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Review

Matrix metalloproteinases: A review of their structure and role in acute coronary syndrome

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Abstract

Objective: To summarize the structure, function, and regulation of matrix metalloproteinases (MMPs) and to review the literature to date on their potential role in the pathogenesis of acute coronary syndromes. **Methods:** A recursive strategy starting with a Medline Search for primary articles in the last decade, followed by identification of additional articles of interest among the cited literature in the primary articles, followed by identification of additional articles of interest cited in the secondary articles. **Results:** MMPs play a central role in many fundamental processes in human health and disease. In vitro evidence suggests that MMP activity may facilitate atherosclerosis, plaque destabilization, and platelet aggregation. Limited evidence from clinical studies supports a role of MMPs in the development of acute coronary syndromes. **Conclusions:** MMP activity likely contributes to the development of acute coronary syndromes and may be an important therapeutic target for future drug development.

Keywords: Acute coronary syndromes; Matrix metalloproteinases

1. Introduction

Matrix metalloproteinases (MMPs) are a family of Zn^{2+}-dependent endopeptidases capable of cleaving components of extracellular matrix [1,2]. This ability to modify the structural integrity of tissues is essential for certain aspects of normal physiology, including embryonic development, cell migration, wound healing, and tissue resorption. MMP modification of integrins and activation of certain cell-signaling cascades also contribute to regulation of platelet function. However, the disregulation or activation of MMP expression is a feature of numerous pathologic conditions, such as tumor metastasis, vascular and cardiac remodeling, and certain rheumatic conditions. Recently, several lines of evidence have implicated MMPs in the rupture of atherosclerotic plaques and subsequent acute coronary syndromes. The purpose of this review is to briefly summarize the structure, function, and regulation of these enzymes, and to review the emerging body of evidence linking MMP activity and plaque rupture, with an emphasis on data in humans with or at risk for acute coronary syndromes.

2. MMP structure and function

MMPs were discovered in 1962, in an effort to establish how the metamorphosing tadpole of a frog lost its tail. Since then, over 66 MMPs (including 20 human MMPs), have been cloned and sequenced [3]. MMPs are found in most living organisms, including the simplest bacteria, suggesting that certain primordial MMPs evolved over 3.5 billion years ago [4,5].

Most MMPs are synthesized and secreted as inactive proenzymes. The majority of MMPs include a propeptide domain with a unique and highly conserved cysteine-containing sequence (‘cysteine switch’) that is capable of

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binding zinc in the catalytic domain, thereby rendering the enzyme inactive [6,7]. Proteolytic disruption of the cysteine–Zn^{2+} bond and removal of the propeptide domain exposes (activates) the catalytic domain. Calcium ions are also required for expression of enzyme activity. The C-terminal hemopexin-like domain has been shown to play a role in substrate binding [7].

MMPs can be categorized into the following groups: collagenases, gelatinases, stromelysins, matrilysins, metalloelastases, and membrane-type matrix metalloproteinases (MT-MMPs). The collagenases include MMP-1, MMP-8, and MMP-13. This group was once thought to be unique in their ability to initiate cleavage of triple helical collagens I, II, and III [8]. The site of cleavage of these fibrillar collagens has been shown to be the Gly–Ile/Leu bond in the collagen α chains, three-quarters of the distance from the amino terminal end of each chain. These fragments then spontaneously denature at physiologic temperature, making them susceptible to further degradation by other members of the MMP family as well as further degradation by the collagenases. Native types IV and V are completely resistant to collagenase activity [8–12].

Gelatinases are composed of the 72-kDa MMP-2 and the 92-kDa MMP-9 [13]. These enzymes are known to cleave native type IV, V, VII, and X collagens and elastin, as well as the products of collagens types I, II and III after proteolysis by collagenases. Recently, research has shown that MMP-2 is also capable of cleaving type I collagen at the same site as the interstitial collagenases, whereas MMP-9 may lack this ability [14].

The stromelysin group is composed of MMP-3, MMP-10, and MMP-11. MMP-3 and MMP-10 have an identical spectrum of activity, but MMP-3 is more potent [15]. Their substrates include proteoglycan core protein, laminin, fibronectin, elastin, as well as nonhelical regions of collagens [15]. MMP-11 has weak, if any, activity against extracellular matrix components; instead its substrates are serine proteinase inhibitors (serpins). Of note, other members of the MMP family have been shown to have activity against some serpins; however, these MMPs have higher affinities for other extracellular matrix molecules [16–18]. MMP-11 is also unique from the other stromelysins in that it is released as an active enzyme, being activated intracellularly by furin, a *trans* Golgi-associated protein [16].

