Bioactive Peptide Signaling Within the Myocardial Interstitium and the Matrix Metalloproteinases

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In response to a prolonged cardiovascular pathophysiological stimulus, a cascade of compensatory structural events occurs within the myocardium. This process occurs as a continuum and has been defined as myocardial remodeling. This remodeling process has been demonstrated within the myocardial compartment after myocardial infarction (MI), with hypertrophy, or in cardiomyopathic disease. Pharmacological interventions targeted at altering the adverse LV myocardial remodeling processes that invariably occur in these cardiac disease states hold therapeutic promise. Although myocardial remodeling is accompanied by changes in the cellular constituents of the LV myocardium, significant alterations in the structure and composition of the extracellular matrix (ECM) also occur. Moreover, it has become increasingly evident that the fibroblast collagen matrix of the myocardial ECM is not a static structure, but rather a dynamic entity that may play a fundamental role in myocardial adaptation to a pathological stress and thereby facilitate the remodeling process. Therefore, identification and understanding of the biological systems responsible for ECM synthesis and degradation within the myocardium holds particular relevance. In this issue, Tsuruda and colleagues1 from the Mayo Clinic report on the interactive effects of brain natriuretic peptide (BNP) and tumor necrosis factor-α (TNF-α) on determinants of fibrillar collagen biosynthesis in cardiac fibroblasts. The results from this study emphasize the fact that the biologically active molecules that are contained within the myocardial interstitium do not act independently, but instead, a summation of signaling events ultimately determines the structure and function of the ECM.

Myocardial Fibroblast

The fibroblast is the most numerous cell type within the myocardium and plays a critical role in the composition and structure of the myocardial ECM. Myocardial fibroblasts are responsive to biochemical and physical stimuli and rapidly proliferate as well as change biological behavior. Changes in myocardial fibroblast form and function contribute significantly to the remodeling process after MI. The increased myocardial fibrillar collagen with pressure overload hypertrophy is due to a combination of increased synthesis and deposition by fibroblasts, which is also accompanied by decreased collagen degradation. The myocardial fibroblast responds to several biochemical factors that are operative in cardiac disease states. For example, oxidative stress induced in myocardial fibroblasts causes the induction of ECM proteolytic enzymes. In addition, localized release of mediators of inflammation such as TNF-α likely modify myocardial fibroblast ECM production/degradation. In the study by Tsuruda and colleagues,1 it was demonstrated that the bioactive peptide, BNP, was released by myocardial fibroblasts and in turn caused an intracellular signaling cascade that reduced fibrillar collagen synthesis and enhanced determinants of ECM degradation. These investigators reported that coinoculation of myocardial fibroblasts with BNP and other biologically active molecules, such as TNF-α or endothelin, amplified the release of ECM degradative enzymes. The response of the myocardial fibroblast cultures to these extracellular ligands was distinctly different when applied individually or in combination. These results emphasize the importance, and ultimately the complexity, of the multiple extracellular signals that are operative within the myocardial interstitium that govern the biological behavior of myocardial fibroblasts.

Matrix Metalloproteinases (MMPs)

The MMPs are a family of zinc-dependent proteases that play a role in normal and pathological myocardial remodeling processes. The MMPs can be classified into subgroups based upon substrate specificity and/or structure and include the collagenases such as MMP-1 and MMP-13, the stromelysins (which include MMP-3), the gelatinases (which include MMP-2 and MMP-9), and the membrane-type MMPs (MT-MMPs). Due to the potent proteolytic capacity of MMPs, regulation of MMP gene expression is under tight control. MMP mRNA expression can be influenced by a variety of chemical agents, neurohormones, corticosteroids, and cytokines. For example, TNF-α can influence MMP gene expression in several cell systems through the formation of transcription factors, which bind to specific response elements on MMP gene promoters. Several MMP promoters contain a nuclear factor-κB binding site (NF-κB), which is also induced by a number of extracellular signals. Although the integration of these transcription factors is a highly complex process, what is clear is that the induction of MMPs is not a uniform process. This is likely due to differences in the location, type, and number of transcription factor binding sites located within each of the MMP gene promoter regions. In the study by Tsuruda and colleagues, MMP release by
myocardial fibroblasts after exposure to exogenous BNP was time and species specific. Moreover, the results from this study demonstrated that the cyclic GMP/protein kinase G receptor transduction pathway influence MMP synthesis and release in cardiac fibroblasts. The investigators demonstrated this through two different approaches. First, the use of a cyclic GMP analogue in the myocardial fibroblasts caused release of MMPs similar to that obtained after stimulation with BNP. Second, interruption of BNP receptor signaling at the level of protein kinase G attenuated myocardial fibroblast release of MMPs. Thus, this study clearly adds to the compendium of receptor transduction pathways that contribute to the release and subsequently the activation of MMPs within the myocardial interstitium. Moreover, although these studies were performed in vitro, these results provide new insight into the multiple mechanisms of action of the natriuretic peptides such as BNP with respect to the LV remodeling process.

