DL, Quigley JP. 1999. Activation of matrix metalloproteinase-9 (MMP-9) via a converging plasmin/stromelysin-1 cascade enhances tumor cell invasion. *J Biol Chem* 274:13066–13076.
- Rivat C, Le Floch N, Sabbah M, Teyrol I, Redeuilh G, Bruyneel E, Mareel M, Matrisian LM, Crawford HC, Gespach C, Attoub S. 2003. Synergistic cooperation between the ap-1 and lef-1 transcription factors in the activation of the matrilysin promoter by the src oncogene: Implications in cellular invasion. *FASEB J* 17.
- Rodriguez-Lopez J, Perez-Pampin E, Gomez-Reino JJ, Gonzalez A. 2006. Regulatory polymorphisms in extracellular matrix protease genes and susceptibility to rheumatoid arthritis: A case-control study. *Arthritis Res Ther* 8.
- Rosenberg GA, Sullivan N, Esiri MM. 2001. White matter damage is associated with matrix metalloproteinases in vascular dementia. *Stroke* 32:1162–1167.
- Rundhaug JE. 2005. Matrix metalloproteinases and angiogenesis. *J Cell Mol Med* 9:267–285.
- SaarialhoKere UK, Vaalamo M, Puolakkainen P, Airola K, Parks WC, KarjalainenLindsberg ML. 1996. Enhanced expression of matrilysin, collagenase, and stromelysin-1 in gastrointestinal ulcers. *Am J Pathol* 148:519–526.
- SaarialhoKere UK, Crouch EC, Parks WC. 1995. Matrix metalloproteinase matrilysin is constitutively expressed in adult human exocrine epithelium. *J Invest Dermatol* 105:190–196.
- Sato H, Takino T, Okada Y, Cao J, Shinagawa A, Yamamoto E, Seiki M. 1994. A matrix metalloproteinase expressed on the surface of invasive tumor-cells. *Nature* 370:61–65.
- Sato H, Okada Y, Seiki M. 1997. Membrane-type matrix metalloproteinases (MT-MMPs) in cell invasion. *Thromb Haemost* 78:497–500.
- Schenk S, Quaranta V. 2003. Tales from the cryptic sites of the extracellular matrix. *Trends Cell Biol* 13:366–375.
- Segura-Valdez L, Pardo A, Gaxiola M, Uhal BD, Becerril C, Selman M. 2000. Upregulation of gelatinases a and b, collagenases 1 and 2, and increased parenchymal cell death in copd. *Chest* 117:684–694.

- Sivak JM, Fini ME. 2002. MMPs in the eye: Emerging roles for matrix metalloproteinases in ocular physiology. *Prog Retin Eye Res* 21:1–14.
- Sorsa T, Tjaderhane L, Salo T. 2004. Matrix metalloproteinases (MMPs) in oral diseases. *Oral Dis* 10:311–318.
- Stamenkovic I. 2003. Extracellular matrix remodelling: The role of matrix metalloproteinases. *J Pathol* 200:448–464.
- Sternlicht MD, Lochter A, Simpson CJ, Huey B, Rougier JP, Gray JW, Pinkel D, Bissell MJ, Werb Z. 1999. The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. *Cell* 98:137–146.
- Stetler-Stevenson WG. 1990. Type iv collagenases in tumor invasion and metastasis. *Cancer Metastasis Rev* 9:289–303.
- Stetler-Stevenson WG. 1999. Matrix metalloproteinases in angiogenesis: A moving target for therapeutic intervention. *J Clin Invest* 103:1237–1241.
- Suarato A, Angelucci F, Caruso M, Scolar A, Volip D, Zamai M. 2002. Polymeric conjugates of antitumor agents patent PCT WO 02/07770 A2.
- Tam EM, Morrison CJ, Wu YI, Stack MS, Overall CM. 2004. Membrane protease proteomics: Isotope-coded affinity tag ms identification of undescribed mt1-matrix metalloproteinase substrates. *Proc Natl Acad Sci USA* 101:6917–6922.
- Tauro JR, Gemeinhart RA. 2004. Peptide-based targeted activation of platinates by matrix metalloproteinases. *Neuro-Oncol* 6:335–336.
- Tauro JR, Gemeinhart RA. 2005. Matrix metalloproteinase triggered delivery of cancer chemotherapeutics from hydrogel matrices. *Bioconjugate Chem* 16:1133–1139.
- Terada T, Iwai M, Kawakami S, Yamashita F, Hashida M. 2006. Novel peg-matrix metalloproteinase-2 cleavable peptide-lipid containing galactosylated liposomes for hepatocellular carcinoma-selective targeting. *J Control Release* 111:333–342.
