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Matrix Metalloproteinase Inhibitors as Investigational and Therapeutic Tools in Unrestrained Tissue Remodeling and Pathological Disorders

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Abstract

Matrix metalloproteinases (MMPs) are zinc-dependent proteolytic enzymes that degrade various proteins in the extracellular matrix (ECM). MMPs may also regulate the activity of membrane receptors and post-receptor signaling mechanisms, and thereby affect cell function. The MMP family includes collagenases, gelatinases, stromelysins, matrilysins, membrane-type MMPs and other MMPs. Inactive proMMPs are cleaved by other MMPs or proteases into active MMPs, which interact with various protein substrates in ECM and cell surface. MMPs regulate important biological processes such as vascular remodeling and angiogenesis, and may be involved in the pathogenesis of cardiovascular disorders such as hypertension, atherosclerosis, and aneurysm. The role of MMPs is often assessed by measuring their mRNA expression, protein levels, and proteolytic activity using gel zymography. MMP inhibitors are also used to assess the role of MMPs in different biological processes and pathological conditions. MMP activity is regulated by endogenous tissue inhibitors of metalloproteinases (TIMPs), and the MMP/TIMP balance could determine the net MMP activity, ECM turnover, and tissue remodeling. Also, several synthetic MMP inhibitors have been developed. Synthetic MMP inhibitors include a large number of zinc binding globulins (ZBGs), in addition to non-ZBGs and mechanism-based inhibitors. MMP inhibitors have been proposed as potential tools in the management of osteoarthritis, cancer, and cardiovascular disorders. However, most MMP inhibitors have broad-spectrum actions on multiple MMPs and could cause undesirable musculoskeletal side effects. Currently, doxycycline is the only MMP inhibitor approved by the Food and Drug Administration. New generation biological and synthetic MMP inhibitors may show greater MMP specificity and fewer side-effects, and could be useful in targeting specific MMPs, reducing unrestrained tissue remodeling, and the management of MMP-related pathological disorders.

Keywords

aneurysm; angiogenesis; atherosclerosis; extracellular matrix; hypertension; remodeling; TIMP

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CONFLICT OF INTEREST

None

1. INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that degrade various proteins in the extracellular matrix (ECM). The first MMP was discovered in 1962 as a collagen proteolytic activity during the degradation of ECM proteins and resorption of the tadpole tail.[1] The MMP family has now grown to at least 28 enzymes. With the exception of MMP-7, MMP-23 and MMP-26, most members of the MMP family share sequence homology with MMP-1 (collagenase 1), and a common core structure typically consisting of a propeptide, a catalytic metalloproteinase domain, a hinge region, and a hemopexin domain.[2–5] MMPs are commonly classified on the basis of their domain organization and substrate preference into collagenases, gelatinases, stromelysins, matrilysins, membrane-type (MT)-MMPs and other MMPs.[6,7]

MMPs degrade various ECM substrates including collagen, elastin and laminin. MMPs may also interact with various bioactive molecules on the cell surface and G-protein coupled receptors (GPCRs), and thereby affect the cellular environment and signaling.[8,9] MMPs play a role in cell proliferation, migration (adhesion/dispersion), differentiation, angiogenesis, and tissue healing and repair. MMPs may also be involved in cell apoptosis, and the inflammatory and immune response.[10]

MMPs are regulated at different levels including mRNA expression, post-translational modification of the MMP protein, and stimulation of their enzymatic activity by various endogenous and exogenous activators. The proteolytic activity of MMPs is also regulated by endogenous tissue inhibitors of metalloproteinases (TIMPs).[7] MMP/TIMP imbalance could affect the net MMP activity, ECM turnover, and tissue remodeling, and could lead to metabolic and immune diseases, cancer, and cardiovascular disorders such as hypertension, atherosclerosis, and aneurysm.[6]

Changes in MMP expression/activity have been proposed as potential biomarkers for the diagnosis and prognosis of certain pathological disorders. MMP inhibitors have also been utilized to reverse the effects of MMPs and to assess whether MMPs play a role in a specific biological process or pathological condition. MMP inhibitors have also been evaluated as potential pharmacological tools in the management of osteoarthritis, cancer, and cardiovascular disorders. In addition to endogenous TIMPs, synthetic MMP inhibitors have been developed and include broad-spectrum and relatively specific MMP inhibitors.

In this chapter, we will discuss reports from Pubmed and other databases to provide an overview of the different MMP inhibitors and their potential effects in various biological processes and pathological conditions. We will briefly describe the MMP-substrate interaction as it would help to understand the interaction between TIMP and other inhibitors with the MMP molecule. We will then describe the different categories of MMP inhibitors. We will provide examples of the role of MMPs in tissue remodeling and biological processes in humans, experimental animals, blood vessels and vascular cells, and discuss how MMP inhibitors could be used to assess the role of MMPs in these processes, and as potential pharmacological tools in the management of cardiovascular disease, and other disorders such as cancer

2. MMP–SUBSTRATE INTERACTION

ECM segregates tissues from each other, provides anchorage and support for cells, regulates cell migration and intercellular communication, and provides a local depot for growth factors released by different cells. ECM proteins and other components provide a structural scaffold for tissue support, cell migration, differentiation and signaling, as well as epithelialization and wound repair. ECM has three main components; fibers, proteoglycans and polysaccharides. Fibers are largely glycoproteins that include collagen, which is the main ECM protein, and elastin, which is not glycosylated and provides plasticity and flexibility to certain tissues such as the arteries, lungs and skin. Laminin is a glycoprotein localized in the basal lamina of the epithelium. Fibronectin is a glycoprotein used by cells to bind to ECM, and can modulate the cytoskeleton to facilitate or hinder cell movement. Proteoglycans have more carbohydrates than proteins, and attract water to keep the ECM hydrated. Proteoglycans also facilitate binding of growth factors to the ECM milieu. Syndecan-1 is a proteoglycan and integral transmembrane protein that binds chemotactic cytokines during the inflammatory process. Other ECM proteins include glycoproteins such as vitronectin, aggrecan, entactin, fibrin and tenascin, and polysaccharides such as hyaluronic acid.[11]

MMPs regulate tissue remodeling and promote degradation of various ECM proteins. Collagen and elastin are essential for structural integrity of the vascular wall and are major MMP substrates. Collagen has various subtypes including collagen I, II, III, IV, V, VI, VII, VIII, IX, X, and XIV. Different MMPs break down various collagen subtypes with different efficacies. Other MMP substrates include aggrecan, entactin, fibronectin, gelatin, laminin, tenascin, and vitronectin. MMPs can also degrade myelin basic protein and casein. Casein is digested by different proteinases, and like gelatin, is used to measure MMP activity in gel zymography assays.[11]

MMPs catalytic activity requires zinc (Zn^{2+}) and a water molecule flanked by three conserved histidine residues and a conserved glutamate, with a conserved methionine acting as a hydrophobic base to support the structure surrounding the catalytic Zn^{2+}. During MMP-substrate interaction, Zn^{2+} is penta-coordinated with a substrate's carbonyl oxygen atom, one oxygen atom from the MMP glutamate-bound water, and the three MMP conserved histidines. This forms an oxy-anion transition state that can polarize the glutamic acid's oxygen atom, proximate the substrate scissile C-N bond, and induce it to act as reversible electron donor. This allows the substrate scissile bond to break, releasing the N-terminal portion of the substrate and forming an MMP-carboxylate complex. Another free H_2O is taken up, releasing the remaining carboxylate portion of the substrate and the free MMP.[12–16] Collectively, upon binding of the substrate, the Zn^{2+}-bound water attacks the substrate carbonyl group, and the transfer of protons through the conserved glutamate to the nitrogen of the scissile bond results in peptide cleavage.[17,18] Alternatively, Zn^{2+} may be penta-coordinated with a substrate's carbonyl oxygen atom, two oxygens from the MMP conserved glutamate, and two of the three conserved histidines. One oxygen from glutamate then performs a nucleophilic attack and breakdown of the substrate.[19]

The specificity of the MMP-substrate interaction depends on specific subsites or pockets (S) within the MMP molecule that interact with corresponding substituents (P) in the substrate. The MMP S1, S2, S3, …Sn pockets on the right side of Zn^{2+} and the primed S1', S2', S3', …Sn' pockets on the left side of Zn^{2+} confer binding specificity to the substrate P1, P2, P3, … Pn and primed P1', P2', P3', … Pn' substituents, respectively.[16] The MMP S1' pocket is the most critical for substrate specificity and binding. Among different MMPs, the MMP S1' pocket is extremely variable, and may be shallow (e.g. MMP-1 and MMP-7), intermediate (e.g. MMP-2, MMP-9, and MMP-13), or deep (e.g. MMP-3, MMP-8, and MMP-12).[12–14] The MMP S2' and S3' pockets are shallower than the S1' pocket, and, therefore, more exposed to solvents.[14] Second to the S1' pocket, the MMP S3 pocket is also important for substrate specificity.[2]

3. REGULATION OF MMP EXPRESSION/ACTIVITY

MMPs are regulated at multiple levels including transcription, secretion, activation of the zymogen proMMP form, inhibition by tissue inhibitors of metalloproteinases (TIMPs) and internalization by endocytosis. Hypoxia promotes MMP-2 and MMP-9 mRNA expression.[20] Extracellular MMP inducer (EMMPRIN, CD147, Basigin) is a widely expressed membrane protein of the immunoglobulin superfamily that has been implicated in tissue remodeling and in pathological conditions such as atherosclerosis, aneurysm, heart failure, osteoarthritis and cancer. High volume mechanical ventilation causes acute lung injury and is associated with upregulation of MMP-2, MMP-9, MT1-MMP and EMMPRIN mRNA expresion.[21] EMMPRIN, MMP-2, MT1-MMP and MT2-MMP are also overexpressed in venous leg ulcers, where unrestrained activation of MMPs could lead to excessive degradation of ECM proteins.[22]

MMPs are synthesized as pre-proenzymes and the signal peptide is removed during translation to generate proMMPs. ProMMPs have a 'cysteine switch' motif PRCGXPD in which the cysteine residue coordinates with the Zn^{2+} ion in the catalytic domain, keeping the proMMP in the inactive form.[23] Activation of proMMPs often takes place extracellularly by other MMPs or other proteases. For example, MMP-3 can transform proMMP-1 into active MMP-1.[24] ProMMP-2 activation takes place on the cell surface by most MT-MMPs, but not MT4-MMP,[25] a process that also requires TIMP-2.[26,27] ProMMP-2 forms a complex with TIMP-2 through their C-terminal domains, thus permitting the N-terminal inhibitory domain of TIMP-2 to bind to MT1-MMP on the cell surface. The cell surface-bound proMMP-2 is then activated by another MT1-MMP molecule that is free of TIMP-2. The MT1-MMP bound to TIMP-2 can act as a "receptor" for proMMP-2. The MT1-MMP-TIMP-2-proMMP-2 complex is then presented to an adjacent free MT1-MMP for activation.[28] The level of TIMP-2 may determine whether MT1-MMP cleaves its own substrate or activates proMMP-2.[29] Other MMPs such as membrane-bound MMP-11, MMP-23, and MMP-28 may be activated intracellularly via the endopeptidase furin, which selectively cleaves paired base residues.[30–33]

Oxidants produced by leukocytes and other cells can activate MMPs by oxidation of the prodomain thiol followed by autolytic cleavage. ProMMPs can be activated by reactive oxygen species (ROS).[34–37] ROS derived from foam cells can activate proMMP-2. Also,

nitric oxide (NO) may activate proMMP-9 during cerebral ischemia by reacting with the thiol group of the cysteine switch and forming an S-nitrosylated derivative.[37] MMPs can be activated by thiol-modifying agents such as 4-aminophenylmercuric acetate, mercury chloride, and N-ethylmaleimide, oxidized glutathione, sodium dodecyl sulfate, and chaotropic agents by disturbing the cysteine-Zn^{2+} interaction at the cysteine switch. MMPs can also be activated by low pH and warm temperature.[38] Other MMPs such as MMP-9 are activated mainly by plasmin.[39] MMP-7 is activated by both MMP-3 and hypochlorous acid, a product of myeloperoxidase in macrophages of atherosclerotic plaques. MMP-7 can in turn activate MMP-1.[34,40]

4. TISSUE INHIBITORS OF METALLOPROTEINASES (TIMPs)

MMPs are inhibited by both endogenous and exogenous inhibitors. TIMPs are endogenous MMP inhibitors that bind MMPs in a 1:1 stoichiometry (Fig. 1).[2,12] TIMPs have an N-terminal domain (125 aa) and C-terminal domain (65 aa); each containing 3 disulfide bonds. The N-terminal domain folds as a separate unit and is capable of inhibiting MMPs.[41,42] The Cys1 is important for chelating the active site Zn^{2+} with its N-terminal α-amino group and carbonyl group, thereby expelling the water molecule bound to the catalytic Zn^{2+}. The TIMP molecule wedges into the active-site cleft of MMP in a manner similar to that of the substrate (Fig. 2). Four homologous TIMPs have been identified and termed as TIMP-1, TIMP-2, TIMP-3 and TIMP-4. TIMP-1 and TIMP-3 are glycoproteins, while TIMP-2 and TIMP-4 do not contain carbohydrates. TIMPs can inhibit multiple MMPs with different efficacies. For example, TIMP-2 and -3 inhibit MT1-MMP and MT2-MMP, whereas TIMP-1 is a poor inhibitor of MT1-MMP, MT3-MMP, MT5-MMP and MMP-19.[43] Also, while TIMP-1 and TIMP-2 bind MMP-10 (stromelysin-2), the binding is 10-fold weaker than that to MMP-3 (stromelysin-1).[44] TIMP-1 has a threonine-2 (Thr2) residue that interacts with the MMP S1’ pocket in a manner similar to that of a substrate P1’ substituent, largely determining the affinity to MMP-3. Substitutions at Thr2 affect the stability of the TIMP-MMP complex and the TIMP specificity to different MMPs. For instance, substitution of Thr2 by alanine results in a 17-fold decrease in the ability of TIMP-1 to bind MMP-1 compared with MMP-3.[45]

