Thoracic aortic aneurysms (TAAs) carry significant morbidity and mortality (5, 7, 9) and form through dynamic remodeling of the aortic extracellular matrix (ECM), resulting in the disruption of elastin filaments and disordered collagen deposition (7). The primary mediators of this structural degradation and reorganization belong to a single family of zinc-dependent endopeptidases: matrix metalloproteinases (MMPs) (3). Numerous studies (10, 13, 24, 34) have demonstrated with both human specimens and animal models that the gelatinase subclass (MMP-2 and MMP-9) displays elevated production and activity in both abdominal and thoracic aneurysm disease. In a well described animal model of abdominal aortic aneurysm (AAA), macrophage-derived MMP-9 was found to work in concert with mesenchymal cell-derived MMP-2 to induce aortic dilatation (24). Using a similar model in the thoracic aorta, a previous study (19) from this laboratory reported elevated MMP-9 levels localized to mesenchymal cells in the absence of inflammatory cell infiltration. These data suggested that there may be inherent differences between the thoracic and abdominal aorta that contribute to the unique mechanisms that drive aortic dilatation (31).

Studies (1, 15, 28, 40) examining human aneurysm specimens from both AAAs and TAAs have demonstrated increased abundance of a unique membrane-bound MMP, membrane-type 1 MMP (MT1-MMP), suggesting that this enzyme may play a significant role in aneurysm development. MT1-MMP is a class I transmembrane protein that has the potential to contribute to aneurysm development in three ways (18). First, MT1-MMP plays an essential role in the activation of MMP-2. Pro-MMP-2 is secreted into the extracellular space as an inactive propeptide, where it is activated by removal of the pro-domain through proteolytic cleavage mediated by an activational complex consisting of pro-MMP-2, tissue inhibitor of metalloproteinase (TIMP)-2, and MT1-MMP (16, 29). It has been recently reported that AAA growth is attenuated in TIMP-2 knockout mice, suggesting that MT1-MMP-dependent activation of pro-MMP-2 is essential during aneurysm development (39). Second, like other extracellular proteases, MT1-MMP possesses gelatinolytic and collagenolytic activity that can directly degrade the structural ECM (18). Finally, in addition to structural ECM proteins, MT1-MMP can also release ECM-sequestered matrixines, cytokines, and growth factors that can further act to drive ECM remodeling (18, 27). Thus, MT1-MMP sits at the crossroads of multiple pathways capable of modifying the aortic ECM and, therefore, may provide an important target for therapeutic intervention. The present study tested the hypothesis that MT1-MMP expression, abundance, and activity would be elevated during TAA development and that this protease would be produced primarily by mesenchymal cells within the thoracic aorta.

METHODS

Experimental design. TAAs were induced in approximately equal numbers of male and female (8–12 wk old) C57BL/6J mice, which were then randomly assigned for terminal study at 2, 4, 8, or 16 wk post-TAA induction (n = 15 mice at 2 and 8 wk postinduction and 18
were intubated, and a surgical plane of anesthesia was maintained by previously described (14). Briefly, after anesthetic induction, mice in Buffer RLT (QIAGEN, Valencia, CA) using a Qiagen Tissuelyser snap frozen, and stored at

The thoracic aorta was then excised. Tissue designated for quantitative then increased to 5%, and mice were euthanized under deep anesthesia on a 4–12% bis-Tris gradient gel and fractionated by electrophoresis. Proteins were then transferred to nitrocellulose membranes (0.45 μm, Bio-Rad) and incubated in antiserum corresponding to MMP-2 [0.4 μg/ml, catalog no. IM33, EMD Biosciences (Calbiochem), La Jolla, CA], MT1-MMP (0.4 μg/ml, catalog no. AB815, Millipore Biosciences, Temecula, CA), and TIMP-2 (0.5 μg/ml, catalog no. AB38973, Abcam, Cambridge, MA). Antisera were diluted in 5% nonfat dry milk-PBS. The secondary peroxidase-conjugated antibody was then applied (1:5,000, 5% nonfat dry milk-PBS), and signals were detected with a chemiluminescent substrate (Western Lightning Chemiluminescence Reagent Plus, PerkinElmer). Film development was performed in a Konica SRX-101A medical film processor, and the 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 unoperated control homogenates.

