Matrix Metalloproteinases
Regulation and Dysregulation in the Failing Heart

Francis G. Spinale

Abstract—Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes responsible for myocardial extracellular protein degradation. Several MMP species identified within the human myocardium may be dysregulated in congestive heart failure (CHF). For example, MMPs that are expressed at very low levels in normal myocardium, such as collagenase-3 (MMP-13) and the membrane-type-1 MMPs, are substantially upregulated in CHF. However, MMP species are not uniformly increased in patients with end-stage CHF, suggesting that a specific portfolio of MMPs are expressed in the failing myocardium. With the use of animal models of CHF, a mechanistic relationship has been demonstrated with respect to myocardial MMP expression and the left ventricular (LV) remodeling process. The tissue inhibitors of the MMPs (TIMPs) are locally synthesized proteins that bind to active MMPs and thereby regulate net proteolytic activity. However, there does not appear to be a concomitant increase in myocardial TIMPs during the LV remodeling process and progression to CHF. This disparity between MMP and TIMP levels favors a persistent MMP activation state within the myocardium and likely contributes to the LV remodeling process in the setting of developing CHF. The elucidation of upstream signaling mechanisms that contribute to the selective induction of MMP species within the myocardium as well as strategies to normalize the balance between MMPs and TIMPs may yield some therapeutic strategies by which to control myocardial extracellular remodeling and thereby slow the progression of the CHF process. (Circ Res. 2002;90:520-530.)

Key Words: myocardial remodeling • heart failure • matrix metalloproteinases
• matrix metalloproteinase inhibitors

The left ventricular (LV) myocardial remodeling process that occurs in various settings of congestive heart failure (CHF) has historically been attributed to intrinsic changes in the cardiac myocyte. However, it is now recognized that important changes also occur within the extracellular matrix (ECM) of the myocardium, contributing to the remodeling process. Moreover, it has become increasingly evident that the myocardial ECM is not a static structure but, rather, a dynamic entity that may play a fundamental role in myocardial adaptation to pathological stress and thereby facilitate remodeling. Specifically, in both human and animal studies, it has been reported that alterations in the collagen interface, both in structure and composition, occur within the LV myocardium, which, in turn, may influence LV geometry.1–14 The myocardial ECM contains a fibrillar collagen network, a basement membrane, proteoglycans and glycosaminoglycans, and bioactive signaling molecules. The myocardial fibrillar collagens, such as collagen types I and III, ensure structural...
integrity of the adjoining myocytes, provide the means by which myocyte shortening is translated into overall LV pump function, and are essential for maintaining alignment of the myofibrils within the myocyte through a collagen–integrin–cytoskeletal myofibril relation.1–5,8–14 The complexity of the ECM can begin to be appreciated through examining the fibrillar network of porcine myocardium subjected to maceration digestion (Figure 1). The ECM forms a continuum between different cell types within the myocardium and provides a structural supporting network to maintain myocardial geometry during the cardiac cycle. Disruption or discontinuities within the fibrillar ECM network will result in a loss of normal structural support and continuity, resulting in myocyte fascicles being subjected to abnormal stress-and-strain patterns during the cardiac cycle, which, in turn, will result in changes in myocardial geometry and function. The identification and understanding of the enzyme systems responsible for ECM degradation within the myocardium has particular relevance in the progression of CHF. The purpose of the present review is 3-fold: (1) to present a brief overview of a proteolytic system within the myocardium that likely contributes to ECM remodeling, ie, the matrix metalloproteinases (MMPs); (2) to examine the results from basic studies that have used pharmacological and genetic strategies to provide a cause-and-effect relationship with respect to MMP activation and the LV remodeling process; and (3) to demonstrate how this system is upregulated or, arguably, dysregulated in patients with CHF.

