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REVIEW

Matrix metalloproteinases – an overview

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Abstract: Matrix metalloproteinases (MMPs, matrixins) are a family of secreted and membrane-bound zinc-dependent endopeptidases that have the combined capacity to degrade all the components of the extracellular matrix. These enzymes have a common zinc-binding motif (HEXXHXXGXXH) in their active site and a conserved methionine turn following the active site. MMP enzymes are strongly involved in a kaleidoscope of normal, pathological, physiological, and biological processes such as embryogenesis, normal tissue remodeling, wound healing, and angiogenesis, and in diseases such as atheroma, arthritis, cancer, and tissue ulceration. MMPs play a significant role in vascular remodeling. Broad-spectrum metalloproteinase inhibitors as potential therapeutics have been developed to explore the involvement of MMPs in various diseases.

Keywords: inflammation, wound healing, tissue remodeling, matrix metalloproteinases inhibitors, disease

Introduction

Proteases can be classified into serine proteases, cysteine proteases, aspartate proteases, and metalloproteases based on their residue or cofactor essential in catalysis. Metzincins are a universally expressed family of multidomain zinc (II)-dependent endopeptidases[1,2] whose members include metalloproteases such as matrix metalloproteinases (MMPs),[3] a disintegrin and metalloproteases (ADAMs),[4,5] the ADAMs with a thrombospondin-like motifs (ADAMTS),[6] the bacterial serralysins,[4] and proteases such as the astacins (including the meprins).[7] The selectivity of MMPs depends on the nature of the zinc-binding group that can have a significant effect in a relevant pathophysiological end point.[8] The metzincin superfamily is distinguished by a highly conserved motif containing 3 histidines that bind to zinc at the catalytic site and a conserved methionine that sits beneath the active site.[9] In addition to metzincins participation in the digestion of intake proteins and tissue development, maintenance, and remodeling, they are also involved in highly specific cleavage events to activate or inactivate themselves or other (pro) enzymes and bioactive peptides.[10]

MMPs have been found in vertebrates, invertebrates, and plants. They are distinguished from other endopeptidases by their dependence on metal ions as cofactors, their ability to degrade extracellular matrix (ECM), and their specific evolutionary DNA sequence. Plant MMPs are conserved proteolytic enzymes found in a wide range of monocotyledonous and dicotyledonous plant species. They play crucial roles in many aspects of plant physiology including growth, development, and the response to stress such as pathogen attack.[11]

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MMPs and their classification

On the basis of substrate specificity and homology, MMPs can be divided into 6 groups: collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs (MT-MMPs), and other MMPs[12–14] (Table 1). In all secreted MMPs, except MMP-7 and MMP-26, the catalytic domain is followed by a C-terminal hemopexin-like domain contributing to substrate and tissue inhibitor of metalloproteinase (TIMP) binding, proteolytic activity, and membrane activation; in most cases, there is a connecting hinge region between these domains. In MT-MMPs, the C-terminal domain attaches the molecule to the plasma membrane.[13,14] A type 2 transmembrane MMP (MMP-23) has cysteine array and immunoglobulin-like domains instead of the conserved hemopexin-like domain. It is expressed as an integral membrane zymogen with an N-terminal signal anchor and regulated by a single proteolytic cleavage for both activation and secretion.[15] MMPs share a common domain structure and are involved in the degradation of various ECM and nonmatrix components.[16,17] Their structural, molecular, and biochemical approaches are involved in various disease processes.[18] Researchers reported that the hemopexin domain was required specifically for tissue invasion events later in metamorphosis but not for tracheal remodeling, a finding with potential implications for inhibitor therapies.[19] There is no MMP-4, MMP-5 or MMP-6, and MMP-18 is a *Xenopus* enzyme.

Enzyme Commission classification of MMPs

As with all other enzymes, MMPs have an EC classification (Table 1), although this lags well behind the MMP designation.[20] As a system of enzyme nomenclature, every Enzyme Commission (EC) number is associated with a recommended name for the respective enzyme. Every enzyme code consists of the letters "EC" followed by 4 numbers separated by periods. Such numbers represent a progressively better classification of the enzyme. The EC classification is based on the reactions that enzymes catalyze. Six general categories have been defined. The first number specifies broad categories: EC 1 – oxidoreductases, EC 2 – transferases, EC 3 – hydrolases, EC 4 – lyases, EC 5 –isomerases, EC 6 – ligases. Within each of these broad categories, the MMP enzymes are further differentiated by a second number that more specifically defines the substrates on which MMPs act. For example, the first 2 EC number 3.4 specify substrate peptides C–N. Individual enzymes in each subclass are further defined by a third and a fourth number. For example, interstitial collagenase (MMP-1) has the code EC 3.4.24.7, whose components indicate the following groups of enzymes: EC 3.4 are hydrolases that act on peptide bonds forming 2 products from a substrate by hydrolysis. The collagens are the major components of bone and cartilage, and MMPs are the only known mammalian enzymes capable of degrading them. The collagenases are capable of degrading triple-helical fibrillar collagens from the N-terminus into distinctive 3/4 and 1/4 fragments. More specifically, cleavage of the triple helix of collagen at approximately three-fourths of the length of the molecule from the N-terminus at 775-Gly ┼ Ile-776 or 775-G ┼ I 776 in the α-1(I) chain occurs.

The basic concepts and the protocols to detect and purify all the members of MMPs family and their inhibitors have been reported.[21] Crystal structures of MMPs provided the exact domain organization, polypeptide fold, and main specificity determinants.[22,23] MMPs have specific domain structures, minimally consisting of a propeptide and a catalytic domain (MMP-7 and MMP-26), commonly with the addition of a hemopexin-like, 4-bladed, β-propeller domain (C-terminus; Figures 1–3) connected by a linker or hinge region (MMP-1, MMP-3, MMP-8, MMP-11, MMP-12, MMP-13, MMP-18, MMP-19, MMP-20, MMP-21, MMP-27, and MMP-28). These additional domains are important in substrate recognition and for inhibitor binding.[24–26] Others have these features plus a fibronectin-like domain of 3 type II repeats (MMP-2 and MMP-9), a transmembrane region and a short cytoplasmic "tail" (MMP-14, MMP-15, MMP-16, and MMP-24), or a glycosylphosphatidyl anchor (MMP-17 and MMP-25). The propeptide of the MMPs contains a "cysteine switch" motif, PRCGXPD, in which the cysteine residue interacts with the catalytic zinc domain to maintain inactivity by preventing a water molecule, essential for catalysis, from binding to the zinc atom until the propeptide has been removed by proteolysis.[27] The catalytic domains have the zinc-binding motif HEXGHXXGXXH, in which the 3 histidine residues ligate the zinc ion.[10] The exact domain organization of all MMPs has been extensively reviewed.[28]

The identification of 2 classes of MMP-11 substrates suggested that subsite preferences for MMP-11 cleavage were dependent upon other subsite residues.[29,30] Hwang and coworkers[31] have described a "degradomics" method that efficiently identifies substrates of MMP-14 in a complex protein mixture from human plasma proteins. Quantitative proteomics and mass spectrometry can be used to identify protease substrates in the cellular context.[32] Activation

