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Historical perspective of matrix metalloproteases

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1. ABSTRACT

Matrix metalloproteinases (MMPs) were identified as early as 1962. Since this seminal finding, this family of zinc-dependent endopeptidases has been studied extensively. This collective work has resulted in delineation of MMP gene and protein structures, the mechanisms of control of MMPs, the action of MMPs on their substrates, and of course their role in normal physiology and their crucial roles in pathophysiology. Stemming from the discovery that MMPs contribute to arthritis, heart disease, and cancer, amongst other diseases, attempts to develop treatment strategies incorporating MMP inhibition have been undertaken. The results of these endeavours have been mediocre, resulting in few FDA-approved MMP inhibitors mostly due to the broad-spectrum nature of these early inhibitors and unwanted side effects of MMP inhibition. The future of exploitation of MMPs in disease lies in the design of more targeted inhibitors; in order to accomplish this, we must all understand the subtle differences between each MMP and their contextual roles. In this chapter, we aim to overview major topics regarding MMPs and what direction we may go in the future.

2. INTRODUCTION

As early as 1949, comparison of the architecture of rapidly progressing tumors and slow-growing tumors revealed stark differences (1). These differences were postulated to be related to depolymerization of the basement membrane by soluble factors, potentially derived from stromal cells (1). Collagen degradation in mammalian tissue was initially described by Woessner in 1962 (2), but the field of study of extracellular matrix (ECM) remodeling proteins was pioneered by the study conducted by Gross and Lapiere, also published in 1962 (3). The work appeared in the *Proceedings of the National Academy of Sciences* and described the ability of a single, soluble factor derived from certain tadpole tissue explants to lyse purified collagen *ex vivo* (3). Prior to the 1962 study by Gross and Lapiere, ECM remodeling in the mammalian context was a poorly understood phenomenon and had been hypothesized to be related to lysosomal protease activity (2,4)_. The landmark discovery of a soluble collagenase led to a shift in ideology about ECM degradation and tissue remodeling. The study of these isolated proteolytic enzymes over the following decades uncovered vast amounts of information on how proteins of the extracellular space are degraded and maintained.

In the years following the publication of the work of Gross and colleagues, various collagenases and procollagenases were isolated from a variety of

animal tissues. They were isolated from cultures derived from skin wounds, regenerating newt limbs, gingivae, bone, postpartum rat uterus, and immune components (5-10)_. Not only were proteases isolated from a number of tissues and cell cultures, but they were also increasingly associated with certain pathologies. Some of the earliest diseases to be associated with ECM remodeling include periodontal disease (gum resorption) and cholesteatoma (7). Eventually, it was also found that collagenases could be derived from rheumatoid synovium and metastatic tumor explants (11-14). As it became increasingly apparent that these isolated proteases were critical to degrading ECM and participated in both physiological and pathophysiological processes, studies to purify, characterize, and define the mode of action of these proteases were undertaken. This issue of *Frontiers in Bioscience* seeks to explore the myriad of functions these proteases play in normal physiology and disease. Here in this section we provide an overview of basic structure and functions of ECM remodeling proteases, specifically the matrixins, or matrix metalloproteinases (MMPs).

3. CLASSIFICATION OF MATRIX METALLOPROTEINASES

The MEROPS database is a large repository of information regarding families of proteases (15,16). This particular tool allows for search of peptidases and their groupings, along with substrates, inhibitors, and known cleavage sites. According to the MEROPS system, MMPs are classified in the clan MA and fall into the M10 family, which is the metalloendopeptidase family. The classification of metalloendopeptidases into a group called the 'metzincins' within the endopeptidase superfamily was initially proposed in 1993 (17, 18). The metzincin family is characterized by two major features, the requirement of a coordinated Zn^{2+} in the catalytic active site and the methionine turn (Met-turn) (17). The sub-families that comprised the metzincin family at that time included the astacins, adamalysins, serralysins, and matrixins (17). Each of these families could be distinguished from one another based on the residue (Z) that is found at the end of the conserved zinc-binding sequence (HEXXHXXGXXHZ) (*i.e.:* Glu (astacins), Asp (adamalysins), Ser in (matrixins), and Pro in (serralysins)). Recently, another mechanism by which to classify metallopeptidases has been proposed (19). Cerdá-Costa *et al.* suggest that metalloproteinases should first be divided by whether they are dimetalate (containing two catalytic metals) or mononuclear metallopeptidases. By this system, MMPs fall into the subclass mononuclear metallopeptidases, the tribe zincins, the clan metzincins, and the family of matrixins (19). The tribe zincins includes any peptidase that contains the motif HEXXH, with the clan metzincins including the conserved zinc binding site HEXXHXXGXXHZ and the Met-turn (19).

4. MATRIX METALLOPROTEINASE PROTEIN STRUCTURE

MMPs are calcium-dependent, Zn^{2+} containing endopeptidases. They can either be soluble or membrane-bound and are found in most kingdoms of life. There are 23 human MMPs and they can be divided into groups: true collagenases, gelatinases, stromelysins, elastases, and membrane-type MMPs (20,21). True collagenases are defined as those proteases that can cleave native collagen, a triple helical protein, at a particular site across all three chains resulting in ¾ and ¼ fragments (22). Gelatinases are those MMPs that primarily cleave denatured collagen and gelatins, while stromelysins have a more extensive specificity, including serpins and various ECM components, but do not cleave interstitial collagen (20, 22). Matrilysins also target ECM components and a variety of cell surface molecules, but is most often found intracellularly. Membrane-type MMPs have collagenolytic abilities and may also cleave cell surface molecules, considering they are activated intracellularly and arrive at the membrane in an active form (20). Despite these groupings, several MMPs fall outside of these categories and contain other unique features.

MMPs from vertebrates are modular, containing particular elements that are similar amongst all of the family members (Figure 1). A small signaling peptide (~20 amino acids), a propeptide region (~80 amino acids), a calcium and zinc dependent catalytic domain (~165 amino acids), a linker region of varying residue length (may determine what substrates the MMP can accommodate), and a hemopexin-like domain (PEX) (~200 amino acids) (22). Certain MMPs also contain additional domains, including fibronectin-like repeats (MMP-2, -9), immunoglobulin-like domains, and vitronectin-like repeats. Further, members of the family that are membrane-bound may contain either a glycosyl phosphatidylinositol linkage signal or transmembrane and cytoplasmic tail domains (MMP-14, MMP-17, MMP-24, and MMP-25) (Figure 1).

4.1. The signal peptide

The signaling peptide region, previously referred to as the pre-domain, is variable amongst proteins but commonly consists of a positively charged N-terminal segment, followed by a hydrophobic section, and finally a polar C-terminal segment (23). This peptide interacts with the signal recognition particle (SRP) and eventually the signal recognition peptide receptor at the target, *i.e.:* endoplasmic reticulum (24, 25). This is a general phenomenon for secretory proteins and MMPs are no exception. Specific mutations within the signal peptide of MMPs have been associated with disease (26). The inability of a mutated signal peptide to interact with the SRP disrupts correct protein trafficking. For example, a mutation in the hydrophobic region of the signal peptide of MMP-14 results in decreased cell surface expression

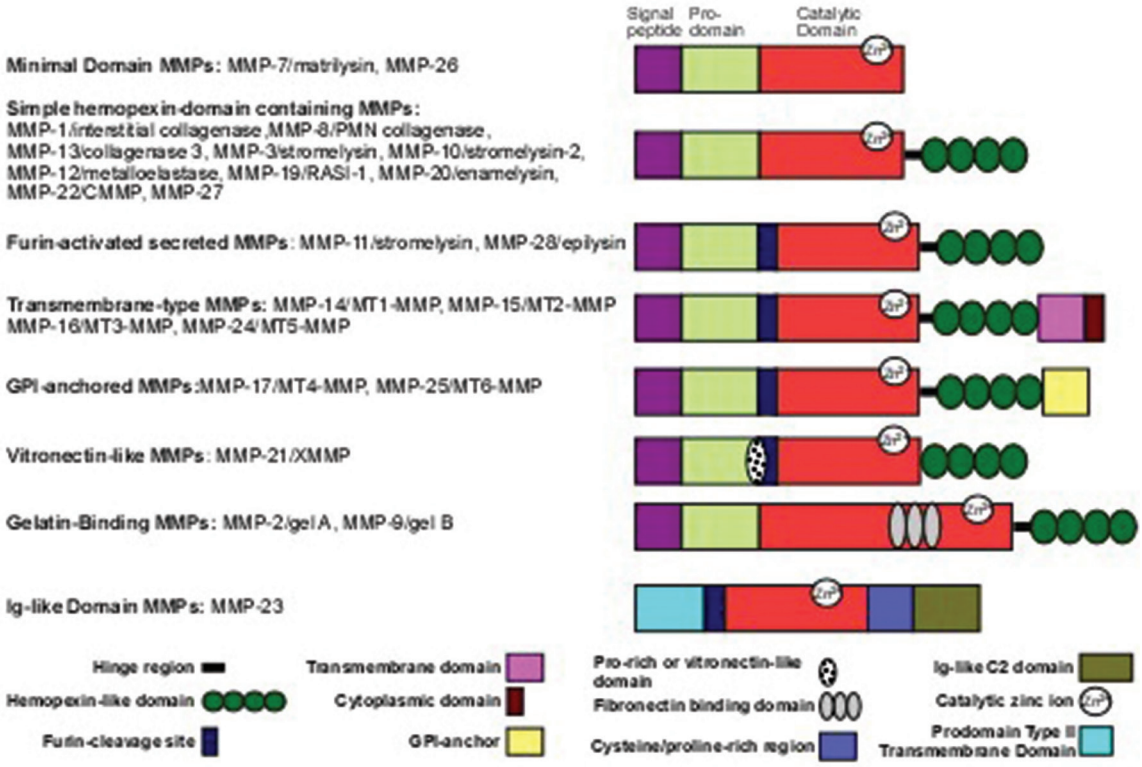

Figure 1. The MMP family. The MMP family share certain conserved domains, particularly the catalytic domain. Figure 1 demonstrates the MMPs based on structural features of the protein.

and reduced ability to activate MMP-2 and remodel extracellular matrices which presents as an osteolytic disorder, as proper skeletal formation and maintenance requires the action of MMPs (23).

4.2. The propeptide domain

The propeptide domain is crucial to regulation of MMP activity, as it serves to block access to the catalytic domain; matrixins are usually secreted in zymogen form and require activation. The propeptide domain is included in even the most structurally simple of MMPs (MMP-7) and is composed of three alpha chains with flexible connecting loops (20). The prodomain is a stable domain because the three helices tend to form a hydrophobic core via interactions with one another, despite being connected by flexible loops (27, 28). Jozic, *et al.* suggest that the bundle formation of the three helices in the domain are ideal for the sequential activation that has been documented to occur in MMPs, which includes a proteolytic event that cleaves the prodomain and an autocatalytic event that completes the removal (22, 27). The connective loops in this domain are highly susceptible to proteolysis; indeed in MMP-1, -2, and -3 the first step in zymogen activation is cleavage of the loop between helix 1 and 2 (H1 and H2) (28, 29). Additionally, some MMPs contain a furin-like consensus site that is acted upon in the trans-Golgi network by furin. The cleavage of these zymogens by furin during transport allows for delivery of active enzyme to the cell surface in the case of the MT-MMPs or the extracellular space in the case of MMP-11 and -28 (28).

The prodomain includes a highly conserved amino acid sequence that is contained within the prodomain, PRCGXPD (30, 31). Early evidence presented by Sanchez-Lopez, *et al.*¸ indicated that mutation of the region around the autocatalytic site in rat transin increased the activation of the zymogen (32). Later, it was determined that mutation of the cysteine residue results in a zymogen with a higher proclivity for activation (33); modification of the sulfhydryl group also releases the cysteine switch (34). The sulfhydryl group of the cysteine located in the highly conserved amino acid sequence of the prodomain region functions to occupy the catalytic Zn^{2+} ion and prevent the necessary water molecule from interacting with the ion (35). The so-called “cysteine-switch” mechanism of inhibition explains the ability of organomercurials and proteolytic cleavage to activate MMPs. Ion chelating agents such as EDTA can activate MMPs because of the removal of Zn^{2+} from the cysteine (36); organomercurial agents and detergents such as sodium dodecyl sulfate results in a change in protein conformation that allows for release of the cysteine switch (36, 37). Activity of proteases such

as urokinase-type plasminogen activator (uPA), furin convertases, and other active MMPs release the Zn^{2+} by physically removing the prodomain and therefore the critical cysteine residue (31,38-44).

Not only is the cysteine residue crucial to the ability of prodomain to perform its inhibition function, mutational studies have found that there are other amino acid residues in the region that play a role. The arginine and glutamate that function to form a salt bridge have also been shown to be critical to the activation state of MMPs, as mutations of these residues disallows activation by the mercurial compound 4-aminophenylmercuric acetate (APMA) (30, 45, 46). Also, another study of MMP-3 implicated the tyrosine and leucine residue in the ability of the prodomain to efficiently shield enzyme activity (47). Expression of single mutants for these residues resulted in expression of active enzyme (47).

Unique to proMMP-1, the prodomain also interacts with the hemopexin domain. The prodomain backbone of MMP-1 is similar to other MMPs but the orientation of the prodomain toward the hemopexin domain differs (27). When the proMMP-1 structure is compared to the active MMP-1 structure, it appears that the changed orientation of the prodomain minimizes the gap that exists between the hemopexin and catalytic domain in activated MMP-1 (27). This phenomenon may physically prevent substrate from entering the active site, as the hemopexin domain would be rearranged such that the active site residues are more accessible for substrate (48). MMP-26 also holds an exception to the general prodomain rules in that its prodomain consensus sequence varies by one amino acid residue, namely a histidine immediately before the critical cysteine (49, 50). Replacement of the histidine residue with the traditional arginine does not allow for APMA induced activation, suggesting MMP-26 latency is not maintained through the prodomain (50).

Interestingly, and counterintuitively, the prodomain of membrane associated MMPs, such as MT1-MMP, have also been implicated in the activity of the enzyme (51). MT1-MMP requires the prodomain for activation of MMP-2; loss of the prodomain does not interfere with the catalytic activity MT1-MMP against gelatin substrates, but does in fact reduce the ability of MMP-2 to be activated (52). Specifically, a conserved region of the prodomain, ^{42}YGYL^{45} contributes to the folding and function of the prodomain as an activator of MMP-2 (53).

4.3. The catalytic domain

The catalytic domain is highly conserved amongst the MMPs and the domain structures between the MMPs are very similar, nearly matching in their 3-dimensional structure (20, 22). The spherical catalytic domain extends over a 40 angstrom diameter and the shallow active site is situated at the front surface (22). The substrate binding groove contains a flat, unprimed side and a narrow primed side which contains the specificity pocket, S1' (54). The catalytic domain is composed of three alpha-helices and a five-stranded twisted beta-sheet (betaI-betaV) (22,28). The strands in the beta-sheet are parallel in orientation to one another, with the exception of the betaIV. The alphaA backing helix and the alphaB active site helix are in close proximity to the beta-sheet; the contacts between the helices and sheets are hydrophobic and therefore form a hydrophobic core. Several of the loops connecting each beta-strand extend away from the surface of the sheet and these are unique to MMPs as compared to other members of the metzincins (22). The loops participate in coordinating the non-catalytic ions, specifically the second Zn^{2+} ion and up to three Ca^{2+} ions, which serve to provide structure to the loops (22,28,48). This domain is split by the active site asymmetrically, resulting in a larger N-terminal region (average 127 residues, often termed "upper") and a smaller C-terminal region (average 37 residues, often termed "lower") (22).