**MMPs and Tissue Inhibitors**

**MMP Generic Structure and Function**
The MMPs have been demonstrated to play a pivotal role in normal tissue remodeling processes, such as tissue morphogenesis and wound healing.15–20 This proteolytic system degrades a wide spectrum of ECM proteins and is constitutively expressed in a large number of cell and tissue types. Although MMPs likely play important roles in normal tissue remodeling, increased MMP expression has been identified in
pathological processes, such as tumor angiogenesis and metastasis, rheumatoid arthritis, vascular neointimal hyperplasia, and plaque rupture.\textsuperscript{15–22} The MMPs constitute a family of zinc-dependent enzymes that currently number >20 species.\textsuperscript{15,16} There are two principal types of MMPs: those that are secreted into the extracellular space and those that are membrane bound. The secreted MMPs constitute the majority of known MMP species and are released into the extracellular space in a latent or proenzyme state (proMMP). Activation of these latent MMPs is required for proteolytic activity, which can be achieved through enzymatic cleavage of the propeptide domain. Serine proteases, such as plasmin, as well as other MMP species can convert proMMPs to active enzyme.\textsuperscript{23,24} Thus, rapid amplification of MMP activity can occur after an initial enzymatic step. The cleavage of the propeptide domain results in a conformational change and exposure of the catalytic domain to the ECM substrate. There is a significant degree of homology within the catalytic domain of MMP species, and substrate specificity is determined by the large extracellular binding domain at the C-terminus of the enzyme.\textsuperscript{15,16,25} The secreted MMPs bind to specific ECM proteins on the basis of the sequence of this C-terminus and, therefore, are in very close juxtaposition to the future proteolytic substrate. In other words, the latent MMP “docks” to the ECM proteins and remains enzymatically quiescent until activation. Thus, a pool of recruitable MMPs exists within the ECM and provides a means for the rapid induction of proteolytic activity. Furthermore, because these soluble MMPs bind to specific proteins and at specific protein sequences, the activation of MMPs can occur in specific patterns within the ECM.

**MT-MMPs: Localized MMPs With Multiple Functions**

A recently described class of MMPs is the membrane-type MMPs, or MT-MMPs.\textsuperscript{26} The MT-MMPs constitute a novel class of MMPs for several reasons. First, MT-MMPs are membrane bound and, therefore, provide a focalized area for ECM proteolytic degradation. Second, during trafficking to the cell membrane, MT-MMPs undergo intracellular activation through a proprotein convertase pathway.\textsuperscript{24,26} Thus, unlike other classes of MMPs, MT-MMPs are proteolytically active once inserted into the cell membrane. Third, MT-MMPs contain a substrate recognition site for other MMP species and, therefore, constitute an important pathway for activation of other MMPs within the ECM.\textsuperscript{23,24} It has been demonstrated that MT1-MMP proteolytically processes the proforms of the gelatinase MMP-2 and the interstitial collagenase MMP-13. Fourth, the MT-MMPs do not appear to be under the influence of local inhibitory control, inasmuch as the tissue inhibitors of the MMPs (TIMPs) fail to effectively bind to MT-MMPs.\textsuperscript{26} There are now 6 different MT-MMPs that have been cloned, and they appear to be expressed in both normal and diseased tissue.\textsuperscript{26–31} A number of cell types within the myocardium express MT-MMPs, which include fibroblasts, vascular smooth muscle, and cardiac myocytes. The most well-characterized MT-MMP is MT1-MMP, and it has been the focus of several in vitro and in vivo studies.\textsuperscript{26} It has been demonstrated that MT1-MMP degrades fibrillar collagens and a wide range of ECM glycoproteins and proteoglycans. Thus, the MT-MMPs are an area of active research and likely play an important role in ECM degradation localized to the basement membrane and cell-cell contact points.