Table 1 Classification of matrix metalloproteinase enzymes

Sr No.	MMP	No. of class	Enzyme	EC No.	Substrate(s)	Chromosome location (human)
1	MMP-1	Collagenases	Collagenase-1	EC 3.4.24.7	Collagens (I–III, VII, VIII, and X), gelatin, aggrecan, L-selectin, IL-1β, proteoglycans, entactin, ovostatin, MMP-2, MMP-9	11q22-q23
2	MMP-8	Collagenases	Collagenase-2/neutrophil collagenase	EC 3.4.24.34	Collagens (I–III, V, VII, VIII, and X), gelatin, aggrecan, fibronectin	11q21-q22
3	MMP-13	Collagenases	Collagenase-3	EC 3.4.24.B4 (preliminary BRENDA-supplied EC number)	Collagens (I–IV, IX, X, and XIV), gelatin, plasminogen, aggrecan, perlecan, fibronectin, osteonectin, MMP-9	11q22.3
4	MMP-18	Collagenases	Collagenase-4		Type I collagen	
5	MMP-2	Gelatinases	Gelatinase-A	EC 3.4.24.24	Gelatin, collagen IV–VI, X, elastin, fibronectin	16q13
6	MMP-9	Gelatinases	Gelatinase-A	EC 3.4.24.35	Collagens (IV, V, VII, X, and XIV), gelatin, entactin, aggrecan, elastin, fibronectin, osteonectin, plasminogen, MBP, IL-1β	20q11.2-q13.1
7	MMP-3	Stromelysins	Stromelysin-1	EC 3.4.24.17	Collagens (III–V, and IX), gelatin, aggrecan, perlecan, decorin, laminin, elastin, casein, osteonectin, ovostatin, entactin, plasminogen, MBP, IL-1β, MMP-2/TIMP-2, MMP-7, MMP-8, MMP-9, MMP-13	11q23
8	MMP-10	Stromelysins	Stromelysin-2	EC 3.4.24.22	Collagens (III–V), gelatin, casein, aggrecan, elastin, MMP-1, MMP-8	11q22.3-q23
9	MMP-11	Stromelysins	Stromelysin-3		Unknown (casein)	22q11.2
10	MMP-17	Stromelysins	Homology tostromelysin-2 (51.6%)			
11	MMP-7	Matrilysins	Matrilysin (PUMP)	EC 3.4.24.23	Collagens (IV, X), gelatin, aggrecan, decorin, fibronectin, laminin, elastin, casein, transferrin, plasminogen, MBP, β_4-integrin, MMP-1, MMP-2, MMP-9, MMP-9/TIMP-1	11q21-q22
12	MMP-26	Matrilysins	Matrilysin-2		Collagen IV, fibronectin, fibrinogen, gelatin, α(1)-proteinase inhibitor	11p15
13	MMP-14	MT-MMP	MT1-MMP (membrane type)	EC 3.4.24.80	Collagens (I–III), gelatin, casein, fibronectin, laminin, vitronectin, entactin, proteoglycans, MMP-2, MMP-13	14q11-q12
14	MMP-15	MT-MMP	MT2-MMP		Fibronectin, entactin, laminin, aggrecan, perlecan; MMP-2	16q13-q21
15	MMP-16	MT-MMP	MT3-MMP		Collagen III, gelatin, casein, fibronectin, MMP-2	8q21
16	MMP-17	MT-MMP	MT4-MMP			12q24.3
17	MMP-24	MT-MMP	MT5-MMP		Fibronectin, but not collagen type I or laminin	20q11.2
18	MMP-25	MT-MMP	MT6-MMP		Progelatinase A	16p13.3
19	MMP-12	Other enzymes	Macrophage metalloelastase	EC 3.4.24.65	Collagen IV, gelatin, elastin, casein, fibronectin, vitronectin, laminin, entactin, MBP, fibrinogen, fibrin, plasminogen	11q22.2-q22.3
20	MMP-19	Other enzymes	RASI 1		Type I collagen	12q14
21	MMP-20	Other enzymes	Enamelysin		Amelogenin, aggrecan, COMP	11q22.3

(*Continued*)

Table 1 (*Continued*)

Sr No.	MMP	No. of class	Enzyme	EC No.	Substrate(s)	Chromosome location (human)
22	MMP-21	Other enzymes	MMP identified on chromosome 1			
23	MMP-22	Other enzymes	MMP identified on chromosome 1			11q24
24	MMP-23	Other enzymes	From human ovary cDNA			1p36.3
25	MMP-28	Other enzymes	Epilysin			17q11.2
26	MMP-29		Unnamed			

Abbreviations: MMP, matrix metalloproteinases; IL, Interleukin.

is thought to be induced by an oxidative modification of the thiol residue, resulting in disruption of the Cys–zinc interaction and subsequent cleavage of the propeptide domain by autoactivation.[33]

Marchenko and Strongin[34] have reported the discovery, cloning, and characterization of *MMP*-28 cDNA gene, and the broad range of expression in carcinomas cells, as well as in normal adult and fetal tissues, suggested an important functional role for *MMP*-28. The remodeling of endometrial matrices is a necessary process in the coordination of gestational progress, and MMP-1, MMP-2, and MMP-9 are considered to play important roles in this process in cows and other mammalians. *MMP*-2 gene and its protein expression during peri-implantation coincided with ECM degradation in cows. MMP-2 also participated as a regulatory factor for placental release during labor, and its function may be adjusted by other MMP-related molecules, stimulators, and inhibitors.[35]

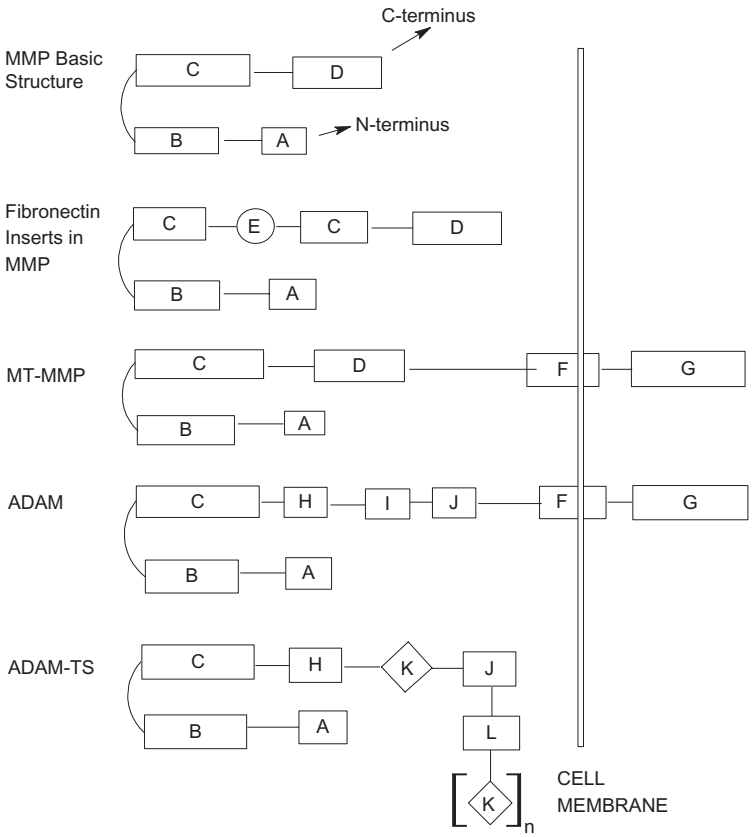

Figure 1 Domain structure of metzincins proteases – schematic representation. A: Pre domain (signal peptide); B: prodomain; C: catalytic domain; D: hemopexin-like domain; E: fibronectin type II insert; F: transmembrane domain; G: cytoplasmic tail; H: disintegrin domain; I: cysteine-rich domain; J: EGF-like domain; K: thrombospondin type I-like repeat; K: spacer region.
Abbreviations: MMP, matrix metalloproteinase; MT-MMP, membrane-type MMP; ADAM, a disintegrin and metalloprotease; ADAM-TS, ADAMs with thrombospondin-like motifs.

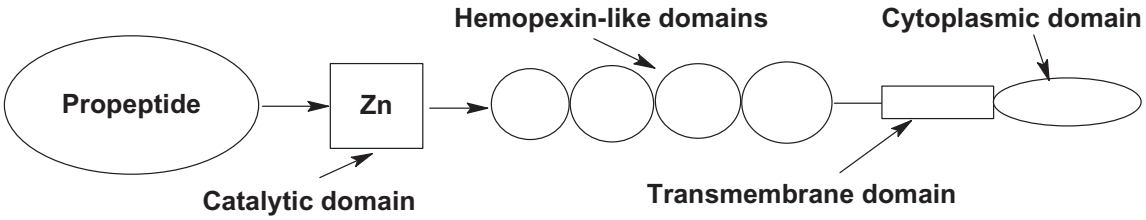

Figure 2 MMPS: general structure.