The large LbetaValphaB loop leads from strand betaV to the active helix, alphaB. The alphaB helix contains the first half of the conserved zinc-binding sequence HEXXHXXGXXH, meaning it has the first two histidines required for coordinating the catalytic Zn^{2+} in addition to the glutamate that participates in catalysis and ends with a turn allowed by the glycine residue (22). The turn is crucial for the third histidine to make contact with the zinc ion. Following the conserved Zn^{2+} binding sequence is a loop that leads to the methionine, which is termed the "Met-turn" (17). This is a highly conserved 1, 4 beta-turn within the metzincins, however it has been shown not to be necessary for proper folding and function of the enzyme (28,55). The loop following the Met-turn is the "specificity loop" that leads to the final helix of the catalytic domain (alphaC) (22, 28). This loop is required for distinguishing substrates and imparts a level of specificity to each MMP.

While all MMPs share this general catalytic domain structure, there are certain other elements found in particular MMPs. For example, the transmembrane MMPs have an additional eight residues forming what is known as the "MT-loop" that is found within the loop connecting beta-sheets II and III (LbetaIIbetaIII) (20, 22). The MT-loop plays a critical role in the ability of some transmembrane type MMPs (not MT4-MMP or MT6-MMP) to activate MMP-2 via interactions with TIMP-2 (56,57). Additionally, the MT-loop of MT1-MMP has been documented to affect the ability of MT1-MMP to localize to cell adhesions and influence cell invasion without directly affecting the ability of the enzyme to catalyze gelatin degradation or induce cell migration (58). Each of these MMPs have slightly different MT-loops, with the exception of the Pro-Tyr residues at the N-terminus,

and therefore potentially dictate the differential functions of the MT-MMPs, although it is currently unclear exactly which substrates would interact with the loop segment (58).

MMP-2 and MMP-9, both gelatinases, contain three fibronectin-like repeats within the LbetaValphaB helix, however these fibronectin-like repeats are oriented away from the enzyme not disturbing the structure of the catalytic domain (22,28). The structure of the fibronectin-like domain with three individual repeats implies that they may be separate binding sites (28). This domain can be expressed on its own and has the ability to interact with basement membrane components such as collagen for degradation and proteoglycans containing heparin sulfate, implying these interactions can contribute to extracellular localization (59). Conversely, deletion of the fibronectin-like region of MMP-2 results in impaired degradation of collagen and gelatin (60). Gelatin binding capacity is also conferred to MMP-9 via these fibronectin-like repeats (61).

4.4. Hinge/linker region

The hinge (or linker) region is a stretch of amino acids that follow the catalytic domain and leads to the hemopexin domain. This region may vary from 15 to 65 residues, depending on which MMP is in question (48). The structure of the linker plays a role in the ability for MMPs to process their substrates, as mutation of the proline rich region has been documented to reduce the ability of MMP-8 to degrade collagen (62). The linker has been purported to make contacts with both the catalytic domain and the hemopexin domain and serves to stabilize their arrangement and contribute to proper enzyme function (48). Recent evidence indicates that the linker region's flexibility is crucial to mediating the conformational change required for interaction between the catalytic and hemopexin –like domain and therefore, proper catalysis and specificity (63,64).

4.5. Hemopexin-like domain

The hemopexin domain is a four-bladed beta-propeller structure that begins with a cysteine residue and ends with another; the two cysteines form a disulfide bond to establish the propeller-like structure (48). Mutation of these cysteines results in loss of the proper structure, and therefore function (65). Centrally located within this region are three water molecules and three ions, including Ca^{2+}, Na^{+}, and Cl^{-} , which may be crucial to structure stabilization (48,66,67). For example, use of chelating agents against the hemopexin domain of MMP-2 results in the loss of binding to fibronectin and heparin (65, 68). The hemopexin-like domain has been shown to be crucial to substrate recognition and specificity (collagenases), while in other cases loss of or lack of the hemopexin-like domain does not substantially inhibit the enzymes (MMP-7, -12, and -26) (28,65,69). In the case of MMP-2, deletion of the hemopexin-like domain leads to lack of collagenolytic activity but retains catalytic activity against substrates such as casein or small synthetic peptides (65). Recently, the hemopexin-like domain has been implicated in the ability of MMPs to orient collagen for catalysis by binding two of the three strands of collagen, mediated by particular residues, allowing the third strand to be oriented into the active site (70). Because the hemopexin-like domain of MMPs can mediate interaction with substrate, it has become a target for inhibition in recent years (71-74).

4.6. Membrane-type matrix metalloproteinases

MT-MMPs are primarily responsible for pericellular degradation of ECM components and activation of soluble MMPs. They are also in a prime position to cleave other cell surface proteins. All membrane-type MMPs include an RXR/KR motif at the C-terminus of the pro-domain that serves as a proprotein-convertase site and allows for cleavage of the zymogen to the active state. MT1-MMP, MT2-MMP, MT3-MMP, and MT5-MMP have all been documented to have single-pass transmembrane domains that span approximately 20 amino acids (41, 75-79). The transmembrane domain is followed by a small intracellular cytoplasmic tail.

MT4-MMP and MT6-MMP are membrane-type MMPs that share most of the same domains as other MT-MMPs, but are membrane bound via a glycophosphatidylinositol anchor (GPI-anchor) at the C-terminus of the hydrophilic stalk region of the these proteases (77,79-82). The incorporation of a GPI-anchor includes removal of the small hydrophobic tail that follows the stalk region found in MT4-MMP and MT6-MMP (79,83). The transamidase enzymes operate in the endoplasmic reticulum and MT4-MMP and MT6-MMP are therefore processed in transit to the plasma membrane (79). The GPI-anchor is finally attached to the C-terminal of the protein via an amide link to phosphoethanolamine (79). The GPI-anchor allows for these proteases to be localized to lipid rafts and participate in signal transduction, potentially by directly modifying components of the lipid raft (79). These MMPs do not have an intracellular domain however their small stalk region does contain some unique characteristics. There are several cysteine residues present in the stalk region and they are reported to mediate the disulfide linkages that results in dimerization that plays a role in MT6-MMP stability (79,84).

4.7. Additional domains

The furin recognition domain is a sequence of amino acids that allows for intracellular cleavage of certain MMPs, which may result in secretion or delivery to the cell surface of active enzyme, as proprotein convertase activity can release the pro-domain (40). MT-MMPs, including MT1-MMP, MT2-MMP, MT3-MMP, and MT5-MMP contain furin recognition domains (85-87). Interestingly, it has been suggested that cleavage of

MT1-MMP by a convertase is not required for its MMP-2 activating activity (85). MT5-MMP contains a classic furin-recognition site located between the prodomain and the catalytic domain, but it also contains another site that is sensitive to convertase activity. Cleavage of this secondary site may result in loss of MT5-MMP from the plasma membrane and the resultant shedding equates to loss of the MT5-MMP function at the cell surface (87). MT4-MMP and MT6-MMP, the GPI-anchored membrane proteases, also contain a furin recognition site and can be activated by the proprotein convertase (77,88). MMP-23 is also integrated into the plasma membrane (type II transmembrane protein) and includes a furin-recognition site which is sensitive to proprotein convertases (24). MMP-11 and MMP-28 also harbor the furin-recognition site and can be proteolytically processed and activated by cleavage (40,89). Not only can furin act as an activator for MMP-28, but also as a chaperone for secretion, assigning a non-proteolytic role to furin for MMP modulation (90). Interestingly, proMMP-2 can be cleaved in the *trans*-Golgi network by furin, but this cleavage activity results in an inactive enzyme (91).

Originally found in *Xenopus laevis*, XMMP, was identified as a novel MMP that contained a signal peptide, a furin-recognition domain, as well as the typical catalytic domain and hemopexin-like domain, although there is no evidence of a hinge region in the human homologue MMP-21 (92,93). Following this discovery, the human orthologue MMP-21 was cloned (94). Uniquely, XMMP contains a vitronectin-like domain between the propeptide region and the furin-recognition domain which is not found in the human orthologue (94). The human counterpart of XMMP contains a proline-rich insert between the propeptide and furin-recognition domains (93).

MMP-23 contains all of the major domains of the other MMPs, including the prodomain, the catalytic domain, the conserved Met-turn, and a hinge region (95,96). The prodomain of the enzyme contains the conserved Cys and Pro residue for the cysteine-switch latency mechanism, but lacks the surrounding conserved residues (95). Strikingly, downstream of the catalytic domain, there exists a Cys-array and the C-terminal portion of this enzyme contains an Ig-like C2-type fold (96). The Cys-array, also known as the toxin-like domain, has been documented to influence certain calcium channels (97). The Ig-like fold within MMP-23 may be responsible for substrate interaction, analogous to the hemopexin-like domain in most other MMPs, as Ig-like C2 domains are normally responsible for protein-protein interactions (97). The N-terminal signal anchor of MMP-23 results in localization to the membrane as a type II transmembrane protein (as opposed to C-terminal transmembrane domains or GPI-anchors of other MT-MMPs) and cleavage by a proprotein convertase results in activation/secretion (24).

4.8. Posttranslational modifications

MMPs may also be post-translationally modified. There is evidence in MT1-MMP that the palmitoylation of Cys^{574} in the cytoplasmic tail contributes greatly to the activity of this MMP by mediating internalization (98). Furthermore, differences in glycosylation have been determined to affect protease stability; differential O-glycosylation of MT1-MMP leads to slower protease turnover (99,100). Not only has glycosylation of this protease been linked to turnover rate, but also has been found to influence the autocatalysis of MT1-MMP. MT1-MMP with reduced glycosylation in the linker regions has the ability to autocatalyze, implying that glycosylation may play a crucial role in protease stability (101). Interestingly, the glycosylation pattern of MT1-MMP has been linked to a particular cytoplasmic tail motif, dileucine^{572}, which may be responsible for mediating interactions with proteins necessary for proper glycosylation (102). O-glycosylation of MT1-MMP may also be a mechanism of control, as differential glycosylation of the linker region impedes the recruitment of TIMP-2 necessary for the ternary MMP-2 activation complex without significantly affecting collagenolytic activity (100). Relatedly, MT6-MMP also exhibits O-glycosylation (79).

Approximately 85% of the glycosylation found on MMP-9 is of an O-linked nature (103,104). The heavily O-linked glycosylation domain of the linker region (OG domain), in addition to the cysteine residue found in this area, is critical in mediating interactions with TIMP-1 and MMP-9's cargo receptors LRP-1 and megalin (104). These interactions are crucial as they can limit the availability of activated MMP-9. N-glycosylation patterns have also been associated with some MMPs. The pro- form of MMP9 also has N-glycosylation sites and this carbohydrate modification is purported to serve as protection from autocatalysis, similarly to O-glycosylation (105). MMP-3 and MMP-8 share the same N-linked glycosylation site as MMP-9 (106). MMP-1 also contains N-glycosylation sites located just outside of the signal peptide domain (107).

Another post-translational modification associated with MMPs is phosphorylation. MT1-MMP undergoes Src mediated phosphorylation at the cytoplasmic tail in cells expressing Src kinase or those stimulated with sphingosine-1-phosphate (S1P) (108). The MMP-2 zymogen has several phosphorylation sites and the phosphorylation status of this protein influences enzymatic activity (109). Further studies indicate that glycosylation of MMP-2 results in a shift in conformation that may be directly linked to activity (110).

5. THE MECHANISM OF ACTION OF MATRIX METALLOPROTEINASES

As outlined by Overall, catalysis by MMPs require binding of the substrate to the active site, a compatible

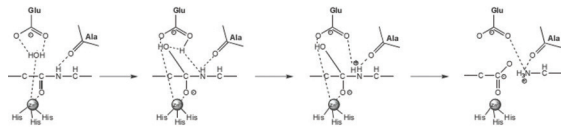

Figure 2. General catalytic mechanism of MMPs. Based on structural data, a general mechanism of action has been delineated for MMP catalytic activity. Reproduced with permission from (212).

specificity pocket (S1') that defines the active site, the catalytic Zn^{2+} properly coordinated with the presence of the catalytic glutamate, and binding of substrates to sites outside the catalytic site (exosites) (65). The target peptide binds to the active site by forming hydrogen bonds between the substrate and active site in an antiparallel fashion (65). Once properly oriented, the substrate can be hydrolyzed.

The reaction carried out by matrixins is considered an ordered, single-displacement reaction that follows Michaelis-Menten kinetics and is optimal at a neutral pH. The mechanism by which MMPs can cleave their substrates has been established based on structures gathered through the years characterizing thermolysin from *Bacillus thermoproteolyticus* and bovine carboxypeptidase A and later structures of MMPs in complex with substrates and inhibitors (19). From available structures in 1988, Matthews stated the thermolysin active site contained a zinc ion coordinated by three amino acid residues and a solvent molecule (111). The solvent molecule is bound by the Zn^{2+} ion and increases the nucleophilicity of the solvent. The carbonyl oxygen is polarized by the catalytic metal. Once this occurs, the scissile carbonyl carbon is available for attack by the solvent. The solvent proton then transfers to the general base, glutamate. This gem-diolate intermediate is a tetrahedral intermediate that is stabilized by surrounding amino acids. The final products are formed by bond cleavage and transfer of protons to the new amino terminal created by the enzyme's cleavage activity (Figure 2).

6. MMP EVOLUTION

It is suggested that the origins of MMPs dates back to the appearance of bacteria, as *B. fragilis* has a peptide sequence for metal-containing enzymes and plants have also been found to encode MMPs (112-114). The signal peptide (pre-domain), pro-domain, and catalytic domains appear in all MMPs, which suggests the primordial gene from which the MMPs derive contained coding for these domains (115). The additional fibronectin and hemopexin domains are in distinct exons and it is likely the gelatinases obtained the fibronectin-like and type-V collagen-like domains by domain insertions (115). MMP-9 likely derived from MMP-2 via gene duplication and then domain insertion, as it contains the additional type-V collagen-like domain (115). Not only do the MMPs share similar protein structure, but they also have similar gene structure. The MMPs exon-intron junctions are similar, suggesting they were in existence before the separate enzymes (116). Insertions such as the fibronectin-like repeats or the vitronectin-like domain appear to be a result of exon shuffling (116). Based on phylogenic trees generated for the MMP family, the earliest event was acquisition of the cysteine-switch, followed by insertion of the vitronectin-like domain (with a subsequent loss of it from matrilysin), and then insertion of the fibronectin-like domains (116). Extension of the hinge region of MMP-9 was one of the later events in MMP evolution, distinguishing MMP-9 from MMP-2 (116).

