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Temporal and Spatial Expression of Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases Following Myocardial Infarction

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Abstract

Following a myocardial infarction (MI), the homeostatic balance between matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) is disrupted as part of the left ventricle (LV) response to injury. The full complement of responses to MI has been termed LV remodeling and includes changes in LV size, shape and function. The following events encompass the LV response to MI: 1) inflammation and LV wall thinning and dilation, 2) infarct expansion and necrotic myocyte resorption, 3) accumulation of fibroblasts and scar formation, and 4) endothelial cell activation and neovascularization.1, 2 In this review, we will summarize MMP and TIMP roles during these events, focusing on the spatiotemporal localization and MMP and TIMP effects on cellular and tissue-level responses. We will review MMP and TIMP structure and function, and discuss specific MMP roles during both the acute and chronic phases post-MI, which may provide insight into novel therapeutic targets to limit adverse remodeling in the MI setting.

Keywords

myocardial infarction; left ventricular remodeling; matrix metalloproteinases; tissue inhibitors of metalloproteinases; review

1. Introduction

A. MMP nomenclature, structure and activation

Matrix Metalloproteinases (MMPs), also known as matrixins, are zinc-dependent enzymes that can both cleave extracellular matrix (ECM) components as well as non-ECM substrates. Currently, the MMP family is composed of 25 proteinases that can be categorized into five groups based on their *in vitro* substrate preferences: collagenases, gelatinases, stromelysins, matrilysins, and membrane-type MMPs. The collagenases (MMP-1, -8, -13) can cleave fibrillar type collagens. The gelatinases (MMP-2, -9) can degrade gelatins. The stromelysins (MMP-3, -10) and matrilysins (MMP-7, -26) are broad-spectrum proteinases, and the membrane-type MMPs (MT-MMPs) are anchored to the plasma membrane.3, 4, 5, 6 These classifications are somewhat arbitrary and substrate preferences overlap for all of these groups. For example, MMP-14 is assigned to the membrane type subgroup but can also be classified as a collagenase.7 MMP-9 was first described as only being able to process collagen that was denatured or already cleaved by collagenases; however, recent literature

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has shown that MMP-9 can process full length interstitial collagens in addition to a broad array of other substrates.[8, 9]

Enzymes assigned to the MMP family share sequence homology to MMP-1, including a conserved cysteine switch motif heptapeptide PRCGXPD and the zinc-binding motif HEXGHXXGXXH found in the catalytic domain.[10] The general MMP structure contains a prodomain of approximately 80 amino acids that maintains the enzyme in its zymogen latent form, a catalytic domain of 160 to 170 amino acids, and a hemopexin-like domain of approximately 210 amino acids that coordinate protein-protein interactions (Figure 1).[11] Most MMPs also contain a signal sequence (17–20 amino acids) that targets the proteins for secretion into the extracellular space.[11] All MMPs contain a 50 amino acid zinc-binding region within the catalytic domain that interacts with the prodomain to maintain the enzyme in its zymogen form.[11] In addition, some MMPs contain specialized amino acid modules that support substrate recognition. MMP-2 and MMP-9, for example, contain three conserved fibronectin type II repeats in their catalytic domain that increase their affinity to gelatin, laminin and collagenous substrates.[12] Unique to MMP-9 is an extended linker region located between the catalytic domain and the hemopexin domain, and this linker region contains a collagen V-like motif that is heavily glycosylated to enhance substrate affinity and selectivity.[13, 14]

MMPs are preserved in a latent form through a bond between cysteine 73 in the prodomain and zinc in the catalytic domain that physically obstructs the catalytic site of the enzyme.[11] The cysteine is the key amino acid in this mechanism; therefore, activation of MMPs is referred to as the cysteine switch mechanism of activation.[15] MMP activation involves cleavage of approximately 10 kDa from the N-terminus that includes both signal sequence and prodomain. The exceptions to the rule that MMPs are extracellularly activated are MMP-11 and the membrane type MMPs (MMP-14, -15, -16, and -17), and these MMPs contain a furin cleavage sequence to allow intracellular activation.[11] *In vitro* activators of MMPs include p-amino-phenol-mercuric acetate (APMA) and sodium dodecyl sulfate (SDS). SDS is able to activate MMPs without cleaving the 10 kDa prodomain, which explains why zymograms are able to detect distinct pro and active MMP forms. *In vivo*, MMP activators include other MMPs, serine proteases, trypsin, and tissue kallikrein.[16] In addition, modifications such as S-glutathiolation produced by peroxinitrite and glutathione can activate pro-MMPs.[17] MMPs can also be activated by MMP cascades. For example, MMP-3 is known as the universal activator that can autocatalyze itself or be activated by serine or cysteine proteases. In turn, active MMP-3 can cleave pro-MMP-9, and MMP-9 can also be activated by MMP-2.[18–20] Activated MMP-14 can activate MMP-13, which in turn can activate MMP-9.[20, 21] Similarly, pro-MMP-2 activation can be tightly regulated and localized to the cell membrane by MMP-14 in a spatial and temporal manner that requires binding of TIMP-2 to MMP-14.[22] MMP-14 forms homodimers through the hemopexin domain as part of the activation of pro-MMP-2.[23] Therefore, there are multiple examples of MMP interdependence.

