Cardiac-Restricted Overexpression of Membrane Type-1 Matrix Metalloproteinase in Mice
Effects on Myocardial Remodeling With Aging

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Background—The direct consequences of a persistently increased myocardial expression of the unique matrix metalloproteinase (MMP) membrane type-1 (MT1-MMP) on myocardial remodeling remained unexplored.

Methods and Results—Cardiac-restricted MT1-MMPexp was constructed in mice using the full-length human MT1-MMP gene ligated to the myosin heavy chain promoter, which yielded approximately a 200% increase in MT1-MMP when compared with age/strain-matched wild-type (WT) mice. Left ventricular (LV) function and geometry was assessed by echocardiography in 3-month (“young”) WT (n=32) and MT1-MMPexp (n=20) mice and compared with 14-month (“middle-aged”) WT (n=58) and MT1-MMPexp (n=35) mice. LV end-diastolic volume was similar between the WT and MT1-MMPexp young groups, as was LV ejection fraction. In the middle-aged WT mice, LV end-diastolic volume and ejection fraction was similar to young WT mice. However, in the MT1-MMPexp middle-aged mice, LV end-diastolic volume was ≈43% higher and LV ejection fraction 40% lower (both P<0.05). Moreover, in the middle-aged MT1-MMPexp mice, myocardial fibrillar collagen increased by nearly 2-fold and was associated with ≈3-fold increase in the processing of the profibrotic molecule, latency-associated transforming growth factor binding protein. In a second study, 14-day survival after myocardial infarction was significantly lower in middle-aged MT1-MMPexp mice.

Conclusions—Persistently increased myocardial MT1-MMP expression, in and of itself, caused LV remodeling, myocardial fibrosis, dysfunction, and reduced survival after myocardial injury. These findings suggest that MT1-MMP plays a mechanistic role in adverse remodeling within the myocardium. (Circ Heart Fail. 2009;2:351-360.)

Key Words: matrix □ myocardial remodeling □ ventricular function □ aging

Left ventricular (LV) remodeling is generically defined as changes in myocardial architecture and structural composition, which in turn will affect overall LV geometry and function. Although the LV remodeling process evokes changes within both the cellular and extracellular compartment, recent studies have demonstrated that changes in extracellular structure and composition occur with LV remodeling.1–6 Specifically, the induction and activation of a family of matrix proteases, termed the matrix metalloproteinases (MMPs), have been demonstrated to occur in patients and animals and are related to the degree of LV remodeling.1–6 Moreover, using transgenic and pharmacological approaches, a cause-effect relationship has been demonstrated between the induction of MMPs and the LV remodeling process.2,7–9 However, there are a large number of MMP types, which are expressed within the myocardium, and a unique functionality may exist for each of these MMP types with respect to the LV remodeling process. One of the more unique MMP types, which has been identified within the human myocardium, is the membrane type (MT) MMPs of which the MT1-MMP subtype has been the most studied.3,10–15 A significant increase in the myocardial levels of MT1-MMP has been identified in patients with LV failure, and the relative magnitude of this increase was greater than that of any other MMP subclass.3 In animal models, MT1-MMP myocardial levels are increased early and appear coincident with adverse LV remodeling.1–5 However, a direct causative relationship between persistently increased myocardial levels of MT1-MMP and the LV remodeling process has not been established. The central hypothesis of this study was that a persistent and selective increase of MT1-MMP within the myocardium would result in LV structural remodeling.
dysfunction, and an inability to respond to a pathological stimulus, such as myocardial infarction (MI).

Methods

Overview and Rationale

A murine construct of cardiac-restricted overexpression of MT1-MMP was developed and then used to examine LV function and geometry as a function of age. Determinants of LV matrix remodeling in this construct was examined by measuring indices of transforming growth factor (TGF)-β signaling,16–21 MMP, and tissue inhibitor of MMP (TIMP) levels through combined immunohistochemical/biochemical approaches. To determine whether persistent induction of MT1-MMP altered the response to a pathological stimulus, survival after a MI was examined.

