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The compendium of matrix metalloproteinase expression in the left ventricle of mice following myocardial infarction

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Kaminski AR, Moore ET, Daseke MJ 2nd, Valerio FM, Flynn ER, Lindsey ML. The compendium of matrix metalloproteinase expression in the left ventricle of mice following myocardial infarction. Am J Physiol Heart Circ Physiol 318: H706–H714, 2020. First published February 21, 2020; doi:10.1152/ajpheart.00679.2019.—Matrix metalloproteinases (MMPs) are proteolytic enzymes that break down extracellular matrix (ECM) components and have shown to be highly active in the myocardial infarction (MI) landscape. In addition to breaking down ECM products, MMPs modulate cytokine signaling and mediate leukocyte cell physiology. MMP-2, -7, -8, -9, -12, -14, and -28 are well studied as effectors of cardiac remodeling after MI. Whereas 13 MMPs have been evaluated in the MI setting, 13 MMPs have not been investigated during cardiac remodeling. Here, we measure the remaining MMPs across the MI time continuum to provide the full catalog of MMP expression in the left ventricle after MI in mice. We found that MMP-10, -11, -16, -24, -25, and -27 increase after MI, whereas MMP-15, -17, -19, -21, -23b, and -26 did not change with MI. For the MMPs increased with MI, the macrophage was the predominant cell source. This work provides targets for investigation to understand the full complement of specific MMP roles in cardiac remodeling.

NEW & NOTEWORTHY To date, a number of matrix metalloproteinases (MMPs) have not been evaluated in the left ventricle after myocardial infarction (MI). This article supplies the missing knowledge to provide a complete MI MMP compendium.

extracellular matrix; fibroblast; macrophage; matrix metalloproteinase; myocardial infarction

INTRODUCTION

In response to myocardial infarction (MI), the myocardium responds by undergoing a repair process that starts with a robust inflammatory response and ends with scar formation (11, 13). Wound healing ranges from formation of a stable scar to progression to heart failure (11, 13). During the inflammatory response, necrotic myocytes and damaged extracellular matrix (ECM) components from the ischemic area are enzymatically broken down. Removal is governed by proteases, in particular the matrix metalloproteinases (MMPs). ECM breakdown provides the platform on which new ECM is deposited to form the infarct scar (11, 13). The goal of this study was to evaluate all MMPs that had not previously been evaluated in the left ventricle after MI. To date, MMP-1A, -1B, -2, -3, -7, -8, -9, -12, -13, -14, and -28 have been identified in myocardium and examined in MI mice (6, 16–19, 23, 26, 27, 33, 37). In addition, all four tissue inhibitors of metalloproteinases (TIMPs) have been evaluated in MI (5, 16, 22, 41, 44). We hypothesized that MMP-10, -11, -15, -16, -17, -19, -23A, -23B, -24, -25, -26, and -27 may change in protein expression during MI remodeling.

METHODS

Tissue collection. The samples used for immunoblotting and multiplex immunohistochemistry were previously collected from other projects and included in the mouse Heart Attack Research Tool (mHART) database and tissue bank (10). All animal procedures were performed in compliance with the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at the University of Mississippi Medical Center. The mice used were wild-type C57BL/6J 3- to 6-mo-old mice (3 males and 3 females for each time point). MI was induced by permanently occluding the coronary artery according to the Guidelines for Experimental Models of Ischemia and Infarction and as previously described, and mice were given buprenorphine (0.5 mg/kg) before the surgery (9, 20, 25, 37, 48). Echocardiography was performed under isoflurane anesthesia using the Vevo 2100 (Visual Sonics, Toronto, ON, Canada) as previously described and outlined in the Guidelines for Measuring Cardiac Physiology in Mice (9, 20, 30, 37). Heart rates were >400 beats/min and were not different among groups (ANOVA P = 0.47). The left ventricle (LV) was sliced into three sections. The apex and base sections were separated into remote left ventricle control (LVC) and infarct (LVI; including border) zones and snap-frozen for immunoblotting. The mid-papillary section was fixed in zinc-formalin and paraffin-embedded for histological evaluation. Infarct area was measured by staining with 1% 2,3,5-triphenyltetrazolium chloride and calculating the volume percentage of LV that was infarcted.