B. TIMP structure, MMP inhibition, and non-MMP related functions

MMPs are endogenously inhibited by the tissue inhibitors of metalloproteinases (TIMPs), a family comprised of four members, TIMP-1, -2, -3, and -4. All four TIMPs have a secretory signal sequence, 12 conserved cysteines, a conserved hallmark sequence (VIRAK), and a similar molecular mass that ranges from 24 to 28 kDa.[24, 25] Because of the close molecular weights among the TIMPs, immunoblotting or ELISA are the best ways to quantify TIMP levels. Some of the early reverse zymography experiments that report TIMP-1 levels cannot rule out that other TIMP family members were included in the quantitation, and interpretation of these data should take this into consideration. TIMPs have a high affinity for the catalytic domain of active MMPs, thereby blocking the MMP catalytic site in a 1:1

molar ratio. Moreover, some TIMPs can also form complexes with pro-MMPs to tightly regulate MMP function prior to activation.24 For example, TIMP-1 can bind pro-MMP-9 while TIMP-2 can bind pro-MMP-2.25 MMP/TIMP ratios are commonly used as a relative stoichiometric representation of MMP activity; however, this raises the interesting question as to whether MMPs require activation to be functionally relevant. Dufour and colleagues showed that pro-MMP-2 and pro-MMP-9 use a non-proteolytic mechanism that involves homodimerization to enhance cell migration, and the hemopexin domains of both MMPs facilitated homodimer formation.26 Therefore, it appears that pro-MMPs can have functional roles that do not require the MMP to be activated. In addition, reporting results as ratios (e.g., MMP-9 to TIMP-1 ratios) assumes that TIMP-1 binding to MMP-9 is the only relevant MMP/TIMP pairing in the system. Given that there are 25 MMPs and 4 TIMPs, and that all TIMPs can inhibit all MMPs, this rationale remains to be proven valid.

Independent of action on MMPs, TIMPs can elicit diverse biological responses. The Mann laboratory has shown that adenoviral-induced over-expression of all 4 TIMPs increases cardiac fibroblast cell proliferation and differentiation to myofibroblasts. Further, TIMP-2 expression increases cardiac fibroblast collagen synthesis and TIMP-3 influences the balance between cell survival and cell death increasing the incidence of apoptosis.27 Thus, TIMP functions are complex and not merely related to MMP inhibition.

C. MMP function post-MI: ECM and non-ECM substrates

MMPs coordinate tissue development, repair, and regeneration by modulating the ECM network to regulate cell adhesion, migration, proliferation, and epithelial-mesenchymal transition as well as other cellular responses.22 Similar to a specific role during development, MMPs and TIMPs follow a spatio-temporal pattern during repair of the myocardium in the post-MI setting. 22, 28, 29 Figure 2 illustrates the temporal progression of LV wall thinning and collagen deposition through day 28 post-MI in the mouse. At each stage, MMPs regulate the tissue response to MI by acting on a myriad of substrates.

MMPs modulate multiple responses (e.g. inflammation and angiogenesis) post-MI by processing ECM and non-ECM substrates. For example, we have previously demonstrated that MMP-7 regulates arrythmogenesis post-MI by cleavage of the gap junction connexin-43. As a consequence, MMP-7 null mice had improved non-rupture survival rates and displayed favorable electrical conduction and increased levels of connexin-43 post-MI. 30 In Table 1, we have listed a sampling of non-collagen ECM and non-ECM substrates regulated by MMPs.

2. MMPs and TIMPs post-MI

Cytokines, chemokines, growth factors and bioactive peptides can regulate MMP and TIMP expression and activation.31 For example, in an autocrine manner, brain natriuretic peptide produced by cardiac fibroblasts induces MMP-1, -2, -3, -14, and TIMP-2 expression.32 At the transcriptional level, pro-inflammatory cytokines, tumor necrosis factor alpha (TNFα) and interleukin 1β (IL-1β), present during remodeling induce MMP-1, -3, -7, -9, -13 and TIMPs -1, -2.31 In the MI setting, transforming growth factor beta ($TGF\beta_1$) inhibits MMP-1 expression and attenuates MMP-1-induced myocyte injury and death, while matricellular proteins such as thrombospondin-1 can regulate activation of latent MMP-2 and MMP-9.33, 34

After chronic permanent occlusion in humans and animal models, the levels of MMPs -1, -2, -3, -7, -8, -9, -12, -13, -14 as well as all 4 TIMPs have been reported to respond to cardiac tissue repair stimuli.35 A literature search reveals that MMP-2 and MMP-9 are the most highly studied MMPs in cardiovascular research. However, this is due to the technical fact

that historically zymograms were the first way to observe MMP activity, and gelatin zymography was the easiest method to perform. Therefore, there is not necessarily a direct correlation between publication numbers and particular MMP biological importance. The roles of MMPs -10, -11, and -15 through -28 have not been evaluated post-MI (Table 2). Because most of these newer MMPs have commercially available antibodies, we should expect to see an increase in the publications focused on these additional MMPs.