TIMPs are widely distributed in many tissues and organs. A change in either MMP or TIMP levels could alter the MMP/TIMP ratio and cause a net change in specific MMP activity. MMP inhibition by TIMPs would decrease degradation of ECM proteins. On the other hand, serine proteinases such as neutrophil elastase could inactivate TIMPs, spare MMPs from inhibition by TIMPs, and in turn favor breakdown of ECM proteins.[46,47]

In addition to inhibiting MMPs, TIMPs can inhibit a broader spectrum of metalloproteinases. TIMP-1 inhibits a disintegrin and metalloproteinase-10 (ADAM-10) while TIMP-2 inhibits ADAM-12.[48,49] TIMP-3 has a much broader metalloproteinase inhibition profile including ADAM-10, ADAM-12, and ADAM-17 as well as a disintegrin and metalloproteinase with thrombospondin motif ADAMTS-1, ADAMST-2, ADAMST-4 and ADAMST-5.[49–52] This broad-spectrum metalloproteinase inhibition by TIMP-3 is best illustrated by the observation that TIMP-3 ablation in mice is associated with emphysema-

like alveolar damage and faster apoptosis of mammary epithelial cells after weaning, whereas TIMP-1 or TIMP-2-null mice do not exhibit such abnormalities.[53,54]

5. OTHER BIOLOGICAL AND PLEIOTROPIC INHIBITORS OF MMPS

In addition to endogenous TIMPs, α2-Macroglobulin is another endogenous MMP inhibitor found in blood and tissue fluids. MMP activity is partly regulated by α2-macroglobulin and related proteins. Human α2-Macroglobulin is a glycoprotein consisting of four identical subunits. α2-Macroglobulin is a wide-spectrum proteinase inhibitor that inhibits most endopeptidases including MMPs, by entrapping them within the macroglobulin. The complex is then rapidly internalized and cleared by endocytosis via low density lipoprotein receptor-related protein-1.[55]

Other proteinase inhibitors may inhibit specific MMPs, although their mechanism of action is unclear.[56] For instance, a secreted form of β-amyloid precursor protein or a C-terminal fragment of procollagen C-proteinase enhancer protein can inhibit MMP-2. Reversion-inducing-cysteine-rich protein with kazal motifs (RECK) is a glycosyl phosphatidylinositol (GPI)-anchored glycoprotein expressed in many cells including vascular smooth muscle cells (VSMCs), and has been shown to inhibit MMP-2, MMP-9 and MMP-14 when expressed in transfected human fibrosarcoma-derived cell line HT1080.[57] Tissue factor pathway inhibitor-2 is a serine proteinase inhibitor that can inhibit MMP-1 and MMP-2.[58]

Monoclonal antibodies have high specificity and affinity for specific MMPs and can detect MMPs in the body fluids and tissues.[6] Monoclonal antibodies REGA-3G12 and REGA-2D9 react specifically with MMP-9, and do not cross-react with MMP-2. MMP inhibition by REGA-3G12 involves the catalytic domain and not the Zn^{2+} binding region or the fibronectin region. REGA-1G8 is less specific and cross reacts with serum albumin. Patients with Crohn's disease suffer from recurring fistulae, and MMP-9, a type IV collagenase, is upregulated in crypt abscesses and around fistulae, suggesting a role of MMP-9 in fistula formation. Interestingly, in a mouse heterotopic xenograft model of intestinal fibrosis, treatment with anti-MMP-9 monoclonal antibody reduced collagen deposition and hydroxyproline content in day-14 intestinal grafts, suggesting reduced fibrosis. Anti-MMP-9 antibody may be a promising therapeutic strategy for fibrosis-related complications of inflammatory bowel disease.[59]

The hemopexin domain could also be a potential target for MMP antibodies. The hemopexin domain of MMP-1 is essential for the specificity of its catalytic domain to cleave collagen. Also, MMP-2 is localized at extracellular sites by its fibronectin domains and MT1-MMP (MMP-14) requires the hemopexin domain for cell surface clustering and ability to activate proMMP-2.[60] The hemopexin domain can also be used to target specific MMP substrates, and prevent their degradation by MMPs. Studies have generated glutathione-S-transferase (GST) fusion proteins containing MMP-9 hemopexin domain or truncated forms corresponding to specific structural blades (B1–B4) of the MMP-9 hemopexin domain. GST-MMP-9 hemopexin domain inhibited MMP-9-dependent degradation of gelatin, but not other MMP-9 substrates such as a fluorogenic peptide, αB crystalline, or nonmuscular actin. The MMP-9 hemopexin domain may shield gelatin and specifically prevent its binding to

and degradation by MMP-9. Of note, GST-MMP-9 hemopexin domain also abolishes the degradation of gelatin by MMP-2, confirming that it is not an MMP-9 antagonist. ELISA assays demonstrated that GST-B4 and GST-B1 specifically bound to gelatin. These findings suggest new functions of MMP-9 hemopexin domain attributed to blades B4 and B1 and should help in designing specific inhibitors of gelatin degradation.[61]

Small interference RNA (siRNA) specific to certain MMPs have been developed and can be used in assessing the role of a specific MMP in a biological process. For instance, MMP-2 siRNA inhibits the transcriptional product of MMP-2.[62] Targeted delivery of MMP siRNA could decrease MMP expression and unrestrained ECM turnover and tissue remodeling in localized pathological conditions such as aneurysm, varicose veins, osteoarthritis and tumors. For instance, specific inhibition of either MMP-2 or MT1-MMP by specific shRNAs hampers melanoma cell migration and invasion.[63] Gene therapy has shown some success in animal models, and with the design of efficient and safe gene delivery into target tissues downregulation of MMPs using siRNA or overexpression of TIMPs may have clinical applications.[64]

Sulodexide (SDX) is a highly purified glycosaminoglycan containing fast-moving heparin fraction (80%) and dermatan sulfate (20%). SDX has pro-fibrinolytic, anti-thrombotic, anti-inflammatory and endothelial protective activity in the vascular system that could be partly related to its effects on MMPs. SDX decreases MMP-9 secretion from white blood cells without MMP prodomain displacement,[65] and may specifically inhibit proteases with cysteine residues such as MMP-2 and MMP-9.[66]

The intracellular signaling pathways and the upstream inducers and downstream transcription factors that affect MMP or TIMP mRNA expression may serve as potential targets for MMP inhibition. Studies have generated an anti-EMMPRIN antibody directed against a specific epitope that successfully inhibited the production of MMP-9 in tumor cell-macrophage *in vitro* co-culture systems. The EMMPRIN antibody also inhibited *in vivo* tumor progression in both the RENCA renal cell carcinoma and CT26 colon carcinoma subcutaneous tumor models, and reduced tumor size and number of metastatic foci in the 4T1 orthotopic model. This was achieved by inhibiting angiogenesis as assessed by immunohistochemical staining for the endothelial marker CD31, by inhibiting tumor cell proliferation as assessed by the staining for Ki-67, and by enhancing tumor cell apoptosis as assessed by the TUNEL assay. The EMMPRIN antibody also recruited more macrophages into the tumor, and skewed the tumor microenvironment for macrophages from TGF-β-dominated anti-inflammatory microenvironment to a less immunosuppressive one, thus allowing improved ability of stimulated macrophages to perform antibody-dependent cell cytotoxicity and to kill tumor cells. These findings suggest that EMMPRIN antibody maps the epitope capable of inducing MMPs, and place EMMPRIN as a potential target to modulate MMPs in cancer therapy.[67] Blockade of mitogen-activated protein kinase (MAPK), NF-κB or activator protein (AP)-1 has shown some efficacy *in vitro* and in animal models of arthritis, and these effects may be partly due to changes in MMP expression.[68] Also, biologics may block inflammatory cytokines and reduce MMP expression in different tissues. Statins may inhibit MMPs through pleiotropic effects. For instance, atorvastatin inhibits MMP-1, MMP-2, and MMP-9 expression in human retinal pigment epithelial

cells,[69] and MMP-1, MMP-2, MMP-3, and MMP-9 secretion from rabbit macrophages and cultured rabbit aortic and human saphenous vein VSMCs.[70] Also, in a rat model of heart failure, pravastatin suppressed the increase in myocardial MMP-2 and MMP-9 activity.[71]

6. SYNTHETIC MMP INHIBITORS

Divalent ions can influence MMP release and activity. Cu^{2+} ion decreases the secretion of MMP-2.[72] Deep sea water components such as Cu^{2+}, Mg^{2+}, and Mn^{2+} inhibit proliferation and migration of cultured rat aortic smooth muscle cells (RASMCs) by inhibiting not only extracellular signal–regulated kinase (ERK1/2) and MAPK kinase (MEK) phosphorylation, but also MMP-2 activity,[73] a mechanism that may involve interference with Zn^{2+} binding at the MMP catalytic active site. Zn^{2+} chelators deprive MMPs from the Zn^{2+} ion critical for their activity.[74] MMP inhibition can also be achieved via a Zn^{2+} binding group, e.g. hydroxamic acid, carboxylic acid, or sulfhydryl group. Other approaches to inhibit MMPs are through non-covalent interaction with sites on the MMP backbone such as the S1', S2', S3', and S4' pockets to which the MMP inhibitor side chains bind in a fashion similar to that of the substrate P1', P2', P3', and P4' substituents. The efficacy and specificity of inhibition are determined by which pockets are blocked for a given MMP.[75] Several synthetic MMP inhibitors have been developed and some of them have been evaluated as investigational or therapeutic tools for degenerative diseases and vascular disorders (Table 1).[14] However, because of the inherent flexibility in the MMP active-site, accurate modeling of specific MMP–inhibitor complexes has been severely limited.[76]

The crystal structure of the MMP-11 catalytic domain during the interaction with a phosphinic inhibitor mimicking a D,L-peptide has suggested that the MMP-11 S1' pocket forms a tunnel running through the enzyme. This open channel is filled by the MMP inhibitor P1' group which adopts a constrained conformation to fit the MMP-11 S1' pocket, together with two water molecules interacting with the MMP-11 specific residue Gln215. The presence of a water molecule interacting with one oxygen atom of the MMP inhibitor phosphinyl group and the proline residue of the MMP Met-turn suggests how the intermediate formed during proteolysis may be stabilized. Furthermore, the hydrogen bond distance observed between the methyl of the phosphinic group and the carbonyl group of Ala182 mimics the interaction between this carbonyl group and the amide group of the cleaved peptidic bond. This crystal structure provides a good model to study the mechanism of proteolysis by MMPs.[77]

The following sections provide brief description of different classes of MMP inhibitors. For detailed information and the original references regarding the different categories of MMP inhibitors, their MMP specificity, IC_{50} or K_i, and their potential use in certain pathological conditions, the reader is referred to other reviews.[11,78]

6.1. Derivatives of Early ZBGs

Because MMPs require catalytic Zn^{2+} for their activation and cleavage of their substrates, the design of MMP inhibitor has traditionally utilized Zn^{2+} binding globulin (ZBG) (Table 1). ZBGs displace the Zn^{2+}-bound water molecule and inactivate the MMP enzyme.[79] ZBG

also acts as an anchor to lock the MMP inhibitor in the MMP active site and direct the backbone of the inhibitor to enter the MMP substrate-binding pockets.[14]

Early MMP inhibitors included hydroxamic acids (ZBG1), carboxylates (ZBG2), thiols, and phosphonic acids (phosphorus-based ZBGs).[17] Hydroxamic acids derivatives were preferred because of the relative ease of their synthesis, and their strong binding to MMPs.[80–83] The effectiveness of hydroxamates stems from the hydrogen bonding between the heteroatoms of the ZBG and the neighboring conserved amino acids in the MMP active site. Some hydroxamate- and carboxylate-based MMP inhibitors show some selectivity to certain MMPs.[84–88] However, while hydroxamates are potent MMP inhibitors, they have poor oral bioavailability, inhibit multiple MMPs, and therefore cause musculoskeletal side effects.[89,90]

Hydroxamic acid derivatives include succinyl, sulfonamide, and phosphinamide hydroxamates.[75,91,92] Batimastat (BB-94), marimastat (BB-2516), and ilomastat (GM6001) are broad spectrum succinyl hydroxamates with a collagen mimicking structure that inhibits MMPs by bidentate chelation of the active site Zn^{2+}.[75,93] Other ZBGs include carboxylic acids, sulfonylhydrazides, thiols, aminomethyl benzimidazole-containing ZBGs, phosphorous-based ZBGs, nitrogen-based ZBGs, and heterocyclic bidentate chelators.[14,17,94]

Hydrazide (ZBG3) and sulfonylhydrazide (ZBG4) analogs of the hydroxamate MMP inhibitor illomastat have been developed (Table 1).[95] Sulfonylhydrazide 9 is a potent inhibitor of MMP-1, MMP-2, and MMP-9.[96] Mercaptosulfide inhibitors (ZBG8) target MMP-14. MMP inhibitors with phosphorus-based ZBGs show improved MMP selectivity. Inhibitor 18 is a potent phosphonate inhibitor with relative selectivity for MMP-8 (neutrophil collagenase).[97] Other phosphorus-based ZBGs include carbamoyl phosphonate ZBG (ZBG9).[14]

