Membrane-Associated Matrix Proteolysis and Heart Failure

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Abstract: The extracellular matrix (ECM) is a complex entity containing a large portfolio of structural proteins, signaling molecules, and proteases. Changes in the overall integrity and activational state of these ECM constituents can contribute to tissue structure and function, which is certainly true of the myocardium. Changes in the expression patterns and activational states of a family of ECM proteolytic enzymes, the matrix metalloproteinases (MMPs), have been identified in all forms of left ventricle remodeling and can be a contributory factor in the progression to heart failure. However, new clinical and basic research has identified some surprising and unpredicted changes in MMP profiles in left ventricle remodeling processes, such as with pressure or volume overload, as well as with myocardial infarction. From these studies, it has become recognized that proteolytic processing of signaling molecules by certain MMP types, particularly the transmembrane MMPs, actually may facilitate ECM accumulation and modulate fibroblast transdifferentiation; both are critical events in adverse left ventricle remodeling. Based on the ever-increasing substrates and diversity of biological actions of MMPs, it is likely that continued research about the relationship of left ventricle remodeling in this family of proteases will yield new insights into the ECM remodeling process and new therapeutic targets. (Circ Res. 2013;112:195-208.)

Key Words: extracellular matrix • fibroblast • heart failure • matrix metalloproteinases • myocardial remodeling • transforming growth factor

The clinical presentation of heart failure (HF) is generally that of a common set of clinical signs and symptoms. However, the underlying mechanical and structural basis responsible for the development of the HF syndrome is quite diverse. Identifying the causal mechanisms responsible for the development and progression of the HF process is crucial for the purposes of developing preventive and therapeutic strategies for this disease. Essential milestones in the development and progression of HF include changes in the structure, composition, and geometry of the left ventricular (LV) myocardium, which has been generically termed LV remodeling. There are fairly distinct patterns of LV remodeling that occur and are dependent on the initial pathophysiological stimulus but, once instigated, LV remodeling is an important predictor for the development and progression of HF.1-3 LV remodeling entails changes in the structure and function of the cardiocyte, the vascular compartment, and extracellular matrix (ECM), whereby changes within all of these entities occur as a continuum during the initiation and progression to HF. There is no question that fundamental defects in cardiocyte function, structure, and viability play a major role in the development and progression to HF. However, there is growing recognition that the ECM mediates both mechanical and biological signals that contribute to this process.6-15 For example, the ECM provides the critical interface for force transmission and alignment of myocardial fascicles, and it also provides the substrate for transmembrane adhesion of cardiocytes.11,13-16 Thus, a loss of normal ECM structure and function can directly alter transduction of contractile force and intracellular signaling of cardiocytes, which in turn will change LV systolic function. In contrast, excessive ECM accumulation can directly alter myocardial passive stiffness properties, which will directly affect LV diastolic function.11,17 In certain instances, significant heterogeneity in ECM remodeling occurs, whereby a loss of normal ECM structure and function is accompanied by abnormal ECM accumulation, which can impair both LV systolic and diastolic functions.18-20 Through transgenic, pharmacological, and clinical observational studies, it is apparent that changes in ECM structure and function directly contribute to the adverse LV remodeling process, which accompanies the development and progression of HF.2,3,11,15,17,21-35 Finally, the dynamic nature of the ECM in terms of trafficking, processing, and compartmentalizing critical biological molecules that modulate myocardial growth and function, from

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growth factors to microRNAs (miRs), is becoming appreciated.6–8,24,27,30,56–61 Accordingly, the overall goal of this review is to provide a current perspective on a specific aspect of ECM proteolysis as it relates to the LV remodeling process and the progression to HF.

LV Remodeling Patterns and HF

For the purposes of this review, some generalizations about the structural basis of the HF process have been made, and an oversimplification can provide a means of classifying some commonalities with respect to LV remodeling. Classification of the LV remodeling process includes: (1) the pressure over-loaded myocardium (such as that with hypertension or aortic stenosis), which causes LV hypertrophy and can give rise to increased collagen accumulation (ie, fibrosis);9,10,17,19,31,34,37–44 (2) the volume overloaded myocardium (such as that with valvular regurgitation, aortocaval fistulae, and dilated cardiomyopathies), which is characterized by significant LV dilation and potentially a loss of normal collagen matrix structure and function;20–28,32,47–49,52,53 and (3) the injured myocardium, most notably that of myocardial infarction (MI), whereby a heterogeneous remodeling process can occur simultaneously within the LV myocardium, giving rise to myocardial hypertrophy and fibrosis within the remote viable region, as well as mural wall thinning, expansion, and loss of normal collagen matrix structure and function within the MI region.20–28,32,47–49,52,53 These generalized LV remodeling categories are utilized for the purposes of putting into context how ECM proteolytic activity may cause a feed-forward process to accelerate the development and progression of LV remodeling. Specifically, this review first provides a brief overview of a refined perspective on the structure and function of the ECM and of the proteases and pathways that affect LV remodeling. Then, a more focused review is provided on ECM proteolytic systems, the matrix metalloproteinases (MMPs), with particular attention to the transmembrane MMPs and the relation to the LV remodeling process. Some of the main goals of this review is to identify how past concepts about MMPs solely causing ECM degradation was an oversimplification, and to examine new functionalities for this highly diverse family of proteolytic enzymes relevant to LV remodeling and progression to HF.