7. CONTROL OF MATRIX METALLOPROTEINASES

Matrixins are classically associated with matrix degradation, but the repertoire of these proteases has expanded to include other protein substrates such as pro-proteases, clotting factors, adhesion molecules, cryptic growth factors, and cytokines, amongst others, implying that MMP activity can have a huge impact on a wide range of processes (22). Therefore, the function of MMPs are tightly regulated at the level of transcription, post-transcriptional modification, post-translational modification, production in the zymogen form (requiring activation), non-specific inhibition by alpha$_2$-macroglobulin, and co-expression of tissue inhibitors of metalloproteinases (TIMPs); these mechanisms collectively serve to limit the activity of the proteases (19,22). Frequently, these mechanisms of inhibition are dysregulated under pathological conditions, which contributes to worsening of disease (22).

Under normal conditions, most MMPs are expressed at low levels, with transcription being highly regulated. One major mechanism of control is the tissue-type restriction of MMP expression. Only certain types of MMPs are expressed in particular tissues, implying that contextual cues dictate which MMP will be expressed (117-119). Active cytokines and growth factors are able to modulate MMP expression, positively or negatively. Frequently, cytokines and growth factors induce or activate *c-fos* and *c-jun*, which can dimerize and bind AP-1 at MMP promoter sites and encourage transcription (118). However, it is found there can also be a more complex control of MMP expression by growth factors. For example, TGF-beta can have differential effects by repressing *MMP-1*expression but can induce MMP-13 (120). Core binding factor-1 (Cbfa1) is associated with expression of MMP-13 in osteoblasts after TGF-beta exposure (121). Stabilized beta-catenin has also been implicated in transcription of matrilysin, which is significant in that dysregulation of beta-catenin activity in colonic cancers is a major determinant of carcinogenesis (122). The MT1-MMP promoter contains regulatory elements including an

Sp1 binding site, 4 CCAAT/enhancer-binding protein (C/EBP) sites, hypoxia responsive elements, and a regulatory region which contains an Ets site which binds Elf-1 and E1AF (123-125). Stromelysin-3 transcription has been reported to be controlled by the C/EBP sites and retinoic acid-responsive elements (RAREs) (126). p53 has also been implicated in the control of MMP expression. Highlighting the complexity of MMP control by transcription factors, p53 has been associated with increased MMP-2 transcription while it is a repressor of MMP-1 transcription (127,128). Overall, there are several transcription factors that can promote transcription of MMPs derived from a variety of signaling pathways, with each MMP having a particular combination of sites in their promoters (129). Single-nucleotide polymorphisms (SNPs) have also been implicated in controlling MMP expression. While most SNPs are functionally ineffective, certain polymorphisms in the promoter of MMPs can lead to disease susceptibility (130). Polymorphisms in the promoter of MMP-3, MMP-9, MMP-1, and MMP-12 contribute to atherosclerosis, aneurysm, and cancer invasion and metastasis (131). SNPs in the MT1-MMP promoter region have been identified as well, with two sites being identified and associated with a downregulation of MMP-14 transcription in renal disease (125). The MMP-2 promoter also contains polymorphisms that can contribute to transcription factor binding through changes in the Sp1 site (132). Interestingly, SNPs may also mediate the ability of microRNAs to negatively regulate MMP expression by altering the microRNA recognition sequence within the mRNA (133). Several microRNAs have been associated with modulation of MMP mRNA levels (134). MMP mRNA longevity can also be modified to control expression of the enzymes. TGF-beta treatment has been found to increase the mRNA stability of MMP-9 (135). Cortisol treatment of osteoblasts also results in an increase in mRNA stability of collagenase (136). These mechanisms are most likely indirect, by affecting the mRNA modifying proteins. Epigenetic controls have also been documented to influence MMP expression (129). DNA methylation of the MMP-9 promoter has been associated with suppressed transcriptional activity and decreased MMP-9 expression (137). Hypermethylation treatment results in a decrease of MMP-2, along with other cancer associated proteins such as VEGF and uPA (138). An additional level of control derives from the modification of histones (129). Histone acetyltransferases serve to add acetyl groups to the histone proteins, which loosens the chromatin and allows for transcriptional machinery to access promoter regions, and this phenomenon has been documented in the case of MMP-1 and MMP-9 (129). Furthermore, the MT1-MMP/MMP-2/TIMP-2 activation axis is regulated by DNA methylation and histone modification (139). Post-translational efficiency has also been associated with regulation of MMPs, particularly MMP-9 (140,141).

Post-translational modification is required for MMPs in that they must release their pro-domain in order to be fully active. This often requires the action of other proteases in the extracellular space, such as fellow MMPs or serine proteases. Specifically, in the case of MMP-2, MT1-MMP/TIMP-2 complexing is required to activate the soluble MMP-2 (51,142). In the case of MMPs that contain furin-like recognition domains (RXK/RR), intracellular proprotein convertases activate the proteins in the Golgi as they are transported in order to get to their final destination (40).

Initially identified in human skin fibroblasts as an inhibitor of collagenase, tissue inhibitors of matrix metalloproteinases (TIMPs) are naturally occurring inhibitors of MMPs that may have co-evolved with the development of connective tissue (143,144). Four TIMPs have been identified in humans (1-4) and are small glycoproteins that consist of two domains (145). They include an inhibitory N-terminal domain and a C-terminal domain that are each structurally stabilized by three disulfide-bound loops (146). These loops are critical to the stability of TIMPs and mutation of the residues severely restricts the ability of these inhibitors to function (147,148). The N-terminal region is composed of a five-stranded beta-barrel with Greek key motif in addition to alpha-helices (144). TIMPs bind to MMPs in a 1:1 ratio and each have a certain specificity for each MMP and expression pattern (145). These endogenous inhibitors tend to operate by binding the C-terminal portion of the MMP, or the hemopexin-like domain, and can also bind the zymogen form of MMPs (145). The differences in amino acid residues that exists in the hemopexin-like domain and TIMPs likely causes the differential affinities observed for the MMPs amongst the TIMPs (145). For example, TIMP-2 and -3 are capable of inhibiting MT-MMPs efficiently, unlike TIMP-1 (144). TIMP-1 is unique compared to the other TIMPs because it is glycosylated; this glycosylation may fine-tune the interaction of the TIMP with the active site and influence the effectiveness of the TIMP-1 (149). Colorectal cancer patients were found to have increased TIMP-1 in circulation, but the protein glycosylation profile was similar to that of normal, healthy donors, indicating it is not a good biomarker for diagnostic purposes (150). *In vitro* studies indicate, however, that aberrant glycosylation on TIMP-1 can result in increased invasiveness, indicating a loss of inhibitory function (151). Furthermore, TIMPs can form complexes with proteins that perform in a non-inhibitory manner. TIMP-2 binding to MT1-MMP allows for pro-MMP-2 to be activated by drawing proMMP-2 in the vicinity of a second, active MT1-MMP molecule (144). The TIMPs have also been associated with activities unrelated to MMP restriction (144). For example, TIMPs have been implicated in growth promotion in certain cell types, despite the role of TIMPs in reducing tumor cell growth (152-156). In fact, collective evidence suggests that TIMP-1 acts in a biphasic manner in reference to cancer progression. It has been documented that in early stages of tumor development, TIMP-1 may contribute

to proliferation and anti-apoptotic behavior, while in late stage of disease, it acts in an inhibitory manner (149).

Alpha-2 macroglobulin also acts as an inhibitor of MMPs (157). It is a relatively abundant serum plasma protein and is the major MMP inhibitor in circulation (118). Alpha-2 macroglobulin complexing with MMPs results in receptor-mediated endocytosis, indicating that alpha-2 macroglobulin inhibition may result in an irreversible inhibition (due to clearance) as opposed to the reversible interactions between TIMPs and MMPs (118). Another endogenous inhibitor of the MMPs includes the C-terminal fragment of procollagen C-terminal proteinase enhancer (PCPE), which is a protein that was originally documented to enhance the function of the procollagen C-terminal proteinase/bone morphogenic protein-1 (PCP/BMP-1) (158,159). Other mechanisms of control also exist for MMPs, which include pericellular segregation of the MMPs and the dependence upon substrate presence. This spatial and temporal control of MMPs limits their activity within their particular milieu (118).

8. MMPS UNLEASHED: MATRIX METALLOPROTEINASE'S ROLES IN DISEASE

Collectively, the family of MMPs is capable of remodeling the ECM by cleaving components such as various collagens, laminins, and fibronectin, amongst others. Additionally, MMPs have been shown to activate cryptic growth factors embedded in the ECM, for example, TGF-beta. These proteases have a broad spectrum of substrates and therefore play a crucial role in development, signaling, and apoptosis (22). Work from Woessner in 1976 and Jeffreys in 1983 demonstrated that procollagenases can be recovered from normal, postpartum uterine tissue and primary cultures of the uterine tissue (160,161). This was some of the first work that demonstrated a role for MMPs in normal tissues. MMPs were ultimately found to play a critical role in the development of the reproductive system and the remodeling required for menstruation, pregnancy, and parturition (162). Further work addressing MMPs roles in the normal inflammatory response and development were undertaken. Shipley, *et al.*, demonstrated that mice lacking macrophage metalloelastase have diminished ability to degrade ECM and have abrogated ability to invade tissues, as they normally would during wound repair (163). Attempts to identify the role of MMP-2 in the processing of the beta-amyloid precursor protein demonstrated that MMP-2 knockout mice were viable, but lagged in growth compared to wild-type from postnatal day 3 to adulthood (164). This implicated a role for MMPs in growth. Relatedly, MMP-9-null animals survive through the embryonic stage and develop into fertile adults, but were found to have a delay in bone ossification due to a lack of proper vascularization of bone growth plates, providing more evidence that MMPs play an important role in development (165). Even more striking is that the global loss of MT1-MMP expression results in viable animals with severe developmental defects, including osteopenia, arthritis, and skeletal dysplasia (166). Not only were these animals severely deformed, they also displayed a significantly shortened lifespan (166). Each of these pieces of evidence suggests that MMPs play important roles in a variety of non-pathogenic, physiological conditions.

Unfortunately, because of their ability to affect several crucial processes, these proteins have been associated with various pathologies such as arthritis, cancer and metastasis, and cardiovascular disease, just to name a few. Collagenase activity can be observed in synovial fluid collected from patients suffering from arthritis (167), suggesting that these MMPs are playing a role in the destruction of the joint by destruction of collagen (168,169). At the time of this work, it was unclear the source of these degrading enzymes, but it was later found that the source of these MMPs in the synovial fluids of arthritis patients were in fact the lymphocytes present in the affected joint (170). Since these early works, several proteases (including MMPs) have been associated with arthritis progression, however few effective treatments exist for this disease (reference (171) has an overview of proteases involved in arthritis).

Expression of MMPs has been correlated with cancer progression, relapse, and survival in multiple types of cancer, including lung carcinoma, colorectal cancer, and breast cancer (73,172-176). In support of the notion that MMPs contribute to tumor formation and progression, mice lacking MMP-7 expression have a reduced incidence of tumor formation of intestinal adenoma in the *Min* mouse model (177). In 1980, Liotta, *et al.*, demonstrated that there is a correlation between metastatic behavior of cells and the overexpression of collagenases, implicating MMPs ECM disruption capacity in the aggressive behavior of transformed cells (178). Importantly, not only have the tumor-derived MMPs been associated with primary tumor development and progression, but cells in the stroma were found to participate in tumor progression by expressing MMPs which contribute to tumor cell invasiveness (179, 180). The impact of MMPs on tumor progression not only lies in the contribution of MMPs to tumor cell invasion, but also to angiogenesis (181). Itoh, *et al.*, demonstrated that lack of MMP-2 can abrogate the ability of cancer cells to induce angiogenesis (182).

Metabolic syndrome, including obesity, insulin resistance, hypertension, and dyslipidemia, is a risk factor for cardiovascular disease (21). Both genetics and lifestyle can influence development of metabolic syndrome, but ultimately cardiac health is affected negatively by incurring metabolic syndrome. Protease activity has been found to play a role in cardiac health as MMPs are induced by various aspects of metabolic

disorder and influence cardiometabolic diseases, atherosclerosis and heart disease, insulin-resistance-associated cardiac disease, as well as hypertensive heart disease (21,183-186). Upregulation or downregulation of particular MMPs can influence atherosclerosis lesion formation, rupture, and platelet aggregation, amongst other aspects of vascular disease (187). It has been shown a significant association of MMP-2 and MMP-9 with atherosclerotic lesion formation and remodeling (188). Macrophage and smooth muscle cell-derived MMP-9 has been associated with atherosclerotic plaque rupture, which can eventually lead to myocardial infarction and sudden cardiac arrest (189,190). Furthermore, the cholesterol component of the atherosclerotic lesion may induce macrophage derived factors, such as cytokines, which can increase MMP expression or reduce the local expression of TIMPs providing an imbalance in proteolytic activity which contributes to plaque rupture by weakening the structure of the atherosclerotic lesion (191,192). Not only do MMPs play a role in various aspects of cardiovascular diseases, but they also influence patient response after treatment. For example, a rabbit model of stenting an artery post-injury results in collagen deposition and increased gelatinase activity, with use of an MMP inhibitor resulting in decreased hyperplasia (193). MMP-9 and MMP-2 have also been implicated in the remodeling associated with abdominal aortic aneurysms (194). Furthermore, local overexpression of TIMP-1 has been found to thwart aortic aneurysm rupture (195).

This brief introduction to MMP's roles in disease makes it clear that the dysregulation of these MMPs and their endogenous inhibitors can be detrimental. Because of the ability of MMPs to affect a plethora of disease processes, including but not limited to the above mentioned diseases, MMPs became the prime target for drug development in multiple fields of disease study.

9. REIGNING THEM IN: EARLY SUCCESSES AND MAJOR FAILURE IN TARGETING MMPS

Evidence of the ability of MMPs to contribute to disease progression accumulated and concomitantly, evidence that inhibiting MMPs could contribute to disease regression or delay progression also accrued. Experiments demonstrated that upregulation of the endogenous MMP inhibitor TIMP-1 in cancer cells resulted in a smaller number of metastatic lesions as compared to control cells in an experimental metastasis model (196,197). While the overexpression of TIMP-1 in this case does not affect the extravasation of the cells into the secondary site, reduction in metastasis is thought to be a result of the inability of the cells to efficiently alter ECM at the site of metastasis (198, 199). While TIMPs function to inhibit MMPs, the role of MMPs and TIMPs in tumor progression is complex and frequently cancer staging counterintuitively correlates with a high level of TIMP expression (200). Therefore, TIMPs themselves are not particularly good candidates for therapeutic agents; they are small peptides and can actually perform dual functions (201).