MT1-MMP Overexpression and Aging

A myocardial-restricted overexpression construct of MT1-MMP (MT1-MMPexp; α-myosin heavy chain promoter [major histocompatibility complex] linked to full-length human MT1-MMP) was established in mice from the FVB background strain. The MT1-MMP full-length human gene sequence (GenBank 793762 accession 90925; 2369 bp) was cloned into the α-major histocompatibility complex construct (courtesy of Jeff Robbins, University of Cincinnati, Cincinnati, Ohio). Clone 26, Genbank 71441), The incorporation of the major histocompatibility-complex MT1-MMP construct was confirmed by using a polymerase chain reaction protocol from tail clip DNA. Three independent lines of MT1-MMPexp mice were developed and after backcrossing and stable breeding patterns, ~50% from each litter were MT1-MMPexp positive. The MT1-MMPexp negative mice were used as reference, wild-type (WT) siblings. The MT1-MMPexp mice displayed no obvious phenotypic abnormalities. MT1-MMPexp and WT mice were maintained until 3 months (“young”; 3.0±0.1 months) or 12 to 18 months (“middle aged”; average age, 14±1 months) of age and then randomized to undergo LV functional assessment and myocardial sampling or to undergo surgical induction of MI. This latter age category was chosen because past studies have identified that after ~12 months old, mice begin to display age-dependent changes in wound healing and myocardial remodeling.22,23 Terminal procedures were performed under isoflurane anesthesia. A precalibrated 4 mm (Vevo 660, VisualSonics, Toronto, Canada). Using long-axis views, LV end-diastolic volume, posterior wall thickness, ejection fraction, and mass were computed. Follow-up which, the mice were positioned on a feed-back temperature compartment, were digitized and the number of ASMA positive cells were computed.

Histomorphometry

LV sections (5 μm) were stained with picro-sirus red for fibrillar collagen and the percent area of collagen within the LV computed.19 For the subsequent MI studies, LV sections were stained with hematoxylin-eosin for measurement of MI size using computer-assisted planimetry (Sigma Scan, Media Cybernetics, Bethesda, Md), where MI size was expressed as a percent of the total LV area. To compute the relative density of alpha-smooth muscle actin (ASMA) positive cells within the interstitium, reflective of myofibroblasts,25 parallel LV sections were incubated with anti-ASMA (AB5694; 1:200 dilution) overnight at 4°C, and specifically bound antisera was visualized by a peroxidase reaction (Vector Laboratories Peroxidase Substrate Kit, SK4100, Burlingame, Calif). The LV sections were imaged at a final magnification of 20×, and 10 random fields within the midmyocardial region, devoid of any vascular compartment, were digitized and the number of ASMA positive cells were computed.

MT1-MMP Immunohistochemistry

Frozen LV sections (7 μm) were fixed in ice-cold acetone for 5 minutes, washed, blocked with 10% normal goat serum (Sigma), and then in the primary MT-MMP antisera (AB815; 1:250 dilution) overnight at 4°C. The LV sections were then vigorously washed and incubated with a secondary antisera (AlexaFluor 488:1:250 dilution, Molecular Probes, Wash), cover-slipped (VECTASHIELD Mounting Medium, Vector Laboratories, Burlingame, Calif) and imaged using confocal microscopy (Zeiss LSM 510; Plan-Apochromat 63X/1.4; 493/519 nm excitation/emission) as well as by difference interference contrast. In a second protocol, dual staining for both MT1-MMP and ASMA was performed in which the secondary antisera used for ASMA localization was at different excitation/emission wavelengths (650 nm/668 nm, AlexaFluor 647; 1:250 dilution).

Myocardial MMP/TIMP Levels

Substrate zymography was performed to assess the relative content of the gelatinases, MMP-2 and MMP-9. A positive control was used in all zymography measurements (2 μg, MMP-2/9 SE-244/237, Biomol, Plymouth Meeting, Pa). Immunoblotting was performed for MMP-13, the predominant rodent interstitial collagenase as well as for TIMP-1, -2 and -4.13 For the immunoblotting studies (10-μg protein), antisera (1:2500 dilution) corresponding to MMP-13 (3533, BioVision; MT1-MMP (AB221, Millipore), TIMP-1 (AB8122), TIMP-2 (AB801), or TIMP-4 (AB8221). For the MT1-MMP immunoblotting and activity assays, LV myocardium was homogenized in ice-cold 250-mmol/L sucrose–20-mmol/L 3-[(N-morpholino)-proanesulfonic acid (MOPS) buffer. The homogenate was centrifuged (100 000g, 1 hour), and the membrane fraction resuspended in buffer. Positive controls for MMP-13 (CC068 Millipore), MT1-MMP (CC1043), TIMP-1 (CC1062), TIMP-2 (CC1064), and TIMP-4 (CC1066) were included in every assay.

MT1-MMP Activity Assay

LV myocardial extracts (50 μg) were incubated with a specific MT1-MMP fluorogenic substrate (MMP-14 Substrate I, Cat. No. 444238; Calbiochem), which has been validated previously.10 The LV myocardial extracts were incubated in ice (37°C, 2 hours) in the presence and absence of the MT-MMP substrate, and excitation/emission recorded (328/400, FluoroStar Galaxy, BMG Labtech Inc, NC). To convert the fluorescent readings from this in situ assay to relative MT1-MMP activity, a recombinant active MT1-MMP construct (MT1-MMP Catalytic Domain, Cat. No. 475935; 2369 bp) was used in a parallel set of reactions.