Human dental pulp has been shown to express high levels of MMP-13 RNA.[36] Sulkala and coworkers[37,38] results revealed that MMP-13 has been expressed and synthesized in pooled pulp samples of sound and carious teeth. cDNA microarrays demonstrated extremely high MMP-13 mRNA expression in pooled pulp samples of sound and carious teeth, with less pronounced expression of MMP-16 (MT3-MMP) and TIMP-1. The wide-scale expression of MMPs and TIMPs by mature human odontoblasts and pulp tissue suggested their participation in dentin matrix organization prior to mineralization, and that growth factors may further control dentin matrix modeling by differentially regulating individual MMPs.[39–41]

Ganea and colleagues[42] described the structural features of MMPs, with special emphasis on their interaction with specific inhibitors. The results on the isolation and characterization of the human MT1-MMP gene and its promoter indicated that the human MT1-MMP promoter has distinctive structural and functional features compared with other MMP genes, which may lead to a unique expression pattern and regulation during physiological and pathological processes.[43]

The conserved sequence (42)YGYL(45) has been reported in the propeptide domain of all 6 members of the MT-MMP subfamily, which was required for intramolecular chaperone function of these intrinsic membrane proteinases.[44]

The structure of a complex of the catalytic domain of MMP-1 with the N-terminal inhibitory domain of human TIMP-1 at 2.54 Å resolution was reported.[45] Mouse anti-human MMP-12 antibody (Catalog # MAB917) binding to forms of human MMP-12, containing the C-terminal hemopexin-like domain only, was reported. Moreover, this antibody does not recognize the fully mature, 20 kDa, proteolytic MMP-12 entity, where the C-terminal domain has been lost.[46]

Activation of MMPs

The experimental tools to study MMP activation *in vitro* and in cellular model systems have been discussed using the activation of proMMP-13 and proMMP-2.[47] MMPs can be activated *in vitro* by many mechanisms, including organomercurials, chaotropic agents, and other proteases.[48] A cysteine-sulfhydryl group within the conserved

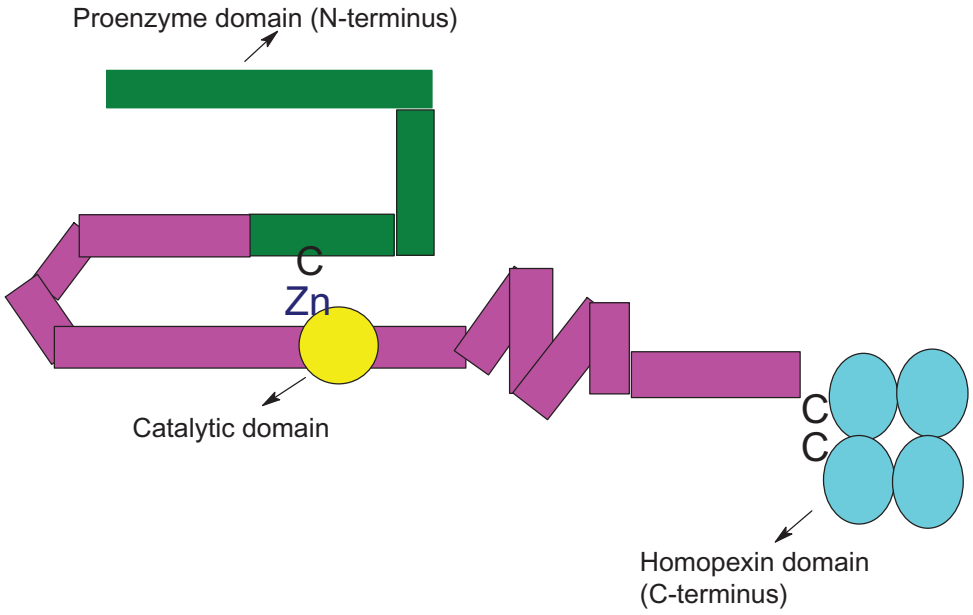

Figure 3 Propeptide interaction with the catalytic domain through a conserved cysteine residue (C) and the Zn^{2+} ion in the catalytic pocket (the so-called cysteine switch).

"PRCGXPD" motif in the propeptide domain of latent MMPs formed a bridge with the catalytic zinc, thereby preventing enzymatic activity; activation occurs when this linkage is disrupted by physical or chemical means.[49] Hyperhomocysteinemia activated myocyte mitochondrial MMP [MT-MMP-9] and induced mitochondrial permeability transition, leading to myocyte mechanical dysfunction by agonizing cardiomyocyte *N*-methyl-D-aspartate receptor-1.[50] MMP-2 is emerging as an important signaling protease implicated in the proteolytic regulation of various intracellular proteins in myocardial oxidative stress injury.[51] A brief description of activation reagents for collagenases has been reported.[52]

Cdc42 mediated the effects of vascular endothelial growth factor on activation of MMP-2 via p38 and actin cytoskeleton reorganization.[53] A recent study has demonstrated that NO is incapable of directly activating proMMP-9; however, S-nitrosylation of MMP-9 propeptide by NO donors is unrelated to their ability to regulate MMP-9 activity.[54] Hypoxia reduced MMP-2 activation and subsequent invasion of human cardiac myofibroblasts by reducing membrane expression of MT1-MMP to delay healing after myocardial infarction was reported.[55] Evidence from rodent models concerning the regulation of various cytokines, chemokines, and MMPs in response to ischemia suggested that gelatin-degrading MMPs mediated the neuroinflammatory response to H-I in the developing brain.[56] A representative zymography protocol for the study of MMPs has been reported.[57]

Certain MMPs expressed collagenase and gelatinase activity, thereby indicating inhibition of these enzymes by a herbal drug and significant anti-arthritic and/or wound healing activity.[58] Cardiac MMPs stimulated by the sympathomimetic action of angiotensin II aggravated chamber diastolic stiffening in models of subacute heart failure. Paolocci et al reported that MMP inhibition prevented such stiffening by favorably modulating high-energy phosphate stores more than by effects on matrix remodeling. Further, interaction between MMP activation and high-energy phosphate metabolism has an important role in mediating diastolic dysfunction.[59] Within the human myocardial interstitium, MMP proteolytic activity has been reported as a dynamic process under conditions such as myocardial arrest and reperfusion.[60]

ECM remodeling in the colon after exposure to ionizing radiation and the role of MMP subtypes specialized in basement membrane degradation were reported.[61] Using transient transfection system with a c-maf construct and MMP-13 promoter-luciferase constructs with specific mutations in transcription factor binding sites, Li et al found that c-maf significantly enhanced MMP-13 promoter activity via the AP-1 site. In addition, it has been shown by gene suppression with RNA interference technology that c-maf downregulation leads to a reduced expression of MMP-13.[62] Benzyl isothiocyanate has potential as an antimetastatic agent [63] as protein levels of MMP-2, MMP-9, and urokinase-plasminogen activator were reduced in a concentration-dependent manner. Free fatty acids augmented insulin stimulation of the MMP/TIMP balance of 3 proatherogenic MMPs and increased activities of 2 MAPKs (JNK and p38 MAPK), both responsible for stimulation of the production of proinflammatory cytokines followed by increased degradation of ECM was reported.[64] Losartan treatment was associated with normalization of MMP-1 activity and collagen content in volume overloaded hearts.[65] MMP-3 may be used to improve the adhesive properties of restorative materials because treatment of dentin surfaces by MMP-3 increased the penetration of the resin into the dentin tubules.[66] Zucker and colleagues reported that approximately 40% of plasma specimens from healthy individuals had detectable levels of the MMP-2:TIMP-1 complexes using specific antibodies. Further, they demonstrated binding between recombinant proMMP-2 and TIMP-1 proteins. However, the explanation for the presence of plasma proMMP-2:TIMP-1 complexes in selected healthy individuals remains to be determined.[67]

