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Matrix Metalloproteinase (MMP)-9: a proximal biomarker for cardiac remodeling and a distal biomarker for inflammation

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Abstract

Adverse cardiac remodeling following myocardial infarction (MI) remains a significant cause of congestive heart failure. Additional and novel strategies that improve our ability to predict, diagnose, or treat remodeling are needed. Numerous groups have explored single and multiple biomarker strategies to identify diagnostic prognosticators of remodeling progression, which will improve our ability to promptly and accurately identify high-risk individuals. The identification of better clinical indicators should further lead to more effective prediction and timely treatment.

Matrix metalloproteinase (MMP-9) is one potential biomarker for cardiac remodeling, as demonstrated by both animal models and clinical studies. In animal MI models, MMP-9 expression significantly increases and is linked with inflammation, diabetic microvascular complications, extracellular matrix degradation and synthesis, and cardiac dysfunction. Clinical studies have also established a relationship between MMP-9 and post-MI remodeling and mortality, making MMP-9 a viable candidate to add to the multiple biomarker list.

By definition, a proximal biomarker shows a close relationship with its target disease, whereas a distal biomarker exhibits non-targeted disease modifying outcomes. In this review, we explore the ability of MMP-9 to serve as a proximal biomarker for cardiac remodeling and a distal biomarker for inflammation. We summarize the current molecular basis and clinical platform that allow us to include MMP-9 as a biomarker in both categories.

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Keywords

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1. Introduction

Despite significant advancements in risk prediction, cardiovascular disease remains a leading cause of death (Roger, et al., 2011). Myocardial infarction (MI) is one of the most highly prevalent cardiovascular diseases, with over 1.2 million Americans being diagnosed with MI annually. While short-term one month survival rates have dramatically improved over the last 30 years, post-MI remodeling progressing to heart failure remains a significant clinical issue. This issue is further fueled by increased incidences of obesity, metabolic syndrome, and diabetes, all of which exacerbate the cardiac remodeling response (Horwich & Fonarow, 2010; Roger, et al., 2011). Because heart failure is associated with substantial morbidity and mortality, as well as an impaired quality of life (Goldberg, Ciampa, Lessard, Meyer, & Spencer, 2007), improved methods to identify at risk patients before they develop heart failure is a primary goal. MI modulates several biological pathways that converge in the remodeling response, which is characterized by changes in left ventricle (LV) size, shape, and function (M. L. Lindsey & Zamilpa, 2010; Pfeffer & Braunwald, 1990).

Several plasma or serum proteins have been characterized in the context of heart failure, and these are broadly classified as markers of LV remodeling. Included in the list are extracellular matrix (ECM) markers- collagen, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs); inflammatory markers- C-reactive protein (CRP), tumor necrosis factor α, and interleukins (IL)- 1, 6, and 18; oxidative stress markers- homocysteine and myeloperoxidase; neurohormonal activation markers- renin, angiotensin II, and aldosterone; myocyte injury markers- cardiac specific troponins and creatine kinase; and myocyte stress markers- brain natriuretic peptide (BNP) and N-terminal pro-BNP (Braunwald, 2008; Fertin, et al., 2012; Maisel, et al., 2002; Opdenakker, et al., 2001; Tang, et al., 2007; Velagaleti, et al., 2010). To date, a myriad of candidate circulating biomarkers have been examined as LV remodeling or heart failure predictors, but the use of one biomarker to accurately assess disease diagnosis, stage, and progression has not been successful and is not expected to be fruitful. To illustrate this point, while average BNP levels are higher in patients with heart failure, individual levels vary from 100–1400 ng/ml. BNP shows a wide spectrum of values and does not stratify with heart failure stage, and BNP responses to heart failure treatments are influenced by comorbidities such as renal failure (Lang & Mancini, 2007). This variation is so large that BNP cannot effectively separate patients with and without heart failure (Maisel, et al., 2002). A more successful approach will likely be to use a multi-marker panel profiling scheme to assess markers during each category (diagnosis, stage, progression) from the initial MI event to progressive remodeling to the development of heart failure. Of the analytes that have been examined, MMPs provide several candidate biomarkers.

MMPs are zinc-dependent endopeptidases that cleave several ECM proteins and as such modulate outcome of various physiological and pathological processes including MI, atherosclerosis and congestive heart failure. In addition to structural ECM components, MMP substrates also include a multitude of ligand and receptor substrates such as cytokines, chemokines, growth factors, and adhesion molecules that alter cellular migration, adhesion, and activation. MMPs, therefore, exert a strong influence on cardiac remodeling through multiple mechanisms (M. L. Lindsey, 2004; M. L. Lindsey & Zamilpa, 2010; Sternlicht & Werb, 2001). MMPs are endogenously inhibited by the tissue inhibitors of metalloproteinases (TIMPs), a family comprised of four members, TIMP-1, -2, -3, and -4.

Pre-clinical and clinical studies in the post-MI setting indicate that MMP-1, -2, -3, -7, -8, -9, -12, -13, and -14 and TIMP-1, -2 -3, and -4 are relevant to MI and LV remodeling (Hansson, et al., 2011; M. L. Lindsey & Zamilpa, 2010; Rohde, et al., 1999; Yarbrough, et al., 2003; Zamilpa & Lindsey, 2010).

