The process of left ventricular (LV) remodeling has been shown to be an important predictor of morbidity and mortality in patients with heart failure. Therefore, identifying the cascade of molecular and cellular events that contribute to LV remodeling is likely to provide new and novel targets for preventing disease progression in heart failure. In this issue of Circulation, Li and colleagues\(^1\) report that changes in the relative abundance of tissue inhibitors of the metalloproteinases (TIMPs) occur with the development of end-stage human heart failure, thus raising the important possibility that alterations in the extracellular matrix of the failing heart may contribute to disease progression in heart failure. The purpose of this editorial was to place the findings of the study by Li et al, as well as those of recently published reports, in perspective with what we know about myocardial failure. The purpose of this editorial was to place the findings of the study by Li et al, as well as those of recently published reports, in perspective with what we know about myocardial failure.

Myocardial Extracellular Matrix Remodeling
The extracellular matrix of the heart includes a fibrillar collagen network, a basement membrane, and proteoglycans. The myocardial fibrillar collagens (such as collagen types I and III) ensure structural integrity of adjoining myocytes, provide the means by which myocyte shortening is translated into overall LV pump function, and have been postulated to be essential for maintaining alignment of myofibrils within the myocyte through a collagen–integrin–cytoskeletal-myofibril relation. The collagen matrix in the myocardium may be conceptualized as scaffolding in which collapse of the individual components of the scaffold may not necessarily result in an absolute loss of collagen content but rather in a loss of structural integrity. Recent scanning electron micrographs by Rossi and colleagues\(^2\) served as an elegant reminder that the collagen matrix in the myocardium is a 3-dimensional structure that supports individual myocytes. Small cuts or breaks in the overall structure of the collagen matrix, as in a scaffold, will result in a loss of continuity and therefore of normal function. In both human and animal studies, changes in LV geometry and function have been associated with changes in the fibrillar collagen network.\(^3-5\)

Thus, untoward changes in myocardial collagen alignment, structure, and support may be a fundamental structural mechanism that contributes to the progressive LV dilation and remodeling that occurs during the progression of CHF. Increased fibrillar collagen accumulation, or fibrosis, has been recognized to occur in a number of cardiac disease states. However, it is important to recognize that this so-called “replacement fibrosis” is most likely an end-stage process that occurs during or after destruction of the fine fibrillar collagen weave that normally surrounds myocytes. Thus, the myocardial collagen remodeling that occurs in progressive LV failure is due to abnormalities not only in collagen synthesis but also in degradation. Accordingly, the myocardial extracellular matrix can be considered to be in a dynamic equilibrium dependent on families of molecules that favor matrix degradation as well as families of molecules that tend to inhibit matrix degradation. Indeed, recent clinical and basic studies of heart failure have clearly demonstrated that a number of extracellular degradative enzymes, collectively called the matrix metalloproteinases (MMPs), exist within the failing myocardium.\(^1,4-7\)

Matrix Metalloproteinases
The MMPs have been suggested to play an important role in tissue remodeling in both normal and pathological conditions.\(^4-10\) For example, changes in MMP activity and expres-
sion have been observed in organ morphogenesis, menstruation and pregnancy, wound healing and inflammation, and tumor metastasis. The MMPs are secreted by a number of cell types, including fibroblasts, smooth muscle cells, and endothelial cells. More recently, a preliminary study demonstrated that adult mammalian myocytes synthesize and release MMPs.11 The general classification of these MMPs is based on substrate specificity, but several of the MMPs can degrade a number of different matrix components. As of this writing, \( \sim 20 \) MMPs have been identified and characterized.10,12 The classes of MMPs that may have particular relevance to myocardial remodeling are the collagenases, which include MMP-1; the stromelysins, which include MMP-3; the gelatinases, which include MMP-9 and MMP-2; and the membrane-type MMPs. After synthesis, the MMPs are secreted into the extracellular space as a proenzyme, or zymogen. The general structure of the MMPs is centered around a zinc-containing catalytic domain contained within an upstream propeptide region and a C-terminal collagen-binding domain. The propeptide region undergoes proteolytic cleavage to yield the active MMP. The C-terminal region is structurally distinct for each MMP species; it provides the capacity to bind to extracellular substrates and therefore imparts specificity. The activity of MMPs is strictly regulated at 3 levels: transcription, activation, and inhibition/deactivation.

