Left ventricular (LV) hypertrophy represents an initial adaptive remodeling response to pressure overload (PO); however, chronic PO is a progressive process that commonly leads to adverse LV remodeling, including the development of myocardial fibrosis and diastolic dysfunction. Studies that have examined potential causes for this maladaptive response have focused primarily on mechanisms that alter collagen synthesis. However, there is emerging evidence that suggests that proteolytic events, such as those mediated by matrix metalloproteinases (MMPs), may also contribute to the adverse extracellular matrix (ECM) remodeling that occurs in PO. For example, our recent studies demonstrated that PO-induced temporal changes in LV structure and function were associated with a time-dependent increase in the induction, expression, and abundance of a unique membrane type-1 (MT1) MMP. These findings may seem to contradict the canonical notion that MMP proteolytic activity primarily results in collagen degradation; however, MMPs act on a diverse portfolio of structural proteins and signaling molecules. In particular, MT1-MMP may play a critical role in facilitating activation of profibrotic synthetic pathways that result in fibrosis. Previous in vitro studies showed that MT1-MMP can hydrolyze latency-associated transforming growth factor–binding protein (LTBP-1) and activation of transforming growth factor–dependent profibrotic signaling. The present study tested the hypothesis that MT1-MMP plays a direct role in the matrix remodeling response to a left ventricular (LV) pressure overload (PO) stimulus.

**Background**—Although matrix metalloproteinases (MMPs) were initially thought to result primarily in extracellular matrix degradation, certain MMP types, such as membrane type-1 (MT1) MMP, may also be involved in profibrotic cascades through hydrolysis of latency-associated transforming growth factor–binding protein (LTBP-1) and activation of transforming growth factor–dependent profibrotic signaling. The present study tested the hypothesis that MT1-MMP plays a direct role in the matrix remodeling response to a left ventricular (LV) pressure overload (PO) stimulus.

**Methods and Results**—Wild-type (WT) and transgenic mice with cardiac-restricted MT1-MMP overexpression or MT1-MMP reduced expression underwent PO for 4 weeks. PO resulted in a 57% increase in LV mass (no change in LV end diastolic volume, resulting in an increase in the LV mass/volume ratio consistent with concentric remodeling), a 60% increase in MT1-MMP–mediated LTBP-1 hydrolysis and a 190% increase in collagen content in WT mice. Although LV mass was similar among WT, MT1-MMP overexpression, and MT1-MMP reduced expression after PO, significant differences in LV function, MT1-MMP–mediated LTBP-1 hydrolysis, and collagen content occurred. PO in MT1-MMP overexpression increased LTBP-1 hydrolysis (18%), collagen content (60%), and left atrial dimension (19%; indicative of LV diastolic dysfunction) when compared with WT. PO in MT1-MMP reduced expression reduced left atrial dimension (19%), LTBP-1 hydrolysis (40%), and collagen content (32%) when compared with both WT.

**Conclusions**—Despite an equivalent PO stimulus and magnitude of LV myocardial growth, altering MT1-MMP levels caused specific matrix-dependent changes in remodeling, thereby demonstrating a mechanistic role in the development of the maladaptive remodeling and myocardial fibrotic response to PO. (Circ Heart Fail. 2014;7:340-350.)

**Key Words:** hypertrophy, left ventricular matrix metalloproteinases

**Clinical Perspective on p 350**

These findings may seem to contradict the canonical notion that MMP proteolytic activity primarily results in collagen degradation; however, MMPs act on a diverse portfolio of structural proteins and signaling molecules. In particular, MT1-MMP may play a critical role in facilitating activation of profibrotic synthetic pathways that result in fibrosis. Previous in vitro studies showed that MT1-MMP can hydrolyze latency-associated transforming growth factor (TGF)–binding protein (LTBP-1) and release TGF β. TGF is maintained in the ECM in an inactive form through its binding to the LTBPs. LTBP-1 has an MT1-MMP–specific hydrolytic site. Once released, TGF undergoes activation, binds to the TGF receptor, activates intracellular signaling pathways that include phosphorylation of SMAD2 (pSMAD2), pSMAD2 translocation to the nucleus, and an increase collagen expression. Thus, this MT1-MMP–dependent proteolytic activity provides 1 potential mechanism by which the PO causes activation of profibrotic...
synthetic pathways that result in fibrosis. Therefore, the purpose of the present study was to test the hypothesis directly that MT1-MMP plays a mechanistic, cause, and effect role in the development of the maladaptive remodeling and myocardial fibrotic response to PO. This hypothesis was tested by altering MT1-MMP expression and abundance using transgenic mice that overexpress MT1-MMP and transgenic mice with reduced expression of MT1-MMP in a murine model of chronic PO created by transverse aortic constriction (TAC).

