Epilysin (Matrix Metalloproteinase-28) Joins the Matrix Metalloproteinase Team on the Field of Postmyocardial Infarction Remodeling

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Although significant advancements in the treatment of acute coronary syndromes have occurred, myocardial injury culminating in a myocardial infarction (MI) remains a significant and problematic outcome. The initial cellular and extracellular events that occur soon after an MI are those fairly prototypical to a wound-healing response, characterized by a robust expression of a number of inflammatory mediators, inflammatory cells, and eventually the emergence of a fibroblast phenotype, the myofibroblast. However, a divergence in this prototypical wound response occurs at later post-MI time periods. Notably, rather than a well-formed quiescent scar, continued extracellular matrix (ECM) turnover and instability can occur, ultimately yielding a process of infarct expansion and left ventricular (LV) dilation. Indeed, the magnitude of this post-MI LV dilation, presumably secondary to infarct expansion and matrix instability, is a recognized determinant of clinical outcomes.1,2 The post-MI period is one characterized by a number of complex events that are both time and cell type dependent, and these events are likely relevant to both the MI region and the viable myocardium, in terms of post-MI remodeling. Of particular interest and potential therapeudic relevance are biological cascades that contribute to the instability of the ECM in the post-MI context. In this issue of Circulation Research,3 Ma and colleagues identify how a specific proteolytic pathway can significantly alter critical signaling and cell-dependent events within the ECM, and in turn, alter the course of post-MI remodeling.

Matrix Metalloproteinases—Beyond Matrix Degradation

The initial description of a matrix-degrading enzyme was based on the seminal observations, whereby the excised tadpole tail degraded a collagen gel—hence the term collagenase.4 Over 23 matrix metalloproteinase (MMP) types are expressed in humans, and the distribution, functionality, and substrates are diverse. The MMPs were classified based on recognized substrates, and although this nomenclature no longer holds relevance to substrate specificity, it is still commonly used to group the MMP types. This classification scheme would include the Collagenases, such as MMP-1, MMP-13, and MMP-8; the Gelatinases, which include MMP-2 and MMP-9; the Stromelysins, which include MMP-3; the Membrane Type MMPs, which would include MMP-14; and the ever-growing classification of Others, which include matrilysin (MMP-7), enamelysin (MMP-20), and epilysin (MMP-28). This is hardly an exhaustive list of MMP types that are likely expressed within the mammalian myocardium, but does provide a framework for identifying studies that have been performed using murine transgenic constructs of certain MMP types in the context of post-MI remodeling.5,6 Transgenic deletion of either gelatinase MMP-9 or MMP-2 reduced the degree of LV dilation early on after MI.5,6 Targeted deletion of MMP-7 appeared to affect myocardial conduction pathways and proteins in the context of post-MI remodeling.7 Cardiac restricted overexpression of MMP-14 worsened post-MI remodeling, ECM stability, and survival.8 However, the findings from these past transgenic studies should not be interpreted to mean that MMP deletion favorably affects post-MI remodeling, whereas MMP overexpression exacerbates adverse post-MI remodeling. In the present study by Ma et al,3 MMP-28 gene deletion did not yield a long-term favorable effect on LV remodeling, function, or survival post-MI; in fact, these outcomes were exacerbated in MMP-28 null mice. In a study from the same laboratory as that of the present report,9 transgenic overexpression of MMP-9 was unexpectedly associated with a favorable effect on LV remodeling and function early post-MI. Thus, uniformly ascribing a specific MMP type as a negative factor throughout the post-MI remodeling process is likely an oversimplification, and the dynamic changes in substrate processing by certain MMP types during the evolution of the post-MI remodeling process must be considered.

Although historically considered to be enzymes that degraded specific structural proteins of the ECM (ie, collagen and basement membrane components), the proteolytic substrates for MMPs is now recognized to be extremely diverse.10,11 These substrates include the processing of latent biological signaling molecules to an active state, release of membrane-bound cytokines, processing active signaling molecules to an inactive state, and activation of other proteolytic enzymes, including other MMPs. This diversity in substrate processing by specific MMP types is likely to be of significant relevance in the context of myocardial remodeling, such as after MI. The studies described by Ma et al underscore...
the diverse functionality of certain MMP types through the use of a proteomic profiling approach and identified certain proteins that may be specifically regulated by MMP-28 post-MI. Although these findings do not infer that these proteins were specifically processed by MMP-28, the fact that levels for the gelatinase MMP-9 were modified by MMP-28 gene deletion does suggest that tight interactions exist between MMP types. Furthermore, this present report provided findings to suggest that MMP-28 gene deletion likely affected signaling pathways relevant to cell differentiation as well as maturation relevant to the post-MI remodeling process. These findings provide further evidence that specific MMP induction and activation can potentially influence a number of pathways pertinent to overall myocardial form and function.

