A Matrix Metalloproteinase Induction/Activation System Exists in the Human Left Ventricular Myocardium and Is Upregulated in Heart Failure

Francis G. Spinale, MD, PhD; Mytsi L. Coker, BA; Lena J. Heung, BS; Brian R. Bond, PhD; Himali R. Gunasinghe, BS; Takuma Etoh, MD, PhD; Aron T. Goldberg, MD; James L. Zellner, MD; A. Jackson Crumbley, MD

**Background**—Matrix metalloproteinases (MMPs) contribute to matrix remodeling in disease states such as tumor metastases. Extracellular matrix metalloproteinase inducer (EMMPRIN) has been reported to increase MMP expression, and membrane-type MMP or MT1-MMP has been implicated to activate MMPs. The present study examined whether and to what degree EMMPRIN and MT1-MMP were expressed in human left ventricular (LV) myocardium as well as the association with MMP activity and expression in dilated cardiomyopathy (DCM).

**Methods and Results**—LV myocardial zymographic MMP activity increased by 2-fold with both nonischemic DCM (n=21) and ischemic DCM (n=16) compared with normal (n=13). LV myocardial abundance of MMP-9 was increased with both forms of DCM. MMP-2 and MMP-3 were increased with nonischemic DCM. MMP-1 levels were decreased with both forms of DCM. EMMPRIN increased by 250% and MT1-MMP increased by 1000% with both forms of DCM.

**Conclusions**—Increased LV myocardial MMP activity and selective upregulation of MMPs with nonischemic and ischemic forms of DCM occurred. Moreover, a local MMP induction/activation system was identified in isolated normal human LV myocytes that was upregulated with DCM. The control of MMP activation and expression in the failing human LV myocardium represents a new and potentially significant therapeutic target for this disease process. (Circulation. 2000;102:1944-1949.)

**Key Words:** myocardium • remodeling • metalloproteinases • cardiomyopathy

Left ventricular (LV) remodeling is an important contributory event in the progression to end-stage congestive heart failure (CHF). However, the underlying cellular and molecular basis for LV myocardial remodeling in patients with severe CHF remains poorly understood. As in most tissue remodeling processes, LV myocardial remodeling with CHF is accompanied by changes in the structure and composition of the collagen matrix.1–4 Matrix metalloproteinases (MMPs) are an endogenous family of zinc-dependent enzymes that have been identified to be responsible for matrix remodeling in several disease states.

**Methods**

**Patients**

Human LV myocardium was obtained from explanted hearts of patients (age 35 to 64 years) undergoing total orthotopic heart transplantation secondary to nonischemic (idiopathic; n=21) or ischemic (n=16) DCM. Sections of the LV free wall were snap-frozen in liquid nitrogen and stored at −70°C until use. Nonischemic DCM was identified by preoperative presentation and ischemic DCM confirmed by coronary catheterization studies. Patients with confirmed myocarditis were excluded from this study. In the patients with DCM, medications included digoxin (95%), diuretics (98%), ACE inhibitors (78%), β-adrenergic antagonists (9%), and calcium channel blockers (4%). The treatment penetration was equivalent in both forms of DCM (χ²=2.97, P=0.226). Normal LV myocardial samples (n=13; age 16 to 22 years) were obtained from donor hearts not matched for transplantation or used for valve harvest (Cryolife, Inc). For the isolated myocyte studies, myocardial biopsies were obtained from 6 patients with normal LV ejection fraction (>60%) undergoing elective coronary artery bypass surgery as described previously.5 Patient consent was obtained for all myocardial samples used in the study, and protocols were approved by the Medical

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From the Medical University of South Carolina, Charleston.

Correspondence to Francis G. Spinale, MD, PhD, Cardiothoracic Surgery, Room 625, Strom Thurmond Research Bldg, 770 MUSC Complex, Medical University of South Carolina, 114 Doughty St, Charleston, SC 29425.

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Proteases such as trypsin or plasmin. In the present study, trypsin was used to activate enzyme moieties and/or facilitate the removal of endogenous inhibitors. To express the zymographic results in terms of MMP activity, the standard curve was used to express LV myocardial MMP zymographic activity in picograms per hour.

Myocardial Zymography and MMP/TIMP Measurements

LV myocardial samples were homogenized and concentrated to prevent MMP proteolytic activation and degradation as described previously. Basal LV gelatinase activity was examined by substrate specific gelatin zymography because detergent exposure, for example, SDS, facilitates MMP enzymatic activation by unfolding. Total recruitable LV gelatinase activity was assessed similarly after preactivation of LV myocardial extracts with trypsin. It has been demonstrated previously that the stepwise activation of MMPs can be elicited by serine proteases such as trypsin or plasmin.