The sole member of the matrilysin group is MMP-7, which was first characterized from a human rectal carcinoma cell line. It has greater activity than the other MMPs against versican, a chondroitin sulfate proteoglycan that is particularly abundant in atherosclerotic plaque, and also degrades other common stromelysin substrates [17,19,20]. MMP-7 is also thought to be less susceptible to tissue inhibitors of MMPs (TIMPs) [21,22].

MMP-12, or metalloelastase, is a 22-kD MMP with activity against elastin. In addition to its activity on elastin, MMP-12 is capable of degrading other components of the extracellular matrix, including proteoglycans, fibronectin, laminin, vitronectin, type IV collagen, and heparin sulfate [23].

The MT-MMPs are similar in structure to the soluble MMPs in that they contain the propeptide region with the conserved cysteine switch, the zinc-catalytic domain, and the hemopexin-like domain near their C terminus. They also contain a short amino acid sequence between their propeptide and catalytic domains that serves as a mechanism for their intercellular activation. Furthermore, the MT-MMPs also contain 75–100 amino acid extensions at their C-terminus with a hydrophobic region serving as a transmembrane domain [24]. Four different membrane-type MMPs have been identified (MT-MMP 1, MT-MMP 2, MT-MMP 3, and MT-MMP 4). These MMPs have been shown to readily degrade gelatin, fibronectin, laminin, vitronectin, and dermatan sulfate proteoglycan, and also are initiators of activation of MMP-2 [25,26]. MT1-MMP also has been shown to specifically cleave native type-I and type-III collagens into 3/4 and 1/4 length fragments [26].

3. Regulation of MMP activity

MMP activity is regulated at three levels: gene transcription, posttranslational activation of zymogens, and interactions of secreted MMPs with inhibitors [27]. It is thought that for most MMPs (excluding MMP-2), the key step to regulation is at the level of transcription [3] (Table 1). MMP gene expression is regulated through the interaction

Table 1
Transcriptional regulation of MMPs[a]

	Hypoxia	Reoxygenation	OXLDL	IL-1	IL-1B	TNF-α	TGF-B_1	CD40-L	Thrombin	Refs
MMP-1			+	+		+		+		[29,31,41]
MMP-2	+/−	+			+		+		+	[30,37,38]
MMP-3					+	+		+	+	[29,30,37]
MMP-8					+	+		+		[35]
MMP-9			+			+	+	+		[29,34,36,40,43–45]
MMP-12					+	+	−			[31]
MMP-13					+					[30]
MMP-14		+	+	+		+				[38,125]

[a] LPS, Chlamydia HSP60, SDF-1, PPARγ, HDL, and several growth factors have also been shown to modify expression of at least one MMP.

of transcription factors, and co-activators and co-repressor proteins with *cis*-acting elements in the promoter region of MMP genes. The mechanism by which gene transcription is mediated is thought to be through a prostaglandin E_2 (PGE_2)–cAMP dependent pathway. G-proteins have been implicated in this pathway [28]. Transcriptional activation can be stimulated by a variety of inflammatory cytokines, hormones, and growth factors, such as interleukin-1β (IL-β), IL-6, tumor necrosis factor-α (TNF-α), epidermal growth factor, platelet-derived growth factor (PDGF), and basic fibroblast growth factor [29–32]. Other factors involved in upregulation include *Chlamydia pneumoniae* heat shock protein 60 (HSP60), which induces TNF-α and MMP-9; and CD40 ligand, which induces MMP-1, MMP-3, and MMP-8 [33–35]. Insulin, through influencing the binding of activator protein-1 (AP-1), has a stimulatory effect on the expression of MMP-12 [23]. Hyperglycemia can increase MMP-9 activity in vascular endothelial cells; however, this effect could be secondary to the effects of increased insulin [36]. Thrombin has been shown to upregulate MMP-2 and MMP-3 mRNA [37]. Prolonged hypoxia, greater than 24 h, has been shown to increase MMP-2 mRNA expression, whereas shorter durations decrease its expression. Re-oxygenation after short periods of hypoxia upregulates MMP-2 and MT1-MMP mRNA expression [38]. Oxidized LDL increases expression of MMP-1, MMP-9, and MMP-14 [39–41].