ZBGs have a net negative charge that prevents them from penetrating the cell and restricts their actions to the extracellular space, and therefore reduces their cell toxicity.[98] Some ZBGs have shown relative selectivity for MMP-2 and have been evaluated in tissue and animal models of angiogenesis and tumor invasion. Compound 20 is more specific to MMP-2 than MMP-1, MMP-3, MMP-8, and MMP-9. In a murine model of metastatic melanoma, intraperitoneal administration of compound 20 at 50 mg/kg/day for three weeks caused 55% reduction in lung metastasis.[99]

Compound 21 is a carbamoyl phosphonate MMP inhibitor with greater selectivity to MMP-2 and MMP-9 than MMP-1, MMP-3, MMP-8, MMP-12, and MMP-13. Compound 21 inhibits cell invasion in Matrigel assay in a concentration-dependent fashion, and prevents tumor colonization in a murine melanoma model when administered orally or intraperitoneally. Compound 21 reduces tumor growth and metastasis in a murine model produced by implantation of human prostate tumor cells in immunodeficient mice. Compound 21 is water soluble at physiological pH and does not cause acute toxic effects at the doses used in the murine models.[98]

Research has been directed toward the development of MMP inhibitors with increased selectivity toward specific MMPs. The development of highly specific synthetic active-site-

directed MMP inhibitors necessitates identifying the specific structural features of each individual MMP that can be exploited to obtain the desired selectivity. Some ZBGs could have the potential to be used clinically if their potency and selectivity toward specific MMPs are enhanced and their targets are better-defined using site-specific delivery.[100] For example, a series of biphenyl sulfonamide carboxylate MMP inhibitors with high selectivity for MMP-13 were designed for treatment of osteoarthritis.[100] Also, the carboxylic acid scaffold of those MMP inhibitors was used to develop selective MMP-12 inhibitors for treatment of chronic obstructive pulmonary disease,[101] Selective hydroxamic acid inhibitors of MMP-2 have been developed as potent anti-angiogenic agents, and inhibitor 7 is the most selective MMP-2 inhibitor of this series.[86] Another hydroxamate MMP inhibitor with specificity towards MMP-3 was designed for treatment of chronic non-healing wounds.[88] Other ZBGs have been developed to improve selectivity, bioavailability, and pharmacokinetics, and include oxygen, nitrogen, and sulfur donor–atom ligands and monodentate, bidentate, and tridentate chelators.

6.2. Nitrogen-based ZBGs

Nitrogen-based ZBGs (ZBG10–16) have binding preference to late transition metals and improved selectivity towards Zn^{2+}-dependent enzymes.[102,103] Compound 22 is an adequate inhibitor of MMP-9 with little effects on MMP-1, MMP-2, and MMP-12. Nitrogen-based ZBGs such as the pyrimidine-2,4,6-trione and dionethione inhibitors have been studied extensively. The pyrimidine-2,4,6-trione group is found in many FDA-approved drugs including barbiturates, and the metabolic disposition and bioavailability of these compounds have been well-studied.[104] Pyrimidine-2,4,6-trione MMP inhibitors have shown relative specificity toward gelatinases and potential usefulness as anticancer drugs.[105] As part of the development of osteoarthritis drugs, pyrimidine-2,4,6-trione MMP inhibitors have been optimized to inhibit MMP-13,[106–109] and have shown 100-fold selectivity for MMP-13 over MMP-2, MMP-8, and MMP-12.[108]

Compound 23 was evaluated for its anti-angiogenic, anti-invasive, and anti-tumorigenic, activity. At concentrations as low as 10 nM, compound 23 shows anti-cancer efficacy in both *in vitro* and *in vivo* models, and inhibits tumor invasion by 85%.[110]

6.3. Heterocyclic bidentate ZBGS

Heterocyclic bidentate chelators ZBG20–30 were developed as MMP inhibitors.[94] Compared with hydroxamic acids. heterocyclic bidentate ZBGs have better biostability and tighter Zn^{2+} binding due to ligand rigidity and, in some cases, the presence of sulfur donor atoms.[111,112] Heterocyclic bidentate ZBGs are more potent in inhibiting MMP-1, MMP-2, and MMP-3 than acetohydroxamic acid,[94] and show low toxicity in cell viability assays.[113] Compound 25 is a pyrone-based MMP inhibitor that is more selective toward MMP-3 than MMP-1 and -2.[114] Compound 26 is a potent inhibitor of MMP-2, MMP-3, MMP-8 and MMP-12, with less effects on MMP-1, MMP-7, MMP-9, and MMP-13.[115] In a rat model of cardiac ischemia/reperfusion injury, treatment of the heart with compound 26 (5 μM) recovered more than 80% of the heart's original contractile function compared with 50% in control nontreated hearts.[115]

Other ZBGs include 6-, 7-, and 8-membered heterocyclic chelators as 1-hydroxy-2-piperidinone, 1-hydroxyazepan-2-1, 1-hydroxyazocan-2-1, and 1-hydroxy-1,4-diazepan-2-1.[116] Compound 27 is highly selective to MMP-1 and moderately selective to MMP-3. Compound 27 has a 47 hour half-life when administered intravenously at 2 mg/kg in rats, and has been shown to reduce brain edema in a mouse model of cerebral ischemia/reperfusion injury produced by transient occlusion of mid-cerebral artery.[116]

Just as changes in the ZBG can alter the MMP selectivity, changes in the point of attachment of the ZBG to the backbone of the MMP inhibitor can also change its potency and selectivity. For instance, compound 30 has an IC_{50} of 240 nM against MMP-3,[114,115] while its structural isomer compound 32 shows weaker ~30% inhibition even at 100 μM concentration.[117]

6.4. Tetracycline-based MMP inhibitors

Tetracyclines are antibiotics that can chelate Zn^{2+} ion and thereby inhibit MMP activity.[75] Doxycycline is a semi-synthetic tetracycline that inhibits MMP-2 and MMP-9.[118] Chemically modified tetracyclines have been developed to inhibit MMP activity.[118] Chemically modified tetracyclines are preferred over conventional tetracyclines because they reach higher plasma levels for prolonged periods of time, and therefore require less frequent administration, and cause less gastrointestinal side effects when administered orally for a chronic disorder. COL-3 or metastat is a chemically modified tetracycline that has a tetracycline scaffold with unsubstituted positions C4-C9, and is a potent MMP inhibitor.[119] Although tetracyclines are relatively weak Zn^{2+} chelators and inhibitors of MMP activity, they could affect MMP expression,[119] and their effects on MMP synthesis may contribute to their potential benefits in rheumatoid arthritis.[120]

6.5. Non-zinc-binding MMP inhibitors

Some MMP inhibitors do not have a ZBG and hence do not bind the catalytic Zn^{2+} ion (Table 1).[121–128] Because the Zn^{2+} active site is the most conserved feature in all MMPs, it has been thought that minimizing the interaction with the catalytic Zn^{2+} ion would improve the inhibitor selectivity toward different MMPs. Non-zinc-binding MMP inhibitors show a noncompetitive mechanism of inhibition.[125] These MMP inhibitors bind to and lock the MMP active site into a specific conformation that is less favorable for substrate binding. Non-zinc-binding MMP inhibitors show high selectivity to MMP-13 and have shown therapeutic potential in animal models of osteoarthritis.[121,128] Compound 37 inhibits MMP-13, but not MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-12, MMP-14, or MMP-17. The flexibility of the MMP-13 molecule relative to other MMPs may provide a favorable and accessible conformation for interaction with compound 37 that is not available in other MMPs.[123,128] Although these MMP inhibitors show high degree of MMP selectivity that could minimize the side effects associated with broad-spectrum MMP inhibitors, it is not clear whether their selectivity is due to their non-ZBG properties or other factor(s).[123,128]

Of note, non-zinc binding MMP inhibitors are hydrophobic. The hydrophobicity of these MMP inhibitors is important for maintaining sufficient inhibitor-MMP interaction to

produce high potency. However, hydrophobicity also decreases the water solubility of the MMP inhibitor. Because most of these non-zinc-binding MMP inhibitors are highly potent and show relative selectivity, studies have attempted to improve their water solubility and other biochemical properties.[121] Derivatives have been developed to modify the solvent-exposed portions of the MMP inhibitor while maintaining its hydrophobic core structure.[124]

Compound 37 has shown promising results in animal models of osteoarthritis. In MMP-13-induced rat model of knee joint cartilage damage, compound 37 was effective at doses as low as 0.1 mg/kg. Also, in rat model of surgically induced knee cartilage damage, compound 37 administered orally twice daily at 30 mg/kg resulted in a 68% reduction in the cartilage lesion. Fibroplasias were absent in joints of rats treated with compound 37, but were observed in rats treated with broad-spectrum MMP inhibitors.

6.6. Mechanism-Based MMP Inhibitors

Mechanism-based MMP inhibitors such as SB-3CT (compound 40) coordinate with the MMP Zn^{2+}, thus allowing the conserved MMP Glu202 to perform a nucleophilic attack and form a covalent bond with the inhibitor.[14] When compared with the traditional Zn^{2+} chelating MMP inhibitors, the covalent bond prevents dissociation of the MMP inhibitor, and therefore decreases the rate of catalytic turnover and the amount of MMP inhibitor needed to saturate the MMP active site.[129] SB-3CT and its successors have shown therapeutic potential, and more selective MMP inhibitors may be developed through covalent modifications with the MMP active site.[14]

SB-3CT is a selective inhibitor of MMP-2 and MMP-9. The structure of SB-3CT is relatively simple, as reflected by its low molecular weight. The mechanism of MMP inhibition by SB-3CT is similar to that of a “suicide substrate” in which a functional group is activated, leading to covalent modification of the MMP active site.[129] SB-3CT shows slow-binding kinetics with MMP-2, MMP-3, and MMP-9, that reach equilibrium between the MMP, the inhibitor and the MMP–inhibitor complex within seconds to minutes. Slow-binding inhibition also contributes to slow dissociation rate of the MMP-inhibitor complex.[130] Of note, following 95% inhibition, MMP-2 regains 50% of its activity after 3 days dialysis, indicating some degree of reversibility and thus distinguishes SB-3CT from the irreversible mechanism of a true suicide inhibitor.[129,130] The selectivity of SB-3CT stems from the difference in the binding kinetics for various MMPs, and may be related to its inhibition of MMP-2 and MMP-9 via a slow-binding mechanism and inhibition of MMP-14 through competitive inhibition.[131]

In preclinical studies, SB-3CT has shown potential benefits in reducing brain damage caused by cerebral ischemia. SB-3CT showed anti-cancer effects in T-cell lymphoma and prostate cancer models.[132–134] In *in vitro* Matrigel tests SB-3CT at 1 μM concentration reduced the invasion ability of human prostate cancer cells by 30%.[133] SB-3CT also reduced angiogenesis and intraosseous tumor growth in a bone metastasis model of prostate cancer.[133] In a mouse model of T-cell lymphoma, SB-3CT caused dose-dependent reduction in the number of liver metastases.[135] At 50 mg/kg/day, SB-3CT inhibited liver metastases by 73% and reduced the colony size of the metastases, while treatment with the broad-spectrum MMP inhibitor batimastat was associated with increased metastasis in the same tumor

model. SB-3CT also provided neuronal protection in a murine model of stroke.[136] In mice treated with SB-3CT either prior to or 2 h following ischemia induced by occlusion of right middle cerebral artery, the infarct volume was decreased to 30% of the control. Administration of SB-3CT was protective up to 6 hours after the ischemic event in mice. Also, neurological behavioral scores evaluated 24 hours after reperfusion showed improvement in SB-3CT -treated compared with control non-treated mice, and the improvement was correlated with the reduction in the brain infarct volume.

Although SB-3CT (compound 40) shows marked *in vivo* activity, it undergoes rapid metabolism, and a metabolite of the parent compound may be responsible for its *in vivo* activity.[137,138] Compound 43 shows slow-binding kinetics with MMP-2, MMP-9, and MMP-14 and is a more potent inhibitor of MMP-2, MMP-3, MMP-7, MMP-9, and MMP-14 than compound 40.[139] Analysis of the different MMP inhibitor metabolites led to the design of derivatives with better *in vivo* stability and prolonged systemic effects.[139] Compound 45 is a slow-binding inhibitor of MMP-2 and MMP-9, but a competitive inhibitor of other MMPs. Compound 45 is more potent for MMP-9 than MMP-2, and its metabolites are 75% more stable and show longer systemic effects than those of compound 40.

SB-3CT and its successors may have clinical potential, and the use of mechanism-based, slow-binding inhibitors may provide a new approach to improve selectivity of MMP inhibitors. Other covalent modifications in the MMP active site may lead to better MMP selectivity.[48]

Given the accessibility of secreted MMPs such as MMP-2 and membrane-tethered MMPs such as MT1-MMP, they represent ideal targets for specific inhibition by small molecules. Thiirane-based ND-322 is a novel small-molecule and selective MMP-2/MT1-MMP inhibitor that has been shown to reduce melanoma cell growth, migration and invasion, and to delay metastatic dissemination. ND-322 may represent a new inhibitor in the repertoire of treatments of melanoma.[63]

Even with the marked improvements in the design of MMP inhibitors, doxycycline remains the only FDA-approved MMP inhibitor.[89,90,140,141] Another major limitation of MMP inhibitors is that they cause musculoskeletal side effects in the form of joint stiffness, pain, inflammation, and tendinitis.[14,90,142,143]

7. MMP Inhibitors as Investigational Tools in Biological Processes

MMPs play a role in many biological processes including tissue remodeling and growth as well as tissue defense mechanisms and immune response. Increased expression of MMPs has been documented during different stages of mammalian development, from embryonic implantation[144] to the morphogenesis of different tissues including lung, bone and mammary gland.[145,146] Other biological processes such as tissue repair and wound healing are associated with increased expression of MMPs.[147] The role of MMPs in these biological processes has been supported by reversal of the effects of MMPs by MMP inhibitors.

