TRANSLATIONAL PHYSIOLOGY

The history of matrix metalloproteinases: milestones, myths, and misperceptions

Rugmani Padmanabhan Iyer,1,3,4,5 Nicolle L. Patterson,1,3,4,5 Gregg B. Fields,1,2 and Merry L. Lindsey1,3,4,5

1San Antonio Cardiovascular Proteomics Center, The University of Texas Health Science Center at San Antonio, San Antonio, Texas; 2Torry Pines Institute for Molecular Studies, Port Saint Lucie, Florida; 3Barshop Institute of Longevity and Aging Studies, The University of Texas Health Science Center at San Antonio, San Antonio, Texas; 4Division of Geriatrics, Gerontology and Palliative Medicine, Department of Medicine, The University of Texas Health Science Center at San Antonio, San Antonio, Texas; and 5Division of Cardiology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas

Submitted 30 July 2012; accepted in final form 13 August 2012

Iyer RP, Patterson NL, Fields GB, Lindsey ML. The history of matrix metalloproteinases: milestones, myths, and misperceptions. Am J Physiol Heart Circ Physiol 303: H919–H930, 2012. First published August 17, 2012; doi:10.1152/ajpheart.00577.2012.—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.

review; matrix metalloproteinases; MMP; cardiovascular disease; myocardial infarction

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 MMPs 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 viewpoints, 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. In this paper, 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).

When compared to MMP-1, MMP-2 was identified as a higher molecular mass species (72 kDa) with gelatinolytic activity, whereas MMP-3 was identified as a lower molecular mass 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 (29) in 1985 and subsequently named stromelysin. 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 enzymatic activity that was not robustly influenced by treatment with phorbol esters, whereas MMP-1 and MMP-3 production were both greatly enhanced with stimulation.
Myths and Misperceptions in the MMP Field

Over the years, several myths and misperceptions have arisen in the MMP field, in part because of a lack of understanding and in part because of 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. 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 an archaic technology. Another myth...
Table 2. A selection of MMP and TIMP null phenotypes in mice

<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 <em>Pseudomonas aeruginosa</em> 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>↑ Intestinal 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>-25, -27</td>
<td>Mouse model available but phenotype not yet published or observed</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>Left ventricular dilation, cardiomyocyte hypertrophy</td>
</tr>
<tr>
<td>TIMP-4</td>
<td>↑ neutrophil infiltration</td>
</tr>
</tbody>
</table>

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). *There are two MMP-1 genes in mice, which are MMP-1a and MMP-1b. SMC, smooth muscle cell; Th2, T-helper 2.

was that one needed to measure active MMP in the sample in order for it to be relevant. In fact, MMPs do not need to be active to be functionally relevant. Aikawa and colleagues (37) showed that MMP-13 and Wnt compete for binding to lipoprotein receptor-related protein 5/6, without the activation of MMP-13. Nath and colleagues (32) showed that MMP-1 interacts with integrins to alter Akt phosphorylation without the activation of MMP-1. Furthermore, Bannikov and colleagues (7) demonstrated that pro-MMP-9 in the presence of substrate has enzymatic activity without the loss of the 10-kDa prodomain. Strongin (143) has reported that binding of TIMP-2 to the hemopexin domain of catalytically inactive MMP-14 induces MAPK activation and cell growth. 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 because of 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 with 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 regard 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

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 hapatocarcinogenesis</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>
IL-1β, α1-proteinase inhibitor, pro-lysyl oxidase, and other MMPs (1-13) (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 vascular endothelial growth factor (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 because of 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 tenasin (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, whereas both MMPs are present in several additional cell types (61, 128).

**Activation.** The idea that MMPs are only activated extracellularly is a misconception. The extracellular activation of MMPs, converting proenzymes to active forms, does occur and was first reported in 1972 (64). Thougouinova et al. (149) showed that chymase deficient mice do not have activated MMP-9, suggesting that chymase is a major in vivo activator. 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, we state that it is important to note that there are several exceptions to the extracellular activation rule. MMP-11, MT-MPPs, 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 (5, 72) have shown that MMP-2 has intracellular substrates in cardiac myocytes, including troponin. MMP-2 can proteolyze citrate synthase, a glycolytic enzyme, in the cytoplasm (20). Similarly, MMP-9 and MMP-11 can break down cytoskeletal proteins actinin and actin (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 (98) have shown that TIMPs stimulate fibroblast proliferation as well as the phenotypic differentiation into myofibroblasts. Vanhouette and Heymans (159) have written a nice review on the MMP-independent effects of TIMPs.

