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Assigning matrix metalloproteinase roles in ischaemic cardiac remodelling

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Abstract

Matrix metalloproteinases (MMPs) and their endogenous inhibitors have been studied in the myocardium for the past 2 decades. An incomplete knowledge base and experimental design issues with inhibitors have hampered attempts at translation, but clinical interest remains high because of strong associations between MMPs and outcomes after myocardial infarction (MI) as well as mechanistic studies showing MMP involvement at multiple stages of the MI wound-healing process. This Review focuses on how our understanding of MMPs has evolved from a one-dimensional early focus on measuring MMP activity, monitoring MMP:inhibitor ratios, and evaluating one MMP–substrate pair to the current use of systems biology approaches to integrate the whole MMP repertoire of roles in the left ventricular response to MI. MMP9 is used as an example MMP to explain these concepts and to provide a template for examining MMPs as mechanistic mediators of cardiac remodelling.

The wound-healing response to myocardial infarction (MI) includes a robust inflammatory response followed by scar formation[1,2]. The extracellular matrix (ECM) provides both structural and signalling reactions to MI that span a wide range of outcomes in animal models and humans[3,4]. Excessive inflammation and ECM deposition can increase left ventricular stiffness, a conduit for heart failure and arrhythmias. By contrast, an insufficient repair response can lead to left ventricular aneurysm or rupture. Mechanical properties of the infarct scar are an important determinant of outcomes after MI, and a balance is needed to produce a sufficient remodelling response to MI[5].

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Competing interests

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Matrix metalloproteinases (MMPs) are a family of 25 enzymes that proteolytically process both ECM and inflammatory proteins, making MMPs highly relevant to cardiac remodelling after MI[6,7]. Each ECM protein is processed by at least one MMP; as such, MMP activity directly shapes the scar structure after MI[8]. In addition to MMP9, MMP1, MMP2, MMP3, MMP7, MMP8, MMP12, MMP13, MMP14, and MMP28 have been evaluated in the plasma or left ventricle of animals and humans[3,6,7,9,10]. A summary of the similarities and differences between MMP family members is provided in TABLE 1. Of the MMPs that have been evaluated in the circulation and myocardium following MI, MMP9 is the most studied, and we have garnered substantial information on MMP9 promoter activity, polymorphisms in humans, effects of post-translational modifications, and consensus sequences for substrates cleaved by this enzyme[7,11,12] (FIG. 1). With the exception of MMP2, only one isoform has been reported for each of the other MMPs. In addition to the full-length form of MMP2 that localizes to both extracellular and intracellular spaces, cardiomyocytes produce an amino-terminal-truncated 65 kDa form of MMP2 in response to oxidative stress that lacks the secretory and pro-domain sequences[13,14]. This amino-terminal-truncated MMP2 localizes to the mitochondria and triggers the transcription of inflammatory and proapoptotic genes. At the same time, extra-cellular MMP2 processes ECM substrates, such as type I collagen, to facilitate the migration of inflammatory cells into the infarct region6. This arrangement illustrates how an MMP can regulate cell physiology and inflammation from inside and outside the cell.

The net effect of an MMP at a given time and place (local cell and global myocardial region) depends on the availability of substrates for processing and the presence of endogenous inhibition that limits MMP activity. As such, MMPs can stimulate ECM synthesis or degradation, can be pro-inflammatory or anti-inflammatory, and can promote or prevent angiogenesis, depending on the substrate context[11,15]. This Review does not attempt to describe all MMPs, their substrates, and the tissue inhibitors of metalloproteinases (TIMPs) present in the myocardium under different conditions. Instead, this Review refers to resources where this information is readily available, and discussion is focused on concepts that will drive the cardiac MMP research field forwards. Rather than attempting to summarize our current knowledge of all MMPs, MMP9 is used to set the historical context for the evaluation of MMPs in the left ventricle and provide a template for assigning MMP causation in cardiac remodelling. Although concentrated on MMP9, this template has broad applicability to other MMPs and to other proteases.

Assessment of cardiac MMPs

Early approaches.

MMPs were first measured in the pathological myocardium in the mid-1990s[15]. Early examinations had three major goals: measuring MMP presence and activity, monitoring MMP:TIMP ratios, and correlating one MMP to one substrate. Historically, MMPs were evaluated using enzyme-based activity assays (in vitro cleavage and zymography), primarily because of a lack of antibodies. Zymography is a technique whereby a substrate (most commonly gelatin or casein) is incorporated into an SDS-polyacrylamide gel, which allows the molecular weight of the protease to be observed[16]. Gelatin is a substrate for MMP2 and

MMP9, whereas casein is a substrate for MMP3; therefore, zymograms using these substrates preferentially favour the evaluation of these respective MMPs. Both pro and active forms can be visualized because MMPs in the presence of substrate can be activated by a conformational change that does not remove the pro-domain[17]. As a consequence of the technological ease of using gelatin zymography to measure activity levels compared with other substrates such as casein (which has solubility issues), MMP2 and MMP9 became over-represented in the myocardial literature. Zymogram results are at risk of being overinterpreted because zymography is a TIMP-free environment except where the inhibitor localizes (due to separation away during gel electrophoresis), and, therefore, the technique might not measure actual in vivo activity; instead, zymography measures activity potential. Some research groups have addressed this issue by using in situ zymography to measure activity in the setting of the MI environment[18].

Current approaches.

Since the development of a multitude of antibodies for each MMP and TIMP, including antibodies to specific regions (such as the pro, hinge, or catalytic domains), the use of immunoblotting is now the preferred method for assessing MMP relative concentrations in tissue and plasma. Pro and active MMP can be distinguished by differences in molecular weight of about l0 kDa owing to cleavage of the pro-domain. Note that measuring leukocyte-derived MMPs (including MMP7, MMP8, MMP9, MMP12, MMP14, and MMP28) in serum is not appropriate because circulating leukocytes produce MMPs, and the clotting process artificially elevates MMP concentrations by stimulating secretion[15].