7.1. MMP Inhibitors and Role of MMPs in Smooth Muscle Relaxation

Studies have suggested that MMPs via PI_3K and ATP synthesis may transactivate EGFR and contribute to the α-adrenergic receptor-induced vascular tone. Inhibition of the expression of MMP-2 or MMP-7 blunted the phosphorylation of Akt by PI_3K and thus inhibited the response to phenylephrine in rat mesenteric artery.[148] We have shown that phenylephrine-induced contraction of rat aorta is inhibited ~50% by MMP-2 and ~70% by MMP-9.[149] The inhibitory effects of MMP-2 and MMP-9 on phenylephrine contraction were reversible upon washing out the MMPs, supporting specificity of the effects of MMPs. The MMP-induced inhibition of aortic contraction was concentration- and time-dependent, and reversible suggesting that the actions of MMPs are not solely due to irreversible degradation of ECM protein. Also, the inhibitory effects of MMPs on VSM contraction are not likely due to degradation of phenylephrine or the α-adrenergic receptors because MMPs also inhibit prostaglandin F2α-induced contraction, suggesting that the effects of MMPs are not specific to a particular agonist/receptor, but likely involve direct effects on common VSM contraction pathway(s) downstream from receptor activation.

VSM contraction is triggered by increases in Ca^{2+} release from the intracellular stores and Ca^{2+} entry from the extracellular space. MMPs do not inhibit phenylephrine-induced contraction in Ca^{2+}-free solution, suggesting that they do not inhibit the Ca^{2+} release mechanism from the intracellular stores. On the other hand, MMPs inhibit phenylephrine-induced Ca^{2+} influx in rat aortic rings.[149] The mechanism by which MMPs inhibit Ca^{2+} entry could involve direct effects on the Ca^{2+} channels. MMPs may also affect K^+ channels. MMP-2 causes relaxation of rat inferior vena cava (IVC) that is abolished by blockers of the large conductance Ca^{2+}-activated K^+ channels such as iberiotoxin, suggesting a role of VSM hyperpolarization.[150] MMPs are known to induce collagen degradation and produce Arg-Gly-Asp (RGD)-containing peptides, which could bind to $\alpha_v\beta_3$ integrin receptors and inhibit Ca^{2+} entry into VSM.[151] MMPs may also stimulate protease-activated receptors (PARs) and activate signaling pathways that could lead to blockade of VSM Ca^{2+} channels.[152] This is supported by reports that proteases such as thrombin activate PARs and promote endothelium-dependent VSM relaxation by inhibiting Ca^{2+} influx.[153] Thus while MMPs may affect VSM contraction through modulation of surface membrane ion channels, further studies are needed to define the role of integrins and PARs as possible molecular mechanisms via which MMPs could inhibit VSM contraction.

We have shown that MMP-2 and MMP-9 cause inhibition of Ca^{2+} entry-dependent mechanisms of contraction not only in rat aorta,[149] but also in rat IVC.[154] Our studies support that MMPs are expressed in both the arterial and venous system and could have significant effects on the arterial and venous structure and function. However, the findings in certain arteries should not be generalized to other arteries in the systemic circulation or specialized arteries such as the coronary and cerebral arteries. Also, veins differ from arteries in their structure and function, and the effects of MMPs on the veins should not be generalized to the arteries. Veins have few layers of VSMCs compared to several layers in the arteries. Also, venous and arterial VSMCs originate from distinct embryonic locations and are exposed to different pressures and hemodynamic conditions in the circulation.[155] Studies have shown that while cell migration and MMP-2 and MMP-9 levels could be

similar in cultured saphenous vein VSMCs and internal mammary artery VSMCs, venous VSMCs exhibit more proliferative and invasive capabilities than arterial VSMCs.[156] Other studies have shown that MMP-2 expression is greater in cultured human saphenous vein VSMCs than human coronary artery VSMCs. In contrast, the expression of MMP-3, MMP-10, MMP-20, and MMP-26 is greater in coronary artery than saphenous vein VSMCs.[155] Similarly, TIMPs may show different expression levels in veins versus arteries. For instance, the levels of TIMP-1, TIMP-2, and TIMP-3 are greater in cultured human saphenous vein than coronary artery VSMCs.[155] These observations highlight the importance of further studying the differences in the expression/activity of MMPs and TIMPs in veins versus arteries and in venous versus arterial disease.

7.2. MMP Inhibitors and Role of MMPs in Smooth Muscle Migration

MMPs play a role in VSMC migration. In rat aortic smooth muscle cells (RASMCs) cultured on collagen I gel to mimic ECM, exposure to interstitial flow enhanced cell motility. Upregulation of MMP-1 enhanced flow-induced cell motility, while the MMP inhibitor GM-6001 attenuated flow-induced cell migration. ERK1/2 phosphorylation and increased expression of activator protein-1 (AP-1) transcription factors c-Jun and c-Fos appear to be involved in MMP-mediated enhancement of flow-induced cell motility.[157] Young human ASMCs produce active MMP-2 and show a greater migratory capability than aged cells. The activation of pro-MMP-2 in young cells is likely due to an increase in MT1-MMP. In contrast, aged cells produce only the inactive zymogen proMMP-2 form. Upregulation of TIMPs could also reduce MMP-2 activity in aged cells. Interestingly, treatment of young cells with TIMP-1 and TIMP-2 leads to a migratory behavior that mimics that of aged cells.[158] MMP-2 activation may be involved in chemokine-induced chemotaxis in monolayers of human VSMCs.[159] Also, MMP-2 knockout decreases VSMC migration and neointima formation in the mouse carotid ligation model (Table 2).[160,161]

MMP-9 may also be involved in VSMC migration. Tanshinone IIA, a major constituent of Salvia miltiorrhiza bunge, inhibits tumor necrosis factor-α (TNF-α)-induced human ASMC migration, partly through inhibition of MMP-9 activity. Tanshinone IIA also inhibits TNF-α-induced ERK and c-jun phosphorylation, and NF-κB and AP-1 DNA-binding.[162] Suppression of MMP-9 expression by downregulation of NF-κB may also mediate the inhibitory effects of curcumin on migration of human ASMCs.[163] Also, MMP-9 knockout is associated with reduced VSMC migration and neointima formation in mouse models of filament loop injury[164] and carotid artery occlusion (Table 2).[165]

Disruption of the basement membrane is required for VSMC migration.[166] MMPs degrade the basement membrane and in turn facilitate ECM-integrin interactions, leading to activation of focal adhesion kinase (FAK) and increased cell migration. MMPs also cause fragmentation of membrane components such as type I collagen, thus creating new integrin-binding sites. Growth factor receptors, cadherins and integrins mediate signalling pathways that play a role in reorganization of the cytoskeleton in preparation for cell migration.[167,168] MMPs cleave E-cadherin in epithelial cells, VE-cadherin in endothelial cells and N-cadherin in VSMCs,[169,170] thus dissolve adherence junctions and free the cells to move and migrate.

MMPs not only facilitate migration by promoting proteolysis of ECM proteins, but could also directly enhance cell migration. MMP-1 promotes growth and invasion of cells by binding to and cleavage of PAR-1, which reveals a tethered ligand that initiates signaling via a GPCR and stimulates cell migration.[171] This mechanism may allow the cells to sense a proteolytic environment and actively move towards an area of degraded matrix.

MMP inhibitors have been useful in demonstrating the effect of MMPs on VSMC migration. Gene transfer of TIMPs reduces VSMC migration *in vitro* and reduces neointma formation and intima thickening in *in vivo* models of vascular injury. TIMPs 1–4 delivered directly or by gene transfer inhibit migration of SMCs *in vitro*[172,173] and reduce neointima formation in human saphenous vein organ culture.[174] TIMP gene transfer also preserves the tunica media basement membrane and inhibits VSMC migration to the intima. Synthetic MMP inhibitors inhibit migration of VSMC in cultured baboon arterial explant,[175] and early VSMC migration in the rat model of carotid balloon injury.[176] Collectively, experimental evidence supports that MMPs enhance VSMC migration via their proteolytic degradation of ECM proteins as well as direct cellular effects, and MMP inhibitors could reverse or reduce VSMC migration.

7.3. MMP Inhibitors and Role of MMPs in Smooth Muscle Proliferation

In addition to their role in facilitating VSMC migration, MMPs may regulate VSMC proliferation. VSMC proliferation at sites of endothelial cell injury and subsequent lipid deposition play a role in atheroma formation, and MMPs appear to be involved in these processes. Pretreatment of human ASMCs with ethanol extract of Buddleja officinalis attenuates high-glucose-induced cell proliferation by suppressing MMP-9 activity.[177] Also, MMP-9 knockout is associated with inhibition of VSMC proliferation in mouse model of filament loop arterial injury.[164] Of note, MMP-9 knockout is not associated with decreased VSMC proliferation in mouse model of carotid artery occlusion,[165] likely due to compensatory activation of other proteases.[178]

MMPs could regulate VSMC proliferation via several mechanisms. MMPs could promote permissive interactions between VSMCs and various components of ECM. Integrin-mediated pathways may be essential for stimulation of VSMC proliferation.[179,180] MMPs may free growth factors from attachment to ECM components or cell surface so that they can act on their receptors. Heparin-binding growth factors such as fibroblast growth factor-1 (FGF-1) and FGF-2, are potent mitogens for VSMCs that are released through the action of MMPs on ECM proteoglycans.[7] Together with ADAMs, MMPs could facilitate the release of cell surface heparin-bound epidermal growth factor (HB-EGF), which in turn stimulates VSMC proliferation.[181,182] MMPs also activate transforming growth factor-h (TGF-h) by cleaving off the latency-associated peptide.[183] MMPs can also liberate active insulin-like growth factor-1 (IGF-1) by degrading its binding proteins. Together with signals from FAK, these processes upregulate and/or stabilize key regulators of the cell cycle. Dismantling of cadherin-catenin complex occurs in balloon-injured rat carotid arteries leading to increased expression of the cell cycle gene cyclin D1 which stimulates VSMC proliferation.[184] MMP-induced cadherin shedding promotes dissolution of adherens junctions and translocation of

h-catenin to the nucleus where it acts as a transcription factor to further promote cell proliferation.[168,170]

MMP inhibitors have been useful to assess the role of MMPs in VSMC proliferation. Some studies have reported excess neointima formation in rat model of carotid arteries balloon injury after treatment with the MMP inhibitor GM-6001.[185,186] Other studies have shown that synthetic MMP inhibitors inhibit VSMC proliferation *in vitro*.[170,187] Also, inhibition of MMPs is associated with decreased N-cadherin shedding, increased cell membrane N-cadherin, decreased h-catenin nuclear translocation and decreased proliferation of cultured human VSMCs. Tetracycline-based MMP inhibitors reduce VSMC migration and neointima formation in rat model of carotid artery balloon injury.[176,188] Collectively, experimental evidence largely points to a stimulatory effect of MMPs on VSMC proliferation, and reversal of this effect by MMP inhibitors.

7.4. MMP Inhibitors and Role of MMPs in Angiogenesis

Angiogenesis is the process of forming new blood vessels. Angiogenesis requires degradation of the vascular basement membrane and ECM remodeling in order to allow endothelial cells to migrate into the surrounding tissue. Angiogenesis plays a role in several biological processes and pathological conditions including the progression of atherosclerotic plaques and tumor growth.[189,190] MMPs mediate the effects of several pro-angiogenic factors by virtue of their proteolytic activity. Angiogenic growth factors such as FGF, TGF-α, TGF-β, TNF-α, vascular endothelial growth factor (VEGF) and angiogenin are secreted by endothelial cells and other cells, and act in an autocrine or paracrine fashion to promote angiogenesis. The expression of MMP-1, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-13, and MMP-19 is up-regulated more than 1.5-fold in human umbilical vein endothelial cells (HUVECs) treated with VEGF. VEGF induces MMP-10 expression possibly via PI_3K and MAPK pathways.[191] MMPs take part in remodeling of the basement membrane and degradation of various components of ECM necessary for angiogenesis. MMPs also enhance angiogenesis by detaching pericytes from the vessels, releasing ECM-bound angiogenic factors, exposing cryptic pro-angiogenic integrin binding sites in ECM, generating promigratory ECM component fragments, and cleaving endothelial cell-cell adhesions.

MT1-MMP plays a specific role in angiogenesis.[192,193] Semaphorin 4D is overexpressed in cancers and promotes neovascularization upon stimulation of its Plexin-B1 receptor on endothelial cells. MT1-MMP targets semaphorin 4D and releases it from its inactive membrane bound form to act in a paracrine manner on endothelial cells.[194] MT1-MMP-dependent TGF-β signaling may also be involved in prostaglandin E_2-induced endothelial cord formation in cultured HUVECs[195].