Latency TGF Binding Protein (LTBP)-1 Processing, TGF-R1 and Smad-2

One of the initial critical steps for matrix-bound TGF to become a competent profibrotic signaling molecule is through the proteolytic release from the LTBP-1.16,21 In light of the fact that LTBP-1 is initially a high-molecular weight protein, which is subsequently proteolytically processed to low molecular weight,16,21 LV extracts (20 μg of total protein) were loaded onto 3% to 8% Tris Acetate gels
in vitro studies, referent WT myocardial extracts (n
LTBP-1 could be proteolytically processed by MT1-MMP. For these
sc-2215, Santa Cruz Biotechnology, Santa Cruz, Calif) was used.
MT1-MMP catalytic domain (0.5 to 3
incubated at 37°C for 2 hours, with increasing concentrations of the
LTBP-1 (SC28133; 1:200). In all these studies, a
rigidly maintained in a protease inhibitory cocktail (150 mmol/L,
EDTA: 1 mmol/L, phenylmethylsulfonyl fluoride: 1 mmol/L, apro-
tinin: 1 mg/mL, leupeptin: 1 mg/mL, pepstatin). Immunoblotting was
performed for LTBP-1 (SC28133; 1:200). In all these studies, a
control and aging groups using an ANOVA and pairwise compari-
dences performed by a Bonferroni adjusted
values were computed as a percent of control values where the

to a Gaussian distribution, subjected to ANOVA and finally to
between-group differences in these values were compared using ANOVA followed
Bonferroni adjusted t test. For the morphometric data (percent
collagen, ASMA density), the data were first confirmed to conform to a
Gaussian distribution, subjected to ANOVA and finally to
Tukey’s test for mean separation. For the survival portion of the
study, survival curves were constructed using Kaplan–Meier proba-
bility estimates and 14-day post-MI survival compared using a χ²
analysis. Values of P<0.05 were considered statistically significant.
All statistical procedures were performed using the STATA statisti-
cal software package (Statacorp, College Station, Tex). Results are
presented as mean±SEM. Final sample sizes for each protocol/
experiment are indicated in the figure legend or table. The authors
had full access to the data and take full responsibility for its integrity.

Results

LV Function and Geometry

LV function measurements were performed under equivalent,
ambient heart rates (Table). LV systolic pressure was equiva-
lent across the WT and MT1-MMPexp groups, as well as
between young and middle-aged mice. However, LV end-di-
astolic pressure and wall thickness were increased in both
middle-aged WT and middle-aged MT1-MMPexp groups.
LV end-diastolic volume and ejection fraction were similar
between young WT and MT1-MMPexp groups, and was
unchanged in the middle-aged WT group. However, LV
end-diastolic volume was increased and ejection fraction
reduced in the middle-aged MT1-MMPexp group. Maximal
LV elastance was similar between young WT and MT1-
MMPexp groups, was decreased in the middle-aged MT1-MMPexp
and was unchanged in the middle-aged WT group. LV mass
was increased in the middle-aged WT group and was
increased further in the middle-aged MT1-MMPexp
group.

Fibrillar Collagen Content and ASMA Density

Representative full LV sections from the young and middle-
aged WT and MT1-MMPexp groups under bright field to
illustrate the significant changes in LV geometry, and follow-
ing picro-sirius staining and polarized light imaging to
demarcate the myocardial fibrillar collagen are shown in
Figure 1. Relative LV fibrillar collagen was increased in the
young MT1-MMPexp group when compared with young WT
values (0.95±0.14%, 0.53±0.12%, respectively, P<0.05). In

| Table. Effects of Age on LV Geometry and Function With MT1-MMP Overexpression |
|---------------------------------|---------------------------------|
| WT                             | MT1-MMPexp                      |
| Heart rate, bpm                |                                |
| 478±8                          | 478±6                           |
| 494±9                          | 486±7                           |
| LV peak systolic pressure, mm Hg|                                |
| 98±3                           | 104±2                           |
| 99±4                           | 93±9                            |
| LV end-diastolic pressure, mm Hg|                                |
| 6±1                            | 10±1*                           |
| 7±1                            | 11±2*                           |
| Posterior wall thickness, mm    |                                |
| 0.78±0.01                      | 0.98±0.02*                      |
| 0.84±0.03                      | 0.94±0.04*                      |
| End-diastolic volume, μL        |                                |
| 43.7±1.7                       | 43.5±0.9                        |
| 42.8±2.1                       | 60.3±3.3*                       |
| Ejection fraction, %           |                                |
| 61.2±1.1                       | 61.0±0.9                        |
| 56.3±2.2                       | 37.3±2.1†                       |
| Emass, mm Hg/mL                |                                |
| 7.5±0.7                        | 4.5±0.6*                        |
| 7.4±1.9                        | 12.4±3.8                        |
| LV mass, mg                    |                                |
| 105.1±3.3                      | 136.7±3.2*                      |
| 109.8±5.8                      | 162.3±9.8†                      |
| Sample size, n                 |                                |
| 32                             | 58                              |
| 20                             | 35                              |

Values presented as mean±SEM.
*P<0.05 versus young WT.
†P<0.05 versus middle-aged WT.