The current preferred method to assess in vivo MMP activity is to show substrate cleavage. Cleavage can be assessed in an unbiased proteomics approach, for example, by comparing 2D gels from wild-type and knockout mouse left ventricular tissue after MI. This approach was used to identify fibronectin as an in vivo MMP7 and MMP9 substrate[19,20]. Using an MMP-specific substrate catalogue, a targeted approach can also be used to focus on substrates present in the post-MI left ventricle at the time of evaluation. The simplest method for this approach is immunoblotting, showing full-length and smaller substrate fragments. MMP specificity can be established by subtracting out MMP deletion or inhibition patterns to remove false-positives caused by nonspecific staining. Mass spectrometry can be used to sequence the immunoblot bands and map substrate cleavage sites. This approach assumes that the substrate catalogue is sufficiently inclusive and that the most relevant MMP substrates in MI have been identified. This assumption is likely to be true for MMP9, which has been extensively evaluated, but is probably not true for MMP12 and MMP28, which have little available information on substrates relevant to MI.

MMP data are often expressed as a ratio of MMP:TIMP values, particularly in clinical research. For example, MMP9 preferentially binds to TIMP1 and, for this reason, MMP9:TIMP1 ratios are often reported[15]. This oversimplifies the in vivo setting, where multiple MMPs interact with all four TIMPs. In addition to TIMPs, α2-macroglobulin prevents MMP activity in the circulation. Therefore, MMP:TIMP ratios should be interpreted with caution.

Finally, each MMP has a variety of substrates that it can proteolytically process, and each substrate can be cleaved by at least two different MMPs. For example, type I collagen (full-length and denatured) is an MMP9 substrate also processed by MMP1, MMP8, MMP13, and MMP14 (REFS[11,12,21]), illustrating both competition and compensation across MMPs and their substrates. A more detailed summary of the history of MMPs, in terms of the major breakthroughs, myths that have arisen, and misperceptions that have been made in data analysis has been published previously[15].

Establishing causal roles

In order for an MMP to have a causal role in cardiac remodelling after MI, the concentration of the MMP must increase or decrease following MI, have effects on cardiac remodelling when inhibited or overexpressed, operate through actions that can be mimicked in vitro by Mi-relevant cell types, and involve substrates whose proteolysis modulates aspects of cardiac remodelling[22]. Using these four criteria, we have created a template for establishing MMP causality (FIG 2). Using MMP9 as an example for MMP involvement in Mi-induced cardiac remodelling, we describe the current evidence for each criterion (TABLE 2.).

Criterion 1: MMP9 levels increase after MI.

According to this criterion, the first stage of observation is that the effect on cardiac remodelling should be in direct proportion to the change in MMP concentration. The effect is U-shaped rather than linear, as low and high MMP9 concentrations have similar end-organ outcomes, albeit through different mechanisms[8,23–26]. Ample evidence exists for this first observation stage, given that the MMP9 level is elevated in plasma and left ventricle after MI in mice, rats, hamsters, rabbits, pigs, sheep, dogs, and humans[11,12,15,22]. In humans, high plasma MMP9 levels are predictive of cardiovascular mortality in individuals with coronary artery disease[27,28]. The post-MI increase in MMP9 levels could be due to an increase in the number of MMP9-producing cells or to an increase in the amount of MMP9 produced per cell. Although some evidence is available for the second option, ample supporting evidence exists across species of a large infiltration of neutrophils and macrophages into the myocardium during the first days after MI. In addition to MMP9, levels of MMP2, MMP7, MMP8, MMP12, and MMP14 increase in the left ventricle after MI owing to increases in the numbers of leukocytes, endothelial cells, and fibroblasts as well as increases in the production MMPs per cell[6].

In some cases, there might not be a net change in myocardial expression of an MMP, but the difference in cell-type expression is relevant. This situation is seen for MMP28, for which baseline expression is high in cardiomyocytes and post-MI expression is high in macrophages[29]. Net MMP28 levels, therefore, decrease early after MI owing to cardiomyocyte loss, but the concentration of macrophage-derived MMP28 increases during the shift in cellular source of MMP28. With membrane-bound MMPs, including MMP14, which is expressed in cardiomyocytes, macrophages, and fibroblasts, a sharp increase in MMP expression in one cell type might mask a decrease in another cell type. Evaluating the cellular sources of MMPs provides an additional layer of information.

The second stage of observation for this criterion is that MMP9 proteolyses ECM substrates relevant to cardiac remodelling. Hundreds of in vitro MMP9 substrates have been identified, but few have been shown to be processed in vivo by MMP9. The MEROPS peptidase database provides a useful resource for proteases, inhibitors, and identified substrates. The list of confirmed in vivo MMP9 substrates includes type I collagen and fibronectin, as well as ATP-citrate synthase, platelet glycoprotein 4, and osteopontin[19,26,30,31].

Although most MMPs (with the exception of MMP11, MMP14, and MMP28) are secreted and require extracellular activation, we identified ATP-citrate synthase as an intracellular MMP9 substrate[30]. *Mmp9* deletion increased ATP-citrate synthase activity after MI, resulting in reduced inflammation and improved cardiac physiology by preserving mitochondrial superoxide dismutase [Mn] levels and mitochondrial function. In addition to MMP9, intracellular substrates have been identified for MMP2, MMP3, MMP13, and MMP14 (REF.[30]].

Osteopontin is a known in vitro substrate for MMP9, as well as MMP2, MMP3, and MMP7 (REF.[31]). Proteomic analysis of left ventricular post-MI tissue combined with cleavage assays on peptides that span the cleavage site revealed that osteopontin is cleaved in vivo at three sites (at amino acids 151–152, 193–194, and 195–196). Osteopontin fragments upstream and downstream of these sites increased the rate of cardiac fibroblast migration, indicating that cleavage generates peptide fragments with biological activity. MMPs also generate collagen and fibronectin fragments that stimulate new production of these ECM proteins owing to feedback signalling[11,12,19].