Several factors are also known to inhibit MMP gene expression. Some of these inhibitors include indomethacin, corticosteroids, and interleukin-4 (IL-4) [2,30,42]; their suppression can be restored by the exogenous addition of PGE_2 or cAMP [28]. Peroxisome proliferator-activated receptor gamma (PPARγ), a ligand-activated nuclear receptor transcription factor, is expressed in vascular smooth muscle cells and macrophages and its activation has been demonstrated to inhibit MMP-9 mRNA and protein expression. Therefore, ligands of PPARγ, e.g., prostaglandin D_2 metabolite and troglitazone, decrease MMP expression [43]. However, not all MMPs react similarly to the same stimulus and the impact of various factors can be cell-specific. For example, transforming growth factor-β1 (TGF-β1) has been shown to inhibit MMP-12 expression in human peripheral blood macrophages, although in human monocytes it increases expression of MMP-2 and MMP-9 [31,44]. Similarly, stromal cell-derived factor (SDF-1) has been shown to reduce MMP-9 expression in monocytes of patients with unstable angina; however in human megakaryocytes it increases expression and release of MMP-9 [44,45].

MMP gene transcription can also be modified by promoter region sequence variants [46]. For example, a C to T change at position −1562 in the promoter region of the gelatinase B (MMP-9) gene, influences MMP-9 expression [47]. The T variant has greater promoter activity than the C allele secondary to preferential binding of a repressor protein to the C allele. Similarly, a functionally significant 5A/6A polymorphism has been reported in the promoter region of the MMP-3 gene [48]. The 5A allele has been shown in vitro to have a two-fold increase in promoter activity than the 6A variant. This is thought to be secondary to a preferential binding of a repressor protein to the 6A allele [49]. Several studies of these, and other MMP promoter region polymorphisms, have been associated with various aspects of cardiovascular disease (see below), although in most cases the precise mechanisms have not been elucidated.

4. Activation

Activation of latent zymogens can occur intracellularly, at the cell surface by MT-MMPs, in the extracellular space through the action of other proteases, or even by previously activated MMPs through a process called stepwise activation (Fig. 1). Both stromelysin-3 (MMP-11) and MT1-MMP (MMP-14) can be activated intracellularly. These MMPs contain a motif of basic amino acids upstream of the catalytic domain that are thought to act as endoproteolytic processing signals for furin via the trans-Golgi network [50,51]. They are processed to active proteinases through a process involving post-translational endoproteolysis, further processed by furin via the trans-Golgi network and then secreted in an active form [24].

The main mechanism of pro-MMP-2 activation involves the zymogen forming a complex at the cell surface with MT1-MMP and TIMP-2 and a neighboring MT1-MMP cleaving pro-MMP-2 at the pro-domain [52]. This process is thought to be mediated by integrin binding to the hemopexin domain of pro-MMP-2 which facilitates this process [52]. Several integrins have been shown to be involved in this process, including α2β1and αVβ3 [53,54]. In addition, MT-MMP, facilitated by MMP-2, can activate the zymogen form of MMP-13 [55].

Certain proteases, especially plasmin, are thought to be the major contributors in the initiation of extracellular stepwise activation via the cysteine switch [6]. Pro-MMP-2 is thought to lack the cleavage sites in the amino-terminal domain required for activation by plasmin [56]. However, reactive oxygen compounds that undergo reactions with thiol groups are known to activate pro-MMP-2 and are postulated to act through the ‘cysteine switch’ mechanism previously described [57]. Two coagulation factors, thrombin and factor Xa, have been demonstrated to activate pro-MMP-2 [56,58,59]. Several MMPs can also perform the first step in the stepwise activation of pro-MMP-2. MMP-3 has been shown to activate the zymogen form of MMP-1, MMP-7, MMP-8, MMP-9, and MMP-13 [20,27,60,61]. Once activated, MMP-7 can also activate proMMP-1, 9, and MMP-13, thereby producing a cascade-like effect on MMP activation. Similarly, MMP-2 activates proMMP-9 [62] and MMP-12 has been shown to activate pro-MMP-2 and MMP-3 [2].