Myocardial Immunoblotting

Past studies identified a cell-surface protein in specific tumor cell lines that caused the induction of MMP expression termed extracellular matrix metalloproteinase inducer (EMMPRIN). Membrane-type MMP (MT1-MMP) is a recently discovered MMP that has the capacity to activate other MMPs, including collagenase-3 (MMP-13). Accordingly, relative EMMPRIN, MT1-MMP, and MMP-13 levels were measured in normal and DCM myocardial samples by immunoblotting. A positive control was included in all immunoblots (MMP-13: CC068, MT1-MMP: CC1042, Chemicon; EMMPRIN: Human Breast Cancer Preparation).

Myocyte Isolation and Immunohistochemistry

The LV myocardial biopsy was placed in a collagenase solution within a microtiration system developed by this laboratory. LV myocytes were washed, incubated with primary antisera for MT1-MMP (1:300) or EMMPRIN (1:20) overnight at 4°C, washed, and incubated with conjugated goat-FITC antisera (1:60; 1 hour, 25°C). LV myocytes were double-stained with α-actinin (1:100) and nuclei identified by propidium iodide with confocal microscopy as described previously. Colocalization of the cytoskeletal-sarcomeric-associated protein α-actinin, MT1-MMP, and EMMPRIN were performed by means of confocal microscopy (Olympus Fluoview BX50WI).

Data Analysis

All quantitative data with respect to MMP activity, MMP/TIMP abundance, and EMMPRIN abundance are shown in the Table.

<table>
<thead>
<tr>
<th>Human LV Myocardial Zymographic Activity, MMP Abundance, and EMMPRIN Abundance</th>
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*P<0.05 vs normal. †P<0.05 vs DCM. ‡Computed as percentage of normal.

Results

All quantitative data with respect to MMP activity, MMP/TIMP abundance, and EMMPRIN abundance are shown in the Table.

MMP Zymographic Activity in Normal and DCM Human Myocardium

LV myocardial MMP zymographic activity was detected in all human LV myocardial samples at 50 to 90 kDa, consistent with the molecular weights of several MMP species including MMP-2 and MMP-9 (Figure 2). Moreover, MMP zymographic activity was increased in both forms of DCM. LV myocardial MMP zymographic activity after trypsin activation increased by >3-fold from basal values (Figure 2). Total activity was linear with increasing MMP-2/MMP-9 concentrations [y=0.133×10⁻³(x)-0.059; r=0.99, P<0.05, SEE=0.0179]. This standard curve was used to express LV myocardial MMP zymographic activity in picograms per hour.

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recruitable MMP zymographic activity was higher in both nonischemic and ischemic DCM. There was no relation between increased MMP activity in DCM patients when stratified with respect to pharmacological treatment (P = 0.9634).

MMP Abundance in Normal and DCM Human Myocardium

MMP-9 was increased in both ischemic and nonischemic DCM. MMP-2 was increased 2-fold in nonischemic DCM but was unchanged in ischemic DCM. MMP-1 was reduced in both nonischemic and ischemic DCM. A robust immunoreactive signal was observed for collagenase 3 (MMP-13) in normal human myocardium (Figure 3) and was increased with both forms of DCM. Stromelysin (MMP-3) was increased in nonischemic DCM but was unchanged with ischemic DCM.

MMP Inhibition in Normal and DCM Human Myocardium

A mechanism for controlling MMP activity in normal tissue is through a family of closely related inhibitors known as tissue inhibitors of MMPs, or TIMPs. One of the more ubiquitous TIMPs, TIMP-1, has been demonstrated in LV myocardium previously. With both nonischemic and ischemic DCM, TIMP-1 abundance was similar to normal values. However, actual MMP-1/TIMP-1 complex abundance was reduced with nonischemic DCM and was further reduced with ischemic DCM.

Local MMP Induction and Activation in Normal and DCM Myocardium

EMMPRIN levels, as measured by immunoblotting, were increased in both nonischemic and ischemic DCM (Figure 3). Relative EMMPRIN levels were increased in ischemic DCM when compared with nonischemic DCM. MT1-MMP, as measured by immunoblotting (Figure 3), was localized at ~50 kDa. MT1-MMP was substantially increased in ischemic and nonischemic DCM.