Myocardial ECM Redefined and MMP Relevance

The emergence of studies about the biology and function of MMPs in the progression of HF has resulted in an overall reappraisal of the ECM in terms of normal myocardial structure and function and contributory roles in adverse remodeling processes. The myocardial ECM historically was considered to be a relatively static structure composed of fibrillar collagens with relatively slow turnover rates, but it is now apparent that the ECM is a complex and dynamic entity. All of these ECM constituents are vulnerable to proteolytic processing directly or indirectly by MMPs,62–66 which, in turn, alters the interactions between these different structural and biologically active interstitial molecules, thereby determining the overall structure and function of the ECM. Changes in the structure and function of the ECM hold physiological implications in terms of LV systolic and diastolic performance. With regard to systolic function, biological modeling and structure–function studies have demonstrated that the ECM plays a critical role in mechanotransduction of sarcomere shortening into myocardial contractile force.11,12,15,16,67 For example, activation of endogenous MMPs acutely altered fibrillar collagen content in papillary muscles and directly affected active tension development, whereas the contractility of myocytes isolated from these preparations was unaffected.16 When examined from a biophysical–mechanical basis, it is clear that the ECM contributes to stiffness properties of muscle in general and holds particular relevance to the myocardium.9–12,17,19,39,42,44,67–70 For example, correlative studies, such as those reported by Borg et al,71 demonstrated that species-specific differences in the structure and composition of fibrillar collagen were associated with changes in myocardial stiffness. In clinical studies of LV hypertrophy secondary to a prolonged pressure overload, significant collagen accumulation was directly associated with indices reflective of increased LV myocardial stiffness.9,10,37–44,68,69 In basic mechanistic studies, inhibition of fibrillar collagen cross-linking, for example, directly reduced myocardial stiffness properties.70,72 The effects on the stiffness modulus are nonlinear;12,15,16,67,69 therefore, small changes in 1 direction or the other in terms of collagen content and architecture will result in significant and physiologically relevant changes in LV myocardial stiffness and, in turn, overall LV diastolic performance. Thus, proteolytic alterations of the ECM, such as those through the induction and activation of the MMPs, not only would contribute to overall LV myocardial architecture but also would directly affect LV myocardial systolic and diastolic functions.

The ECM is also an important entity in terms of providing for the deposition, processing, trafficking, and transmembrane transduction of biological signals.6–8,13,14,30,57,73–81 The classic ligand-transmembrane receptor–intracellular transduction pathway is dependent on the rate and extent that a ligand can traverse the ECM and interact with the extracellular domain of the cognate receptor. Moreover, the release of exosomes containing mRNA or miRs into contact with the ECM, as well as the direct release of miRs into the interstitial space, can provide a cell–cell communication pathway.9,12 The MMPs and associated proteases process transmembrane receptors, including integrins, and thereby alter receptor transduction
MMPs: A Small Family on a Large Tree

Although this review focuses on the certain MMP types in terms of LV remodeling, it must be recognized that this is but 1 family of the large class of metzincin proteases; a highly diverse set of proteolytic enzymes exists within the ECM. In terms of relevance and interaction with the MMPs, a family of associated proteinases would include the disintegrin and metalloproteinases (ADAMs) and the ADAM with thrombospondin motifs.73,74 For example, ADAM-10 processes membrane-bound TNF to a soluble form and, in turn, can cause the induction of MMPs.73,74 Because a number of MMPs can process membrane-bound TNF as well, this can form a potent, localized induction loop within the myocardium. Among a number of other substrates, ADAM with thrombospondin motifs are engaged in proteolytic removal of the procollagen N-terminal domain, a critical step in collagen maturation. These particular ADAM with thrombospondin motifs therefore also would reduce the susceptibility of newly formed collagen to proteolytic degradation and, in turn, would increase collagen stability and cross-linking. In light of the fact that certain transmembrane-type MMPs can be colocalized to ADAM/ADAM with thrombospondin motifs and integrin complexes,13,14,73,74 it is likely that a strong interplay exists between these families of ECM proteinases.

The initial description of a matrix-degrading enzyme was based on the seminal observations whereby the excised tadpole tail degraded a collagen gel, hence the term collagenase.84 There are now >23 MMP types expressed within humans, and the distribution, functionality, and substrates are diverse, as discussed in several broader reviews.52,66,85-89 Moreover, the term MMP function yielded only 10 publications in 1990, whereas this same search term yielded >2200 publications in 2011 alone (PubMed, National Library of Medicine, search performed July 14, 2012). Thus, to provide focus, only those MMP types that have been most intensely investigated with respect to LV remodeling are discussed. Initially, the MMPs were classified based on recognized substrates, and although this nomenclature no longer holds relevance, it is still commonly used to group the MMP types. This classification scheme includes the following: collagenases, such as MMP-1, MMP-13, and MMP-8; the gelatinases, which include MMP-2 and MMP-9; the stromelysins/matrilysins, which include MMP-3 and MMP-7; and the membrane-type MMPs, which include MMP-14. This is not the exhaustive list of MMP types that are likely expressed within the mammalian myocardium. Moreover, this list does not include MMP types that are relevant within the vasculature, such as MMP-12. Nevertheless, this simplified list and classification scheme provide a suitable context in which to examine the current field in terms of LV remodeling. This review focuses on proteolytic interactions of the MMPs, such as the membrane-type MMPs (ie, MMP-14), with particular attention to the differential biological effects and substrates relevant to the LV remodeling that occurs in pressure overload, volume overload, and MI.