Upregulation of MMPs has been positively linked to tumor size and the increased angiogenic and metastatic potential of tumors. Expression of MMP-2 and MMP-9 and VEGF is positively correlated to tumor size, depth of invasion, lymphatic and venous invasion, lymph node metastasis, and microvessel density of gastric carcinomas.[196] MMP-2 mediates the angiogenic effect of pituitary tumor transforming gene expression in HEK293 cells.[197]. Downregulation of MMP-2 decreases tumor-induced angiogenesis in cultured

human microvascular endothelial cells. MMP-2 inhibition causes apoptotic cell death *in vitro*, and suppresses tumor growth of pre-established U-251 intracranial xenografts in nude mice.[198] Overexpression of MMP-9 in human breast cancer MCF-7 cells results in increased tumor angiogenesis, tumor growth, and VEGF/VEGFR-2 complex formation.[199] MMP-9 may also be involved in FGF-2/FGFR-2 pathway in the mouse angiogenesis model,[200] and downregulation of MMP-9 expression inhibits tumor growth in nude mice.[201] Also, MMP-3 mediates matriptase/MT-SP1-induced tumor growth and angiogenesis by enhancing ECM degradation in tumor cell microenvironment.[202]

While angiogenic factors can induce MMP expression in endothelial and stromal cells, MMPs can in turn enhance the availability/bioactivity of angiogenic factors. Degradation of ECM releases ECM/basement membrane-sequestered angiogenic factors such as VEGF, bFGF and TGF-β. MMP-1 and MMP-3 degrade perlecan in endothelial cell basement membranes to release b-FGF. Connective tissue growth factor forms an inactive complex with VEGF165. and cleavage of connective tissue growth factor by MMP-1, MMP-3, MMP-7, or MMP-13 releases active VEGF165. MMP-2, -3, and -7 degrade the ECM proteoglycan decorin and release latent TGF-1, and MMP-2 and MMP-9 cleave the latency-associated peptide to activate TGF-β1.[203].

In support of a role of MMPs in angiogenesis, dormant tumors may secrete TIMPs to prevent the tumor from switching to the angiogenic phenotype and thereby arrest tumor growth.[204,205] We should note that MMPs may exert anti-angiogenic effects through the generation of endogenous angiogenesis inhibitors by proteolytic cleavage of certain collagen chains and plasminogen. MMP-9 mediates tamoxifen-induced increase in endostatin and thus decreases angiogenesis in hormone dependent ovarian cancer.[206] MMP-7, MMP-9 and MMP-12 may block angiogenesis by converting plasminogen to angiostatin, a potent angiogenesis antagonist. MMP-14 cleaves endoglin, a TGF-β co-receptor, and thus inhibits its angiogenic effect.[207] Thus MMPs are important regulators of angiogenesis with an overall tendency towards stimulation, and MMP inhibitors reverse these angiogenic effects.

7.5. MMP Inhibitors and Role of MMPs in Cell Apoptosis

Apoptosis is a form of cell death that involves activation of the intracellular cysteine proteases, caspases. Apoptosis of VSMCs plays a role in attenuating intimal thickening and destabilizing atherosclerotic plaques.[208,209] Several factors promote apoptosis including death signals originating from outside the cell as well as intracellular factors such as DNA damage, cell cycle status and the levels of the tumor suppressor p53.[209] MMP-7 is involved in the cleavage of N-cadherin and modulation of VSMC apoptosis. In contrast, survival signals maintain VSMC viability even in the face of a pro-apoptotic environment. Survival pathways are closely linked to those triggering proliferation and therefore could be influenced by MMPs. Survival factors such as platelet-derived growth factor (PDGF), HB-EGF and IGF-1 act via tyrosine kinase receptors to stimulate the PI_3K/Akt pathway. MMP-2, MMP-7 and MMP-9 cleave cell surface pro-HB-EGF and liberate the soluble active growth factor which binds to EGF-R and promotes growth.[181,210] In human coronary VSMCs, oxidized low density lipoprotein (oxLDL) and 4-hydroxynonenal activate PDGFR-β and the ERK1/2 pathway and in turn increase the production of MMP-1.[211] MMP-1,

MMP-2, MMP-8 and MMP-9 degrade members of the IGF binding protein family and thereby increase the bioavailability of IGF-1 and its anti-apoptotic effects.[7]

Cell–matrix contacts promote VSMC survival, and their disruption leads to apoptosis in a process originally termed anoikis.[212] ECM–integrin interactions trigger FAK activation and induce the p53 survival signaling pathway.[213,214] MMP production appears to favor FAK activation and hence survival signaling. On the other hand, excess MMPs could degrade ECM proteins or integrins and promote anoikis.[215] MMPs may also modulate apoptosis by cleaving death ligands such as TNF-α and Fas ligand and their receptors. MMP-1, MMP-2, MMP-8, MMP-9 and MMP-13 and the MT-MMPs 14, 15, 16 and 17 can cleave pro-TNF-α.[7,216] Similarly, MMP-7 sheds Fas-L from the cell surface.[217,218] Caspase-mediated cleavage of the DNA repair enzyme poly(ADP-ribose) polymerase is an important step in apoptosis. MMP-2 has been localized in the nuclei of isolated cardiac myocytes and may be involved in cleaving nuclear poly(ADP-ribose) polymerase.[219]

TIMP-3, but not TIMP-1 or TIMP-2, stimulates apoptosis in many cell types including VSMCs.[173,218] TIMP-4 also stimulates VSMC apoptosis.[220] Thus, MMPs appear to regulate VSMC apoptosis and promote cell survival via several pathways, and MMP inhibitors could oppose the effects of MMPs on cell survival and promote apoptosis.

8. MMP INHIBITORS AS POTENTIAL TOOLS IN PATHOLOGICAL CONDITIONS

Altered MMP expression/activity and the resulting MMP/TIMP imbalance could cause unrestrained tissue remodeling in multiple pathological conditions including autoimmune and inflammatory disorders, osteoarthritis and cancer. MMP/TIMP imbalance has also been implicated in cardiovascular disorders such as hypertension. atherosclerosis and aneurysm.

8.1. MMP Inhibitors and Role of MMPs in Hypertension

Hypertension is a multifactorial disorder involving alterations in the renal, neuronal and vascular control mechanisms of blood pressure. Hypertension is often associated with vascular remodeling and rearrangement of various components of the vascular wall including ECM. The elevated plasma levels of some MMPs in hypertension have suggested that the underlying pathophysiology may involve excessive elastolysis or accumulation of collagen degradation products in the vascular wall.[221] Several MMPs and TIMPs may be involved in the vascular remodeling associated with hypertension. Increased MMP activity could result in increased degradation of elastin relative to collagen leading to decreased elasticity. On the other hand, decreased TIMP-1 activity could lead to accumulation of poorly cross-linked immature and unstable fibrin degradation products, resulting in misdirected deposition of collagen.[221] Some studies have shown a correlation between MMP levels and hypertension (Table 3). Other studies have shown low levels of MMPs and high levels of TIMPs levels in hypertension and suggested that decreased degradation of collagen type I could play a role in the development of hypertension (Table 3). Studies have compared the effects of early and late hypertension on ECM remodeling in Dahl rats of different age groups: young salt-resistant (control), young salt-sensitive (early hypertension),

middle-age salt-resistant (aging), and middle-age salt-sensitive (late hypertension). In the early phase of hypertension, several MMPs decreased, TIMP-1 increased, and total collagen increased, consistent with increased fibrosis. MMP-8 activity decreased in young salt-sensitive rats. Also, MMP-14 correlated positively with changes in left ventricular mass in early hypertension. In contrast, late hypertension was associated with increased MMP-8 and MMP-14 and decreased total collagen levels. These findings suggest downregulation and upregulation of MMPs at early versus late stages of hypertension.[222] We should note that ECM remodeling in response to pressure overload is a dynamic process involving both ECM accumulation and degradation, and, in addition to the stage of hypertension, antihypertensive treatment may further modulate collagen metabolism.

In addition to regulation of ECM turnover, MMPs could affect vascular remodeling in hypertension via other cellular mechanisms. MMPs may mediate EGFR transactivation induced by excessive stimulation of GPCRs such as α_1-adrenergic receptors which in turn promote the synthesis of contractile proteins in VSMCs and thereby contribute to vasoconstriction and hypertension. Also, in fructose treated rat model of acquired systolic hypertension and insulin resistance, the insulin-resistant VSMCs showed increased expression/activity of MMP-2 and MMP-7, EGFR, the contractile proteins myosin light chain (MLC) kinase and MLC-II, and their transcriptional activators possibly through activation of ERK1/2. Disruption of MMP-EGFR signaling normalized the increased expression of contractile proteins and their transcriptional activators in insulin-resistant VSMCs and arteries and prevented the development of hypertension in fructose treated rats.[223] Also, in a study comparing the effects of treatment with angiotensin II (AngII) for 10 days in wild-type and MMP-9 knockout mice, baseline blood pressure was equivalent in both phenotypes, but AngII treatment increased systolic blood pressure to a greater extent in MMP-9 knockout than wild-type mice. In response to AngII treatment, the carotid artery pressure-diameter relationship and arterial compliance were increased in wild-type, but reduced in MMP-9 knockout mice. Also, maximal carotid artery diameter was greater in wild-type versus MMP-9 knockout mice. AngII treatment induced MMP-2 and increased carotid media thickness equally in both phenotypes. On the other hand, AngII treatment induced MMP-9 and enhanced MMP-9 *in situ* gelatinase activity only in wild-type mice, and vessels from these mice produced more collagen I breakdown products than MMP-9 knockout mice. Conversely, staining for collagen IV was enhanced in vessels from AngII-treated MMP-9 knockout mice. These findings suggest that the onset of AngII-induced hypertension is accompanied by increased MMP-9 activity in conductance vessels, MMP-9 deficiency results in vessel stiffness and increased pulse pressure, and MMP-9 activation may have a beneficial role in early hypertension by preserving vessel compliance and alleviating the increase in blood pressure.[224]

MMP/TIMP imbalance in blood vessels, particularly in the intima and media, may account for the increased proteolytic activity and maladaptive vascular remodeling in hypertension. Increased levels of MMP-2, MMP-9, and MMP-14 and enhanced gelatinolytic activity were observed in the aortas of two kidney-one clip (2K-1C) rat model of hypertension. Doxycycline treatment for 8 weeks attenuated 2K-1C hypertension, prevented the increase in the aortic intima and media thicknesses, attenuated the increases in MMP-2, MMP-9, and MMP-14 in the intima and media, but did not change the levels of TIMPs 1–4.[225] MMP-2

may contribute to arterial remodeling in early hypertension by decreasing the actin-binding protein calponin-1. The absence of calponin is associated with VSMC phenotype switch, and leads to VSMC migration and vascular remodeling. In a study of Sham-operated and 2K-1C rat model of hypertension, MMP-2 activity was increased in aortas from 2K-1C rats at 1 and 2 weeks of hypertension, followed by increased VSMC proliferation, and these effects were abolished by treating 2K-1C rats with doxycycline. Increased aortic media to lumen ratio started in 2K-1C rats at 1 week of hypertension, and was established by 2 weeks. MMP-2 and calponin-1 co-localized in the cytosol of VSMCs. Aortas from 2K-1C rats showed a decrease in calponin-1 protein levels but not calponin-1 mRNA expression, at 1 week of hypertension, and doxycycline treatment prevented the decrease in calponin-1 protein level. Conversely, calponin-1 was upregulated in 2K-1C rats at 2 weeks of hypertension. These findings suggest that MMP-2 may contribute to the post-translational decrease in calponin-1, and thereby contribute to hypertension-induced maladaptive arterial remodeling.[226] In VSMCs from spontaneously hypertensive rats, TNF-α increased cell migration and MMP-9 expression. Upregulation of MMP-9 was transcriptionally regulated at the AP-1 and NF-κB sites in the MMP-9 promoter, suggesting a role for increased VSMC proliferative capacity, G1 to S-phase cell-cycle progress, and MMP-9 expression in the vascular remodeling in hypertension.[227] Studies in Dahl salt-sensitive rats fed high-salt diet for 6 weeks have shown that intraperitoneal treatment with the MMP inhibitor GM6001 1.2 mg/kg body weight on alternate days for 4 weeks reduced blood pressure. MMP-9 expression and activity were reduced in cerebral vessels of GM6001-treated Dahl salt-sensitive rats. GM6001 treatment ameliorated oxidative/nitrosative stress and tight junction proteins in cerebral vessels of Dahl salt-sensitive rats, suggesting restoration of vascular integrity. These findings suggest that inhibition of MMP-9 attenuates high blood pressure and hypertension-associated cerebrovascular pathology in salt-sensitive hypertension.[228] Also, in spontaneously hypertensive rats, induction of acute hypertension by AngII was associated with post-transcriptional activation of vascular MMP-7, transcription of myocardial ADAM-12, a major metalloproteinase implicated in cardiac hypertrophy, and overexpression of downstream hypertrophy marker genes. Knockdown of MMP-7 attenuated hypertension, inhibited ADAM-12 expression, and prevented cardiac hypertrophy.[229] In addition to cardiac hypertrophy, MMPs may also play a role in other hypertensive complications such as intracranial hemorrhage.[225,230,231]