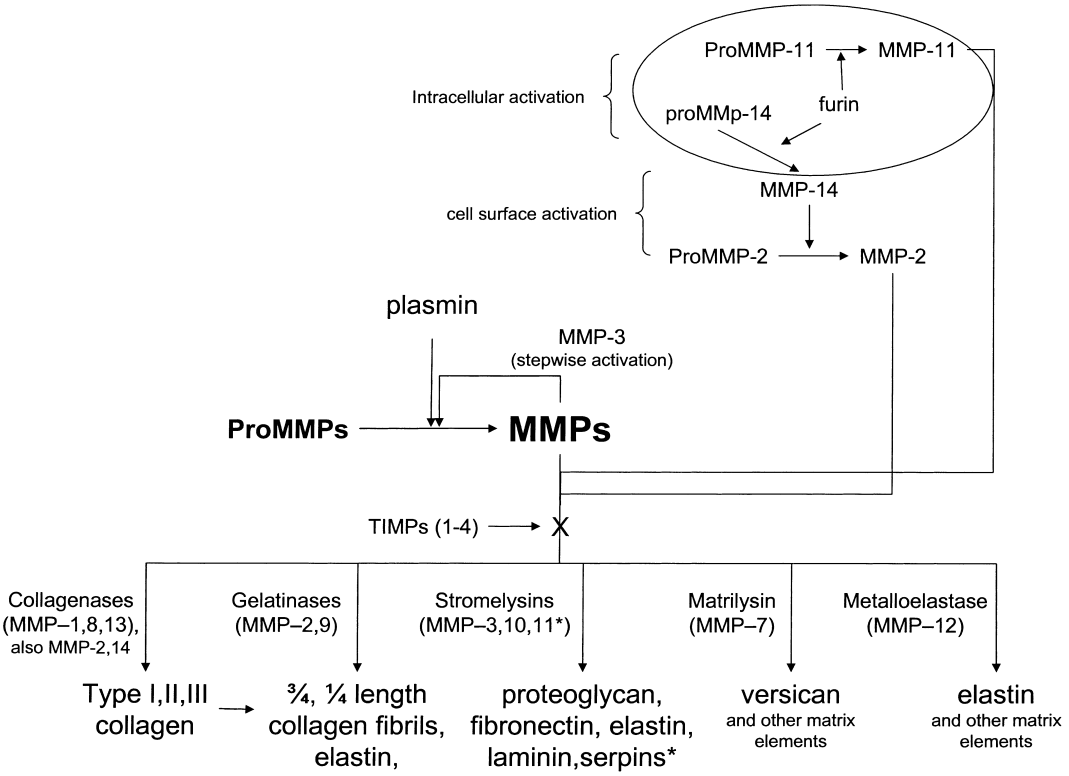

Fig. 1. Schematic summary of MMP activation and activity.

MMP activity is also regulated by tissue-specific inhibitors, of which there are four known tissue inhibitors of metalloproteinases (TIMP-1, TIMP-2, TIMP-3, and TIMP-4) (Fig. 1). The TIMPs are secreted by a variety of cell lines including smooth muscle cells and macrophages and their activity is increased by PDGF and TGF-β and either increased or decreased by different interleukins [63]. Tissue factor pathway inhibitor-2 (TFPI-2) also reduces activity of MMP-2 and MMP-9 and decreases the ability of MMP-1 and MMP-13 to degrade triple-helical collagen [35].

5. Effects of cardiovascular medications on MMP activity

Several therapies commonly prescribed for patients with coronary disease may influence MMP function, see Table 2. Nitroglycerin, frequently used in the symptomatic relief of stable and unstable angina, has been shown to increase the expression and the activity of MMP-2, MMP-7, and MMP-9 in isolated human macrophages; nitroglycerin was also shown to decrease TIMP-1 protein and mRNA levels [64]. Similarly, heparin has been shown to induce MMP-1 and MMP-2 at both the mRNA and the protein level [65]. Recent evidence has shown rosiglitazone decreases levels of MMP-9 through the previously described mechanism of binding to PPARγ [66]. The calcium channel blockers, amlodipine and diltiazem, have been shown to increase the activity of MMP-1 and MMP-2 in cultured human vascular endothelial cells; however, all three subclasses of calcium channel blockers have been shown to upregulate IL-6 mRNA, and IL-6 has been shown to increase TIMP-1 [67]. Furthermore, amlodipine has been shown to decrease MMP-1 levels in IL-1β-induced human endothelial cells [68]. The net effect of ACE inhibitors on MMP expression is unclear. Decreased MMP-1 activity has been shown with angiotensin II therefore predicting that ACE inhibitors may increase the activity of MMP-1 [69]. Losartan has been shown to increase MMP-2 activity in human vascular smooth cells [70].

