The History of Matrix Metalloproteinases (MMPs):
Milestones, Myths, and (Mis)Perceptions

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Abstract
Since the discovery of tadpole collagenase in 1962, the matrix metalloproteinase (MMP) family has emerged as a significant proteinase group with recognized effects on the cardiovascular system. Over the last 40 years, many milestones have been achieved, from the identification of the first MMP, to the generation of the first MMP cDNA clone and null mouse, to the clinical approval of the first MMP inhibitor. Over the years, a few myths and misunderstandings have interwoven into the truths. In this review, we will discuss the major milestones of MMP research, as well as review the misinterpretations and misperceptions that have evolved. Clarifying the confusions and dispelling the myths will both provide a better understanding of MMP properties and functions and focus the cardiovascular field on the outstanding research questions that need to be addressed.

keywords: review, matrix metalloproteinases, MMP, cardiovascular disease, myocardial infarction
**Introduction**

Since 1962, the matrix metalloproteinase (MMP) family has been extensively studied in a multitude of animal and tissue models, and the first review article with matrix metalloproteinases in the title was written by Henning Birkedal-Hansen in 1988 (12). MMPs have been evaluated using the expertise of many different disciplines, including biochemistry, cell biology, pathology, immunology, physiology, and computational biology and from many different disease view points, including arthritis, cancer, periodontal disease, and cardiovascular disease.

While this is a general MMP review, we will focus on cardiovascular and inflammatory aspects, as our own investigations focus on these areas and they will be of greater appeal to this audience. Below, we will summarize the major milestones in the MMP research field, as well as discuss the myths and misperceptions that have arisen throughout the years. Finally, we end with discussion on where the cardiovascular MMP field is headed.

**Milestones**

Table 1 lists the major milestones in MMP research. Before the first MMP (collagenase/MMP-1) was identified, an initial milestone was the study by Woessner in 1962 showing that a protein enzyme in mammalian uterus could degrade collagen (165). Later that year, Jerome Gross and Charles Lapiere were the first to identify an MMP using a biochemical approach (60). They showed that the anuran tadpole had strong collagenolytic activity in the skin, gut, and gills, tissues that underwent the most radical remodeling during metamorphosis. This discovery was the first step into a field that would grow exponentially in the following years. In 1966, MMP-1 was purified from tadpole tail fin and back skin (106).

Compared to MMP-1, MMP-2 was identified as a higher molecular weight species (72 kDa) with gelatinolytic activity, while MMP-3 was identified as a lower molecular weight species (54 kDa) with proteoglycan and casein degrading activity. MMP-2 was first described and isolated in the 1970s and initially denoted as 72-kDa type IV collagenase/gelatinase A (166). MMP-2 was sequenced by Goldberg and colleagues (31) and purified from human rheumatoid synovial...
fibroblasts and characterized by the Nagase laboratory (109). MMP-3 neutral proteinase activity was first described in the 1970s (166), and the enzyme was purified and described as a proteoglycanase in 1983 (55). MMP-3 was also isolated and purified from rabbit synovial fibroblasts by the Werb laboratory in 1985 and subsequently named stromelysin (29). MMP-3 was shown in 1987 to be an activator of pro-MMP-1 (105). When these three MMPs were identified and characterized, it was noted the MMP-2 had constitutive expression that was not robustly influenced by treatment with phorbol esters, whereas MMP-1 and MMP-3 production were both greatly enhanced with stimulation.

As additional MMPs were identified, the members of the family were frequently recognized by more than one name. In the late 80’s, Ed Harris, Jr. and colleagues first proposed using the name matrix metalloproteinase for this family of enzymes. Subsequently, the International Union of Biochemistry and Molecular Biology designated the family with the unique name Matrix Metalloproteinases and assigned each family member with an enzyme number. By 1991, MMP-1, -2, -3, -7, -8, -9, and -10 as well as TIMP-1 and -2 had been named and characterized. The MMP family has grown to current total of 25 members, although not all of these are found in humans. The MMP family has been divided into five sub groups – collagenases, gelatinases, matrilysins, stromelysins, and membrane type (MT-) MMPs. These groups are based on sequence homology and in vitro ECM substrate characteristics (95).

To be classified as an MMP family member, the enzyme should meet the following requirements: 1) proteolysis of at least one ECM component; 2) catalysis dependent on zinc at the active site; 3) activation by proteinases or organomercurials; 4) inhibited by ethylenediaminetetraacetic acid (EDTA), 1,10-phenanthroline, and one of the tissue inhibitors of metalloproteinases (TIMPs); and 5) cDNA has sequence homology to MMP-1. An original qualification, that the proteinase be secreted in a pro-form, no longer holds, as MMP-11 and -28 are intracellularly activated by furin and are secreted in active forms and the membrane bound MMPs are not necessarily secreted (112).
MMPs were first classified by their *in vitro* ECM substrate characteristics, although there was no real logic for why particular substrates were tested over others. Some of the more abundant ECM proteins, such as type I collagen, fibronectin, and laminin, were tested for many but not all MMPs. The first 10 MMPs have had broad substrate characterization, while for later MMPs (for example, MMP-28) only a few substrates have been identified or examined. This limited substrate classification has led to a number of misperceptions and oversimplifications in understanding the diversity of MMP functions.