8.2. MMP Inhibitors and Role of MMPs in Atherosclerosis

Atherosclerosis is a multifactorial vascular disease. Dysfunctional endothelium recruits different inflammatory pathways leading to intimal differentiation, VSMC proliferation, ox-LDL deposition, platelet activation and aggregation, and the formation of an atheroma of fat, collagen and elastin with a thin fibrous cap. Dysregulated ECM metabolism may contribute to vascular remodeling during the development and complications of atherosclerotic lesions. Enhanced MMP expression has been detected in the atherosclerotic plaque, and activation of MMPs appears to facilitate atherogenesis, platelet aggregation and plaque destabilization.[232,233] MMP-1, MMP-2, MMP-3, MMP-7, MMP-9 and MMP-12 are produced by SMCs and macrophages in the arterial wall, and are highly expressed in atherosclerotic lesions.[234,235] Also, the plaques' shoulders and regions of foam cell accumulation display increased expression of MMP-1, MMP-3 and MMP-9. Activated

gelatinases have been found in plaque extracts, and gelatinolytic and caseinolytic activities have been detected in atherosclerotic areas, but not in unaffected arterial tissue.[236] Importantly, low-fat diet is associated with reduced plaque proteolysis and decline in MMP-1 levels and macrophage content.[237] Patients on hemodialysis develop atherosclerosis rapidly and show evidence of fibrinolysis/proteolysis imbalance in their plasma, and MMP-2 may play a role in the development of atherosclerosis in these patients.[238] Also, the urinary levels of MMP-9 and TIMP-1 are elevated in patients with coronary artery disease and acute coronary syndrome compared with healthy volunteers.[239] Plasma levels of MMP-1, MMP-3, and MMP-7 are higher in patients with high compared with those with low intima-media thickness. MMP-7 is positively associated with carotid calcification,[240] and plasma levels of MMP-8 are positively associated with the occurrence of carotid plaques.[241] MMP-10 is induced by C-reactive protein in endothelial cells, and is overexpressed in atherosclerotic lesions. Also, high serum levels of MMP-10 are associated with increased inflammatory markers, increased carotid intima-media thickness and atherosclerotic plaques.[242]

Certain genetic variants of MMPs have been associated with the progression and complications of atherosclerosis. In a 3-year coronary atherosclerosis study, the 6A active variant of the MMP-3 promoter was correlated with progression of coronary arterial lumen narrowing[243] and acute myocardial infarction.[244] In a study on 139 patients with coronary artery disease and 119 healthy subjects, MMP-3 5A/6A genetic variant was associated with coronary artery disease, and the PON1 variant was associated with the number of diseased coronary vessels.[245] In a subgroup of the Etude Cas-Temoin de l'Infarctus du Myocarde (ECTIM) study of acute myocardial infarction, the more active T allele of an MMP-9 functional promoter polymorphism (C1562T) was more common in patients with 3-coronary vessel disease, but did not predict myocardial infarction.[246] In a study of 1127 patients, higher serum levels of MMP-9 were associated with the T allele, but did not predict cardiovascular death.[247] Other studies have shown associations of MMP-9 genotypes with different stages of carotid artery atherosclerosis.[248] Interestingly, MMP-9 ablation reduced the size of atherosclerotic lesion in $ApoE^{-/-}$ mice.[249] Also, in the mouse carotid artery ligation model, the plaque burden was reduced in hypercholesterolemic MMP-9 knockout compared with wild-type mice.[250]

MMPs contribute to the pathophysiology of atherosclerosis through several processes and pathways. Vascular inflammation is an important factor in the atherogenic process that has been shown to promote MMP expression. In a study enrolling 18 patients with stable angina, 14 patients with unstable angina and non-ST-segment elevation myocardial infarction, 14 patients with ST-elevation myocardial infarction, and 16 healthy controls, the progression of coronary artery disease was paralleled with increased MMP-9/TIMP-1 ratio in circulating CD14+ monocytes and in the serum. Similar imbalance in the expression of MMP-9 and TIMP-1 was observed in monocyte-derived macrophages within the atherosclerotic plaques.[251] Cholesterol lowering 3-HMGcoA reductase inhibitors decrease the expression of various MMPs in atheromatous plaques by reducing vascular inflammation.[252] For example, rosuvastatin inhibits the expression of MMP-2 and MMP-9.[253]

VSMC migration and proliferation are also involved in atheroma formation. MMPs enhance VSMC migration to atherogenic areas where they proliferate and in turn increase the size of

the lesion. In RASMCs, the herb Salvia miltorrhia extract inhibits VSMC migration in part through downregulation of both MMP-9 and TNF-α.[254]. In a study using mice with genetically modified collagen that resists digestion by MMP collagenases, in an atherogenic background, the lesion size was similar in collagenase-resistant and control mice, but collagen was more abundant and SMC number was decreased in the intimal lesions of collagenase-resistant mice. These findings suggest a role for MMP-mediated collagenolysis in regulating collagen turnover and SMC proliferation in the atheromatous plaque.[255]

MMP-1 may mediate ox-LDL induced activation of the PDGFR-β and ERK1/2 atherogenic pathways.[211] Ox-LDL also activates MMP-2 through upregulation of MT1-MMP and increases in oxidative radicals generated by the xanthine/xanthine oxidase complex.[256] AngII plays a role in the pathogenesis of atherosclerosis. AngII increases the expression of MMP-9 in VSMCs via angiotensin type 1 (AT1) receptor and NF-κB pathways.[257] collectively, these studies have shown an association between MMPs levels, genetic variants of certain MMPs and the atherosclerotic process.

Because MMPs degrade ECM proteins and because increased MMP levels and activity has been detected in vulnerable atherosclerotic plaques, it has been proposed that MMPs reduce the strength of the fibrous cap and contribute to plaque rupture. High-mobility group box 1 is an intracellular gene regulator protein produced by activated VSMCs that causes the progression and increases the vulnerability of atherosclerotic lesions to rupture by increasing the expression of MMP-2, MMP-3 and MMP-9.[258] Areas of atherosclerotic plaque rupture show a decrease in VSMCs and increased macrophage-derived foam cells. Studies have compared brachiocephalic artery plaque instability in apoE/MMP-3, apoE/MMP-7, apoE/MMP-9, and apoE/MMP-12 double knockout mice with their age-, strain-, and sex-matched apoE knockout controls, and concluded that MMP-12 supported lesion expansion and destabilization. MMP-7 had no effect on plaque growth or stability, although it was associated with decreased VSMCs in plaques, while MMP-3 and -9 appeared to play protective roles, limiting plaque growth and promoting a stable plaque phenotype.[259] MMP-1, MMP-12 and MMP-13 derived from intimal macrophages have been suggested to play a role in both plaque initiation and progression.[260] On the other hand, transgenic mice that specifically express MMP-1 in macrophages show smaller plaques and no evidence of plaque rupture when compared with control littermates.[261] This is likely because these mice have altered MMP-1 from birth, which could reduce collagen accumulation. MMP-3 also appears to have a dual role. Mice lacking both MMP-3 and ApoE show extensive atheromas, but reduced aneurysm formation.[262] MMP-3 deficiency is associated with increased collagen and fewer macrophages in plaques, which could contribute to greater stability of atheromatous plaques. Adenoviral gene transfer of TIMP-1 into $ApoE^{-/-}$ mice 6 weeks after commencing a high-fat diet reduced both lesion size and macrophage content, supporting the concept that MMPs adversely affect the stability of established plaques.[263] Estrogen supplementation especially late after menopause may destabilize established plaques, in part due to estrogen's ability to upregulate MT1-MMP without a corresponding increase in TIMP-2, and consequently increased activation of MMP-2.[264]

Although the MMP-induced degradation of ECM proteins could contribute to plaque instability, the ability of MMPs to promote VSMC migration and proliferation may

contribute to atherosclerotic plaque cap growth and stability. MMPs such as MMP-2, MMP-9, MMP-13 and MMP-14 release growth factors such as TGF-h and VEGF that are stored in ECM.[265] MMP-9 releases VEGF bound to proteoglycans in ECM, enhancing its bioavailability and thereby influencing plaque neovascularization. Collectively, evidence suggests dual role for MMPs in intimal thickening and athrogenesis as well as atherosclerotic plaque rupture.[178] Further studies may allow targeting of individual MMPs with specific MMP inhibitors to limit the growth of the atherosclerotic lesions and promote their stability.

Atherosclerosis in the coronary arteries could lead to acute coronary syndrome including unstable anginas and myocardial infarction. Studies have shown an association between MMPs and the development of acute coronary syndrome.[266] A case-control study on 261 patients who had suffered a myocardial infarction and 194 healthy controls, all Spanish male smokers, showed that MMP-1 promoter polymorphisms are associated with the risk of early myocardial infarction.[267] MMP-2 and -9 were elevated following acute myocardial infraction in 91 patients compared to 172 control subjects with stable coronary artery disease. Higher early levels of MMP-9 were also associated with the extent of left ventricular remodeling and circulating white blood cell levels.[268] Increased MMP expression is also observed after coronary angioplasty, suggesting a potential role of MMPs in coronary artery restenotic lesions.[269]

TIMPs may play a role in atherosclerosis. TIMP-4 is detected in cardiovascular tissue areas populated by inflammatory macrophages and CD3+ T cells. Human lymphocytes, monocytes, macrophages and mast cells produce TIMP-4. In advanced atherosclerotic lesions, TIMP-4 is detected around necrotic lipid cores, whereas TIMP-3 is detected within and around the core regions indicating different roles in inflammation-induced cell apoptosis and ECM turnover.[270] In a study on 238 men, TIMP-1 was positively associated with carotid intima-media thickness and carotid-femoral pulse-wave velocity.[271] In a study evaluating multiple carotid wall characteristics, including wall thickness, lumen area, calcium area, lipid core, and fibrous cap measures, for associations with plasma MMPs 1, 2, 3, 7, 8, and 9 and TIMP-1, the fibrous cap thickness was greater in individuals with elevated TIMP-1 levels. Also, TIMP-1 was positively associated with measures of lipid core.[240] Of note, TIMP-1 deficiency produces macrophage-rich lesions with active proteinases and medial destruction in ApoE$^{-/-}$ mice.[272] TIMP-1-deficient mice show 30% smaller atherosclerotic lesions but increased aneurysm formation compared with control mice.[273] However, pharmacological MMP inhibitors did not appear to affect the lesion size in atheroma-prone mice.[274,275]

The levels of MMPs and TIMPs in early post-myocardial infarction period may provide an estimate of the extent of cardiac damage and remodeling. MMP-9 and TIMP-1 correlate with echocardiographic parameters of left ventricular dysfunction after acute myocardial infarction and may identify patients at risk of subsequent left ventricular remodeling and adverse prognosis.[276] MMP inhibitors have not been used extensively in cardiovascular clinical trials partly because cancer trials showed side effects such as tendinitis (possibly due to inhibition of ADAMs), lack of efficacy, and possible adverse effects.[142] In one clinical trial, 100 patients requiring carotid endarterectomy were randomized to receive 200 mg/d

doxycycline or placebo for 2 to 8 weeks before surgery. Carotid plaques retrieved by endarterectomy showed that doxycycline penetrated the atherosclerotic plaques, achieved acceptable tissue levels, and reduced MMP-1 levels, but had no effect on atheroma progression.[277] Also, most animal studies of post-angioplasty or in-stent stenosis have shown little or only short-term beneficial effects of MMP inhibitors.

8.3. MMP Inhibitors and Role of MMPs in Aneurysm

MMPs may play a role in the pathophysiology of thoracic aortic aneurysm (TAA) and abdominal aortic aneurysm (AAA). MMPs have been associated with aneurysm growth and rupture. Also, there is an association between certain haplotypes of MMP-1, MMP-3, MMP-7, MMP-12 and MMP-13 and the risk of coronary artery aneurysms in patients with Kawazaki disease.[278]

MMP/TIMP imbalance may promote TAA formation.[279] High levels of MMP-2 and MMP-9 have been demonstrated in patients with TAA, with MMP-9 predominantly expressed in the faster-growing anterior wall of the aneurysm while MMP-2 is higher in the slower-growing posterior wall.[280] Also, a study of 28 patients with degenerative TAA, 60 patients with thoracic aortic dissection, and 111 control subjects has shown an association between a genetic variant of MMP-9 (8202A/G), TAA and dissection.[281] Interestingly, different sets of MMP/TIMP imbalances were detected within TAA of patients with bicuspid versus tricuspid aortic valves.[282] Experimental studies have shown that the expression of MT1-MMP, which is important for macrophage-mediated elastolysis, increases progressively after induction of TAA in mice.[283,284] Elevated levels of MMP-2, MMP-8, MMP-9 and MMP-12 are detected at various stages of TAA development in mice.[285] Also, induction of TAA formation in rats is associated with increased levels of MMP-2 and MMP-9, and ADAM-10 and ADAM-17.[286] In the mouse model of Marfan syndrome, TAA was prevented in mice treated with the MMP inhibitor doxycycline, while mild aneurysm was evident in mice treated with the β-blocker atenolol. Doxycycline improved elastic fiber integrity, normalized aortic stiffness, and prevented vessel weakening. Also, the impaired vascular contraction and endothelium-dependent relaxation observed in the nontreated and atenolol-treated mice was improved with doxycycline.[287]

MMPs could also play a role in the pathophysiology of AAA. Artic aneurysm shows several histopathological changes in tunica intima and media including accumulation of lipids in foam cells, extracellular free cholesterol crystals, calcifications, thrombosis, ulcerations and rupture of the vascular layers. There is also an adventitial inflammatory infiltrate. Degradation of tunica media is a major pathophysiological feature in AAA. Loss of elastin could be an initiating event in AAA formation, and loss of collagen causes continued expansion of the aneurysm wall. Medial neovascularization is characteristic of established AAA and involves proteolytic degradation of ECM by MMPs to facilitate endothelial cell proliferation and migration. Studies demonstrated upregulation of pro-angiogenic cytokines and increased medial neovascularization at the aneurysm rupture edge. Increased collagen turnover could be a major factor in growth and rupture of AAA.[288]