Activation of MMPs by EMMPRIN

High level of EMMPRIN (extracellular MMP inducer) also called CD147 expression has been shown to correlate with both MMP expression and cancer invasive potential.[68] Guo and coworkers have found that EMMPRIN not only stimulated the production of MMP-1 but also formed a complex with MMP-1 at the tumor cell surface.[69] Zhou and colleagues suggested that the expression of CD147 was upregulated during the differentiation of monocyte THP-1 cells to macrophage cells, and CD147 induced the secretion and activation of MMP-2 and MMP-9 and enhanced the invasive ability of THP-1 cells.[70] Potential regulators that influence EMMPRIN level and its MMP inducing activity include growth factors, hormones, glycosylation, and membrane shedding.[71] Schmidt and coworkers suggested that *Chlamydophila pneumoniae* induced MMP activity directly in monocytes through an EMMPRIN-dependent pathway and indirectly in smooth muscle cells via monocyte-derived cytokines.[72]

EMMPRIN induced, in the same cellular model, both MMPs and the serine protease urokinase plasminogen activator, whose concerted action in the breakdown of ECM during various physiopathological situations has been reported.[73]

In addition, EMMPRIN also promoted myofibroblasts' differentiation and tissue contraction through the induction of α smooth muscle actin, thus expanding on the mechanism by which EMMPRIN remodels ECM.[73] Zavadzkas et al[74] found that the selective induction of specific MMPs concomitant with the chronic myocardial overexpression of EMMPRIN was sufficient to produce adverse left ventricular remodeling and heart failure.[75]

EMMPRIN was involved in the inflammatory responses in the artery wall, and the inflammation and MMPs have been shown to play a critical role in the atherosclerotic lesion development and progression.[76] Attia and colleagues reported that EMMPRIN inhibited differentiation of myoblasts by an MMP-dependent activation of TGFb, suggesting that EMMPRIN inhibition may represent a novel strategy in the treatment of muscular degenerative disorders.[77]

The potential of therapeutic strategies targeting CD147 and CD44 to prevent prostate cancer metastasis and to overcome drug resistance has been reported.[78] Xie et al[79] indicated the involvement of EMMPRIN in the early stage of tooth germ development and morphogenesis, possibly by regulating the expression of MMP genes.

Activation of MMPs by uPA and its receptor uPA pathway

Stromal fibroblast promoted pancreatic cancer metastasis via activation of the uPA-plasminogen-MMP-2 cascade as uPA expression and fibroblastic uPAR expression were correlated with liver metastasis of human pancreatic cancer.[80] Cheng et al[81] provided a link between the lipopolysaccharide-induced cardiac dysfunction and the extracellular signal-regulated kinase 1/2 signaling pathway that mediated the upregulation of uPA, MMP-2, and MMP-9. Downregulated expression of maspin (a serpin and tumor suppressor gene), upregulated expression of uPA, and MMP-7 roles in the invasion and metastasis of gastric carcinoma may serve as effective markers of the biopathological behavior of gastric tumor.[82] Gondi and Rao discussed the therapeutic potential of small interfering RNA-mediated targeting of uPAR-uPA system and MMPs as therapeutic agents for the treatment of cancer.[83]

MMPs in plants

The origin of the MMP family of enzymes and their cognate inhibitors predates the divergence of plants and animals.[84] Genes encoding MMPs have been cloned from several plant species, including soybean, cucumber, and the model legume *Medicago trunculata*, and have also been identified in sugarcane. In *Arabidopsis thaliana*, a family of 5 very similar intronless MMP genes has been identified encoding proteins with the same characteristic domain structure as animal MMPs.[85] Results suggested that MMP activity may be involved in ECM modification, facilitating the cell division and expansion required during seed development, germination completion, and subsequent seedling establishment.[86] An MMP-like protein from *M. truncatula* (MtMMPL1) has been shown to be involved in the establishment of symbiotic interactions with *Sinorhizobium meliloti*.[11] Its total phenolic content may influence MMP-9 activity, and in this aspect, some of the plants with higher phenolic content exhibited various biological activities that could serve as potent inhibitors of the aging process in the skin.[87] Triterpenoid saponins 3-O-[O-beta-D-glucopyranosyl-(1→2)-O-beta-D-glucopyranosyl] oleanolic acid from the whole plants of Viola ibukiana Makino showed MMP-1 expression inhibition activities in a dose-dependent manner.[88] Two plant MMPs purified from soybean (*Glycine max*) leaves[89] and buckwheat (*Fagopyrum esculentum*) seeds[90] have been characterized. Evidence indicated that GmMMP2 may play a role in a novel defense mechanism in plants against pathogenic infections.[91]

MMPs in insects

Two types of peptidic metalloproteinase inhibitors have been identified in insects, one inhibiting microbial metalloproteinases and contributing to innate immunity, and the other putatively mediating regulation of endogenous MMPs during metamorphosis.[92] Results from the Tribolium model insect indicated that MMPs regulated tracheal and gut development during beetle embryogenesis, pupal morphogenesis, and innate immune defense reactions, thereby revealing the evolutionarily conserved roles of MMPs.[93] A mechanism mediated by a baculovirus-encoded fibroblast growth factor has been proposed that signals a previously undescribed stepwise cascade of protease activation wherein MMPs activate effector caspases, leading to remodeling of basal lamina lining tracheal cells associated with the intestine and culminating in the establishment of efficient systemic infections.[94]

MPs and polymorphisms

Minematsu et al reported a single-nucleotide polymorphism, a significant increase in allelic frequency of the C-1562T in MMP-9, in a Japanese smoker population with emphysema compared with matched smokers without emphysema.[95] Zhou and colleagues[96] reported that the same polymorphism in MMP-9 is associated with susceptibility to chronic

obstructive pulmonary disease (COPD) in the Han population of south China. However, in studies reported by Joos et al[97] polymorphisms in MMP-1 and MMP-12, but not MMP-9, were identified as being associated with a rate of decline in lung function of 590 continuing smokers who were chosen for having the fastest and slowest 5-year rate of deterioration in pulmonary task. Hirano et al[98] reported several polymorphisms of the TIMP-2 gene, which were significantly higher in COPD patients compared with control subjects, thereby possibly decreasing the level of TIMP-2 protein in these patients and leading to an increase in pulmonary MMPs.[99] The genetic evaluation by association study demonstrated that the MMP-13 gene, at least in part, contributed to the development of coronary artery lesions in Kawasaki disease.[100]

Wagner et al[101] tested 2 different MMP-9 DNA polymorphisms, a CA repeats and a cytosine to thymidine transition in the promotor sequence, for frequency in 52 patients with cervical artery dissection and compared the results with those of 52 healthy controls. No differences were found in the allelic distribution of either polymorphism, thereby indicating that alleles of these well-characterized functional polymorphisms of MMP-9 gene were not associated with structural alterations in the matrix of vessels of patients with cervical artery dissection.[101]

Zhai and coworkers genotyped 7 polymorphisms [*MMP*-1 -1607 1G/2G (rs1799750), *MMP*-2 C-1306T (rs243865) and C-735T (rs2285053), *MMP*-3 -1612 5A/6A (rs3025058), *MMP*-9 C-1562T (rs3918242), *MMP*-12 G-82A (rs2276109), and *MMP*-13 G-77A (rs17860523)] in 434 incident patients with hepatocellular carcinoma and 480 controls to explore MMP polymorphisms having any bearing on the risk of HCC.[102]