Immunoblotting. Immunoblotting was performed according to the published guidelines (3). For first-pass assessment, the samples for each time were pooled together to allow the complete time course of remote and infarct region groups to all be run on one gel. By densitometry, MI time points that showed peak expression were selected for individual variability analysis. Total protein (10 μg) samples were run on 4–12% Criterion XT Bis-Tris precast gels (Bio-Rad, Hercules, CA) and were transferred onto Trans-Blot Turbo Transfer Pack Nitrocellulose Membranes (Bio-Rad). The membranes were stained with Pierce Reversible Protein Stain Kit for nitrocellu-

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Fig. 1. Myocardial infarction (MI) confirmation. Echocardiography on day 0 before MI and on days 1, 3, 5, 7, 14, and 28 after MI revealed increases in left ventricle (LV) end systolic (A) and diastolic (B) dimensions (mm) and decreases in fractional shortening (%; C) and LV infarct wall thickness (mm; D). E: Infarct size was determined by 1% 2,3,5-triphenyltetrazolium chloride staining to determine the % total LV that was infarcted. F: Lung weight (dry; mg) increased beginning at MI day 7; n = 6 (3 males/3 females) for each time point. Comparisons were made by 1-way ANOVA with Newman-Keuls post-test. *P < 0.05 vs. day 0.

MMP, matrix metalloproteinase.

| MMP     | Company/Cat. No.        | Dilution |
|---------|-------------------------|----------|
| MMP-8   | Abcam/ab81286           | 1:1,000  |
| MMP-9   | R&D Systems/AP09        | 1:1,000  |
| MMP-10  | MyBioSource/MBS207749   | 1:1,000  |
| MMP-11  | MyBioSource/MBS9449414  | 1:1,000  |
| MMP-15  | MyBioSource/MBS250010   | 1:1,000  |
| MMP-16  | MyBioSource/MBS9130916  | 1:1,000  |
| MMP-17  | MyBioSource/MBS2528233  | 1:1,000  |
| MMP-19  | MyBioSource/MBS7044316  | 1:1,000  |
| MMP-21  | Epitomics 19551         | 1:1,000  |
| MMP-23B | MyBioSource/MBS220284   | 1:2,000  |
| MMP-24  | MyBioSource/MBS9202434  | 1:1,000  |
| MMP-25  | MyBioSource/MBS126850   | 1:1,000  |
| MMP-26  | MyBioSource/MBS236784   | 1:500    |
| MMP-27  | MyBioSource/MBS2517647  | 1:1,000  |

**Statistical analysis.** Data were analyzed according to the recommendations of the Statistical Considerations in Reporting Cardiovascular Research and are presented as means ± SE for echocardiography and SD for all other data (28). Statistical analysis was performed using GraphPad Prism 7 software. Echocardiography and necropsy from mice were used as positive controls. Chemiluminescence visualization was conducted by incubating the membrane with Amersham ECL Prime Western Blotting Detection Reagent (GE Healthcare) for 5 min and visualizing the blot with an ImageQuant LAS 4000 and ImageQuantTL V8.1 software (GE Healthcare).

Multiplex immunohistochemistry imaging. MI LV sections (n = 6) fixed in 10% zinc-buffered formalin, paraffin-embedded, and sectioned at 5 μm were used from the mHART biobank (10). MI day 1 LV was used for MMP-10, MMP-11, MMP-16, and MMP-24. MI day 7 LV was used for MMP-25, and MI day 3 LV was used for MMP-27. Naïve LV sections (n = 6) were compared against each respected MMP. Sections were stained with specific MMP antibody (1:100; Table 1), as described previously (38, 39). Neutrophils were stained with a neutrophil-specific antibody (1:100, ab21595; Abcam, Cambridge, UK). Macrophages were stained with a macrophage-specific antibody (1:100, CL8943AP; Cedarlane, Burlington, ON, Canada). Cell nuclei were stained with 4’,6-diamidino-2phenylindole (DAPI).

