A fundamental objective of cardiovascular medicine is the early detection of structural changes within the heart and vasculature that would be harbingers of disease. For example, a much greater emphasis has been placed on identifying patients with underlying changes in left ventricular (LV) myocardial structure before the onset of occult LV dysfunction and symptomatic heart failure. These changes in LV myocardial form and function have been generically termed LV remodeling, and an important research direction is to identify critical pathways that contribute to this adverse remodeling process. Although significant past research efforts have been made to identify changes in cardiocyte structure and function, LV remodeling is a multifactorial process involving both cellular and extracellular pathways. An evolving concept is that a significant interplay exists among biological signaling molecules, transmembrane proteins, and proteases within the myocardial interstitial space, which in turn promotes LV remodeling. In the present issue of Circulation, a pair of studies is presented that further demonstrate the diversity of proteolytic pathways.

The matrix metalloproteinases (MMPs) were once considered a small set of proteases with a rather limited role in terms of degrading structural proteins within the interstitium. However, as the number of MMPs and the portfolio of proteolytic substrates continue to grow, so does the recognized functionality of these proteases in the myocardial remodeling process. The generalized nomenclature for the MMPs was initially established on what was considered to be the fundamental proteolytic targets for each MMP class; hence the use of collagenses for the MMP types MMP-1, -8 and -13 and the gelatinases (processing of denatured collagen) for MMP-2 and MMP-9. However, this MMP nomenclature should be considered one of convenience and not one of proteolytic relevance. These MMP types, such as the collagenses and the gelatinases, are synthesized and released from all myocardial cell types into the interstitial space in a nonactive proenzyme, which in turn requires proteolysis and removal of the prodomain at a specific sequence to yield a functional catalytic domain and a fully active MMP. Through the use of in vitro mapping techniques and proteolytic assays, as well as in vivo substrate measurements, it has become clear that active MMPs process a large number of both structural and signaling proteins within the myocardial interstitial space. A class of MMPs that is uniquely different is the membrane-type MMPs, such as MT1-MMP, which is a transmembrane protease with both an intracellular domain and an extracellular domain, and which is fully functional once trafficked to the cell membrane. All myocardial cell types express MT1-MMP, including robust levels in cardiocytes and fibroblasts. A large number of structural proteins, transmembrane adhesion molecules, and signaling moieties are processed by MT1-MMP. The wide range of substrates and biological import of MT1-MMP is further exemplified in that this is the predominant pathway by which proMMP-2 is processed to fully active MMP-2.

This proMMP-2 activation cascade highlights the complexity and tightly orchestrated interactions between MMPs and tissue inhibitors of metalloproteinase (TIMPs). Specifically, pro-MMP-2 and TIMP-2 form a complex that results in removal of the prodomain of MMP-2 by MT1-MMP yielding active MMP-2 (the Figure). This form of MMP activation cascade can then yield a very potent and focused pericellular proteolysis. In particular, MT1-MMP and MMP-2 can alter cell–matrix and cell–cell interactions through degrading the cell–matrix binding substrate or by directly processing transmembrane binding proteins, the integrins. Although TIMP-2 facilitates MMP-2 activation, TIMP-2 can in turn bind to the active form of MMP-2 at a different site and cause MMP-2 inhibition (the Figure). Thus, the activation and inhibition of MMP-2, one of the more ubiquitous and abundant MMP types, is facilitated by TIMP-2 and demonstrates how differences in location and context of specific members of the proteolytic cascade can yield distinctly different and opposing events within the interstitial space. The studies presented by Westermann et al (gene deletion of MMP-2) and Kandalam et al (gene deletion of TIMP-2) underscore that a critical balance and set of essential interactions exist within the interstitial space between MMPs and TIMPs with respect to the myocardial biological response after a pathophysiological stimulus.
MMP–TIMP Interactions: New Insights and Targets

