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Review Article

Myocardial matrix metalloproteinase-2: inside out and upside down

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ABSTRACT

Since their inaugural discovery in the early 1960s, matrix metalloproteinases (MMPs) have been shown to mediate multiple physiological and pathological processes. In addition to their canonical function in extracellular matrix (ECM) remodeling, research in the last decade has highlighted new MMP functions, including proteolysis of novel substrates beyond ECM proteins, MMP localization to subcellular organelles, and proteolysis of susceptible intracellular proteins in those subcellular compartments. This review will provide a comparison of the extracellular and intracellular roles of MMPs, illustrating that MMPs are far more interesting than the one-dimensional view originally taken. We focus on the roles of MMP-2 in cardiac injury and repair, as this is one of the most studied MMPs in the cardiovascular field. We will highlight how understanding all dimensions, such as localization of activity and timing of interventions, will increase the translational potential of research findings. Building upon old ideas and turning them inside out and upside down will help us to better understand how to move the MMP field forward.

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Introduction

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases responsible for cleaving protein substrates, with the most commonly identified substrates being extracellular matrix (ECM) proteins.[1] MMPs are involved in both physiological processes, such as embryogenesis and organogenesis during development, and in pathological processes such as inflammation, metastasis, and tissue remodeling.[2–4] The general structure of an MMP includes an inhibitory pro-peptide domain, a zinc-containing catalytic domain, a linker peptide, and a hemopexin domain.[2] The majority of MMPs are synthesized in an inactive pro-MMP form, and the MMP can be activated by

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the removal of the approximately 10 kDa pro-peptide, which exposes the zinc-binding catalytic domain. While the disruption of the pro-domain is generally required for MMP activation, recent studies have reported that post-translational modifications of MMPs, in the absence of the removal of the pro-domain, can also result in MMP activation.[3] (Fig. 1.)

The broad family of MMPs can be further classified into collagenases (MMP-1, MMP-8, MMP-13, and MMP-18), gelatinases (MMP-2 and MMP-9), stromelysins (MMP-3, MMP-10, and MMP-11), matrilysins (MMP-7 and MMP-26), and membrane-type MMPs (MMP-14, MMP-15, MMP-16, MMP-17, MMP-24 and MMP-25), a classification system that is loosely based on initial ECM substrate screens. An original criteria to being classified as an MMP was the ability of the enzyme to proteolytically process at least one ECM protein.[2,5] The first non-ECM substrates to be identified were cytokines, and one of the first to make the list was interleukin-1β which is cleaved into its active form by several MMPs, including MMP-2 and -9.[6,7] A multitude of cytokines, chemokines, and growth factors are now known to be substrates of extracellular MMPs.

Approximately a decade ago, the Schulz laboratory first showed that MMP-2 targeted intracellular proteins, and proteolysis of these intracellular proteins mediated myocardial ischemia-reperfusion (I/R) injury.[8] (Fig. 2) Specifically, they found that proteolytic degradation of the thin myo filament protein troponin I (TnI) by MMP-2 was directly involved in the acute contractile defect of isolated rat hearts exposed to I/R. This article initiated a cascade of research focused on intracellular roles for MMPs. Subsequently, degradomics experiments used the power of proteomics and bioinformatics approaches to reveal a wide variety of potential intracellular MMP substrates.[9–11]

Myocardial ischemia is defined as the sustained loss of oxygen, resulting from an obstruction in coronary blood flow. When ischemia lasts beyond a critical window of 20–30 minutes, myocyte necrosis ensues. Irreversible ischemic damage resulting from ischemia for longer periods of time is defined as myocardial infarction (MI).[12] Optimal therapy for MI includes reperfusion strategies (whether mechanical or thrombolytic) to restore blood flow to the ischemic region. Reperfusion, while beneficial to the patient, also initiates a robust inflammatory response that can extend myocardial damage.[13]

In the myocardium, MMPs regulate both physiological and pathophysiological processes. MMP-2 is one of the most studied of all identified MMPs, as it is found in almost all cardiac cells and, in part, due to its ease of evaluation. This review will focus on the roles of MMP-2 in cardiac pathology (namely MI and I/R injury), which will serve as a template for other MMPs known to be elevated in these same models.