Differential Expression and Effects of MMPs in Pressure Overload

In general, whether because of systemic hypertension or a fixed outflow obstruction such as aortic stenosis, LV pressure overload causes significant myocardial growth, paralleled by increased ECM accumulation, particularly fibrillar collagen.9,10,17,19,41,68,69 This would lead to the assumption that MMP expression, and in turn ECM proteolysis, would be reduced with LV hypertrophy and ECM accumulation. However, in contrast to canonical thought, certain MMP types are actually induced with LV hypertrophy and ECM accumulation, notably with the progression of diastolic dysfunction, whereas other MMP types are reduced or relatively unchanged.31,38,40-44,49,90 Some of the basic and clinical studies that provide support for a multidimensional role of MMPs in LV pressure overload and the progression to HF are summarized.

LV Pressure Overload and Transmembrane MMP Interactions

Cyclic strain has been shown to induce a number of MMPs, such as the gelatinase MMP-2.91 In myocardial biopsies performed in patients with LV pressure overload secondary to aortic stenosis, increased MMP-2 expression and activity were identified.90 In patients with LV hypertrophy and HF with a history of hypertension, plasma levels of MMP-2 were significantly increased compared with age-matched control subjects.10,40-42,44 For example, in patients with established LV hypertrophy, the relative levels of plasma MMP-2 could be utilized in a diagnostic model with respect to progression to HF.44 Moreover, a meta-analysis demonstrated that elevated plasma MMP-2 levels were a consistent finding in patients with LV hypertrophy and developing HF.41 In a murine model of LV pressure overload, genetic deletion of MMP-2 reduced the degree of myocardial fibrillar collagen accumulation and improved indices of LV diastolic function.92 It is well-established that the primary mechanism for conversion of pro-MMP-2 to active MMP-2 is through complex formation with the membrane-type MMP. MMP-14-86,88,93 MMP-14 expression is sensitive to changes in mechanical load, whereby increased wall tension proliferated MMP-14 promoter activity in vitro.94 Increased myocardial MMP-14 expression has been identified in patients with LV pressure overload.95 In animal models of LV pressure overload, an early and sustained induction of MMP-14 has been identified.31,90,95 For example, increased MMP-14 promoter activity and subsequently MMP-14 proteolytic activity have been reported after the induction of LV pressure overload in mice.92 Moreover, these studies have identified an association between changes in myocardial ECM remodeling, particularly increased collagen accumulation with MMP-14 induction.31,90,95 These observations would suggest that increased MMP-14 contributes to adverse ECM remodeling, which is a pivotal structural event in terms of LV pressure overload and progression to HF. The pathways
by which MMP-14 contributes to adverse ECM remodeling likely include facilitating proteolysis of interstitial molecules directly (such as integrins), amplification of active MMP-2 causing ECM instability, and abnormal architecture, as well as through enhancing profibrotic signaling pathways. As discussed in a subsequent section, an important proteolytic relationship likely exists between MMP-14 and the subsequent activation of the profibrotic signaling molecule TGF-β,

which has particular relevance in the context of LV pressure overload.

LV Pressure Overload Causes Diversity in MMP Induction

Although certain MMP types, such as MMP-2 and MMP-14, appear to be uniformly increased with LV pressure overload, other MMP types, such as the interstitial collagenase MMP-13, have been reported to be decreased.

For example, detectable plasma levels of MMP-13 were reduced in patients with LV hypertrophy and HF.

Other MMP types, such as MMP-1 and MMP-3, appear to be either unchanged or reduced with LV pressure overload.

Interestingly, transgenic expression of human MMP-1 in mice (this MMP type is absent in rodents) and induction of LV pressure overload resulted in a relative reduction in myocardial fibrillar collagen content and improved indices of LV function.

These findings suggest that the loss of normal constitutive levels of certain MMP types, or failure of an induction of certain MMP types with LV pressure overload, may facilitate abnormal ECM accumulation and adverse myocardial remodeling. This process does not occur in isolation, and key matricellular proteins and signaling molecules, some of which are identified in a preceding section, significantly change with LV pressure overload, as do the constitutive inhibitors of the MMPs that are described briefly in a subsequent section. It also must be recognized that the induction/repression of certain MMP types is likely to be a dynamic process and therefore would be a function of both time and magnitude of the LV load.

MMPs in Volume Overload/Cardiomyopathies

In terms of significant LV dilation and ECM remodeling that are hallmarks of the dilated cardiomyopathies and volume overload states, a robust expression and activation of MMPs have been clearly identified.

For example, in patients with end-stage dilated cardiomyopathy, myocardial samples obtained at the time of transplantation revealed significantly higher levels of MMPs from all of the representative MMP classes.

The MMPs elevated in patients with dilated cardiomyopathy included MMPs associated with an inflammatory response such as MMP-7, MMP-8, and MMP-9. Of note, the most robust levels for any of the MMP types were those of MMP-14.

On the contrary, relative levels for MMP-1 were reduced in dilated cardiomyopathy samples. In a more recent clinical study of stress-induced cardiomyopathy (takotsubo), a similar reduction in plasma MMP-1 levels was observed.

One of the limitations of these clinical observations is that the measurements were performed when severe HF had become manifest and, therefore, whether and to what degree these changes contributed to disease progression remained unclear.

Volume Overload and MMP Induced ECM Proteolysis: Evidence From Animal Models

Using animal models of LV dilation and dysfunction, a mechanistic role for MMP induction in this process has emerged. Using the rapid pacing model of developing dilated cardiomyopathy, an early and time-dependent induction of MMPs has been reported, which paralleled changes in LV geometry and function. More importantly, the early MMP induction and LV dilation preceded any significant change in isolated myocyte contractility, suggesting that MMP-mediated ECM remodeling is an early structural event in this LV remodeling process. In the canine model of volume overload secondary to mitral regurgitation, LV dilation was accompanied by an early and robust increase in MMP-9, followed by a persistent increase in relative MMP-2 levels, as assessed by substrate zymography. In the rodent model of aortocaval fistula, the progression of LV dilation and dysfunction is concordant with myocardial MMP induction.

