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Review article

Matrix metalloproteinases as input and output signals for post-myocardial infarction remodeling

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ABSTRACT

Despite current optimal therapeutic regimens, approximately one in four patients diagnosed with myocardial infarction (MI) will go on to develop congestive heart failure, and heart failure has a high five-year mortality rate of 50%. Elucidating mechanisms whereby heart failure develops post-MI, therefore, is highly needed. Matrix metalloproteinases (MMPs) are key enzymes involved in post-MI remodeling of the left ventricle (LV). While MMPs process cytokine and extracellular matrix (ECM) substrates to regulate the inflammatory and fibrotic components of the wound healing response to MI, MMPs also serve as upstream signaling initiators with direct actions on cell signaling cascades. In this review, we summarize the current literature regarding MMP roles in post-MI LV remodeling. We also identify the current knowledge gaps and provide templates for experiments to fill these gaps. A more complete understanding of MMP roles, particularly with regards to upstream signaling roles, may provide new strategies to limit adverse LV remodeling.

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1. Introduction

According to the European Cardiovascular Disease Statistics (www.ehnheart.org/cvd-statistics.html), each year cardiovascular disease causes over 4 million deaths in Europe. In the United States, approximately 6 million Americans currently suffer from heart failure, and the high 5-year mortality rate of 50% results in an annual health care cost of >$34 billion [1]. In 70% of heart failure cases, myocardial infarction (MI) is the underlying etiology [2–9]. While immediate reperfusion of the occluded artery is an optimal therapeutic strategy for ST segment elevation MI, the number of MI patients that are not reperfused (due to late presentation or other exclusion criteria) accounts for approximately 250,000 new patients with permanently occluded arteries each year in the United States [1]. Therefore, adverse remodeling of the left

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ventricle (LV) following MI remains a significant cause of congestive heart failure.

Matrix metalloproteinases (MMPs) are a family of 25 proteolytic enzymes that regulate extracellular matrix (ECM) turnover and inflammatory signaling. While about half of the MMPs have been measured in the post-MI LV and several MMPs (e.g., MMP-2, −7, −9, −12, −14, and −28) have been studied in detail, there remains significant knowledge gaps [10–19]. To provide a framework for this review, we will use the four postulates for cardiac metalloproteinases actions (CarMA) previously developed to define the iterative process for proving MMP causality in post-MI LV remodeling [20]. The four postulates are: 1) the MMP increases post-MI; 2) the MMP stimulates cell signaling *in vitro*; 3) modulating MMP levels alters LV remodeling; and 4) MMP proteolytic products recapitulate at least partial components of MMP functions. Note that the four postulates match the order and substance of the Koch's postulates to establish a cause and effect relationship between a microbe and a disease, rather than by how much evidence is currently available to support the postulate. Postulates 1 and 3 have the most literature available to date.

Below, we list the postulate, followed by the published evidence to support it, followed by the critical knowledge gaps that need to be addressed to provide mechanistic insight into MMP roles in the post-MI LV. We give examples of studies performed in mice with genetic deletions of MMP-7, MMP-9, MMP-14, or MMP-28 or inhibitors against MMP-12. We also discuss a few misconceptions that have arisen along the past 20 years of cardiac MMP research. We end with discussion of how the identified knowledge gaps can be filled, providing example experimental design templates including considerations for controls and potential limitations. While each of the four postulates has been shown for about half of the MMPs, there remains a lack of complete understanding for all MMPs. In addition, multiple MMPs have not been evaluated for even the first postulate. While we discuss each of the postulates to provide a comprehensive review, the second postulate (that MMPs stimulate cell signaling directly) provides an exciting new avenue of research that is only now coming to light.

2. CarMA Postulate 1: The MMP increases post-MI

This is the smoking gun postulate; the first evidence that must be shown is that the MMP is in a location and at a concentration high enough to have an effect. We and others have shown that MMP-2, −7, −8, −9, −12, and −14 increase post-MI [12,21–33]. In particular, MMP-9 and MMP-12 have been shown to increase at both the gene and protein level, and both pro- and active protein forms are elevated post-MI [12,34]. Both increase in the infarct at day 1 post-MI and remain elevated through days 5–7 post-MI [12,34].

In addition to measuring total amounts of mRNA or protein expression, it is also important to measure quality and location. With the exceptions of MMP-11, MMP-14, and MMP-28, most MMPs are secreted as pro-enzymes that need to be activated for substrate cleavage [35,36]. Understanding if the MMP observed is in a pro, active, or inactivated/inhibited state provides knowledge useful for assessing function. Monitoring location and cell source are also important components. For example, total MMP-28 concentration actually decreases post-MI, due to the significant loss of cardiomyocytes that are the major pre-MI source of MMP-28 in the myocardium [37]. At the same time, the amount of MMP-28 contributed by the macrophage increases with the infiltration of macrophages into infarct. This postulate, therefore, is fulfilled for macrophage-derived MMP-28.