For the most part, MMPs are secreted from the cell as proMMPs and are activated extracellularly by tissue or plasma proteinases. The first step in activation involves cleavage of a part of the propeptide, and complete activation occurs with removal of the entire propeptide by the MMP intermediate or by other active MMPs (Nagase, Visse, & Murphy, 2006). MMPs can also be activated *in vitro* by treatment with organomercurial compounds, urea, SH reagents, and chaotropic agents, which chemically perturb the proMMP to alter its structure and permit activitiy without loss of the 10 kD pro-domain. Other exogenous MMP activators include oxidants such as HOCl and $ONOO^-$, which activate proMMPs by reacting with the cysteine in the propeptide. This activation process can also take place *in vivo*, under inflammatory conditions (Gu, et al., 2002; Peppin & Weiss, 1986). On the other hand the major endogenous MMP inhibitor in serum is α2-macroglobulin and in tissue are the TIMPs (Sorokin, 2010).

In 2001, an NIH working group standardized the definition of a biomarker as any characteristic that can be objectively evaluated as an indicator of a normal biological process, a pathological process, or a pharmacological responses to therapeutic intervention (Biomarkers Definitions Working Group, 2001; Vasan, 2006). The American Heart Association released a scientific statement focused on the importance for developing biomarkers to enhance diagnostic methods and provide surrogate measures of treatment efficacy (Balagopal, et al., 2011; Fortmann, et al., 2004; Hlatky, et al., 2009; Richards, 2009; Smith, et al., 2004; Vasan, 2006). Because no single biomarker will likely provide sufficient information to predict disease progression, the next step is to identify the combination of markers that improve risk prediction beyond what is currently available. A combination biomarker strategy can also be used strategically to make go or no-go decisions that will accelerate drug discovery (Krishna & Wagner, 2010). An essential biomarker, by definition, would modulate both the target response as well as distal events related to disease outcome (Krishna, Herman, & Wagner, 2008; Krishna & Wagner, 2010).

In this review, we provide rationale for using MMP-9 as a biomarker. We will discuss its effectiveness as a proximal biomarker for cardiac remodeling (one that shows a close relationship with its target disease) and a distal biomarker for inflammation (one that exhibits non-targeted disease modifying outcomes). We provide a logic model by which to evaluate the inclusion of MMP-9 as a candidate marker for post-MI remodeling that may also serve as a template to evaluate other candidate markers.

2. Methods of review

We searched PubMed for all papers that included MMP-9, which was over 13,000 papers. We then focused the search by articles published in the past 1, 2, 3, 5, or 10 years. Subsequently, we added inflammation, cardiac remodeling, cardiovascular, myocyte, fibroblast, neutrophils, or leukocytes to the MMP-9 keyword search (each term was searched individually with MMP-9). We included all clinical reports, review articles, journal articles, clinical trial reports, meta-analysis studies, randomized controlled trials, and original research manuscripts that were published in English. The numbers of manuscripts with these key words are shown in Figure 1.

3. Pre-clinical and clinical studies: MMP-9-mediated proximal effects (Figure 2)

3.1 Post-MI LV healing phases

Following MI, both the infarcted region as well as the remote non-infarcted zone undergo cardiac remodeling as a part of the wound healing response (Pfeffer & Braunwald, 1990). The LV healing response can be divided into two overlapping phases, the inflammatory and reparative phases. The first phase, the inflammatory phase, is characterized by the robust release of inflammatory mediators and degradation of ECM that occurs in the setting of myocyte necrosis. During the inflammatory phase, cardiomyocyte death triggers the rapid activation of the complement system, which induces free radical production and activates the toll-like receptor-mediated pathway. The second phase, the reparative phase, is characterized by fibroblast proliferation and release of fibrosis-promoting cytokines that contribute positively to scar formation, with the net result being increased ECM synthesis and deposition (Frantz, Bauersachs, & Ertl, 2009).

The inflammatory and fibrotic pathways share several components. For example, both pathways involve activation of nuclear factor kappa-B (NF-κB) in infiltrating and resident myocardial cells to stimulate the expression of cytokines, chemokines, growth factors, and adhesion molecules. Among these factors are IL-1β, tumor necrosis factor α, monocyte chemotactic protein (MCP)-1/(CCL2), and intercellular adhesion molecule 1, which stimulate and facilitate leukocyte intravasation into the infarct region. During permanent occlusion, neutrophil infiltration occurs primarily during days 1–3 post-MI, while macrophage infiltration occurs primarily during days 3–7 post-MI. During reperfusion, the kinetics and amplitude of the inflammatory response shifts, such that both neutrophils and macrophages enter the tissue simultaneously as soon as reperfusion is initiated. The transition from the inflammatory to reparative phase is associated with the activation of pathways that turns off inflammation and promotes ECM scar formation (Dobaczewski, Gonzalez-Quesada, & Frangogiannis, 2010; Frantz, et al., 2009). Targets involved in inflammatory events and reparative processes will be central components of a successful LV remodeling biomarker discovery and drug target development.