Results of another study suggested that the polymorphisms *MMP*-7 A-181G, *MMP*-8 C-799T, and *MMP*-21 C572T may not play a major role in mediating susceptibility to hepatocellular carcinoma.[103] Genetic factors play a role in COPD. Haq et al genotyped 26 single-nucleotide polymorphisms, providing comprehensive coverage of reported SNP variation, in *MMP*-1, *MMP*-9, and *MMP*-12 from 977 COPD patients and 876 nondiseased smokers of European descent and evaluated their association with disease alone and in haplotype combinations. Results showed that haplotypes of 2 single-nucleotide polymorphisms in *MMP*-12 (rs652438 and rs2276109) showed an association with severe or very severe disease.[104] Mutant alleles for *MMP-9* 2003 G>A and *MMP-2* −735 C>T are associated with reduced risk for allograft rejection, improved allograft survival in North Indian transplant recipients, and could serve as an ideal marker to predict pretransplant allograft outcome.[105]

Polymorphisms of the MMP-1 promoter may confer increased risk for idiopathic pulmonary fibrosis and reveal a putative gene–environment interaction between the −755 MMP-1 polymorphism and smoking in this disease.[106] To evaluate the effects of the MMP-3 -1171 and TIMP-1 372 T>C polymorphisms on the modified risk of ankylosing spondylitis, genotypes of 241 patients with AS and 241 controls were identified, and the findings highlighted the importance of the MMP-3 and TIMP-1 genes as crucial elements in ankylosing spondylitis development.[107] Pannu et al investigated the genetic association between polymorphisms in MMP-2 and MMP-9 and sporadic intracranial aneurysms. Results showed the involvement of MMP-9, but not MMP-2, in the pathogenesis of intracranial aneurysms.[108] Dos Reis and colleagues investigated the correlation between MMP-1, MMP-2, MMP-7, and MMP-9 polymorphisms with susceptibility to prostate cancer, and classic prognostic parameters of prostate cancer. Based on Gleason score, the polymorphic homozygote genotype of MMP-9 was more common in Gleason 6 or less tumors ($P = 0.003$), whereas a polymorphic allele in the MMP-2 gene was more common in Gleason 7 or greater tumors ($P = 0.042$). Thus, MMP-1 and MMP-2 protected against prostate cancer development, and MMP-9 was related to higher risk, in contrast.[109] MMP-2 genetic variants have been reported as an important mediator of functional outcome after stroke.[110] Gene–occupation interaction might play a certain role in exaggerating lumbar disc degeneration. In this regard, there is a possibility that subjects who carry mutant alleles 5A of MMP-3 and/or A of VDR-Apa are more vulnerable to lumbar disc degeneration, when they are exposed to whole-body vibration and/or bending and twisting under ergonomic loads.[111] Based on functional −1562 C/T polymorphism studies, MMP-9 gene may be mediating the relation between neuropsychiatric illnesses (schizophrenia, bipolar mood disorder, multiple sclerosis) and with cardiovascular disease and cancer.[112] MMP-2 -1306CC genotype increased the risk of developing acute myocardial infarction.[113] Analysis of the MMP family revealed that MMP-8 is often mutated in melanoma.[114]

Four polymorphisms in the promoter region of MMP-2, MMP-7, and MMP-9, which are MMP-2 −1306 C>T, MMP-2 −735 C>T, MMP-7 −181 A>G, and MMP-9 −1562 C>T, have been reported to be functional and may contribute to genetic susceptibility to cancers. Meta-analysis suggested that MMP-2 −1306 C>T, MMP-2 −735 C>T, and MMP-7 −181 A>G may play allele-specific roles in cancer development, while MMP-9 −1562 C>T may not be a major risk factor for most cancer types.[115]

MMPs and MMP inhibitors

An MMP inhibitor inhibits MMPs. They may be both endogenous and exogenous. The endogenous metalloproteinases are TIMPs. Exogenous MMP inhibitors include hydroxamic acid derivatives such as batimastat (BB-94), marimastat (BB-2516),[116] and SM-25453.[117,118] A highly specific thiirane gelatinase inhibitor SB-3CT blocked MMP-9 activity, including MMP-9-mediated laminin cleavage.[119] The structures of sarafotoxins and endothelins show an interesting topological similarity to the core of the metalloproteinase interaction sites of TIMPs. The effects of *N*-acetylation and other changes, as well as docking studies, supported the hypothesis that the engineered sarafotoxins bind to MMPs in a manner analogous to the TIMPs.[120] So far only Periostat (doxycycline hyclate, a nonspecific MMP inhibitor) has been approved for periodontal disease.[121] MMP enzymes can be inhibited by low doses of doxycycline below the levels likely to disrupt the oral flora.[122]

MMPs are inactivated by TIMP-1, TIMP-2, TIMP-3, and TIMP-4, which act by forming a 1:1 complex with the catalytic zinc in the MMPs site.[18] TIMPs may be either secreted as soluble proteins (TIMP-1, TIMP-2, and TIMP-4) or bound to ECM components (TIMP-3).[123] Structurally, TIMPs contain 2 domains. The N-terminal domain binds to the active site of active metalloproteases via a 1:1 noncovalent interaction, blocking the access of substrates to the catalytic site. In addition, The C-terminal domain of TIMP-1 and TIMP-2 binds to the hemopexin-like domain of proMMP-9 and proMMP-2, respectively. The latter binding is essential for the cell surface activation of MMP-2 by MMP-14.[124] The recent development of selective and nonselective inhibitors of MMPs provided new therapeutic possibilities for the treatment of inflammatory diseases.[125] The biological functions of TIMPs and their occurrence in disease have been reported.[126] Snoek-van Beurden and von den Hoff have reported zymographical techniques for the analysis of MMPs and TIMPs.[127]

TIMP-1

Myocardial ECM is highly susceptible to ischemic injury in acute myocardial infarction, and elevation of TIMP-1 may be a surrogate marker for increased ECM turnover.[128] TIMP-1, besides its MMP-inhibitory functions, appears to have independent influence on cell growth and apoptosis.[129] The findings that high tumor tissue levels of TIMP-1 might be associated with reduced benefit from classical adjuvant chemotherapy should be validated in larger prospective studies.[130] High levels of TIMP-1 in tumor tissue extracts were significantly associated with a poor prognosis in patients with primary breast cancer.[131] TIMP-1 binding to CD63 inhibits cell growth and apoptosis, and thus TIMPs function in a contextual fashion so that the mechanism of action depends on the tissue microenvironment.[132] On certain cell types, TIMP-1 and TIMP-2 can exhibit growth factor-like activity, and they inhibited the tumorigenic and metastatic phenotype in cancer cells.[133]

TIMP-2

In melanocytic cells, TIMP-2 gene expression may be regulated by microphthalmia-associated transcription factor.[134] The expression level of TIMP-2 protein can directly modulate the nuclear factor-κB pathway in human melanoma cells.[135] TIMP-2 regulated MMP-2 activity not only on the cell surface but also in the extracellular environment.[136] Increasing TIMP-2 in glioblastoma patients may potentially cause adverse effects, particularly in tumors containing high levels of MT1-MMP and MMP-2.[137]

TIMP-3

Black found that mice lacking TIMP-3 developed inflamed livers, and that the cause of this inflammation was an increase in TNF activity.[138] The structure revealed that TIMP-3 exhibited a fold similar to those of TIMP-1 and TIMP-2 and interacted through its functional binding edge, which consisted of the N-terminal segment and other loops, with the active-site cleft of TNF-converting enzyme in a manner similar to that of MMPs.[139] Recent findings suggested a novel therapeutic target to be explored for the improvement of cognitive function in humans based on the first evidence of TIMP-3 involvements in cognitive function and hippocampal MMP activity in mice.[140]