MI

In these studies, old WT and old MT1-MMP mice underwent LV
echocardiography, after which a thoracotomy was performed, the
LV visualized, and the main left coronary artery ligated (8.0
Neurilon, Ethicon, K801). The intraoperative mortality (first 24
hours) was 15% and similar between groups. The mice were
followed for 14 days post-MI at which time a second echocar-
diogram was performed and the LV harvested for histomorphom-
etry and MT1-MMP measurements.

Data Analysis

LV function and geometry was compared between the referent
control and aging groups using an ANOVA and pairwise compar-
sions performed by a Bonferroni adjusted t test. The zymographic/
immunoreactive signals were analyzed using densitometric methods
(Gel Pro Analyzer, Media Cybernetics) to obtain 2-dimensional
integrated optical density values. The integrated optical density
values were then computed as a percent of control values where the
control values were set to 100% and comparisons performed by a
separate t test. For the MMP immunoassays, a Winsorized mean was
used if extreme values existed in the data set. Between-group
differences in these values were compared using ANOVA followed
Bonferroni adjusted t test. For the morphometric data (percent
collagen, ASMA density), the data were first confirmed to conform to a
Gaussian distribution, subjected to ANOVA and finally to
(EA03785, Invitrogen, Carlsbad, Calif). The LV extracts were
rigidly maintained in a protease inhibitor cocktail (150 mmol/L,
EDTA: 1 mmol/L, phenylmethylsulfonyl fluoride: 1 mmol/L, apro-
tinin: 1 mg/mL, leupeptin: 1 mg/mL, pepstatin). Immunoblotting was

the middle-aged WT group, fibrillar collagen was increased compared with respective young WT values (0.82±0.08%, P<0.05) and was increased by >2-fold in the middle-aged MT1-MMPexp group (2.26±0.59%, P<0.05). The density of positive ASMA interstitial cells in the young WT and middle-aged WT groups were similar (25±2 versus 23±1 cells/mm²), increased in the young MT1-MMPexp groups (65±5 mm², P<0.05) and remained elevated in the middle-aged MT1-MMPexp group (43±1 mm², P<0.05).

MT1-MMP Immunohistochemistry
LV sections were first examined for MT1-MMP relative content and distribution using confocal fluorescence microscopy (Figure 2). A clear and definitive signal for MT1-MMP could be observed along cardiac myocytes in the young WT LV sections. In the middle-aged WT group, the intensity for MT1-MMP staining increased along the sarcolemmal-matrix interface. This type of distribution is consistent with the transmembrane characteristics of MT1-MMP.14,15 The greatest immunofluorescent signal was observed in both the young and middle-aged MT1-MMPexp sections, with robust staining along the myocyte-matrix interface. LV sections were next subjected to dual immunofluorescence in which sections were stained for MT1-MMP as well as for ASMA. A positive signal for ASMA was observed within interstitial cells in all LV sections, consistent with the myofibroblast phenotype (Figure 2).25 Moreover, in the MT1-MMPexp sections, the increased MT1-MMP levels were spatially associated with these ASMA positive interstitial cells.
MMP/TIMP Levels and MT1-MMP Activity

Representative MT1-MMP immunoblots of LV myocardial membrane extracts are shown in Figure 3. MT1-MMP levels were increased in the middle-aged WT mice compared with the young WT mice. MT1-MMP levels were increased by ~2-fold with cardiac-restricted MT1-MMPexp. Total MT1-MMP levels were also increased in the middle-aged WT group compared with the young WT group. Using the same LV myocardial extracts, MT1-MMP proteolytic activity was assessed using a specific fluorogenic substrate. Using increased concentrations of a recombinant MT1-MMP construct (8 to 125 ng/mL) with a known catalytic activity resulted in a linear relationship with respect to fluorescence emission ($y = 529x$, $r^2 = 0.98$, $P < 0.001$). Myocardial MT1-MMP activity was significantly increased in both the young and middle-aged MT1-MMP overexpression groups compared with respective WT control values ($P = 0.028$ and $P = 0.020$, respectively; young = 3 months, middle aged [MA] = 14 months). $^*P < 0.05$ versus young WT values. $^#P < 0.05$ versus respective MA WT values.