HMG-CoA reductase inhibitors have been shown to affect MMP expression. In one study, fluvastatin and lovastatin inhibited MMP-1 expression in endothelial cells [71]. Interestingly, pravastatin did not have this effect. The authors speculated that this difference was secondary to the more lipophilic HMG-CoA reductase inhibitors (fluvastatin and lovastatin) being more permeable through endothelial cell membranes. Also fluvastatin and simvastatin have been shown to decrease the secretion of MMP-9 from human macrophages in vitro [72]. In contrast, in one small clinical study pravastatin did demonstrate a decrease in MMP-2 and an increase of TIMP-1 in human carotid plaques [73] compared to placebo. Recently, Cipollone et al. demonstrated COX-2/mPGES and MMP-2 and MMP-9 were decreased in human carotid plaques in patients on simvastatin [74]. Although limited effects have been previously reported, Luan et al. recently found that multi-

Table 2
Effects of cardiovascular medication on MMP levels

	Nitroglycerin	Rosiglitazone	Amlodipine	Losartan	Statin	Estrogen
Collagenases						
MMP-1			?[a] [67]		↓ [68]	↓ [79]
MMP-8						
MMP-13						
Gelatinases						
MMP-2	↑ [64]			↑ [70]	↓ [73,74]	↑ [77]
MMP-9	↑ [64]	↓ [66]			↓ [72,74]	↑ [80]
Stromelysin						
MMP-3						
MMP-10						
MMP-11						
Matrilysin						
MMP-7	↑ [64]					
Metalloelastase						
MMP-12						

[a] Amlodipine has been shown to increase MMP activity in human vascular endothelial cells [67], but decreased activity in IL-1 beta-induced endothelial cells [68].

ple statins inhibited MMP-3 activity in rabbits (in addition to MMP-1 and 9) [75].

Estrogen receptor-α and -β have been previously identified in vascular smooth muscle, including aorta and coronary vessels. Estradiol has been shown to increase levels and activity of MMP in mesangial cells [76]. Estrogen increases MMP-2 activity in rat mesenteric arteries [77]. It has also been reported that raloxifene, a selective estrogen receptor modulator, increases the production of MMP-1 in monocytes [78]. In human coronary endothelial cells, high concentrations of estradiol decrease MMP-1 activity in vitro and this effect is enhanced with the addition of progestins [79]. The clinical evidence regarding estrogen action and MMPs is sparse. A small study showed serum levels of MMP-9 to be increased in most women taking conjugated equine estrogen alone or with a progestin, a result thought to be secondary to estrogen's inhibition of PAI-1 [80]. In contrast, progesterone, through secondary mechanisms in stromal cells, limits MMP activity [81]. These effects of estrogen and progesterone may have contributed to the unexpected increase in CHD events observed in women treated with hormone therapy in the Women's Health Initiative [82].

COX-2 inhibition may also inhibit MMP expression. As previously described, MMP production in macrophages has been shown to be through a PGE_2/cAMP dependent pathway. Theoretically, COX-2 inhibition could attenuate this pathway. However, the Vioxx Gastrointestinal Outcomes Research (VIGOR) and Celecoxib Long-term Arthritis Safety Study (CLASS) trials showed an increase, rather than a decrease in ACS in patients on COX-2 inhibitors compared to subjects not taking these or other non-steroidal anti-inflammatory medications [83]. Another target of MMP inhibition has been demonstrated in animal models of adenovirus-mediated TIMP gene transfer. Infection with TIMP-2 has been shown to decrease SMC invasiveness and to delay neointimal development in rats and to reduce lesion area in apoE-deficient mice [84,85].

6. MMPS and atherosclerosis

Several lines of evidence indicate that MMPs influence the process of atherosclerotic lesion formation (Table 3). On one hand, MMP activity may contribute to the pathogenesis of atherosclerosis by facilitating migration of vascular smooth muscle cells through the internal elastic lamina into the intimal space, where they proliferate and contribute to plaque formation [11,86,87]. On the other hand, MMP activity may diminish plaque volume by degrading extracellular matrix in the intima [78]. Studies using various genetic manipulations in animal models have been used to determine which MMPs are relevant in the progression of atherosclerosis. In one study of TIMP-1-deficient mice (in which MMP activity is increased), reduced atherosclerotic plaque size was noted [88]. In contrast, in another study by the same investigators, mice fed a high-cholesterol diet for 30 days (MMP-3 knockout mice) had increased aortic plaque size [89]. Galis et al. used an MMP-9 knockout mouse carotid artery model to demonstrate that a MMP-9 deficiency leads to a decrease in intimal hyperplasia and lumen loss, but an accumulation of interstitial collagen [27]. The authors speculated that MMP-9 inhibition could increase the mechanical stability of arteries by increasing their collagen content and decreasing lumen loss.