Four major MMP milestones were the (i) identification of the tissue inhibitors of metalloproteinases (TIMPs), (ii) elucidation of MMP proenzyme forms, (iii) structural determination of MMPs, and (iv) creation of MMP null mice. The first TIMP, TIMP-1, was described in 1972, and four TIMPs have been identified to date (10). Additional natural MMP inhibitors that have been identified include α2 macroglobulin, tissue factor pathway inhibitor, the membrane-anchored glycoprotein RECK, and MMP pro-domains (107). The recognition of MMP zymogen forms and subsequent propeptide removal as part of the MMP activation process was key to understanding MMP regulation (27, 104, 123, 141).

In 1994, the first crystal structure of human fibroblast collagenase catalytic domain was published by the Longley laboratory (97). The crystal structure was solved at 2.4 Å resolution in the form of a collagenase-inhibitor complex. The first MMP null was generated in the Matrisian laboratory - the targeted disruption of the MMP-7 gene - in 1997 (164). Taconic has a knockout repository website (www.taconic.com) that lists all of the available MMP null models. Current lists of null and transgenic MMP mice available, with selected phenotype information, are provided in Tables 2 and 3.

**Myths and Misperceptions in the MMP field**

Over the years, several myths and misperceptions have arisen in the MMP field, in part due to a lack of understanding and in part due to unclear literature. We list here several of these misunderstandings, in an attempt to clarify the facts.
Gelatin Zymography. The statement that gelatin zymography is the best way to measure MMPs is a misunderstanding. While it is true that MMPs can be measured by zymography, several myths have mutated from this idea. One myth is that a zymogram is the only way to measure MMP activity. The reason why zymograms were prominently used in the first 30 years of MMP research was that MMP antibodies were not widely available. With the current large choice in MMP antibodies that recognize both pro and activated forms, the zymogram is for the most part now an archaic technology. Another myth was that one needed to measure active MMP in your sample in order for it to be relevant. In fact, MMPs do not need to be active to be functionally relevant. Krane showed that MMP-13 and Wnt compete for binding to LRP5/6, without the activation of MMP-13 (37). Nagase showed that MMP-1 interacts with integrins to alter Akt phosphorylation, without the activation MMP-1 (32). Furthermore, Bannikov and colleagues demonstrated that pro-MMP-9, in the presence of substrate, has enzymatic activity without the loss of the 10 kDa pro-domain (7). Strongin has reported that binding of TIMP-2 to the hemopexin domain of catalytically inactive MMP-14 induces MAPK activation and cell growth (143). Thus pro-MMPs can be functionally active and should not be ignored or undervalued. For MMP studies in cardiovascular research, we recommend that immunoblotting and immunohistochemistry be used to measure MMP levels, coupled with in vivo MMP imaging techniques described below.

MMP-2 and -9. The statement that MMP-2 and MMP-9 are the most important MMPs is a myth. A literature search will clearly demonstrate that MMP-2 and MMP-9 have the most number of publications. These two MMPs, however, are not necessarily more significant than other MMPs simply because they are more frequently measured. The popularity of MMP-2 and -9 stems from earlier days when zymography was the method of choice to measure MMP levels and activity, due to a lack of available antibodies for immunoblotting approaches (95). Because gelatin zymography was much more technically accessible than zymography using casein or other substrates, MMP-2 and -9 were the easiest MMPs to measure (154). As a result, the
number of articles evaluating MMP-2 and -9 are a log fold higher in number compared to other
MMPs, particularly some of the newer family members.

Currently, many other MMPs are being studied in the cardiovascular system, particularly
MMP-7 and MMP-14 (28, 94, 140, 172) Beyond noting expression levels, there remains a large
knowledge gap in regards to roles of additional MMP family members in cardiovascular disease
processes. Included in the list of MMPs that have not been fully analyzed for substrate profiles,
cellular localization, and biological roles are MMP-11, MMP-20, and MMP-28 (99).

Substrates. The statement that MMPs only process ECM proteins is a misunderstanding.
MMPs degrade not only ECM, but non-ECM substrates as well. The ability to cleave non-ECM
proteins, such as cell surface membrane proteins, is an important mechanism to regulate
cellular functions. Proteolysis can stimulate or deactivate intracellular signaling pathways, such
as apoptosis and autophagy pathways (23, 142, 163)). The challenge is to identify which are the
most important MMP substrates. For example, in addition to gelatin (denatured collagen), MMP-
2 also degrades the ECM substrates elastin, fibronectin, and aggrecan, as well as non-ECM
substrates IL-1\(\beta\), \(\alpha\)1 proteinase inhibitor, pro-lysyl oxidase, and other MMPs (-1, -9 and -13)
(22, 93). ECM substrates of MMP-9 include aggrecan, types I, II, III, IV, V, VII, X, and XI
collagen, elastin, fibronectin, galectin-3, laminin, link protein, secreted protein acidic and rich in
cysteine, and vitronectin (Table 4) (122). MMP substrate analysis is further complicated by the
fact that MMPs may require cofactors for substrate cleavage. MMP-9 cleaves VEGF, in the
presence but not the absence of heparin (88). An interesting website that provides information
on substrates, including putative substrates, is the Center on Proteolytic Pathways
(http://cpp.burnham.org/metadot/index.pl) and some of the MMP-9 putative substrates are listed
in Table 5. While this website lists 375 substrates for MMP-9, it is not all inclusive since type I
collagen is not listed (85).