A. Cell specific expression of MMPs post-MI

MMP-1 (57/52 kDa latent and 49/37 kDa active forms) was first identified as fibroblast collagenase. MMP-1 is abundant in normal LV and is expressed by fibroblast-like cells and endothelial cells.36, 37 Karl Weber's team was the first to report increased MMP-1 levels in the rat LV post-MI.38 MMP-1 activity within the infarct region begins at day 2, reaches a maximum at day 7 and begins to decline through day 14 when the activity returns control levels. Fibroblast-like cells express MMP-1 mRNA beginning at day 7 post-MI suggesting that MMP-1 transcription could be triggered by depletion of latent MMP-1.39, 40 This data was somewhat controversial at the time, as many groups had reported that adult rodents do not express MMP-1.41 Carlos Lopez-Otin's group has since demonstrated that rodents express slightly different MMP-1 genes compared to humans. These two genes, termed McoI-a and Mcol-b, are homologous to human MMP-1 and are primarily expressed during murine embryogenesis.42 In humans, MMP-1 protein decreases in LV tissue extracts from patients with dilated cardiomyopathy.43

MMP-2 (72 kDa latent and 66/62 kDa active forms) was first named gelatinase a. MMP-2 is detected in normal murine LV extracts and is expressed by cardiomyocytes, endothelial cells, vascular smooth muscle cells, and fibroblasts.6, 44, 45 Immunolocalization of MMP-2 within the cell shows MMP-2 within the sarcolemmal membrane and nucleus of cardiomyocytes.46 In a transgenic mouse model where the MMP-2 promoter is fused to the β-galactosidase reporter, MMP-2 expression is present at day 1 post-MI and continues through day 7 in fibroblasts.47 Increased MMP-2 activity is detected at day 2, peaks at day 7 and declines to control levels by day 14 in the rat LV infarct 39 Similar to the MMP-2 profile during development, in the rabbit MI model, MMP-2 protein increases modestly in response to MI.48 MMP-2 is constitutively detected at high levels in normal and control sham LV, which suggests a homeostatic role for MMP-2 in the heart.37, 43 In 1 to 6-day old infarct tissue extracted from humans, MMP-2 is detected in myocytes, fibroblasts, endothelial, smooth muscle, and inflammatory cells.49 In human cardiomyopathy LV extracts, no differences in MMP-2 levels are detected when compared to normal extracts.43 While MMP-2 levels show a comparatively modest change in the post-MI setting, exciting work by Schulze and colleagues has revealed novel intracellular roles for MMP-2. This team has identified myosin light chain and troponin I as intracellular MMP-2 substrates.50 Therefore, quantitation of total levels may not be as informative as localization studies for MMP-2.

MMP-3 (59/57 kDa latent and 48 kDa active forms) was first named stromelysin-1. Cardiac fibroblasts, cardiomyocytes and macrophages express MMP-3, and in normal tissues including human and murine LV extracts MMP-3 (57 kDa and 48 kDa) expression is detected.44, 45, 51, 52 In the experimental rabbit MI model, MMP-3 enzymatic activity detected in cardiomyocytes begins at day 2, peaks at day 4, and declines by day 14.48 In human LV cardiomyopathic extracts, MMP-3 increases in dilated LV when compared to normal controls.43

MMP-7 (28 kDa latent and 19 kDa active forms) was first named matrilysin and is the smallest MMP member. MMP-7 is found in murine LV infarcts and is expressed by cardiomyocytes and macrophages.30, 44 The role of MMP-7 has been studied at 7 days and

8 weeks post-MI. In mouse studies of 7 day MI, expression and activity of MMP-7 increases in cardiomyocytes found in the remote tissue and within macrophages that have infiltrated the infarct region.30 In sheep, MMP-7 expression decreases in the remote and infarct regions 8 weeks after MI, and like the mouse MI model, MMP-7 expression is robust in remote regions.53

MMP-8 (64 kDa latent and 58 kDa active forms) was originally named neutrophil collagenase, leading to the misconception that MMP-8 was only expressed in neutrophils. Macrophages express MMP-8, and low levels of MMP-8 mRNA and protein (58 kDa) are found in control and remote murine and porcine myocardium.44, 52, 54, 55 Post-MI, an increase in MMP-8 mRNA is detected at 6 hours and peaks after 12 hours in the infarcted myocardium.55 MMP-8 protein expression increases at 2 weeks post-ligation and persists through week 16, suggesting that MMP-8 is actively participating in late remodeling events. 56 In the sheep MI, MMP-8 expression is conserved in the remote areas but peaks in the infarct areas at 8 weeks post-MI.53 Interestingly, in humans, elevated MMP-8 mRNA levels are detected in dilated cardiomyopathy LV samples, and increased activity is found in 1–6 day post-MI ruptured ventricles indicating early activation of MMP-8 may contribute excessive collagenolysis that results in rupture.49, 57