3.2 MMP-9 expression in post-MI cardiac remodeling

Table 1 highlights basic science studies evaluating LV MMP-9 levels in animal models, while Table 2 highlights clinical trials examining LV MMP-9 levels in humans. MMP-9 is expressed in cardiac myocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, neutrophils, macrophages, and fibroblasts (Coker, et al., 2001; Hasty, et al., 1990; Heymans, et al., 1999; Kawakami, et al., 2004; M. Lindsey, et al., 2001; M. L. Lindsey, Escobar, Mukherjee, et al., 2006; Opdenakker, et al., 2001; Porter & Turner, 2009; van den Borne, et al., 2009). MMP-9 was first described as being able to process only collagen that was first denatured or already cleaved by collagenases such as MMP-1. Recent literature, however, has shown that MMP-9 can process full length interstitial collagens (Egeblad & Werb, 2002; Lauer-Fields, et al., 2008). Further, MMP-9 does not require an activation cleavage step to proteolyze substrates. Pro-MMP-9, in the presence of substrate, has enzymatic activity without the loss of the 10 kDa pro-domain (Bannikov, Karelina, Collier, Marmer, & Goldberg, 2002).

MMP-9 activates several chemokines, including CXCL5, CXCL6, and CXCL8, and contributes to the release of cell surface receptors (e.g., tumor necrosis factor-α receptor) to alter the local microenvironment (Van Den Steen, et al., 2003). MMP-9 also has several inflammatory response elements, including activator protein-1, specificity protein-1, and NF-κB sites that makes it highly responsive to inflammatory stimuli (Benbow &

Brinckerhoff, 1997; M. L. Lindsey & Zamilpa, 2010). In the mouse, rat, pig, rabbit, and dog models of MI, MMP-9 levels are consistently increased in the infarct region (Ducharme, et al., 2000; Etoh, et al., 2001; Heymans, et al., 1999; M. Lindsey, et al., 2001; M. L. Lindsey, Escobar, Dobrucki, et al., 2006; M. L. Lindsey, et al., 2005; Romanic, Burns-Kurtis, Gout, Berrebi-Bertrand, & Ohlstein, 2001; Tao, Cavasin, Yang, Liu, & Yang, 2004). In mouse, rabbit, and pig MI models, pharmacological MMP inhibition reduces LV dilation and preserves cardiac function (Chancey, Brower, Peterson, & Janicki, 2002; Mukherjee, et al., 2003; Rohde, et al., 1999; Spinale, et al., 1999a, 1999b). Mice with targeted deletion of the MMP-9 gene show attenuated LV dilation after experimental MI accompanied by decreased collagen accumulation (Ducharme, et al., 2000; Heymans, et al., 1999). Interestingly, however, MMP-9 deletion also stimulates neovascularization in the post-MI infarct region (M. L. Lindsey, Escobar, Dobrucki, et al., 2006). This suggests that MMP-9 serves both beneficial and detrimental roles in the post-MI response.

A striking increase in MMP-9 activity is found at days 1 to 4 in the infarct region, and this increase corresponds with neutrophil and macrophage infiltration (Ducharme, et al., 2000; Heymans, et al., 1999; Hudson, et al., 2006; Ramani, et al., 2004; Tao, et al., 2004). Mukherjee *et al* demonstrated that MMP-9 promoter transcripts with a β-galactosidase reporter show MMP-9 promoter activity at day 3 post-MI that peaked at day 7 (Mukherjee, et al., 2010). The earlier initial increase in MMP-9 protein levels seen at day 1 post-MI is due to the release of pre-formed MMP-9 from infiltrating neutrophils, where it is stored in gelatinase granules (Mukherjee, et al., 2010).

Kelly and colleagues provided insight into the complexity of MMP-9 in terms of its having both beneficial and detrimental roles during post-MI remodeling (Kelly, et al., 2007). They found that increased early levels of MMP-9 associated with both neutrophil numbers and the extent of LV remodeling, indicating that MMP-9 from the neutrophil has an overall detrimental effect. In contrast, increased late levels of MMP-9 associated with preservation of LV function, indicating that MMP-9 after the initial wound healing phase may serve an overall beneficial effect. The temporal profile of MMP-9, in addition to its magnitude, is an important consideration. Post-MI, the establishment of new blood vessel networks is needed to supply oxygen to the highly metabolically active infarct area (Post, Laham, Sellke, & Simons, 2001; Sim, Zhang, Shim, Lim, & Ge, 2002). Of note, MMP-9 deletion enhanced neovascularization in the post-MI setting in mice, suggesting that targeted strategies to inhibit MMP-9 early post-MI might improve rather than impair angiogenesis (M. L. Lindsey, Escobar, Dobrucki, et al., 2006).

Recent advances in mass spectrometry–based proteomic approaches and new emerging technologies hold particular promise for unbiased discovery and subsequent validation of novel biomarkers of cardiovascular disease (Gerszten, Asnani, & Carr, 2011). As an example of such an approach, Zamilpa *et al* identified multiple proteins that are differentially expressed in the infarct region of MMP-9 null mice compared to wild type mice. Among previously known *in vitro* MMP-9 substrates, fibronectin was validated as an *in vivo* MMP-9 substrate in the post-MI setting (Zamilpa, et al., 2010).