In a similar murine model of volume overload, transgenic deletion of MMP-9 attenuated the degree of LV dilation and dysfunction. Using broad-spectrum MMP pharmacological inhibition at the onset of the pathological stimulus (either rapid pacing or fistula), the progression of LV dilation and dysfunction attenuated significantly and reduced indices of ECM turnover and instability. Taken together, these findings provide a cause–effect relationship between MMP proteolytic activity and LV dilation with a volume overload stimulus.

LV Dilation and Volume Overload; MMP and Signaling Interactions

In the aortocaval fistula model, the MMP induction–activation cascade is likely facilitated by a resident interstitial cell, the mast cell. There is a strong inter relationship between bioactive signaling molecules within the ECM, such as the cytokine TNF and MMP induction/activation. Mast cells can secrete significant amounts of MMPs as well as TNF, and both of these pathways have been implicated in volume overload–induced remodeling. As such, it is likely that cross-talk occurs between interstitial cell types, such as the mast cell and fibroblast, in terms of cytokine processing and signaling, which in turn would perpetuate the LV remodeling process in terms of MMP induction and ECM proteolytic events. Interruption of mast cell function directly or inhibition of the TNF signaling pathways in turn reduced the magnitude of LV remodeling with volume overload. Interestingly, the magnitude of LV remodeling and MMP induction with volume overload appear to be estrogen-dependent. Uniformly, the initiation of LV dilation with a volume overload stimulus is a robust increase in active MMP-2 and, although not directly measured in these past investigations, would putatively suggest a concordant induction of MMP-14. Through in silico mapping, the MMP-14 promoter contains estrogen response elements, and estrogen exposure directly modifies MMP-14 levels within the myocardium. In the estrogen receptor knockout mouse, MMP-14 expression was increased, as was MMP-2 activation. These observations support the hypothesis that estrogen may modify...
MMP-14 expression and, as a consequence, the proteolytic events that facilitate ECM remodeling and LV dilation with a volume overload stimulus.

**MMPs in MI**

With regard to LV remodeling, post-MI remodeling may hold the greatest complexity in terms of the temporal and regional heterogeneity of events occurring within the ECM.4,5,18-20,109-111 Nevertheless, through both clinical observational studies and the use of transgenic/pharmacological studies, it is quite apparent that MMPs play pivotal roles in both the requisite healing process after myocardial injury and the adverse remodeling process commonly termed infarct expansion.21-28,32,47-49,52,53,55,56,108,112-114 In the early time period after MI (first 72 hours), a more classical wound healing response occurs with the appropriate amplification of cytokines, influx of inflammatory cells, and proliferation/transdifferentiation of fibroblasts to a myofibroblast phenotype.109,110,115-117 A diverse number of MMP types released from both inflammatory cells and myofibroblasts facilitate proteolysis of ECM components and allow for nascent scar formation.109,110,115-117 However, unlike the prototypical wound healing response in more static tissue interfaces, continuous and persistent release of MMPs occur well beyond this initial wound healing period. There is a shift in MMPs synthesized primarily by the expanded population of transdifferentiated myofibroblasts,16,117 which results in ECM instability, particularly at the infarct-viable myocardial border.4,5,20 However, the remote viable myocardium also undergoes significant ECM remodeling, which includes proliferation of fibroblasts, MMP induction, and fibrillar collagen accumulation.18,19,24,26,32,47-49,52,109,110 Thus, the early elaboration of certain MMP types that appear to facilitate a physiological wound healing response are followed by a more maladaptive induction of MMPs within the MI, border, and remote myocardium, which in turn contribute to adverse remodeling within all of these regions. The challenge that remains is to identify those MMP types that facilitate an appropriate wound healing response vs those that contribute to the development of post-MI remodeling and the progression to HF.

**Post-MI Remodeling and MMP Induction**

Some of the first studies to demonstrate a mechanistic relationship between MMP activation and LV remodeling after MI were performed in rodents.23,57 For example, Heymans et al23 demonstrated in different transgenic constructs a relationship between MMP activation and post-MI remodeling. Rohde et al53 reported pharmacological MMP inhibition in mice attenuated the invariable LV dilation that occurred after surgically induced MI. Transgenic deletion of MMP-9, MMP-2, or MMP-7 in murine constructs of MI all have been shown to alter the post-MI remodeling process, but in some particularly unique ways.21,22,26,27 For example, targeted deletion of MMP-7 appeared to affect myocardial conduction pathways and proteins, and underscored the diversity of substrates that are likely relevant in the context of post-MI remodeling.27,118 Using transgenic constructs that facilitated MMP activation along with pharmacological rescue provided further support for a mechanistic relationship between MMP induction and activation, as well as adverse post-MI remodeling.23,25 Finally, direct molecular imaging approaches in murine models of MI have been performed, which provided a temporal map of MMP induction and activation within the LV during the remodeling process.24,47 For example, using transgenic MMP reporter constructs, increased MMP-2 and MMP-9 promoter activation was identified within the MI and border zones early post-MI, but interestingly, increased MMP-2 promoter activation was identified within the viable remote region as well as in the atrium.26,47 Taken together, these murine studies have provided novel insight into those MMP types that may be causative in LV remodeling, as well as the regional and temporal aspects of MMP induction post-MI.