Proximity of MMP9 to its substrate can vary both temporally and spatially. Temporally, MMP9 cleaves substrates derived from dying cardiomyocytes and neutrophils early after MI and later cleaves substrates derived from macrophages and fibroblasts. Therefore, tracking the time course of both the MMP level and its substrate portfolio is important in assigning probability of substrate cleavage. Spatially, MMP9 and its substrates can come from either the same or distinct cellular sources. Collagen is predominantly produced by reparative cardiac fibroblasts, which do not highly express MMP9, whereas IL-8 and MMP9 are both derived from neutrophils, and osteopontin and MMP9 are both derived from macrophages[11]. This precision in MMP and substrate localization helps to time wound-healing events. For example, collagen degradation is stimulated during the inflammation period after MI and is not stimulated during scar formation. In addition to ECM, MMP9 proteolyses a large number of inflammatory mediators and growth factors, including angiogenic stimulators and fibroblast activators. A sample of known MMP9 substrates and the effect of MMP9 cleavage on their activity is provided (TABLE 3).

Criterion 2: MMP9 inhibition or overexpression has effects on cardiac remodeling.

Effects of MMP deletion or inhibition on cardiac remodelling after MI have been evaluated for MMP2, MMP7, MMP9, MMP12, MMP14, and MMP28 (REF.[6]). Ample evidence shows that this criterion is met for MMP9 (REF.[11]). These studies highlight the complexity of the MMP9 system because both *Mmp9* deletion and *Mmp9* overexpression specifically in macrophages yield positive effects on cardiac remodelling after MI[11,25]. Likewise, *Mmp9* deletion from birth in all cells has a net beneficial effect on cardiac remodelling after MI,

whereas MMP9 inhibition started at 3h after MI (a time that mimics early clinical intervention) has a net detrimental effect on cardiac structure and physiology[24]. *Mmp9*$^{-/-}$ mice had no overt cardiac phenotype in the absence of myocardial injury, but proteomic evaluation identified 34 proteins with differential levels in plasma compared with wild-type mice. Baseline differences between wild-type and *Mmp*$^{-/-}$ mice, therefore, might explain the striking differences in outcomes after MI.

In addition to showing evidence for this criterion through the use of mouse models of MMP modification or inhibition, the use of TIMP animal models is another approach to assess MMP effects[32]. The results of the combined studies are reconciled by considering MMP9 effects on individual substrates as part of the whole system. For example, the list of MMP9 effects includes activating beneficial factors and deactivating detrimental factors (such as releasing latent transforming growth factor (TGF)-β-binding protein and inactivating platelet glycoprotein 4) as well as deactivating beneficial factors and activating detrimental factors (such as activating IL-lβ and inhibitors of angiogenesis). Therefore, the net effect of MMP9 is beneficial when the first group predominates and is detrimental when the second group predominates. Understanding the interplay between substrates helps to design inhibition or overexpression strategies based on timing and location.

A corollary of this criterion is that therapies that reduce MMP9 activity should also have an effect on cardiac remodelling, which has been shown for statins, angiotensin-converting enzyme inhibitors, endothelin-receptor antagonists, and β-blockers[3,7,22]. Measuring MMP9 gene and protein expression is a common output for studies examining the responses of genetically modified mice to MI. Evaluating MMP9 is an important way to connect intracellular effects to the extracellular environment and changes in left ventricular physiology.

Criterion 3: MMP9 actions can be mimicked in vitro by MI-relevant cell types.

This criterion incorporates cell signalling aspects and considers how ECM is bridged with intracellular changes. To date, the majority of work in this area considers MMP9 as an upstream stimulus with an indirect role. For example, after MI, IL-8 is a potent inducer of MMP9 release from the gelatinase granules within neutrophils, and high IL-8 and MMP9 levels have both been associated with worse outcomes after MI. MMP9 has been used to stimulate macrophages directly to produce a mixed transition state of M1–M2 polarization[33]. Specifically, levels of C-C motif chemokine 5 (CCL5) are higher and those of CCL3, IL-lβ, IL-6, and TGFβ are lower in MMP9-stimulated macrophages. MMP12 has been used to stimulate apoptosis in neutrophils isolated from the blood[34]. Whether this effect occurs as a result of MMP12 acting as a ligand to stimulate receptor-mediated cell signalling or as an upstream proteolytic mediator of signalling has not been investigated.

Another indirect way in which this criterion is demonstrated is through effects on cell physiology. Transgenic mice that overexpress *Mmp9* specifically in macrophages show differences in macrophage phenotypes at day 7 after MI[25]. Transcriptomic analysis of macrophages isolated from the infarct and border regions in these mice showed reduced levels of vascular endothelial growth factor A, platelet-derived growth factor A, and TGFβ3, together with elevated levels of TIMP4, revealing potential indirect influences of *Mmp9*

overexpression on fibroblasts and endothelial cells. With the exception of latent TGFβ3, which is activated by MMP2 and MMP9, whether these factors are MMP substrates has not been evaluated. As another example, MMP9 processes platelet glycoprotein 4 into several fragments that prevent neutrophil apoptosis and reduce macrophage phagocytosis by diminishing cell-surface-associated platelet glycoprotein 4 (REFS[24,26,35]). MMP9 deficiency, therefore, stimulates neutrophil removal by activating apoptosis and promotes tissue clearance by activating macrophage phagocytosis.

Criterion 4: Proteolysis of MMP9 substrates modulates cardiac remodeling.