**MMP Endogenous Inhibitory Control**

The activated MMPs undergo autocatalysis, resulting in lower molecular weight forms and, ultimately, in inactive protein fragments.\textsuperscript{23,24} Another important control point of MMP activity is through the presence of an endogenous class of low-molecular-weight molecules called TIMPs.\textsuperscript{32–36} Four different TIMP species have been identified and bind to activated MMPs in a 1:1 stoichiometric ratio. Furthermore, certain TIMPs bind to proMMPs and thereby form MMP-TIMP complexes. The functional significance of these proMMP-TIMP complexes remains incompletely understood, but they may actually facilitate MMP activation.\textsuperscript{23,24,34} For example, it has been demonstrated that TIMP-2 forms a complex with membrane-type MMPs and that this complex enhances the activation of proMMP-2.\textsuperscript{24} One of the better characterized TIMPs is TIMP-1, which binds with great affinity to activated MMPs. TIMP-4 appears to have a predominant distribution within the myocardium.\textsuperscript{36} However, the significance of the myocardial expression of TIMP-4 within the myocardium remains unclear. In addition to binding to MMPs, TIMPs appear to influence cell growth and metabolism in vitro.\textsuperscript{37,38} Thus, TIMPs may have multiple biological effects with respect to MMP activity within the myocardium, which would be relevant to the LV remodeling process.

**Myocardial MMPs: Present and Future**

The classification of MMPs was originally determined by substrate specificity, but as the characterization of this enzyme system has proceeded, a great deal of substrate crossover between MMP classes and species has been identified. Nevertheless, a general classification and numbering scheme has been developed for the MMPs, and a comprehensive table can be consulted from several recent comprehensive reviews.\textsuperscript{15,16} The interstitial collagenases (such as MMP-1 and MMP-13), the stromelysins (such as MMP-3), and the gellatinases (such as MMP-2 and MMP-9) have been demonstrated within the mammalian myocardium and are presented in the Table. However, it is very likely that this list will be expanded, particularly for members of the MT-MMP family as well as other novel MMP species. An important direction of future research will be to carefully examine the portfolio of MMPs expressed in the mammalian myocardium and to compare and contrast potential differences in myocardial MMP expression between species. The full complement of myocardial MMPs present in normal human and other mammalian species must be determined to develop appropriate experimental models and designs regarding myocardial MMP expression and activation in the LV remodeling process.

**Pathways for MMP Induction**

MMP mRNA expression can be influenced by a variety of chemical agents, neurohormones, corticosteroids, and cytokines.\textsuperscript{37–40} For example, tumor necrosis factor-α (TNF-α), a
### Matrix Metalloproteinases Identified in Human Myocardium

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<thead>
<tr>
<th>Name</th>
<th>Number</th>
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<td>Collagenases</td>
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<tr>
<td>Interstitial</td>
<td>MMP-1</td>
<td>Collagens I, II, III, VII, and basement membrane components</td>
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<td>Collagenase 3</td>
<td>MMP-13</td>
<td>Collagens I, II, and III</td>
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<td>Gelatinases</td>
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<td>Gelatinase A</td>
<td>MMP-2</td>
<td>Gelatins, collagens I, IV, V, VII, and basement membrane components</td>
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<td>Gelatinase B</td>
<td>MMP-9</td>
<td>Gelatins, collagens IV, V, XIV, and basement membrane components</td>
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<td>Stromelysins</td>
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<td>Stromelysin 1</td>
<td>MMP-3</td>
<td>Fibronectin, laminin, collagens III, IV, IX, MMP activation</td>
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<td>Membrane-type MMPs</td>
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<td>MT1-MMP</td>
<td>MMP-14</td>
<td>Collagens I, II, III, fibronectin, laminin-1: activates proMMP-2 and proMMP-13</td>
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cytokine, can influence MMP gene expression in several cell systems through the formation of transcription factors that bind to specific response elements on MMP gene promoters. One common response element contained within the promoter region of certain MMP genes is the activator protein-1 (AP-1) site. Other factors, such as retinoids, transforming growth factor-β, and glucocorticoids, are thought to inhibit MMP gene expression by several mechanisms, such as by sequestering unbound AP-1 proteins.