The MMP-14 substrate repertoire also reflects the complexity of MMP substrates. MMP-14
null mice have a more severe phenotype than MMP-2 null mice, indicating that MMP-2 is not the
only relevant substrate of MMP-14 (45). MMP-14 is also a potent collagenase, and MMP-14 null mice have increased collagen deposition (45). Furthermore, MMP-2 null and collagen resistant double mutant mice recapitulate the MMP-14 null phenotype, indicating that both MMP-2 and collagen are critical substrates of MMP-14 (44).

**Common names.** MMPs, such as collagenase or metalloelastase, were originally named based on the major substrate cleaved, but this does not mean that they only process collagen or elastin. In the early years, new MMPs were named primarily for the substrate cleaved. MMP-1 was named collagenase and MMP-2 was named gelatinase. MMP-1, however, also cleaves tenascin and aggrecan (22). MMP-7 was first called putative metalloproteinase-1 or punctuated metalloproteinase (PUMP) due to its truncated size, and this has led to the misstatement that MMP-7 is a putative MMP. MMP-12 was called metalloelastase but also cleaves fibronectin and tenascin (41). MMP-14 is well known as a membrane bound MMP (MT1-MMP), but it is a highly relevant and often ignored collagenase (147).

**Cell specificity.** It is a myth that MMPs have cell specificity. MMPs were often named based on the cell type from which they were first identified. MMP-8 (neutrophil collagenase) was identified as a collagen-digesting protease present in neutrophils (65, 153). This led to the incorrect assumption by some that MMP-8 was a neutrophil marker. However, further research has shown that MMP-8 is expressed in other cells, including macrophages and endothelial cells (130). Similarly, MMP-9 was first coined neutrophil gelatinase and MMP-12 was known as macrophage metalloelastase, while both MMPs are present in several additional cell types (61, 128). Table 6 shows the variety of cell types that express particular MMPs.

**Activation.** The idea that MMPs are only activated extracellularly is a misunderstanding. The extracellular activation of MMPs, converting pro-enzymes to active forms, does occur and was first reported in 1972 (64). Tchougounova showed that chymase deficient mice do not have activated MMP-9, suggesting that chymase is a major *in vivo* activator (149). Plasmin, heparin, and oxidants can activate MMPs in the extracellular environment (22). Additionally, many MMPs
(MMP-3, in particular) are activators of other MMPs (22). MMP-14 processes MMP-2 as well as MMP-13, to give it both direct and indirect collagenolytic activity (40). Having said this, it is important to note that there are several exceptions to the extracellular activation rule. MMP-11, MT-MMPs, and MMP-28 contain furin cleavage sequences and can be activated intracellularly (45).

Intracellular functions. The idea that MMPs only work extracellularly is a myth. MMPs can also degrade proteins in the cytoplasm, mitochondria, and nucleus. Schultz and colleagues have shown that MMP-2 has intracellular substrates in cardiac myocytes, including troponin (5, 72). MMP-2 can proteolyze citrate synthase, a glycolytic enzyme, in the cytoplasm (20). Similarly, MMP-9 and MMP-11 can break down the cytoskeletal proteins actinin and actin (20). While these may not be typical substrates during normal homeostatic regulation, these substrates likely play important roles in the myocardial response to ischemia and reperfusion.

TIMP functions and specificity. The concept that TIMPs only function to inhibit MMPs is a misconception. In addition to being MMP inhibitors, TIMPs have growth factor functions (67). Mann and colleagues have shown that TIMPs stimulate fibroblast proliferation as well as the phenotypic differentiation into myofibroblasts (98). Vanhoutte and Heymans have written a nice review on the MMP-independent effects of TIMPs (159).

The idea that TIMP-4 is the cardiac specific TIMP is a misunderstanding. When TIMP-4 was first cloned, it was reported as being abundantly present in the heart and present at only very low levels in other tissues (59). Based on this report, TIMP-4 was coined the cardiac specific TIMP and was even reported as the cardiac specific inhibitor of MMPs. Subsequently, Leco et al showed that TIMP-4 is robustly expressed in brain, testis, and skeletal muscle (87, 136).