TIMP-4

Human TIMP-4 has been shown to bind to MMP-1, MMP-2, MMP-3, MMP-7, and MMP-9.[141] The use of the plasma concentration of the TIMP-4 as a biomarker for the diagnosis of heart failure has been reported.[142] Researchers reported a cardiopulmonary vasculature-specific role of TIMP-4 activation in systemic sclerosis.[143] TIMP-4 might play an important role in glandular secretion, endometrial tissue remodeling, and invasion of the trophoblast cells by regulating MMPs in a localized manner in the uteri of rhesus monkey during early pregnancy.[144] The information available for TIMP-4 provided a putative structural model to propose potential relevant directions toward solving its function and role in diseases such as cancer.[123] Results demonstrated upregulation of TIMP-4 in human cardiovascular disorders exhibiting

inflammation.[145] Radomski et al[146] have shown that TIMP-4 is the major inhibitor of MMPs in human platelets and also partially inhibited both platelet aggregation and recruitment. Pilka et al[147] have implicated TIMP-4 in human implantation, showing that endometrial TIMP-4 expression was high at midcycle and in hyperplasia but downregulated in malignant tumors. Koskivirta et al[145] have reported upregulation of TIMP-4 expression in human cardiovascular disorders exhibiting inflammation. Lee et al[148] have reported that TIMP-4 is the most potent endogenous inhibitor of MMP-26, and both proteins may play an integral role during the conversion of high-grade prostatic intraepithelial neoplasia to invasive adenocarcinoma in human prostate.

Other aspects of MMPs vs TIMPs

A strategy offered a novel method for the functional classification of MMPs on the basis of the characteristic profiles obtained using the diverse set of inhibitors.[149] A method has been reported for assessing the selectivity of inhibitors against multiple enzymes.[150]

Vanhoutte and Heymans highlighted the MMP-independent biological properties of the 4 TIMP family members, as well as cell-specific findings related to the process of cardiac remodeling, disease, and failure.[151] The analysis of MMPs and TIMPs may contribute to the improvement of orthodontic treatment regimens.[152] Immunohistochemical staining of formalin fixed, paraffin-embedded tissue sections of oral submucous fibrosis for MMP-1, MMP-2, MMP-9, and their tissue inhibitors TIMP-1 and TIMP-2 recorded statistically significant increase in the levels of stromal expression of MMP-1, MMP-2, MMP-9, TIMP-1 and TIMP-2.[153] A study has been reported, which can help to design novel inhibitors against gelatinase A.[154]

Results on local disturbances in the expression of MMPs and their inhibitors in the skin of patients with systemic sclerosis suggested that altered expression of MMPs/TIMPs may contribute to the development of local inflammatory infiltrated and tissue fibrosis in systemic sclerosis.[155] Scientists have described the substrate specificity, domain structure and function, the activation of proMMPs, the regulation of matrixin activity by TIMPs, and their pathophysiological implication.[14] The widespread expression of MMPs and TIMPs by mature human odontoblasts and pulp tissue suggested that they may participate in dentin matrix organization prior to mineralization, and that growth factors may further control dentin matrix modeling, not by regulating the synthesis of type I or III collagens as previously believed but rather by differentially regulating each MMPs and TIMPs.[156]

Clinical significance of MMPs

Abnormal activity of MMPs has been implicated in many disease processes. MMPs are considered key players in the regulation of both cell–cell and cell–ECM interactions, and the elucidation of their potential as drug targets in disease or as important features of the repair process will be dependent upon careful analysis of their role in different cellular locations and at different disease stages.[157]

MMPs and diabetic nephropathy

Dysregulation of MMPs and TIMPs may contribute to the development of nephropathy. Recent studies suggest that elevations in blood sugar may abnormally affect MMP enzyme activity. MMPs role in the pathogenesis of diabetic nephropathy (DNP) has been reported.[158] MMP-9 has been shown to be involved in the development of DNP, and trypsin-MMP cascade was involved in the pathogenesis of DNP.[159] The expression of MMP-9 in the kidney of mice with DNP was enhanced.[160] DNP was related with profound decrease of serum TIMP-2, TIMP-1, and MMP-2. An approximately 2-fold increase of MMP-9/TIMP-1 and MMP-2/TIMP-2 ratio was found in DNP when compared with diabetes with normal renal function.[161] Gharagozlian and coworkers have reported that serum levels of both MMP-9 and MMP-2 were significantly higher in subjects with type 1 diabetes compared to controls; however, no differences was observed between the 2 groups in the levels of TIMP-1 or TIMP-2, respectively.[162]

Sen and coworkers tested the hypothesis that induction of PPARγ by ciglitazone decreased tissue homocysteine level, thereby providing a protective role against DNP. In addition, glomerular MMP-2 and MMP-9 activities, as well as TIMP-1 expression, were increased strongly in diabetic mice and normalized with ciglitazone treatment. Interestingly, TIMP-4 expression was opposite to that of TIMP-1 in diabetic and ciglitazone-treated groups. Sen and coworkers further suggested that DNP exacerbated glomerular tissue level of homocysteine, and this caused further deterioration of glomerulus; ciglitazone, however, protected DNP in part by activating PPARγ and clearing glomerular tissue homocysteinemia.[163] Cornish and colleagues have reported that in human subjects, diabetes resulted in decreased TIMP-1 and MMP-1 proteins in renal glomeruli.[164]

Abdominal aortic aneurysms and MMPs

Increased MMP-12 levels in the abdominal aortic aneurysms (AAAs) implicate this protease in AAAs pathogenesis. Plasminogen-regulated macrophage migration in

inflammation via activation of MMP-9 may be an attractive approach to regulate inflammatory responses and AAA development.[165] The presence of activated MMP-9 and MMP-2 might contribute to the degradation of the ECM proteins that occurs during the development of aneurysms.[166] The majority of AAAs are small (<5.5 cm) and asymptomatic, and the natural history of AAAs is characterized by a low risk of rupture and exponential expansion, as the aortic diameter increases. MMPs as systemic biochemical markers of AAAs may contribute to diagnosis of unsuspected AAAs or to the surveillance of patients with small AAAs.[167] Patients with plasma levels of MMPs that did not return to basal levels were more prone to develop endoleaks and aneurysm expansion. However, MMPs appear to remain elevated for at least 3 months in open surgery while they return to baseline more rapidly following endovascular graft implantation.[168] Biomarkers identified for AAA include MMP-9.[169] Endovascular repair should be reserved for a subset of patients whose anatomy allows stent implantation, and those who following thorough investigations and information, accept the late inconvenience of the endovascular approach.[170] Circulating MMP-9 concentrations were found higher in patients with AAA than those in subjects without AAA.[171] Increased level of plasma MMPs values were reported in patients with thoracic aortic aneurysms, along with reduced tissue endogenous inhibitor-1 expression. Successful endovascular aneurysm repair results in MMP value normalization, whereas high levels persist in patients with endoleaks.[172] The levels of MMP-1, MMP-3, and MMP-9 increased immediately after surgery in patients operated for acute type A aortic dissection, whereas the levels of MMP-2 decreased. However, at 24 hours postoperatively, levels of MMP-1, MMP-2, and MMP-9 were almost equal to the preoperative ones.[173] An imbalance in the MMPs-to-TIMPs activity ratio may underlie the pathogenesis of vascular diseases such as AAAs, varicose veins, hypertension, and preeclampsia.[174]

Patients with acute aortic dissection, who have abdominal pain, have significantly higher serum MMP-9 levels than patients with surgical acute abdomen.[175] Increased expression of angiogenic factors such as MMPs are associated with the formation of cerebral arteriovenous malformations. Plasma MMP-9 levels are significantly elevated over controls at baseline, increased significantly immediately after surgery, and decreased to pretreatment levels during follow-up.[176]