Methods

Overview and Rationale
To determine whether and to what extent modulation in MT1-MMP abundance alters the myocardial response to PO, 2 transgenic mouse lines were created: MT1-MMP overexpression (OE) and MT1-MMP reduced expression (RE); Friend virus B-type (FVB) wild-type (WT) mice served as controls. PO was created in these 3 groups of mice using TAC (PO); mice that did not undergo TAC (non-PO) were also studied in all 3 groups. The experimental response variables were chosen for the following reasons: first, to demonstrate that the transgenic constructs resulted in a change in MT1-MMP activity and a change in MT1-MMP-dependent LTBP-1 hydrolytic potential; second, to determine whether changes in MT1-MMP activity resulted in the activation of profibrotic signaling changes in the pSMAD2/SMAD2 ratio and in collagen I A1 (Col1A1) and collagen III A1 (Col3A1) expression were assessed; third, to determine whether these changes in profibrotic signaling resulted in changes in LV structure, diastolic function, and ECM fibrillar collagen content; and fourth, to determine whether these changes in MT1-MMP, LV structure, and function altered survival rates.

Transgenic Mouse Models
Mice with myocardial-restricted expression of human MT1-MMP (MT1-OE) were developed on an FVB background by placing the heavy chain promoter, as previously described.17 The presence of human MT1-MMP was confirmed by real-time polymerase chain reaction (RT-PCR) using human-specific MT1-MMP primers. A stable, viable colony of this transgenic line was established. Mice served as controls. PO was created in these 3 groups of mice using the American Society of Echocardiography criteria.21 LV mass was normalized to body weight. LAD was used to reflect chronic changes in LV diastolic pressure; LAD increases as a function of sustained increased LV diastolic pressure (ie, an integration of pressure over time).22,23 The aortic pressure gradient created by PO was measured using the modified Bernoulli equation: Pressure gradient=4(Vpeak)2, where Vpeak is the peak Doppler velocity across the TAC.2

Echocardiography
Echocardiographic measurements were made using a 40 MHz mechanical scanning transducer (707B) and a Vevo 770 echocardiograph (VisualSonics, Inc, Toronto, Canada). LV dimension, volume, wall thickness, ejection fraction (EF), mass, and left atrial dimension (LAD) were measured using the American Society of Echocardiography criteria.21 LV mass was normalized to body weight. LV myocardial collagen content was examined in LV myocardial sections (5-μm thick) stained with picrosirius red. A qualitative assessment of collagen content was made from birefringence light microscopy, and experimental groups were compared quantitatively based on collagen volume fraction (CVF), defined as the area stained by picrosirius red divided by the total area of interest.20,24 Fields with large blood vessels were excluded from the analysis. Areas examined were distributed throughout the myocardium from subendocardial to subepicardial and excluded the epicardial surface.

Cardiomyocyte cross-sectional area was measured using previously published techniques in the 6 groups of mice in the current study. Five cells in 10 random fields were measured in each mouse.

MT1-MMP Activity Assays
Myocardial MT1-MMP activity was directly measured in LV myocardium from each mouse using 2 MT1-MMP–specific quenched fluoroogenic substrates previously described.25,26 One substrate, MMP-14 Substitute I (catalog no. 444258, Calbiochem), contained a peptide sequence representing a synthetic MT1-MMP–specific cleavage site.17,25,27 The second custom-designed substrate, SCJI-1 (AnaSpec), contained a LTBP-1 peptide sequence representing an endogenous MT1-MMP–specific hydrolysis site.17,25 The LV myocardial extracts (50 μg) were incubated (37°C) in the presence and absence of each MT1-MMP substrate, and excitation/emission were recorded (328/400 nm for the synthetic MT1-MMP substrate, 340/485 nm for the endogenous LTBP-1 substrate FluoroStar Galaxy; BMG Labtech) continuously for ≤20 hours and compared with a standard curve using active recombinant MT1-MMP.27 Several in vitro validation studies were previously performed to demonstrate the specificity of each MT1-MMP substrate.27

Immunoblot Studies for Protein Abundance
SMAD2 and pSMAD2 protein abundance were determined in LV myocardial samples from non-PO or 4-week PO WT, MT1-OE, and MT1-RE mice using previously published methods.27 LV samples were thawed and transferred to cold extraction/homogenization buffer (buffer volume: 1:6 [wt/vol]) containing 10 mmol/L cacodylic acid (pH 5.0), 0.15 mol/L NaCl, 10 mmol/L ZnCl2, 1.5 mmol/L NaN3, and 0.01% Triton X-100 (vol/vol) and homogenized. Ten micrograms of the supernatant were fractionated on a 4% to 12% bis-tris gel. Proteins were transferred to nitrocellulose membranes (0.45 μm; Bio-Rad Laboratories, Hercules, CA) and incubated in antisera corresponding to total SMAD2 and pSMAD2 (nos. 5339 and 3101, respectively; Cell Signaling Technology, Beverly, MA). Antisera were diluted in 5% nonfat dry milk-PBS. A secondary

Pressure Overload
The methods used to create TAC have been previously described.20 All mice were between 12 and 14 weeks of age at the time of PO. Mice were studied 4 weeks after PO. Twenty mice underwent PO in the WT and MT1-RE groups, and 40 mice underwent PO in the MT1-OE group. All PO mice had a minimum pressure gradient of 100 mmHg across the TAC. Ten mice did not undergo TAC and served as non-PO controls in each of the 3 groups. In each group discussed above, equal numbers of male and female mice were assigned; thus, 50% of each group was male and 50% were female.
peroxidase-conjugated antibody was then applied (1:5000; 5% non-fat dry milk-PBS), and signals were detected with a chemiluminescent substrate (Western Lightning Chemiluminescence Reagent Plus, Perkin-Elmer). Band intensity was quantified using Gel-Pro Analyzer software (version 3.1.14; Media Cybernetics, Silver Spring, MD) and reported as the percent change from the non-PO control homogenates.