The idea that certain TIMPs inhibit specific MMPs is a myth. While there is some selectivity, there is a lot of confusion about TIMP affinities for MMPs. While TIMP-1 can inhibit all MMPs except MMP-14 efficiently, TIMP-1 has greater affinity for MMP-9 over MMP-2 and TIMP-2 has greater affinity for MMP-2 than MMP-9 (111). TIMP-3 can inhibit MMPs and non-MMP
proteases, including ADAM17 and ADAMTS-4 and -5 (14). There is some cell specificity for TIMPs. For example, TIMP-1 is the only TIMP expressed by neutrophils (118). While MMP-8, MMP-9, and TIMP-1 are all expressed by neutrophils, each is predominantly localized to a distinct granule or vesicle within the neutrophil (118). The majority of MMP-8 localizes to specific granules, MMP-9 to gelatinase granules, and TIMP-1 to secretory vesicles (73). Neutrophils can be sequentially activated, such that the specific granules are released, followed by the gelatinase granules, and finally the secretory vesicles to provide exquisite regulation (48). The Quigley laboratory has shown that, due to this differential release, it is possible for neutrophils to release TIMP-free MMP-9, but this does not mean that neutrophils do not express TIMP-1 (4).

**Therapeutic potential.** The idea that all MMPs serve negative functions is a myth. The myth in the field of MMPs is that all MMPs have adverse effects and hence need to be blocked equally, in all cases, at all times. TIMPs and many synthetic small molecule inhibitors have been designed to target MMPs in cancer, arthritis, and cardiovascular disease (33, 43). MMP inhibitor trials have often failed for several reasons, including (i) drugs were tested in patients with advanced disease, while animal model studies had shown effectiveness of MMP inhibitors in early stage disease, (ii) doses were not adequate, (iii) combination therapies should have been applied, as MMP inhibitors are not cytotoxic, and (iv) broad spectrum inhibition profiles resulted in off-target activities, inhibition of anti-target MMPs, and subsequent musculoskeletal syndrome (51, 113, 160). Peterson has reviewed the need to identify more specific and selective MMP inhibitors (116). Along these lines, several selective inhibitors have been reported for MMP-13 (9, 15, 24, 46, 56, 58, 66, 70, 71, 84, 89, 117, 120, 125) and MMP-14 (40, 145, 171), and the Fields laboratory has developed transition state analogy, triple-helical peptide inhibitors that are selective for MMP-2/MMP-9 and collagenolytic MMPs (82, 83, 85).

There is still interest in the potential of MMP inhibitors to treat cardiovascular disease (6), but we need to understand the biology before this will be successful. Van Lint showed in a lethal hepatitis model that survival curves differ for MMP-2, -3, or -9 null mice, indicating different
timeline of responses for the different MMPs (158). We now know that some MMPs actually have protective roles (96). For example, MMP-1, -2, -7, -9, -14, and -17 are MMP targets (should be blocked) in cancer, but MMP-3, -8, -9, -12, -14, and -19 are anti-targets (should not be blocked). Notice that both MMP-9 and MMP-14 are targets and anti-targets for cancer. Angiogenesis inhibitors angiostatin, endostatin, and tumstatin can be produced by the action of MMP-9 on plasminogen, type XVIII collagen, and type IV collagen, respectively (39, 100, 113). MMP-9 inhibition may be effective in early stage disease [when it facilitates tumor development and releases vascular endothelial growth factor (VEGF)] but antagonistic in advanced disease (36, 39, 51, 76, 113). MMP-14 has been assigned as the collagenase critical for tumor cell migration and invasion (76, 127). However, inhibition of MMP-9 or MMP-14 cleavage and inactivation of CXCL12 may promote metastasis (113). TIMP-1 is a marker of fibrosis, but TIMP-1 and TIMP-3 double null mice show increased fibrosis (75, 77). In response to LPS, MMP-7 and -8 null mice are almost completely protected, MMP-2, -3, -12, and -13 null mice show some protection, and MMP-9 and -19 show no difference in response (157, 158). MMP-7, -8 and -9 all process IL-1β, an initiator of the LPS response, but MMP-7 and -8 degrade while MMP-9 activates IL-1β.

These myths and misunderstandings illustrate the overlapping roles of MMPs in cardiac remodeling. Because MMP functions have temporal, spatial, and cell specific contexts, a more detailed understanding of the functional consequences of MMP actions is needed before we can fully and effectively appreciate the complexities of MMP biology.

Current Perspectives and Future Directions

Going forward, the three major areas that need to be focused on are MMP functions during cardiovascular disease, MMP regulation, and MMP imaging in vivo (Figure 1).

1. MMP functions during cardiovascular disease

More information is needed on when and where MMPs and TIMPs are expressed, which is not as simple as it sounds. MMPs are often measured at one time point, but changes over time
and space need to be considered. Expression patterns are very relevant for cardiovascular
diseases that are a continuum of responses, such as during the formation of atherosclerosis,
after myocardial infarction, or during the progression to heart failure. Not only do cell types
come and go, but cells differentiate over time, and cell differentiation stages can affect MMP
and TIMP expression. For example, fibroblasts stimulated with platelet derived growth factor
(PDGF) express MMPs -1, -2, -3, -11, -14 and TIMPs -1 and 2, but not MMP-9 (86). If the
fibroblast is differentiated into a myofibroblast, however, MMP-9 is produced in response to
PDGF stimulation (86). MMP-9 is expressed in macrophages, but not in circulating monocytes.
The literature is very unclear on this, as many studies using isolated monocytes adhere the cells
to plastic, which means that these cells are macrophages and not monocytes when examined.
Little is known about how MMP and TIMP levels fluctuate with time and location.