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. (87, 136) showed that TIMP-4 is robustly expressed in brain, testis, and skeletal muscle.
The idea that certain TIMPs inhibit specific MMPs is a myth. While there is some selectivity, there is a great deal of confusion about MMP affinities for TIMPs. Whereas 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-9 than MMP-9 (111). TIMP-3 can inhibit MMPs and non-MMP proteases, including A disintegrin and metalloproteinase domain-containing protein 17 and A disintegrin and metallopeptidase with thrombospondin type 1 motif-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 specific granule or vesicle. 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 (4) has shown that, because of 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.

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 1) drugs were tested in patients with advanced disease, whereas animal model studies had shown effectiveness of MMP inhibitors in early stage disease; 2) doses were not adequate; 3) combination therapies should have been applied, as MMP inhibitors are not cytotoxic; and 4) broad spectrum inhibition profiles resulted in off-target activities, inhibition of anti-target MMPs, and subsequent musculoskeletal syndrome (51, 113, 160). Peterson (116) has reviewed the need to identify more specific and selective MMP inhibitors. 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 analogs, 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 et al. (158) showed in a lethal hepatitis model that survival curves differ for MMP-2, -3, or -9 null mice, indicating different time line of responses for the different MMPs. 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 antitargets (should not be blocked). Notice that both MMP-9 and MMP-14 are targets and antitargets 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 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

Table 6. **MMP and TIMP cell expression (known cardiovascular cell expression)**

<table>
<thead>
<tr>
<th>MMP</th>
<th>Additional Names</th>
<th>Cell Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1</td>
<td>Collagenase-1; fibroblast collagenase</td>
<td>Endothelial, fibroblasts, macrophages</td>
</tr>
<tr>
<td>-2</td>
<td>Gelatinase A; 72-kDa type IV collagenase</td>
<td>Endothelial, fibroblasts, platelets, T lymphocytes</td>
</tr>
<tr>
<td>-3</td>
<td>Stromelysin-1</td>
<td>Endothelial, fibroblasts, macrophages, vascular smooth muscle</td>
</tr>
<tr>
<td>-7</td>
<td>Matrilysin</td>
<td>Macrophages</td>
</tr>
<tr>
<td>-8</td>
<td>Collagenase-2; neutrophil collagenase</td>
<td>Neutrophils, endothelial, fibroblasts</td>
</tr>
<tr>
<td>-9</td>
<td>Gelatinase B; 92-kDa type IV collagenase</td>
<td>Neutrophils, endothelial, eosinophils, macrophages, T lymphocytes</td>
</tr>
<tr>
<td>-10</td>
<td>Stromelysin-2</td>
<td>Fibroblasts, T lymphocytes</td>
</tr>
<tr>
<td>-11</td>
<td>Stromelysin-3</td>
<td>Fibroblasts</td>
</tr>
<tr>
<td>-12</td>
<td>Macrophage elastase</td>
<td>Fibroblasts</td>
</tr>
<tr>
<td>-13</td>
<td>Collagenase-3</td>
<td>Fibroblasts</td>
</tr>
<tr>
<td>-14</td>
<td>MT1-MMP</td>
<td>Fibroblasts, macrophages</td>
</tr>
<tr>
<td>-15</td>
<td>MT2-MMP</td>
<td>Fibroblasts, macrophages</td>
</tr>
<tr>
<td>-16</td>
<td>MT3-MMP</td>
<td>Fibroblasts, macrophages, vascular smooth muscle</td>
</tr>
<tr>
<td>-17</td>
<td>MT4-MMP</td>
<td>Eosinophils, lymphocytes, monocytes</td>
</tr>
<tr>
<td>-18</td>
<td>Xenopus laevis collagenase-4</td>
<td>Xenopus expression only</td>
</tr>
<tr>
<td>-19</td>
<td>RAS1-1</td>
<td>Vascular smooth muscle, endothelial, monocytes</td>
</tr>
<tr>
<td>-20</td>
<td>Enamelysin</td>
<td>Endothelial</td>
</tr>
<tr>
<td>-23</td>
<td>CA-MMP</td>
<td>Unknown</td>
</tr>
<tr>
<td>-24</td>
<td>MT5-MMP</td>
<td>Unknown</td>
</tr>
<tr>
<td>-25</td>
<td>MT6-MMP</td>
<td>Neutrophils, monocytes</td>
</tr>
<tr>
<td>-26</td>
<td>Matrilysin-2</td>
<td>B lymphocytes</td>
</tr>
<tr>
<td>-27</td>
<td>CMMP/MMP-22</td>
<td>Fibroblasts</td>
</tr>
<tr>
<td>-28</td>
<td>Epilysin</td>
<td>Cardiomyocytes, macrophages, T lymphocytes</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>Collagenase inhibitor</td>
<td>Leukocytes, fibroblasts, mesenchymal stem cells, vascular smooth muscle</td>
</tr>
<tr>
<td>TIMP-2</td>
<td></td>
<td>Fibroblasts, macrophages, vascular smooth muscle</td>
</tr>
<tr>
<td>TIMP-3</td>
<td></td>
<td>Fibroblasts, pericytes</td>
</tr>
<tr>
<td>TIMP-4</td>
<td></td>
<td>Cardiomyocytes, lymphocytes, macrophages, mast cells, vascular smooth muscle</td>
</tr>
</tbody>
</table>