Known ECM substrates and novel non-ECM extracellular substrates

MMP substrates identified to date can be grouped into extracellular and intracellular proteins (Table 1). The MEROPS database provides a resource for information about many proteinases, including MMPs, as well as the proteins that inhibit them (http://merops.sanger.ac.uk/). Analysis of the cleavage site sequences across substrates provides information on consensus sequences. For example, the consensus sequence of the MMP-2 cleavage site is XPXX↓(L/I)XXX (where X is any amino acid), based on evaluation of 3413 cleavages. Note that the consensus cleavage sites are based primarily on early data gathered on the premise that MMPs target only ECM proteins. While they are useful for *in silico* identification of putative substrates, using only these sequences will generate many false positive and negative hits. A small set of predicted substrates for MMP-2, including both intracellular and extracellular substrates, have been derived from *in vitro* and *in silico* cleavage assays.[14,15] Of note, several of these substrates are processed by MMP-2, as well as other MMPs, suggesting that there is some redundancy built into this system. Redundancy, therefore, should be a

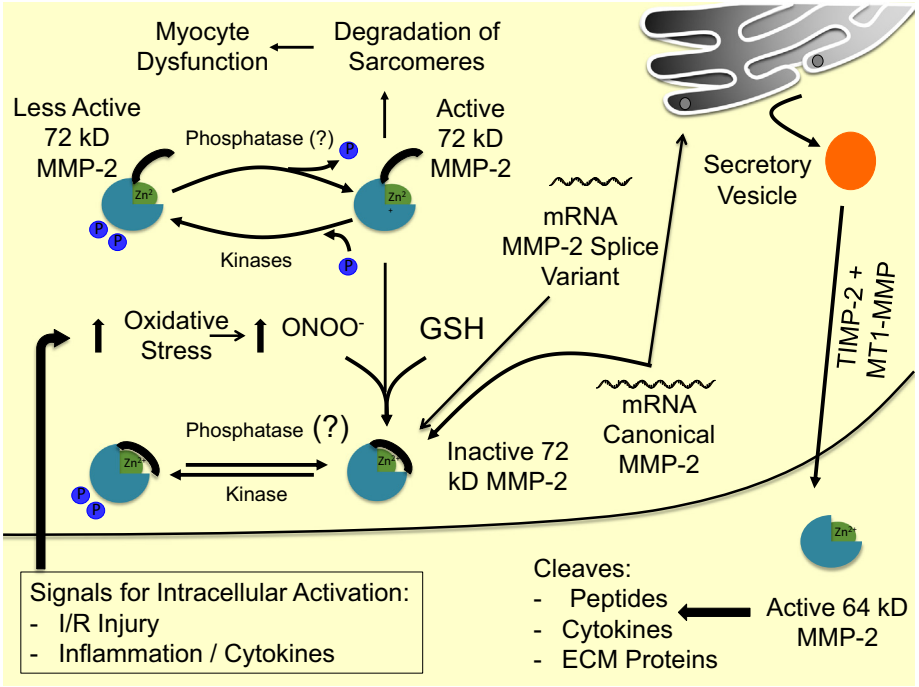

Fig. 1. MMP-2 targets to the cytosol of the myocyte, where it undergoes post-translational modifications. The 72 kDa form of MMP-2 can be activated extracellularly by proteases (e.g., plasminogen or MMP-14) to yield a 64 kDa active form. The 72 kDa form of MMP-2 can also be activated intracellularly by its S-glutathiolation, which requires the biosynthesis of peroxynitrite induced by ischemia/reperfusion or by pro-inflammatory cytokines. This activation occurs in the presence of glutathione and does not require proteolytic removal of the pro-domain. This active 72 kDa MMP-2 form targets susceptible intracellular proteins within the cardiomyocyte to induce contractile dysfunction. MMP-2 targets to the cytosol by at least two mechanisms: 1) An MMP-2 splice variant in cardiomyocytes lacks the secretory signal sequence, resulting in its intracellular retention. 2) The canonical MMP-2 signal sequence is inefficient, yielding MMP-2 that targets to the cytosol and to the endoplasmic reticulum for secretion. In addition, MMP-2 has been shown to be phosphorylated at several sites, which modulates its activity. GSH, glutathione; $ONOO^-$, peroxynitrite.