In order to fully understand MMP function in physiology and pathology, the identification of
the most important substrates is needed. In a complex environment of multiple MMPs and
multiple substrates, we need to know which of the possible permutations provide the driving
influences. For a particular MMP, we need to know which substrates it prefers; for a particular
substrate, we need to know the affinity profile for all of the different MMPs that process that
substrate. While we need to have more information on the substrate catalogues for the
individual MMPs, we also need to know what the most critical substrates are. For MMP-9,
collagen, galectin-3, and VEGF have been mentioned as critical substrates, but in the
myocardium fibronectin is another very relevant substrate that is often overlooked (170). There
is a need for competitive in vitro assays that better reflect the complex combinations of many
MMPs and many substrates seen in biological systems. This will help to identify the substrates
that propel remodeling, which can be useful predictors of outcome.

We spend a great deal of time worrying about how other MMPs, other proteases, or TIMP
levels increase or decrease to compensate when a particular MMP or TIMP is deleted or
overexpressed, but the presence of a net effect indicates that compensation does not really
matter for that phenotype. The only concern is whether an MMP function would be masked by
the appearance of the MMPs that would not be typically seen in a normal response, which could
have implications when pharmacological inhibitors are applied. To confirm that a substrate is
responsible for a phenotype, we need experiments where exogenously cleaved substrate is
added back in to rescue the phenotype. If MMP null and substrate null show the same
phenotype, this suggests the substrate is downstream of that MMP. Additionally, substrate
cleavage in vitro does not matter as much as cleavage in vivo. The generation of more complex
null models is required to understand MMP functions more specifically. This will help in
identifying which MMPs to target, which MMPs to stimulate (anti-target), and how or if one MMP
regulates the other.

Attention should be given to the functions of the different MMPs, as well as the potentially
different functions for different forms of the same MMP. For example, MMP-9 has monomeric,
homodimeric, and heterodimeric forms, yet we know little about the differences between these
forms. MMP-14 dimerizes in a self-activation process, but the roles of MMP-9 dimerization as
well as MMP-9 binding to TIMP-1 or neutrophil gelatinase associated lipocalin have not been
examined.

MMPs and TIMPs will not likely be useful biomarkers for diagnosis or prognosis, when used
as a single indicator. Several MMPs and TIMPs, including MMP-1, MMP-2, MMP-3, MMP-9,
TIMP-1, TIMP-2, and TIMP-4 have been proposed as indicators of cardiac injury (3, 21, 90, 119,
135, 148), but these need to be evaluated in combination with other markers. All of the above
studies will also help us to identify which MMPs to inhibit or promote. Once this is accomplished,
more selective and specific MMP inhibitors can be designed and tested.

2. MMP Regulation

Several microRNAs (miR) have been shown to regulate MMPs at the translational level (35,
137). For example, miR-21 regulates MMP-2 by upregulating phosphatase and tensin homolog
levels in fibroblasts (151). MMP-2 is regulated by miR-29b, MMP-9 is regulated by miR-29b and
miR-491-5P, and MMP-13 is regulated by miR-27b (2, 26, 169). MMP-14 is regulated by miRNA-9 and MMP-16 is downregulated by miR-146b (167, 173). While we have some knowledge about which miRs alter which MMPs, a more detailed understanding of miR regulation of MMPs is required before we can translate its use in cardiovascular diagnostics and therapeutics.

The TIMPs and other endogenous MMP inhibitors are most often studied as output measurements, meaning that little is known beyond whether the TIMP increases or decreases. TIMP-1 and TIMP-4 are the most frequently studied TIMPs in the cardiovascular field, and more information is needed on the time and space changes in all of the TIMPs. TIMPs are also known to have roles independent of MMP inhibition, but details on how these functions regulate cardiovascular pathology are needed.

3. MMP Imaging

MMP imaging has greatly advanced in the last 10 year, and most imaging projects have focused on macrophage MMPs. The FRET reporter LaRee1, based on the MMP-12 preferential cleavage site sequence Pro-Leu-Gly-Leu-Glu-Glu-Ala, has been used to measure in vivo MMP-12 in macrophages in a mouse pulmonary inflammation model (30). The MMPSense™ probe (with the sequence Gly-Gly-Pro-Arg-Gln-Ile-Thr-Ala-Gly) showed MMP-2 and -9 upregulation in atherosclerotic plaques by visualizing probe accumulation via fluorescent molecular tomography (38). To distinguish between resting versus activated macrophages, Suzuki et al. constructed a probe containing an MMP-9-cleavable linker with the sequence Val-Pro-Leu-Ser-Leu-Tyr-Ser-Gly (146). The probe binds to the scavenger receptor-AI and becomes internalized by activated macrophages upon MMP-9 cleavage and release of the trigger factor (146). rLuc technology may be a useful reporter in animal models, as the reporter does not suffer from auto fluorescent artifacts and is widely used for in vivo imaging.