3.3 MMP-9 effects on LV rupture

LV wall rupture is one of the more serious complications, accounting for 5 to 31% of all inhospital MI deaths (Figueras, Cortadellas, & Soler-Soler, 2000). While rupture rates in humans have fallen due to the success of reperfusion, the incidence of LV ruptures remains at 0.5%–1.4% (Lopez-Sendon, et al., 2010). LV ruptures are more frequent in patients with STEMI (0.9%) than patients with other acute coronary syndromes (NSTEMI, 0.17%; unstable angina, 0.25%) (Lopez-Sendon, et al., 2010). In C57BL/6J male mice, the 7 day post-MI survival rate is approximately 60%, and about one in three deaths will occur as a

result of rupture (Gao, Xu, Kiriazis, Dart, & Du, 2005; Yang, et al., 2008; Zamilpa, et al., 2011). Survival in female mice is about 90% at day 7 post-MI, with about one in ten deaths occurring as a result of rupture. Gender studies in 129sv mice showed that males have higher MMP-9 activity in the infarct region associated with increased inflammatory cell infiltration, as well as increased MMP-9 expression in circulating peripheral blood mononuclear cells (Fang, Du, Gao, & Dart, 2010). MMP inhibition using the CP471, 474 inhibitor significantly reduced both rupture incidence and MMP-9 activity in mice, supporting a role of MMP-9 in the pathogenesis of rupture. In humans, increased MMP-9 levels have been detected in ruptured human ventricles (van den Borne, et al., 2009). LV rupture in human and mice share an association between rupture rates and the accumulation of inflammatory cells, as well as a common location at the border zone. LV rupture in human and mice are disparate in the influence of sex on rupture rates (Gao, et al., 2005). In the clinical setting, the risk of post-MI rupture is higher in females than males (Figueras, et al., 2000; Reardon, et al., 1997).

3.4 Clinical studies: MMP-9 is a biomarker for cardiac remodeling

Blankenberg and colleagues performed the first comprehensive clinical study that implicated MMP-9 as a novel prognostic biomarker for individuals at increased risk for CV mortality (Blankenberg, et al., 2003). MMP-9 correlated with the acute-phase reactant proteins IL-6, hs-CRP, and fibrinogen, indicating that MMP-9 could have its own pathophysiological significance in cardiovascular mortality. Squire and colleagues extended these studies to demonstrate that, in humans, higher MMP-9 correlated with larger LV volumes and greater dysfunction following MI (Squire, Evans, Ng, Loftus, & Thompson, 2004). The Vasan team examined patients from the Framingham Heart Study and found that plasma MMP-9 levels associated with increased LV diastolic dimensions and increased wall thickness (Sundstrom, et al., 2004). Hlakty and colleagues showed that circulating MMP-9 levels independently associated with acute MI but not stable angina (Hlatky, et al., 2007). MMP-9 levels correlated with LV enlargement, lower ventricular ejection fraction, and persistent adverse LV remodeling in chronic systolic heart failure patients (Yan, et al., 2006). Fertin et al examined 112 correlations among 52 different biomarkers and LV remodeling indices. The most consistent biomarkers associated with LV remodeling were related to ECM turnover or neurohormonal activation. Among the biomarkers, MMP-9, collagen peptides, and B-type natriuretic peptide were prominent biomarkers that predicted adverse LV remodeling after MI (Fertin, et al., 2012). Of note, several polymorphisms have been evaluated within the MMP-9 gene and have been shown to influence gene expression (B. Zhang, et al., 1999). Specifically, the C1562T allele associates with increased MMP-9 plasma concentrations, whereas the R279Q polymorphism had no effect on plasma levels but associated with future CV events. The 279 amino acid where these polymorphisms occur resides in the catalytic domain of the MMP-9 enzyme, suggesting that MMP-9 activity levels may be higher in patients with the R279Q polymorphism (Shipley, et al., 1996; Tanner, et al., 2011). Combined, these studies offer strong evidence for a proximal role of MMP-9 in LV remodeling. In heart failure patients, serum carboxy-terminal telopeptide of procollagen type I, carboxy-terminal telopeptide of procollagen type I, and amino-terminal propeptide of procollagen type III are all elevated and serve as indicators of diastolic dysfunction. In these same patients, serum MMP-9 levels are elevated, suggesting increased degradation of myocardial collagen (Martos, et al., 2007). This particular study elegantly demonstrated the role of MMP-9 in stimulating LV remodeling in hypertensive and diastolic heart failure patients.

3.5 MMP-9 roles in inflammation: neutrophils, macrophages, and lymphocytes are cell sources of MMP-9

MMP-9 is secreted by neutrophils early post-MI, and by macrophages, lymphocytes, and fibroblasts at later phases post-MI (Figure 3). In neutrophils, MMP-9 is synthesized during bone marrow granulocyte differentiation and is released following neutrophil activation (Jonsson, et al., 2011). Fang *et al* quantified MMP-9 levels in peripheral blood mononuclear cells that were differentiated into macrophages *in vitro* (Fang, et al., 2007). Circulating cells isolated at day 4 after MI in 129sv mice showed increased MMP-9 levels compared to cells isolated from the sham mice. Peripheral blood mononuclear cells isolated from patients with acute MI and differentiated to macrophages also produced a higher amount of MMP-9 compared to cells isolated from patients with stable angina or healthy controls, indicating that macrophages are an important cellular source of plasma MMP-9 (Fang, et al., 2010).

3.6 MMP-9 effects on inflammatory chemokines and cytokines

MMP-9 modulates leukocyte function through a number of cytokine-mediated mechanisms. MMP-9 can process pro-IL-1β into active IL-1β and can truncate IL-8 into a more active form. As both IL-1β and IL-8 can stimulate MMP-9 degranulation from neutrophils, providing an important positive feedback loop for neutrophil activation and chemotaxis (Opdenakker, et al., 2001).