Early synthetic inhibitors were designed such that they mimicked MMP substrates based on amino acid sequence of triple helical collagen cleavage site (MMP-1); the inhibitors mimic the collagenase cleavage site (Gly-Ile or Gly-Leu) (202). The inhibitors should interact with the active site and inhibit MMPs by chelating the catalytically active Zn^{2+} and are generally most effective if the sequence mimics the P1' and P2' site (202). The hydroxamic acid derivatives function to coordinate the catalytic zinc with the two oxygen atoms in its sequence and hydrogen bonding of the nitrogen of the hydroxamate and the carbonyl of the enzyme backbone help to stabilize the interaction with the inhibitor, in addition to van der Waals forces (201). According to Whittaker, *et al.,* there are four classes that MMP inhibitors can fall into based loosely on structure, including succinyl hydroxamates, sulfonamide hydroxamates, non-hydroxamates, and natural products (202). Carboxylates, organoborate, and dithiolate are also Zn^{2+} chelating groups, but the hydroxamic acid derivatives tend to contribute to a more potent inhibition (201).

Hydroxamic acid derivatives, namely batimastat and marimastat (Figure 3), were some of the first promising peptidomimetic MMPIs (201). Batimastat (BB-94) is a low molecular weight, reversible inhibitor of MMPs, which is broad spectrum in nature and was identified in early *in vitro* studies as decreasing tumor burden in various models of cancer and significantly reducing metastatic spread, and ultimately increased survival rates of involved animals (203-205). Inhibition of hemangioma growth was observed in an *in vitro* model, and it was postulated that angiogenesis was abrogated with MMP inhibition which is a positive effect considering tumor reliance on generating neovasculature (206). Intrapleural injection to patients was more effective against early-stage tumors, reducing angiogenesis and tumor burden, but late-stage disease treatment elicited no benefit (207). The side effects included fatigue and nausea in addition to increased liver enzymes (207). Batimastat is not orally available, however its relative marimastat (BB-2516) is an orally bioavailable drug (202). While this drug did advance to clinical trials, side effects such as severe musculoskeletal pain sidelined the progress of this drug (208). MMP inhibitors designed to target the active site were also designed to harbor carboxylate groups for the purpose of zinc chelation (202); these inhibitors tended not to be as potent as their hydroxamate relatives. Like batimastat, these compounds were not orally available. The thiol zinc-binding groups were also deemed effective against MMPs, performing only slightly worse than hydroxamate inhibitors (202). Phosphorous-based zinc-binding groups have also been explored for the purpose of MMP inhibition, and phosphinic acid inhibitors have been found

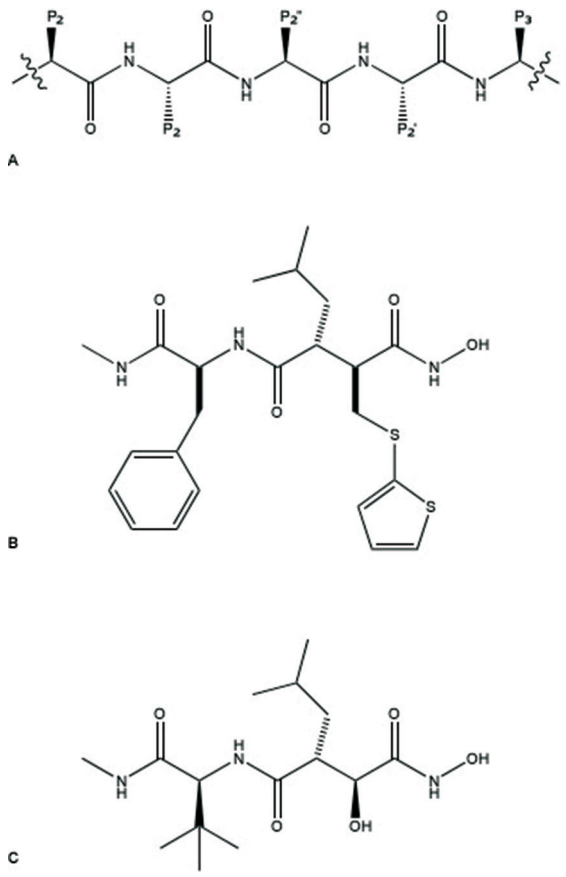

Figure 3. Collagen and the early peptidomimetic inhibitors. (A) Representation of the collagen backbone. (B) Batimastat (BB-94) (PubChem ID: CID5362422) (C) Marimastat (BB-2516) (Pubchem ID: CID119031)

to be the most potent of the phosphorous-based inhibitor designs. Non-peptidomimetic inhibitors were developed for the purpose of attempting to circumvent the problems observed with the peptidomimetic compounds and clinical trials with BAY 12-9566 showed some promising results regarding reduced angiogenesis and lung metastases, but the side effects included liver and kidney toxicities and anemia (207). Bisphosphonates have been explored as MMPIs, as they are capable of inhibiting several MMPs by acting as a zinc-chelator, by reducing MMP expression, and preventing MMP activation and breakdown (207).

Natural products have also been explored for the purpose of discovering novel MMP inhibitors. One of the most successful MMP inhibitors has been the tetracycline derivatives. Tetracycline's non-antimicrobial properties include inhibition of MMPs (209). In order to avoid unwanted antibiotic action, chemically modified tetracyclines have been developed (210). This family of tetracyclines inhibit MMPs as they are able to inhibit inflammatory cascades that can cause MMP overexpression (210). In addition to this indirect method of inhibiting MMP activity, tetracyclines are capable of directly inhibiting the protease (209). One of the most successful inhibitors of MMPs, doxycycline, is FDA-approved for use in the case of periodontal disease. Furthermore, pigment epithelium-derived factor is a naturally occurring serpin that has demonstrated anti-angiogenic and anti-metastatic properties and may have potential to be used as a biological treatment (207).

Overall, the mechanism of inhibition by synthetic MMPIs has been relatively broad spectrum and that is the primary reason for failure of these drugs in clinical trials, with the exception of a few successes. The ability of these MMPIs to inhibit multiple MMPs can result in inhibition of MMPs during normal physiological processes and, unfortunately, can also inhibit MMPs that contribute to disease prevention, *i.e.:* anti-tumoral MMPs (MMP-3, -8, -9) (207). Incurring painful musculoskeletal side effects is another major reason for trial failure. Other reasons for failure of Phase III clinical trials with MMPIs include dosing issues (doses for healthy volunteers as opposed to cancer patients) and study design, specifically the inclusion of only late-stage disease sufferers. Despite these failures, a list of over fifty clinical trials is currently on record at clinicaltrial.gov that involve MMPIs. Furthermore, there are currently many new strategies being developed for MMP inhibition in various kinds of diseases, yet many are still in *in vitro* settings. For example, work has been undertaken to identify MMP-specific inhibitors, including those for MMP-9 and MMP-14. A peptide that mimics the MMP-9 PEX domain blade IV that mediates homodimerization of the protease has been developed; this peptide effectively reduces cell migration by blocking the requisite dimerization of MMP-9 (71,211). Furthermore, a novel, small-molecule inhibitor of MMP-9 that selectively targets the PEX domain based on docking studies using the ZINC 2007 database has been identified (72). The best performing hit reduced cell migration and invasion and was shown to reduce tumor growth and metastasis in an orthotopic model (72). MMP-14 has also been targeted in a similar fashion; two of the outer blades of the PEX domain (blade I and blade IV) are responsible for interaction with CD44 (another cell surface molecule) and MMP-14 (73). Synthetic peptides that mimic these regions of MMP-14 prevent hetero- and homo-dimerization. These peptides were effective at reducing the ability of MMP-14 expressing cells to migrate and invade, and ultimately, were able to prevent metastases in a mouse model (73). Despite these developments in specific MMP inhibitor discovery, MMPs remain a prime target in the context of many diseases and the race for novel inhibitors continues.

10. THE FUTURE OF MATRIX METALLOPROTEINASES

Historically, MMPs were identified on the basis of their ability to modify ECM; we now understand that

the activities of MMPs are more nuanced than that alone. They affect growth factors and cytokines, induce cell signaling, and ultimately contribute to the progression of multitudes of diseases. Targeted inhibition of MMPs currently seems to be the main avenue of exploration in development of novel inhibitors and there is still much to be learned about the subtle ways MMPs are controlled and our ability to manipulate them. The remainder of this special review edition of ***Frontiers in Biosciences*** details the importance of MMPs in major diseases and the current outlook on each field. The effects of MMPs are broad and various in nature; a deeper understanding of the proteolytic and non-proteolytic activities of MMPs will be crucial in developing more effective, targeted therapies in many of the diseases that MMPs are critical players in.

11. REFERENCES

1. I. Gersh and H. R. Catchpole: The organization of ground substance and basement membrane and its significance in tissue injury, disease and growth. *Am J Anat*, 85(3), 457-521 (1949) DOI: 10.1038/290457a0
2. J. F. Woessner, Jr.: Catabolism of collagen and non-collagen protein in the rat uterus during post-partum involution. *Biochem J*, 83, 304-14 (1962) DOI: 10.1056/NEJMra022567
3. J. Gross and C. M. Lapiere: Collagenolytic activity in amphibian tissues: a tissue culture assay. *Proc Natl Acad Sci U S A*, 48, 1014-22 (1962) DOI: 10.1242/jcs.01134
4. H. Birkedal-Hansen: From tadpole collagenase to a family of matrix metalloproteinases. *J Oral Pathol*, 17(9-10), 445-51 (1988) DOI: 10.1007/s10545-011-9330-8
5. H. C. Grillo and J. Gross: Collagenolytic activity during mammalian wound repair. *Dev Biol*, 15(4), 300-317 (1967) DOI: 10.1016/0022-5193(67)90079-3
6. H. C. Grillo, C. M. Lapie´be, M. H. Dresden and J. Gross: Collagenolytic activity in regenerating forelimbs of the adult newt (Triturus viridescens). *Developmental Biology*, 17(5), 571-583 (1968) DOI: 10.1016/j.molmed.2010.04.007
7. H. M. Fullmer and W. Gibson: Collagenolytic activity in gingivae of man. *Nature*, 209(5024), 728-9 (1966) DOI: 10.1038/nbt793
8. H. M. Fullmer and G. S. Lazarus: Collagenase in bone of man. *J Histochem Cytochem*, 17(12), 793-8 (1969) DOI: 10.1016/j.bbamcr.2008.06.021
9. J. J. Jeffrey and J. Gross: Collagenase from rat uterus. Isolation and partial characterization. *Biochemistry*, 9(2), 268-73 (1970) DOI: 10.1126/science.7678183
10. G. S. Lazarus, R. S. Brown, J. R. Daniels and H. M. Fullmer: Human granulocyte collagenase. *Science*, 159(3822), 1483-5 (1968)
11. M. Abramson: Collagenolytic activity in middle ear cholesteatoma. *Ann Otol Rhinol Laryngol*, 78(1), 112-24 (1969) DOI: 10.2337/db11-0194
12. M. Abramson and C.-C. Huang: Localization of collagenase in human middle ear cholesteatoma. *Laryngoscope*, 87(5), 771-791 (1977) DOI: 10.1016/j.cell.2013.01.041
13. L. A. Liotta, S. Abe, P. G. Robey and G. R. Martin: Preferential digestion of basement membrane collagen by an enzyme derived from a metastatic murine tumor. *Proc Natl Acad Sci U S A*, 76(5), 2268-72 (1979)
14. T. Salo, L. A. Liotta and K. Tryggvason: Purification and characterization of a murine basement membrane collagen-degrading enzyme secreted by metastatic tumor cells. *J Biol Chem*, 258(5), 3058-63 (1983) DOI: 10.1038/ng1180
15. N. D. Rawlings, A. J. Barrett and A. Bateman: MEROPS: the database of proteolytic enzymes, their substrates and inhibitors. *Nucleic Acids Res*, 40(Database issue), D343-50 (2012) DOI: 10.1056/NEJM199404073301403
16. N. D. Rawlings, M. Waller, A. J. Barrett and A. Bateman: MEROPS: the database of proteolytic enzymes, their substrates and inhibitors. *Nucleic Acids Res*, 42(Database issue), D503-9 (2014) DOI: 10.1007/s10545-011-9338-0
17. W. Bode, F.-X. Gomis-Rüth and W. Stöckler: Astacins, serralysins, snake venom and matrix metalloproteinases exhibit identical zinc-binding environments (HEXXHXXGXXH and Met-turn) and topologies and should be grouped into a common family, the

'metzincins'. *FEBS Letters*, 331(1–2), 134-140 (1993)
DOI: 10.1074/jbc.M708444200

18. N. D. Rawlings and A. J. Barrett: Evolutionary families of peptidases. *Biochem J*, 290 (Pt 1), 205-18 (1993)
DOI: 10.1038/oby.2008.253

19. N. Cerdà-Costa and F. Xavier Gomis-Rüth: Architecture and function of metallopeptidase catalytic domains. *Protein Sci*, 23(2), 123-144 (2014)
DOI: 10.1016/j.diabres.2011.01.010

20. H. Nagase, R. Visse and G. Murphy: Structure and function of matrix metalloproteinases and TIMPs. *Cardiovasc Res*, 69(3), 562-573 (2006)

21. Y. Hua and S. Nair: Proteases in cardiometabolic diseases: Pathophysiology, molecular mechanisms and clinical applications. *BBA - Molecular Basis of Disease*(0) (2014)
DOI: 10.1186/1758-5996-3-32

22. C. Tallant, A. Marrero and F. X. Gomis-Rüth: Matrix metalloproteinases: Fold and function of their catalytic domains. *BBA - Molecular Cell Research*, 1803(1), 20-28 (2010)
DOI: 10.1016/S0168-8227(98)00110-7

23. B. R. Evans, R. A. Mosig, M. Lobl, C. R. Martignetti, C. Camacho, V. Grum-Tokars, M. J. Glucksman and J. A. Martignetti: Mutation of membrane type-1 metalloproteinase, MT1-MMP, causes the multicentric osteolysis and arthritis disease Winchester syndrome. *Am J Hum Genet*, 91(3), 572-6 (2012)
DOI: 10.1371/journal.pmed.0030442

24. D. Pei, T. Kang and H. Qi: Cysteine Array Matrix Metalloproteinase (CA-MMP)/MMP-23 Is a Type II Transmembrane Matrix Metalloproteinase Regulated by a Single Cleavage for Both Secretion and Activation. *J Biol Chem*, 275(43), 33988-33997 (2000)

25. P. Walter and A. E. Johnson: Signal Sequence Recognition and Protein Targeting to the Endoplasmic Reticulum Membrane. *Annu Rev Cell Biol*, 10(1), 87-119 (1994)
DOI: 10.1007/s10863-009-9211-0

26. H. Jarjanazi, S. Savas, N. Pabalan, J. W. Dennis and H. Ozcelik: Biological implications of SNPs in signal peptide domains of human proteins. *Proteins*, 70(2), 394-403 (2008)

27. D. Jozic, G. Bourenkov, N.-H. Lim, R. Visse, H. Nagase, W. Bode and K. Maskos: X-ray Structure of Human proMMP-1: NEW INSIGHTS INTO PROCOLLAGENASE ACTIVATION AND COLLAGEN BINDING. *J Biol Chem*, 280(10), 9578-9585 (2005)
DOI: 10.1007/s000590050004

28. K. Maskos: Crystal structures of MMPs in complex with physiological and pharmacological inhibitors. *Biochimie*, 87(3–4), 249-263 (2005)
DOI: 10.1016/j.ijcard.2013.12.014