Radiotracers have also been developed to monitor MMP activation in a murine model of post-infarction remodeling (144). Initial studies were performed with an ¹¹¹In-labeled MMP-
targeted radiotracer (\(^{111}\)In-RP782) and a negative control enantiomeric compound (\(^{111}\)In-RP788). The experiments were performed in control mice and in mice 1 week post surgically-induced myocardial infarction (MI). Subsequent \textit{in vivo} imaging studies utilizing micro-single photon emission computed tomography/CT imaging studies with an analogous \(^{99m}\)Tc-labeled MMP-targeted radiotracer (\(^{99m}\)Tc-RP805) and \(^{201}\)TI demonstrated good biodistribution and clearance kinetics. Myocardial uptake in the MI region was found to be 5-fold increased, and a significant 2-fold increase in myocardial activity in remote regions was also detected. This finding suggested activation of MMPs in regions remote from the MI. This approach holds potential clinical usefulness as a diagnostic tool for \textit{in vivo} localization of MMP activation and tracking of MMP-mediated post-MI remodeling. A radiotracer to monitor the development and calcification of aortic plaques has been designed, as the MMP activity in atherosclerotic lesions is associated with plaque instability (108). The amount of uptake was also proportional to plaque size, which may make this a good method for future clinical noninvasive assessment of the extent of expression of various MMPs. With these tools in hand, imaging can be used to track the \textit{in vivo} expression of MMPs, which will be useful both to monitor disease progression and therapeutic efficacy.

In conclusion, MMP research has come a long way in the last five decades. This review article has summarized the milestones, dispelled the common myths, and focused future directions to help translate MMP biology to therapeutic applications for cardiovascular disease.

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**Figure Legend**

**Figure 1. Key future areas of research for the cardiovascular MMP field.** MMP function and regulation remain to be investigated from multiple perspectives, including needs for better understanding of time and expression pattern, substrate specificity patterns, and the role of MMPs, TIMPs, or substrates as markers of disease progression or treatment efficacy. MMP imaging techniques provide a promising avenue for future research.
Table 1. Major Milestones in MMP Research. The first ten milestones are general MMP milestones, while the last five relate to MMPs in cardiovascular pathophysiology.

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<th>Milestone</th>
<th>Investigators</th>
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<td>Enzyme degradation of collagen described</td>
<td>Woessner (165)</td>
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<tr>
<td>First MMP described; MMP-1 purified</td>
<td>Gross and Lapiere (60, 106)</td>
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<td>MMP-1 purified</td>
<td>Gross and Lapiere</td>
</tr>
<tr>
<td>MMP-2 and -3 identified, isolated, and sequenced</td>
<td>Woessner, Nagase, Goldberg, Reynolds, and Werb (29, 31, 55, 109, 166)</td>
</tr>
<tr>
<td>MMP-3 as activator of pro-MMP-1</td>
<td>Docherty (105)</td>
</tr>
<tr>
<td>MMP term coined</td>
<td>Harris (110)</td>
</tr>
<tr>
<td>TIMP-1 and RECK identified as natural MMP inhibitors</td>
<td>Jeffrey, Noda, and Murphy (10, 107, 132)</td>
</tr>
<tr>
<td>MMP zymogen and mechanism of activation</td>
<td>Multiple investigators (27, 104, 123, 141)</td>
</tr>
<tr>
<td>Crystal structure of collagenase catalytic domain solved</td>
<td>Longley (97)</td>
</tr>
<tr>
<td>MMP-7 is the first MMP null mouse generated</td>
<td>Matrisian (164)</td>
</tr>
<tr>
<td>MMP diversity and expression in human heart failure</td>
<td>Spinale (42, 138, 139)</td>
</tr>
<tr>
<td>MMP diversity and expression in atheromatous plaque</td>
<td>Libby and Lee (91)</td>
</tr>
<tr>
<td>Plaque rupture and inflammation</td>
<td>Galis (54)</td>
</tr>
<tr>
<td>MMP roles in inflammatory and fibrotic responses to myocardial infarction</td>
<td>Lindsey (95)</td>
</tr>
<tr>
<td>MMP imaging <em>in vivo</em></td>
<td>Sinusas (174)</td>
</tr>
</tbody>
</table>
Table 2. A Selection of MMP and TIMP Null Phenotypes in Mice. Of note, most MMP null mice are viable and fertile and show phenotypes only under stressed conditions (17, 19, 34, 47, 49, 53, 68, 69, 74, 92, 99, 121, 124, 133, 164).