Transcription of MMPs is usually influenced in a similar manner by a given factor from cell to cell, but there are several exceptions in which a response is cell specific. For example, interleukin-1 induces the expression of MMP-1 and MMP-3 in fibroblasts but not in keratinocytes, and transforming growth factor-β suppresses MMP-9 production in fibroblasts but induces its production in keratinocytes. Identified on the MMP-9 gene promoter is another transcription factor–binding site known as the nuclear factor-kB site. Studies have demonstrated a requirement of this site in the upregulation of MMP-9 by inflammatory cytokines in human fibroblasts. It was observed in several cell systems that exposure to phorbol esters, which increase protein kinase C intracellular pathways, also increases MMP mRNA transcription. Past studies have identified a transmembrane protein that induces the expression of specific MMPs in vitro. The nomenclature of this protein is primarily that of the extracellular MMP inducer protein (EMMPRIN). The most active area of EMMPRIN investigation has been with respect to tumor invasion, a process in which increased EMMPRIN expression has been localized to areas with intense remodeling activity or to highly invasive tumors.

An important avenue of future research will be not only to define the signaling pathways that regulate MMP expression but also to determine the ensemble of extracellular stimuli responsible for inducing the MMP species that are obligatory in the LV remodeling process. Moreover, past in vitro studies have suggested the intriguing possibility that an identical set of extracellular stimuli present within tissue may cause a very different and cell-specific induction of MMPs. Inasmuch as the myocardium is composed of multiple cell types, these cell-specific responses may hold particular relevance in elucidating the biochemical basis for remodeling within the myocardial interstitium as well as the coronary vasculature.

### Myocardial MMPs in Animal Models: Proof of Concept

#### Increased Myocardial MMP Levels Are Time Dependent During Heart Failure

A clear cause/effect relationship between MMPs and the LV remodeling process has been demonstrated through the use of animal models of developing CHF and transgenic models and through the use of pharmacological MMP inhibition studies.

In a pacing LV-failure animal model, a time-dependent increase in myocardial MMP levels has been demonstrated to accompany the progression of LV dilation and dysfunction. These changes in myocardial MMP levels were accompanied by alterations in ECM structure (Figure 2). Moreover, this past study demonstrated that the increased MMP levels and myocardial remodeling preceded significant changes in isolated myocyte contractile dysfunction. These observations suggest that the induction of myocardial MMPs is an early event in the development of LV remodeling and pump dysfunction with chronic rapid pacing.

In the Syrian cardiomyopathic hamster model, in vitro MMP activity was increased and noted to be particularly elevated in cardiomyopathic LV samples in which significant cardiac remodeling had occurred. With the use of the spontaneously hypertensive heart-failure (SHHF) rat model, myocardial MMP levels were shown to increase during the transition from compensated LV hypertrophy to the decompensated dilated phenotype. Thus, several animal model systems have demonstrated a time-dependent relationship between increased MMP expression and activity, the myocardial remodeling process, and the progression to failure.

#### Alterations in MMP/TIMP Levels in LV Hypertrophy

An important cause of clinical heart failure is diastolic dysfunction secondary to LV hypertrophy. More specifically, LV hypertrophy can cause reduced myocardial compliance and thereby impede adequate filling during diastole. Abnormalities in the structure and the composition of the ECM have been demonstrated to contribute to myocardial compliance and, in turn, to influence specific determinants of diastolic function. Evidence exists supporting the concept that diminished myocardial MMP activity can facilitate collagen accumulation in developing hypertrophy. In the spontaneously hypertensive rat, the development of compensated hypertrophy is associated with increased myocardial TIMP levels, which would imply reduced MMP activity. Because the spontaneously hypertensive rat model progresses to decompensation and LV failure, myocardial TIMP levels fall below normal levels, which would favor increased MMP activity. Thus, it is likely that a time-dependent spectrum of myocardial MMP activation occurs with the development of pressure-overload hypertrophy and contributes to the overall remodeling process. In support of this postulate,
studies completed by this laboratory have demonstrated time-dependent changes in myocardial MMP levels after an acute and prolonged pressure-overload stimulus. In these studies, acute pressure overload induced myocardial MMP expression and a resultant increase in zymographic activity. However, with a prolonged pressure overload, MMP zymographic activity began to normalize and was accompanied by changes in TIMP levels. In volume-overload states, such as with mitral regurgitation or aortocaval fistula, increased myocardial MMP levels and zymographic activity have been reported. For example, in a rat model of volume overload, increased myocardial MMP zymographic activity was associated with changes in LV volumes and mass. These studies would suggest that the early induction of myocardial MMP activity occurs with an overload stimulus that, in turn, would alter extracellular myocyte support. These changes in extracellular fibrillar support and architecture would, in turn, facilitate alterations in myocyte size and geometry, which is the structural basis of hypertrophy.