Originally, MMPs were classified based on the cell type where the MMP was first observed or the substrates first used to evaluate activity, and this led to confusion [20]. For example, MMP-1 was first named collagenase, but this MMP also processes tenascin and aggrecan into fragments [20]. MMP-8 was first termed neutrophil collagenase, whereas subsequent studies have shown macrophages to be robust expressors of this MMP and additional MMP-8 substrates include aggrecan, fibrinogen, CXCL5, and CXCL6 [38–40]. MMP-12 was initially termed macrophage metalloelastase, whereas this MMP also processes osteopontin, and MMP-9 has greater enzyme affinity for elastin cleavage than MMP-12 [16,41–43]. In our own studies, we were surprised to find that post-MI LV neutrophils robustly express MMP-12 at day 1 post-MI [12]. Along with MMPs, the tissue inhibitors of metalloproteinases (TIMPs) have also been misunderstood. While TIMP-4 has been termed the cardiac-specifc TIMP due to its high expression in the myocardium, TIMP-4 is also expressed in the kidney, placenta, colon, and testes [44].

While CarMA postulate 1 is fulfilled for a subset of MMPs, the current knowledge gap 1 is that each MMP has not been evaluated (e.g., MMP-15 and MMP-16) and not all MMPs (e.g., MMP-7 and MMP-28) have been mapped as extensively as others (e.g., MMP-2 and MMP-9).

3. CarMA Postulate 2: MMP stimulates cell signaling *in vitro*

This postulate dictates that MI-relevant cells stimulated with an MMP will display biological functions similar to what is observed during LV remodeling *in vivo*, if that MMP has a causal role. Basically, this postulate raises the possibility that MMPs can serve in direct signaling capacities, which separates this role from that of an enzyme. While there are examples to provide evidence for this postulate, this idea has not been examined in detail.

In vitro, MMP-9 directly activates macrophage polarization to an M1/M2 transition state [45]. MMP-9 stimulation increased the expression of the pro-inflammatory M1 gene Ccl5, but also decreased the expression of M1 markers Ccl3, interleukin (IL)-1β, and IL-6. Transforming growth factor (TGF)β1, an anti-inflammatory M2 marker, was also down-regulated after MMP-9 treatment. MMP-12 stimulates neutrophils *in vitro* to induce expression of the apoptosis markers, CD44, caspase 3, and caspase 8 [12], and MMP-9 also stimulates capase 3 expression in neutrophils [46]. CD44 regulates apoptosis by interacting with hyaluronic acid and is a critical mechanism in wound healing to clear inflammatory cells from injury sites [47,48]. MMP-12 can also process CD44 to generate a 15 kDa fragment, indicating a feedback loop. CD44 cleavage prevents the clearance of the CD44 ligand hyaluronic acid, which is a stimulus for inflammation resolution during wound healing [12]. Combined, these results indicate that MMPs can be used as direct stimulating factors as well as output factors. This is an entirely new concept in MMP biology, and future studies evaluating how MMPs activate cell signaling (e.g., direct binding of receptors such as integrins or indirect effects through processing of substrates) are warranted.

In vivo, MMP-7 has been shown to have direct activity on myocardial electrical activity by cleaving connexin-43 in the C-terminal domain [49]. Infusion of recombinant MMP-7 into the jugular vein of mice induced heart block within 60 min of infusion, concomitant with reduced connexin-43 staining at the myocyte to myocyte borders. This study provides direct evidence that connexin-43 is an *in vivo* substrate of MMP-7 and that its processing results in a pathophysiological phenotype.

Knowledge Gap 2 is that the MMP signaling pathways that regulate cell function have not been mapped. There is a need to identify MMP signaling pathways that regulate post-MI relevant cell functions, including myocyte apoptosis; neutrophil apoptosis and degranulation; macrophage polarization and phagocytosis; and fibroblast proliferation, differentiation, and ECM expression. Included in this knowledge gap is the need to know which receptors are engaged by MMPs and whether the effects are actually directly occuring through receptor engagement and signaling or due to an indirect effect that has not been elucidated (e.g., substrate fragment binding to a receptor or shedding of an inhibitor in the signaling pathway). While this postulate has the largest unknown component, it also is one of the more exciting postulates due to its novelty.