MMP-9 (105 kDa in mouse and 92kDa in human latent and 95 kDa in mouse and 88 kDa in human active forms) was first known as neutrophil gelatinase or gelatinase b. While highly associated with neutrophil and macrophages, MMP-9 is also expressed in cardiac myocytes, fibroblasts, vascular smooth muscle cells and endothelial cells. The 92 kDa form of MMP-9 can be detected in normal LV septum and free wall extracts, albeit at much lower levels than post-MI.44, 45, 58, 59 In the rat MI model, MMP-9 mRNA increases as early as 6 hours after the ligation and peaks after 24 hours.55 MMP-9 promoter transcripts with β-galactosidase reporter show MMP-9 promoter activity at day 3 post-MI and peaks at day 7 within inflammatory cells.47 In the porcine MI model, MMP-9 activity increases 3 hours after coronary occlusion in the infarct region and remote-infarct border.60 In the rabbit model, increases in macrophage-derived MMP-9 are detected as early as 24 hours post-MI. 48 Robust MMP-9 activity is found at days 1 through 4 in infarct and border infarct regions of murine LV that correspond with neutrophil and macrophage infiltration, respectively.40, 58 Moreover, in the rat, MMP-9 activity rises abruptly for the first week after the occlusion and declines by day 14 suggesting MMP-9 enzymatic activities predominate early responses post-MI.39, 56 In line with these findings, increases in MMP-9 are also detected in ruptured human ventricles, and MMP-9 null mice show attenuated remodeling at early time points.49, 61

MMP-12 (54 kDa latent and 45 kDa active forms) was originally found in conditioned medium of mouse peritoneal macrophages and was named macrophage elastase.62 In normal LV tissue extracts from young, middle age and adult mice, MMP-12 (54 kDa) protein is detected; however, in human LV samples of patients with dilated cardiomyopathy the levels of MMP-12 were below detection, suggesting that MMP-12 may serve a protective effect in the myocardium.44, 52

MMP-13 (60 kDa latent and 48 kDa active forms) was first identified as collagenase 3. Cardiac fibroblasts express MMP-13 and is found in normal LV tissue extracts (60 kDa and 48 kDa).44, 45 MMP-13 mRNA remains stable following MI through week 16 in the rat; however, a significant increase in MMP-13 enzyme activity is detected after week 1, peaks at week 2 and returns to baseline at week 8. 25 In the sheep model, MMP-13 levels are stable in the remote areas, but high MMP-13 activity is observed in the infarct 8 weeks after the occlusion.53, 56

MMP-14 (65 kDa latent and 54/45/40 kDa active forms) is also known as MT1-MMP. MMP-14 is expressed by cardiac myocytes and cardiac fibroblasts and all 4 forms have been identified in normal human and murine LV (65, 54, 45, and 40 kDa).[44, 45, 63, 64] Like the proposed MMP-8 role in later remodeling events, a dramatic increase in MMP-14 (MT1-MMP) is detected in the rat during week 16 post-MI.[56] In the sheep MI model, MMP-14 is found in remote and transition regions; however, noticeable increases are observed in infarct areas at 8 weeks post-MI.[53] In humans, MMP-14 mRNA is present in dilated cardiomyopathy.[57]

B. Cell specific expression of TIMPs post-MI

TIMP-1 (29 kDa) co-localizes with MMP-1 in normal myocardium and is expressed by cardiac myocytes and fibroblasts.[37, 44,48, 65] Unlike the increased expression and activity observed with MMPs, a general decrease in TIMP protein levels are observed acutely post-MI. In the rat infarct region, TIMP-1 mRNA increases within fibroblasts 6 hours following ligation and declines after 2 days with no detectable change in remote or sham areas.[39] Interestingly, TIMP-1 protein is reduced for the first week after ischemia and returns to control levels by day 7 in the rabbit.[48] In the sheep, TIMP-1 levels are lower in the transition areas compared to the controls, and the levels are below detection within the infarct.[53] In agreement with these findings, in tissues from patients with ischemic cardiomyopathy TIMP-1 protein was also found reduced.[66] Unexpectedly, in human 1–6 day-old ruptured ventricles, TIMP-1 protein levels are found elevated compared to control non-ruptured MI ventricles.[49]