Each MMP antibody, neutrophil antibody, and macrophage antibody was conjugated to FITC, Cy3, and Cy5 fluorophores (Opal 520, Opal 620, and Opal 690; Perkin-Elmer, Waltham, MA). Images were acquired at ×40 using the Mantra Quantitative Pathology Imaging System (Perkin-Elmer). Five random fields were chosen within the infarct region for analysis. Total MMP, MMP + neutrophil, and MMP + macrophage staining were quantified as percent area of the field using inForm cell analysis (Perkin-Elmer).

MMP mRNA expression in MI macrophages and fibroblasts. To further validate cell source for MMPs and TIMPs, we evaluated MMP mRNA expression in previously collected transcriptomic data sets from macrophages and fibroblasts isolated from the infarct region on MI days 1, 3, and 7 and compared with day 0 no MI LV (38, 39).
variables were compared using one-way ANOVA with Newman-Keuls posttest. Comparisons between two groups were made using unpaired t-test or Mann-Whitney test. MMP-25 was compared with infarct wall thinning by Pearson correlation. Statistical significance was set at $P < 0.05$.

**RESULTS**

Proof of MI was obtained using echocardiography. As shown in Fig. 1, the mice displayed LV dilation by dimensions, impaired myocyte contractility by fractional shortening, and infarct wall thinning. Infarct sizes ranged from ~40 to 50%, and lung mass increased, indicating the development of pulmonary edema.

Rigor and reproducibility assessment. To assess the rigor and reproducibility of our immunoblotting, both operators performed immunoblotting for MMP-8 and MMP-9, which was then compared with previous findings (17, 46). MMP-8 and -9 increased following MI, with the greatest increase in expression of the pro form occurring on day 1 and the active form occurring on day 3. The interperson variation ratios for MMP-8 (Fig. 2) and MMP-9 (Fig. 3) were excellent. Therefore, our results were highly consistent between operators in both technique and analysis and were consistent with past reports (6, 14).

MMP changes with MI. MMP-10, -11, -15, -16, -17, -19, -21, -23B, -24, -25, -26, and -27 were evaluated in the post-MI LV. MMP-10 and -11 (Fig. 4), -16 and -24 (Fig. 5), and -25 and -27 (Fig. 6) were all upregulated with MI. MMP-15 and MMP-17 (Supplemental Fig. S2), as well as MMP-19, MMP-21, MMP-23B, and MMP-26 (Supplemental Fig. S3), were not different over the MI time course. MMP-10 and MMP-11 increased with the highest level of expression in the pro form on MI day 1; the increase in the pro form was not matched with an increase in the active form. MMP-16 increased with the highest level of expression on MI day 1 in both the pro and active forms. MMP-24 exists only in active form and had the highest level of expression on MI day 1. MMP-25 exists only in the active form as well and had the greatest level of expression on MI day 7. By regression analysis, MMP-25 correlated negatively with wall thickness ($r = -0.67, P = 0.02$), indicating that MMP-25 was highest in infarcts that were the thinnest. MMP-27 has only an active form and had the greatest level of expression on MI day 3.

MMP immunohistochemistry. Immunohistochemistry was performed on LV sections using MMP-specific antibodies on day 0 and MI day 1 for MMP-10, -11, -16, and -24, on day 0 and MI day 7 for MMP-25, and on day 0 and MI day 3 for MMP-27 (Table 2). Total MMP-10 did not change in the LV on day 1, with MMP-10 localized within macrophages or neutrophils not changing. MMP-11 increased on MI day 1, and whereas localization within the macrophages did not change, MMP-11 within the neutrophil decreased. Total MMP-16 increased, with MMP-16 localized within macrophages but not neutrophils. Total MMP-24 increased with MI, and MMP-24