**Differential MMP Induction After MI**

**Refining MMP Targets**

Using large animal post-MI models and MMP profiling, past studies have demonstrated that certain MMP types, such as MMP-1, were significantly reduced within the MI region, whereas other MMP types, such as MMP-14, were significantly increased within all regions post-MI.49,49,111 These past observations suggest that certain MMP types, such as MMP-1, may not be contributory to the adverse LV remodeling process post-MI, and pharmacological MMP–1 sparing inhibitors were examined in preclinical HF models.48,50 Using appropriate dosing titration, the plasma concentrations of these MMP inhibitors effectively would be an order of magnitude lower than that necessary to achieve MMP-1 inhibition, but would effectively inhibit MMP types such as MMP-2, MMP-9, and MMP-14.50 The main finding from these studies was that it was not necessary to perform global MMP inhibition, and that targeting a potential subset of MMP types may be an effective strategy.119 Moreover, these past studies suggested that pharmacological MMP inhibition is not necessary immediately after MI but can be successfully instituted after the completion of the initial wound healing period.48 The relative distribution of MMP-14 within the LV at 14 days post-MI (when, presumably, resolution of the acute wound healing phase has subsided) and the relations to cardiocytes and fibroblasts, respectively, are shown in Figure 1. It can be appreciated that MMP types, such as MMP-14, are ubiquitously expressed in a number of cell types, including myocytes, fibroblasts, and endothelial cells. Within the remote myocardium, MMP-14 is expressed within myocytes and fibroblasts and likely contributes to a prothrombotic response within this region. On the contrary, MMP-14 induction appears to be robust within proliferating fibroblasts in both the border and MI regions, where significant and persistent ECM proteolytic activity and turnover occur. This suggests that MMP-14 and subsequent activation of soluble MMP types, such as MMP-2, would cause continued ECM instability within these regions post-MI and thereby contribute to LV remodeling, dilation, and eventually dysfunction. This is but 1 illustration that emphasizes how the development of future strategies to interrupt the adverse LV remodeling process post-MI will need to be region-specific, temporally specific, and MMP type-specific.
It is apparent that MMP induction and activation are not all or none processes in terms of ECM proteolysis and, in fact, certain MMP types may actually facilitate ECM synthesis and accumulation, that is, fibrosis. One of the MMP types with a diverse proteolytic portfolio that likely plays a role in ECM degradation and instability, as well as ECM profibrotic signaling, is MMP-14. Uniformly, increased myocardial levels of MMP-14 have been identified in LV remodeling with pressure overload, a dilated LV phenotype, or post-MI. Although most certainly MMP-14 is not the only MMP type with a multiplicity of actions, this particular MMP is discussed for the purpose of illustration and is schematically presented in Figure 2. First, MMP-14 is localized to the cell membrane surface and provides for focal ECM degradation of basement membrane components, and thereby alters cell–cell interactions. Second, MMP-14 likely works in a cooperative manner with ADAMs to cause integrin proteolysis and shedding. Third, MMP-14 directly causes proteolysis of a number of ECM constituents, which in turn results in the formation of smaller fragments (ie, matricryptins), and thereby alters a number of intracellular signaling cascades. Fourth, as stated previously, MMP-14 constitutes a predominant pathway by which other soluble MMP types, such as MMP-2, are activated within the ECM. Finally, MMP-14 can directly facilitate ECM synthesis by enhancing and amplifying profibrotic signaling molecules, such as TGF.

The diversity of biological events that can be induced in whole or in part by MMP-14 also may provide insight into how 1 MMP type may have significantly different effects on ECM structure and function in the context of the different LV remodeling patterns. For example, in LV pressure overload, the signals and substrates for MMP-14 include profibrotic signaling molecules and processing of growth factors. In LV volume overload, the mechanical and biological signals likely coalesce to provoke increased release of soluble MMP types such as MMP-2, which in turn would be processed by MMP-14. In post-MI remodeling, it is likely that these MMP-14–mediated events that are operative in both pressure and volume overload are occurring simultaneously, but in a region-specific and time-specific manner.