Cardiac fibroblasts are a rich source of TIMP-2 (28 kDa) in normal myocadium.[44, 45] TIMP-2 protein levels in the rat do not change in response to MI for the first week, but TIMP-2 protein shows dual peaks at weeks 2 and 16 after MI.[56] In the sheep MI model, TIMP-2 protein levels remain similar to control in the transition areas; however, TIMP-2 levels in the infarct fall below detection levels.[53] In humans, TIMP-2 mRNA and protein levels remain unchanged in the failing human ventricle.[66]

TIMP-3 (24 kDa) is detected in normal murine LV extracts.[44] In sheep, TIMP-3 protein is found at significantly lower levels in the infarct regions at 8 weeks post-MI than in control or remote regions. [53] Similarly, in failing human hearts resulting from ischemic cardiomyopathy, TIMP-3 levels decrease in ventricular tissue.[66] TIMP-3 deletion accelerates cardiac remodeling post-MI by promoting matrix degradation (MMP-9 levels were higher) and inflammatory cytokine expression (TNFα specifically).[67] This suggests that TIMP-3 over-expression may prove beneficial in the LV remodeling process.

TIMP-4 (28 kDa glycosylated and 24 kDa forms) is expressed by cardiomyocytes and is abundant in normal, human and murine myocardium.[44, 67] A common misconception is that TIMP-4 is a cardiac-specific TIMP. However, TIMP-4 is also highly expressed in brain, ovary, and skeletal muscle.[68] After ischemic cardiomyopathy, TIMP-4 transcript levels remain stable in humans.[66] In the rat MI model, TIMP-4 mRNA remains unchanged, but TIMP-4 protein levels decrease at one and eight weeks post-MI.[56] Similarly in the sheep MI model, TIMP-4 protein is reduced at 8 weeks post-MI in the left ventricle infarct regions.[53]

3. Permanent occlusion MI model versus ischemia/reperfusion MI model

In this section, we will discuss factors in common and distinct between the permanent occlusion MI and reperfused MI models to provide mechanistic insight into the differential MMP and TIMP expression in these two settings. In Table 3 we have listed similarities and differences between permanent occlusion and ischemia/reperfusion MI models. Of note, the

majority of studies use rodent models and the evaluation of MMPs and TIMPs in humans and large animal models remains a fertile field.

In ischemia/reperfusion MI models, MMP and TIMP profiles diverge from the profiles observed with permanent occlusion and these differences may help to explain the ultimate benefit of reperfusion on myocardial wound repair and LV function.69 Alterations in ECM expression and deposition as well as modifications to the inflammatory response kinetics are observed after reperfusion and contribute to the generation of a more organized scar within the infarct region.

A. MI size, mortality rate and LV remodeling

In comparing permanent occlusion to reperfusion, the Entman laboratory showed that for mice, when infarct sizes are similar (30% at 24 hours), permanent occlusion results in more infarct expansion.69 Mortality rate in the reperfused group was about 30% lower than total occlusion at 28-day post-MI. LV ejection and filling velocities returned to control levels after 2 weeks of reperfusion, while values remained suppressed with permanent occlusion.69, 70 Reperfusion reduces the degree of remodeling, as the gradual LV wall thinning observed with permanent ligation is attenuated with reperfusion, with a benefit seen beginning at day 4 post-MI and persisting through day 28.69, 70 Moreover, LV dilation significantly decreases in reperfusion models and the percent lumen surface is lower at days 4, 14 and 28 post-MI compared with permanent occlusion MI.70 In both models, the vasculature within the infarct region begins to regress 48 hours after ligation, but reperfusion stimulates more robust endothelial cell proliferation resulting in augmented angiogenesis compared to permanent occlusion.70

B. Inflammatory response and cellular infiltration

With respect to inflammation and cellular infiltration, similarities and differences are evident between permanent occlusion and reperfusion. The messenger RNA levels of IL-6 and $TGF\beta_1$ are reduced early after reperfusion, and although the levels return to baseline in both models after 24 hours, IL-1β, TNFα, and $TGF\beta_1$ increase again by day 7 in the permanent occlusion setting.71 Although cytokine levels are generally decreased with reperfusion, inflammatory cell influx is paradoxically increased. Reperfusion heralds in a much earlier inflammatory cell response, bringing in both neutrophils and macrophages simultaneously. In contrast, permanent occlusion results in an orchestrated influx of neutrophils followed by macrophages. Compared to permanent occlusion, reperfusion resulted in higher neutrophil and macrophages numbers, a prolonged presence of neutrophils, and increased myocyte remnant clearance.70 In line with this finding, in the canine MI model of reperfusion, neutrophil influx is 80% increased compared to the canine permanent occlusion model.72 In addition to differences in inflammatory cells, the percent of myofibroblasts in the permanent occlusion model was significantly higher at day 7 compared with the reperfused infarct.70