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).
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 null mice 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, whereas 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 (Fig. 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 (MI), 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 express MMP-1, -2, -3, -11, -14 and TIMP-1 and -2, but not MMP-9 (86). If the fibroblast is differentiated into a myofibroblast, however, MMP-9 is produced in response to platelet-derived growth factor 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.

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 phenotype. 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 (antitarget), 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. 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.

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 phos-
phatase 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.

**MMP imaging.** MMP imaging has greatly advanced in the last 10 years, and most projects have focused on imaging macrophage MMPs. The fluorescence resonance energy transfer 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. (146) constructed a probe containing an MMP-9-cleavable linker with the sequence Val-Pro-Leu-Ser-Leu-Tyr-Ser-Gly. 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 postinfarction remodeling (144). Initial studies were performed with an 111In-labeled MMP-targeted radiotracer ([111In-RP782] and a negative control enantiomeric compound ([111In-RP788]. The experiments were performed in control mice and in mice 1-wk postsurgically-induced MI. Subsequent in vivo imaging studies using micro-single photon emission computed tomography/computer tomography imaging studies with an analogous 99mTc-labeled MMP-targeted radiotracer ([99mTc-RP805] and 201Tl demonstrated good biodistribution and clearance kinetics. Myocardial uptake in the MI region was found to be fivefold increased, and a significant twofold 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 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 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.

**GRANTS**

We acknowledge support from R01-CA-098799 and the Multiple Sclerosis National Research Institute (to G. B. Fields) and from NHLBI HHSN 268201000036C (N01-HV-00244) for the San Antonio Cardiovascular Proteomics Center and R01-HL-075360, the Max and Minnie Tomerlin Voelcker Fund, and the Veteran’s Administration (Merit) (to M. L. Lindsey).

**DISCLOSURES**

M. L. Lindsey has received grant funding from Novartis and has current grant funding from Amnion Pharmaceuticals, Inc., and Canopus Corporation. Both projects are unrelated to this paper.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


Bister VO, Salmela MT, Karjalainen-Lindsberg ML, Uria J, Lohi J, Brew K, Nagase H.
Blavier L, Lazaryev A, Dorey F, Shackleford GM, DeClerck YA.

Bister VO, Salmela MT, Karjalainen-Lindsberg ML, Uria J, Lohi J, Brew K, Nagase H.
Blavier L, Lazaryev A, Dorey F, Shackleford GM, DeClerck YA.


Tissue inhibitor of metalloproteinase-1 (TIMP-1) is an independent predictor of all-cause mortality, cardiac mortality, and myocardial infarction. Circulation 113: 5178–5183, 2006.


Lec JCO, Apht SS, Taniguchi GT, Hawkes SP, Khokha R, Schultz GA, Edwards DR. Murine tissue inhibitor of metalloproteinase-4...
HISTORY OF MMPs

Review
H928