**Gene Expression Analysis**

Changes in Col1A1 and Col3A1 expression were examined in all myocardial samples using a RT-PCR method.

**RNA Isolation**

Myocardial homogenates were subjected to RNA extraction (RNeasy Fibrous Tissue Mini Kit; Qiagen, Valencia, CA), and the quantity and quality of the RNA were determined (Experion Automated Electrophoresis System; Bio-Rad Laboratories) using previously published methods.\(^1\),\(^2\),\(^7\),\(^24\)

**Real-Time Polymerase Chain Reaction**

RNA (1 μg) was reverse transcribed to generate cDNA (iScript cDNA Synthesis Kit; Bio-Rad Laboratories). The cDNA was amplified using gene-specific primer/probe sets (TaqMan Universal PCR Master Mix; catalog no. 4364321; Applied Biosystems, Foster City, CA) using single-color RT-PCR (MyiQ; Bio-Rad Laboratories). The specific primer/probe sets (Applied Biosystems) were Col1A1 and Col3A1 (catalog nos. Mm00801666-g1 and Mm01254476-m1). Negative controls were run to verify the absence of genomic DNA contamination (reverse transcription control) and the absence of overall DNA contamination in the PCR system and working environment (template control). Results from RT-PCR methods are presented as % change from FVB WT non-PO control.

**Statistical Analysis**

Measurements were analyzed before (non-PO) and 4 weeks after PO in WT, MT1-OE, and MT1-RE mice using a 2-way ANOVA; if significant, pairwise comparisons were made using Tukey test to adjust for multiple comparisons. Survival curves were constructed using Kaplan–Meier probability estimates and survival at 4 weeks was just for multiple comparisons. Survival curves were constructed using a least squares linear regression analysis. Values of P<0.05 were considered statistically significant. All statistical procedures were performed using the STATA statistical software package (StataCorp, College Station, TX). Results are presented as mean±SEM. Final sample sizes for each protocol/experiment are indicated in the Table. The authors had full access to the data and take full responsibility for its integrity. All animal procedures were performed in accordance with institutional guidelines.

### Results

**Survival**

PO produced by TAC caused a 40% mortality rate in the WT mice, 12 of the 20 assigned to this group survived to the 4-week study point (Figure 1). Modulation of MT1-MMP abundance altered the response to PO. PO in the MT1-OE mice caused an 80% mortality rate (9 of the 40 assigned to this group survived to the 4-week study point), whereas PO in the MT1-RE mice caused a 20% mortality rate, 16 of the 20 assigned to this group survived to the 4-week study point (Figure 1).

There were no mortalities in the non-PO control mice; all 10 assigned to each group survived to the 4-week study point. WT, MT1-OE, and MT1-RE mice that did not undergo TAC were followed for 4 weeks, from age 12 to 16 weeks. There were no mortalities in these 3 non-PO mice (Figure 1).

**Structure and Function**

LV structure, systolic function, and diastolic function data in the absence of PO (non-PO) and after 4 weeks of PO in WT, MT1-OE, and MT1-RE mice are presented in the Table. First, the results in the Table highlight the effects of PO on structure, function, and signaling. Second, the results highlight the effects of modulation of MT1-MMP abundance on the response to PO specifically by examining 3 comparisons: differences between FVB WT PO data (column 2 in the Table) versus MT1-OE PO data (column 4), FVB WT PO data (column 2) versus MT1-OE PO data (column 4), and FVB WT PO data (column 2) versus MT1-MMP Overexpression data (column 3).

<table>
<thead>
<tr>
<th></th>
<th>FVB Wild-Type</th>
<th>MT1-MMP Overexpression</th>
<th>MT1-MMP Reduced Expression</th>
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<tbody>
<tr>
<td></td>
<td>Non-PO</td>
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<td>Non-PO</td>
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<tr>
<td>Body weight, g</td>
<td>24±1</td>
<td>28±1</td>
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<td>LV mass, mg</td>
<td>74±2</td>
<td>132±3*</td>
<td>101±5†</td>
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<td>LV wall thickness, mm</td>
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<td>1.1±0.03*</td>
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<tr>
<td>LV mass/body weight, mg/g</td>
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<td>4.7±0.1*</td>
<td>3.5±0.1†</td>
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<td>LV cardiomyocyte CSA, μm²</td>
<td>260±10</td>
<td>400±14*</td>
<td>325±21†</td>
</tr>
<tr>
<td>LV end diastolic dimension, mm</td>
<td>3.5±0.1</td>
<td>3.5±0.1*</td>
<td>3.7±0.1</td>
</tr>
<tr>
<td>LV end diastolic volume, μL</td>
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<td>47.9±1.5</td>
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<tr>
<td>LV mass/volume, mg/μL</td>
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<tr>
<td>LV fractional shortening, %</td>
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<td>33±1*</td>
<td>31±1†</td>
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<td>LV ejection fraction, %</td>
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<td>59±1*</td>
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<td>LA diameter, mm</td>
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<td>2.0±0.3†</td>
</tr>
<tr>
<td>Sample size (male/female)</td>
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<td>12 (6/6)</td>
<td>10 (5/5)</td>
</tr>
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</table>