**Disruption of Normal Myocardial MMP/TIMP Levels Causes LV Remodeling**

Several transgenic models have been constructed to disrupt normal myocardial MMP and TIMP levels. A loss of MMP inhibitory control through TIMP-1 gene deletion has been shown to cause LV dilation in mice. In another study, cardiac-restricted overexpression of the interstitial collagenase (MMP-1) resulted in changes in the myocardial ECM structure, which was accompanied by alterations in LV function. It has been shown that the deletion of the MMP-9 gene in mice alters the course of LV remodeling after myocardial infarction (MI). For example, Heymans et al demonstrated that a loss of function of certain MMPs in transgenic mice can be associated with increased myocardial rupture early after MI. The role of MMP expression and activation with respect to MI has been the subject of a past review in Circulation Research.

**Exogenous MMP Inhibition Modulates the LV Remodeling Process**

Pharmacological MMP inhibition has been used in several animal models of LV dysfunction. For example, MMP inhibitor treatment with chronic rapid pacing attenuated the degree of LV dilation that invariably occurs in this condition.
model. Moreover, the degree of ECM disruption and disorganization that was observed in the untreated pacing CHF group was attenuated with MMP inhibition (Figure 2). In the SHHF rat model, MMP inhibition resulted in an attenuation of LV dilation and improved pump function. In that model, the transition from a compensated LV hypertrophic state to LV dilation and dysfunction occurred in rats aged 9 to 13 months. Accordingly, SHHF rats were treated with a broad-spectrum MMP inhibitor beginning at 9 months of age and were compared with age-matched untreated SHHF rats. As expected, significant LV dilation and myocardial remodeling occurred at 13 months of age in the SHHF rats compared with normal rats (Figure 2). However, MMP inhibition significantly attenuated the degree of LV dilation that occurred in this SHHF model. The reduction in the degree of LV dilation with MMP inhibition was accompanied by a preservation of LV pressure development. In the mouse MI model, MMP inhibition has also been shown to reduce the degree of post-MI LV dilation. Thus, broad-spectrum MMP inhibition has been demonstrated to modulate the LV remodeling process in several different animal systems.

**Myocardial MMPs in Human CHF**

**Emergence of MMP Species in Patients With CHF**

Myocardial MMP species abundance and indices of the MMP activation state have been assessed in end-stage human heart failure. Several studies have reported increased MMP zymographic activity, likely reflecting increased abundance of the gelatinases (MMP-2 and MMP-9), in myocardial samples from patients with cardiomyopathic disease. For example, Gunja-Smith et al reported a robust increase in MMP zymographic activity in the failing human myocardium that was accompanied by a reduction in fibrillar collagen cross-link formation. These biochemical events would favor ECM degradation within the cardiomyopathic myocardium. In addition, MMP species that possess the capacity to degrade a wide spectrum of ECM components are increased in end-stage human CHF. For example, increased levels of MMP-3 have been identified in human cardiomyopathic myocardium. Inasmuch as MMP-3 can deag a number of collagens and basement membrane components as well as play a role in the activation of other MMPs, increased abundance of this particular MMP species may result in significantly increased proteolytic degradation within the myocardial ECM. Interstitial collagenase MMP-13 is expressed at very low levels within normal myocardium but is significantly increased in end-stage CHF. MMP-13 is most strongly associated with aggressive tumor metastases; this finding suggests that this MMP species may be selectively expressed in pathological remodeling processes.