In the post-MI setting, the overexpression of human CRP in mice results in more severe LV remodeling with increased LV dilation, a greater extent of LV dysfunction, and more prominent cardiomyocyte hypertrophy and fibrosis than their littermate controls (Mano, et al., 2011). The CRP transgenic mice also display enhanced macrophage infiltration into the infarct region, at rates that are directly proportional to increased MCP-1 expression and MMP-9 activity (Takahashi, et al., 2010). Increased CRP, therefore, leads to increased macrophage accumulation through a direct MMP-9 role.

3.7 MMP-9 inhibitors

It is well established that an increased expression of MMP-9 associates with the pathological status in a wide range of inflammatory diseases, including MI, rheumatoid arthritis, liver fibrosis, and periodontal disease. A pathogenic role of MMP-9 in tissue breakdown and remodeling during aggressive tumor growth and angiogenesis is also established. Because of past failures with global non-specific MMP inhibitors, the current focus in the MMP inhibitor drug discovery arena is to develop inhibitors specific for particular MMPs.

The main structural requirement of an MMP inhibitor is the zinc binding group (ZBG) that chelates the active-site zinc ion. Tandon and Sinha applied a docking and molecular dynamics approach to study the binding of inhibitors to the active site of MMP-9. Three categories of zinc binding groups were chosen: 1) sulfonamide hydroxamate, 2) thioester, and 3) carboxylic moieties. Out of these three categories, the thioester based zinc binding moiety provided the most promising docking scores compared to the other two groups (Tandon & Sinha, 2011).

Gutierrez and colleages demonstrated that the MMP-9 inhibitor doxycline attenuated *Trypanosoma cruzi* infection induced cardiac injury (Gutierrez, et al., 2008). These results indicate that MMP-9 inhibition in myocarditis mollifies inflammation to increase survival in mice (Gutierrez, et al., 2008). Of interest, doxycycline is the only FDA-approved MMP inhibitor currently on the market (Lee, et al., 2004; Y. Zhang, et al., 2012).

Pharmacological inhibition of MMPs has been effective in limiting tissue damage after MI in animal models. Villarreal et al observed that short-term treatment of doxycline reduced

adverse LV remodeling and improved LV function in male Male Sprague-Dawley rats (Villarreal, et al., 2003). MMP inhibition in humans, however, has not been as successful, as broad-spectrum MMP inhibitors showed adverse secondary effects on the musculoskeletal system that were linked to the non-selective nature of these inhibitors (Creemers, Cleutjens, Smits, & Daemen, 2001; Spinale, 2002). .

4. MMP-9 distal effects on other inflammatory diseases (Figure 2)

4.1 Atherosclerosis

Atherosclerosis is an inflammatory disease characterized by plaque formation and artery wall thickening as a result of the accumulation of lipids. Atherosclerosis mainly affects vein grafts, arterial blood vessels, and also includes the accumulation of macrophages, low-density lipoproteins, plasma proteins that transport cholesterol, and triglycerides (Ross, 1999).

Konstantino *et al* have reviewed the role of MMP-9 in the pathophysiology of atherosclerosis and plaque rupture (Konstantino, et al., 2009). While other MMPs (including MMPs -1,-2,-3,-7,-8,-10,-11,-12, and -13) have been evaluated, MMP-9 has been the most studied MMP in atherosclerosis pathology (Konstantino, et al., 2009). Despite the number of studies that demonstrate increased MMP-9 levels in the atherosclerotic lesion, few studies have been designed to determine the causal roles of MMP-9 or to explore the clinical applicability of MMP-9 inhibition. Mechanistic studies in apolipoprotein E (Apo E)-null mice model provide conflicting insight on MMP-9 roles in plaque formation. Lutton *et al* showed that after 25 weeks of a cholesterol-rich diet, Apo E / MMP-9 double-null mice had 70% smaller sized plaques with less collagen and macrophage content compared with Apo E null / MMP-$9^{+/+}$ mice, suggesting that MMP-9 deficiency protects from plaque development (Luttun, et al., 2004).

Conversely, Johnson *et al* demonstrated a larger lesion area and increased macrophage content in Apo E / MMP-9 double-null mice compared with Apo E null / MMP-$9^{+/+}$ mice after 8 weeks of a high-fat diet, indicating that MMP-9 deficiency promoted rather than impaired atherosclerosis progression (Johnson, George, Newby, & Jackson, 2005). The inconsistent results could be ascribed to a variation in timing of lesion measurements, as Johnson *et al* determined the lesion size after 8 weeks of a high-fat diet, whereas Lutton *et al* assessed the lesion size after 25 weeks of a cholesterol-rich diet. The concept that MMP function can switch from deleterious to beneficial can be explained by a shift in substrate availability, since net MMP activity is determined by what substrates are processed. To support this idea, Nooijer *et al*. demonstrated that negative effects of MMP-9 overexpression on plaque stability appear to be more prominent in advanced atherosclerotic plaques (de Nooijer, et al., 2006). Advanced plaques showed more significant features of vulnerable plaque with a high incidence of intraplaque hemorrhage (de Nooijer, et al., 2006). The overexpression of activated MMP-9 in macrophages induced substantial plaque disruption in advanced atherosclerotic lesions of Apo E null mice, revealing that enhanced macrophage MMP-9 proteolytic activity can induce acute plaque disruption. MMP-9, therefore, is a therapeutic target for stabilizing rupture-prone plaques that are in the advanced stage. The fact that MMP-9 and macrophages co-exist in vulnerable plaques highlights the role for MMP-9 in this process. Future studies examining the temporo-spatial dynamics of MMP-9 expression during plaque development and destabilization are required to fully understand the significance of MMP-9 activity in atherosclerosis. (Gough, Gomez, Wille, & Raines, 2006). The cholesterol lowering drug statins (e.g. simvastatin, atorvastatin, and pravastatin) downregulate 3-hydroxy-3-methylglutaryl coenzyme A to improve plaque quality in atherosclerotic patients. Statins reduce macrophage accumulation and collagen degradation

by reducing CD40 ligand/CD40 and expression of the adhesion molecule VCAM-1 (Libby & Aikawa, 2003).