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**Fig. 2. Variability assessment of matrix metalloproteinase (MMP)-8 immunoblotting. A**: time course immunoblot replicate 1. **B**: time course immunoblot replicate 2. **C**: replicate 1 compared with replicate 2 showing the interperson/variation ratio. **D**: normalized densitometry for pro (P) and active (A) MMP-8 on day 0 (D0) 0 and day 1 (D1); $n = 6$ (3 males/3 females) for each time point. D0 to myocardial infarction (MI) D1 comparisons were made by t-test. LVC, left ventricle control; LVI, left ventricle infarct.
localized within macrophages increased and MMP-24 within neutrophils did not change. Total MMP-25 increased, with MMP-25 localizing within macrophages increasing and MMP-25 within neutrophils not changing. Total MMP-27 increased but did not increase in macrophages or neutrophils. These results indicate that a strong proportion of MI MMP protein expression is localized to the macrophage. These results are also consistent with MMP expression in MI neutrophils being low due to degranulation of vesicles to release the MMPs.

**MMP expression in MI macrophages and fibroblasts by RNAseq.** Transcriptomics were previously performed on LV macrophages isolated from LV on day 0 and MI days 1, 3, and 7 (results summary in Table 3, with full individual results in Supplemental Table S1) (38). Of the 24 MMPs evaluated, 17 showed differential expression in infarct macrophages. MMP-8, -9, and -25 show prominent increases in gene expression on MI day 1, matching the immunoblotting results. For MMP-10, -11, -16, -24, and -27, immunoblotting showed increased expression, and macrophage expression showed no change or decreases in gene expression. This indicates that the increases in MMP-10, -11, -16, -24, and -27 shown by immunoblotting were not due to macrophage as a source, or there was a mismatch between macrophage gene and protein expression.

Transcriptomics were previously performed on LV fibroblasts isolated from LV on day 0 and MI days 1, 3, and 7 (results summary in Table 4, with full individual results in Supplemental Table S2) (39). Of the 24 MMPs evaluated, five showed differential expression in infarct macrophages. MMP-16 shows prominent increases in gene expression on MI day 3 in fibroblasts, whereas immunoblotting for LV MMP-16 showed increased expression on day 1. There was no difference or no change in expression of MMP-10, -11, -16, -24, and -27 in fibroblasts. This indicates that the increases in MMP-10, -11, -16, -24, and -27 shown by immunoblotting were not due to fibroblasts as a source, or there was a mismatch between fibroblast gene and protein expression.

**DISCUSSION**

The goal of this study was to evaluate the MMP family members that had not been previously examined in the MI LV. We validated our immunoblotting approach using MMP-8 and MMP-9 as controls and evaluated MMP-10, -11, -15, -16, -17, -19, -21, -23B, -24, -25, -26, and -27 on day 0 and MI days 1, 3, 5, 7, 14, and 28. The most salient findings of this study were that 1) MMP-10, -11, -16, -24, -25, and -27 are newly identified MMPs increased after MI by immunoblotting; 2) MMP-15, -17, -19, -21, -23B, -24, -25, -26, and -27 on day 0 and MI days 1, 3, 5, 7, 14, and 28. The most salient findings of this study were that 1) MMP-10, -11, -16, -24, -25, and -27 are newly identified MMPs increased after MI by immunoblotting; 2) MMP-15, -17, -19, -21, -23B, and -26 did not change with MI from initiation through MI day 28; and 3) the macrophage was the predominant source of the MMPs by immunohistochemistry and by transcriptomic analyses. Previously, information on expression of 13 MMPs has been reported. We add to the literature information on an additional 14 MMPs to complete the MMPs in the MI LV compendium.

MMP-8 and -9 were evaluated as positive control experiments, as both have previously been shown to increase after MI (6). We showed excellent reproducibility both between the
High MMP-11 activity on MI trophils and macrophages are not the major source of MMP-11. Immunohistochemistry showed no difference in MMP-11 activity between M1 and M2 anti-inflammatory macrophages.