**Increased MT1-MMP in Human CHF: A Local Proteolytic and Activation System**

In addition to soluble MMP species, certain MT-MMPs appear to be increased in the failing human myocardium. MT1-MMP has been demonstrated to be expressed in normal human LV myocytes and is significantly increased within the myocardium of patients with dilated cardiomyopathy compared with nonfailing myocardial samples (Figure 3). The induction of MT1-MMP in the failing myocardium may result in significantly increased proteolytic activity at the extracellular surface of the sarcolemma and thereby alter myocyte adhesion to the basement membrane. This will result in a focal loss of myocyte continuity with the ECM and diminish the capacity of sarcomere shortening to be efficiently translated into muscle contraction. With the use of immunofluorescent staining, MT1-MMP has been colocalized to the cell membrane and, therefore, may be in proximity to the ECM-binding proteins, the integrins. Thus, increased MT1-MMP activity at the level of the myocyte may alter integrin engagement to the ECM and, in turn, significantly affect myocyte cytoskeletal architecture and intracellular signaling. The importance of integrin signaling in the maintenance of normal myocardial structure and function was the subject of a recent review in *Circulation Research*. Finally, MT1-MMP can activate proMMP-13 to an active form and thereby amplify local proteolytic activity of this MMP species. Thus, increased expression of the MT-MMPs during the development of CHF may significantly contribute to alterations in myocardial ECM structure and composition as well as alter myocyte intracellular signaling pathways. In light of these observations, important future directions will be to define the exact role that the MT-MMPs play in the cell-remodeling process and to determine which specific MT-MMP species are increased in patients with CHF.

**Discordant Myocardial MMPs and TIMPs in Heart Failure**

Although a number of MMP species are expressed within the human myocardium, not all of these MMPs are upregulated in end-stage CHF. Specifically, the abundance of interstitial collagenase-1, or MMP-1, is significantly reduced in patients with cardiomyopathic disease. Furthermore, differential levels of MMP-2 have been observed in CHF myocardium of ischemic or nonischemic origin. These observations suggest that selective induction of MMP species occurs within the failing human myocardium. The molecular basis for a selective portfolio of MMPs to be increased within the failing human myocardium is likely due to the type, degree, and duration of the specific extracellular stimuli that are present.

**Loss of Endogenous MMP Inhibitory Control in Human Failing Myocardium**

A loss of TIMP-mediated inhibitory control has been reported in post-MI remodeling and in dilated cardiomyopathy. In the rat MI model, MMP mRNA levels increased early after MI, but this increase was not associated with a concomitant increase in TIMP mRNA levels. In an in vitro system of ischemia and reperfusion, TIMP-1 expression was reduced in the early reperfusion period. These findings suggest that increased MMP expression and activation coupled with a loss of endogenous MMP inhibitory control occur early in the post-MI period and contribute to post-MI remodeling. In patients with dilated cardiomyopathy, a reduction in relative myocardial TIMP levels and/or alterations in MMP/TIMP binding have been reported. Li et al provided evidence to suggest that changes in the MMP/TIMP stoichiometric ratio occurred with end-stage cardiomyopathic disease. Specifically, TIMP-1 and TIMP-3 levels were reduced.
in the cardiomyopathy samples, whereas TIMP-2 levels were unchanged compared with levels in nonfailing samples. Additionally, in both ischemic and nonischemic cardiomyopathy, an absolute reduction in MMP-1/TIMP-1 complex formation has been observed. Taken together, these results suggest that an imbalance occurs between the induction of MMPs and TIMPs within the failing myocardium that would favor persistent MMP activity, ECM proteolysis, and continued myocardial remodeling.