Epilysin (MMP-28)—An Addition to the Team Roster

In terms of our understanding the biology of MMPs in regards to the myocardial remodeling process, the vast majority of science on this subject is extrapolated from cancer research.12–14 The initial studies and potential relevance of MMP-28 in pathological remodeling, inflammation, and disease progression is no exception.15–18 Specifically, although MMP-28 appears to have a wide tissue-cell expression pattern under normal conditions, increased MMP-28 expression has been identified in gastric carcinomas.15 However, this is not a uniform observation, whereby expression studies in advanced squamous cell and colon carcinomas have identified a marked reduction in MMP-28.17,18 In dermal wound healing, a clear temporal expression pattern for MMP-28 has been identified, which further suggests that the functional role of MMP-28 in tissue-remodeling processes is context- and time-dependent.17 In the study by Ma et al, an improvement in LV function was observed in the MMP-28 null mice very early post-MI, which was then followed by progressive worsening of LV geometry, function, and survival. These investigators identified that certain patterns of acute/chronic inflammation were altered with MI and MMP-28 gene deletion, and the modulation of specific inflammatory pathways may be of particular importance with respect to this MMP type.19

The structure of MMP-28 is not unlike other soluble MMP types, such as the gelatinases MMP-2/MMP-9, in terms of containing certain prototypical domains.17 First, MMP-28 contains a prodomain that must be cleaved to yield a proteolytically competent MMP. Second, MMP-28 contains a catalytic domain that is of a typical metalloprotease sequence. Third, MMP-28 contains both a hinge and hemopexin domain likely involved in ECM binding and substrate recognition. However, MMP-28 also contains a recognition sequence for the proprotein convertase furin, which implies that this MMP type is likely activated intracellularly.20 This is a rather uncommon feature for soluble MMP types that are normally considered to be released into the interstitial space in an inactive pro-MMP form.13,19 Another MMP type that undergoes this type of intracellular furin-mediated activation is the MMP-14, and in fact, a highly conserved and specific sequence within the prodomain of MMP-28 and MMP-14 has been identified.17 This posttranslational processing step is not the only relevant convergence of these MMP types, in that coexpression of MMP-28 and MMP-14 has been identified in certain carcinomas.17,18 Moreover, MMP-28 and MMP-14 have been shown to contribute to epithelial–mesenchymal transition in carcinoma cells.16 As discussed in a subsequent paragraph, this may hold particular importance in terms of the observations made by Ma et al17 regarding MMP-28 gene deletion and fibroblast phenotype post-MI.

The inflammatory signaling cascades and cell types that are expressed in the post-MI period are of critical importance, in terms of initiating an appropriate wound-healing response. However, it is also likely that persistent activation of certain inflammatory signaling pathways may actually contribute to the adverse post-MI remodeling process.21 Of particular interest and relevance in the study by Ma et al,17 that in MMP-28 null mice, macrophage activation and polarization was significantly reduced when compared with wild-type mice after MI. In a mouse model of pseudomonas pneumonia,19 it was demonstrated that macrophage recruitment to the lung was actually enhanced with MMP-28 deletion, but macrophage maturation and neutrophil recruitment was altered. Taken together, these studies suggest that MMP-28 plays an important role in the expression/processing of factors necessary for macrophage chemotaxis—a critical cellular event in the post-MI period. Indeed, the study by Ma et al,17 using a large cytokine-profiling array, identified that a specific profile of cytokines expressed post-MI were differentially altered by MMP-28 gene deletion. However, which of these cytokines or signaling pathways is directly processed by MMP-28, and which of these pathways is an indirect consequence of MMP-28 gene deletion remains to be determined. Nevertheless, this present report and past studies suggest that MMPs are not simply a downstream consequence of a generalized inflammatory process, but rather are critical factors in the overall regulation of the pattern and type of inflammatory cascades that are evoked in both the early and late post-MI periods.