MMP-10 is heavily secreted by macrophages during acute inflammation in damaged or infected tissue. During infection with Pseudomonas aeruginosa, MMP-10 is responsible for mitigating the inflammatory actions of M1 macrophages as well as activating M2 anti-inflammatory macrophages.

MMP-24, also known as MT5-MMP, is a membrane-type MMP expressed in the nervous system. Importantly, MMP-24 cleaves collagen I, IV, V, VII, and X, laminin, aggrecan, fibronectin, and tenascin. This widespread degradative effect upon multiple extracellular matrix substrates suggests that MMP-16 alone may have a more influential role in directing LV remodeling than previously predicted.

MMP-25, also known as MT6-MMP, is a membrane-type MMP expressed in lung, spleen, and leukocytes. MMP-25 substrates include fibrin, fibronectin, collagen I, and collagen IV. In the context of MI, pro-inflammatory N1 neutrophils contribute to LV wall thinning after MI by generating large amounts of MMP-12 and MMP-25. MMP-25 showed highest expression on MI day 1 and independently tracked with infarct wall thinning on MI day 7. Therefore, MMP-25 may be relevant to neutrophil prolongation of pro-inflammation.

MMP-27 has a unique COOH-terminal extension, causing it to be retained in the endoplasmic reticulum. MMP-27 immunolocalizes with CD45, CD163, and CD206 in macrophages during acute inflammation in damaged or infected tissue. Although MMP-10 is not constitutively expressed in healthy tissue, it is heavily secreted by macrophages during acute inflammation in damaged or infected tissue. During infection with Pseudomonas aeruginosa, MMP-10 is responsible for mitigating the inflammatory actions of M1 macrophages as well as activating M2 anti-inflammatory macrophages.

In cardiac tissue, TIMP-4 regulates MMP-10 during the process of LV remodeling, and in end-stage heart failure patients myocardial samples revealed a positive correlation between upregulation of MMP-10 and LV dilation, indicating that MMP-10 influenced ECM structure. Inhibition of MMP-10, accordingly, may help to prevent LV dilation. Substrates of MMP-11 include collagen IV and VI, fibronectin, α2-macroglobulin, and insulin-like growth factor-binding protein 1. MMP-11 is regulated in M2 macrophages compared more so than M1 macrophages. Immunohistochemistry showed no difference in MMP-11 localized in macrophages and a decrease in neutrophils, whereas total MMP-11 increased, suggesting that neutrophils and macrophages are not the major source of MMP-11 in MI. High MMP-11 activity on MI day 1 in M1 macrophages suggests that MMP-11 may be involved in pro-inflammatory signaling or ECM turnover, which are hallmarks of the inflammatory phase.

MMP-16, also known as MT3-MMP, had highest expression on MI day 1 in both the pro and active forms, which suggests that it may have an active role in early myocardial remodeling.

MMP-16 may have an indirect role through its activation of MMP-9 and, more importantly, MMP-2. MMP-2 cleaves collagen I, IV, V, VII, and X, laminin, aggrecan, fibronectin, and tenascin. This widespread degradative effect upon multiple extracellular matrix substrates suggests that MMP-16 alone may have a more influential role in directing LV remodeling than previously predicted.

MMP-11 activity on MI day 1 in the infarcted zone. Although MMP-10 and -11 increased on MI day 1 in the infarcted zone. Although MMP-10 is not constitutively expressed in healthy tissue, it is heavily secreted by macrophages during acute inflammation in damaged or infected tissue. During infection with Pseudomonas aeruginosa, MMP-10 is responsible for mitigating the inflammatory actions of M1 macrophages as well as activating M2 anti-inflammatory macrophages. In cardiac tissue, TIMP-4 regulates MMP-10 during the process of LV remodeling, and in end-stage heart failure patients myocardial samples revealed a positive correlation between upregulation of MMP-10 and LV dilation, indicating that MMP-10 influenced ECM structure. Inhibition of MMP-10, accordingly, may help to prevent LV dilation. Substrates of MMP-11 include collagen IV and VI, fibronectin, α2-macroglobulin, and insulin-like growth factor-binding protein 1. MMP-11 is regulated in M2 macrophages compared more so than M1 macrophages. Immunohistochemistry showed no difference in MMP-11 localized in macrophages and a decrease in neutrophils, whereas total MMP-11 increased, suggesting that neutrophils and macrophages are not the major source of MMP-11 in MI. High MMP-11 activity on MI day 1 in M1 macrophages suggests that MMP-11 may be involved in pro-inflammatory signaling or ECM turnover, which are hallmarks of the inflammatory phase.