### Potential Molecular Mechanisms for Discordant Myocardial MMP/TIMP Levels

There are a number of signaling pathways that likely contribute to increased myocardial MMP expression and discordant TIMP levels. The transmembrane protein EMMPRIN has been shown to induce the production of several MMPs in vitro and has been localized to human LV myocytes (Figure 3). It has been established that EMMPRIN exists in the normal human LV myocardium and is significantly increased in DCM. Representative levels of EMMPRIN within the nonfailing and cardiomyopathic myocardium are shown in Figure 3. Interestingly, in vitro studies have demonstrated that although EMMPRIN induced MMP expression, it did not influence the basal expression of TIMP-1. A similar pattern of expression has been observed with cardiomyopathy in which increased EMMPRIN levels were associated with increased levels of certain MMP species but in which TIMP-1 levels remained unchanged or actually

**Figure 3.** Top left, Normal isolated human LV myocytes were isolated by using previously described techniques; they were subjected to immunofluorescent staining for α-actinin, MT1-MMP, or EMMPRIN. The isolated myocytes used for immunostaining demonstrated a normal rod-shaped morphology, and sarcomeric banding could be readily appreciated. A strong immunofluorescent signal was detected in myocytes for both MT1-MMP and EMMPRIN. Bottom left, Immunoblotting for MT1-MMP and EMMPRIN was performed in sarcolemmal preparations from normal human myocardium and from cardiomyopathic human myocardium. A significant increase in MT1-MMP and EMMPRIN was present in cardiomyopathic samples. (Quantitative results from these studies are presented in Spinaile FG, Coker ML, Heung LJ, Bond BR, Gunasinghe HR, Etoh T, Goldberg AT, Zeiner J, Crumbley AJ. A matrix metalloproteinase induction/activation system exists in the human left ventricular myocardium and is upregulated in heart failure. Circulation. 2000;102:1944–1949, by permission of the American Heart Association ©2000). 

**Figure 3.** Bottom left, Immunoblotting for MT1-MMP and EMMPRIN was performed in sarcolemmal preparations from normal human myocardium and from cardiomyopathic human myocardium. A significant increase in MT1-MMP and EMMPRIN was present in cardiomyopathic samples. (Quantitative results from these studies are presented in Spinaile FG, Coker ML, Heung LJ, Bond BR, Gunasinghe HR, Etoh T, Goldberg AT, Zeiner J, Crumbley AJ. A matrix metalloproteinase induction/activation system exists in the human left ventricular myocardium and is upregulated in heart failure. Circulation. 2000;102:1944–1949, by permission of the American Heart Association ©2000). Right, Schematic diagram of the components of the MMP induction/activation system identified within the human myocardium. The membrane-bound extracellular MMP inducer protein (EMMPRIN) was localized to the LV myocyte and has been demonstrated previously to cause upregulation of several MMP species. The MMPs are synthesized and released in a zymogen or proMMP form. The proMMPs are activated within the extracellular space by a number of proteolytic pathways. However, MT-MMPs, such as MT1-MMP, have been demonstrated to activate MMPs, resulting in very localized sites of MMP activation. The upregulation of this local MMP induction/activation system may yield important pathways that contribute to the LV remodeling process in cardiomyopathic disease states.
decreased. Thus, increased EMMPRIN expression may induce certain MMP species without a concomitant increase in TIMPs, which would result in an MMP/TIMP stoichiometry favoring ECM proteolysis. Physical stimuli, such as stress and strain, also likely induce MMP expression within the failing myocardium. In cardiomyopathic patients, chronic unloading of the LV through the use of ventricular assist devices was associated with a reduction in LV chamber dilation and myocardial MMP levels. These clinical observations suggest that the persistently elevated LV myocardial wall stress, which is a common feature of cardiomyopathy, can augment MMP levels. The specific pathways that transduce physical stimuli into intracellular cues for MMP expression remain to be identified, but transmembrane systems, such as the integrins, are likely to play a role. Future studies that define the interrelationship between integrin signaling and MMP expression and activation are likely to yield critical information on how physical stimuli are translated into the maladaptive remodeling process in CHF.