MMP-2 and MMP-9 appear to play a role in AAA formation.[289,290] Patients with AAA show elevated plasma levels of MMP-2 and MMP-9 in the range of 0.06–0.6 μg/

ml.[289,291,292] MMP-9 is the most abundantly expressed MMP in AAA and is produced mainly by the aneurysm-infiltrating macrophages.[293] The plasma level and aortic wall expression of MMPs are especially elevated in patients with imminent aneurysm rupture. In a study examining circulating levels of MMPs in non-ruptured and ruptured AAA immediately prior to open repair, MMP-1 and MMP-9 levels were elevated in the plasma of patients with ruptured AAA versus non-ruptured AAA. A 4-fold elevation in preoperative plasma levels of MMP-9 were associated with non-survival at 30 days from rupture surgery compared with patients surviving for greater than 30 days.[294] Secretion of MMP-2 and MMP-9 by human ASMCs is enhanced in tissues of AAA in response to hypoxia.[295] MMP-2 and MMP-9 appear to be necessary to induce experimental AAA formation in mice,[296] and targeted gene disruption of MMP-9 in mice suppresses the development of AAA.[297] MMP-8 may also have a role in AAA formation. MMP-8 levels are higher in infrarenal aortic biopsies taken from AAA compared with normal aorta. Immunohistochemistry localized MMP-8 to mesenchymal cells within the adventitia of the aortic wall. On the other hand, the levels of TIMP-1 and TIMP-2 were lower in AAA than in normal aortic samples. The high levels of MMP-8 and low levels of TIMP-1 and TIMP-2 in aortic aneurysms may represent a favorable environment for collagen degradation and aneurysm formation and expansion.[298]

MMPs may serve as potential biomarkers for estimation of aneurysmal area and proteolytic activity.[299] Plasma MMP-9 levels are correlated with aneurysmal size and expansion.[300] In a study measuring the levels of MMP-9 in peripheral venous blood from 25 patients with AAA, 15 patients with atherosclerotic occlusive disease, and 5 control subjects, plasma MMP-9 levels were directly correlated with the amount of MMP-9 produced within the aneurysm tissue. Elevated MMP-9 levels were observed in one half of patients with AAA and less than 10% of those with atherosclerotic occlusive disease. Importantly, plasma MMP-9 levels decreased substantially after aneurysm repair.[291] A meta-analysis of data on 580 AAA cases and 258 controls concluded that an elevated MMP-9 has 48% sensitivity and 95% specificity as a diagnostic screening test for the presence of AAA.[301] However, normal MMP-9 levels may not exclude the presence of AAA (negative predictive value, 52%). Also, some studies showed no significant correlation between serum levels of MMP-9 and AAA diameter,[302–305] or between the plasma and aneurysm wall levels of any MMP or TIMP and AAA diameter,[306] making it important to further explore the validity and accuracy of MMP-9 and other MMPs as investigational tools of AAA.

Studies have investigated whether genetic variants of MMPs are associated with AAA risk. A study in 51 patients with AAA and 48 controls showed that variations in MMP-2 gene do not contribute to the development of AAA.[307] In contrast, a study enrolling 414 AAA patients and 203 control subjects showed an association between the T allele of the C-1562T functional promoter polymorphism of the MMP-9 gene and AAA formation.[308] Another study enrolling 146 AAA patients and 156 healthy individuals showed no association between MMP-9 and AAA.[309] A meta-analysis of 6 gene polymorphisms (ACE I/D, MTHFR+677C>T, MMP9-1562C>T, IL-1β/3953C>T, eNOS 4a/4b and TIMP-1/+434C>T) reported in multiple case control studies, showed that 3 of these polymorphisms, ACE RR 1.33 [95% CI 1.20–1.48], MTHFR RR 1.14 [1.08–1.21] and MMP-9 RR 1.09 [1.01–1.18], were associated with a significant risk of AAA.[310]

The mechanism of action of MMPs in aneurysm formation has largely been attributed to their proteolytic effects on ECM proteins and subsequent weakening of the aortic wall. MMP-2 has the greatest elastolytic activity and is produced mainly by VSMCs and fibroblasts.[311] Additional inhibitory effects of MMP-2 and MMP-9 on Ca^{2+}-dependent mechanism of aortic VSM contraction may play a role in the early development of aneurysm.[149] MMP-9 is a more potent inhibitor of aortic contraction than MMP-2, consistent with the dominant MMP-9 expression in AAA wall.[293] Aortic VSM contractile function may contribute to the structural integrity of the aortic wall and limit its tendency to dilate in response to pulsatile forces generated with each cardiac cycle. Atrophy of the tunica media and depletion of VSMCs are consistent histological findings in AAA.[312] Also, disruption of structural integrity of the tunica media e.g. in chronic aortic dissection, often leads to late aneurysm formation. MMP-induced inhibition of VSM contraction may function synergistically with MMP-induced degradation of ECM, causing further weakening of the aortic wall and aneurysm formation.

Studies have investigated the effects of MMP inhibitors on AAA growth and rupture. Small randomized clinical trials suggested favorable effects of doxycycline on retarding AAA expansion.[313] One study demonstrated that two weeks doxycycline treatment in patients with advanced AAA resulted in reduction of aortic wall neutrophil and cytotoxic T-cell content, and suppression of the inflammatory cytokines IL-6 and IL-8 and transcription factors AP-1, C/EBP and STAT3.[314] In another study, patients undergoing endovascular AAA repair were randomized to doxycycline or placebo for 6 months following the procedure. Plasma MMP-9 decreased below baseline in doxycycline treated patients while there was an insignificant increase in the placebo group. In patients with endoleaks at 6 months, plasma MMP-9 increased in 83% of the placebo group, but in only 14% of doxycycline-treated group. Among endoleak-free patients with AneuRx or Excluder endografts, doxycycline caused greater decreases in maximum aortic diameter and the aortic neck dilatation than placebo.[300] Thus, MMP inhibitors may provide an alternative therapeutic tool in AAA.

9. CONCLUDING REMARKS

MMPs play an important role in ECM metabolism and other biological processes, and increased MMP expression/activity has been associated with unrestrained tissue remodeling in autoimmune and vascular disease as well as cancer. MMPs, TIMPs and the MMP/TIMP ratio could provide an estimate of the extent of tissue remodeling in various biological processes and pathological conditions. One challenge is that studies often focus on measuring certain MMPs or TIMPs, and it is important to not generalize the findings to other MMPs and TIMPs. Tissue remodeling is a dynamic process, and an increase in one MMP in a certain region may be paralleled by a decrease of other MMPs in other regions. Also, because of the differences in the proteolytic activities of different MMPs towards different substrates, the activities of MMPs may vary during the course of cardiovascular disease. Therefore it is critical to examine different MMPs and TIMPs in various regions and at different stages of the disease.

MMP inhibitors have been used as investigational tools to determine the role of MMPs in biological processes and pathological conditions. Synthetic MMP inhibitors include ZBG, non-ZBG, and mechanism-based inhibitors. MMP inhibitors may represent potential therapeutic tools in the management of osteoarthritis, cancer and cardiovascular disease. However, most of the currently available MMP inhibitors show little specificity toward different MMPs. Also, most of the clinical trials of MMP inhibitors were not very successful, in part due to the lack of MMP inhibitor efficacy.[315] The low efficacy of MMP inhibitors in clinical trials may be related to the design of the study where MMP inhibitors were often used as single agent therapy in patients with advanced disease, beyond the stage when these compounds could have been effective.[142] Studies in animal models have suggested that MMP inhibitors could be effective in preventing the development and progression of early disease, but may have little effect in advanced stages of the disease. A promising preclinical proof-of-principle study tested whether early treatment with a selective MMP inhibitor between the time of diagnosis and definitive surgery, the so-called "window-of-opportunity," can inhibit breast cancer metastasis and thereby improve survival. The 4T1 mouse model of aggressive mammary carcinoma was treated with SD-7300 (SC-81490), an oral inhibitor of MMP-2, MMP-9, and MMP-13, starting after the initial detection of the primary tumor. Seven days later, the primary tumors were excised and analyzed for MMP activity, and the SD-7300 treatment was discontinued. After 4 weeks, the animals were sacrificed and their lungs were analyzed histologically for number of metastases and metastatic burden (metastases' area/lung section area). SD-7300 treatment inhibited 70% to 80% of tumor-associated MMP activity, reduced lung metastasis number and metastatic burden by 50% to 60%, and increased survival, relative to control vehicle. These findings suggest that treatment of early invasive breast cancer with selective MMP inhibitors can lower the risk of recurrence and increase long-term disease-free survival.[316]

Another limitation of MMP inhibitors is that they may cause musculoskeletal side effects including joint stiffness, inflammation, pain, and tendinitis.[143] Classic MMP inhibitors may have poor selectivity to specific MMPs and could cause additional biological side effects.[75] New approaches have attempted to address some of the drawbacks of classic MMP inhibitors. Specific antibodies could target the MMP active site in a more specific fashion and could identify sites on the MMP molecule that determine their extracellular location and substrate specificity. Specific MMP siRNA could inhibit the expression of specific MMP mRNA. Despite the marked advances in the design of MMP inhibitors, therapeutic inhibition of MMPs remains a challenge, and the tetracycline antibiotic doxycycline is the only FDA-approved MMP inhibitor.[317] New MMP inhibitors with better selectivity could improve the specificity of MMP inhibitors, target specific MMPs in relevant pathological conditions, and mitigate some of the side effects. Targeted delivery of MMP inhibitors locally in the vicinity of the lesion could also minimize their systemic undesirable effects. Co-delivery of drug and gene is a new strategy in cancer therapy. Studies have utilized folate-targeted star-shaped cationic copolymer consisting of β-cyclodextrin and poly(L-lysine) dendron to co-deliver docetaxel chemotherapy and MMP-9 siRNA for treatment of nasopharyngeal carcinoma. Codelivery of docetaxel/MMP-9 siRNA induced more apoptosis and decreased invasive capacity of HEN-1 nasopharyngeal carcinoma cells. *In vivo* assays showed that codelivery of docetaxel/MMP-9 siRNA inhibited HNE-1 tumor growth and

decreased expression of the cell proliferation marker proliferating-cell nuclear antigen (PCNA), suggesting that it could be a promising strategy for treatment of localized tumors such as nasopharyngeal carcinoma.[318]

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ABREVIATIONS

AAA	abdominal aortic aneurysm
ADAM	a disintegrin and metalloproteinase
AngII	angiotensin II
ECM	extracellular matrix
EGF	epidermal growth factor
ERK	extracellular signal–regulated kinase
FAK	focal adhesion kinase
FGF	fibroblast growth factor
GPCR	G-protein coupled receptor
HUVECs	human umbilical vein endothelial cells
IGF	insulin-like growth factor
IVC	inferior vena cava
MAPK	mitogen-activated protein kinase
MMP	matrix metalloproteinase
NO	nitric oxide
oxLDL	oxidized low density lipoprotein
PARs	protease-activated receptors
PDGF	platelet-derived growth factor
RASMCs	rat aortic smooth muscle cells
TAA	thoracic aortic aneurysm
TGF	transforming growth factor
TIMP	tissue inhibitor of metalloproteinases

TNF-α	tumor necrosis factor-α
VEGF	vascular endothelial growth factor
VSM	vascular smooth muscle
VSMCs	VSM cells
Zn^{2+}	zinc
ZBG	zinc binding globulin

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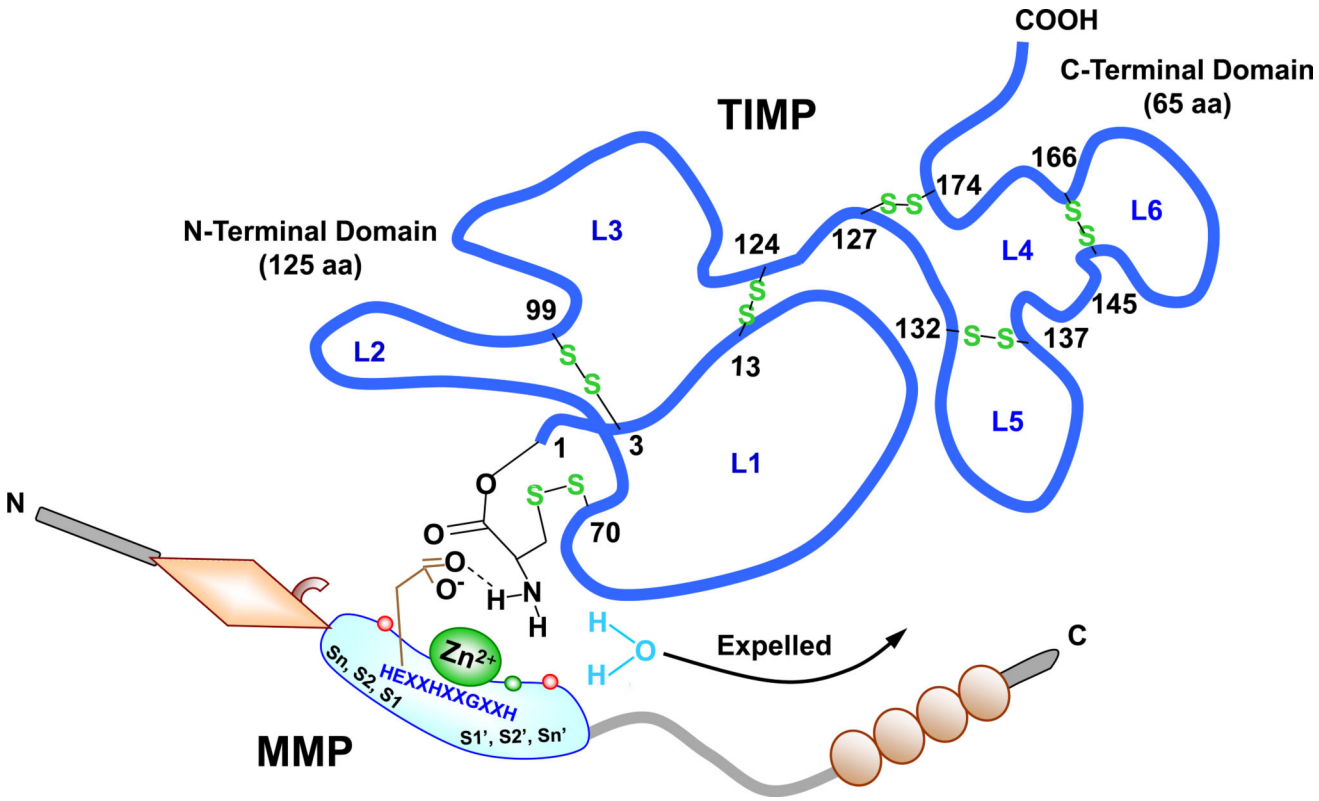

Fig. 1.