Table 3
Non-membrane bound MMPs with significance in atherosclerosis

	Activity in plaques	Elevated in ACS	Elevated in PCI	References
Collagenases				
MMP-1	+			[12,98]
MMP-8	+			[102]
MMP-13	+			[12]
Gelatinases				
MMP-2	+	+	+	[98,113,118–120]
MMP-9	+	+	+	[98,113,114,118]
Stromelysin				
MMP-3	+			[98]
MMP-10	+			[15]
MMP-11	+/−			[16]
Matrilysin				
MMP-7				
Metalloelastase				
MMP-12				
Membrane bound				
MMP-14			+	[119]

MMPs may also facilitate positive remodeling of the artery wall through digestion of the external elastic lamina, thereby minimizing luminal encroachment of accumulating plaque. In one study intravascular ultrasound was used in conjunction with directional coronary atherectomy to demonstrate increased MMP-3 staining in human coronary arteries undergoing positive remodeling [90]. Another study used hearts obtained within 24 h post mortem from patients who did not die from coronary artery disease. Immunostaining revealed MMP-2 and MMP-9 to be more prevalent in plaques of expansively remodeled vs. constrictive remodeled segments of atherosclerotic coronary arteries [91].

Finally, not all of the clinical data are consistent with respect to MMPs and extent of atherosclerosis. Noji et al. investigated circulating levels of MMPs in premature coronary atherosclerosis. In this study 53 male patients who had one or more significant stenosis (>50% of diameter) were compared to 133 similar subjects thought to be free from atherosclerosis by history, rest EKG, and exercise tolerance test. Patients with unstable presentation or diabetes were excluded from this study. Blood levels of MMP-9 were found to be significantly higher in the patients with known CAD. In contrast, CAD patients had lower levels of MMP-2 and MMP-3. The design of this study was limited by not having control subjects with angiographically proven normal coronary anatomy [92].

7. In vitro evidence linking MMP activity with plaque rupture

There is a rapidly expanding body of evidence suggesting that acute coronary syndromes may also be influenced by MMPs through degradation of the fibrous cap of vulnerable atherosclerotic lesions. The tensile strength of the fibrous cap protecting the plaque from disruption is mainly derived from collagen types I and III; however, the fibrous cap also contains elastin and proteoglycans [87,93–95]. The accumulation of macrophage-derived foam cells in atherosclerotic lesions correlates with increased local release of MMPs and a thin fibrous cap [29,96].

Much of the existing data implicating MMPs in plaque rupture has been obtained from patients undergoing coronary atherectomy and carotid endarterectomy. In one such report, specimens from patients with unstable angina showed a 70% increase in intracellular MMP-9, indicating active synthesis, compared to specimens from patients with stable angina [97]. MMP-2 (gelatinase A) is highly activated in coronary plaques, and its activation is correlated with plaque calcification [92,98,99]. Similarly, plaques from patients undergoing carotid endarterectomy that were thought to be unstable(patients symptomatic within 1 month of surgery) stain higher for MMP-9 than plaques from patients that were thought to be stable [100]. Another study of carotid endarterectomy samples reported increased levels and activity of MMP-1 and MMP-13 in atheromatous plaques compared to fibrous lesions [12]. MMP-1 expression is also increased in regions of high mechanical stress in human coronary lesions [101]. MMP-7 and MMP-12 are thought to play an important role in plaque rupture secondary to their production in atherosclerotic plaques by foam cells that reside along the perimeter of lipid cores. More recently, MMP-8 (previously neutrophil collagenase) was reported to be expressed by human vascular endothelial cells, smooth muscle cells, and macrophages [102].

Furthermore, its activity is increased in the shoulder regions of atheromatous plaques that are most vulnerable to rupture [91,102]. Of the group of interstitial collagenases, MMP-8 has been previously determined to have the most activity against collagen type I (the major contributor to the tensile strength of fibrous plaques) and has been found to be increased in shoulder regions of atheromatous plaques [102]. MMP-3 is expressed by cells of atherosclerotic plaques, but not by cells of normal arteries [15,98,103,104]. MMP-11 has been demonstrated in human atherosclerotic plaques and has been proposed to have implications outside of matrix breakdown through action on the coagulation cascade and fibrinolytic activity [16]. Finally, MMP-14 has been demonstrated in human atherosclerotic plaques co-localizing with MMP-2 in the media underlying fibrous and lipid-rich regions [39].

Additional evidence linking MMPs to plaque rupture can be seen from data linking cyclooxygenase (COX) and prostaglandin E_2 synthase (PGES) to MMP production. As previously described, production of MMPs by macrophages occurs through a PGE_2/cAMP-dependent pathway. This was demonstrated by Cipollone et al., using plaques from patients undergoing carotid endarterectomy. The samples were classified as symptomatic and asymptomatic from clinical evidence of recent transient ischemic attack or stroke. The plaques that were symptomatic compared to asymptomatic were shown to have significantly more abundant immunoreactivity for MMPs 2 and 9, as well as PGES and COX-2 [105].