Metalloproteinases and cancer

MMPs have come to represent important therapeutic and diagnostic targets for the treatment and detection of human cancers. The status of the MMP inhibitors currently in US Food and Drug Administration-approved clinical trials has been presented.[177] So far there is no promising drug-target therapy that has been evolved for the MMPs in potentially malignant and malignant lesions of the head and neck.[178] MMP-9 has been suggested as a tumor marker in diagnosis but not in prognosis of esophageal cancer.[179] According to Choi and coworkers, MMP-2, MMP-9, and hypoxia-inducible transcription factor (HIF-1α) expression could be used as a prognostic marker in papillary thyroid microcarcinoma.[180] Of the MMP-14/TIMP-2/MMP-2 complex, MMP-14 was the factor most significantly associated with the outcome of breast cancer and was an independent factor of poor overall survival when adjusted for clinical prognostic factors, but not for certain ancillary markers.[181] Sun and Stetler-Stevenson showed that TIMP-2 overexpression was sufficient to increase the nuclear factor-κB activity and protect cells from apoptosis.[182] Enhancement of MMP-9 expression was reported in gastric cancer compared to normal mucosa.[183]

MMP-7 can be a useful serum marker to show disease activity in malignant ovarian tumors.[184] The current understanding of MMP-2 and MMP-9 expression and activity in precancer and cancer lesions of cervical uterine has been reported.[185] Circulating MMP-9 and TIMP-1 were inferior to circulating tumor markers, carbohydrate antigen 19-9 (CA19-9), for detecting pancreatic ductal adenocarcinoma and did not improve the diagnostic accuracy when combined with CA19-9.[186] Recent observations showed that expression of MMP-13 promoted the survival of squamous carcinoma cells.[117] Furthermore, results indicated a potential clinical significance of serum TIMP-1 and MMP-9 measurements in the diagnosis and prognosis of patients with pancreatic cancer, respectively.[187]

(-)-Epigallocatechin-3-gallate suppressed salivary gland tumors by inhibiting metastasis through β1 integrin-mediated signaling.[188] Currently, many MMP inhibitors (MMPIs) are under development for treating different malignancies, and many MMP promoter polymorphisms have been reported in malignant tissues.[24] The progress of MMPIs from marine natural products has been reported and saccharoids, flavonoids, polyphones, and fatty acids are the most important groups of MMPIs derived from marine natural products.[189]

Using real-time polymerase chain reaction analysis, Walsh and coworkers examined the metamorphic RNA levels of MT1-MMP, MT3-MMP, TIMP-2, TIMP-3, and a potent gelatinase (Gel-A) that can be activated by the combinatory activity of a MT-MMP and a TIMP. In the metamorphic tail and intestine, the RNA levels of TIMP-2 and MT1-MMP

mirror each other and closely resemble that of Gel-A as all 3 are elevated during periods of cell death and proliferation. Conversely, MT3-MMP and TIMP-3 do not have similar RNA level patterns nor do they mimic the RNA levels of the other genes examined. Interestingly, TIMP-3, which has been shown to have antiapoptotic activity, is found at low levels in tissues during periods of apoptosis.[190]

MMPs vs chronic wounds and inflammation

A high level of MMP-1 was found essential to wound healing, while an excess of MMP-8 and MMP-9 was harmful. In addition, the MMP-1/TIMP-1 ratio has been reported as a predictor of wound healing in diabetic foot ulcers.[191–193] Kilpadi et al reported that wound fluids from pressure ulcer patients in home health or extended care settings treated with negative pressure wound therapy had decreased levels of MMP-3, MMP-9, and MMP-3:TIMP-1 ratios during the initial weeks of treatment compared with baseline.[194] Doxycycline and tetracyclines also inhibited MMP activity. *In vitro*, these have the ability to disrupt bacterial biofilms that are often present in chronic wounds. However, although the efficacy of these antibiotics has been demonstrated in periodontal disease, studies are needed to test their efficacy in chronic wounds.[195]

Lung disease and MMPs

Li et al[196] have elucidated signaling mechanisms operative in human bronchial epithelial and explained the disease-enhancing MMP-1 gene activation in response to diesel exhaust particles. The expression of MMP-2 and MMP-9 in lung disease and the role these gelatinases may play in disease progression were reported.[197] Rather than an individual MMP, a complex network of MMPs, together with inflammatory cytokines and other mediators, results in the distinct phenotype of inflammatory diseases, such as asthma, and has been reported in a particular disease.[198] The minor allele of a single-nucleotide polymorphisms in MMP12 (rs2276109) is associated with a positive effect on lung function in children with asthma and in adults who smoke.[199]

Chronic obstructive pulmonary disease and MMPs

Evidence suggested that asthma and COPD share common pathogenetic pathways.[200] MMP-12 is potentially an important therapeutic target for the treatment of COPD.[201] MMPs play both direct and indirect roles in matrix destruction associated with emphysema and may indirectly influence cytokine release and angiogenesis that could in turn influence the development and progression of COPD.[202] Based on the analysis of MMPs/TIMPs, Wong et al[198] speculated that in a particular disease, it may be a complex network of MMPs, rather than an individual MMP, together with inflammatory cytokines and other mediators results in the distinct phenotype of inflammatory diseases, such as COPD.

MMPs and malaria

Significant alterations in expression of the mRNA of 9 MMPs, 5 MT MMPs, TACE, and the 4 TIMPs were observed in different organs during cerebral malaria (CM). mRNAs of MMP-3, MMP-8, MMP-13, and MMP-14 in the spleen, MMP-8, MMP-12, MMP-13 and MMP-14 in the liver, and MMP-8 and MMP-13 in the brain increased. The increase in MMP expression in the brain was significantly less pronounced after infection of C57Bl/6 mice with the noncerebral strain Plasmodium berghei NK65, but it was similar in CM-susceptible C57Bl/6 and CM-resistant Balb/C mice upon infection with Plasmodium berghei ANKA. Furthermore, in comparison with C57Bl/6 mice, a larger increase in TIMP-1 and a marked >30-fold induction in MMP-3 were found in the brain of Balb/C mice, suggesting possible protective roles for TIMP-1 and MMP-3.[203]

MMP-9, able to disrupt the basal lamina, is possibly involved in the generation of hallmarks of CM, such as blood–brain barrier endothelium dysfunction, localized hemorrhages, and extravasation of phagocytic cells and parasitized red blood cells into brain tissues.[204] The effect of 3 months of chloroquine treatment on serum MMP-9 and TIMP-1 in patients with systemic lupus erythematosus suggested that chloroquine treatment may affect the MMP network.[205] TIMP-1 association with signs and symptoms of severe malaria and elevated MMP-8 levels were found in patients with severe or uncomplicated Plasmodium falciparum malaria.[206]

Central nervous system disease and MMPs

Agrawal and colleagues observed that MMPs go bad in neurological conditions, likely aided by the sudden and massive upregulation of several MMP members, and MMP inhibitors have therapeutic potential early after central nervous system (CNS) injury.[207] In ischemic brain injury, MMPs are implicated in various stages of the disease and may have a detrimental or beneficial role depending on the stage of brain injury.[208] Hu and coworkers[209] indicated that less-frequent injections of PEG minocycline-liposomes are an effective

alternative pharmacotherapy to daily minocycline injections for the treatment of CNS autoimmune diseases. Results suggested that inhibition of MMP-28 may be beneficial under conditions of dysmyelination.[210]

Cardiovascular disease and MMPs

Cardiovascular disease (CVD) is the leading cause of death, and the role of MMPs and the potential of their inhibitors in *de novo* atherosclerotic plaque destabilization, arterial remodeling, restenosis after balloon angioplasty and stenting, aneurysm formation, and heart failure have been reported.[211] Aortic stiffness is related to MMP-9 levels and serum elastase activity, not only in isolated systolic hypertension but also in younger, apparently healthy individuals, thereby suggesting that elastases including MMP-9 may be involved in the process of arterial stiffening and development of isolated systolic hypertension.[212] The role of MMPs and their inhibitors during embryonic cardiovascular development and the relation of these to the pathophysiology of adult cardiovascular diseases were reported.[213] MMP-1 serum levels were found associated with total plaque burden, but a specification of plaque morphology was not allowed.[214] Aquilante et al[215] observed that the metabolic syndrome and smoking were independently associated with elevated serum MMP-8 concentrations. A positive association between plasma Hg and circulating net MMP-9 and MMP-2 activities was reported. These findings provide a new insight into the possible biological mechanisms of Hg toxicity, particularly in CVDs.[216] During acute coxsackievirus B3 infection, MMP-9 halted virus propagation in the heart, promoted proper immune infiltration and remodeling, and preserved cardiac output.[217] Expression of MMP-1 mRNA may be correlated with the pathogenesis and activity of atherosclerosis in systemic lupus erythematosus.[218]