In the first series of studies, purified LV myocyte sarcolemmal preparations were prepared and immunoblotting was performed (Figure 4). A robust signal was obtained in LV sarcolemmal preparations for both MT1-MMP and EMMPRIN in both forms of DCM. MT1-MMP and EMMPRIN were localized by immunofluorescence to human LV myocytes by confocal microscopy (Figure 5). Colocalization of both MT1-MMP and EMMPRIN to the LV myocyte sarcolemma was observed. A punctate and well-defined colocalization with α-actinin was also observed with MT1-MMP.

Discussion

Although the activation of MMPs has been identified to occur in a number of disease states, whether and to what degree of activation of this local proteolytic system occurs in failing human myocardium as the result of DCM remain unknown. There were 3 significant findings of the present study regarding myocardial MMP activity and expression in severe end-stage human DCM. First, MMP zymographic activity was increased in DCM and was accompanied by increased levels of certain MMP species, including the emergence of unique MMPs without a compensatory increase in the levels of the endogenous inhibitors of MMPs (TIMPs). Second, a local induction/activation system was localized to the LV myocyte sarcolemma that consisted of EMMPRIN and MT-MMP. Third, this local inducer/activation system was significantly upregulated in DCM. These findings provide new mechanistic insight into the cellular and molecular basis for...
fibrillar collagens such as collagen type I and III. Thus, the MMP-1 and MMP-13 within the myocardium include the report to demonstrate the emergence of this MMP species in microscopy was performed to localize muscle cells. Moreover, neutrophils have also been reported to MMP-9 is synthesized by myocytes, fibroblasts, and smooth laminin and fibronectin. Past studies have demonstrated that MMPs posses the capacity to degrade a number of interstitial proteins including basement membrane components such as laminin and fibronectin. Past studies have demonstrated that MMP-9 occurred in both nonischemic and ischemic DCM, because of a high affinity for this substrate. However, these MMPs possess the capacity to degrade a number of interstitial proteins including basement membrane components such as laminin and fibronectin. Past studies have demonstrated that MMP-9 is synthesized by myocytes, fibroblasts, and smooth muscle cells. Moreover, neutrophils have also been reported to be a potential source of MMP-9. Increased myocardial levels of MMP-9 occurred in both nonischemic and ischemic DCM, but MMP-2 was increased only in nonischemic DCM. The MMPs contain certain regulatory DNA sequences within the promoter region that bind transcription factors that are influenced by a number of intracellular signaling pathways. The development of DCM is accompanied by increased neurohormonal system activity such as increased levels of norepinephrine and tumor necrosis factor-α. These bioactive molecules can induce formation of transcription factors, which will bind to the MMP promoter region. MMP-9 and MMP-2 contain dissimilar promoter sequences and regulatory elements. Thus, the different levels of MMP-2 with nonischemic and ischemic forms of DCM probably are due to the differences in the underlying cause of the disease process, which in turn may result in the stimulation of different intracellular signaling pathways and selective activation of MMP-2. Although further studies regarding the regulatory mechanisms of MMP expression in the myocardium are warranted, results from the present study demonstrated that MMPs are differentially regulated in end-stage DCM.

MMP-1 induction may depend on cooperative binding of the PEA-3 element and the formation of the transcription factor AP-1, whereas stromelysin, or MMP-3 transcription, can occur independent of the AP-1 response. Thus, increased extracellular stimuli such as neurohormones and cytokines, which occur with DCM, may induce differential levels of MMP-1 and MMP-3 expression. In the present study, MMP-1 levels were reduced in both forms of DCM, whereas MMP-3 levels were increased in nonischemic DCM. MMP activation can be achieved through a final common enzymatic pathway requiring MMP-3. Moreover, MMP-3 can participate in the activation cascade required to achieve activation of MMP species relevant to the LV remodeling process. Thus, the increased MMP-3 expression that occurred in nonischemic DCM may be an important contributory mechanism for the LV remodeling in this form of heart failure.

TIMPs bind to the active site of the MMPs by blocking access to extracellular matrix substrates. The MMP/TIMP complex is formed in a stoichiometric 1:1 molar ratio and forms an important endogenous system for regulating MMP activity in vivo. Although up to 4 TIMPS have been identified to date, the most well characterized of these endogenous inhibitors of MMPs are TIMP-1 and TIMP-2. TIMP-1 and TIMP-2 form a complex with several MMPs, which include MMP-1 and MMP-9. In the present study, there was no change in absolute TIMP-1 levels with DCM, but a reduction in MMP-1/TIMP-1 complex formation occurred. One contributory factor for the reduction in the levels of this specific MMP/TIMP complex was the absolute reduction in MMP-1 levels with DCM. TIMP-1 binds to other MMPs such as proMMP-9, which slows the activation process of this MMP species. In both forms of DCM, MMP-9 levels were increased without a concomitant increase in TIMP-1 levels. Thus, the ratio of MMP-9 to TIMP-1 was reduced, which in turn may have contributed to the increased MMP zymographic activity and the reduction in MMP-1/TIMP-1 complex formation observed with DCM. Although the role of other myocardial TIMPs remains to be defined, the focus of this study as well as past reports was MMP induction and activation.