8. MMMPS and platelet aggregation

The rupture of atheromatous plaques allows dissection of blood into the intima and subsequently the lipid-rich pool. A sequence of events ensues, including platelet aggregation and thrombus formation, which can compromise arterial patency and result in acute coronary syndrome [86]. Platelet adhesion occurs through the actions of von Willebrand factor (vWF) via its interactions with the two major platelet receptors, glycoprotein (GP)Ib and GPIIb/IIIa (αIIbβ3 integrin). GPIb is involved with platelet adhesion, while GPIIb/IIIa also mediates subsequent steps of platelet aggregation [106].

Some MMPs have been demonstrated to be involved in platelet aggregation. MMP-1 is located at the plasma membrane of platelets where it modifies αIIbβ3 integrin, thereby inducing intracellular tyrosine phosphorylation events and priming platelets for aggregation [107]. MMP-1 also redistributes β3 integrins to discrete areas on the cell periphery and co-localizes in cell contact sites [107]. MMP-2 has been localized to the cytosolic compartment of human platelets and is translocated to the platelet surface and released during platelet aggregation [108]. MMP-2 also potentiates vWF-induced GPIb expression and platelet adhesion [109]. Similarly, MMP-2 amplifies the pro-aggregatory effects of collagen on platelets through a mechanism thought to be independent of aspirin and thromboxane [110]. In contrast, some MMPs have been shown to have inhibitory effects on platelet aggregation. Very high concentrations of MMP-2 as well as MMP-9 have been shown to inhibit platelet aggregation [110,111]. Although MMPs have been shown to have effects on platelets, platelets have also been shown to have effects on MMP secretion [112]. MMP-9 is synthesized by human monocytes when they are coordinately adherent to collagen and platelets. Thus, the effects of MMPs on platelet aggregation are still unclear; however these effects could demonstrate a snowball effect with MMPs upregulating platelets and platelets causing MMP secretion by other cells. It still remains to be seen whether selective MMP inhibition could be an adjunct to existing anti-platelet therapy used in acute coronary syndromes.

9. Association of MMPS with acute coronary syndrome

Although convincing data exist demonstrating the association of MMPs with atheromatous plaques and co-localization of MMPs in the shoulder region of vulnerable lesions, a direct association with actual plaque rupture is less established.

Kai et al. measured MMP-2 and MMP-9 levels in 50 patients (22 with acute myocardial infarction [AMI], 11 with unstable angina, 17 with stable angina and 17 normal volunteers) [113]. The normal subjects had no known cardiovascular disease, hypertension, or diabetes mellitus. Peripheral venous blood was drawn on hospital days 1, 3, and 7. Serial MMP levels were not obtained in the patients who received thrombolytics or underwent angioplasty. MMP-2 levels were increased two-fold in the unstable angina and AMI groups vs. the stable angina and controls and were sustained over the 7 day period. Initially, levels of MMP-9 were greatest in the unstable angina group, followed by the AMI group; however, these elevated levels decreased to the same level as controls after 7 days of follow-up.

Another clinical study compared levels of MMP-9 and TIMP-1 in patients with angiographically identified lesions in the left anterior descending artery vs. normal subjects [114]. In the patients with AMI ($n=20$), 12 had complete occlusion of the left anterior descending artery and eight had subtotal occlusion (defined as TIMI-1 or TIMI-2 flow). The unstable angina group ($n=9$) had angina at rest and a highly stenotic lesion in the left anterior descending artery. The stable angina group ($n=17$) had no angina symptoms at rest but a highly stenotic lesion in the left anterior descending artery. Blood samples were obtained from the aorta and the great cardiac vein within 12 h of onset of symptoms in the myocardial infarction group and within 48 h in the unstable angina group. In both the AMI and unstable angina groups, the great cardiac vein–aortic root difference was significantly increased for MMP-9 and

TIMP-1 compared to the other two groups. Therefore it was concluded that during ACS there is increased production of MMP-9 and TIMP-1 in coronary arteries.