29. K. Suzuki, J. J. Enghild, T. Morodomi, G. Salvesen and H. Nagase: Mechanisms of activation of tissue procollagenase by matrix metalloproteinase 3 (stromelysin). *Biochemistry*, 29(44), 10261-10270 (1990)
DOI: 10.1016/j.nutres.2013.02.005

30. A. J. Park, L. M. Matrisian, A. F. Kells, R. Pearson, Z. Y. Yuan and M. Navre: Mutational analysis of the transin (rat stromelysin) autoinhibitor region demonstrates a role for residues surrounding the "cysteine switch". *J Biol Chem*, 266(3), 1584-90 (1991)
DOI: 10.1073/pnas.061038798

31. D. G. Vartak and R. A. Gemeinhart: Matrix metalloproteases: underutilized targets for drug delivery. *J Drug Target*, 15(1), 1-20 (2007)
DOI: 10.1161/01.RES.88.5.529

32. R. Sanchez-Lopez, R. Nicholson, M. C. Gesnel, L. M. Matrisian and R. Breathnach: Structure-function relationships in the collagenase family member transin. *J Biol Chem*, 263(24), 11892-9 (1988)
DOI: 10.1161/CIRCRESAHA.109.212753

33. L. J. Windsor, H. Birkedal-Hansen, B. Birkedal-Hansen and J. A. Engler: An internal cysteine plays a role in the maintenance of the latency of human fibroblast collagenase. *Biochemistry*, 30(3), 641-7 (1991)
DOI: 10.1161/CIRCHEARTFAILURE.108.812099

34. L. C. Chen, M. E. Noelken and H. Nagase: Disruption of the cysteine-75 and zinc ion coordination is not sufficient to activate the precursor of human matrix metalloproteinase 3 (stromelysin 1). *Biochemistry*, 32(39), 10289-10295 (1993)
DOI: 10.1161/01.CIR.97.1.12

35. H. E. Van Wart and H. Birkedal-Hansen:

The cysteine switch: a principle of regulation of metalloproteinase activity with potential applicability to the entire matrix metalloproteinase gene family. *Proc Natl Acad Sci U S A*, 87(14), 5578-82 (1990)
DOI: 10.1073/pnas.94.2.514

36. E. B. Springman, E. L. Angleton, H. Birkedal-Hansen and H. E. Van Wart: Multiple modes of activation of latent human fibroblast collagenase: evidence for the role of a Cys73 active-site zinc complex in latency and a "cysteine switch" mechanism for activation. *Proc Natl Acad Sci U S A*, 87(1), 364-8 (1990)
DOI: 10.1093/geronj/11.3.298

37. J. Blaser, V. Knauper, A. Osthues, H. Reinke and H. Tschesche: Mercurial activation of human polymorphonuclear leucocyte procollagenase. *Eur J Biochem*, 202(3), 1223-30 (1991)

38. C. S. He, S. M. Wilhelm, A. P. Pentland, B. L. Marmer, G. A. Grant, A. Z. Eisen and G. I. Goldberg: Tissue cooperation in a proteolytic cascade activating human interstitial collagenase. *Proc Natl Acad Sci U S A*, 86(8), 2632-6 (1989)
DOI: 10.1073/pnas.85.17.6465

39. N. Ramos-DeSimone, E. Hahn-Dantona, J. Sipley, H. Nagase, D. L. French and J. P. Quigley: Activation of matrix metalloproteinase-9 (MMP-9) via a converging plasmin/stromelysin-1 cascade enhances tumor cell invasion. *J Biol Chem*, 274(19), 13066-76 (1999)
DOI: 10.1007/978-1-61779-998-3_9

40. D. Pei and S. J. Weiss: Furin-dependent intracellular activation of the human stromelysin-3 zymogen. *Nature*, 375(6528), 244-7 (1995)
DOI: 10.1074/jbc.275.5.3343

41. H. Sato, T. Takino, Y. Okada, J. Cao, A. Shinagawa, E. Yamamoto and M. Seiki: A matrix metalloproteinase expressed on the surface of invasive tumour cells. *Nature*, 370(6484), 61-5 (1994)
DOI: 10.1111/j.1474-9726.2011.00723.x

42. R. Fridman, M. Toth, D. Pena and S. Mobashery: Activation of progelatinase B (MMP-9) by gelatinase A (MMP-2). *Cancer Res*, 55(12), 2548-55 (1995)
DOI: 10.1007/s00125-008-1054-4

43. V. Knauper, B. Smith, C. Lopez-Otin and G. Murphy: Activation of progelatinase B (proMMP-9) by active collagenase-3 (MMP-13). *Eur J Biochem*, 248(2), 369-73 (1997)
DOI: 10.1073/pnas.0501559102

44. V. Knauper, L. Bailey, J. R. Worley, P. Soloway, M. L. Patterson and G. Murphy: Cellular activation of proMMP-13 by MT1-MMP depends on the C-terminal domain of MMP-13. *FEBS Lett*, 532(1-2), 127-30 (2002)
DOI: 10.1073/pnas.93.26.15364

45. G. Galazka, L. J. Windsor, H. Birkedal-Hansen and J. A. Engler: APMA (4-aminophenylmercuric acetate) activation of stromelysin-1 involves protein interactions in addition to those with cysteine-75 in the propeptide. *Biochemistry*, 35(34), 11221-7 (1996)
DOI: 10.1126/science.3201231

46. S. Das, M. Mandal, T. Chakraborti, A. Mandal and S. Chakraborti: Structure and evolutionary aspects of matrix metalloproteinases: A brief overview. *Mol Cell Biochem*, 253(1-2), 31-40 (2003)
DOI: 10.1038/331717a0

47. B. D. Freimark, W. S. Feeser and S. A. Rosenfeld: Multiple sites of the propeptide region of human stromelysin-1 are required for maintaining a latent form of the enzyme. *Journal of Biological Chemistry*, 269(43), 26982-26987 (1994)
DOI: 10.1042/BJ20021594

48. S. Iyer, R. Visse, H. Nagase and K. R. Acharya: Crystal Structure of an Active Form of Human MMP-1. *J Molec Biol*, 362(1), 78-88 (2006)

49. H. I. Park, J. Ni, F. E. Gerkema, D. Liu, V. E. Belozerov and Q.-X. A. Sang: Identification and Characterization of Human Endometase (Matrix Metalloproteinase-26) from Endometrial Tumor. *J Biol Chem*, 275(27), 20540-20544 (2000)
DOI: 10.1093/hmg/3.1.13

50. N. D. Marchenko, G. N. Marchenko and A. Y. Strongin: Unconventional activation mechanisms of MMP-26, a human matrix metalloproteinase with a unique PHCGXXD cysteine-switch motif. *J Biol Chem*, 277(21), 18967-72 (2002)
DOI: 10.1073/pnas.88.23.10614

51. J. Cao, M. Hymowitz, C. Conner, W. F. Bahou

and S. Zucker: The Propeptide Domain of Membrane Type 1-Matrix Metalloproteinase Acts as an Intramolecular Chaperone when Expressed in transwith the Mature Sequence in COS-1 Cells. *J Biol Chem*, 275(38), 29648-29653 (2000)

52. J. Cao, M. Drews, H. M. Lee, C. Conner, W. F. Bahou and S. Zucker: 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(52), 34745-52 (1998)
DOI: 10.1007/s13311-013-0177-6

53. M. Pavlaki, J. Cao, M. Hymowitz, W.-T. Chen, W. Bahou and S. Zucker: A Conserved Sequence within the Propeptide Domain of Membrane Type 1 Matrix Metalloproteinase Is Critical for Function as an Intramolecular Chaperone. *J Biol Chem*, 277(4), 2740-2749 (2002)
DOI: 10.1038/nature02517

54. R. P. Verma and C. Hansch: Matrix metalloproteinases (MMPs): chemical-biological functions and (Q)SARs. *Bioorg Med Chem*, 15(6), 2223-68 (2007)
DOI: 10.1083/jcb.200712101

55. G. S. Butler, E. M. Tam and C. M. Overall: The Canonical Methionine 392 of Matrix Metalloproteinase 2 (Gelatinase A) Is Not Required for Catalytic Efficiency or Structural Integrity: PROBING THE ROLE OF THE METHIONINE-TURN IN THE METZINCIN METALLOPROTEASE SUPERFAMILY. *J Biol Chem*, 279(15), 15615-15620 (2004)
DOI: 10.1042/BJ20040561

56. W. R. English, B. Holtz, G. Vogt, V. Knäuper and G. Murphy: Characterization of the Role of the "MT-loop": AN EIGHT-AMINO ACID INSERTION SPECIFIC TO PROGELATINASE A (MMP2) ACTIVATING MEMBRANE-TYPE MATRIX METALLOPROTEINASES. *J Biol Chem*, 276(45), 42018-42026 (2001)
DOI: 10.1136/jmedgenet-2013-101604

57. R. Lang, M. Braun, N. E. Sounni, A. Noel, F. Frankenne, J. M. Foidart, W. Bode and K. Maskos: Crystal Structure of the Catalytic Domain of MMP-16/MT3-MMP: Characterization of MT-MMP Specific Features. *J Molec Biol*, 336(1), 213-225 (2004)
DOI: 10.1136/jmedgenet-2013-101604

58. A. M. Woskowicz, S. A. Weaver, Y. Shitomi, N. Ito and Y. Itoh: MT-LOOP-dependent Localization of Membrane Type I Matrix Metalloproteinase (MT1-MMP) to the Cell Adhesion Complexes Promotes Cancer Cell Invasion. *J Biol Chem*, 288(49), 35126-35137 (2013)
DOI: 10.1073/pnas.85.17.6465

59. B. Steffensen, U. M. Wallon and C. M. Overall: Extracellular matrix binding properties of recombinant fibronectin type II-like modules of human 72-kDa gelatinase/type IV collagenase. High affinity binding to native type I collagen but not native type IV collagen. *J Biol Chem*, 270(19), 11555-66 (1995)
DOI: 10.1126/science.1112125

60. G. Murphy, Q. Nguyen, M. I. Cockett, S. J. Atkinson, J. A. Allan, C. G. Knight, F. Willenbrock and A. J. Docherty: Assessment of the role of the fibronectin-like domain of gelatinase A by analysis of a deletion mutant. *J Biol Chem*, 269(9), 6632-6 (1994)
DOI: 10.1093/nar/gkp100

61. I. E. Collier, P. A. Krasnov, A. Y. Strongin, H. Birkedal-Hansen and G. I. Goldberg: Alanine scanning mutagenesis and functional analysis of the fibronectin-like collagen-binding domain from human 92-kDa type IV collagenase. *J Biol Chem*, 267(10), 6776-81 (1992)
DOI: 10.1038/ng1778

62. H. Tsukada and T. Pourmotabbed: Unexpected crucial role of residue 272 in substrate specificity of fibroblast collagenase. *J Biol Chem*, 277(30), 27378-84 (2002)
DOI: 10.1038/ng1769

63. G. F. Fasciglione, M. Gioia, H. Tsukada, J. Liang, R. Iundusi, U. Tarantino, M. Coletta, T. Pourmotabbed and S. Marini: The collagenolytic action of MMP-1 is regulated by the interaction between the catalytic domain and the hinge region. *J Biol Inorg Chem*, 17(4), 663-72 (2012)
DOI: 10.1007/s00401-012-1001-9

64. V. Knäuper, A. J. P. Docherty, B. Smith, H. Tschesche and G. Murphy: Analysis of the contribution of the hinge region of human neutrophil collagenase (HNC, MMP-8) to stability and collagenolytic activity by alanine scanning mutagenesis. *FEBS Letters*, 405(1), 60-64 (1997)
DOI: 10.1093/hmg/ddn437

65. C. M. Overall: Molecular determinants of metalloproteinase substrate specificity: matrix metalloproteinase substrate binding domains, modules, and exosites. *Mol Biotechnol*, 22(1), 51-86 (2002)
DOI: 10.1091/mbc.E07-05-0404

66. J. Li, P. Brick, M. C. O'Hare, T. Skarzynski, L. F. Lloyd, V. A. Curry, I. M. Clark, H. F. Bigg, B. L. Hazleman, T. E. Cawston and D. M. Blow: Structure of full-length porcine synovial collagenase reveals a C-terminal domain containing a calcium-linked, four-bladed β-propeller. *Structure*, 3(6), 541-549 (1995)
DOI: 10.1128/MCB.24.22.9823-9834.2004

67. A. M. Libson, A. G. Gittis, I. E. Collier, B. L. Marmer, G. I. Goldberg and E. E. Lattman: Crystal structure of the haemopexin-like C-terminal domain of gelatinase A. *Nat Struct Mol Biol*, 2(11), 938-942 (1995)
DOI: 10.1073/pnas.1008924107

68. U. M. Wallon and C. M. Overall: The Hemopexin-like Domain (C Domain) of Human Gelatinase A (Matrix Metalloproteinase-2) Requires Ca2+ for Fibronectin and Heparin Binding: BINDING PROPERTIES OF RECOMBINANT GELATINASE A C DOMAIN TO EXTRACELLULAR MATRIX AND BASEMENT MEMBRANE COMPONENTS. *J Biol Chem*, 272(11), 7473-7481 (1997)
DOI: 10.1371/journal.pone.0040879

69. G. Murphy, J. A. Allan, F. Willenbrock, M. I. Cockett, J. P. O'Connell and A. J. Docherty: The role of the C-terminal domain in collagenase and stromelysin specificity. *J Biol Chem*, 267(14), 9612-8 (1992)

70. L. H. Arnold, L. E. Butt, S. H. Prior, C. M. Read, G. B. Fields and A. R. Pickford: The Interface between Catalytic and Hemopexin Domains in Matrix Metalloproteinase-1 Conceals a Collagen Binding Exosite. *J Biol Chem*, 286(52), 45073-45082 (2011)

71. A. Dufour, S. Zucker, N. S. Sampson, C. Kuscu and J. Cao: Role of Matrix Metalloproteinase-9 Dimers in Cell Migration: DESIGN OF INHIBITORY PEPTIDES. *J Biol Chem*, 285(46), 35944-35956 (2010)

72. A. Dufour, N. S. Sampson, J. Li, C. Kuscu, R. C. Rizzo, J. L. DeLeon, J. Zhi, N. Jaber, E. Liu, S. Zucker and J. Cao: Small-Molecule Anticancer Compounds Selectively Target the Hemopexin Domain of Matrix Metalloproteinase-9. *Cancer Res*, 71(14), 4977-4988 (2011)
DOI: 10.1023/B:MCBI.0000009880.94044.49

73. K. Zarrabi, A. Dufour, J. Li, C. Kuscu, A. Pulkoski-Gross, J. Zhi, Y. Hu, N. S. Sampson, S. Zucker and J. Cao: Inhibition of Matrix Metalloproteinase 14 (MMP-14)-mediated Cancer Cell Migration. *J Biol Chem*, 286(38), 33167-33177 (2011)
DOI: 10.1128/MCB.23.15.5409-5420.2003