MMPs are secreted in a proenzyme form and require proteolytic cleavage for activation, most notably by serine

![Figure 5. Normal isolated human LV myocytes were subjected to immunofluorescent staining with MT1-MMP or EMMPRIN and/or sarcomeric protein α-actinin. Isolated myocytes used for immunostaining demonstrated normal rod-shaped morphology; sarcomeric banding could be readily appreciated. Strong immunofluorescent signal was detected in myocytes for MT1-MMP, EMMPRIN, and α-actinin. Left. Immunostaining and confocal microscopy was performed to localize α-actinin (red) and MT1-MMP (green) as well as colocalization of these proteins. Nuclei were identified by DNA staining. MT1-MMP localized to sarcolemna and was associated with sarcomeric banding pattern. Right. More diffuse pattern for EMMPRIN (green) was observed in LV myocytes; however, more robust staining was observed along sarcolemna (lower power images: ×600, bar=3 μm; higher power images: ×1000, bar=1 μm). Substitution with nonimmune antisera abolished this fluorescent staining pattern.](http://circ.ahajournals.org/lookup/fig/10.1161/CIRCULATIONAHA.107.712209)
MMP Induction and Activation

Although serine proteases such as plasmin and trypsin contribute to MMP activation through proteolytic cleavage of the C-terminal propeptide domain, recent studies have demonstrated that the membrane-bound MMPs, MT-MMPs, can induce local MMP activation. MT1-MMP has been clearly shown to induce local MMP activation in an isolated LV myocyte. Given that the present study has identified both EMMPRIN and MT1-MMP in LV myocytes, and this laboratory routinely performs isolated LV myocyte studies, future directions will entail a more careful examination of this local induction and activation system in an isolated LV myocyte system.

proteases. The present study demonstrated heightened MMP activity in DCM by zymography that increased after pretreatment with the serine protease trypsin. Endogenous myocardial MMP activation by serine proteases could involve chymase, which has been demonstrated to be increased during both pressure- and volume-overload states. There are problematic issues that surround in vitro zymographic measurements that prevent direct extrapolation to in vivo LV myocardial MMP activity. First, this assay does not provide a measure of total MMP content and species identification. Second, the zymographic assays were performed under optimal enzymatic conditions and substrate availability and in the absence of the influence of TIMPs. These issues were addressed in part by the present study through the direct measurement of MMP and TIMP abundance and MMP/TIMP complex formation. Another limitation of the present study is that MMP zymographic activity was measured in end-stage DCM. Whether increased MMP activity exists in milder forms of heart failure remains to be established. Finally, although the entire complement of LV myocardial cells, including fibroblasts, smooth muscle cells, endothelial cells, and the LV myocyte itself have been demonstrated to produce MMPs, the focus of the present study was first, to examine global MMP expression and activity in whole myocardial tissue, and second, to determine the existence of a local cellular MMP induction and activation system in LV myocardium. Given that the present study has identified both EMMPRIN and MT1-MMP in LV myocytes, and this laboratory routinely performs isolated LV myocyte studies, future directions will entail a more careful examination of this local induction and activation system in an isolated LV myocyte system.

A large portfolio of extracellular stimuli can alter the expression of MMPs in various cell systems. In severe end-stage congestive heart failure such as DCM, it is likely that a number of neurohormonal and cytokine signaling cascades contribute to alterations in myocardial MMP expression and activity. However, it remained unclear whether a local MMP induction system existed at the level of the LV myocyte and whether this system may be upregulated in DCM. EMMPRIN is a 58-kDa, membrane-bound protein that has been identified in both normal (keratinocytes) and diseased human tissue (breast and lung carcinoma). Exposure of human fibroblasts to recombinant EMMPRIN caused an induction of MMP-1, MMP-2, and MMP-3. Whether and to what degree other MMPs are induced by EMMPRIN remain to be defined. Basal expression of EMMPRIN has been reported in a number of tissue types, suggesting multiple roles for this transmembrane protein. The present study clearly defined increased abundance of EMMPRIN in failing human myocardium. This provides circumstantial evidence that EMMPRIN may facilitate MMP expression in DCM myocardium. Identification of extracellular binding domains that interrupt EMMPRIN function would be an important avenue of future study that may provide a definitive approach for detecting the role of EMMPRIN in myocardial remodeling. In the present study, EMMPRIN was identified in normal human LV myocardium. With confocal microscopy, a definitive staining pattern for EMMPRIN was localized to the LV myocyte sarcolemma. Using multicellular culture systems, it has been postulated that EMMPRIN induces MMP expression by a cell-cell interaction or a paracrine-mediated effect. Increased myocardial EMMPRIN levels were observed in both forms of DCM and could be localized to the LV myocyte sarcolemma. Thus, increased sarcomemal levels of EMMPRIN in DCM may enhance MMP induction through this cell-cell interaction.