A third study evaluated the levels of MMP-1 and MMP-2 in acute MI defined as ST segment elevation on the ECG and plasma creatine kinase level more than double the normal value [115]. Plasma and peripheral blood mononuclear cells (PBMCs) were evaluated every 4 h until the maximum CK level was determined and then on days 1, 7, 14, and 21. It was determined that plasma MMP-1 levels were undetectable or low and not significantly altered from controls. On the other hand, MMP-2 levels increased over time in MI patients and became significantly higher than those of control subjects by days 14 and 21. In contrast, MMP-1 production by PBMCs in culture increased with time and was significantly higher than controls, while MMP-2 was undetectable. This study demonstrates elevated plasma MMP-2 levels and activity and increased MMP-1 production by PBMCs in culture in the subacute phase of AMI. This data may implicate these MMPs in post-MI complications, but not plaque rupture. Hirohata et al. examined serum concentrations of MMP-1 and TIMP-1 in 13 consecutive patients after their first MI who underwent successful reperfusion [116]. Blood was sampled on the day of admission and then on days 2–5, 7, 14, and 28. These investigators reported significant time-dependent increases in both MMP-1 and TIMP-1 levels that peaked at day 14 (Table 3).

Recently, Blankenberg et al. reported a strong and independent association between plasma levels of MMP-9 and subsequent 4.1 year risk for fatal CHD events among 1127 subjects with established coronary disease [117]. This association was independent of conventional cardiovascular risk factors, but attenuated after adjustment for CRP, IL-6, fibrinogen, and IL-18 levels. In contrast to previous studies, this study included a large number of subjects and demonstrated a prospective relationship between levels and subsequent events.

There remain several limitations of the currently available clinical studies of MMPs and acute coronary events. For instance, the extent to which plasma levels or activity of MMPs reflect levels or activity within atherosclerotic lesions remains unclear. Furthermore, as discussed above, thrombin generation can also activate MMP expression. Thus, elevated levels of MMP could merely be a consequence, rather than a cause of intravascular thrombosis.

10. MMPS and restenosis

Balloon injury of the vascular wall during percutaneous coronary interventions (PCI) initiates a sequence of events that may lead to stenosis. It has been demonstrated in a rat model of carotid artery stenosis that smooth muscle cell proliferation peaks 2 days after injury and SMCs appear in the intima 4 days after injury [84]. A possible contributor to this process is digestion and remodeling by MMPs. MMP expression has been shown to be upregulated following balloon injury and to a greater extent following stenting. For example, balloon injury has been shown to increase MMP-2 and MMP-9 in carotid pig arteries [118]. Jenkins et al., showed increased expression of MMP-2 in the neointima following balloon injury to the rat carotid artery and that the activated form of MMP-2 coincides with VSMC migration and is preceded by an increase in MMP-14 [119]. Similar data are also available from clinical studies in subjects undergoing PCI. Hojo et al. demonstrated increased MMP-2 expression and activity in the coronary circulation following angioplasty and that a significant positive correlation exists between MMP-2 levels post angioplasty and the degree of angiographic restenosis [120] (Table 3).

Studies have also looked at the potential role of the inhibition of MMPs for the prevention of restenosis. De Smet et al. demonstrated a reduction in late luminal loss by MMP inhibition with Batimastat in pigs following balloon angioplasty [121]. This study demonstrated MMP inhibition to reduce constrictive remodeling but not inhibition of neointima formation. Similarly Li et al. studied the effects of the MMPI, gm6001 in rabbit iliac arteries post-angioplasty and found that 1 week of treatment significantly reduced the extent of intimal hyperplasia and collagen accumulation [122]. However, Cherr et al. studied the effects of RO113-2908, a broad-spectrum MMP inhibitor, and no reduction in intimal hyperplasia or constrictive remodeling was observed in cynomolgus monkeys post-angioplasty [123].

Several investigators have also explored the role of gene therapy as a means to inhibit MMP activity after PCI. Turunen et al. showed that TIMP-1 gene transfer reduces intimal thickening in a restenosis model in rabbits [124]. A similar study in rat SMCs infected with adenovirus-mediated TIMP-2 showed decreased SMC invasiveness and delayed neointimal development following carotid angioplasty [84].

11. Conclusion

In summary, MMPs are a complex group of proteinases with important roles in cardiovascular physiology and pathology. Their role in acute coronary syndromes is unfolding as we obtain more data implicating their presence in vulnerable regions of plaque formation, their ability to degrade fibrous caps of atheroma, their effects on platelet aggregation, and their role in post-angioplasty restenosis. However, the net effect of MMP activity in the pathogenesis of atherosclerosis is less clear. More research is needed to better determine the role of MMPs and platelet aggregation; although it is already hypothesized that experimental MMP inhibitors regulate platelet aggregation through mechanisms other than those used by

aspirin and GPIIb/IIIa inhibitors. Furthermore, the effects of MMPs on coronary angiogenesis and neovascularization must also be considered before their role in CAD can be fully understood. This could result in additional therapies for clinicians to use in treating patients with coronary disease.

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