4.2 Rheumatoid arthritis

Rheumatoid arthritis is a systemic inflammatory disorder that affects synovial joints. Patients with rheumatoid arthritis are more prone to atherosclerosis and have increased risks for MI and stroke (Symmons & Gabriel, 2011). The synovial fluid from patients of rheumatoid arthritis contains increased levels of MMP-9 (Ahrens, Koch, Pope, Stein-Picarella, & Niedbala, 1996; Yoshihara, et al., 2000). Proteolytic degradation of articular cartilage is one of the early features of the disease and is mediated by an increased activity of MMP-3, -8, and -9 (Tchetverikov, et al., 2003). In particular, MMP-9 cleavage of aggrecan releases multiple neo-epitopes that stimulate an immune response to both initiate the pathogenesis and aggravate the progression (Ram, Sherer, & Shoenfeld, 2006). MMP-9 increases in various autoimmune diseases such as systemic lupus erythematosus, Sjogren's syndrome, systemic sclerosis, multiple sclerosis, and polymyositis (Ram, et al., 2006). Therefore, MMP-9 is considered an important target for therapy in autoimmune diseases.

4.3 Cancer

Cancer is a disease of dysregulated tissue growth. As cancer progresses, the uncontrolled growth often metastasizes and becomes invasive, wherein the tumor cells spread to other locations in the body via the lymphatic system or the bloodstream. MMPs are involved in many cancer-related processes including invasion, metastasis, angiogenesis, and cell proliferation (Egeblad & Werb, 2002). Although a number of MMPs (MMPs -2, -7, -9, -11, and -14) are readily detected in most tumor types at all stages, the pattern of expression of other MMPs (MMP-1,-3 -8,-13) varies considerably by tumor type and stage.

Roy *et al* reviewed the role of specific MMPs as novel biomarkers in different types of cancer such as breast (MMP-1,-9,-13), pancreas (MMP-2,-7,-9), lung (MMP-1,-7,-9), bladder (MMP-2,-9), colorectal (MMP-1,-2,-7,-9,-13), ovarian (2,-9,-14), prostate (MMP-2,-9) and brain (MMP-2,-9) (Roy, Yang, & Moses, 2009). MMP-9 is in common to each of the above-mentioned cancers and has been proposed as an overarching biomarker of cancer. Further, MMP-9 has been shown to have epigenetic regulation that may provide additional biomarker candidates.

Cancer and cardiovascular disease intersect, as treatment strategies to fight cancer often induce cardiac dysfunction. One example of this is the use of anthracyclines (e.g., doxorubicin) as an anti-oncogenic therapy. Anthracyclines have known cardiotoxic side effects, including a significant activation of MMP-9 (Goetzenich, et al., 2009). The inflammatory component is also a strong connection between these two diseases.

4.4 Periodontal disease

Periodontal disease is broadly classified into two subgroups: periodontitis and gingivitis. Periodontitis is an inflammatory disease that mainly affects the supporting tissues of the teeth leading to the progressive destruction of connective tissue attachments to alveolar bone. Gingivitis is a non-destructive inflammatory disease characterized by an increased build-up of plaque on tooth surfaces. Longtime untreated gingivitis progressing to periodontitis is the most destructive form of periodontal disease. MMP-8 and MMP-9 are major diagnostic markers that have been well described in periodontal disease (Ramseier, et al., 2009). Periodontal disease shows a multifaceted pattern and progresses as a feed forward continuum of infection and inflammatory dysregulation with subsequent bone loss. Specific biomarkers, including MMP-8, MMP-9, IL-1β IL-6, and type I collagen pyridinoline cross-

linked telopeptide (ICTP), have been used for periodontal disease identification (Ramseier, et al., 2009).

4.5 Diabetes mellitus and vascular complications

Diabetes mellitus stimulates a strong the immune system response by upregulating specific cytokines, chemokines, and leukocyte populations to contribute to increased vascular cell apoptosis and tissue fibrosis during plaque formation (Donath & Shoelson, 2011). The increase in macrophage numbers associates with reduced collagen content and MMP-9 overexpression in human diabetic plaques (Cipollone, et al., 2003). Furthermore, advanced glycation end products (the product of non-enzymatic glycation reactions stimulated by increased circulating glucose levels) stimulate COX-2/PGES-1 expression and induce MMP-9 synthesis in macrophages (Cipollone, et al., 2003; Kadoglou & Liapis, 2004). Diabetes, therefore, exacerbates the inflammatory response in atherosclerosis.

Abdominal aortic aneurysms (AAAs) are a chronic degenerative condition associated with a risk of vessel wall rupture. AAAs develop due to the progressive degradation of aortic wall elastin and collagen, and an increase in the local production of MMP-9 has been implicated in this process. The FDA approved MMP-9 inhibitor doxycycline reduces MMP-9 expression in human vascular wall cell types and in AAA tissue explants *in vitro*. Patients administered with doxycycline also show suppressed MMP-9 expression in the AAA tissue (Kadoglou & Liapis, 2004; Thompson & Baxter, 1999).