Changes in MMP mRNA levels can be influenced by a wide variety of chemical agents, neurohormones, and cytokines, as well as by changes in cytoskeletal architecture and basement membrane adhesion.5-13 It is likely that protein kinase C (PKC) is involved in the intracellular induction of MMP transcription, because the exposure of several different cell systems to phorbol esters, which increase PKC, causes increased MMP mRNA expression.10,12 Thus, increased levels of catecholamines, angiotensin II, and endothelin, which in turn can cause a receptor-mediated increase in PKC within a number of cell types in the myocardium, may cause increased levels of several species of myocardial MMPs in the failing heart. Biologically active peptides and cytokines, such as tumor necrosis factor-\( \alpha \) (TNF-\( \alpha \)) and interleukin-1, have been demonstrated to increase MMP transcription in several cell systems.13 Given that increased levels of such cytokines as TNF-\( \alpha \) have been identified in patients with LV failure, it is possible that proinflammatory cytokines contribute to LV remodeling through upregulation of specific myocardial MMPs. The promoter regions of the MMP-1 and the MMP-3 genes have some common regulatory DNA sequences, but there are several additional upstream response elements in the MMP-3 gene.8,13 Thus, increased extracellular stimuli, such as neurohormones and cytokines, may induce differential levels of MMP-1 and MMP-3 expression. The gelatinases MMP-9 and MMP-2 also contain dissimilar promoter sequences and regulatory elements.8,13 This laboratory reported previously that MMP-3 and MMP-9 levels were increased in dilated cardiomyopathy, whereas MMP-1 levels were decreased and MMP-2 levels unchanged, suggesting that there is differential regulation of myocardial MMP expression in the failing human heart.7

Because MMPs are secreted primarily in an inactive or pro-MMP form, MMP activation occurs after secretion into the extracellular space. Thus, an important control point for MMP activity is proteolytic processing of the pro-MMPs. The propeptide of the MMP contains a cysteine switch sequence that enfolds the zinc atom of the catalytic site.12 MMP activation requires dissociation of the zinc-cysteine interaction through proteolytic cleavage of the propeptide sequence or changes in the cysteine switch conformation by chemical perturbations. A large number of proteases and organonemecurials have been shown to activate the MMPs from thezymogen form in vitro.8,12 It has been demonstrated that serine proteases such as trypsin, plasmin, or urokinase generate an identical form of active MMP.12 Thus, a potentially important upstream mechanism for activation of latent MMPs is the urokinase/plasminogen cascade. One of the most frequently studied of the MMPs with respect tozymogen activation is the interstitial collagenase MMP-1. The first step in MMP-1 activation is proteolytic cleavage ahead of the cysteine residue, which results in a partially active intermediate form that is then quickly converted to the active form by autolytic means or through cleavage by the MMP stromelysin, or MMP-3.8,10,12 Thus, an important regulatory step in overall MMP activation involves the expression and activational state of MMP-3. Increased ECM levels and activity of MMP-3 have been reported to occur in both humans and animals with LV dilation and failure.6,7 Several studies have demonstrated that MMP zymographic activity is increased with end-stage cardiomyopathic disease in patients.1,4,7 In the study by Li et al,1 MMP proteolytic activity after preactivation with a phorbol ester and the serine protease trypsin was increased in cardiomyopathic samples based on in vitro zymography. This finding is consistent with past studies and suggests that a greater proportion of recruitable MMP exists within the failing myocardium. However, it must be recognized that the in vitro zymographic approach usually requires preactivation of the MMP preparations and electrophoretic separation of proteins and therefore provides only an index of potential MMP activational states that may exist in vivo.