It has become abundantly clear that MMPs process a large portfolio of structural and signaling proteins critical for the maintenance of normal tissue structure and function, and dysregulation of this diverse family of MMPs can initiate and promulgate pathological remodeling in disease states such as inflammation, cancer, and cardiovascular disease. The initial development of pharmacological MMP inhibitors was based on the concept that MMPs were a relatively generic family of proteases that degraded matrix, and thereby inhibition would cause favorable effects in the context of pathological remodeling processes such as myocardial infarction. However, any LV remodeling process, particularly that of myocardial infarction, has an inflammatory component necessary for facilitating the wound healing response, and MMPs likely play an important role in this early inflammatory/wound healing response. Matrix metalloproteinases such as MT1-MMP and MMP-2 have been shown to process cytokines such as tumor necrosis factor-α and growth factors such as transforming growth factor β that are operative in the LV remodeling process. Using a murine model of myocarditis and a transgenic MMP-2 null mouse (MMP-2−/−), Westermann and colleagues demonstrated that after viral infection, LV function and survival worsened in MMP-2−/− mice. Moreover, the degree of myocardial inflammation and injury was amplified with MMP-2 gene deletion, and that this was likely due to an interruption in the normal processing and turnover of critical chemokines, such as monocyte chemoattractant protein-3 (MCP-3). Through both in vitro enzymatic assays and in vivo neutralizing antibody studies, results are provided to suggest that MMP-2 plays a critical role in MCP-3 proteolysis and inactivation and thereby is necessary to quell the inflammatory storm induced in this viral myocarditis model. The present study underscores how induction and activation of MMP-2 may actually play an antinflammatory role. There are several conclusions that can be drawn from this study. First, because of the ubiquitous expression profiles, high abundance, and importance in the maintenance of normal tissue structure and function, suppression of MMP-2 through either pharmacological or genetic approaches in the context of LV remodeling may not yield beneficial effects. Second, integrated examination of LV remodeling and MMP substrates yielded an important protease-signaling interaction between MMP-2 and MCP-3, and facilitating MCP-3 proteolysis may yield a novel target in myocardial wound healing and inflammation.

In the study by Kandalam and colleagues, the complementary end of the MMP-2/TIMP-2 activation/inhibition cascade was examined. Using a murine model of myocarditis and a transgenic MMP-2 null mouse (MMP-2−/−), Westermann and colleagues demonstrated that after viral infection, LV function and survival worsened in MMP-2−/− mice. Moreover, the degree of myocardial inflammation and injury was amplified with MMP-2 gene deletion, and that this was likely due to an interruption in the normal processing and turnover of critical chemokines, such as monocyte chemoattractant protein-3 (MCP-3). Through both in vitro enzymatic assays and in vivo neutralizing antibody studies, results are provided to suggest that MMP-2 plays a critical role in MCP-3 proteolysis and inactivation and thereby is necessary to quell the inflammatory storm induced in this viral myocarditis model. The present study underscores how induction and activation of MMP-2 may actually play an antinflammatory role. There are several conclusions that can be drawn from this study. First, because of the ubiquitous expression profiles, high abundance, and importance in the maintenance of normal tissue structure and function, suppression of MMP-2 through either pharmacological or genetic approaches in the context of LV remodeling may not yield beneficial effects. Second, integrated examination of LV remodeling and MMP substrates yielded an important protease-signaling interaction between MMP-2 and MCP-3, and facilitating MCP-3 proteolysis may yield a novel target in myocardial wound healing and inflammation.
Diverse LV remodeling in this murine model of POH are likely multifactorial. First, the proMMP-2/TIMP-2 complex will not be formed, and thereby removes a potentially abundant substrate for MT1-MMP. Second, unlike TIMP-1, which is a poor inhibitor of MT1-MMP, TIMP-2 effectively inhibits MT1-MMP catalytic activity. Thus, TIMP-2 deletion likely caused unbridled MT1-MMP activity within the myocardial interstitial space, particularly with a superimposition of a pathological stimulus, and the results from Kandalam and colleagues would support this postulate. MT1-MMP is a highly potent and diverse MMP type and, with respect to LV growth and fibrosis, likely plays an important role in profibrotic signaling pathways. Thus, increased MT1-MMP activity, which was a consequence of TIMP-2 deletion, may actually result in accelerated myocardial fibrosis in POH. The results from this study also underscore that a critical balance exists not only between MMP/TIMP binding but also likely between TIMP types. The 4 TIMPs arise from distinctly different gene products and transduction pathways, and for this reason can have uniquely different effects on tissue growth and function. In addition to having different affinities to different active MMPs, TIMPs modulate cell growth, differentiation, and viability. Thus, shifts in the relative balance of the different TIMP types within the myocardial interstitium, particularly in the context of relevant stimuli such as POH, would likely alter cardiocyte as well as fibroblast biological behavior. A differential increase in plasma levels of TIMPs occurs in clinical forms of POH whereby the changes in plasma TIMP-2 and TIMP-3 were relatively modest when compared with TIMP-1 and TIMP-4. The results from the present murine study and past clinical observations would suggest the intriguing possibility that an imbalance in the relative TIMP types with POH may actually facilitate adverse LV remodeling such as fibrosis. There are several potential targets and directions that arise from the study relative to POH and TIMP-2 deletion. First, MT1-MMP, through both proMMP activation and profibrotic signaling, may present as a more specific target in the context of adverse LV remodeling. Second, targeted TIMP augmentation, in order to normalize the relative balance between TIMP types in cardiac disease states such as POH, may yield a favorable response, such as reducing matrix accumulation.