MMP-28 and MMP-14—A Cross-Road to Fibroblast Transdifferentiation Post-MI?

Although the ECM within the MI region was considered to be a relatively inert and a cellular entity, this region contains a myriad of signaling molecules, cell types, and an ever-changing microenvironment. Of particular interest is the predominance and proliferation of the myofibroblast within the MI region. The fibroblast is a cell of mesenchymal origin, and whereas the source(s) of the transdifferentiated fibroblasts within the MI region is a subject of active investigation, this transdifferentiation process holds similar biological features to that of epithelial–mesenchymal transition observed in cancer.14,22–24 Although a number of carefully orchestrated events appear to be necessary for this transdifferentiation process, it appears that transforming growth factor (TGF) β is a central element.14,16,23,24 MMP-28 has been shown to potentially regulate epithelial–mesenchymal transition through a loss of matrix adhesion and cell–cell contact through proteolysis of the adhesion receptor E-cadherin.16 It has also been demonstrated that MMP-14 likely plays a critical role in TGF-mediated activation and signaling through targeted proteolysis of the TGF latency binding protein, and thereby facilitating a TGF signaling cascade.5,25 Interestingly, in vitro studies have demonstrated that the transient induction
of MMP-28 and a persistent induction of MMP-14 appear to occur in a coordinated fashion whereby the epithelial cell phenotype dissipated, and the cells displayed a more proliferative and invasive phenotype. Using the conventional marker for fibroblast transdifferentiation to a myofibroblast phenotype, α-smooth muscle actin, Ma et al identified a significant reduction in smooth muscle actin-positive fibroblasts within the MI region in MMP-28 null mice. Using smooth muscle actin as a marker for fibroblast transdifferentiation may hold some limitations, and whether and to what degree these fibroblast phenotype changes altered the structure and function of the ECM within the MI remains to be completely understood. Nevertheless, the observations made by Ma et al clearly demonstrated an association between changes in myofibroblast density to that of reduced collagen content within the MI region of the MMP-28 null mice. These observations coupled with those from past epithelial–mesenchymal transition studies lead to a postulate regarding how certain MMP types can directly influence fibroblast phenotype within the remodeling myocardium post-MI (Figure). Specifically, the initial influx of inflammatory cells into the MI region, such as macrophages, causes a robust release of MMP-28 and contributes to a further loss of cell adhesion, such as that of fibroblast–ECM binding. The study by Ma et al supports the concept that a shift in expression of MMP-28 within viable myocytes to that of macrophages occurs in the early post-MI period. In parallel, an early and robust induction of MMP-14 occurs within the fibroblasts in the MI region, and ex vivo expression/activity studies support this spatiotemporal expression pattern. This, in turn, will cause a localized induction of TGF and contribute to fibroblast transdifferentiation, a biological milestone in the post-MI remodeling process. Although this proposed cascade of events is speculative and oversimplified, it does afford the opportunity to once again examine carefully the basic research regarding MMP functionality in cancer and the potential translation of these findings to relevant tissue remodeling processes, such as those that occur post-MI.

**Summary**

Studies such as that by Ma et al challenge the concept that the induction of MMPs after MI is uniformly an adverse biological event; the MMP type, time, and context must be carefully considered. What is further underscored from the study by Ma et al is that certain MMP types should remain unopposed, and the timing of MMP interference should be carefully considered. It is likely that these considerations along with other study design issues contributed to the equivocal results observed in an initial trial utilizing a fairly broad-based MMP inhibition strategy in post-MI patients. The challenge that remains is to identify those MMP types that facilitate an appropriate wound-healing response versus those that contribute to the development of adverse post-MI remodeling and ultimately the progression to heart failure. The induction of MMPs, with MMP-28 as no exception, is regulated at the transcriptional, posttranscriptional, and posttranslational level. Thus, selective targeting of MMP types at one or more of these levels of regulation in the post-MI context will likely yield relevant therapeutic targets. Of particular interest in terms of MMP-28 and arguably of MMP-14 is how manipulation of the intracellular convertase step of activation may modify the cascade of biological events post-MI. Although much remains to be done, the once held concept that MMPs serve a rather monotonous function, that is, structural matrix protein degradation, has clearly been dispelled.

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None.

**References**


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