A number of ECM fragments are produced by MMPs and have been summarized previously, including a schematic of the major protease families generating ECM bioactive fragments and a list of the receptors that interact with these fragments[36]. This fourth criterion is best illustrated in the evaluation of the collagen-derived matricryptin, C-1158/59 (REF.[21]). This collagen fragment is generated by both MMP2 and MMP9, and is further degraded to inactivity by MMP9; as such, levels of this fragment are low when MMP9 concentrations are high. When exogenously infused after MI in mice, the peptide stimulates neovascularization and prevents left ventricular dilatation. A template for how to identify and then evaluate MMP9 substrate cleavage products for biological activity is given (FIG. 3). Findings to fulfil this criterion highlight several concepts: not all cleavage products generated by MMP9 proteolysis promote adverse cardiac remodelling, and focusing on the substrate rather than the MMP provides hierarchical information on where that substrate falls in the preference ranking for a particular MMP. For example, major substrates are expected to recapitulate many, if not all, aspects of cardiac remodelling affected by the MMP, whereas minor substrates have little or no effect. Whether a particular substrate has a preference for one MMP over others can be shown by examining affinity constants of binding and by competition binding assays.

In addition to binding substrates for cleavage, MMP9 can bind to proteins and form complexes. For example, MMP9 binds to neutrophil gelatinase-associated lipocalin (NGAL) in humans but not in mice[37]. This difference is due to low homology between human and mouse forms of NGAL; in particular, human NGAL has a cysteine at amino acid 87 that allows the MMP9–NGAL complex to form. Understanding how MMPs form nonproteolytic complexes with other proteins and what consequences this binding has is the subject of ongoing investigation. This example highlights the need to examine MMP effects across species.

Integrative effects of MMP9 after MI

On the basis of the information gathered to address these four criteria, our current knowledge of MMP9 roles in cardiac remodelling after MI can be assembled (FIG. 4). Some highlights of our knowledge base include the following four points. First, a number of known inputs stimulate MMP9 production, including cytokines and chemokines present in the myocardium after MI. In addition to MMP9, other inflammation-responsive MMPs include MMP3, MMP7, MMP8, MMP10, MMP12, MMP14, and MMP28 (REF.[38]). Second, MMP9 is produced by a variety of cell types, including cardiomyocytes, endothelial cells,

fibroblasts, and leukocytes. Of these cells, leukocytes (neutrophils and macrophages) are the largest producers of MMP9 in the infarct zone[18,39]. Neutrophils and macrophages also contribute to the production of inflammation-responsive MMPs. Although cardiac fibroblasts can produce MMP9, these cells are not a major source of MMP9 at day 7 after MI. Third, MMP9 is regulated at multiple steps, which highlights the need for tight control of MMP activity. Regulation of MMP9 activity occurs at the levels of synthesis, secretion, activation, inhibition, and substrate availability. Fourth, MMP9 actions influence molecular, cellular, and tissue aspects of cardiac remodelling: all scales are involved.

Strategies for MMP9 inhibition

MMP inhibition has been under investigation since the early 1990s and has evolved from general MMP inhibition that blocked activity of other protease families (with adverse consequences) to more selective inhibition of a few MMPs to even more selective inhibition of specific MMPs[9]. When a specific MMP9 inhibitor was administered at 3h after MI in mice, the effect was impaired wound healing[24]. The next generation of MMP inhibition might involve the consideration of particular substrates as targets, rather than a specific MMP.

To date, the only FDA-approved MMP inhibitor is doxycycline, which inhibits MMP2 and MMP9. Effects of doxycycline treatment in patients with acute MI and left ventricular dysfunction were evaluated in the TIPTOP trial[40]. When given at a very specific time frame and at a low, subantimicrobial dose (l00 mg twice daily for 7 days, started immediately after percutaneous intervention; $n = 110$), doxycycline reduced infarct size and left ventricular end-diastolic volumes. The low dose of the inhibitor used suggests that there is a hierarchy of MMP preference for particular substrates, such that low doses can distinguish profiles of proteolysis. This concept is an avenue that has not been explored. MMP inhibition with doxycycline can also produce indirect or off-target effects, some of which are beneficial. For example, TIMP2 levels are elevated with doxycycline treatment, illustrating a potential feed-forward mechanism whereby the presence of one inhibitor expands the MMP-inhibition capacity by upregulating other inhibitors with broader specificity[41]. In addition, evaluating MMP inhibitors in the setting of MMP polymorphisms is understudied. Several *MMP9* polymorphisms have been identified that increase MMP9 activity (FIG. 1b), and the *MMP9* 1562 C/T polymorphism has been associated with increased incidence of MI, at least in some ethnic groups[6]. The effects of MMP inhibition in individuals with *MMP9* polymorphisms remain to be investigated.

Future directions

Of the ten MMPs that have been evaluated after MI (criterion 1), our team has contributed to the understanding of MMP7, MMP9, MMP12, and MMP28 (REF.[6]). The 15 other MMPs have not even been evaluated for criterion 1, and several of the MMPs in the evaluated list (MMP1, MMP3, MMP8, and MMP13) have not been examined beyond criterion 1. MMP1 has two isoforms in the mouse (MMP1a and MMPlb), which complicates interspecies evaluation. One future direction is to develop the MMP knowledge map for each of these MMPs, including using matridomics to catalogue all the ECM components modified by the

MMP and degradomics to map substrate cleavage sites[22,42,43]. In terms of rigour and reproducibility, using established guidelines for antibody, MI, and cardiac physiology experiments will help to combine results from different investigators and laboratories[44–46]. Suggested experiments to fill in the gaps for each of the criteria are provided in TABLE 4.

Note that MMP9 is an example — not a prototype — MMP because each MMP has distinct substrate profiles and actions. For example, galectin 3 is known to be processed only by MMP9, whereas fibronectin is processed by various MMPs (MMP2, MMP7, MMP9, MMP12, MMP13, and MMP14). The mechanisms by which MMPs interact with each other in the myocardium has not been examined past the point of evaluating which MMPs compensate for the loss of one MMP and which MMPs serve as upstream activators for other MMPs. MMP11 and MMP14 have a functional relationship in cancer, with MMP14 providing pericellular anchoring for MMP11 (REF.[47]). *Mmp9* deletion results in a compensatory increase in cardiac MMP13 levels at baseline[11]. MMP3 and MMP7 are considered general MMP activators that can cleave the pro-domains of a number of MMPs; the effects of MMP3 and MMP7, therefore, are both direct on substrate cleavage and indirect on activation of other MMPs[6]. Type I collagen is predominantly cleaved by MMP14, but MMP2, MMP8, MMP9, and MMP13 can also cleave type I collagen[48]. Membrane-bound MMP14 can be solubilized with preserved proteolytic activity in cancer cell lines[49], and this shedding provides a mechanism for one cell to exert paracrine effects on another. The effects of post-translational modifications, beyond pro-domain release to activate the MMP, need to be considered. In particular, the effects of glycosylation on MMP activity and functions are just beginning to be evaluated[50].