Figure 3. A, LV myocardial membrane extracts from the young WT (n = 12), middle-aged WT (n = 10), young MT1-MMP overexpression (MT1-MMP; n = 10), and middle-aged MT1-MMP (n = 10) mice were subjected to MT1-MMP immunoblotting. Total MT1-MMP myocardial levels, which includes the full-length and truncated active forms (55 kDa), increased by over 2-fold with cardiac-restricted MT1-MMPexp. Total MT1-MMP levels were also increased in the middle-aged WT group compared with the young WT group. B, Using the same LV myocardial extracts, MT1-MMP proteolytic activity was assessed using a specific fluorogenic substrate. Using increased concentrations of a recombinant MT1-MMP construct (8 to 125 ng/mL) with a known catalytic activity resulted in a linear relationship with respect to fluorescence emission ($y = 529x$, $r^2 = 0.98$, $P < 0.001$). Myocardial MT1-MMP activity was significantly increased in both the young and middle-aged MT1-MMP overexpression groups compared with respective WT control values ($P = 0.028$ and $P = 0.020$, respectively; young = 3 months, middle aged [MA] = 14 months). $^*P < 0.05$ versus young WT values. $^#P < 0.05$ versus respective MA WT values.

Figure 4. A, LV myocardial extracts from the young WT (n = 12), middle-aged WT (MA; n = 10), young MT1-MMP overexpression (MT1-MMP; n = 10), and middle-aged MT1-MMP (n = 10) mice were subjected to gelatin zymography. Total MMP-2 levels (72 and 64 kDa forms) increased in both the young and middle-aged MT1-MMP mice compared with WT ($P = 0.002$ for both comparisons), with much higher levels of the active form of MMP-2 (64 kDa; $P = 0.002$ and $P = 0.003$, respectively). No change in MMP-9 was observed in the middle aged or MT1-MMP groups. Positive recombinant controls for MMP-2 and MMP-9 were included on all zymograms. B, MMP-13, the predominant rodent collagenase, and tissue inhibitors of MMPs, TIMP-1, TIMP-2, and TIMP-4 were determined by immunoblotting on the same LV myocardial extracts. MMP-13 increased in both the middle-aged WT and middle-aged MT1-MMP groups ($P = 0.007$ and $P = 0.002$, respectively). Relative TIMP-1 levels were significantly reduced in the middle-aged MT1-MMP group ($P = 0.035$). TIMP-2 levels were increased in the young MT1-MMP group ($P = 0.048$), and relative TIMP-4 levels were increased in the young MT1-MMP group ($P = 0.043$) and in both the middle-aged WT and MT1-MMP groups ($P = 0.047$, $P = 0.041$; young = 3 months, middle aged = 14 months). $^*P < 0.05$ versus young WT values. $^#P < 0.05$ versus respective middle-aged WT values.)

groups. The TIMP-1 levels were reduced in the middle-aged MT1-MMPexp group, whereas the TIMP-2 levels were increased in the young MT1-MMP group. The TIMP-4 levels were increased in the middle-aged WT group and in both the
young and middle-aged MT1-MMPexp groups. The MT1-MMP proteolytic activity was assessed using a specific fluorogenic substrate and validated by increasing concentrations of a recombinant MT1-MMP construct (with a known catalytic activity; Figure 3). Myocardial MT1-MMP activity was increased by 2-fold in both the young and middle-aged MT1-MMPexp groups.

**LTBP-1 Processing, TGF-R1 and Smad-2**

Positive immunoreactive bands corresponding to the full length (180 kDa) and proteolytically processed form (60 kDa) of LTBP-1 were observed in all LV myocardial extracts (Figure 5). Total myocardial levels of LTBP-1 were increased in the middle-aged WT group and both MT1-MMP groups. The lower molecular weight form of LTBP-1 was increased to the greatest degree in the middle-aged MT1-MMP group. Using increasing concentrations of a recombinant MT1-MMP catalytic domain, a relative reduction in the 180 kDa form of LTBP-1 and emergence of the 60 kDa form was observed in LV myocardial extracts (Figure 5). These results provided in vivo and in vitro evidence of MT1-MMP-mediated proteolytic processing of LTBP-1. Accordingly, we examined whether a specific cleavage site for MT1-MMP exists within LTBP-1 in silico, which in turn would yield the appropriate LTBP-1 fragment obtained from these immunoblotting experiments. The full-length sequence for LTBP-1 (National Center for Biotechnology Information; AAI30290.1) was examined for MT1-MMP-mediated proteolytic specificity. Three potential peptides were identified: A176: SGRSENIRTA, A42: SGRIGFLRTA, and B175A: SGAAHMHTYA. The peptides were aligned to each individual sequence of interest to identify potential binding/cleavage sites using the CLUSTAL W+ alignment algorithm with the BLOSSUM scoring matrix. Sequences demonstrating positive peptide alignment were analyzed for domain structure using the Simple Modular Architecture Research Tool to assess putative cleavage sites. Peptide A176 demonstrated a high concordance with LTBP-1 and significant alignment for MT1-MMP specific cleavage. Moreover, these in silico mapping experiments predicted a LTBP-1 cleavage product of 60 kDa, which was obtained from the in vivo/in vitro experiments.