MT1-MMP quenched fluorogenic peptide activity assay. MT1-MMP activity was determined using an MT1-MMP-specific quenched fluorogenic peptide assay. Briefly, equal amounts of each aortic homogenate (15 μg) from normal and TAA-induced mice were incubated for 60 min at 37°C with a quenched fluorogenic peptide that contains an MT1-MMP-specific cleavage site [MCA-Pro-Leu-Ala-Cys(p-OMeBz)-Trp-Ala-Arg(Dpa)-NH2, EMD Biosciences]. Upon cleavage of the peptide by active MT1-MMP, the quenching group becomes sufficiently separated from the fluoroscein group, allowing a fluorescent signal to be emitted. Fluorescence was measured and recorded on a fluorescent microplate reader (Fluostar Galaxy BMG Labtechnologies, Cary, NC) and compared with a standard curve using active recombinant MT1-MMP. Results were normalized to the amount of GAPDH present in each sample, as determined by ELISA assay (catalog no. 3401, Bioo Scientific, Austin, TX).

Immunohistochemistry and cellular colocalization. Three independent tissue sections (3 μm), ~50 μm apart, collected from control or TAA-induced animals were analyzed by immunohistochemical staining for MT1-MMP and one cell type-specific marker for fibroblasts, discoidin domain receptor 2 (DDR2). Aortic tissue sections were deparaffinized and rehydrated with deionized water. Dako citrate antigen retrieval (catalog no. S1699; Dako North America, Carpinteria, CA) was performed for 30 min at 99°C, the tissue was cooled to room temperature, and the slides were subsequently washed with water followed by 0.025% Triton X-100 in Tris-buffered saline (TBS). BSA (3%)-TBS was applied for 2 h to block the tissue, and it was then incubated with rabbit polyclonal antibody to MT1-MMP (1:250, catalog no. ab38971, Abcam) or goat polyclonal antibody to DDR2 (1:100, catalog no. sc-7555, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. After incubation, tissue sections were washed with 0.025% Triton X-100 in TBS and then incubated in 0.3% H2O2 in TBS for 30 min to block endogenous peroxidase activity. Tissue sections were then incubated with peroxidase-conjugated goat anti-rabbit antibody (1:250, catalog no. 6101, Vector Labs, Burlingame, CA) or peroxidase-conjugated rabbit anti-goat antibody (1:250, catalog no. BA-5000, Vector Labs) to identify positive MT1-MMP and DDR2 antibody binding, respectively. Sections were washed again with 0.025% Triton X-100 in TBS and then incubated with DAB reagent for 30 s (catalog no. SK-4100, Vector Labs). Sections were then dehydrated, cleared, coverslipped, and analyzed by light microscopy.

To determine the colocalization of MT1-MMP and DDR2, tissue sections were blocked with 3% BSA-TBS and first incubated over-
ELEVATED MT1-MMP DURING TAA DEVELOPMENT

night with the goat polyclonal antibody to DDR2 (1:100, catalog no. SC7555, Santa Cruz Biotechnology). Sections were then washed and incubated with peroxidase-conjugated rabbit anti-gold antibody (1:250, catalog no. BA-5000, Vector Labs) as described above. After a 30-min incubation, slides were developed in DAB reagent for 90 s. Tissue sections were then blocked again in 3% BSA-TBS for 1 h followed by a second overnight incubation with the rabbit polyclonal antibody for MT1-MMP (1:250, catalog no. AB8315, Millipore Biosciences). After being washed as described above, tissue sections were incubated with alkaline phosphatase-conjugated gold anti-rabbit antibody (1:250, ABC-AP reagent, catalog no. AK-5001, Vector Labs) for 30 min and visualized using the Vector blue chromagen (catalog no. SK-5300, Vector Labs). Tissue sections were then dehydrated, cleared, coverslipped, and analyzed by light microscopy.

Multiple tissue sections from control and 16-wk TAA-induced mice (8 sections each, separated by at least 50 μm) were immunostained with MT1-MMP antibody. Digital images were recorded with a Zeiss AxioCam MRc mounted on a Zeiss Axiostarplus 2 using a ×63 objective. Multiple high-power fields (control: n = 27 and 16 wk post-TAA induction: n = 51) were subjected to analysis by two methods: 1) cells were counted and expressed per square millimeter of tissue area and 2) positive MT1-MMP staining was analyzed using classical stereological techniques.