Values presented as Mean±SEM. BW indicates body weight; CSA, cross-sectional area; FVB, Friend virus B-type; LA, left atrium; LV, left ventricular; MT1-MMP, membrane type-1 matrix metalloproteinase; Non-PO, mice that did not undergo transverse aortic constriction; and PO, transverse aortic constriction–induced pressure overload.

*P<0.05 vs strain-matched non-PO.
†P<0.05 vs condition-matched FVB.
‡P<0.05 vs condition-matched MT1-MMP overexpression.
MT1-RE PO data (column 6), and MT1-OE PO data (column 4) versus MT1-RE PO data (column 6). All other additional statistical comparisons are presented in the Table.

Effects of PO
In WT mice, PO was associated with a 57% increase in LV mass, no change in LV end diastolic volume, and a 79% increase in the LV mass/volume ratio consistent with the development of concentric remodeling (Table). In addition, PO was associated with a slightly decreased EF (but EF did not fall below the normal range) and a 59% increase in LAD consistent with the development of diastolic dysfunction (Table).

Changes in cardiomyocyte cross-sectional area that resulted from PO and transgenic constructs closely paralleled changes in LV mass described above, indicating that the level of hypertrophy at the LV chamber level was based on the level of hypertrophy at the cellular level (Table).

Effects of Modulation of MT1-MMP Abundance
At baseline, in the absence of PO, MT1-OE resulted in small but significant changes in LV structure and function. MT1-OE mice had significantly increased LV mass, no change in LV end diastolic volume, increased LV mass/volume ratio, slightly decreased EF (but still within the normal range), and increased LAD when compared with WT mice (Table). By contrast, at baseline, in the absence of PO, MT1-RE resulted in no significant changes in LV structure or function when compared with WT mice (Table).

After 4 weeks of PO, there were some similarities and some differences in structure and function in the MT1-OE mice when compared with those in the WT mice. After 4 weeks of PO, the LV mass was similar in the MT1-OE mice versus WT mice. However, because PO caused an increase in LV end diastolic volume in MT1-OE (but not in WT), MT1-OE mice had a smaller LV mass/volume ratio than the WT mice (2.8±0.1 in WT PO versus 2.2±0.2 in MT1-OE-PO; *P<0.01; Table). In addition, after 4 weeks of PO, EF was 30% lower in the MT1-OE mice versus WT mice. Finally, after 4 weeks of PO, LAD was 20% higher in the MT1-OE mice versus WT mice (Table).

By contrast, after 4 weeks of PO, LV structure and systolic function in the MT1-RE mice was similar to the WT mice, but both LV diastolic function and survival were less adversely affected in the MT1-RE mice than those in the WT mice. After 4 weeks of PO, LV mass, LV end diastolic volume, the LV mass/volume ratio, and EF were similar in the MT1-RE mice versus the WT mice (Table). In addition, after 4 weeks of PO, LAD was 20% smaller in the MT1-RE mice versus WT mice (Table).

MT1-MMP–Dependent Profibrotic Signaling
Effects of PO
In WT mice, PO was associated with a ≈60% increase in MT1-MMP activity, LTBP-1 peptide hydrolysis, and the pSMAD2/SMAD2 ratio (Figures 2–4).

Effects of Modulation of MT1-MMP Abundance
At baseline, in the absence of PO, MT1-OE resulted in an increase in MT1-MMP activity, LTBP-1 peptide hydrolysis, and the pSMAD2/SMAD2 ratio when compared with WT mice (Figures 2–4). By contrast, at baseline, in the absence of PO, MT1-RE mice had a lower MT1-MMP activity, LTBP-1 peptide hydrolysis, and pSMAD2/SMAD2 ratio when compared with WT mice (Figures 2–4).

After 4 weeks of PO, there was a more robust activation of MT1-MMP–dependent profibrotic signaling in MT1-OE mice when compared with that in the WT mice. After 4 weeks of PO, MT1-MMP activity and LTBP-1 peptide hydrolysis were 15% higher in the MT1-OE mice than those in the WT PO mice (Figures 2 and 3). In addition, after 4 weeks of PO, the pSMAD2/SMAD2 ratio was 115% higher in the MT1-OE mice than that in the WT PO mice (Figure 4). By contrast, after 4 weeks of PO, there was a less robust activation of MT1-MMP–dependent profibrotic signaling in MT1-RE mice when compared with that in the WT mice. After 4 weeks of PO, MT1-MMP activity and LTBP-1 peptide hydrolysis were 30% lower in the MT1-RE mice than those in the WT PO mice (Figures 2 and 3). In addition, after 4 weeks of PO, the pSMAD2/SMAD2 ratio was 25% lower in the MT1-RE mice than that in the WT PO mice (Figure 4).