Normal ECM Degradation and Profibrotic Signaling by MMP-14

Through a number of in vitro and proteolytic assays, it has been demonstrated that MMP-14 processes latent TGF binding protein-1, which causes release of biologically active TGF and the binding to local TGF receptor complexes. Further studies have demonstrated that MMP-14 can proteolytically alter the TGF coreceptor, endoglin (CD150), which in turn will modify TGF-mediated signaling. In the context of LV pressure overload, it is likely that MMP-14 contributes to the acceleration of myocardial fibrillar collagen accumulation and the development of diastolic dysfunction. Although remaining associative, it is likely that MMP-14 induces profibrotic signaling through a TGF-dependent pathway. In the volume overload cardiomyopathies, robust MMP-2 activation is a hallmark in the progressive LV dilation and the loss of ECM-mediated support. In this case, it is likely that significantly increased MMP-14–mediated activation
of MMP-2 would be a critical proteolytic pathway in which heightened ECM proteolysis occurs.\textsuperscript{19,46,50,51,54,55,96–98} In the context of post-MI LV remodeling, it is likely that MMP-14 plays critical roles in both ECM proteolysis and instability of the MI region and, at the same time, increases ECM accumulation and fibrosis within the border region.\textsuperscript{24,32,52} In a transgenic mouse model of cardiac-restricted MMP-14 overexpression, adverse post-MI remodeling, such as increased fibrosis, has been observed.\textsuperscript{20,32} In contrast, transgenic-mediated reduction in MMP-14 expression reduced the degree of post-MI remodeling and ECM accumulation.\textsuperscript{32} In both of these past studies, concordant directional changes in MMP-14-mediated latent TGF binding protein-1 hydrolysis were observed. The liberation of TGF from the latency binding proteins results in binding to a transmembrane receptor complex and, ultimately, the phosphorylation of receptor transduction elements, the Smad proteins. In the murine model of MI, the heightened MMP-14-specific latent TGF binding protein-1 hydrolysis was directly associated with increased Smad phosphorylation, indicative of increased TGF receptor stimulation.\textsuperscript{24,32} With either increased or decreased levels of MMP-14, Smad phosphorylation changed in a parallel fashion and, in turn, was associated with concordant changes in fibrillar collagen expression.\textsuperscript{72,32} In the murine model of pressure overload, MMP-14 promoter induction and, ultimately, MMP-14 expression were directly associated with the time-dependent changes in LV myocardial fibrillar collagen expression and accumulation.\textsuperscript{31} Whereas these studies are far from conclusive and the TGF signaling pathway can be complex, these observations and those from other model systems\textsuperscript{76–78} suggest that MMPs such as MMP-14 not only can cause degradation of normal ECM structure and thereby function but also can induce a potent profibrotic signaling cascade, such as through the proteolytic release of TGF (Figure 2). These proteolytic events can be occurring simultaneously in a specific region or in different regions, which in turn would facilitate adverse ECM remodeling.

**Fibroblast Proliferation, Transdifferentiation, and MMP-14**

The diverse actions of MMP-14 in terms of simultaneously causing ECM proteolysis as well as processing biological signaling molecules have received considerable attention with regard to the tissue remodeling and mesenchymal cell transdifferentiation, which occur in cancer.\textsuperscript{78,84–88,116} There are similarities in the signaling pathways that are evoked during this mesenchymal transdifferentiation process to that of fibroblast–myofibroblast transdifferentiation.\textsuperscript{109,110,116,117,123,124} In terms of LV remodeling, such as with pressure overload or MI, it is likely that this transdifferentiation process occurs in that an increased density of myofibroblasts commonly occurs. Although an incomplete definition, myofibroblasts are those that express key markers, such as α-smooth muscle actin.\textsuperscript{117,124} In this case, transformed fibroblasts have been identified with unabated expression of ECM proteolytic enzymes, such as MMP-14.\textsuperscript{125} The specific cell type of origin with respect to the myofibroblast remains unclear and may arise from a stem-cell type, pericyte, or a clonal expansion of endogenous fibroblasts. For example, in models of MI and angiotensin II–induced hypertrophy, it has been identified that mesenchymal stem cells transmigrate, proliferate, and differentiate to a myofibroblast phenotype.\textsuperscript{126,127} The transmigration of mesenchymal cells likely requires an upregulation of MMPs as well as processing of chemokines, whereby MMP-14 may play a role in both of these processes.\textsuperscript{52,84,120,128,129} With aging, cardiac-restricted overexpression of MMP-14 increased cell markers of myofibroblast transformation as well as myocardial fibrosis.\textsuperscript{24} TGF has been shown to play a role in mesenchymal transdifferentiation.\textsuperscript{116,130} Taken together, these observations suggest that MMPs, such as MMP-14, can contribute to selective degradation of basement membrane components and activation of biological signaling molecules—all critical events that contribute to fibroblast transdifferentiation and expansion in the context of LV remodeling. However, this remains an intriguing postulate, and additional research in this area is required.

**Regulation of MMPs as Targets for Myocardial Remodeling**

In light of the potent biological effects of MMP proteolytic activity, there are critical control points in terms of MMP transcription, synthesis, and post translational modification that hold potential for therapeutic targeting.

**Transcriptional Activity**

MMP transcriptional activity is influenced by upstream biological signals that are operative in the context of LV remodeling and HF, which include bioactive molecules such as neurohormones and cytokines.\textsuperscript{30,80,86–88,131,132} Thus, pharmacological approaches that modify these signaling pathways likely modify MMP transcription. MMP induction also is likely to be sensitive to mechanical signals, such as load, and it has been shown that an acute increase in LV afterload can induce MMPs, such as MMP-2 and MMP-14. Moreover, there are possible important interactions and integrations between mechanical and biological signals that ultimately influence MMP induction. For example, an acute increase in LV load caused a robust increase in interstitial MMP activity that could be modified, to some degree, by angiotensin II receptor inhibition.\textsuperscript{31} After an acute MI in pigs, a similar pharmacological approach has been shown to modify myocardial MMP induction.\textsuperscript{132} The importance of MMP transcriptional activity in terms of LV remodeling and progression to HF also can be demonstrated from clinical studies of MMP polymorphisms.\textsuperscript{133–135} For example, a genetic variation within the MMP-9 promoter (C-1562T), which results in higher MMP-9 transcriptional activity, was associated with elevated plasma MMP-9 levels and worsened post-MI survival.\textsuperscript{134} Although these polymorphism studies can be problematic with respect to direct causality in specific MMP induction, it does emphasize the potential mechanistic importance of MMP promoter activity. Using promoter–reporter constructs,\textsuperscript{29,35} it may be possible to determine which signaling pathways play a predominant role in type-specific MMP transcriptional activity in the context of LV remodeling. Another likely and potentially unique mechanism for MMP transcriptional activation is through the extracellular matrix metalloproteinase inducer (EMMPRIN) protein.\textsuperscript{136,137}
Significantly elevated myocardial levels of EMMPRIN have been identified in patients with significant LV remodeling and HF and is also increased in patients after MI. In both of these clinical observational studies, increased expression of EMMPRIN was coincident with MMPs likely contributory to the LV remodeling process, including MMP-2, MMP-9, and MMP-14. In a transgenic model of cardiac restricted overexpression of EMMPRIN, increased myocardial levels of MMP-2 and MMP-14 occurred and resulted in adverse LV remodeling as a function of age. However, it remains uncertain how EMMPRIN causes MMP transcription in the context of LV remodeling and to what degree this signaling pathway contributes to the progression of HF.