C. MMP, TIMP and ECM differences

Because reperfusion increases cell infiltration kinetics post-MI, MMP and TIMP levels are likely altered between the two models. Carlyle and colleagues used the coronary artery ligation model in the rat, with and without delayed reperfusion following 150 min occlusion, to quantify MMP activity up to 7 days post-MI.73 MMP-1, -2, and -9 were measured in the infarct region, and all 3 were elevated in the permanent occlusion model. Delayed reperfusion attenuated levels of each. In pig models of *in vivo* and *ex-vivo* ischemia/reperfusion, MMP-1 activity is markedly increased in the infarct regions compared to non-infarct controls.38, 54 In both permanent occlusion and reperfusion models, no apparent changes in MMP-2 activity are observed early after reperfusion in the porcine, rat or canine

MI models.59, 74, 75 Beginning at day 7, MMP-2 activity is attenuated with reperfusion and is approximately 60% lower than levels in the permanent occlusion model. MMP-9 activity, which increases up to 100-fold with permanent occlusion, is reduced by 55% and 84% in the infarct region of reperfused myocardium at 24 and 48 hours, respectively, even though the neutrophil numbers increase with reperfusion.59, 73 In agreement with these findings, MMP-9 activity in a canine model of reperfusion increases in the infarct compared with remote non-infarct controls and sham LV.76 During moderate ischemia and reperfusion (90 min ischemia/90 min reperfusion) in pigs, MMP-9 increases 70% in the ischemic regions.74, 75

After 90 min ischemia followed by 120 min reperfusion in a canine model, Sawicki and colleagues found that TIMP-3 decreases in the infarct zone but TIMP-4 levels were unchanged.75 During moderate ischemia followed by reperfusion, TIMP-1 was not detected and TIMP-2 and TIMP-3 were present in both ischemic and non-ischemic tissue.74

In addition to the improved clearance of necrotic cardiomyocytes observed with reperfusion, earlier collagen deposition is also seen in the reperfused mouse MI model.70 In the rat infarct, reperfusion accelerates fibronectin expression compared with non-reperfused models.73 After 24 hours of reperfusion, fibronectin protein levels double in reperfused infarct. In *ex vivo* porcine hearts, reperfused 2 hours after occlusion, fibronectin and osteopontin levels both increase in the infarct region.54 Together, these data indicate that fibronectin expression responds before changes to collagen expression are detected, suggesting that fibronectin may be an earlier marker of remodeling.

4. Therapeutic treatments that regulate MMP and TIMP levels post-MI

We will focus on current therapeutic strategies and how pharmacologic agents such as angiotensin converting enzyme and β adrenergic receptor inhibitors alter MMP and TIMP levels.

Angiotensin converting enzyme inhibitors (ACEi) and angiotensin II type I receptor blockers (ARBs), as well as β-adrenergic receptor (βAR) blockers, are common therapeutic strategies post-MI. Although the mechanisms of action are not entirely clear, each used as a therapeutic treatment can reduce MMP expression and activity. MMP-2 activity in human LV cardiomyopathy extracts is reduced *in vitro* by ACE inhibitors in a dose dependent manner.77 In the rat MI model, ACEi (ramipril) decreases MMP-2 activity to control levels, but increases MMP-1 activity.78 Similarly, in a rat congestive heart failure model, ACEi (trandolapril) reduced MMP-2 activity but induced TIMP-2 levels.79 Yamamoto and colleagues have shown, in a hamster MI model, that ACE inhibitors reduce MMP-9 activity post-MI by directly binding the MMP-9 active site.80 Ramipril also reduces TIMP-2 protein levels but increases TIMP-4 levels 80% above control.78 In a canine ischemia/reperfusion model, administration of valsartan, an ARB, had no significant effect on MMP activity. However, TIMP-3 protein significantly increased in the infarct region.75β-AR blockers also alter MMP expression and activity. In the rat MI model, propranolol significantly increased both MMP-8 and MMP-9 mRNA levels at 6, 12, 24, and 72 h. At the protein level, propanolol significantly reduced the enzymatic activity of MMP-2 after 6 hours and MMP-9 after 12 and 24 hours.55 Propanolol, therefore, may mimic some of the beneficial effects seen with reperfusion.

5. Future Directions & Conclusions

While a lot of information on MMP and TIMP roles in the post-MI setting has been gathered over the past 15 years, additional studies are needed to further clarify the role of MMPs and TIMPs in the post-MI setting (Table 4). For one, we still need to know the expression levels

and roles of MMPs not yet studied. Of the 25 MMPs identified to date, the role of 16 MMPs post-MI remains to be investigated. Additionally, we need to know the full complement of MMPs and TIMPs expressed for each cell type in the post-MI LV. We need to distinguish between data that is absent and negative data indicating a lack of expression. A combination of *in vivo*, *ex vivo*, and *in vitro* tools will need to be utilized to reveal the MMP and TIMP profiles for each cell present during cardiac repair. We also need to know what the aggregate functions for each MMP and TIMP are. In addition, it will be important to know whether the MMP or TIMP is involved in protein processing or modulating cellular processes during remodeling.