Through immunoprecipitation experiments, Berditchevski and colleagues demonstrated that EMMPRIN forms a complex with αβ1 integrin. Functions of integrins include cell-cell adhesion, extracellular matrix-cell adhesion, and transduction of cellular signaling cascades. The coexistence of EMMPRIN and αβ1 integrin suggests that EMMPRIN-mediated MMP induction may be influenced by both the composition of and stress placed on the extracellular matrix. The intracellular signaling pathways by which EMMPRIN facilitates MMP expression remain to be fully elucidated but probably involve tyrosine kinase pathways. The EMMPRIN protein sequence contains a PKC phosphorylation site, which may also be an important intracellular regulatory mechanism (Biology Workbench NCSA, Prosite Database, Board of Trustees, University of Illinois, 1997). Interestingly, in vitro studies have demonstrated that although EMMPRIN induced MMP expression, it did not influence basal expression of TIMP-1. In the present study, a similar pattern of expression...
was observed with DCM in which increased EMMPRIN levels were associated with increased levels of certain MMP species, but TIMP-1 levels remained unchanged. Thus, while remaining speculative, increased EMMPRIN expression in developing DCM may contribute to increased MMP levels in myocardial cells without a concomitant increase in TIMP levels. Increased EMMPRIN and MT1-MMP were increased in LV myocardium of patients with DCM. Upregulation of this local MMP induction/activation system may contribute to LV remodeling process in cardiomyopathic disease states.

Although the causes of CHF are diverse, a common event in the progression of this disease process is LV remodeling, resulting in increased wall stress and subsequent pump dysfunction. An important cause of CHF is DCM, in which significant LV remodeling and changes in chamber geometry are an important prognostic indicator of disease progression. In the present study, patients with DCM were treated with conventional medical therapy (ie, ACE inhibition, diuretics, and -adrenergic antagonists). Such therapeutic approaches for CHF have been focused on modifying LV load or paracrine-mediated effect may influence EMMPRIN-mediated MMP expression. Past studies have identified that isolated LV myocytes synthesize a number of MMP species that are released into extracellular space. MT-MMPs such as MT1–MMP have been demonstrated to activate other MMPs and to degrade extracellular matrix components. MT1–MMP was localized to LV myocyte and thereby provides localized site for MMP activation and matrix degradation. EMMPRIN and MT1–MMP were increased in LV myocardium of patients with DCM. Upregulation of this local MMP induction/activation system may contribute to LV remodeling process in cardiomyopathic disease states.

The present study identified that a local MMP induction and activation pathway exists in the normal human LV myocardium. Membrane-bound extracellular MMP inducer protein (EMMPRIN) was localized to LV myocyte; it has been demonstrated previously that intracellular contact and/or paracrine-mediated effect may influence EMMPRIN-mediated MMP expression. Past studies have identified that isolated LV myocytes synthesize a number of MMP species that are released into extracellular space. MT-MMPs such as MT1–MMP have been demonstrated to activate other MMPs and to degrade extracellular matrix components. MT1–MMP was localized to LV myocyte and thereby provides localized site for MMP activation and matrix degradation. EMMPRIN and MT1–MMP were increased in LV myocardium of patients with DCM. Upregulation of this local MMP induction/activation system may contribute to LV remodeling process in cardiomyopathic disease states.

The concept that direct modulation of the LV myocardial remodeling process through the control of MMP expression and activity would be an important therapeutic strategy in the setting of developing CHF. The present study identified that a local MMP induction and activation pathway exists in the normal human LV myocardium that is upregulated with DCM. Moreover, localization of this system to the LV myocyte suggests that a paracrine loop exists with respect to MMP induction and activation (Figure 6). The local myocardial MMP induction/activation system identified in the present study may represent an important system responsible for LV remodeling in the heart failure process.

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**References**

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