MT1-MMP-targeted microbubble contrast imaging. Targeted microbubble contrast imaging was performed to assess MT1-MMP protein binding in control (n = 6) and TAA-induced (8 wk postinduction, n = 3) mice in real time by high-resolution microultrasound. Mice were anesthetized with 3–5% isoflurane vapor in a closed chamber. Mice were then placed ventrally on a thermostatic platform (37°C), and anesthesia was maintained by delivering 1.5–2.0% isoflurane through a nose cone. The hair over the thorax was depilated, and a layer of ultrasound gel was applied to facilitate imaging. The descending thoracic aorta was identified by two-dimensional ultrasound using a 40-MHz scanning head with a spatial resolution of 30 μm (Vevo 770 System, VisualSonics, Toronto, ON, Canada). Biotinylated MT1-MMP antibody (custom rabbit polyclonal, Thermo Scientific Open Biosystems, Huntsville, AL) was conjugated to the streptavidin-coated microbubble contrast agent (Vevo MicroMarker Target-Ready Contrast Agent Kit, VisualSonics). A 27-gauge butterfly needle attached to polyethylene-10 tubing was then inserted in the lateral tail vein and used to inject 70 μL of the MT1-MMP antibody-conjugated microbubbles over 2 s. This was immediately followed by 50 μL of saline to flush the catheter. In each animal, baseline cineloops were captured before the injection, during the injection (including the first pass of the targeted microbubbles through the descending thoracic aorta), and then at 5-min intervals through 15 min after the injection. Using Vevo 770 software, the baseline (preinjection) cineloop from each animal was processed as a reference to which all other cineloops from the same animal were compared by digital subtraction to identify targeted microbubble contrast binding and localization. MT1-MMP-targeted contrast microbubble binding was then quantified within a region of interest drawn at the site of TAA induction. Results were corrected for region volume between animals and normalized for the total injected microbubble dose, as determined by contrast microbubble binding over a high-powered microscopic image and scored positive when an intersection point fell on a region of increased cellular staining. MT1-MMP immunostaining was then expressed as the percent change from control, and statistical significance was determined by Mann-Whitney rank sum analysis.

RESULTS

Aortic dilation and TAA formation. After the periodontal application of CaCl2, the descending thoracic aortic diameter was increased from baseline at all time points after the TAA induction surgery. At 4 wk post-TAA induction, aortic diameter increased 50 ± 5% over baseline, reaching a true aneurysm by definition. Aortic dilation appeared to plateau between 4 and 8 wk post-TAA induction and then continued to dilate between 8 and 16 wk post-TAA induction, reaching a maximum diameter increase of 72 ± 7% with no mortality due to aneurysm rupture (Fig. 1).

TAA-dependent changes in gene expression. To establish the expression levels MT1-MMP during TAA development, aortic tissue was harvested at each time point after TAA induction, and the gene expression was measured by quantitative real-time PCR. MT1-MMP mRNA levels were elevated by approximately fivefold at 2 wk post-TAA induction but were not different from control at the other time points. Additionally, mRNA expression levels of the pro-MMP-2 activational complex members (MMP-2 and TIMP-2) were also determined. The data revealed elevated MMP-2 mRNA at 2 and 4 wk post-TAA induction, whereas the levels of TIMP-2 mRNA were not significantly altered at any time point (Table 1).

TAA-dependent changes in protein levels. Aortic homogenates from control and TAA-induced mice were subjected to fractionation by electrophoresis, and the protein levels of MT1-MMP and pro-MMP-2 activational complex members were determined. Compared with control animals, MT1-MMP protein abundance increased progressively over the 16-wk time course and was significantly different from control at each time point from 4 to 16 wk post-TAA induction (Fig. 2A). Interestingly, both MMP-2 and TIMP-2 protein levels peaked 2 wk post-TAA induction and then reduced but remained elevated

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compared with control levels at each subsequent time point (Fig. 2, B and C, respectively). The ratio of active to latent MMP-2 displayed a sharp fivefold increase at 2 wk post-TAA induction, remained elevated at 4 wk post-TAA induction (2-fold), and decreased below baseline at 16 wk post-TAA induction, returned to baseline at 8 wk post-TAA induction, and increased again at 16 wk post-TAA induction compared with reference control, and 16-wk values were significantly greater than those at the other time points (*P < 0.001 vs. 2 wk, &P < 0.05 vs. 4 wk, and ++P < 0.05 vs. 8 wk).

Quantification of MT1-MMP activity in aortic tissue. MT1-MMP-specific activity was determined in aortic homogenates from control and TAA-induced mice using a quenched fluorogenic peptide cleavage assay. MT1-MMP activity was increased at 2 wk post-TAA induction, remained elevated at 4 wk post-TAA induction (2-fold), and decreased below baseline at 16 wk post-TAA induction (~48% of control; Fig. 3).