- Thomas P, Khokha R, Shepherd FA, Feld R, Tsao MS. 2000. Differential expression of matrix metalloproteinases and their inhibitors in non-small cell lung cancer. *J Pathol* 190:150–156.
- Timar F, Botyanszki J, Suli-Vargha H, Babo I, Olah J, Pogany G, Jeney A. 1998. The antiproliferative action of a melphalan hexapeptide with collagenase-cleavable site. *Cancer Chemother Pharmacol* 41:292–298.
- Torii K, Iida KI, Miyazaki Y, Saga S, Kondoh Y, Taniguchi H, Taki F, Takagi K, Matsuyama M, Suzuki R. 1997. Higher concentrations of matrix metalloproteinases in bronchoalveolar lavage fluid of patients with adult respiratory distress syndrome. *Am J Respir Crit Care Med* 155:43–46.
- Tsuruda T, Costello-Boerrigter LC, Burnett JC. 2004. Matrix metalloproteinases: Pathways of induction by bioactive molecules. *Heart Failure Rev* 9:53–61.
- Turk BE, Huang LL, Piro ET, Cantley LC. 2001. Determination of protease cleavage site motifs using mixture-based oriented peptide libraries. *Nat Biotechnol* 19:661–667.
- Van Valckenborgh E, Mincher D, Di Salvo A, Van Riet I, Young L, Van Camp B, Vanderkerken K. 2005. Targeting an MMP-9-activated prodrug to multiple myeloma-diseased bone marrow: A proof of principle in the 5t33mm mouse model. *Leukemia* 19:1628–1633.
- Van Wart HE, Birkedal-hansen H. 1990. The cysteine switch—a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc Natl Acad Sci USA*. 87:5578–5582.
- Vu TH, Werb Z. 2000. Matrix metalloproteinases: Effectors of development and normal physiology. *Genes Dev* 14:2123–2133.
- Vu TH, Shipley JM, Bergers G, Berger JE, Helms JA, Hanahan D, Shapiro SD, Senior RM, Werb Z. 1998. MMP-9/gelatinase b is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell* 93:411–422.
- Wang GY, Bergman MR, Nguyen AP, Turcato S, Swigart PM, Rodrigo MC, Simpson PC, Karliner JS, Lovett DH, Baker AJ. 2006. Cardiac transgenic matrix metalloproteinase-2 expression directly induces impaired contractility. *Cardiovasc Res* 69:688–696.
- Weber BHF, Vogt G, Pruett RC, Stohr H, Felbor U. 1994. Mutations in the tissue inhibitor of metalloproteinases-3 (timp3) in patients with sorsbys fundus dystrophy. *Nat Genet* 8:352–356.
- Westermarck J, Kahari VM. 1999. Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J* 13:781–792.
- Whittaker M, Floyd CD, Brown P, Gearing AJH. 1999. Design and therapeutic application of matrix metalloproteinase inhibitors. *Chem Rev* 99:2735–2776.
- Woessner JF. 2002. MMPs and timp3—an historical perspective. *Mol Biotechnol* 22:33–49.
- Wroblewski LE, Noble PJM, Pagliocca A, Pritchard DM, Hart CA, Campbell F, Dodson AR, Dockray GJ, Varro A. 2003. Stimulation of MMP-7 (matrilysin) by helicobacter pylori in human gastric epithelial cells: Role in epithelial cell migration. *J Cell Sci* 116:3017–3026.
- Ye S. 2000. Polymorphism in matrix metalloproteinase gene promoters: Implication in regulation of gene expression and susceptibility of various diseases. *Matrix Biol* 19:623–629.
- Yu Q, Cok SJ, Zeng CB, Morrison AR. 2003. Translational repression of human matrix metalloproteinases-13 by an alternatively spliced form of T-cell-restricted intracellular antigen-related protein (tiar). *J Biol Chem* 278:1579–1584.
- Yu WH, Woessner JF. 2000. Heparan sulfate proteoglycans as extracellular docking molecules for matrilysin (matrix metalloproteinase 7). *J Biol Chem* 275:4183–4191.
- Zhang X, Nothnick WB. 2005. The role and regulation of the uterine matrix metalloproteinase system in menstruating and non-menstruating species. *Front Biosci* 10:353–366.
- Zheng T, Zhu Z, Wang ZD, Homer RJ, Ma B, Riese RJ, Chapman HA, Shapiro SD, Elias JA. 2000. Inducible targeting of il-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *J Clin Invest* 106:1081–1093.
- Zucker S, Vacirca J. 2004. Role of matrix metalloproteinases (MMPs) in colorectal cancer. *Cancer Metastasis Rev* 23:101–117.
- Zucker S, Cao J, Chen WT. 2000. Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment. *Oncogene* 19:6642–6650.