Although changes in both SMAD2 and pSMAD2 occurred in these studies, the changes in the ratio were driven primarily by changes in pSMAD2 (Figure 4).

Collagen Expression and Content
Effects of PO
In WT mice, PO was associated with a 200% increase in Col1A1 expression, a 130% increase in Col3A1 expression, and a 190% increase in CVF when compared with those in WT PO mice (Figures 5 and 6).

Effects of Modulation of MT1-MMP Abundance
At baseline, in the absence of PO, MT1-OE resulted in an increase in Col1A1 and Col3A1 expression and an increase in CVF when compared with those in WT mice (Figures 5 and 6). By contrast, in the absence of PO, MT1-RE resulted in
a slightly lower Col1A1 and Col3A1 expression and a lower CVF when compared with those in WT mice (Figures 5 and 6).

After 4 weeks of PO, collagen expression was a more robust, and collagen content was larger in the MT1-OE mice when compared with that in the WT mice. After 4 weeks of PO, Col1A1 expression was 275% lower, Col3A1 expression was 220% higher, and CVF was 30% higher in the MT1-OE mice when compared with those in WT mice. By contrast, after 4 weeks of PO, collagen expression was a less robust, and collagen content was smaller in the MT1-RE mice when compared with that in the WT mice. After 4 weeks of PO, Col1A1 expression was 250% higher, Col3A1 expression was 600% lower, and CVF was 40% lower in the MT1-RE mice when compared with those in the WT mice (Figure 5 and 6).

Figure 2. Chronic pressure overload (PO) induced changes in membrane type 1 matrix metalloproteinase (MT1-MMP) activity. A, Representative examples and (B) mean data in Friend virus B-type (FVB) wild-type (WT; n=10 non-PO, 12 PO) vs MT1-MMP overexpression (OE; n=10 non-PO, 9 PO) vs MT1-MMP reduced expression (RE; n=10 non-PO, 16 PO) mice. MT1-OE mice had an increase in MT1-MMP activity and abundance before (non-PO) and after PO when compared with those in WT mice. By contrast, the MT1-RE mice had a significantly lower MT1-MMP activity and abundance before PO and a significantly blunted increase in MT1-MMP activity and abundance after PO when compared with those in both WT and MT1-OE mice. *P<0.05 vs strain-matched non-PO control, +P<0.05 vs condition-matched FVB WT. #P<0.05 vs condition-matched MT1-OE. Non-PO indicates mice that did not undergo transverse aortic constriction.

Figure 3. Chronic pressure overload (PO) induced changes in latency-associated TGF-binding protein (LTBP-1) peptide hydrolytic potential. A, Representative examples and (B) mean data in Friend virus B-type (FVB) wild-type (WT; n=10 non-PO, 12 PO) vs membrane type-1 matrix metalloproteinase (MT1-MMP) overexpression (OE; n=10 non-PO, 9 PO) vs MT1-MMP reduced expression (RE; n=10 non-PO, 16 PO) mice. MT1-OE mice had an increase in LTBP-1 peptide hydrolysis before and after PO when compared with those in the WT mice. By contrast, the MT1-RE mice had a significantly lower LTBP-1 peptide hydrolysis after PO when compared with those in both WT and MT1-OE mice. *P<0.05 vs strain-matched non-PO control, +P<0.05 vs condition-matched FVB WT. #P<0.05 vs condition-matched MT1-OE. Non-PO indicates mice that did not undergo transverse aortic constriction.

Relationships Between MT1-MMP and Structural, Functional, and Signaling End Points

There were direct significant relationships between MT1-MMP activity and left atrial diameter (Figure 7), CVF (Figure 7), and the pSMAD2/SMAD2 ratio. In each case, as MT1-MMP increased, left atrial diameter (r=0.62; P<0.001), CVF (r=0.63; P<0.001), and the pSMAD2/SMAD2 ratio (r=0.52; P<0.01) increased.

Discussion

The current study was designed to define the mechanism by which a specific MMP type, MT1-MMP, directly contributes to the profibrotic response, increased ECM accumulation, and
prospective LV diastolic dysfunction that occurs in chronic PO. Transgenic mice that overexpress MT1-MMP or have reduced MT1-MMP expression underwent PO and yielded a number of novel findings that address this fundamental issue. First, PO was associated with MT1-MMP–dependent profibrotic signaling; PO caused MT1-MMP induction, increased MT1-MMP activity, MT1-MMP–specific LTBP-1 peptide hydrolytic potential, phosphorylation of SMAD2, collagen expression, collagen content, and resulted in diastolic dysfunction. Second, transgenic modulation of MT1-MMP abundance fundamentally changed this PO-induced MT1-MMP–dependent profibrotic signaling response but did not change the magnitude of the hypertrophic response to PO. MT1-OE markedly increased profibrotic signaling; whereas MT1-RE significantly decreased profibrotic signaling; but neither directional modulation of MT1-MMP altered the extent of PO-induced hypertrophy. Thus, the induction of a unique transmembrane proteolytic enzyme provides a fundamental mechanism that influences the myocardial response to PO. Specifically, there is a mechanistic relationship between the changes in MT1-MMP abundance and changes in ECM structure and LV diastolic function that occur during PO. In addition to these and other data underscoring the fact that MMPs have a diversity of both function and substrates, these data provide insight into the specific molecular pathways by which MT1-MMP induction contributes to the ECM remodeling and fibrosis that represent 1 critical event that occurs during the progression from chronic PO to diastolic dysfunction.