Understanding of how one MMP can have a variety of effects on the left ventricle after MI and how a variety of MMPs can each work on the same substrate is needed to understand the complex interplay and to extrapolate the net effect of multiple simultaneous and serial perturbations. Because MMPs work in concert with each other and with other aspects of the cardiac remodelling process, another future direction is to use systems biology approaches to understand MMP interplay within the context of the whole left ventricle. For example, the interconnection between macrophages and neutrophils with ECM and MMPs is an area of active research[51–54]. Additional components for the network include microRNAs and non-coding RNAs, several of which are known to interact with MMP9, as well as the influence of age and sex[23,55–57]. Circadian rhythm effects might need to be taken into consideration because genes encoding circadian clock proteins are connected to MMP expression[58]. For example, *Arntl*$^{-/-}$ mice have elevated MMP2 and MMP9 levels that are coincident with vascular stiffness[59], and expression of aryl hydrocarbon receptor nuclear translocator-like protein 1 (ARNTL) is reduced, whereas expression of MMP1 and MMP9 is increased in macrophages stimulated with *Mycobacterium tuberculosis* infection[60]. In addition, more studies on how MMP9 inhibition might interact in combination with other strategies, such as reperfusion, are needed[61]. The focus of this Review was on MMPs in cardiac remodelling after MI, and consideration needs to be given to MMPs in other cardiovascular conditions (such as ageing) and pathologies, including hypertension (pressure overload) and heart failure with either preserved or reduced ejection fraction[6,62].

Conclusions

MMPs are well-known mediators of cardiovascular pathophysiology. Although past strategies to inhibit MMPs have not been successful (with the exception of doxycycline), very selective and specific MMP inhibitors are now available. These approaches might have limited clinical use owing to the wide variety of substrates proteolysed by each MMP. A more effective strategy might be to target a specific substrate or group of substrates for modification. Regardless of the current constraints of inhibition strategies, MMP9 is an important mediator of cardiac remodelling after MI and is centrally involved in inflammation and repair components of the response. As such, MMP9 will continue to be examined for mechanistic insights into predicting and therapeutically improving outcomes.

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Key points

- Matrix metalloproteinases (MMPs) are not one-size-fits-all enzymes; MMPs overlap in substrate profiles, but each has a distinct role in cardiac remodelling after myocardial infarction.
- MMP9 is the most-studied MMP in cardiac remodelling after myocardial infarction.
- MMP roles are dictated by the substrates they process, and the best way to assess in vivo MMP activity is to show substrate cleavage.
- The mechanisms by which MMPs interact with each other in the myocardium have not been examined beyond which MMPs compensate for the loss of one MMP and which MMPs serve as upstream activators for other MMPs.
- This Review provides a template for examining MMPs as mechanistic mediators of cardiac remodelling.

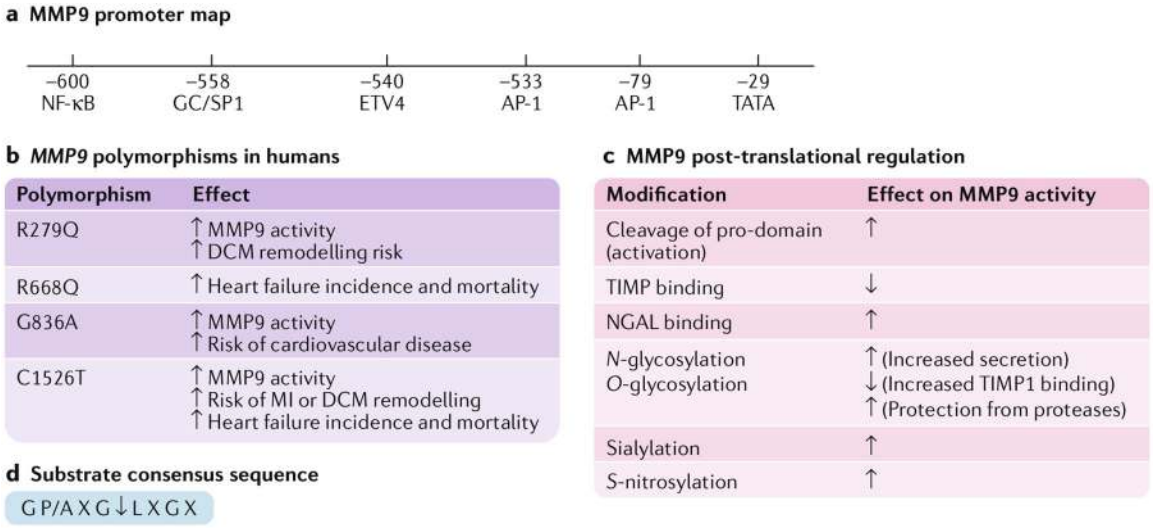

Fig. 1 |. Modifiers of MMP9 expression and activity[12,28,50,63–66].

a | Matrix metalloproteinase 9 (MMP9) promoter map showing promoter elements and binding regions of transcription factors. **b** | Identified human *MMP9* polymorphisms and the effects of these polymorphisms on MMP9 activity. **c** | MMP9 post-translational regulation includes proteolytic activation, binding to inhibitors, increased expression by binding to other proteins, and changes in secretion owing to post-translational modifications. **d** | MMP9 substrate consensus sequence derived from evaluation of known cleavage sites in substrates; MMP9 preferentially cleaves proteins with this sequence. AP-1, activator protein 1; DCM, dilated cardiomyopathy; ETV4, ETS translocation variant 4; GC, GC box; MI, myocardial infarction; NF-κB, nuclear factor-κB; NGAL, neutrophil gelatinase-associated lipocalin; SP1, transcription factor Spl; TATA, TATA box; TIMP, tissue inhibitor of metalloproteinases.