<table>
<thead>
<tr>
<th>MMP</th>
<th>Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1a*</td>
<td>↓ angiogenesis; ↓ tumors</td>
</tr>
<tr>
<td>-2</td>
<td>↑ tumor cell apoptosis</td>
</tr>
<tr>
<td>-3</td>
<td>↓ angiogenesis; ↓ tumors</td>
</tr>
<tr>
<td>-7</td>
<td>↓ Intestinal adenoma formation</td>
</tr>
<tr>
<td>-8</td>
<td>↑ skin tumors; ↑ response in arthritis; ↓ lung fibrosis</td>
</tr>
<tr>
<td>-9</td>
<td>↓ MMP-2 expression, ↓ SMC migration and neovascularization</td>
</tr>
<tr>
<td>-10</td>
<td>↑ inflammation to P. aeruginosa infection</td>
</tr>
<tr>
<td>-11</td>
<td>Accelerated neointima formation in vascular injury model</td>
</tr>
<tr>
<td>-12</td>
<td>Early pulmonary fibrosis and ↓ airway resistance</td>
</tr>
<tr>
<td>-13</td>
<td>↑ Interstitial collagen; defect in growth plate cartilage</td>
</tr>
<tr>
<td>-14</td>
<td>Arthritis; osteopenia; dwarfism; ↓ macrophage infiltration</td>
</tr>
<tr>
<td>-16</td>
<td>↓ growth, ↓ mesenchymal cell viability</td>
</tr>
<tr>
<td>-19</td>
<td>Obesity; ↑ Tenascin C; ↑ Th2 inflammation</td>
</tr>
<tr>
<td>-20</td>
<td>Decreased mineral content; deteriorating enamel organ morphology</td>
</tr>
<tr>
<td>-24</td>
<td>Abnormal mast cell degranulation</td>
</tr>
<tr>
<td>-15, -17, -21, -23, -25, -27</td>
<td>Mouse model available but phenotype not yet published or observed</td>
</tr>
<tr>
<td>-1b*, -18, -22, -26</td>
<td>Mouse model not available</td>
</tr>
<tr>
<td>-28</td>
<td>↑ inflammation and ECM response to cardiac aging</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>↑ remodeling post-myocardial infarction, ↓ adipose in high fat diet</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>Delayed neuronal differentiation, Weak muscles</td>
</tr>
<tr>
<td>TIMP-3</td>
<td>LV dilation, cardiomyocyte hypertrophy</td>
</tr>
<tr>
<td>TIMP-4</td>
<td>↑ neutrophil infiltration</td>
</tr>
</tbody>
</table>

*Note that there are two MMP-1 genes in mice, which are MMP-1a and MMP-1b.
Table 3. A Selection of MMP and TIMP Transgenic Overexpression Phenotypes in Mice (11, 16, 18, 79, 101, 103, 126, 150, 161).

<table>
<thead>
<tr>
<th>MMP</th>
<th>Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1a</td>
<td>Emphysematous changes similar to human emphysema</td>
</tr>
<tr>
<td>-2</td>
<td>Myocyte hypertrophy; systolic dysfunction</td>
</tr>
<tr>
<td>-3</td>
<td>↑ squamous cell carcinoma</td>
</tr>
<tr>
<td>-7</td>
<td>↑ tumor; Protection from pulmonary fibrosis</td>
</tr>
<tr>
<td>-9</td>
<td>↑ susceptibility of carcinogenesis; decreased fibrosis</td>
</tr>
<tr>
<td>-10</td>
<td>Abnormality in wound epithelium organization; abnormal keratinocyte migration</td>
</tr>
<tr>
<td>-11</td>
<td>Death during late embryogenesis in Xenopus laevis oocytes</td>
</tr>
<tr>
<td>-12</td>
<td>↑ arthritic lesions; ↑ macrophage infiltration (rabbit)</td>
</tr>
<tr>
<td>-13</td>
<td>Articular cartilage degradation, joint pathology as observed in osteoarthritis</td>
</tr>
<tr>
<td>-14</td>
<td>Fibrosis, adenocarcinoma</td>
</tr>
<tr>
<td>-15</td>
<td>Mouse model available but phenotype not yet published</td>
</tr>
<tr>
<td>-1b, -8, -16, -17, -18, -19, -20, -21, -22, -23, -24, -25, -26, -27, -28</td>
<td>Mouse models not available</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>↓ cellular proliferation and angiogenesis during hepatocarcinogenesis</td>
</tr>
<tr>
<td>TIMP-2</td>
<td>↓ tumor formation, ↓ angiogenesis, ↑ apoptosis</td>
</tr>
<tr>
<td>TIMP-3</td>
<td>Protection from metabolic inflammation and related metabolic disorders</td>
</tr>
<tr>
<td>TIMP-4</td>
<td>Diastolic dysfunction</td>
</tr>
</tbody>
</table>
Table 4. A selection of ECM and non-ECM MMP-9 substrates (1, 50, 52, 115, 129, 156).

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Effect of cleavage on activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>α2 macroglobulin</td>
<td>↓</td>
</tr>
<tr>
<td>Plasminogen → Angiostatin</td>
<td>↑</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>↑</td>
</tr>
<tr>
<td>Fibroblast Growth Factor Receptor 1</td>
<td>shedding</td>
</tr>
<tr>
<td>Growth Related Oncogene-α</td>
<td>↓</td>
</tr>
<tr>
<td>Intercellular Adhesion Molecule 1</td>
<td>↓</td>
</tr>
<tr>
<td>Interleukin-1β</td>
<td>↑</td>
</tr>
<tr>
<td>Interleukin-8</td>
<td>↑</td>
</tr>
<tr>
<td>Pro-MMP-2, -9, -13</td>
<td>↑</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>↑</td>
</tr>
<tr>
<td>Platelet Factor 4</td>
<td>↓</td>
</tr>
</tbody>
</table>
Table 5. A selection of putative MMP-9 substrates, from the Center on Proteolytic Pathways website (http://cpp.burnham.org/metadot/index.pl) (80, 102).