**Mechanisms of Diastolic Dysfunction: Cellular Versus Extracellular Mechanisms**

Abnormalities in diastolic function are consequent to changes in both the cardiomyocyte and the ECM.13,29–31 Cardiomyocyte abnormalities occur largely in the context of the development of LV hypertrophic growth. PO-induced increase in LV mass and cardiomyocyte hypertrophy have been thought to constitute an initial adaptive mechanism that normalizes or limits the increase in LV systolic wall stress that occurs in PO.2 However, progressive hypertrophy may become maladaptive and result in diastolic dysfunction.1 Unlike cardiomyocyte hypertrophy, ECM fibrosis is always maladaptive and results in diastolic dysfunction.8 These facts add to the complexity of defining which cellular and molecular mechanisms represent the causal mechanism responsible for diastolic dysfunction in PO.

**Figure 4.** Chronic pressure overload (PO) induced changes in the phosphorylation of SMAD2 (pSMAD2)/SMAD2 ratio. A, Representative examples (B) mean data in Friend virus B-type (FVB) wild-type (WT; n=10 non-PO, 12 PO) vs membrane type 1 matrix metalloproteinase (MT1-MMP) overexpression (OE; n=10 non-PO, 9 PO) vs MT1-MMP reduced expression (RE; n=10 non-PO, 16 PO) mice. MT1-OE mice had an increase in the pSMAD2/SMAD2 ratio before and after PO when compared with that in WT mice. By contrast, the MT1-RE mice had a significantly lower pSMAD2/SMAD2 ratio before PO and a significantly blunted increase in the pSMAD2/SMAD2 ratio after PO when compared with those in both WT and MT1-OE mice. *P<0.05 vs strain-matched non-PO control, +P<0.05 vs condition-matched FVB WT. #P<0.05 vs condition-matched MT1-OE. Non-PO indicates mice that did not undergo transverse aortic constriction.

**Figure 5.** Chronic pressure overload (PO) induced changes in collagen 1A1 (Col1A1; A) and collagen 3A1 (Col3A1; B) expression in Friend virus B-type (FVB) wild-type (WT; n=10 non-PO, 12 PO) vs membrane type 1 matrix metalloproteinase (MT1-MMP) overexpression (OE; n=10 non-PO, 9 PO) vs MT1-MMP reduced expression (RE; n=10 non-PO, 16 PO) mice. MT1-MMP–OE mice had an increase in Col1A1 and Col3A1 before (non-PO) and after PO when compared with that in WT mice. By contrast, MT1-RE mice had a significantly lower Col1A1 and Col3A1 before PO and a significantly blunted increase in Col1A1 and Col3A1 after PO when compared with those in both WT and MT1-OE mice. *P<0.05 vs strain-matched non-PO control, +P<0.05 vs condition-matched FVB WT. #P<0.05 vs condition-matched MT1-OE. Non-PO indicates mice that did not undergo transverse aortic constriction.
Fortunately, modulation of MT1-MMP expression in the context of the current study did not alter the hypertrophic response to PO but selectively affected the ECM remodeling response to PO. Thus, MT1-MMP–dependent ECM remodeling represents an independent mechanism causing diastolic dysfunction.

In the current study, the extent of diastolic dysfunction was assessed by examining changes in left atrial size. Left atrial size serves to integrate the increase in LV diastolic pressure over time. There were direct relationships among MT1-MMP activity and left atrial diameter, CVF, and mortality. As MT1-MMP increased, left atrial diameter, CVF, and mortality increased; the converse occurred when MT1-MMP abundance fell. Furthermore, the PO-induced diastolic dysfunction seems to be related to the activation of a TGF-dependent profibrotic signaling pathway. These data are concordant with human and animals studies that suggested that TGF-dependent profibrotic signaling is a consistent finding in chronic PO.3–38 In addition, the current study identified MT1-MMP as a critical factor in the activation of this signaling pathway beginning with MT1-MMP–dependent LTBP-1 proteolysis, TGF release, SMAD phosphorylation, and increased collagen synthesis.