LV murine extracts were next subjected to immunoblotting for TGF-R1 and a common convergence point of the TGF-R1 receptor transduction pathway, Smad-2. Total TGF-R1 levels were increased in the middle-aged MT1-MMP group (Figure 6). A common Smad, Smad-2 in the classical TGF signaling pathway was measured in which both total Smad-2 and the phosphorylated form of Smad-2 were quantified (Figure 6). Total phospho-Smad-2 levels were increased in the middle-aged WT group and both MT1-MMP groups. Moreover, the ratio of phosphorylated/total Smad-2 was increased to the greatest extent in the middle-aged MT1-MMP group.

**Persistent MT1-MMP Myocardial Expression and MI**

In a second study, MI was induced and after the initial 24-hour recovery period, middle-aged WT (n=30) and middle-aged MT1-MMP mice (n=29) were followed for 14 days post-MI (Figure 7). Before MI induction, baseline LV end-diastolic volume (43±1 uL) and ejection fraction (65±1%) in the middle-aged WT group were very similar to those obtained in the initial cohort of middle-aged WT mice (Table). In the middle-aged MT1-MMPexp group LV end-diastolic volume was increased (77±3 uL) and ejection fraction reduced (39±2%) in a similar pattern to the initial cohort of middle-aged MT1-MMPexp mice (P<0.05; Table).
The survival rate for middle-aged WT mice was 57% and was significantly lower in the middle-aged MT1-MMPexp mice (14%, \( \chi^2: 12.5, P = 0.001 \)). Equivalent distribution of post-mortem findings were observed between the WT and MT1-MMPexp groups, where \( \approx 10\% \) of the deaths were due to myocardial rupture at the LV apical region, 70% were due to occult cardiac decompensation as evidenced by significant serous fluid accumulation within the thoracic space, and 20% revealed no significant transudate or serosanguineous fluid in the thoracic space, and therefore, the deaths were presumed to be of an arrhythmic origin. Representative LV full sections under bright field and under polarized light for both MI groups are shown in Figure 1. Computed MI size was equivalent between the middle-aged WT and MT1-MMP groups (35\% \pm 4\%, 38\% \pm 7\%, respectively). At 14 days post-MI, fibrillar collagen was increased in the middle-aged WT group within the MI and remote region when compared with respective young or middle-aged WT values (10.20 \pm 1.26\%, 3.36 \pm 0.14\%, respectively, \( P < 0.05 \)). In the surviving middle-aged MT1-MMPexp MI mice (\( n = 4 \)), fibrillar collagen was increased from referent control values as well as post-MI WT values within the MI and remote regions (19.5 \pm 1.93\%, 6.05 \pm 0.32\%, respectively, \( P < 0.05 \)). Total MT1-MMP levels were increased by 2-fold and the fully proteolytically active form of MT1-MMP (55 kDa) increased by nearly 10-fold in the middle-aged MT1-MMPexp group (Figure 7).

Discussion

Changes in the expression and activity of the large family of matrix MMPs have been well documented in animal models and in clinical studies of LV remodeling.\(^1\)\(^-\)\(^9\) One class of MMPs with a diverse substrate portfolio as well as unique functional aspects is the MT-MMPs of which MT1-MMP can be considered prototypical. Although past studies have associated changes in MT1-MMP levels with adverse LV remodeling,\(^1\)\(^,\)\(^3\)\(^,\)\(^10\)\(^,\)\(^11\) the functional and structural consequences of cardiac-restricted overexpression of MT1-MMP (MT1-MMPexp) has not been explored. In this study, persistent cardiac-restricted MT1-MMPexp was induced in mice and the effects on LV structure and function were examined as a function of age. The unique findings from this set of investigations were 3-fold. First, in the middle-aged MT1-MMPexp mice, significant LV remodeling and systolic dysfunction occurred, which was accompanied by increased proteolytic MT1-MMP activity and collagen content. Second, persistent MT1-MMPexp, was associated with increased proteolytic processing of latency-associated TGF binding protein (ie, LTBP-1), increased TGF receptor-1 density, and increased phosphorylation state of a common transduction convergence point of TGF signaling, Smad-2. Third, MI in the middle-aged MT1-MMPexp mice resulted in worsened post-MI survival and exacerbated collagen accumulation. Taken together, the results from this study suggest that the increased myocardial MT1-MMP levels, equivalent to those
levels observed previously in patients and animals with severe LV failure, directly contributes to adverse LV remodeling and dysfunction, a profibrotic response, and poor adaptation to a pathological stimulus, such as MI.