TIMP-MMP Interaction. TIMP is a ~190 aa protein, with an N-terminal domain (loops L1, 2, and 3) and C-terminal domain (loops L4, 5 and 6), which fold independently as a result of 6 disulfide bonds between 12 specific Cys residues. The N-terminal Cys1-Thr-Cys-Val4 and Glu67-Ser-Val-Cys70 are connected via a disulfide bond between Cys1 and Cys70 and are essential for MMP inhibition, as they enter the MMP active site and bidentately chelate the MMP Zn^{2+}. The carbonyl oxygen and α-amino nitrogen in the TIMP Cys1 coordinate with the MMP Zn^{2+}, which is localized in the MMP molecule via the 3 histidines in the HEXXHXXGXXH motif. The TIMP α-amino group then expels Zn^{2+}-bound H_2O by binding the MMP H_2O binding site and forming a H bond with carboxylate oxygen from conserved MMP Glu202 (E in the HEXXHXXGXXH sequence). TIMP-1 and MMP-3 are used as prototypes. The amino acids involved in Zn^{2+}- and pocket-binding may vary with different MMPs and TIMPs.

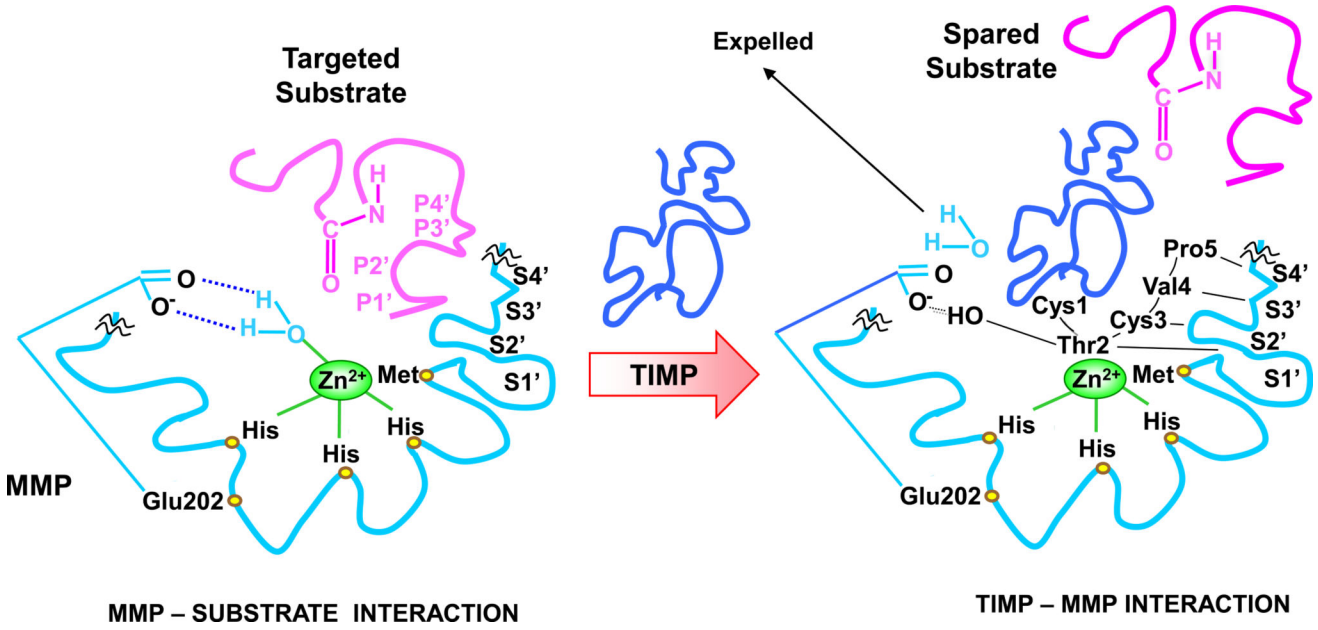

Fig. 2.

MMP Inhibition by TIMP. TIMP Thr2 side chains enter the MMP S1' pocket in a manner similar to that of a substrate P1' substituent, largely determining the affinity to MMP. Thr2 – OH group could also interact with Glu202, further contributing to expelling Zn^{2+}-bound H_2O and preventing substrate degradation. Additionally, the TIMP Cys3, Val4 and Pro5 interact with MMP S2', S3', and S4' pockets in a P2', P3', and P4'-like manner, further preventing substrate binding or degradation. TIMP-1 and MMP-3 are used as prototypes. The amino acids involved in Zn^{2+}- and pocket-binding may vary with different MMPs and TIMPs.

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Table 1

MMP inhibitors, their specificity to MMPs (K_i or IC_{50} <1 nM to 10 μM), and their effects in preclinical trials.

	MMP Inhibitor Category, Number, Chemistry, Other Name	MMP Specificity, IC_{50} or K_i					Preclinical Trial
		<1 nM	1–10 nM	11–100 nM	0.1–1 μM	1–10 μM	
	ZBGs, Hydroxamic Acids						
	Succinyl Hydroxamate						
1	Batimastat (BB-94)		1, 2, 8, 9	3			
	Marimastat (BB-2516)		1, 2, 9, 14	7			
	Ilomastat (GM6001, Galardin)	1, 2, 8, 9, 26	7, 12	3, 14			Glioblastoma, breast, lung, ovarian, and prostate cancer
2	Sulfonamide Hydroxamate, AG3340, Prinomastat	2, 3, 9, 13, 14	1	7			Neovascularization, lung and prostate cancer, uveal melanoma, gliomas
3	Succinyl Hydroxamate		1				
4	RS-104966	13		1			
7	Sulfonamide Hydroxamate	2	8, 9, 14		1, 3	7	Decrease tumor invasion
8	Sulfonamide Hydroxamate		3		2	9	Chronic non-healing wounds
10				2, 3		1	
12						3	
5	**Carboxylic Acids**		13	3, 8	2	7, 9, 14	Osteoarthritis
6			11		3, 12	1, 9, 14	Chronic obstructive pulmonary disease
9	**Sulfonylhydrazides**		2, 9	1	7	3	
16	**Thiol- and Cyclic Mercaptosulfides**	9	2	1, 7, 14	3		
19	**Aminomethyl Benzimidazoles**				11	2, 9, 13	
	Phosphorous-Based						
17	Sulfonamide Phosphonate	8	2	3			

	MMP Inhibitor Category, Number, Chemistry, Other Name	MMP Specificity, IC_{50} or K_i					Preclinical Trial
		<1 nM	1–10 nM	11–100 nM	0.1–1 μM	1–10 μM	
18	Sulfonamide Phosphonate		8	2, 9, 13, 14	1, 3	7	Liver disease, multiple sclerosis, breast cancer
20	Carbamoyl Phosphonate			2			Melanoma
21	Carbamoyl Phosphonate					2	Melanoma, prostate cancer
	Nitrogen-Based						
22	Oxazoline					11	
23	Dionethiones and Pyrimidine-2,4,6 triones, Ro-28-2653		2, 14	8, 9		3	Anti-angiogenic and anti-invasive in tumor models
24	Dionethiones and Pyrimidine-2,4,6 triones	13			2, 9, 12		Osteoarthritis
	Heterocyclic Bidentate Chelators						
25	Terphenyl Backbone, AM-6			3			
26	Biphenyl Backbone, 1,2 -HOPO-2			8, 12	2, 3	13	Cardiac ischemia /reperfusion injury
27	Diphenyl Ether Backbone		2, 9, 13	3		1	Brain edema following ischemia/reperfusion
28	Biphenyl Backbone, Pyrone-based			3, 9, 12	8	2, 13	
29	Biphenyl Backbone, Hydroxypyridinone Derivative					8, 12	
30	Biphenyl Backbone, AM-2			8, 12	−3	−2	
31	Biphenyl Backbone					2, 8, 12, 13	
34	**Non-ZBGs**			13			Osteoarthritis
35				12	2, 8, 13	3, 9	
36						2, 8, 13	
37		13					Osteoarthritis
38	Pyrimidine Dicarboxamide					13	

	MMP Inhibitor Category, Number, Chemistry, Other Name	MMP Specificity, IC_{50} or K_i					Preclinical Trial
		<1 nM	1–10 nM	11–100 nM	0.1–1 μM	1–10 μM	
39	Pyrimidine Dicarboxamide			13			
	Mechanism-Based						
40	Diphenyl Ether Backbone, SB-3CT			2	9, 14	3	Inhibits liver metastasis in T-cell lymphoma and bone metastasis in prostate cancer
42	Diphenyl Ether Backbone, Thiol-Containing			2, 9	14	3	
43	Diphenyl Ether Backbone		2	14	9	3	
45	Diphenyl Ether Backbone		9	2	3, 14		

For details and original references see.[11,78,319,320]

Table 2

Cardiovascular Effects of Gene Ablation of specific MMPs or TIMPs in Mice

MMP/TIMP	Cardiovascular Phenotype	Reference
MMP-2	Reduced neointima formation after vascular injury. Protection from cardiac rupture post-myocardial infarction.	161,321
MMP-9	Reduced neointima formation after vascular injury. Protection from cardiac rupture post-myocardial infarction, vessel stiffness, increased pulse pressure.	161,224,322
MMP-11	Accelerated neointima formation after vascular injury	323
MMP-14	Defective angiogenesis	5,324
TIMP-1	Accelerated neointima formation after vascular injury. Spontaneous cardiac dilatation, increased cardiac dysfunction post-myocardial infarction.	325
TIMP-3	Spontaneous dilated cardiomyopathy	326

Table 3

Representative clinical studies demonstrating the relationship between MMP or TIMP levels and hypertension

Study Year Type	Subjects	Design	Findings	Ref
1998 Clinical Trial	37 patients with essential hypertension, 23 control normotensive subjects	Measure serum levels of carboxy-terminal telopeptide of collagen type I (marker of collagen degradation), MMP-1, TIMP-1, and MMP-1/ TIMP-1 ratio. Repeat measurements after 1 year treatment with the ACE inhibitor lisinopril	No difference in collagen type I levels. Decreased MMP-1 and increased TIMP-1 in hypertensive versus normotensive subjects. Hypertensive patients with left ventricular hypertrophy showed lower free MMP-1 and collagen type I and higher free TIMP-1 than hypertensive patients without left ventricular hypertrophy. Patients treated with lisinopril showed increased serum collagen type I and free MMP-1 and decreased free TIMP-1	327
2003 Clinical Trial	42 hypertensive patients	6 Months treatment with amlodipine	Normalized plasma levels of MMP-9, but not MMP-2	328
2006 Cross Sectional	44 hypertensive patients, 44 controls	Measure plasma levels of MMP-2, MMP-9, and TIMP-1	Higher plasma levels of MMP-2, MMP-9, and TIMP-1 in hypertensive versus control subjects	329
2007 Cross Sectional	202 hypertensive patients, 54 control	Measure carotid-femoral and carotid-radial pulse wave velocity to determine arterial elasticity. Measure serum levels of MMP-9 and TIMP-1 levels by ELISA	Higher serum levels of MMP-9 and TIMP-1 in hypertensive patients versus control subjects. Age, systolic blood pressure, heart rate and TIMP-1 levels were independent predictors of carotid-femoral pulse wave velocity in hypertensive subjects	330
2009 Clinical Trial	33 patients with stage 1 hypertension, 16 age-matched control	Assess serum levels of MMP-9 and TIMP-1 in the hypertensive group before and after 3-month-anti-hypertensive therapy	Pre-treatment serum MMP-9 levels were higher and TIMP-1 levels were lower in hypertensive group versus control. Anti-hypertensive treatment was associated with decreased serum MMP-9 levels and increased TIMP-1 levels	221
2009 Randomized Clinical Trial	595 Non-hypertensive Framingham Offspring Study, participants without prior heart failure or myocardial infarction, mean age 55 years, (360 women)	Measure plasma levels of MMP-9, TIMP-1, and procollagen III N-terminal peptide for 4 years	81 Subjects (51 women) developed hypertension, and 198 (114 women) progressed to higher blood pressure. Subjects with detectable MMP-9 had 1.97-fold higher risk of blood pressure progression than those with undetectable MMP-9. A 1-SD increment of log-TIMP-1 was associated with 50% higher incidence of hypertension and 21% higher risk of blood pressure progression. Individuals in the top TIMP-1 tertile had a 2.15-fold increased risk of hypertension and 1.68-fold increased risk of blood pressure progression relative to the lowest tertile. Plasma procollagen III N-terminal peptide was not associated with hypertension or blood pressure progression.	331
2010 Cross sectional	64 Children (34 males, 30 females)	Measure circulating levels of MMP-2, MMP-9, TIMP-2, insulin-like growth factor-I and insulin growth factor binding protein-3	Circulating levels of MMP-2 and MMP-9 correlate with systolic blood pressure and vascular function. MMP-2 was an independent predictor of systolic blood pressure. MMP-9 was an independent predictor of vascular dysfunction	332