Time post-TAA Induction

![Graph](http://example.com/graph.png)

**Fig. 1.** Change in aortic diameter over time after thoracic aortic aneurysm (TAA) induction. Aortic diameter was determined at baseline and terminal surgery in each animal using digital micrometry. Aortic diameter values were expressed as percent changes from baseline (100%) in each animal. After TAA induction, aortic diameter was elevated at all time points (*P < 0.001 vs. reference control), and 16-wk values were significantly greater than those at the other time points (#P < 0.05 vs. 2 wk, &P < 0.05 vs. 4 wk, and ++P < 0.05 vs. 8 wk).

**DISCUSSION**

Despite surgical intervention, TAAs carry considerable morbidity and mortality. Understanding the molecular pathogenesis of this insidious disease could therefore significantly influence patient survival as well as quality of life. In the present study, a murine model of TAA was used to examine the coordinate changes in MT1-MMP, MMP-2, and TIMP-2 abundance during aortic dilatation in TAA development. It was hypothesized that the induction of TAA would alter the expression, abundance, and activity of MT1-MMP and that this protease would be produced by endogenous mesenchymal cells within the thoracic aortic wall. The unique findings of this study were threefold. First, the distinct proteolytic profiles of MT1-MMP, MMP-2, and TIMP-2 suggest a multifunctional contribution of MT1-MMP toward TAA formation defined by the activation of MMP-2 early in aortic dilatation and through the degradation/release of other ECM substrates such as latent TAA-induced mice versus control animals. MT1-MMP binding was significantly elevated at 10 min (160 ± 33% in TAA-induced mice vs. 75 ± 8% in control mice) and 15 min (217 ± 53% in TAA-induced mice vs. 81 ± 8% in control mice) after the microbubble injection (Fig. 5). Correlation analysis reveal a significant relationship between aortic diameter and MT1-MMP-targeted microbubble binding (Spearman’s rho coefficient = 0.6667, P < 0.05).

**Cellular localization of MT1-MMP.** To identify a cellular source for MT1-MMP production within the aorta during TAA development, aortic tissue sections from control and TAA-induced mice (16 wk postinduction) were immunostained with antibodies specific for MT1-MMP or DDR2, one cell type-specific marker for fibroblasts (12). The immunostaining results revealed that MT1-MMP was localized in a cellular pattern similar to what was seen and previously reported with DDR2 (Fig. 6, top and middle, respectively) (19, 20). MT1-MMP-positive staining was examined using stereological techniques and displayed an elevated number of positively stained cells in 16-wk TAA-induced sections (20 ± 6% increase vs. control, P < 0.05). When the number of cells was expressed per square millimeter of tissue area, 16-wk TAA-induced sections demonstrated a 33.3% increase in the number of positively stained cells [control (n = 27): 1,895 ± 153 cells/mm² tissue vs. TAA induction (n = 51): 2,526 ± 175 cells/mm² tissue; Fig. 6, bottom left]. The sequential staining of tissue sections with DDR2 followed by MT1-MMP demonstrated significant colocalization of MT1-MMP and DDR2 (Fig. 6, bottom right).

**Table 1. Fold increase in aortic gene expression in TAA induction compared with reference control**

<table>
<thead>
<tr>
<th>Gene</th>
<th>2 wk</th>
<th>4 wk</th>
<th>8 wk</th>
<th>16 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mmp14</td>
<td>5.53*</td>
<td>1.30</td>
<td>1.38</td>
<td>1.07</td>
</tr>
<tr>
<td>Mmp2</td>
<td>4.80*</td>
<td>1.66*</td>
<td>1.58</td>
<td>1.40</td>
</tr>
<tr>
<td>Timp2</td>
<td>1.47</td>
<td>1.11</td>
<td>1.09</td>
<td>1.14</td>
</tr>
</tbody>
</table>

Quantitative real-time PCR was used to measure the fold gene expression of matrix metalloproteinase 14, matrix metalloproteinase 2, and tissue inhibitor of metalloproteinase 2 at each time point in thoracic aortic aneurysm (TAA) progression. *P < 0.05 vs. reference control.

**Table 2.** Gene expression and activity of matrix metalloproteinase 14 and matrix metalloproteinase 2 in TAA induction compared with reference control.

**Table 3.** Gene expression and activity of matrix metalloproteinase 14 and matrix metalloproteinase 2 in TAA induction compared with reference control.
ECM-bound growth factors at later time points. Second, MT1-MMP was localized to endogenous fibroblasts or fibroblast-like cells within the aortic wall at all time points during TAA formation, further implicating this cell type as a major mediator of TAA development. Third, high-resolution micro-ultrasound imaging with an MT1-MMP-targeted microbubble contrast agent revealed elevated MT1-MMP protein binding at the site of TAA induction in live animals, suggesting that MT1-MMP may serve as a trackable marker for TAA progression.