Known promoter-binding sequences that appear to be variable among MMP species exist in MMP genes. The elaboration of cytokines, such as TNF-α, can occur in the setting of myocardial ischemia and heart failure and, in turn, may induce MMP expression. For example, exposure of myocardial fibroblasts to TNF-α causes the release of MMP-9 and MMP-13. It is very likely that the basis for the elaboration of these MMP species by TNF-α involves the formation of transcription factors and binding to the AP-1 and nuclear factor-κB promoter regions. More recently, it was demonstrated that in mice with cardiac-restricted overexpression of TNF-α, discordant levels of MMPs and TIMPs occurred. Specifically, during the progression of LV remodeling in the transgenic mice, increased MMP zymographic activity occurred in the absence of a concomitant increase in relative TIMP-1 levels. In addition to TNF-α, other cytokines, such as interleukin-1β, can cause the induction of certain MMP species. Activation of the neurohormonal system, such as the release of catecholamines and angiotensin II, has been demonstrated to induce myocardial MMP release under certain conditions. Neurohormones, such as norepinephrine, endothelin, and angiotensin II, can increase the synthesis of certain MMP species in isolated LV myocytes. Thus, it is likely that a local and concentration-dependent release of bioactive molecules contributes to the imbalance between myocardial MMP and TIMP levels within the remodeling myocardium, although this remains speculative.

**Future Directions**

Orally active nonselective MMP inhibitors, also termed broad-spectrum MMP inhibitors, have been developed and have shown to achieve MMP inhibition at the tissue level. Therefore, modulating MMP activity represents a potential therapeutic target in the context of LV remodeling and CHF. However, there are several problematic issues regarding the use of broad-spectrum MMP inhibitors in the clinical setting of developing CHF. First, long-term inhibition of all MMP species will likely interfere with normal tissue remodeling and extracellular protein turnover. Second, initial clinical experience with broad-spectrum MMP inhibitors in patients with tumor metastases has been associated with undesirable systemic effects. Selective targeting of MMP species that contribute to pathological myocardial remodeling in developing CHF will likely hold greater therapeutic potential. Thus, an important future direction would be to define the specific portfolio of MMPs that are expressed within the failing myocardium and to develop selective targeting strategies to inhibit these MMP species. However, it must be recognized that one important cause of CHF is diastolic dysfunction and that a contributory factor may be an imbalance between myocardial collagen synthesis and degradation. In this context, identifying the systems and pathways that regulate myocardial TIMP levels would provide mechanistic insight as well as potential clues for developing therapeutic strategies to reduce the degree of myocardial fibrosis that can occur with severe LV hypertrophy. Finally, recent studies performed primarily in mice have demonstrated that early disruption of MMP expression and/or activation in an evolving MI may be deleterious to the normal wound-healing process. Specifically, early induction and activation of MMPs may be essential for the wound-healing response, and exogenous MMP pharmacological inhibition may actually worsen myocardial viability in the acute MI period. However, persistently increased myocardial MMP activation after an established MI may contribute to the maladaptive process of infarct expansion. Future studies that identify the temporal profile of MMP species that are expressed within the myocardium during and after an MI and the functional role of these individual MMPs with respect to the remodeling process are necessary. This fundamental temporal information will be necessary for the development of appropriate pharmacological strategies for targeting the portfolio of MMPs responsible for post-MI remodeling. Thus, interventional strategies targeted at modulating the myocardial ECM must be time and disease specific.

Another important area of future research is the development of methods by which to monitor relative MMP levels and activity in patients with CHF. Past studies have documented changes in MMP and TIMP plasma levels after MI and during the development of LV hypertrophy. One possible future approach would be to relate changes in markers of collagen degradation and MMP levels to provide insight into the relative progression of the underlying myocardial remodeling process in CHF patients. However, it must be recognized that the release and activity of MMPs involve a highly compartmentalized process and that plasma MMP levels likely reflect spillover from the interstitial compartment. One future approach will be to construct markers of relative MMP activity that can be detected at the tissue level. Another approach is to use microdialysis techniques to directly interrogate the myocardial interstitial compartment with respect to MMP activity. These improved analytical systems will likely yield important new insights into the regulation and activity of the myocardial MMP system with respect to LV remodeling and the progression of the CHF process.

In conclusion, the myocardial ECM is not a passive entity but is a complex and dynamic microenvironment that represents an important structural and signaling system within the...
myocardium. Improved understanding of the MMP system and how this system is regulated or, arguably, dysregulated in the LV remodeling process will likely provide new insights and strategies for CHF.

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