5 Future directions

Based on the many proximal effects of MMP-9 on cardiac remodeling and the many distal effects of MMP-9 on inflammatory diseases demonstrated in both pre-clinical and clinical studies, the further exploration of MMP-9 inhibitors is justified for the development of novel of cardiovascular agents that may benefit additional inflammatory diseases. Most currently used medications for heart failure (e.g., aldosterone antagonists, diuretics, ACE inhibitors, and beta-blockers) all decrease MMP-9 levels, indicating that screening for MMP-9 targets at early stages may help in the decision making process for cardiovascular drug discovery. While non-specific MMP inhibitor strategies have not proven useful,(Peterson, 2004) a specific inhibitor strategy that targets MMP-9 may prove effective. Several groups, however, are making headway in the MMP specific inhibitor arena (Johnson, et al., 2011; Robichaud, Steffensen, & Fields, 2011).

There are several investigation streams that remain to be explored, before the potential of MMP-9 to serve as a diagnostic marker can be fully realized. These include:

- **a.** MMP-9 specificity and selectivity as a biomarker and comparative advantages over current gold standard biomarkers such as BNP, N-terminal pro-BNP, troponin, or CRP need to be determined.
- **b.** The associations between MMP-9 levels and common risk factors for cardiovascular disease, including obesity, hypertension, smoking, diabetes, and dyslipidemia need to be dissected.
- **c.** Spatial and temporal MMP-9 patterns during the cardiac remodeling continuum and during other inflammatory disease (e.g. cancer, arthritis, and periodontal disease) are needed.
- **d.** The spatial and temporal patterns of MMP-9, compared with the patterns of other MMPs in cardiac remodeling and inflammatory diseases, need to be determined.

e. Standardized procedures and practices are needed for the pre-analytical, analytical, and post-analytical platforms to evaluate MMP-9 performance.

6 Conclusions

Current pre-clinical and clinical documentation strongly support MMP-9 as a panel member in the biomarker list to diagnose or treat the pathophysiology of post-MI ventricular remodeling and congestive heart failure. Immune cells such as neutrophils or macrophages modify many processes in the MI response, and future research focused on biochemical and structural approaches to examine the ECM will llikely provide new information on the remodeling process. Based on the evidence provided, further prospective studies are required to assess the prognostic value of MMP-9 for post-MI remodeling, particularly in comparison with traditional markers.

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Abbreviations

AAA	Abdominal aortic aneurysms
ACS	acute coronary syndrome
BNP	brain natriuretic peptide
CRP	C-reactive protein
ECM	extracellular matrix
IL	Interleukin
LV	left ventricle
MI	myocardial infarction
MMP	Matrix metalloproteinase
TIMP	tissue inhibitor of metalloproteinase

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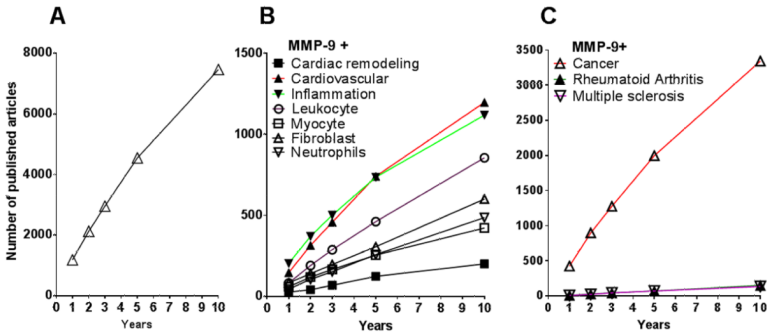


Figure 1.

Research articles and reviews on MMP-9 published in the last decade (2001–2010). A. Total number of articles on MMP-9 published. B. Number of articles published on MMP-9 and cardiovascular diseases, including articles on the proximal effect on cardiac remodeling. C. Number of articles published on MMP-9 in other inflammatory diseases (cancer, arthritis, and multiple sclerosis).

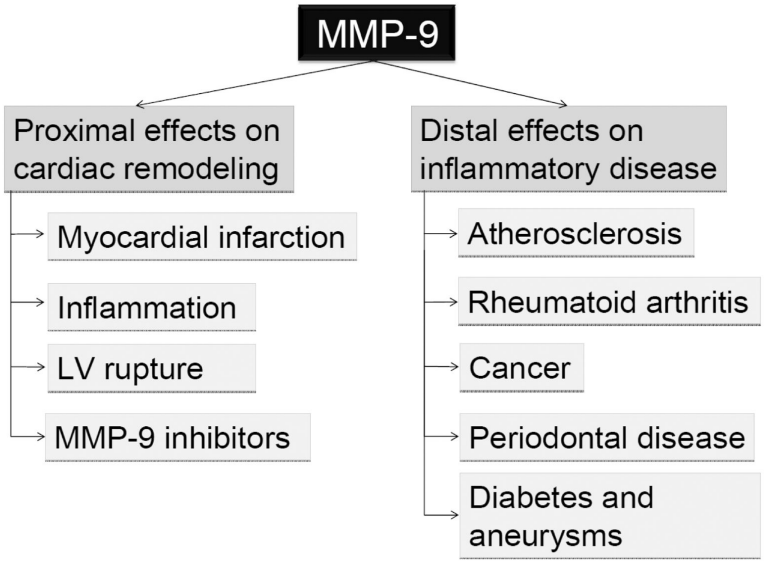
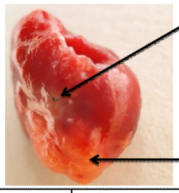


Figure 2.
The effect of MMP-9 is broadly classified into effects proximal and distal to cardiac remodeling. 1. Proximal effects targeted on cardiac remodeling and 2. Distal effects (non-targeted) on inflammatory diseases.