74. A. G. Remacle, V. S. Golubkov, S. A. Shiryaev, R. Dahl, J. L. Stebbins, A. V. Chernov, A. V. Cheltsov, M. Pellecchia and A. Y. Strongin: Novel MT1-MMP small-molecule inhibitors based on insights into hemopexin domain function in tumor growth. *Cancer Res*, 72(9), 2339-49 (2012)
DOI: 10.1091/mbc.E07-12-1287

75. T. Takino, H. Sato, A. Shinagawa and M. Seiki: Identification of the Second Membrane-type Matrix Metalloproteinase (MT-MMP-2) Gene from a Human Placenta cDNA Library: MT-MMPs FORM A UNIQUE MEMBRANE-TYPE SUBCLASS IN THE MMP FAMILY. *J Biol Chem*, 270(39), 23013-23020 (1995)
DOI: 10.1074/jbc.M503062200

76. H. Will and B. Hinzmann: cDNA sequence and mRNA tissue distribution of a novel human matrix metalloproteinase with a potential transmembrane segment. *Eur J Biochem*, 231(3), 602-8 (1995)
DOI: 10.1074/jbc.C200677200

77. X. S. Puente, A. M. Pendás, E. Llano, G. Velasco and C. López-Otín: Molecular Cloning of a Novel Membrane-type Matrix Metalloproteinase from a Human Breast Carcinoma. *Cancer Res*, 56(5), 944-949 (1996)
DOI: 10.1074/jbc.M400920200

78. E. Llano, A. M. Pendás, J. P. Freije, A. Nakano, V. Knäuper, G. Murphy and C. López-Otin: Identification and Characterization of Human MT5-MMP, a New Membrane-bound Activator of Progelatinase A Overexpressed in Brain Tumors. *Cancer Res*, 59(11), 2570-2576 (1999)
DOI: 10.1016/S1567-7249(02)00006-5

79. A. Sohail, Q. Sun, H. Zhao, M. M. Bernardo, J. A. Cho and R. Fridman: MT4-(MMP17) and MT6-MMP (MMP25), A unique set of membrane-anchored matrix metalloproteinases:

properties and expression in cancer. *Cancer Metastasis Rev*, 27(2), 289-302 (2008)
DOI: 10.1186/1741-7007-2-9

80. Y. Itoh, M. Kajita, H. Kinoh, H. Mori, A. Okada and M. Seiki: Membrane Type 4 Matrix Metalloproteinase (MT4-MMP, MMP-17) Is a Glycosylphosphatidylinositol-anchored Proteinase. *J Biol Chem*, 274(48), 34260-34266 (1999)
DOI: 10.1073/pnas.1301951110

81. G. Velasco, S. Cal, A. Merlos-Suárez, A. A. Ferrando, S. Alvarez, A. Nakano, J. Arribas and C. López-Otín: Human MT6-Matrix Metalloproteinase: Identification, Progelatinase A Activation, and Expression in Brain Tumors. *Cancer Res*, 60(4), 877-882 (2000)
DOI: 10.1093/nar/gks266

82. I. A. Radichev, A. G. Remacle, S. A. Shiryaev, A. N. Purves, S. L. Johnson, M. Pellecchia and A. Y. Strongin: Biochemical Characterization of the Cellular Glycosylphosphatidylinositol-linked Membrane Type-6 Matrix Metalloproteinase. *J Biol Chem*, 285(21), 16076-16086 (2010)
DOI: 10.1016/S0014-5793(00)01527-1

83. S. Udenfriend and K. Kodukula: How glycosylphosphatidylinositol-anchored membrane proteins are made. *Annu Rev Biochem*, 64, 563-91 (1995)
DOI: 10.1016/S0891-5849(98)00209-3

84. H. Zhao, A. Sohail, Q. Sun, Q. Shi, S. Kim, S. Mobashery and R. Fridman: Identification and role of the homodimerization interface of the glycosylphosphatidylinositol-anchored membrane type 6 matrix metalloproteinase (MMP25). *J Biol Chem*, 283(50), 35023-32 (2008)
DOI: 10.1083/jcb.200704112

85. J. Cao, A. Rehemtulla, W. Bahou and S. Zucker: Membrane Type Matrix Metalloproteinase 1 Activates Pro-gelatinase A without Furin Cleavage of the N-terminal Domain. *J Biol Chem*, 271(47), 30174-30180 (1996)
DOI: 10.1083/jcb.200906083

86. T. Kang, H. Nagase and D. Pei: Activation of Membrane-type Matrix Metalloproteinase 3 Zymogen by the Proprotein Convertase Furin in the trans-Golgi Network. *Cancer Res*, 62(3), 675-681 (2002)
DOI: 10.1083/jcb.200906084

87. X. Wang and D. Pei: Shedding of Membrane Type Matrix Metalloproteinase 5 by a Furin-type Convertase: A POTENTIAL mechanism for down-regulation. *J Biol Chem*, 276(38), 35953-35960 (2001)
DOI: 10.1126/science.2814477

88. S. A. Shiryaev, A. Y. Savinov, P. Cieplak, B. I. Ratnikov, K. Motamedchaboki, J. W. Smith and A. Y. Strongin: Matrix metalloproteinase proteolysis of the myelin basic protein isoforms is a source of immunogenic peptides in autoimmune multiple sclerosis. *PLoS One*, 4(3), e4952 (2009)
DOI: 10.1016/j.ymeth.2010.03.006

89. S. A. Illman, J. Keski-Oja, W. C. Parks and J. Lohi: The mouse matrix metalloproteinase, epilysin (MMP-28), is alternatively spliced and processed by a furin-like proprotein convertase. *Biochem J*, 375(Pt 1), 191-7 (2003)
DOI: 10.1016/S0925-4439(97)00061-6

90. J. Cao, A. Rehemtulla, M. Pavlaki, P. Kozarekar and C. Chiarelli: Furin directly cleaves proMMP-2 in the trans-Golgi network resulting in a nonfunctioning proteinase. *J Biol Chem*, 280(12), 10974-80 (2005)
DOI: 10.1093/hmg/ddr529

91. M. Pavlaki, S. Zucker, A. Dufour, N. Calabrese, W. Bahou and J. Cao: Furin Functions as a Nonproteolytic Chaperone for Matrix Metalloproteinase-28: MMP-28 Propeptide Sequence Requirement. *Biochem Res Int*, 2011, 630319 (2011)
DOI: 10.1074/jbc.273.36.22983

92. M. Yang, M. T. Murray and M. Kurkinen: A Novel Matrix Metalloproteinase Gene (XMMP) Encoding Vitronectin-like Motifs Is Transiently Expressed in Xenopus laevis Early Embryo Development. *J Biol Chem*, 272(21), 13527-13533 (1997)
DOI: 10.1038/bjc.1972.33

93. G. N. Marchenko, N. D. Marchenko and A. Y. Strongin: The structure and regulation of the human and mouse matrix metalloproteinase-21 gene and protein. *Biochem J*, 372(Pt 2), 503-15 (2003)
DOI: 10.1016/S0092-8674(00)81873-5

94. K. Ahokas, J. Lohi, H. Lohi, O. Elomaa, M.-L. Karjalainen-Lindsberg, J. Kere and U. Saarialho-Kere: Matrix metalloproteinase-21, the human orthologue for XMMP, is expressed

during fetal development and in cancer. *Gene*, 301(1–2), 31-41 (2002)
DOI: 10.1038/nrm3054

95. G. Velasco, A. M. Pendás, A. Fueyo, V. Knäuper, G. Murphy and C. López-Otín: Cloning and Characterization of Human MMP-23, a New Matrix Metalloproteinase Predominantly Expressed in Reproductive Tissues and Lacking Conserved Domains in Other Family Members. *J Biol Chem*, 274(8), 4570-4576 (1999)
DOI: 10.1146/annurev.pharmtox.41.1.367

96. D. Pei: CA-MMP: a matrix metalloproteinase with a novel cysteine array, but without the classic cysteine switch. *FEBS Lett*, 457(2), 262-70 (1999)

97. C. Galea, H. Nguyen, K. George Chandy, B. Smith and R. Norton: Domain structure and function of matrix metalloprotease 23 (MMP23): role in potassium channel trafficking. *Cell Mol Life Sci*, 71(7), 1191-1210 (2014)
DOI: 10.1038/34112

98. N. Anilkumar, T. Uekita, J. R. Couchman, H. Nagase, M. Seiki and Y. Itoh: Palmitoylation at Cys574 is essential for MT1-MMP to promote cell migration. *FASEB J* (2005)
DOI: 10.1016/j.surg.2004.04.007

99. S. Kim, W. Huang, E. P. Mottillo, A. Sohail, Y. A. Ham, M. K. Conley-Lacomb, C. J. Kim, G. Tzivion, H. R. Kim, S. Wang, Y. Q. Chen and R. Fridman: Posttranslational regulation of membrane type 1-matrix metalloproteinase (MT1-MMP) in mouse PTEN null prostate cancer cells: Enhanced surface expression and differential O-glycosylation of MT1-MMP. *Biochim Biophys Acta*, 1803(11), 1287-97 (2010)

100. Y. I. Wu, H. G. Munshi, R. Sen, S. J. Snipas, G. S. Salvesen, R. Fridman and M. S. Stack: Glycosylation broadens the substrate profile of membrane type 1 matrix metalloproteinase. *J Biol Chem*, 279(9), 8278-89 (2004)
DOI: 10.1016/j.ceb.2009.09.004

101. A. G. Remacle, A. V. Chekanov, V. S. Golubkov, A. Y. Savinov, D. V. Rozanov and A. Y. Strongin: O-glycosylation regulates autolysis of cellular membrane type-1 matrix metalloproteinase (MT1-MMP). *J Biol Chem*, 281(25), 16897-905 (2006)
DOI: 10.1073/pnas.072544399

102. T. Ludwig, S. M. Theissen, M. J. Morton and M. J. Caplan: The cytoplasmic tail dileucine motif LL572 determines the glycosylation pattern of membrane-type 1 matrix metalloproteinase. *J Biol Chem*, 283(51), 35410-8 (2008)

103. T. S. Mattu, L. Royle, J. Langridge, M. R. Wormald, P. E. Van den Steen, J. Van Damme, G. Opdenakker, D. J. Harvey, R. A. Dwek and P. M. Rudd: O-Glycan Analysis of Natural Human Neutrophil Gelatinase B Using a Combination of Normal Phase- HPLC and Online Tandem Mass Spectrometry: Implications for the Domain Organization of the Enzyme†. *Biochemistry*, 39(51), 15695-15704 (2000)
DOI: 10.1016/S1097-2765(02)00442-2

104. P. E. Van den Steen, I. Van Aelst, V. Hvidberg, H. Piccard, P. Fiten, C. Jacobsen, S. K. Moestrup, S. Fry, L. Royle, M. R. Wormald, R. Wallis, P. M. Rudd, R. A. Dwek and G. Opdenakker: The Hemopexin and O-Glycosylated Domains Tune Gelatinase B/MMP-9 Bioavailability via Inhibition and Binding to Cargo Receptors. *J Biol Chem*, 281(27), 18626-18637 (2006)
DOI: 10.1016/S0167-5699(97)80014-X

105. L. P. Kotra, L. Zhang, R. Fridman, R. Orlando and S. Mobashery: N-Glycosylation pattern of the zymogenic form of human matrix metalloproteinase-9. *Bioorg Chem*, 30(5), 356-70 (2002)

106. V. Knauper, C. Lopez-Otin, B. Smith, G. Knight and G. Murphy: Biochemical characterization of human collagenase-3. *J Biol Chem*, 271(3), 1544-50 (1996)
DOI: 10.1093/emboj/17.1.37

107. S. M. Wilhelm, A. Z. Eisen, M. Teter, S. D. Clark, A. Kronberger and G. Goldberg: Human fibroblast collagenase: glycosylation and tissue-specific levels of enzyme synthesis. *Proc Natl Acad Sci U S A*, 83(11), 3756-60 (1986)
DOI: 10.1074/jbc.274.42.29905

108. C. Nyalendo, M. Michaud, E. Beaulieu, C. Roghi, G. Murphy, D. Gingras and R. Béliveau: Src-dependent Phosphorylation of Membrane Type I Matrix Metalloproteinase on Cytoplasmic Tyrosine 573: ROLE IN ENDOTHELIAL AND TUMOR CELL MIGRATION. *J Biol Chem*, 282(21), 15690-15699 (2007)
DOI: 10.1074/jbc.M701819200

109. M. Sariahmetoglu, B. D. Crawford, H. Leon, J. Sawicka, L. Li, B. J. Ballermann, C. Holmes, L. G. Berthiaume, A. Holt, G. Sawicki and R. Schulz: Regulation of matrix metalloproteinase-2 (MMP-2) activity by phosphorylation. *FASEB J*, 21(10), 2486-95 (2007)

110. A. L. Jacob-Ferreira, M. Y. Kondo, P. K. Baral, M. N. James, A. Holt, X. Fan and R. Schulz: Phosphorylation status of 72 kDa MMP-2 determines its structure and activity in response to peroxynitrite. *PLoS One*, 8(8), e71794 (2013)
DOI: 10.1083/jcb.153.6.1265

111. B. W. Matthews: Structural basis of the action of thermolysin and related zinc peptidases. *Acc Chem Res*, 21(9), 333-340 (1988)
DOI: 10.1016/S0092-8674(00)80085-9

112. I. Massova, L. P. Kotra, R. Fridman and S. Mobashery: Matrix metalloproteinases: structures, evolution, and diversification. *The FASEB Journal*, 12(12), 1075-1095 (1998)
DOI: 10.1016/S0092-8674(00)00008-8

113. A. A. Franco, L. M. Mundy, M. Trucksis, S. Wu, J. B. Kaper and C. L. Sears: Cloning and characterization of the Bacteroides fragilis metalloprotease toxin gene. *Infect Immun*, 65(3), 1007-13 (1997)
DOI: 10.1038/17135

114. G. McGeehan, W. Burkhart, R. Anderegg, J. D. Becherer, J. W. Gillikin and J. S. Graham: Sequencing and characterization of the soybean leaf metalloproteinase : structural and functional similarity to the matrix metalloproteinase family. *Plant Physiol*, 99(3), 1179-83 (1992)
DOI: 10.1038/35083620
DOI: 10.1038/35084037

115. L. M. Matrisian: The matrix-degrading metalloproteinases. *BioEssays*, 14(7), 455-463 (1992)
DOI: 10.1038/sj.cdd.4401166

116. N. D. Rawlings and A. J. Barrett: (13) Evolutionary families of metallopeptidases. In: *Methods in Enzymology*. Ed J. B. Alan. Academic Press, (1995)
DOI: 10.1074/jbc.M110.215715

117. R. Mohan, W. B. Rinehart, P. Bargagna-Mohan and M. E. Fini: Gelatinase B/lacZ Transgenic Mice, a Model for Mapping Gelatinase B Expression during Developmental and Injury-related Tissue Remodeling. *J Biol Chem*, 273(40), 25903-25914 (1998)
DOI: 10.1038/361365a0