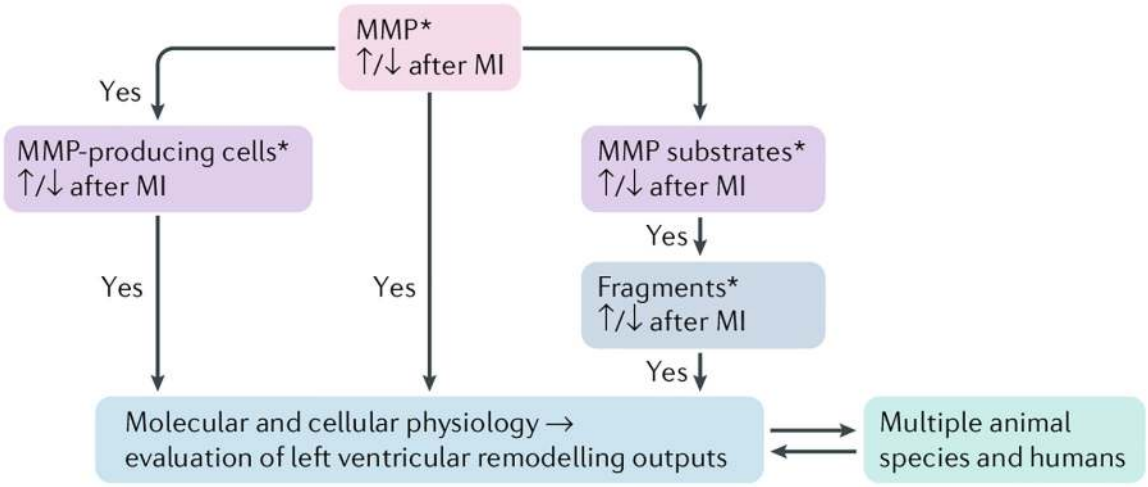

Fig. 2 |. Template for establishing MMP9 causality.

This template highlights that matrix metalloproteinase (MMP)-relevant biomarkers include the MMP, the cell that produces it, the substrates processed, and the proteolytic fragments generated. The iterative process of MMP evaluation spans multiple animal models and evaluation in humans. *Candidate intervention node and biomarker. MI, myocardial infarction.

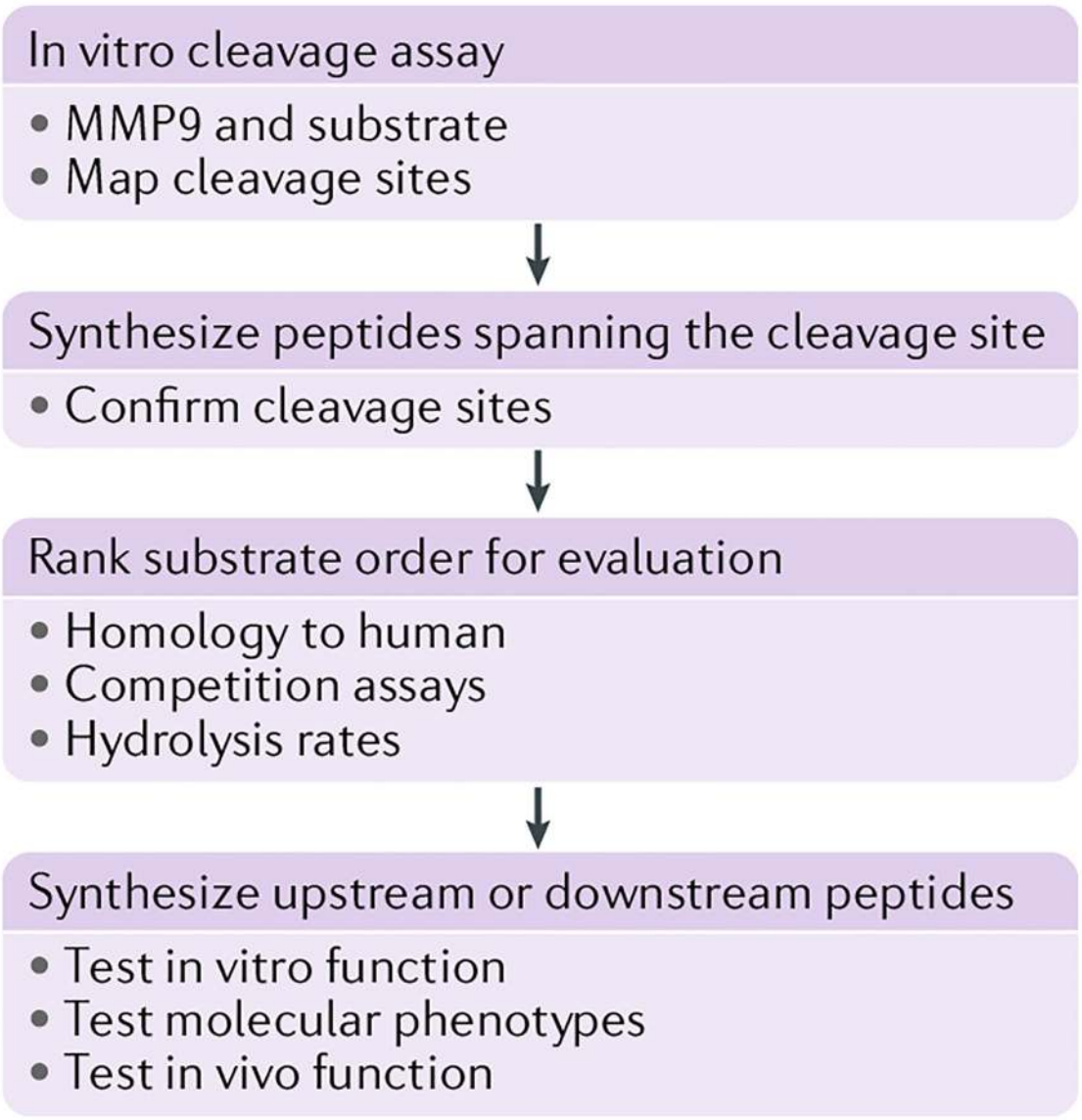

Fig. 3 |. Template for identifying and evaluating extracellular matrix fragments generated by MMP9.