A. infarcted left ventricle



B. time course

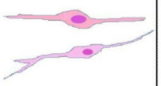
	Early phase	Intermediate phase	Late phase
Time (days)	1-2	3-7	>7
Cells involved	Myocytes Neutrophils	Neutrophils Macrophages Fibroblasts Endothelial cells Smooth muscle cells	Macrophages Fibroblasts Endothelial cells Smooth muscle cells
Major cell contributor of MMP-9	 Neutrophil	 Macrophage	 Fibroblast

Figure 3.

Schematic diagram presenting infarcted left ventricle and cell sources during the different stages of MMP-9 release as result of post-MI cardiac remodeling. A. Infarcted LV at 7 days post-MI. B. Following MI, MMP-9 is released by neutrophils in the early inflammatory phase (days 0–3), macrophages during the late inflammatory phase (days 3–7), and fibroblasts during the remodeling phase. Within the myocardium, cardiac myocytes, endothelial cells, and vascular smooth muscle cells are additional MMP-9 sources.

Table 1

Pre-clinical studies: A selection of articles to summarize the role of MMP-9 in cardiac remodeling

Reference and year	Animal model	Sex	Significant findings
Heymans, et al., 1999	MMP-9 null mice post-MI	Male	↓ cardiac rupture
Ducharme, et al., 2000	MMP-9 null mice post-MI	Male	↓ collagen accumulation and macrophage infiltration ↓ LV dimension ↑ MMP-2, MMP-13, and TIMP-1
Romanic, et al., 2001	Rabbit post-MI	Female	↑ MMP-9 within 24 hours following MI
Lindsey, et al., 2005	Aging CB6F1 mice	Both	↑ LV end-diastolic dimensions and wall thickness in middle aged and old mice. ↑ MMP-9 in old mice
Lindsey, et al., 2005	MMP-9 null mice post-MI	Both	↑ neovascularization post-MI
Mukherjee, et al., 2006	Gelatinase B/ lacZ transgenic mice post-MI	Both	↑ MMP-9 promoter induction at day 3, peaks at day 7
Yang, et al., 2006	C57BL/6J mice post-MI	Male	↑ LV rupture in middle aged mice ↑ LV remodeling and MMP-9 activity
Chiao, et al., 2011	Aging C57/BL6J mice	Both	↑ MMP-9 and MCP-1 levels in plasma and LV ↑ macrophage density in LV with aging

Table 2

Clinical studies: A selection of articles to summarize the role of MMP-9 in cardiac remodeling

Reference and year	Patient population (n)	Location	Significant findings	Conclusion
Blankenberg, et al., 2003	coronary artery disease (1127) *	Germany	MMP-9 higher at baseline in patients with a subsequent fatal CV event	MMP-9 as a novel predictor of future CV mortality
Squire, et al., 2004	acute MI (60) *	UK	MMP-9 peaks at days 1–4 post-MI	MMP-9 present during LV remodeling
Sundström, et al., 2004	previous MI but no heart failure (699) *	USA	Plasma MMP-9 linked to vascular risk factors and echocardiography measurements in males	MMP-9 levels associate with increased LV diastolic dimensions and increased wall thickness
Yan, et al., 2006	symptomatic heart failure with reduced ejection fraction (184) *	Canada and USA	↑ MMP-9 levels correlate with increased LV volumes and reduced LV ejection fraction in patients with heart failure	MMP-9 levels associate with cardiac dysfunction
Hlakty, et al., 2007	acute MI or stable angina (199) *	USA	↑ MMP-9 in acute MI but not stable angina patients	MMP-9 independently associates with development of an acute MI rather than stable angina
Martos, et al., 2007	hypertensive with diastolic dysfunction (86) *	Ireland	↑ MMP-9 in diastolic heart failure	↑ MMP-9 levels associate with active fibrosis
Orn, S et al., 2007	Long-term survivors after MI (52) *	UK and USA	↑ MMP-9 in the acute phase after MI, protective effect during late LV remodeling	No relationship between MMP-9 levels and scar size at any time point after MI
Van den Borne, et al., 2009	autopsy samples of post-MI ruptures (20) ¥	Netherlands	↑ MMP-9 in ruptured LVs	↑ MMP-9 in infarcted area associates with rupture
Hansson, et al., 2011	Uppsala Longitudinal Study of Adult Men (ULSAM)(1082) *	Sweden	↑ MMP-9 and TIMP-1 in men with CV mortality	MMP-9 and TIMP-1 are related to CV mortality risk
Kobayashi, et al., 2011	compare ST elevated ACS and non-ST elevated ACS stable angina patients (266) *	Japan	↑ MMP-9 early post-MI	MMP-9 has higher diagnostic accuracy for ACS than hstroponin

Abbreviations: CV; cardiovascular, LV; left ventricle, ESV; end systolic volume, HF; heart failure, MI; myocardial infarction

* Indicates that MMP-9 was analyzed by ELISA;

¥ indicates MMP-9 was analyzed by zymography or immunocapture activity.