118. M. D. Sternlicht and Z. Werb: How matrix metalloproteinases regulate cell behavior. *Annu Rev Cell Dev Biol*, 17, 463-516 (2001)
DOI: 10.1083/jcb.200704059

119. C. Yan and D. D. Boyd: Regulation of matrix metalloproteinase gene expression. *Jf Cell Physiol*, 211(1), 19-26 (2007)
DOI: 10.1128/MCB.02282-05

120. J. A. Uría, M. G. Jiménez, M. Balbín, J. M. P. Freije and C. López-Otín: Differential Effects of Transforming Growth Factor-β on the Expression of Collagenase-1 and Collagenase-3 in Human Fibroblasts. *J Biol Chem*, 273(16), 9769-9777 (1998)
DOI: 10.1083/jcb.200610042

121. M. J. Jimenez, M. Balbin, J. M. Lopez, J. Alvarez, T. Komori and C. Lopez-Otin: Collagenase 3 is a target of Cbfa1, a transcription factor of the runt gene family involved in bone formation. *Mol Cell Biol*, 19(6), 4431-42 (1999)
DOI: 10.1038/sj.emboj.7600592

122. H. C. Crawford, B. M. Fingleton, L. A. Rudolph-Owen, K. J. Goss, B. Rubinfeld, P. Polakis and L. M. Matrisian: The metalloproteinase matrilysin is a target of beta-catenin transactivation in intestinal tumors. *Oncogene*, 18(18), 2883-91 (1999)
DOI: 10.1016/S1534-5807(01)00055-7

123. J. Lohi, K. Lehti, H. Valtanen, W. C. Parks and J. Keski-Oja: Structural analysis and promoter characterization of the human membrane-type matrix metalloproteinase-1 (MT1-MMP) gene. *Gene*, 242(1-2), 75-86 (2000)
DOI: 10.1016/j.cell.2010.08.017

124. A. D. Moore, C. Hodgkinson, A. Lapenna, F. Zhang, K. Witkowska, F. Liang Ng, S. E. Headland, L. Reynolds, D. Lees, T. Lechertier, A. Milsom, K. Hodivala-Dilke and S. Ye: 203 Hypoxia-inducible Factor-1 Regulates Matrix Metalloproteinase-14 Expression: Underlying Effects of Hypoxia and Statins. *Heart*, 100 Suppl 3, A111-2 (2014)
DOI: 10.1038/cdd.2012.128

125. A. Munkert, U. Helmchen, M. J. Kemper, M. Bubenheim, R. A. Stahl and S. Harendza:

Characterization of the transcriptional regulation of the human MT1-MMP gene and association of risk reduction for focal-segmental glomerulosclerosis with two functional promoter SNPs. *Nephrol Dial Transplant*, 24(3), 735-42 (2009)
DOI: 10.1038/ng.2007.50

126. M.-G. Ludwig, P. Basset and P. Anglard: Multiple Regulatory Elements in the Murine Stromelysin-3 Promoter: EVIDENCE FOR DIRECT CONTROL BY CCAAT/ENHANCER-BINDING PROTEIN β AND THYROID AND RETINOID RECEPTORS. *J Biol Chem*, 275(51), 39981-39990 (2000)
DOI: 10.1074/jbc.M007529200

127. J. Bian and Y. Sun: Transcriptional activation by p53 of the human type IV collagenase (gelatinase A or matrix metalloproteinase 2) promoter. *Mol Cell Biol*, 17(11), 6330-8 (1997)

128. Y. Sun, Y. Sun, L. Wenger, J. L. Rutter, C. E. Brinckerhoff and H. S. Cheung: p53 Down-regulates Human Matrix Metalloproteinase-1 (Collagenase-1) Gene Expression. *JBiol Chem*, 274(17), 11535-11540 (1999)
DOI: 10.1074/jbc.274.17.11535

129. M. Fanjul-Fernández, A. R. Folgueras, S. Cabrera and C. López-Otín: Matrix metalloproteinases: Evolution, gene regulation and functional analysis in mouse models. *BBA - Molecular Cell Research*, 1803(1), 3-19 (2010)
DOI: 10.1016/j.bbamcr.2009.07.004

130. S. Ye: Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. *Matrix Biol*, 19(7), 623-9 (2000)
DOI: 10.1016/S0945-053X(00)00102-5

131. S. Ye, P. Eriksson, A. Hamsten, M. Kurkinen, S. E. Humphries and A. M. Henney: Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression. *J Biol Chem*, 271(22), 13055-60 (1996)
DOI: 10.1074/jbc.271.22.13055

132. S. J. Price, D. R. Greaves and H. Watkins: Identification of Novel, Functional Genetic Variants in the Human Matrix Metalloproteinase-2 Gene: ROLE OF Sp1 IN ALLELE-SPECIFIC TRANSCRIPTIONAL REGULATION. *J Biol Chem*, 276(10), 7549-7558 (2001)
DOI: 10.1074/jbc.M010242200

133. T. Duellman, C. Warren and J. Yang: Single nucleotide polymorphism-specific regulation of matrix metalloproteinase-9 by multiple miRNAs targeting the coding exon. *Nucleic Acids Res*, 42(9), 5518-31 (2014)
DOI: 10.1093/nar/gku197

134. Z. J. Rutnam, T. N. Wight and B. B. Yang: miRNAs regulate expression and function of extracellular matrix molecules. *Matrix Biol*, 32(2), 74-85 (2013)
DOI: 10.1016/j.matbio.2012.11.003

135. I. Sehgal and T. C. Thompson: Novel Regulation of Type IV Collagenase (Matrix Metalloproteinase-9 and -2) Activities by Transforming Growth Factor-β1 in Human Prostate Cancer Cell Lines. *Mol Biol Cell*, 10(2), 407-416 (1999)
DOI: 10.1091/mbc.10.2.407

136. A. M. Delany, J. J. Jeffrey, S. Rydziel and E. Canalis: Cortisol Increases Interstitial Collagenase Expression in Osteoblasts by Post-transcriptional Mechanisms. *J Biol Chem*, 270(44), 26607-26612 (1995)
DOI: 10.1074/jbc.270.44.26607

137. É. Chicoine, P.-O. Estève, O. Robledo, C. Van Themsche, E. F. Potworowski and Y. St-Pierre: Evidence for the role of promoter methylation in the regulation of MMP-9 gene expression. *Biochem Biophys Res Commun*, 297(4), 765-772 (2002)
DOI: 10.1016/S0006-291X(02)02283-0

138. N. Shukeir, P. Pakneshan, G. Chen, M. Szyf and S. A. Rabbani: Alteration of the Methylation Status of Tumor-Promoting Genes Decreases Prostate Cancer Cell Invasiveness and Tumorigenesis *In vitro* and *In vivo*. *Cancer Res*, 66(18), 9202-9210 (2006)
DOI: 10.1158/0008-5472.CAN-06-1954

139. A. V. Chernov, N. E. Sounni, A. G. Remacle and A. Y. Strongin: Epigenetic control of the invasion-promoting MT1-MMP/MMP-2/TIMP-2 axis in cancer cells. *J Biol Chem*, 284(19), 12727-34 (2009)
DOI: 10.1074/jbc.M900273200

140. Y. Jiang and R. J. Muschel: Regulation of Matrix Metalloproteinase-9 (MMP-9) by Translational Efficiency in Murine Prostate

Carcinoma Cells. *Cancer Res*, 62(6), 1910-1914 (2002)

141. M. Fähling, A. Steege, A. Perlewitz, B. Nafz, R. Mrowka, P. B. Persson and B. J. Thiele: Role of nucleolin in posttranscriptional control of MMP-9 expression. *BBA - Gene Structure and Expression*, 1731(1), 32-40 (2005) DOI: 10.1016/j.bbaexp.2005.08.005
142. S. Zucker, M. Drews, C. Conner, H. D. Foda, Y. A. DeClerck, K. E. Langley, W. F. Bahou, A. J. P. Docherty and J. Cao: Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) Binds to the Catalytic Domain of the Cell Surface Receptor, Membrane Type 1-Matrix Metalloproteinase 1 (MT1-MMP). *J Biol Chem*, 273(2), 1216-1222 (1998) DOI: 10.1074/jbc.273.2.1216
143. H. G. Welgus, G. P. Stricklin, A. Z. Eisen, E. A. Bauer, R. V. Cooney and J. J. Jeffrey: A specific inhibitor of vertebrate collagenase produced by human skin fibroblasts. *J Biol Chem*, 254(6), 1938-43 (1979)
144. K. Brew, D. Dinakarpandian and H. Nagase: Tissue inhibitors of metalloproteinases: evolution, structure and function. *BBA - Protein Structure and Molecular Enzymology*, 1477(1–2), 267-283 (2000) DOI: 10.1016/S0167-4838(99)00279-4
145. H. Piccard, P. E. Van den Steen and G. Opdenakker: Hemopexin domains as multifunctional liganding modules in matrix metalloproteinases and other proteins. *J Leukoc Biol*, 81(4), 870-892 (2007) DOI: 10.1189/jlb.1006629
146. R. A. Williamson, F. A. Marston, S. Angal, P. Koklitis, M. Panico, H. R. Morris, A. F. Carne, B. J. Smith, T. J. Harris and R. B. Freedman: Disulphide bond assignment in human tissue inhibitor of metalloproteinases (TIMP). *Biochem J*, 268(2), 267-74 (1990)
147. W. Huang, Q. Meng, K. Suzuki, H. Nagase and K. Brew: Mutational Study of the Amino-terminal Domain of Human Tissue Inhibitor of Metalloproteinases 1 (TIMP-1) Locates an Inhibitory Region for Matrix Metalloproteinases. *J Biol Chem*, 272(35), 22086-22091 (1997) DOI: 10.1074/jbc.272.35.22086
148. N. C. M. Caterina, L. J. Windsor, A. E. Yermovsky, M. K. Bodden, K. B. Taylor, H. Birkedal-Hansen and J. A. Engler: Replacement of Conserved Cysteines in Human Tissue Inhibitor of Metalloproteinases-1. *J Biol Chem*, 272(51), 32141-32149 (1997) DOI: 10.1074/jbc.272.51.32141
149. Y. S. Kim, S. H. Kim, J. G. Kang and J. H. Ko: Expression level and glycan dynamics determine the net effects of TIMP-1 on cancer progression. *BMB Rep*, 45(11), 623-8 (2012) DOI: 10.5483/BMBRep.2012.45.11.233
150. M. Thaysen-Andersen, I. B. Thøgersen, U. Lademann, H. Offenberg, A. M. B. Giessing, J. J. Enghild, H. J. Nielsen, N. Brünner and P. Højrup: Investigating the biomarker potential of glycoproteins using comparative glycoprofiling — application to tissue inhibitor of metalloproteinases-1. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 1784(3), 455-463 (2008) DOI: 10.1016/j.bbapap.2007.12.007
151. Y. S. Kim, S. Y. Hwang, H. Y. Kang, H. Sohn, S. Oh, J. Y. Kim, J. S. Yoo, Y. H. Kim, C. H. Kim, J. H. Jeon, J. M. Lee, H. A. Kang, E. Miyoshi, N. Taniguchi, H. S. Yoo and J. H. Ko: Functional proteomics study reveals that N-Acetylglucosaminyltransferase V reinforces the invasive/metastatic potential of colon cancer through aberrant glycosylation on tissue inhibitor of metalloproteinase-1. *Mol Cell Proteomics*, 7(1), 1-14 (2008) DOI: 10.1074/mcp.M700084-MCP200
152. B. Bertaux, W. Hornebeck, A. Z. Eisen and L. Dubertret: Growth Stimulation of Human Keratinocytes by Tissue Inhibitor of Metalloproteinases. *J Investig Dermatol*, 97(4), 679-685 (1991) DOI: 10.1111/1523-1747.ep12483956
153. T. Hayakawa, K. Yamashita, K. Tanzawa, E. Uchijima and K. Iwata: Growth-promoting activity of tissue inhibitor of metalloproteinases-1 (TIMP-1) for a wide range of cells A possible new growth factor in serum. *FEBS Letters*, 298(1), 29-32 (1992) DOI: 10.1016/0014-5793(92)80015-9
154. J. Bian, Y. Wang, M. R. Smith, H. Kim, C. Jacobs, J. Jackman, H.-F. Kung, N. H. Colburn and Y. Sun: Suppression of *in vivo* tumor growth and induction of suspension cell death by tissue inhibitor of metalloproteinases (TIMP)-3. *Carcinogenesis*, 17(9), 1805-1811 (1996) DOI: 10.1093/carcin/17.9.1805

155. P. Valente, G. Fassina, A. Melchiori, L. Masiello, M. Cilli, A. Vacca, M. Onisto, L. Santi, W. G. Stetler-Stevenson and A. Albini: TIMP-2 over-expression reduces invasion and angiogenesis and protects B16F10 melanoma cells from apoptosis. *Int J Cancer*, 75(2), 246-53 (1998)
DOI: 10.1002/(SICI)1097-0215 (19980119) 75:2<246::AID-IJC13>3.0.CO;2-B

156. A. H. Baker, S. J. George, A. B. Zaltsman, G. Murphy and A. C. Newby: Inhibition of invasion and induction of apoptotic cell death of cancer cell lines by overexpression of TIMP-3. *Br J Cancer*, 79(9-10), 1347-1355 (1999)
DOI: 10.1038/sj.bjc.6690217

157. L. Sottrup-Jensen and H. Birkedal-Hansen: Human fibroblast collagenase-alpha-macroglobulin interactions. Localization of cleavage sites in the bait regions of five mammalian alpha-macroglobulins. *J Biol Chem*, 264(1), 393-401 (1989)

158. J. D. Mott, C. L. Thomas, M. T. Rosenbach, K. Takahara, D. S. Greenspan and M. J. Banda: Post-translational Proteolytic Processing of Procollagen C-terminal Proteinase Enhancer Releases a Metalloproteinase Inhibitor. *J Biol Chem*, 275(2), 1384-1390 (2000)
DOI: 10.1074/jbc.275.2.1384

159. K. Takahara, E. Kessler, L. Biniaminov, M. Brusel, R. L. Eddy, S. Jani-Sait, T. B. Shows and D. S. Greenspan: Type I procollagen COOH-terminal proteinase enhancer protein: identification, primary structure, and chromosomal localization of the cognate human gene (PCOLCE). *J Biol Chem*, 269(42), 26280-26285 (1994)

160. J. G. Weeks, J. Halme and J. F. Woessner, Jr.: Extraction of collagenase from the involuting rat uterus. *Biochim Biophys Acta*, 445(1), 205-14 (1976)
DOI: 10.1016/0005-2744(76)90173-X

161. W. T. Roswit, J. Halme and J. J. Jeffrey: Purification and properties of rat uterine procollagenase. *Archives of Biochemistry and Biophysics*, 225(1), 285-295 (1983)
DOI: 10.1016/0003-9861(83)90032-2

162. D. L. Hulboy, L. A. Rudolph and L. M. Matrisian: Matrix metalloproteinases as mediators of reproductive function. *Mol Hum Reprod*, 3(1), 27-45 (1997)
DOI: 10.1093/molehr/3.1.27