Rheumatoid arthritis, inflammatory diseases, and MMPs

Serum MMP-3 levels in rheumatoid arthritis (RA) patients have been reported to be higher than healthy controls, thereby indicating that it would be valuable to predict bone damage progression, especially in the early stage of RA. Further, it was suggested that MMP-3 could be a useful marker for follow up of anticytokine therapy. In this way, 2 enzyme immunoassay systems for serum MMP-3, namely, PANACLEAR MMP-3 "Plate" and MMP-3 "BS," have been approved in Japan as diagnostic tools for RA. However, these should be converted with the regression coefficient for using MMP-3 value for clinical practice.[219]

Immunofluorometric assay and enzyme-linked immunosorbent assay were used to compare gingival crevicular fluid (GCF) levels of MMP-8 and MMP-13 and TIMP-1 in patient with RA and systemically healthy counterparts with inflammatory periodontal disease.[220] The total amounts of MMP-8 were lower in the healthy control group than in RA-gingivitis, RA-periodontitis, and healthy-periodontitis groups. Similar GCF MMP-8 and MMP-13 levels in patients with RA and systemically healthy counterparts suggested that RA may create a tendency to overproduce these enzymes.[220] All forms of MMP-2 were inhibited by 2-hydroxyethyl methacrylate in a dose-dependent manner, implying MMP-2 inhibition *in vivo*.[221] Relationship between activation of inflammatory cells and tissue remodeling suggested new therapeutic possibilities for the treatment of inflammatory diseases.[222,223] The development of modulators of MMP/TIMP activity could be used as a new class of drugs for the treatment of severe sepsis.[224]

The lack of heparanase expression and activity was accompanied by alterations in the expression level of MMP family members, primarily MMP-2 and MMP-14.[225]

MMPs have been directly implicated in the pathophysiology of many arterial and venous disorders and remain an important potential therapeutic target.[226] The role of MMPs and TIMPs in the pathogenesis of chronic liver disease, as well as their possible use as noninvasive serum markers for inflammation and fibrosis in this pathology, has been reported.[227] Scientists suggested that MMP genes undergo dynamic changes over evolution; therefore, there is an immediate need to investigate whether MMP expression and functions during vertebrate development are conserved.[228] Independent from their antiviral activity, zidovudine and indinavir interfered directly with MMP production in glial cells, thus suggesting the possible therapeutical use in neurological diseases associated with MMPs involvement.[229]

Multiple sclerosis and MMPs

MMPs have been proposed as biomarkers in multiple sclerosis,[230] and MMP-25 is a promising drug target in multiple sclerosis.[231] Buhler et al[232] suggested that MMP-7 may facilitate immune cell access or restimulation in perivascular areas and provided a new therapeutic target to treat this disorder. Sang and colleagues hypothesized that the MMP-9 gene may be mediating the relationship of neuropsychiatric illnesses (schizophrenia, bipolar mood disorder, multiple sclerosis) that are comorbid with CVD and cancer.[233]

Other aspects

Nitric oxide scavenger, AMD6221, could ameliorate the increased generation of nitric oxide and increased MMPs' activities.[234,235] Wu and colleagues reported details regarding diagnostic and therapeutic methods for applying novel MMP-29 polypeptides to the diagnosis, treatment, and prevention of various diseases or disorders related to these polypeptides.[236] Recent studies in mice and flies point to essential roles of MMPs as mediators of change and physical adaptation in tissues, whether developmentally regulated, environmentally induced, or disease associated.[237]

Researchers proposed that increased apparent diffusion coefficient, which is a marker of vasogenic edema, is related to the activity of MMP-2 and MMP-9. MRI provides unique information that can be used to guide tissue studies of white matter injury.[238] MMPs do not properly work as peripheral blood biomarkers without taking into account the preanalytical impact of blood sampling.[239] Studies using MMP inhibitors and MMP knockout mice indicated that MMPs play essential roles in infection and in host defense against infection. Some basic concepts of infections caused by gram-negative bacteria, reviews reports describing MMP expression and inhibition, and studies with MMP-deficient mice in models of infection caused by gram-negative bacteria and of septic shock were reported.[240]

Conclusion and perspectives

MMPs (a multigenic family of proteolytic, zinc-dependent enzymes), displaying multidomain structures and substrate specificities, are involved in both the turnover and degradation of ECM proteins, processing, activation, or deactivation of a variety of soluble factors. Based on their substrate specificity and domain organization, MMPs may be classified as collagenases, gelatinases, stromelysins, membrane type, matrilysins, and other MMPs. Their synthesis and functions are regulated by three major mechanisms including transcriptional activation, post-transcriptional processing, and control of activity by TIMPs.

MMPs have attracted more attention because of their roles in diseases. They are believed to participate in embryonic development, arthritis, angiogenesis, morphogenesis, reproduction, tissue resorption and remodeling, tumor growth, progression, invasion, metastasis through breakdown of ECM, cell surface proteins, processing growth factors, cytokines, and chemokines. MMPs and TIMPs work together to remodel the ECM. TIMPs selectively inhibited certain MMP enzymes. Several classes of structures such as carboxylic acid derivatives; heterocyclic structures; hydroxamate moieties with a peptide, peptidomimetic, or nonpeptide backbone; biphenyl moieties with nonpeptide backbone; and tetracycline analogs are the most common low-molecular-weight compounds that have *in vitro* inhibitory activity against MMPs. These inhibitors can be used for human beings, animals, and other organisms. The recent development of selective and nonselective inhibitors of MMPs provided new insights in the relationship between activation of inflammatory cells and tissue remodeling. A number of MMP and TIMP family members that are differentially expressed in anterior cruciate ligament and medial collateral ligament might be involved in the differential matrix remodeling process, as well as the differential healing ability of anterior cruciate ligament and medial collateral ligament.

Roles of MMPs in various disorders or diseases include the following: cardiopulmonary vasculature-specific role of TIMP-4 activation in systemic sclerosis; MMP-3 and TIMP-4 polymorphisms affected angiographic coronary plaque progression in patients with type 2 diabetes and patients without diabetes; MMP-9 in several aspects of CNS activity; inhibition of the detrimental effect of MMPs with BB-94 following bilateral carotid artery occlusion induced global ischemia; an increased expression of MT1-MMP and TIMP-2 in periodontitis-affected gingival tissues; salivary levels of MMP-8 as biomarkers of periodontitis; increased MMP-9 and decreased TIMP-2 level in autoimmune diseases; elevated MMP-9 and TIMP-1 level in tuberculous meningitis cerebrospinal fluid samples; plasma MMP-1 and TIMP-1 in ulcerative colitis; human cytomegalovirus contribution to atherogenesis through specific effects on MMP-9 activity; TIMP-2 deficiency accelerated adverse post-myocardial infarction remodeling; an increased expression of MT1-MMP and TIMP-2 in periodontitis-affected gingival tissues; TIMP-3 deficiency impaired cognitive function in mice; MMP-9 and MMP-10 role in pterygium formation; TIMP contribution to pterygium invasion inhibition; TIMP-1/MMP-9 ratio in terms of severity and mortality in sepsis; a pathogenic role for MMP-9 in neuropsychiatric disorders such as schizophrenia, bipolar illness, and multiple sclerosis; and synthetic TIMPs for use in cancer prevention and treatment.

Disclosure

The authors report no conflicts of interest in this work.

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