MMP-16, also known as MT3-MMP, had highest expression on MI day 1 in both the pro and active forms, which suggests that it may have an active role in early myocardial remodeling.
phages in the cycling human endometrium (7). CD163 and CD206 are expressed by M2 macrophages (7). In our study, MMP-27 was increased in its active form (58 kDa) on MI day 3, when macrophages are undergoing a metabolic shift and upregulating genes associated with M2 macrophages (38). Whether or not MMP-27 plays a role in phenotypic shift of macrophages has not been examined.

By immunohistochemistry, MMP-11, -16, -24, -25, and -27 increased with MI. These results are consistent with the immunoblotting results. Whereas MMP-10 increased by immunoblotting, a similar increase by immunohistochemistry was not observed. By transcriptomics, the macrophage was not a predominant source of MMP-10, whereas cardiac fibroblasts showed decreased expression with MI. This indicates that the immunoblotting results likely reflect the combination of effects across cell types. Transcriptomics, immunoblotting, and immunohistochemistry combined confirmed increased MMP-11, -16, -24, and -25 in the MI LV and indicated that macrophages were the predominant source of these MMPs.

Further studies on the MMPs that were differentially expressed are needed to better understand the roles of each MMP as well as their functional relationships to each other. There is a need to follow up on the MMPs elevated after MI to determine their specific cause and effect relationship in the left ventricle. MMPs have a number of roles in the myocardium, including processing of extracellular matrix substrates, inflammatory mediators, and growth factors, and the implications of these findings need to be determined (11, 18, 24). In particular, the role of the substrate fragments generated by these MMP should be investigated, as substrate proteolysis is known to release active biopeptides that influence MI remodeling (29).

In conclusion, we add to the current knowledge base that MI yields increased expression of MMP-10, -11, -16, -24, -25, and -27 in the infarct region. The summary of our current knowledge of MMP expression after MI in mice is provided in Table 5.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

Fig. 5. Matrix metalloproteinase (MMP)-16 and MMP-24 myocardial infarction (MI) expression. A: pooled time course expression of MMP-16 (top) and individual analysis from day 0 (D0) to MI day 1 (D1) expressed as densitometry normalized to total membrane stain. B: pooled time course expression of MMP-24 (bottom) and individual analysis from D0 to MI D1 expressed as densitometry normalized to total membrane stain; n = 6 (3 males/3 females) for each time point. D0 to MI D1 comparisons were made by t-test. A, active; LVC, left ventricle control; LVI, left ventricle infarct; P, pro.
Fig. 6. Matrix metalloproteinase (MMP)-25 and MMP-27 myocardial infarction (MI) expression. A: pooled time course expression of MMP-25 (top) and individual analysis from day 0 (D0) to MI day 7 (D7) expressed as densitometry normalized to total membrane stain. B: pooled time course expression of MMP-27 (bottom) and individual analysis from D0 to MI day 3 (D3) expressed as densitometry normalized to total membrane stain; n = 6 (3 males/3 females) for each time point. D0 to MI D1 comparisons were made by t-test. Active; LVC, left ventricle control; LVI, left ventricle infarct.