163. J. M. Shipley, R. L. Wesselschmidt, D. K. Kobayashi, T. J. Ley and S. D. Shapiro: Metalloelastase is required for macrophage-mediated proteolysis and matrix invasion in mice. *Proc Natl Acad Sci U S A*, 93(9), 3942-6 (1996)
DOI: 10.1073/pnas.93.9.3942

164. T. Itoh, T. Ikeda, H. Gomi, S. Nakao, T. Suzuki and S. Itohara: Unaltered secretion of beta-amyloid precursor protein in gelatinase A (matrix metalloproteinase 2)-deficient mice. *J Biol Chem*, 272(36), 22389-92 (1997)
DOI: 10.1074/jbc.272.36.22389

165. T. H. Vu, J. M. Shipley, G. Bergers, J. E. Berger, J. A. Helms, D. Hanahan, S. D. Shapiro, R. M. Senior and Z. Werb: MMP-9/gelatinase B is a key regulator of growth plate angiogenesis and apoptosis of hypertrophic chondrocytes. *Cell*, 93(3), 411-22 (1998)
DOI: 10.1016/S0092-8674(00)81169-1

166. K. Holmbeck, P. Bianco, J. Caterina, S. Yamada, M. Kromer, S. A. Kuznetsov, M. Mankani, P. G. Robey, A. R. Poole, I. Pidoux, J. M. Ward and H. Birkedal-Hansen: MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. *Cell*, 99(1), 81-92 (1999)
DOI: 10.1016/S0092-8674(00)80064-1

167. E. D. Harris, Jr., D. R. DiBona and S. M. Krane: Collagenases in human synovial fluid. *J Clin Invest*, 48(11), 2104-13 (1969)
DOI: 10.1172/JCI106177

168. E. D. Harris, Jr., D. R. DiBona and S. M. Krane: A mechanism for cartilage destruction in rheumatoid arthritis. *Trans Assoc Am Physicians*, 83, 267-76 (1970)

169. E. D. Harris, Jr. and S. M. Krane: An endopeptidase from rheumatoid synovial tissue culture. *Biochim Biophys Acta*, 258(2), 566-76 (1972)
DOI: 10.1016/0005-2744(72)90249-5

170. J. M. Dayer, R. Graham, G. Russell and S. M. Krane: Collagenase production by rheumatoid synovial cells: stimulation by a human lymphocyte factor. *Science*, 195(4274), 181-3 (1977)
DOI: 10.1126/science.188134

171. L. Troeberg and H. Nagase: Proteases involved in cartilage matrix degradation in

osteoarthritis. *Biochim Biophys Acta*, 1824(1), 133-45 (2012)
DOI: 10.1016/j.bbapap.2011.06.020

172. S. J. Urbanski, D. R. Edwards, A. Maitland, K. J. Leco, A. Watson and A. E. Kossakowska: Expression of metalloproteinases and their inhibitors in primary pulmonary carcinomas. *Br J Cancer*, 66(6), 1188-94 (1992)
DOI: 10.1038/bjc.1992.434

173. B. Davies, D. W. Miles, L. C. Happerfield, M. S. Naylor, L. G. Bobrow, R. D. Rubens and F. R. Balkwill: Activity of type IV collagenases in benign and malignant breast disease. *Br J Cancer*, 67(5), 1126-31 (1993)
DOI: 10.1038/bjc.1993.207

174. P. D. Brown, R. E. Bloxidge, N. S. Stuart, K. C. Gatter and J. Carmichael: Association between expression of activated 72-kilodalton gelatinase and tumor spread in non-small-cell lung carcinoma. *J Natl Cancer Inst*, 85(7), 574-8 (1993)
DOI: 10.1093/jnci/85.7.574

175. B. Davies, J. Waxman, H. Wasan, P. Abel, G. Williams, T. Krausz, D. Neal, D. Thomas, A. Hanby and F. Balkwill: Levels of matrix metalloproteases in bladder cancer correlate with tumor grade and invasion. *Cancer Res*, 53(22), 5365-9 (1993)

176. Z. S. Zeng, Y. Huang, A. M. Cohen and J. G. Guillem: Prediction of colorectal cancer relapse and survival via tissue RNA levels of matrix metalloproteinase-9. *J Clin Oncol*, 14(12), 3133-40 (1996)

177. C. L. Wilson, K. J. Heppner, P. A. Labosky, B. L. Hogan and L. M. Matrisian: Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. *Proc Natl Acad Sci U S A*, 94(4), 1402-7 (1997)
DOI: 10.1073/pnas.94.4.1402

178. L. A. Liotta, K. Tryggvason, S. Garbisa, I. Hart, C. M. Foltz and S. Shafie: Metastatic potential correlates with enzymatic degradation of basement membrane collagen. *Nature*, 284(5751), 67-8 (1980)
DOI: 10.1038/284067a0

179. P. Basset, J. P. Bellocq, C. Wolf, I. Stoll, P. Hutin, J. M. Limacher, O. L. Podhajcer, M. P. Chenard, M. C. Rio and P. Chambon: A novel metalloproteinase gene specifically expressed in stromal cells of breast carcinomas. *Nature*, 348(6303), 699-704 (1990)
DOI: 10.1038/348699a0

180. R. Masson, O. Lefebvre, A. Noel, M. E. Fahime, M. P. Chenard, C. Wendling, F. Kebers, M. LeMeur, A. Dierich, J. M. Foidart, P. Basset and M. C. Rio: *In vivo* evidence that the stromelysin-3 metalloproteinase contributes in a paracrine manner to epithelial cell malignancy. *J Cell Biol*, 140(6), 1535-41 (1998)
DOI: 10.1083/jcb.140.6.1535

181. W. G. Stetler-Stevenson: Matrix metalloproteinases in angiogenesis: a moving target for therapeutic intervention. *J Clin Invest*, 103(9), 1237-41 (1999)
DOI: 10.1172/JCI6870

182. T. Itoh, M. Tanioka, H. Yoshida, T. Yoshioka, H. Nishimoto and S. Itohara: Reduced angiogenesis and tumor progression in gelatinase A-deficient mice. *Cancer Res*, 58(5), 1048-51 (1998)

183. S. Uemura, H. Matsushita, W. Li, A. J. Glassford, T. Asagami, K.-H. Lee, D. G. Harrison and P. S. Tsao: Diabetes Mellitus Enhances Vascular Matrix Metalloproteinase Activity: Role of Oxidative Stress. *Circ Res*, 88(12), 1291-1298 (2001)
DOI: 10.1161/hh1201.092042

184. E. Maquoi, C. Munaut, A. Colige, D. Collen and H. R. Lijnen: Modulation of adipose tissue expression of murine matrix metalloproteinases and their tissue inhibitors with obesity. *Diabetes*, 51(4), 1093-101 (2002)
DOI: 10.2337/diabetes.51.4.1093

185. A. K. Death, E. J. Fisher, K. C. Y. McGrath and D. K. Yue: High glucose alters matrix metalloproteinase expression in two key vascular cells: potential impact on atherosclerosis in diabetes. *Atherosclerosis*, 168(2), 263-269 (2003)
DOI: 10.1016/S0021-9150(03)00140-0

186. C. Bouvet, L.-A. Gilbert, D. Girardot, D. deBlois and P. Moreau: Different Involvement of Extracellular Matrix Components in Small and Large Arteries During Chronic NO Synthase Inhibition. *Hypertension*, 45(3), 432-437 (2005)
DOI: 10.1161/01.HYP.0000154680.44184.01

187. A. Papazafiropoulou and N. Tentolouris: Matrix metalloproteinases and cardiovascular

diseases. *Hippokratia*, 13(2), 76-82 (2009)

188. G. Pasterkamp, A. H. Schoneveld, D. J. Hijnen, D. P. de Kleijn, H. Teepen, A. C. van der Wal and C. Borst: Atherosclerotic arterial remodeling and the localization of macrophages and matrix metalloproteases 1, 2 and 9 in the human coronary artery. *Atherosclerosis*, 150(2), 245-53 (2000) DOI: 10.1016/S0021-9150(99)00371-8

189. M. J. Davies and A. C. Thomas: Plaque fissuring--the cause of acute myocardial infarction, sudden ischaemic death, and crescendo angina. *Br Heart J*, 53(4), 363-73 (1985) DOI: 10.1136/hrt.53.4.363

190. D. L. Brown, M. S. Hibbs, M. Kearney, C. Loushin and J. M. Isner: Identification of 92-kD gelatinase in human coronary atherosclerotic lesions. Association of active enzyme synthesis with unstable angina. *Circulation*, 91(8), 2125-31 (1995) DOI: 10.1161/01.CIR.91.8.2125

191. Z. S. Galis, G. K. Sukhova, R. Kranzhofer, S. Clark and P. Libby: Macrophage foam cells from experimental atheroma constitutively produce matrix-degrading proteinases. *Proc Natl Acad Sci U S A*, 92(2), 402-6 (1995) DOI: 10.1073/pnas.92.2.402

192. M. Moreau, I. Brocheriou, L. Petit, E. Ninio, M. J. Chapman and M. Rouis: Interleukin-8 Mediates Downregulation of Tissue Inhibitor of Metalloproteinase-1 Expression in Cholesterol-Loaded Human Macrophages: Relevance to Stability of Atherosclerotic Plaque. *Circulation*, 99(3), 420-426 (1999) DOI: 10.1161/01.CIR.99.3.420

193. C. Li, W. J. Cantor, N. Nili, R. Robinson, L. Fenkell, Y. L. e. Tran, H. A. Whittingham, W. Tsui, A. N. Cheema, J. D. Sparkes, K. Pritzker, D. E. Levy and B. H. Strauss: Arterial repair after stenting and the effects of gm6001, a matrix metalloproteinase inhibitor. *J Am Coll Cardiol*, 39(11), 1852-1858 (2002) DOI: 10.1016/S0735-1097(02)01873-9

194. A. Yamashita, T. Noma, A. Nakazawa, S. Saito, K. Fujioka, N. Zempo and K. Esato: Enhanced Expression of Matrix Metalloproteinase-9 in Abdominal Aortic Aneurysms. *World J Surg*, 25(3), 259-265 (2001) DOI: 10.1007/s002680020062

195. E. Allaire, R. Forough, M. Clowes, B. Starcher and A. W. Clowes: Local overexpression of TIMP-1 prevents aortic aneurysm degeneration and rupture in a rat model. *J Clin Invest*, 102(7), 1413-20 (1998) DOI: 10.1172/JCI2909

196. R. M. Schultz, S. Silberman, B. Persky, A. S. Bajkowski and D. F. Carmichael: Inhibition by Human Recombinant Tissue Inhibitor of Metalloproteinases of Human Amnion Invasion and Lung Colonization by Murine B16-F10 Melanoma Cells. *Cancer Res*, 48(19), 5539-5545 (1988)

197. R. Khokha, M. Zimmer, S. Wilson and A. Chambers: Up-regulation of TIMP-1 expression in B16-F10 melanoma cells suppresses their metastatic ability in chick embryo. *Clin Exp Metastasis*, 10(6), 365-370 (1992) DOI: 10.1007/BF00133464

198. S. Koop, R. Khokha, E. E. Schmidt, I. C. MacDonald, V. L. Morris, A. F. Chambers and A. C. Groom: Overexpression of metalloproteinase inhibitor in B16F10 cells does not affect extravasation but reduces tumor growth. *Cancer Res*, 54(17), 4791-7 (1994)

199. A. R. Nelson, B. Fingleton, M. L. Rothenberg and L. M. Matrisian: Matrix metalloproteinases: biologic activity and clinical implications. *J Clin Oncol*, 18(5), 1135-49 (2000)

200. A. F. Chambers and L. M. Matrisian: Changing Views of the Role of Matrix Metalloproteinases in Metastasis. *J Natl Cancer Inst*, 89(17), 1260-1270 (1997) DOI: 10.1093/jnci/89.17.1260

201. E. Ganea, M. Trifan, A. C. Laslo, G. Putina and C. Cristescu: Matrix metalloproteinases: useful and deleterious. *Biochem Soc Trans*, 35(Pt 4), 689-91 (2007)

202. M. Whittaker, C. D. Floyd, P. Brown and A. J. Gearing: Design and therapeutic application of matrix metalloproteinase inhibitors. *Chem Rev*, 99(9), 2735-76 (1999) DOI: 10.1021/cr9804543

203. B. Davies, P. D. Brown, N. East, M. J. Crimmin and F. R. Balkwill: A synthetic matrix metalloproteinase inhibitor decreases tumor burden and prolongs survival of mice bearing human ovarian carcinoma xenografts. *Cancer Res*, 53(9), 2087-91 (1993)

204. R. G. Chirivi, A. Garofalo, M. J. Crimmin, L. J. Bawden, A. Stoppacciaro, P. D. Brown and R. Giavazzi: Inhibition of the metastatic spread and growth of B16-BL6 murine melanoma by a synthetic matrix metalloproteinase inhibitor. *Int J Cancer*, 58(3), 460-4 (1994) DOI: 10.1002/ijc.2910580326

205. X. Wang, X. Fu, P. D. Brown, M. J. Crimmin and R. M. Hoffman: Matrix metalloproteinase inhibitor BB-94 (batimastat) inhibits human colon tumor growth and spread in a patient-like orthotopic model in nude mice. *Cancer Res*, 54(17), 4726-8 (1994)

206. G. Taraboletti, A. Garofalo, D. Belotti, T. Drudis, P. Borsotti, E. Scanziani, P. D. Brown and R. Giavazzi: Inhibition of angiogenesis and murine hemangioma growth by batimastat, a synthetic inhibitor of matrix metalloproteinases. *J Natl Cancer Inst*, 87(4), 293-8 (1995) DOI: 10.1093/jnci/87.4.293

207. M. B. Alcantara and C. R. Dass: Pigment epithelium-derived factor as a natural matrix metalloproteinase inhibitor: a comparison with classical matrix metalloproteinase inhibitors used for cancer treatment. *J Pharm Pharmacol*, 66(7), 895-902 (2014) DOI: 10.1111/jphp.12218

208. M. Pavlaki and S. Zucker: Matrix metalloproteinase inhibitors (MMPIs): The beginning of phase I or the termination of phase III clinical trials. *Cancer and Metastasis Rev*, 22(2-3), 177-203 (2003) DOI: 10.1023/A:1023047431869

209. L. M. Golub, H. M. Lee, M. E. Ryan, W. V. Giannobile, J. Payne and T. Sorsa: Tetracyclines inhibit connective tissue breakdown by multiple non-antimicrobial mechanisms. *Adv Dent Res*, 12(2), 12-26 (1998) DOI: 10.1177/08959374980120010501

210. B. Pasternak and P. Aspenberg: Metalloproteinases and their inhibitors-diagnostic and therapeutic opportunities in orthopedics. *Acta Orthop*, 80(6), 693-703 (2009) DOI: 10.3109/17453670903448257

211. A. Dufour, N. S. Sampson, S. Zucker and J. Cao: Role of the hemopexin domain of matrix metalloproteinases in cell migration. *J Cell Physiol*, 217(3), 643-51 (2008) DOI: 10.1002/jcp.21535

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