In this study, mice with persistent MT1-MMPexp resulted in severe LV dilation, dysfunction, and hypertrophy as a function of age. To determine whether intrinsic myocardial contractility was affected with MT1-MMPexp, load-independent indices of contractile function were assessed using pressure-conductance volumetry. These studies revealed that LV contractility was reduced as a function of age, but was not further impaired in the MT1-MMPexp mice. These observations would suggest that the reduced LV ejection performance in the middle-aged MT1-MMPexp mice was most likely due to the significant alterations in chamber geometry as well as matrix remodeling. In addition, this study demonstrated that the induction of a pathological stimulus (MI) in these middle-aged MT1-MMP mice was associated with a poor compensatory response defined as a reduced survival. These findings suggest that the persistent induction of MT1-MMP results in a more vulnerable myocardium when exposed to MI.

One of the more unexpected outcomes from these MT1-MMPexp studies was the changes in myocardial collagen content. Total myocardial collagen content was increased by nearly 2-fold when compared with respective WT values in the middle-aged MT1-MMPexp mice. There are several possible factors for this shift in steady-state collagen content with MT1-MMPexp. First, increased MT1-MMP levels would heighten pericellular matrix proteolysis, change local cell–matrix interactions, and thereby affect steady-state synthesis rates. Second, the relative increase in myofibroblasts with MT1-MMPexp would potentially result in increase net collagen synthesis. Third, the increased myocardial collagen content in the MT1-MMPexp mice may be the direct result of the diverse proteolytic profile of this membrane bound MMP. For example, increased myocardial MT1-MMP induction was accompanied by heightened activation in the determinants of the profibrotic signaling pathway: TGF. Full activation and release of TGF into the interstitium requires specific proteolysis of LTBP-1. In this study, increased fibrillar collagen content occurred in the middle-aged WT mice and was associated with increased LTBP-1 processing, TGF-R1 levels, and increased phosphorylation of a critical TGF intracellular signaling molecule, Smad-2. These associative observations suggest that the increased collagen accumulation with aging is likely due, in part to increased processing and activation of the TGF pathway. Through in vivo, in vitro, and in silico approaches, this study provided evidence for a mechanistic link between MT1-MMP proteolytic processing of LTBP-1. A recent in vitro study in endothelial cells also demonstrated that MT1-MMP proteolytic processing of LTBP-1. More importantly, phosphorylation of the intracellular signaling molecule Smad-2 occurred to the greatest degree in the aging MT1-MMPexp mice. Thus, although this study provides only associative data, these unique findings suggest that the induction of MT1-MMP causes LTBP-1 processing and subsequently a profibrotic signaling cascade that culminates in increased myocardial collagen accumulation.