Following identification of a cleavage site with the use of mass spectrometry, an in vitro cleavage assay can be used to confirm substrate processing by matrix metalloproteinase 9 (MMP9). Peptides spanning the cleavage site can be used to confirm cleavage-site location, and competition assays can be used to rank MMP9 preferences for substrates within a mixed pool. Peptides upstream and downstream of the cleavage site can show whether the generated fragments have biological activity. The same approach can be used for other MMPs and other protease families.

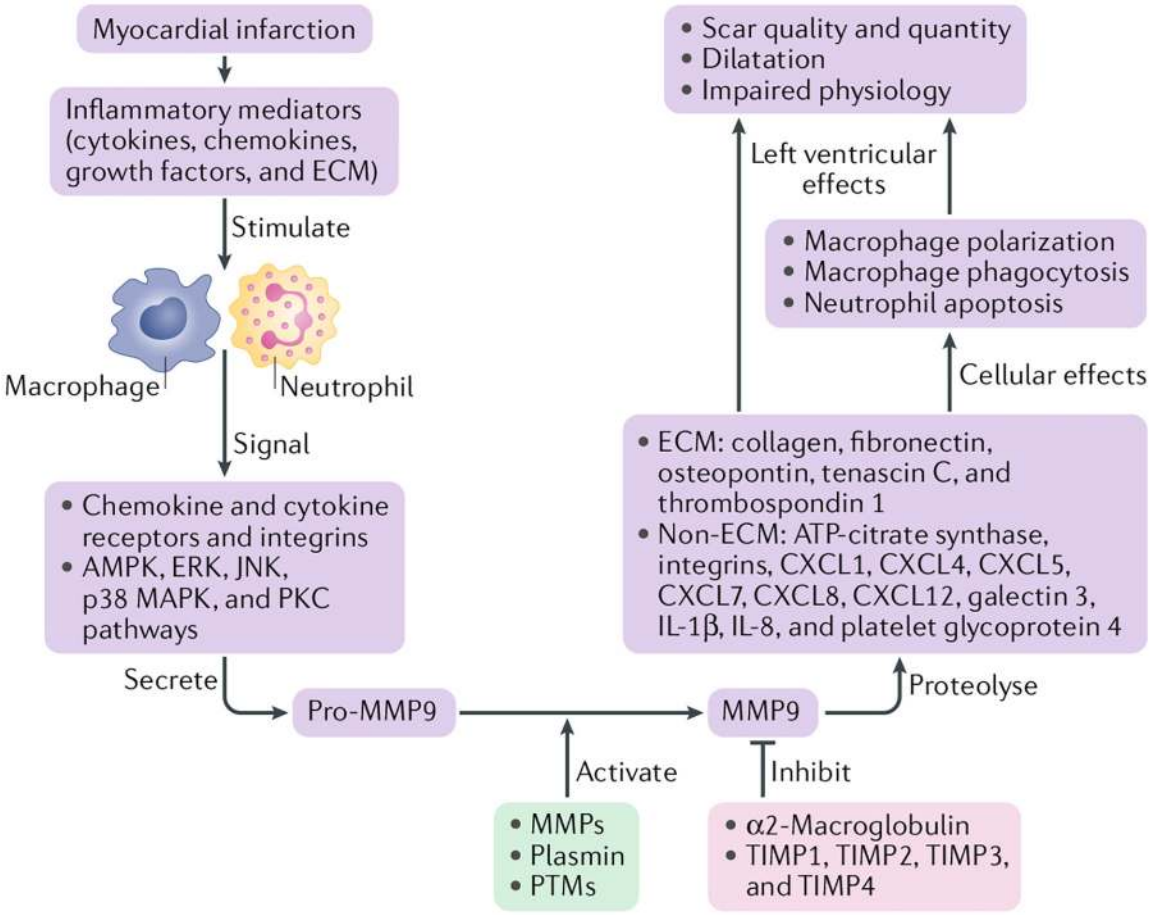

Fig. 4 |. MMP9 roles in cardiac remodelling after myocardial infarction[26,67–74].
The matrix metalloproteinase 9 (MMP9) map includes knowledge of which factors stimulate production and secretion of MMP9, which factors stimulate or inhibit its activation, which substrates can be processed by MMP9, and how cellular and left ventricular physiology are altered. AMPK, AMP-activated protein kinase; CXCL, C-X-C motif chemokine; ECM, extracellular matrix; ERK, extracellular-signal-regulated kinase; JNK, JUN N-terminal kinase; MAPK, mitogen-activated protein kinase; PKC, protein kinase C; PTM, post-translational modification; TIMP, tissue inhibitor of metalloproteinases.