Membrane-Type MMPs and Outside-In/Inside-Out Signaling

A recently described class of MMPs, the membrane type MMPs, constitute a novel group of cell surface-associated MMPs. During trafficking to the cell membrane, MT-MMPs undergo intracellular activation through a proprotein conver-
tase pathway. Thus, unlike other classes of MMPs, MT-
MMPs are proteolytically active once inserted into the cell membrane. In comparison to other MMPs, MT-MMPs are biologically potent proteolytic enzymes. First, MT-MMPs possess a wide portfolio of extracellular substrates resulting in degradation of critical myocardial ECM components. Because the MT-MMPs are transmembrane proteins, they constitute an important point of local ECM proteolytic activity. Second, 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. Thus, MT-MMPs can amplify local MMP activation
states of soluble MMPs and thereby significantly increase ECM degradation. Third, MT-MMPs can process other membrane-bound signaling molecules such as TNF-α. This implies that MT-MMPs can contribute to the release of potent signaling molecules into the myocardial interstitium and activate local receptor systems. Therefore, MT-MMPs constitute an important local proteolytic system within the myocardium with respect to ECM remodeling and extracellular signaling. In a past clinical report, we measured myocardial levels of the MT-MMP species, MT1-MMP in end-stage heart failure and in nonfailing subjects. Whereas MT1-MMP levels were detectable in normal human myocardium, a robust increase was detected in heart failure samples. Thus, MT1-MMP, which is constitutively active within the myocardial ECM, is significantly increased in patients with LV remodeling and heart failure. The study by Tsuruda et al demonstrated that MT1-MMP levels increased in myocardial fibroblasts after exposure to BNP and thereby may be an upstream pathway for inducing this class of MMPs.

The integrins are a family of transmembrane proteins that serve multiple functions with respect to myocardial structure and function. Recent studies have provided evidence that MT-MMPs play a pivotal role in integrin processing and thereby facilitate signaling. Integrins form the binding interface with proteins comprising the basement membrane and therefore directly influence myocyte growth and geometry. Moreover, integrins coalesce at important structural sites within the myocyte and interdigitate with an intracellular signaling cascade system involving focal adhesion kinase. Binding of ECM ligands to the integrins causes activation of intracellular cascades, the formation of transcription factors, and may ultimately lead to MMP gene induction. Moreover, integrin processing and clustering to the cell membrane forms a means by which a cell such as the fibroblast directly interfaces with the ECM and thereby initiates a cascade of signaling events termed outside-in signaling. Taken together, the results from these past studies and the investigation by Tsuruda et al demonstrate that the interstitium is a dynamic entity that involves a number of signaling molecules interacting with cell surface receptors, which in turn induces proteolytic enzymes that can directly modify the ECM.

The ECM is a complex and dynamic microenvironment that represents an important structural and signaling system within the myocardium. Several studies have demonstrated that mechanical strain placed upon myocardial fibroblasts can alter ECM synthesis and degradation. Thus, mechanical signals through integrins, as well as signaling by bioactive molecules such as endothelin, BNP, and TNF-α are summated intracellularly and result in the formation of specific MMPs targeted to the cell membrane as well as released into the local interstitial space (Figure). From the first report by Gross and colleagues regarding the resorption of a tadpole tail, it is now clearly recognized that MMPs play a critical role in normal and pathological tissue remodeling processes. Future research that integrates the upstream transduction pathways that contribute to MMP synthesis and activation will likely provide mechanistic insight into the LV remodeling process as well as yield novel therapeutic targets.

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**References**


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