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Table 3. MI macrophage MMP expression

| Gene  | ANOVA P Value | Newman-Keuls Multiple-Comparisons Test |
|-------|---------------|----------------------------------------|
| Mmp1b | 0.026         | D0–D7 (↑); D1–D7 (↑); D3–D7 (↑)        |
| Mmp2  | 0.002         | D0–D1 (↑); D0–D3 (↓)                   |
| Mmp3  | 0.023         | D0–D3 (↑)                               |
| Mmp8  | <0.0001       | D0–D1 (↑); D0–D3 (↑); D1–D3 (↑); D1–D7 (↓); D3–D7 (↓) |
| Mmp9  | 0.017         | D0–D1 (↑); D1–D3 (↓); D1–D7 (↓)        |
| Mmp10 | 0.723         | ↔ ↔ ↔                                  |
| Mmp11 | 0.033         | D0–D1 (↓); D0–D3 (↓)                   |
| Mmp12 | <0.0001       | D0–D1 (↑); D0–D3 (↑); D0–D7 (↑); D1–D3 (↓); D1–D7 (↓) |
| Mmp13 | <0.001        | D0–D3 (↑); D0–D7 (↑); D1–D3 (↓); D1–D7 (↓) |
| Mmp14 | <0.001        | D0–D1 (↑); D0–D3 (↓); D0–D7 (↓)        |
| Mmp15 | 0.009         | D0–D1 (↓); D0–D3 (↓); D0–D7 (↓)        |
| Mmp16 | 0.283         | ↔ ↔ ↔                                  |
| Mmp17 | 0.374         | ↔ ↔ ↔                                  |
| Mmp19 | <0.001        | D0–D1 (↑); D0–D3 (↓); D0–D7 (↓); D1–D3 (↓); D1–D7 (↓) |
| Mmp21 | 0.372         | ↔ ↔ ↔                                  |
| Mmp23 | 0.004         | D0–D1 (↓); D0–D3 (↓); D1–D7 (↑); D3–D7 (↑) |
| Mmp24 | 0.203         | ↔ ↔ ↔                                  |
| Mmp25 | 0.011         | D0–D1 (↑); D1–D3 (↓); D1–D7 (↓)        |
| Mmp27 | 0.411         | ↔ ↔ ↔                                  |
| Mmp28 | 0.051         | ↔ ↔ ↔                                  |
| Timp1 | <0.0001       | D0–D1 (↑); D0–D3 (↑); D0–D7 (↑); D1–D3 (↓); D1–D7 (↓); D3–D7 (↓) |
| Timp2 | <0.0001       | D0–D1 (↑); D0–D3 (↑); D1–D3 (↓); D1–D7 (↓) |
| Timp3 | 0.005         | D0–D1 (↑); D0–D3 (↑); D0–D7 (↓)        |
| Timp4 | 0.004         | D0–D1 (↓); D0–D3 (↓); D0–D7 (↓)        |

Arrows denote direction of change. D, day; Mmp, matrix metalloproteinase; Timp, tissue inhibitor of metalloproteinase. Sample sizes are n = 4 males for each time (D0 and myocardial infarction (MI) D1, D3, and D7). ↑ Increased or ↓ decreased compared with day 0 (P < 0.05); ↔ no change.