<table>
<thead>
<tr>
<th>Protein</th>
<th>Cleavage Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-1 type II collagen isoform 2</td>
<td>GPKG ANGD</td>
</tr>
<tr>
<td>Complement C1q</td>
<td>GPLG ARGI</td>
</tr>
<tr>
<td>Interleukin-8</td>
<td>LPRS AKEL</td>
</tr>
<tr>
<td>Integrin beta 4 isoform</td>
<td>PRDY ST</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>VAPP PVVL</td>
</tr>
<tr>
<td>SERPINE2 (Plasminogen activator inhibitor)</td>
<td>LPLL LLAS</td>
</tr>
<tr>
<td>Secreted protein acidic and rich in cysteine</td>
<td>GANP VQVE</td>
</tr>
<tr>
<td>Thrombospondin 1</td>
<td>PFHY NP</td>
</tr>
<tr>
<td>Tissue factor pathway inhibitor isoform b precursor</td>
<td>PPLK LMHS</td>
</tr>
</tbody>
</table>
Table 6. MMP and TIMP Cell Expression – known cardiovascular cell expression. Note that the absence of a cell in the list means either that the MMP is not expressed by that cell type or MMP expression in that cell type has not been studied (8, 13, 25, 57, 62, 63, 73, 78, 81, 95, 114, 131, 134, 152, 155, 162, 168).

<table>
<thead>
<tr>
<th>MMP Additional names</th>
<th>Cell expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Collagenase-1; fibroblast collagenase</td>
<td>endothelial, fibroblasts, macrophages</td>
</tr>
<tr>
<td>2 Gelatinase A; 72 kDa type IV collagenase</td>
<td>endothelial, fibroblasts, platelets, T lymphocytes</td>
</tr>
<tr>
<td>3 Stromelysin-1</td>
<td>Endothelial, fibroblasts, macrophages, vascular smooth muscle</td>
</tr>
<tr>
<td>7 Matrilysin</td>
<td>macrophages</td>
</tr>
<tr>
<td>8 Collagenase-2; neutrophil collagenase</td>
<td>neutrophils, endothelial, fibroblasts</td>
</tr>
<tr>
<td>9 Gelatinase B; 92 kDa type IV collagenase</td>
<td>neutrophils, endothelial, eosinophils, macrophages, T lymphocytes</td>
</tr>
<tr>
<td>10 Stromelysin-2</td>
<td>fibroblasts, T lymphocytes</td>
</tr>
<tr>
<td>11 Stromelysin-3</td>
<td>fibroblasts</td>
</tr>
<tr>
<td>12 Macrophage elastase</td>
<td>macrophages, stromal cells,</td>
</tr>
<tr>
<td>13 Collagenase-3</td>
<td>fibroblasts</td>
</tr>
<tr>
<td>14 MT1-MMP</td>
<td>fibroblasts, macrophages</td>
</tr>
<tr>
<td>15 MT2-MMP</td>
<td>fibroblasts, macrophages</td>
</tr>
<tr>
<td>16 MT3-MMP</td>
<td>fibroblasts, macrophages, vascular smooth muscle</td>
</tr>
<tr>
<td>17 MT4-MMP</td>
<td>eosinophils, lymphocytes, monocytes</td>
</tr>
<tr>
<td>18 Xenopus laevis collagenase -4</td>
<td>xenopus expression only</td>
</tr>
<tr>
<td>19 RASI-1</td>
<td>vascular smooth muscle, endothelial, monocytes</td>
</tr>
<tr>
<td>20 Enamelysin</td>
<td>endothelial</td>
</tr>
<tr>
<td>23 CA-MMP</td>
<td>unknown</td>
</tr>
<tr>
<td>24 MT5-MMP</td>
<td>unknown</td>
</tr>
<tr>
<td>25 MT6-MMP</td>
<td>neutrophils, monocytes</td>
</tr>
<tr>
<td>26 Matrilysin-2</td>
<td>B lymphocytes</td>
</tr>
<tr>
<td>27 CMM/MMP-22</td>
<td>fibroblasts</td>
</tr>
<tr>
<td>28 Epilysin</td>
<td>cardiomyocytes, macrophages, T lymphocytes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TIMP</th>
<th>Cell expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 collagenase inhibitor</td>
<td>leukocytes, fibroblasts, mesenchymal stem cells, vascular smooth muscle</td>
</tr>
<tr>
<td>2</td>
<td>fibroblasts, macrophages, vascular smooth muscle</td>
</tr>
<tr>
<td>3</td>
<td>fibroblasts, pericytes</td>
</tr>
<tr>
<td>4</td>
<td>cardiomyocytes, lymphocytes, macrophages, mast cells, vascular smooth muscle</td>
</tr>
</tbody>
</table>
Future Areas for MMP Research

**Function**
- cell and temporal expression
- substrate affinities: competition and specificity
- oligomerization effects
- null and transgenic models

**Regulation**
- translational: miRNA
- post-translational: TIMPs and other inhibitors

**Imaging**
- MMP presence
- MMP activity