Diversity of Myocardial Interstitial Proteolytic Pathways: Conclusions

Using murine models of myocarditis and POH coupled with transgenic deletion of MMP-2 or TIMP-2, respectively, the studies by Westermann et al and Kandalam et al underscore the diversity and complex interactions of proteolytic pathways that exist within the myocardial interstitium. MMP-2 deletion exacerbated inflammation and LV failure in a myocarditis model, revealing that chemokines serve as proteolytic substrates for MMPs. Tissue inhibitor of metalloproteinase 2 deletion in POH accelerated LV hypertrophy and fibrosis, likely by altering relative MT1-MMP activity and increasing matrix accumulation through profibrotic effects. As with any basic studies using murine systems, translational and clinical interpretation should be done with appropriate caution. First, global MMP-2 or TIMP-2 deletion will likely cause a number of biological effects, some compensatory, and thus evaluating the effects of superimposition of a pathological stimulus within these transgenic constructs can be difficult. Second, the acute viral load to induce myocarditis or the abrupt and severe aortic constriction to induce POH in the mouse do not necessarily recapitulate the LV remodeling process and disease course in humans. Nevertheless, the findings from these studies continue to challenge dogma surrounding MMP/TIMP functionality and interactions within the dynamic entity, the myocardial interstitium. These findings provide further evidence suggesting that our expectations that initial MMP pharmacological inhibition studies would yield clinical benefit, without recognition as to timing, context, specificity, and the diversity of proteolytic pathways affected, were extremely optimistic. The recognition and identification of the complex and diverse proteolytic interactions between MMPs and TIMPs within the myocardial interstitium will likely yield relevant targets for both the detection and treatment of early structural events in the LV remodeling process.

Acknowledgments

The author is grateful to Shaun Riffle, MFA, Office of Media Resources, University of South Carolina School of Medicine, for creation of the figure.

Sources of Funding

This work was supported by National Institutes of Health grants HL057952 and HL059165 and the Research Service of the Department of Veterans Affairs.

Disclosures

None.

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**Keywords:** Editorials, fibrosis, fibroblasts, models, experimental, left ventricular remodeling, matrix metalloproteinases
Diversity of Myocardial Interstitial Proteolytic Pathways: Gene Deletion Reveals Unexpected Consequences
Francis G. Spinale

Circulation. 2011;124:2052-2055
doi: 10.1161/CIRCULATIONAHA.111.064071
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2011 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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