Post-transcriptional Regulation

The miRs can directly influence post transcriptional events through a number of molecular interactions, including binding to mRNA and interfering with initiation of translation and accelerating mRNA degradation. Thus, whereas the specificity of certain miRs in terms of mRNA targets remains to be fully developed, it is becoming apparent that certain miRs likely hold relevance to LV remodeling and to the ECM in particular. For example, in a mouse model of MI, changes in relative myocardial miR-29a levels had distinct and directional effects on ECM remodeling in terms of myocardial fibrosis. Other studies have provided direct evidence for a mechanistic relationship between relative miR-29a levels and...
MMP protein content. In other studies, overexpression of miR-133a in a transgenic model reduced the invariable changes within the ECM with LV pressure overload. Although it is likely that specific miRs can directly influence ECM proteolytic pathways by post transcriptional regulation of specific MMPs, it is also likely that changes in ECM composition and structure can, in turn, influence this post transcriptional pathway. Specifically, miRs can be released into the ECM through several mechanisms, including a pathway involving exosomes. The trafficking of miRs within the ECM may constitute an important mechanism of cell–cell communication and, therefore, MMP-mediated changes in ECM structure, function, and composition directly affect this process. In addition, the egress of miRs into the ECM will result in movement into the vascular compartment and allow for profiling specific miR levels through peripheral blood sampling. Using this approach, specific relationships between plasma profiles of specific miRs and MMPs in the context of LV remodeling, such as after an MI, may be possible. Post-translational Control

A critical control point with respect to post translational regulation of MMP activity is through the endogenous set of small-molecular-weight proteins, the tissue inhibitors of MMPs (TIMPs). There are 4 known TIMPs with very high affinity for binding to active MMPs that were initially thought to be of similar functional characteristics. However, there is now clear and specific functionality identified for each TIMP, and this has been the focus of several reviews. In general, however, there appears to be a discordant induction of TIMPs in the context of LV remodeling, which in turn would affect net MMP proteolytic capacity. For example, in LV pressure overload, relatively higher levels of TIMP-1 occur in relation to MMP levels, and this would favor a net reduction in ECM degradation. On the contrary, levels of TIMPs in the context of post-MI remodeling appear to be reduced, which would favor higher net MMP proteolytic activity. However, this is likely a significant oversimplification of the complex interaction between MMPs and TIMPs in terms of LV remodeling. For example, in pressure overload, transgenic-mediated deletion of TIMP-2 or TIMP-3 actually accelerated the progression of LV remodeling and failure. Moreover, TIMPs not only bind to the active MMP domain but also may bind to the pro-MMP domain, which can actually facilitate MMP activation. For instance, pro-MMP-2 and TIMP-2 form a complex that ultimately interacts with MMP-14, yielding active MMP-2. This MMP–TIMP interaction therefore suggests that increased levels of TIMP-2 may actually facilitate MMP activation and ECM proteolysis. Thus, similar to that of MMPs, there is significant functional diversity and complexity of the TIMPs. Understanding these functional interactions between specific MMP and TIMP types will likely yield novel and more specific strategies to regulate ECM proteolysis with LV remodeling.

One of the more exciting and perhaps disappointing findings about the regulation of ECM proteolysis has been in terms of post translational control of MMP activity through pharmacological MMP inhibition. Preclinical models of broadspectrum or MMP-1–sparing MMP inhibitors uniformly demonstrated beneficial effects in terms of affecting early adverse LV remodeling, particularly in the context of post-MI remodeling. In addition to providing further evidence for a cause–effect relationship between MMP activation and adverse LV remodeling, these studies provided the impetus for moving to clinical feasibility. Early clinical trials of pharmacological MMP inhibitors in cancer patients were plagued by adverse systemic effects, notably of a musculoskeletal origin. Furthermore, it also was difficult to determine effective dosing for MMP inhibitors because there was not a biological response variable that could be easily measured. Nevertheless, a clinical trial of MMP inhibition in post-MI patients (Selective Matrix Metalloproteinase Inhibitor to Prevent Ventricular Remodeling After Myocardial Infarction [Prevention of Myocardial Infarction Early Remodeling (PREMIER)]) was undertaken. The primary end point was that of changes in LV volumes at 6 months post-MI, whereby patients were randomized to a single dosing regimen of an MMP-1–sparing inhibitor or placebo. However, the dosing regimen was altered during the course of this study because of concerns about potential musculoskeletal effects. As such, a simulation of the pharmacokinetics of this specific MMP inhibitor and the dosing strategy used in the PREMIER study suggest that a minimum concentration necessary to provide MMP inhibition for a majority of MMP types was not realized. Moreover, the overall net change in the primary response variable, LV end-diastolic volume, was surprisingly low compared with the majority of other post-MI studies (≈10%) and thereby would attenuate the ability to detect a treatment effect. For these reasons, it was not surprising that the results of this initial MMP inhibition study were equivocal. However, because of the clear cause–effect relationship between MMP-mediated ECM proteolysis and the adverse tissue remodeling process in a number of pathological conditions, there is a resurgence of alternative and more targeted MMP inhibitors on the horizon.