It has been demonstrated previously that once MT1-MMP undergoes translational processing and trafficking to the membrane, then a proteolytically competent enzyme exists. MT1-MMPexp studies was the changes in myocardial collagen content. Total myocardial collagen content was increased by nearly 2-fold when compared with respective WT values in the middle-aged MT1-MMPexp mice. There are several possible factors for this shift in steady-state collagen content with MT1-MMPexp. First, increased MT1-MMP levels would heighten pericellular matrix proteolysis, change local cell–matrix interactions, and thereby affect steady-state synthesis rates. Second, the relative increase in myofibroblasts with MT1-MMPexp would potentially result in increase net collagen synthesis. Third, the increased myocardial collagen content in the MT1-MMPexp mice may be the direct result of the diverse proteolytic profile of this membrane bound MMP. For example, increased myocardial MT1-MMP induction was accompanied by heightened activation in the determinants of the profibrotic signaling pathway: TGF. Full activation and release of TGF into the interstitium requires specific proteolysis of LTBP-1.21,29 In this study, increased fibrillar collagen content occurred in the middle-aged WT mice and was associated within increased LTBP-1 processing, TGF-R1 levels, and increased phosphorylation of a critical TGF intracellular signaling molecule, Smad-2. These associative observations suggest that the increased collagen accumulation with aging is likely due, in part to increased processing and activation of the TGF pathway. Through in vivo, in vitro, and in silico approaches, this study provided evidence for a mechanistic link between MT1-MMP proteolytic processing of LTBP-1. A recent in vitro study in endothelial cells also demonstrated that MT1-MMP proteolytically processed LTBP-1. More importantly, phosphorylation of the intracellular signaling molecule Smad-2 occurred to the greatest degree in the aging MT1-MMPexp mice. Thus, although this study provides only associative data, these unique findings suggest that the induction of MT1-MMP causes LTBP-1 processing and subsequently a profibrotic signaling cascade that culminates in increased myocardial collagen accumulation.
amounts of the 68 kDa form would indicate that greater amounts of MMP-2 are being processed from the proform to the active form. These observations provide the first in vivo evidence that selective induction of MT1-MMP within the myocardial compartment in and of itself causes increased levels of an active form of MMP-2. Increased activation of MMP-2 would further contribute to matrix instability and loss of cellular continuity in the MT1-MMPexpp mice. MMP-13 levels, the predominant rodent collagenase, were increased in both the middle-aged WT and MT1-MMPexpp mice. The increased MMP-13 levels in the aging myocardium would in turn, contribute to the instability and disruption of a normally functioning matrix. However, extrapolation of MMP protein levels to enzymatic activity must be done with caution and requires the consideration of a number of posttranslational events including the relative levels of the endogenous MMP inhibitors, the TIMPs. In this study, relative TIMP-1 levels fell in the middle-aged MT1-MMPexpp group, and relative TIMP-2 levels increased in the young MT1-MMPexpp group. In the middle-aged WT and the middle-aged MT1-MMPexpp groups, TIMP-4 levels were increased. Thus, TIMPs do not change in a uniform fashion as a function of age and do not necessarily change in a uniform pattern with changes in relative MMP levels. This observation would suggest that TIMPs are differentially regulated within the myocardial compartment. Moreover, functional studies have identified unique roles for each of these TIMPs in the context of MMP processing, inhibition, and matrix remodeling. Thus, a more comprehensive stoichiometric analysis of MMP and TIMP complexes in this transgenic system with aging would be necessary. In addition, TGF has been shown to upregulate TIMPs. Thus, increased TGF signaling would also contribute altered MMP/TIMP stoichiometry, which in turn would cause a shift in the balance of ECM turnover favoring ECM accumulation, and eventually fibrosis.

Increased myocardial levels of MT1-MMP have been reported previously in the context of LV remodeling in humans and animals. Through the use of microdialysis in a large animal model, it has been demonstrated that increased myocardial MT1-MMP activity occurs very early after ischemia. This study used a cardiac-overexpression model of MT1-MMP, driven by a myosin heavy chain promoter, to induce myocardial MT1-MMP levels to those levels observed in these past studies. However, using the myocyte heavy chain promoter, the preponderance of expression will be restricted to the cardiac myocyte. LV myocardial fibroblasts robustly express MT1-MMP, and increased fibroblast levels of MT1-MMP have been reported in patients with end-stage LV failure. In this study, interstitial density of ASMA positive cells, consistent with myofibroblasts, were increased with MT1-MMP induction and colocalized to the sarcolemmal sites of MT1-MMP expression. Whether increased density or phenotypic transformation of ASMA positive myocardial fibroblasts was a consequence of MT1-MMP overexpression remains to be established. Moreover, whether MT1-MMP induction in fibroblasts as well as in cardiac myocytes may cause a more severe LV phenotype remains to be explored. This study examined the consequences of MT1-MMP overexpression, but targeted down-

regulation of this MMP was not addressed. Thus, based on past studies identifying increased MT1-MMP levels in the failing human myocardium and the results from the present study, more targeted and selective transgenic/pharmacological strategies to selectively interrupt MT1-MMP myocardial expression and activity in the context of LV remodeling would be warranted.

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References


**CLINICAL PERSPECTIVE**

Changes within the extracellular matrix of the myocardium occur as a function of age and can contribute to progressive alterations in left ventricular (LV) geometry and function-termed LV remodeling. Although a cause-effect relationship has been identified between LV remodeling and a family of extracellular proteases-termed matrix metalloproteinases (MMPs), the functional role of individual MMP types with respect to LV remodeling, particularly as a function of age, remained unclear. A novel MMP type, membrane type-1 (MT1-MMP), has been identified previously to be increased in patients with end-stage heart failure. Accordingly, this project induced cardiac overexpression of human MT1-MMP in mice and examined LV function and structure as a function of age. Persistently increased myocardial MT1-MMP expression caused LV remodeling, myocardial fibrosis, and dysfunction with age. These findings suggest that MT1-MMP plays a novel mechanistic role in adverse remodeling within the myocardium through not only enhancing collagen turnover, but likely contributing to the profibrotic pathway. Thus, regulating MT1-MMP activity may provide an important therapeutic approach for modifying myocardial matrix remodeling.
Cardiac-Restricted Overexpression of Membrane Type-1 Matrix Metalloproteinase in Mice: Effects on Myocardial Remodeling With Aging

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