Table 4. MI fibroblast MMP expression

| Gene  | ANOVA P Value | Newman-Keuls Multiple-Comparisons Test |
|-------|---------------|----------------------------------------|
| Mmp1a | 0.730         | ↔ ↔ ↔                                  |
| Mmp1b | 0.325         | ↔ ↔ ↔                                  |
| Mmp2  | 0.061         | ↔ ↔ ↔                                  |
| Mmp3  | 0.010         | D0–D3 (↓); D0–D7 (↑); D1–D3 (↑); D1–D7 (↓) |
| Mmp8  | 0.587         | ↔ ↔ ↔                                  |
| Mmp9  | 0.561         | ↔ ↔ ↔                                  |
| Mmp10 | 0.007         | D0–D3 (↓); D1–D7 (↓)                   |
| Mmp11 | 0.650         | ↔ ↔ ↔                                  |
| Mmp12 | 0.071         | ↔ ↔ ↔                                  |
| Mmp13 | 0.187         | ↔ ↔ ↔                                  |
| Mmp14 | 0.003         | D0–D3 (↓); D0–D7 (↑); D1–D3 (↑); D1–D7 (↑) |
| Mmp15 | 0.221         | ↔ ↔ ↔                                  |
| Mmp16 | 0.044         | D1–D3 (↑)                              |
| Mmp17 | 0.003         | D0–D3 (↓); D0–D7 (↑); D1–D3 (↑); D1–D7 (↑) |
| Mmp19 | 0.779         | ↔ ↔ ↔                                  |
| Mmp21 | 0.722         | ↔ ↔ ↔                                  |
| Mmp23 | 0.053         | ↔ ↔ ↔                                  |
| Mmp24 | 0.090         | ↔ ↔ ↔                                  |
| Mmp25 | 0.244         | ↔ ↔ ↔                                  |
| Mmp27 | 0.157         | ↔ ↔ ↔                                  |
| Mmp28 | 0.841         | ↔ ↔ ↔                                  |
| Timp1 | 0.029         | D0–D7 (↑); D1–D7 (↑)                   |
| Timp2 | 0.260         | ↔ ↔ ↔                                  |
| Timp3 | 0.001         | D0–D1 (↓); D0–D3 (↑); D0–D7 (↑); D1–D3 (↑); D1–D7 (↑) |
| Timp4 | 0.203         | ↔ ↔ ↔                                  |

D, day; Mmp, matrix metalloproteinase; Timp, tissue inhibitor of metalloproteinase. Sample sizes are n = 3 males for each time (D0 and myocardial infarction (MI) D1, D3, and D7). ↑ Increased or ↓ decreased compared with day 0 (P < 0.05); ↔ no change.

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Table 5. Summary of our current knowledge of MMP expression after MI in mice

| MMP  | Activity Post-MI | Peak Protein  |
|------|-----------------|---------------|
| MMP-1A* | Yes (23) | ↑ 7 |
| MMP-1B |               |               |
| MMP-2 | Yes (16) | ↑ 7 |
| MMP-3 | Yes (16) | ↑ 4 |
| MMP-7 | Yes (27) |               |
| MMP-8 | N               | 1/3 |
| MMP-9 | Yes (6, 16–26, 37) | ↑ 3/5 |
| MMP-10 | No            | 1/1 |
| MMP-11 | No          | 1/0 |
| MMP-12 | Yes (19) | ↑ 1–7 |
| MMP-13 | Yes (16) | Time course not performed |
| MMP-14 | Yes (16) | Time course not performed |
| MMP-15 | No          | NA |
| MMP-16 | No          | 1/1 |
| MMP-17 | No          | NA |
| MMP-19 | No          | NA |
| MMP-20† | No | NA |
| MMP-21† | No | NA |
| MMP-23A | No | No pro/1 |
| MMP-23B | No | No pro/1 |
| MMP-24 | No | No pro/7 |
| MMP-26 | No | ↑ 3/28 |
| MMP-27 | No | No pro/3 |
| MMP-28 | Yes (33) | ↑ High expression in myocytes at baseline; goes up (in macrophages) |
| TIMP-1 | Yes (5, 16) | ↑ |
| TIMP-2 | Yes (41) | ↑ 1 |
| TIMP-3 | Yes (44) | ↑ 1 |
| TIMP-4 | Yes (22) | ↓ 1 |

Results from this study are indicated with boldface. NA, not applicable.

* Increased or ↓ decreased compared with day 0 (P < 0.05); ↔ no change.

MMP-1 expressed in rat, human, and porcine myocardial infarction (MI) left ventricle (LV) (23) (MMP-4, -5, and -6 were later found by sequencing to be repeats of MMP-1, -2, and -3 (40); MMP-18, alias for MMP-19); †MMP-20 (enamelysin) (42) and MMP-21 (35) very low to no expression in cardiac tissue.

Spinal FG. Matrix metalloproteinase-7 affects connexin-43 levels, electrical conduction, and survival after myocardial infarction. *Circulation* 113: 2019–2029, 2006. doi: 10.1161/CIRCULATIONAHA.106.612960.

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