**MMP-Induced Proteolytic Versus Profibrotic Signaling**

Data suggest that the pathways by which MT1-MMP contribute to adverse ECM remodeling likely include facilitating localized proteolysis of interstitial molecules directly (such as integrins) causing remodeling, amplification of active soluble MMPs (such as MMP-2) causing ECM instability and abnormal architecture, as well as through enhancing profibrotic signaling pathways (such as TGF-β) causing fibrosis.39–45 TGF-β is maintained in the ECM in an inactive form through its binding to the LTBP. LTBP-1 has an MT1-MMP–specific hydrolytic site. Through a number of in vitro and proteolytic assays, it has been demonstrated that MT1-MMP processes LTBP-1, which releases TGF-β.14,46–48 Once released, TGF-β undergoes activation, binds to the TGF receptor, activates intracellular signaling pathways that include pSMAD. pSMAD2 translocates to the nucleus and regulates transcription in a profibrotic manner, including an increase collagen expression.37,38 The TGF signaling pathway is complex, involves a number of different transmembrane receptors, a number of different SMAD proteins, and has both canonical and noncanonical components including TGF-β(TGFβ-activated kinase 1)/TAK1/p-38 MAPK (mitogen-activated protein kinase) pathway that play an important role in the maladaptive hypertrophy and dysfunction that develops with PO.49 It was beyond the purpose of this study to examine all of these components of TGF signaling pathway; however, data presented in the current study do indicate that PO activates a TGF-associated signaling pathway that is modulated by, if not actually initiated by MT1-MMP. Transgenic modulation of MT1-MMP altered these profibrotic signaling pathways; increased MT1-MMP increasing and decreased MT1-MMP decreasing TGF signaling.

**MT1-MMP in Adverse Myocardial Remodeling**

Previous studies have shown that the specific pattern of ECM remodeling and the functional changes that result from this...
remodeling are critically dependent on the type of hemodynamic overload present. The cellular and molecular mechanisms responsible for these differences in ECM remodeling have not been completely defined. The current study and previous studies have attempted to define a differential pattern of selective MMP induction in pressure versus volume overload. The volume overload that follows a myocardial infarction leads to eccentric remodeling and increased expression and activity of MT1-MMP. Because the LV remodeling is distinctly different in PO when compared with that in volume overload, there is no a priori reason to think that the MT1-MMP/LTBP-1/TGF-β/SMAD signaling pathway would be activated in PO, at least not in the same fashion. However, data from the current study showed that the induction of MT1-MMP occur in both volume and PO. How do these different pathological stimuli lead to an increase in MT1-MMP and how does an increase in MT1-MMP play a cause and effect role in each form of LV remodeling? Although the current study does not address the entirety of this question, it does provide significant new insight into a number of possible answers. The different biophysical stimuli created in pressure versus PO result in an individualized portfolio of context-specific substrates. MT1-MMPs effect on these stimulus-dependent, context-specific, but different substrates results in distinctly different effects on collagen homeostasis and changes in LV structure and function.

For example, volume overload causes significant increases in LV diastolic volume and end diastolic wall stress with less pronounced changes in end systolic wall stress. In vivo and in vitro studies have shown that these changes in mechanical load are sufficient to increase MT1-MMP, MMP-2 (and other soluble MMPs), and a host of inflammatory cytokines (such as TNF). In addition, volume overload generally decreases the tissue inhibitors of MMP (TIMPs). Context-specific substrates, such as MMP-2 and TNF and its downstream proteolytic cascade, are the principle targets of MT1-MMP that result in ECM proteolysis, LV dilation, eccentric remodeling,
and systolic dysfunction. These proteolytic effects are not limited by increased TIMPs. By contrast, PO causes significant increases in end systolic wall stress with less pronounced changes in end diastolic wall stress. The changes in mechanical load lead to not only a increased MT1-MMP, but also a significant increase in TIMPs that act to limit ECM proteolysis. The MT1-MMP–dependent activation of MMP-2 that occurs with PO acts in a concert complex among MMP-2, MT1-MMP, and TIMP-2 to hydrolyze LTBP-1. Thus, PO-induced MT1-MMP induction acts on the context-specific substrate LTBP-1, leading to the activation of TGF and the TGF-dependent profibrotic signaling process. Therefore, differences in biophysical stimuli result in an individualized portfolio of context-specific substrates that allow MT1-MMP to serve as a common mechanism for differential LV remodeling. In addition to profibrotic signaling-induced LV remodeling, selective changes in MMPs, TIMPs, and TGF may induce other pleiotropic effects, such as inflammation, angiogenesis, and apoptosis/autophagy, that may affect LV structural and functional remodeling in response to hemodynamic load.

Other ECM Regulatory Mechanisms

The current study focused specifically on ECM regulatory mechanisms affecting collagen synthesis. However, it is recognized that in addition to synthesis, mechanisms regulating collagen postsynthetic processing and collagen degradation also determine ECM collagen structure, geometry, and content. For example, PO has been shown to increase TIMPs that may act to limit the effects of MMPs and, therefore, decrease collagen degradation. There is no a priori reason to expect that transgenic MT1-MMP modulation will alter TIMP expression. In addition, TIMPs have poor affinity for MT1-MMP but have a uniformly high affinity for soluble MMPs. Therefore, TIMPs provide potent inhibition to soluble MMPs but do not significantly modify MT1-MMP activity. Thus, the aggregate effects of PO-induced increased MT1-MMP activity (not blunted by TIMPs) acting on context-specific substrates, such as LTBP-1 and activating downstream TGF profibrotic cascade, result in myocardial fibrosis.