A better understanding of the diverse directions MMP function can take will help develop novel strategies to target MMP-regulated function by deviating from the traditional inhibition of the catalytic domain and focusing on other enzyme domains (e.g., the hemopexin or pro-domains). Along these lines, more details on the spatiotemporal expression are needed. As we have seen with multiple MMPs, it is important to measure levels at multiple times and places in order to confirm known biological mechanisms and uncover novel functions. As was illustrated by Rick Schulz and his team, MMPs can be found in surprising locations including the nuclei of cardiac myocytes. Targeting nuclear MMPs and substrates can potentially help us develop ways to correct DNA repair errors.46 Interestingly, more intracellular MMP substrates are being identified, and we are increasingly becoming aware of the potential for MMPs to process membrane proteins and other non-traditional substrates. We will need to incorporate these ideas into strategies to develop new inhibitors.

Finally, we need to catalogue the full list of MMP substrates. In order to develop a successful therapeutic treatment, an inclusive list of targets and potential pathways must be provided to understand the global effect of the treatment strategy. In summary, we reviewed the current literature on MMP and TIMP levels, localization, and roles in the post-MI setting. This evaluation reveals new directions that must be taken in future research in order to design more effective MMP inhibition strategies.

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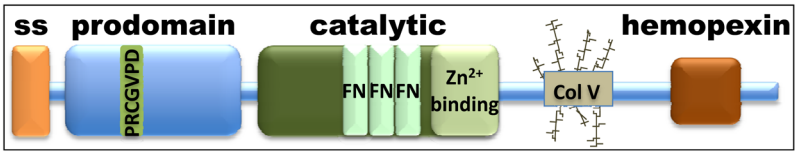

Figure 1.
The general MMP structure contains a signal sequence (ss), a prodomain, a catalytic domain, and a hemopexin-like domain. The catalytic domain contains a zinc-binding region that interacts with the prodomain. For several MMPs (e.g., MMP-2 and MMP-9), the catalytic domain also contains multiple fibronectin type II repeats.

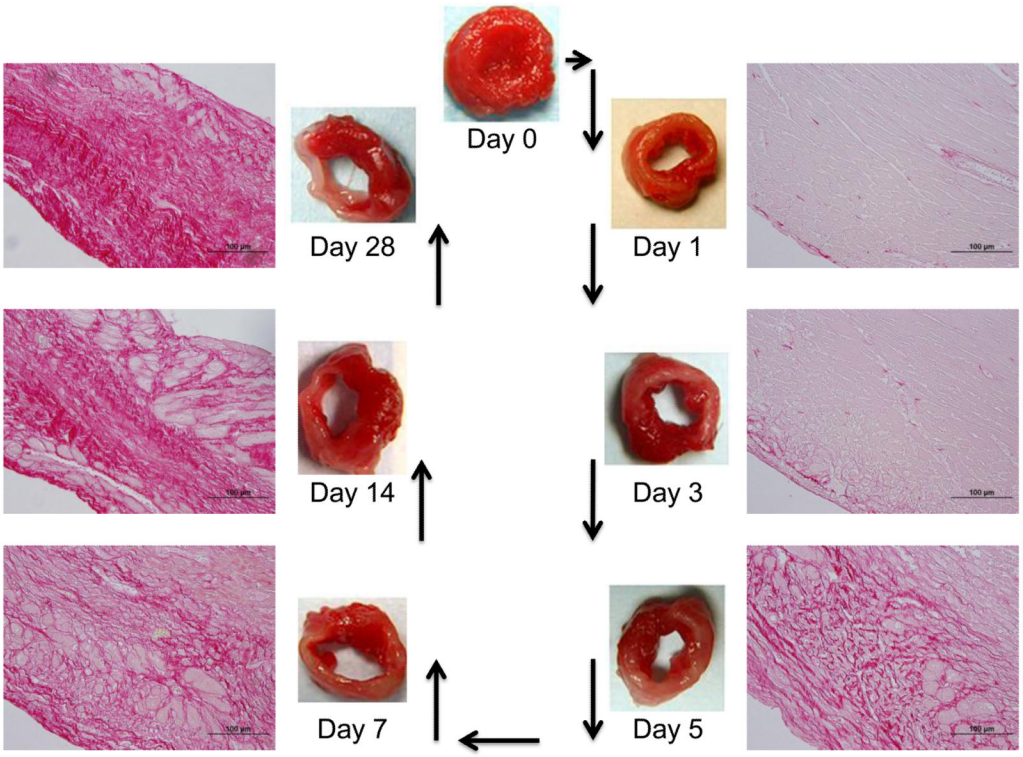

Figure 2.
The temporal progression of LV wall thinning and collagen deposition through day 28 post-MI in the mouse. The LV slices are stained with 1% 2,3,5-triphenyltetrazolium chloride to detect metabolically active tissue. Viable tissue stains red, while infarct tissue remains white. The histological sections are stained with 1% picrosirius red, which stains collagen red.