A final and important control point of MMP activity is the inhibition of activated enzyme. There is an endogenous class of proteins called the tissue inhibitors of the matrix metalloproteinases, or TIMPs. At present, 4 TIMPs have been characterized with respect to being separate gene products and influence the activity of MMPs.8,14-17 The TIMPs are low-molecular-weight proteins (\( \sim 20 \) to 30 kDa) and can complex with high efficiency to activated MMPs. TIMPs bind to the active site of the MMPs by blocking access to the collagen substrate. The MMP/TIMP complex is a tight, noncovalent bond with an extremely high affinity for the MMPs.6,14-17 The TIMPs appear to bind MMPs in a stoichiometric 1:1 molar ratio and occupy the catalytic domain of the activated enzyme. Therefore, TIMPs form an important endogenous system for regulating actual MMP activity in vivo. In light of the potential importance of these inhibitory proteins to modulate MMP activity, the TIMP family of secreted proteins is a field of active interest in many areas of cardiovascular biology.9 The findings by Li et al1 provide evidence to suggest that changes in the stoichiometric
ratio of MMPs to TIMPs have occurred with end-stage cardiomyopathic disease. Specifically, TIMP-1 and TIMP-3 levels were reduced in cardiomyopathic samples, whereas TIMP-2 levels were unchanged compared with control myocardium. It has been demonstrated previously that TIMP-1 and TIMP-2 expression is differentially regulated by a number of external stimuli. For example, such cytokines as TNF-α can modify the expression of TIMP-1 through the induction of nuclear transcription factors, whereas TIMP-2 expression appears to be unaffected by cytokine stimulation. In a previous study, we reported increased MMP-9 with no change in MMP-2 levels, whereas Li et al reported a reduction in TIMP-1 abundance with no change in TIMP-2 levels in cardiomyopathic myocardium. Whether these reported changes in MMPs and TIMPs with end-stage cardiomyopathy reflect alterations in specific pro-MMP/TIMP complexes, MMP stability, and/or activation states remains to be established.

TIMP-3 is somewhat different from other TIMPs in that it is directly bound to components of the extracellular matrix, whereas it is believed that TIMP-1 and TIMP-2 are freely diffusible within the interstitial compartment. Thus, TIMP-3 potentially may modulate MMP activity in a more focal manner than other TIMPs. TIMP-3 transcription is influenced by external stimuli in a similar manner to that of TIMP-1. In consistent with this observation, the study by Li et al reported a reduction in TIMP-3 comparable to that of TIMP-1 in cardiomyopathic myocardium. The final TIMP currently characterized, TIMP-4, has been shown to have a unique expression pattern. Specifically, TIMP-4 mRNA has been detected at low levels in the kidney and colon but is absent in the lung, liver, and brain. Interestingly, the only organ observed to have high expression patterns for TIMP-4 was the myocardium. However, whether TIMP-4 possesses different MMP inhibitory activity within the myocardium remains unclear. Li et al reported that steady-state mRNA levels for TIMP-4, unlike the other TIMPs, were unchanged but protein levels were reduced with ischemic cardiomyopathy. TIMP mRNA half-life is ~60 hours, and whether changes in the stability of TIMP mRNA occur with changes in external stimuli remains to be established. Nevertheless, the observation of discordant mRNA and protein levels for TIMP-4 suggests that posttranscriptional/translational alterations may have occurred with ischemic cardiomyopathy. Because TIMP-4 appears to be expressed selectively in high abundance in the myocardium, future investigations into the function and regulation of this specific TIMP in cardiac disease states would be warranted.

**The Two Faces of TIMPs**

Although TIMPs are considered to be endogenous inhibitors of MMPs, the in vivo function of these proteins may not be straightforward. First, TIMPs may actually participate in the process of MMP activation. Specifically, it has been demonstrated that TIMP-2 forms a complex with species of membrane-type MMPs and that the formation of this complex enhanced the activation of pro-MMP-2. A second role of TIMPs that is independent of modulating MMP activation states is through effects on cell growth. TIMP-1 and TIMP-2 have been shown to stimulate a growth response in fibroblast cell cultures in a concentration-dependent manner. Thus, it is possible that changes in TIMP levels within the myocardium may have multiple biological effects that would be relevant to the cardiomyopathic disease process.

**Summary**

Although it is becoming increasingly clear that disease progression in heart failure is inextricably linked to the process of LV remodeling, the precise constellation of mechanisms that are responsible for the LV remodeling remains unknown. In the foregoing discussion, we have attempted to outline several lines of evidence suggesting that discrete changes in the activity of enzymatic systems responsible for extracellular matrix degradation within the myocardium contribute to the process of LV remodeling in heart failure. The results from several recent studies, including the study by Li et al in this issue of Circulation, demonstrate that myocardial MMP activity is increased in cardiomyopathic ventricles and is associated with changes in the endogenous inhibitors of the MMPs, namely the TIMPs. Therefore, this study, as well as others, adds to the increasing body of circumstantial evidence suggesting that MMP activation contributes to the changes in LV geometry that occur with the progression of dilated cardiomyopathy. In this regard, it will be important in future studies to identify upstream mechanisms for myocardial MMP activation, as well as to develop new strategies for inhibition of MMP expression and activity. Thus, the concept of controlling MMP expression and/or activity as a means for governing LV remodeling represents a new and exciting therapeutic target for treating the failing heart.

**References**


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