Future Directions in MMP Translational Research and Myocardial Remodeling

Diagnostics

One of the initially unsuspected outcomes from MMP translational research was the ability to measure MMPs from a peripheral blood sample. Whereas the sources and origins of changes in peripheral levels of MMPs can be multifactorial, clinical studies have provided evidence that profiling MMPs and TIMPs can hold diagnostic and prognostic significance in the context of HF. As basic science studies of the biological roles of specific MMP types continue, it is likely that a refined set of MMP measurements from a peripheral blood sample would be useful biomarkers for identifying those patients at risk for more rapid and progressive LV remodeling and the development of HF. In addition, it may be possible to utilize these MMP/TIMP profiles in terms of a surrogate marker for assessing and developing dosing regimens. A more direct method for quantifying MMP activity and expression with respect to the LV remodeling process is through imaging. The increased spatial resolution of imaging
Approaches and validation have been reported previously.52 Left ventricular (LV) myocardial perfusion by 201TI is designated as green, and MMP radiotracer uptake is designated as red. Significant MMP radiotracer uptake could be observed within the LV myocardium, indicative of MMP proteolytic activity, at 3 and 14 days post-MI. Moreover, MMP activity appears to occur within normally perfused LV regions at later post-MI time points. Images courtesy of Dr Albert Sinusas, Professor of Medicine and Diagnostic Radiology Director, Cardiovascular Imaging Director, Yale Translational Research Imaging Center, Yale University School of Medicine.

Figure 3. Dual-isotope hybrid single-photon emission computed tomography/cine x-ray computed tomography imaging obtained using Thallium-201 (201TI) and a technetium-99m-labeled matrix metalloproteinase (MMP)-targeted tracer (99mTc-RP805) in a canine model of myocardial infarction (MI) induced by balloon coronary occlusion. The imaging approaches and validation have been reported previously.52 Left ventricular (LV) myocardial perfusion by 201TI is designated as green, and MMP radiotracer uptake is designated as red. Significant MMP radiotracer uptake could be observed within the LV myocardium, indicative of MMP proteolytic activity, at 3 and 14 days post-MI. Moreover, MMP activity appears to occur within normally perfused LV regions at later post-MI time points. Images courtesy of Dr Albert Sinusas, Professor of Medicine and Diagnostic Radiology Director, Cardiovascular Imaging Director, Yale Translational Research Imaging Center, Yale University School of Medicine.

Systems in general, coupled with molecular probes such as an MMP-specific imaging agent, is possible to directly identify areas of increased MMP activity with LV remodeling. For example, initial studies using a radiolabeled MMP tracer in a murine model of MI demonstrated specific and focal uptake within the MI and border zones.57 These initial studies were advanced to large animals, whereby dual single-photon emission computed tomography and cine x-ray computed tomography imaging could be performed and could allow for spatial registration of LV anatomy and MMP activity with MI.52 Representative dual-isotope single-photon emission computed tomography/cine x-ray computed tomography imaging in a large animal model of MI is shown in Figure 3. These imaging approaches will likely further our understanding of the regional and temporal aspects of MMP induction in the context of LV remodeling and allow for serial translational studies to be performed because of the need for myocardial sampling.

Therapeutics

Although broad-based pharmacological MMP inhibitors yielded problematic issues surrounding dosing efficacy as well as significant potential for systemic effects, important research directions in this area would involve more highly selective inhibitors. For example, the in vivo use of a selective MMP-14 inhibitory antibody has been described.145 Another approach is to interfere with MMP substrate binding, rather than directly inhibiting the zinc-dependent catalytic domain.149 Finally, tetracycline derivatives remain a feasible possibility for MMP inhibition and have been advanced clinically in terms of preventing the MMP-mediated tissue remodeling in gingivitis.142,150

Another potential approach is through the use of cell-based, or more targeted, therapies that would obviate the need for systemic delivery. For example, implantation of TIMP-transfected cells altered the time course of post-MI remodeling in rats.151 Other applications include MMP gene transfer through regional placement of biodegradable materials.146,147 Whereas direct targeting of MMP induction and activation remains a potential area for relevant translational research, it also may be possible to exploit this family of proteases in other relevant therapeutic areas. For example, MMP-mediated proteolysis of the ECM may be relevant in terms of the microenvironment and matrix scaffolding for stem cell engraftment.52,153

This review was based on the premise that MMPs act on structural and biological signaling molecules within the ECM. However, there is evidence for intracellular actions of MMPs that hold relevance to LV remodeling and the progression to HF.35,144,150,154 For example, MMP-2 has been localized to cardiocyte mitochondria and was inducible by oxidative stress. Moreover, this past study identified different MMP-2 isoforms existing within the intracellular matrix as opposed to the ECM.154 Thus, not only is there an ever-growing number of MMP types that are expressed within the myocardial with LV remodeling but also there may be isoforms for each of these MMP types. These discrete MMP expression—localization patterns hold relevance in terms of substrate processing, whereby a number of intracellular substrates, both of the cytoskeletal and contractile protein domains, have been identified by degradomic profiling.62–66,118 Based on the ever-increasing substrates and diversity of biological actions of MMPs, it is likely that continued research of the relationship of LV remodeling to this family of proteases will yield new insights into the ECM remodeling process itself and well as new therapeutic targets.

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Disclosure

None.

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