Table 1

Examples of Non-collagen ECM and non-ECM substrates regulated by MMPs 30, 81–98

Substrate	MMP	Regulated Activity
Aggrecan	-3, -8, -14	Inflammation
CD44 (hyaluronan receptor)	-14	Cell Migration
Complement C1q	-1, -2, -3, -9	Neutrophil superoxide production
Connexin 43	-7	Myocyte Electrical Conduction
Fibrinogen	-2, -3, -7, -9, -14	Myocyte Contractility
Fibronectin	-7, -9	Gene Expression
Fibroblast Growth Factor Receptor 1	-2	Mitogenesis and Angiogenesis
Galectin-3	-2, -9	Cell Proliferation and Phagocytosis
Interleukin-1β	-1, -2, -3, -9	Inflammation
Insulin-like Growth Factor Binding Protein-3	-1, -2, -3	Cell Proliferation
Latent TGF-β1, -β2, -β3	-2, -9	Cell Differentiation and Angiogenesis
Laminin	-2, -3, -9, -14	Cell Migration
Monocyte Chemotactic Proteins-1, -2, -4	-1, -3	Cell Chemotaxis
Monocyte Chemotactic Protein-3	-2, -14	Cell Chemotaxis
Myosin Light Chain 1	-2	Contractile Dysfunction
Osteopontin	-2, -3, -7, -9	Cell Adhesion
Serpina 1d	-9	Protease Inhibition
Tenascin-C	-7, -9	MMP Expression
Thrombospondin-1	-9	Angiogenesis
Tumor Necrosis Factor-α	-1, -2, -3, -7, -9	Inflammation
Troponin-I	-2	Cardiac Mechanical Function

Table 2

MMP and TIMP expression in cell types involved in the post-MI LV response.

Cell types involved post-MI	MMPs expressed	MMPs not yet studied
Cardiac myocytes	MMP-1, -2, -3, -7, -9, -1438, 48, 49, 30, 48, 64, 99 TIMP-1, -448, 67	MMPs -10, -11, -15, -16, -17, -18, -19, -20, -21, -22, -23, -24, -26, -27 and -28
Cardiac fibroblasts	MMP-1, -2, -3, -9, -13, -1439, 45, 49 TIMP-1, -239, 45	
Myofibroblasts	MMP-2, -14100	
Neutrophils	MMP-8, -9 59, 101, 102	
Macrophages	MMP-1, -3, -7, -8, -9 -1230, 52, 58	
Endothelial Cells	MMP-2, -949	
VSMCS	MMP-2, -949	

Table 3

Similarities and differences between permanent occlusion and ischemia/reperfusion MI models. [56, 66, 69–71, 73, 75, 103]

	Permanent Occlusion							**Ischemia/Reperfusion**				
(1) Infarct Size at 24 hours	~30%							~30%				
(1) Ejection velocity and filling velocity	Continuous suppression							Returns to level of sham in 14 days				
(1) Ejection Fraction at 14 days	26. ± 3.1%							24.1 ± 4.0%				
(1) Mortality rate at 28 days	35.6%							5.8%				
mRNA levels	1h	3h	d1		d7			1h	3h	d1		d7
(2) IFN-γ	↑	↓	↓		↑			↑	↓	↓		↓
(2) IL-1β	↑	↑↑	↓		↑↑			↑	↑	↓		↓
(2) IL-2	↑↑	↑	↑↑		↑↑			↑	↑	↓		↑
(2) IL-6	↑	↑↑	↓		↑			↑	↓	↓		↓
(2) TGF-β1	↑↑	↑	↓		↑↑			↓	↑	↓		↑
(2) TNF-α	↑	↑	↓		↑↑			↑	↑	↓		↑
Protein levels			d1	d3	d7					d1	d3	d7
(2) MMP-1 (54 kDa)			↓	↑	↑↑					↓	↑	↑
(2) MMP-2 (65 kDa)			↓	↑↑	↑↑					↓	↑	↑
(2) MMP-9 (92 kDa)			↑↑	↓	↓					↑	↓	↓
					d7	d14	d35		3h			
(2) TIMP-1*					↔	↑	↔	(3)	nd			
(2) TIMP-2*					↔	↑	↑	(3)	↑			
TIMP-3						nd		(4)	↓			
(2) TIMP-4*					↔	↓	↔	(3)	↓			
								(4)	↔			

(1) paired study in mouse; (2) rat; (3) pig; (4) dog; h-hours; d- days; w-week; ↑-increased expression; ↑↑ increased compared with ischemia/reperfusion; ↓-decreased expression; * independent studies; ↔-no change; nd- not detected

Table 4

Future Directions

We still need to know:	
1	Expression levels and roles of MMPs not yet studied.
2	Full complement of MMPs and TIMPs for each cell type involved in the post-MI response.
3	Net effect of each specific MMP and TIMP in the post-MI setting.
4	Spatiotemporal changes in MMP activity and TIMP levels.
5	More complete list of substrates for each MMP.