Table 1 |

Comparisons between MMPs in cardiac remodelling after MI

MMP	Cellular expression after MI	MI-relevant substrates		Actions	
		Shared with MMP9	Not shared with MMP9		
MMP2	Cardiomyocytes, endothelial cells, fibroblasts, macrophages, and vascular smooth muscle cells	Complement C1q, fibrinogen, fibronectin, galectin 3, IL-8, laminin, latent TGFp, plasminogen, troponin, TNF, and type I and IV collagen	Fibroblast growth factor receptor 1 and SPARC	•	Owing to high constitutive activity, considered MMP housekeeper that regulates normal tissue turnover[6]
				•	Deletion or inhibition prevents cardiac rupture[75]
				•	Mediates intracellular proteolysis of myocyte sarcomeric proteins[13,14]
				•	Generates laminin fragments that prevent macrophage migration[75]
MMP7	Cardiomyocytes, endothelial cells, and macrophages	Fibrinogen, fibronectin, laminin, MMP1, MMP2, MMP9, tenascin C, TNF, and type IV collagen	Gap junction-α1 protein and SPARC	•	Lateralization of gap junction-a1 protein[76]
				•	Deletion after MI reduces left ventricular dilatation and increases survival owing to improved electrical conduction[76]
MMP12	Endothelial cells, fibroblasts, macrophages, neutrophils, and vascular smooth muscle cells	Fibronectin, heparan sulfate, hyaluronan, laminin, plasminogen, platelet glycoprotein 4, and type I and IV collagen	Osteonectin	•	Platelet glycoprotein 4 cleavage reduces neutrophil apoptosis and macrophage phagocytosis[34]
				•	Type IV collagen cleavage allows macrophage and fibroblast migration[34]
				•	Inhibition initiated after MI increases cardiac rupture rates and delays neutrophil apoptosis to prolong tissue degradation[34]
MMP14	Cardiomyocytes, fibroblasts, and macrophages	Fibrinogen, fibronectin, laminin, periostin, TGFp, tenascin C, and type I collagen	CD44 antigen, MMP2, MMP13, and perlecan	•	Degrades type I collagen and fibronectin to reduce extracellular matrix structural support[48]
				•	Stimulates fibrosis by activating TGFp and periostin[48]
				•	Activates MMP2 through interaction with TIMP2 (REF[48])

MI, myocardial infarction; MMP, matrix metalloproteinase; SPARC, secreted protein acidic and rich in cysteine; TGFβ, transforming growth factor-β;TIMP2, metalloproteinase inhibitor 2; TNF, tumour necrosis factor.

Table 2 |

Evidence for MMP9 involvement in cardiac remodelling after MI[11]

Criteria	Evidence
Criterion 1: MMP9 levels increase after MI	• Increased in plasma (mice and humans)[77–79] • Increased in left ventricular infarct area (mouse, rat, hamster, rabbit, sheep, pig, dog, and human)[18,24,80–86] • Increased in cardiac lymph (dog)[18]
Criterion 2: MMP9 inhibition or overexpression has effects on cardiac remodelling	• Genetic deletion: improves left ventricular physiology and remodelling after MI, improves age-related cardiac dysfunction, and attenuates angiotensin II-induced cardiac fibrosis[80,87,88] • Macrophage-specific overexpression improves left ventricular physiology and remodelling after MI in adult and aged mice[25] • Global MMP inhibition improves cardiac remodelling, but early pharmacological MMP9 inhibition (3 h after MI) worsens cardiac remodelling[24,89]
Criterion 3: MMP9 actions can be mimicked in vitro by MI-relevant cell types	• Decreased neutrophil apoptosis[24] • Decreased macrophage phagocytic capacity[24,26]
Criterion 4: Proteolysis of MMP9 substrates modulates cardiac remodelling	C-1158/59 (a collagen-derived matricryptin) is protective in cardiac remodelling after MI[21]

MI, myocardial infarction; MMP, matrix metalloproteinase.

Table 3 |

MI-relevant MMP9 substrates and effects of proteolysis[12,15]

MMP9 substrate	Effect of proteolysis on the substrate
ATP-citrate synthase[a]	Fragments
CXCL1, CXCL4, CXCL5, CXCL7, and CXCL12	Inactivates
CXCL5 and CXCL8	Activates
Decorin	Degrades
Elastin	Fragments
Endothelin	Activates
Fibronectin[a]	Fragments
Galectin 3	Degrades
IL-1P and IL-8	Activates
Integrins	Solubilizes
Intercellular adhesion molecule 1	Degrades
Laminin	Fragments
Latent transforming growth factor-p-binding protein	Releases
Osteopontin	Activates
Plasminogen (to angiostatin)[a]	Activates
Platelet factor 4	Degrades
Platelet glycoprotein 4[a]	Inactivates
Pro-MMP2, pro-MMP9, and pro-MMP13	Activates
Tenascin C	Degrades
Thrombospondin 1	Fragments
Type I[a], II, III[a], IV, V, XI, and XVI collagen	Fragments

CXCL, C-X-C motif chemokine; MI, myocardial infarction; MMP, matrix metalloproteinase.

[a]Confirmed in vivo (all substrates confirmed in vitro).

Table 4 |

Experiments to address criteria for establishing MMP causality

Criterion addressed	Experiment	Establishes
1	qRT-PCR, immunoblotting, in situ hybridization, and immunohistochemistry of plasma, infarct tissue, or isolated cells	Changes in MMP levels after MI and which cells express the MMP
2	• Gene deletion or overexpression, pharmacological agents (MMP inhibitors), and other blocking strategies (antisense oligonucleotides and small interfering RNA) • Outputs: left ventricular physiology and remodelling, cell physiology, and gene or protein expression	MMP is in the signalling pathway
3	• Stimulate naive cardiac cells with secretome of post-MI, MMP9-modified cells, use proteomics to identify secretome constituents, and use blocking antibodies to assign cause and effect relationships • Outputs: cell physiology and gene or protein expression	Intercellular communication is paracrine
	Cell co-cultures	Intercellular communication requires direct contact
4	Proteomics (matridomics and degradomics) to identify MMP substrates, surface plasmon resonance binding assays for kinetics assessment; might need to consider complexity of effects when two or more MMPs and two or more substrates are involved, which mimics in vivo setting	MMP substrate profile and hierarchy of substrate preferences and MMP preferences
	• Infuse substrate fragments to mimic MMP effects • Outputs: in vitro and in vivo signalling and cell physiology, left ventricular physiology and remodelling	Substrate is downstream of MMP, and proteolysis is necessary and sufficient to recapitulate MMP phenotype

MI, myocardial infarction; MMP, matrix metalloproteinase; qRT-PCR, quantitative real-time polymerase chain reaction.