Future Directions

Using transgenic constructs provide the advantage of being able to increase or decrease the expression of a single gene product; in the current experiment, this was specific to MT1-MMP. However, this change in MT1-MMP expression is life long and may result in unintended secondary changes. In addition, the creation of a PO can only be performed in the presence of a permanent condition of increased or decreased MT1-MMP expression. Thus, the change in MT1-MMP expression cannot be limited to only a period during which PO is induced and cannot be changed after PO is complete. Future studies should be designed to address these limitations using a conditional transgenic mouse model in which an inducible construct can be studied. Alternatively, a systemically applied selective inhibitor of MT1-MMP might be used.

In the murine models used in the current study, there are significant limitations in the ability to determine the cause of death and in performing sufficient surveillance to predict cause and occurrence of mortality. Defining cause of death in murine models is exceedingly difficult. Post mortem examinations are often ambiguous. For example, even when post mortem findings of pericardial and pleural effusion suggest increased LV diastolic filling pressures, primary arrhythmic death may have occurred, blurring the distinction between death caused by heart failure versus arrhythmia. Therefore, the cause of death was not formally investigated in this study. However, the time course of changes in LV structure and function that results from PO induction by TAC was carefully defined in a recent study. In this study, mice underwent echocardiographic studies at 1, 2, or 4 weeks after PO; at each time point, mice underwent terminal studies, thus serial studies in each individual mouse was not performed. In addition, structural and functional changes measured at 5 to 7 days after PO have not been shown to predict mortality rates or mode of death in this murine model. Therefore, serial imaging studies were not performed in the transgenic mice used in the current study. This is clearly an important area for future studies.

Clinical Implications

The transition from PO-induced remodeling to diastolic dysfunction and myocardial fibrosis represents a pivotal development in the clinical course of hypertensive heart disease and Heart Failure with preserved Ejection Fraction (HFpEF). Management strategies for patients with hypertensive heart disease and HFpEF represent a large unmet need and require new directions that target underlying pathophysiologic mechanisms. Our studies show that the murine model of TAC-induced PO causes changes in LV structure and function that are similar to the structure–function changes in hypertensive heart disease and HFpEF. Therefore, the MT1-MMP–dependent changes in profibrotic signaling represent a clinically relevant causal mechanism that may serve as potential therapeutic targets to prevent or reverse PO-induced adverse ECM remodeling. For example, data from the current study suggest that a decrease in MT1-MMP induction, rather than a complete ablation of MT1-MMP, may be sufficient to modify ECM remodeling in PO. Because MT1-MMP is processed intracellularly by a furin-dependent mechanism and released onto the cell surface as a preactivated MMP form, furin inhibitors, currently being developed for oncological application, may hold promise for patients with hypertensive heart disease and HFpEF. Future basic and translational studies should target these therapeutic applications.

Conclusions

PO induction of a unique transmembrane proteolytic enzyme provides a fundamental mechanism influencing the myocardial response to PO. Variations in MT1-MMP did not lead to alterations in the induction of the hypertrophic response to increased stress; however, PO-induced MT1-MMP–dependent profibrotic signaling lead to alterations in interstitial fibrosis and diastolic function. Thus, these data suggest that there is a direct mechanistic relationship between the changes in MT1-MMP and changes in ECM structure and LV diastolic function that occur during PO.

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References
The transition from pressure overload (PO)–induced remodeling to diastolic dysfunction and myocardial fibrosis represents a pivotal development in the clinical course of hypertensive heart disease and HFpEF. Management strategies for patients with hypertensive heart disease and HFpEF represent a large unmet need and require new directions that target underlying pathophysiological mechanisms. Our studies showed that the murine model of transverse aortic constriction–induced PO causes changes in LV structure and function that are similar to the structure–function changes in hypertensive heart disease and HFpEF. In the current study, data suggest that PO-induced membrane type-1 matrix metalloproteinase (MT1-MMP)–dependent profibrotic signaling lead to alterations in interstitial fibrosis and diastolic function and that there is a direct mechanistic relationship between the changes in MT1-MMP and changes in extracellular matrix structure and LV diastolic function that occur during PO. Therefore, the MT1-MMP–dependent changes in profibrotic signaling represent a clinically relevant causal mechanism that may serve as potential therapeutic targets to prevent or reverse PO-induced adverse extracellular matrix remodeling. For example, data from the current study suggest that a decrease in MT1-MMP induction, rather than a complete ablation of MT1-MMP, may be sufficient to modify extracellular matrix remodeling in PO. Because MT1-MMP is processed intracellularly by a furin-dependent mechanism and released onto the cell surface as a preactivated MMP form, furin inhibitors, currently being developed for oncological application, may hold promise for patients with hypertensive heart disease and HFpEF. Future basic and translational studies should target these therapeutic applications.
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