Taken together, this study suggests that MT1-MMP plays a multifunctional role in TAA development and identifies MT1-MMP as a critical target that could be exploited for therapeutic or diagnostic benefits.

Advantages of the murine TAA model. Many investigators have studied aneurysm wall specimens collected at the time of surgical repair to examine structural and biochemical alterations. While informative, this tissue can only provide a snapshot of the histologic transformations and enzymatic activity.

Fig. 2. Quantitative immunoblot analysis was used to measure membrane type-1 (MT1) matrix metalloproteinase (MMP), MMP-2, and tissue inhibitor of metalloproteinase (TIMP)-2 protein abundance. Values are expressed as percent changes from reference control (CON) animals. A: MT1-MMP was elevated at 4 wk post-TAA induction and progressively increased through the 16-wk time course (*P < 0.05 vs. reference control animals). B and C: MMP-2 (B) and TIMP-2 (C) protein levels were increased at all time points after TAA induction (*P < 0.05 vs. reference control animals). Values of the 4-, 8-, and 16-wk time points were significantly different from peak protein abundance observed at 2 wk (#P < 0.05 vs. 2 wk).
occurring at that current stage of the disease. Alternatively, murine aneurysm models have allowed the examination of the rate of aortic dilation, progression of matrix remodeling, and temporal pattern of gene expression during aneurysm growth (3, 19, 37). The present study used a CaCl₂-induced murine aneurysm model that has been well established in both the abdominal and thoracic aorta (6, 11, 14, 24). Previous work from Basalyga and coworkers (4) demonstrated that the calcification of vascular elastic fibers induced MMP production by an as-yet-undescribed mechanism, suggesting that the early MMP induction initiates aortic dilatation and aneurysm development. The present study followed mice for 16 wk after CaCl₂ treatment and documented a 72% increase in aortic diameter over the 16-wk time course in the absence of atherosclerosis and a significant inflammatory component. Demonstrating that this model system results in a true TAA lends credibility to the subsequent biochemical and imaging experiments carried out at intermediate time points and, therefore, elevates the potential to identify therapeutic targets that could influence aneurysm progression.

**MT1-MMP in TAA progression.** The potential role for MT1-MMP in TAA development has become increasingly apparent due to evidence of enhanced activity in several human and animal studies of aneurysm disease (8, 15, 28, 35, 40). Based on this enzyme’s known substrates and activity, it has been suggested to contribute to TAA development through enhanced activation of MMP-2 (8). Hence, this study investigated the temporal pattern of production for all three components of the pro-MMP-2 activational complex. Consistent with work from another group (32), MMP-2 mRNA and protein levels increased concordantly in this model during early TAA development, as evidenced by the near fivefold increase in the ratio of active to latent MMP-2 during early aortic dilatation. These data suggested that components of the pro-MMP-2 activational complex should likewise be elevated during that time period. Interestingly, mRNA levels of MT1-MMP and TIMP2 did not mirror that of MMP-2 throughout the 16-wk time course. At 2 wk post-TAA induction, MT1-MMP mRNA levels were increased but returned to baseline at subsequent time points. MT1-MMP protein levels, however, remained unchanged from control at 2 wk post-TAA induction but then progressively increased through 16 wk post-TAA induction. TIMP-2 mRNA levels, on the other hand, remained unchanged at all time points, whereas protein levels were markedly elevated 2 wk post-TAA induction and remained elevated, albeit at a lower level, for the remainder of the study. These observed disparities

Fig. 4. MT1-MMP activity and functional significance. A: activity of MT1-MMP was measured in aortic homogenates from normal and TAA-induced mice using an MT1-MMP-specific quenched fluorogenic peptide activity assay. The results demonstrated elevated MT1-MMP activity (in ng·mg GAPDH⁻¹·h⁻¹) at 2, 4, and 16 wk post-TAA induction (*P < 0.05 vs. reference control animals; #P < 0.05 vs. 2 wk). B: pairwise regression analysis was performed between MT1-MMP activity (in ng·mg GAPDH⁻¹·h⁻¹) and the time-dependent change in aortic diameter (in mm). The results demonstrated a significant correlation (r = 0.4142, P = 0.0205), suggesting that MT1-MMP activity is required for aortic dilatation during TAA development. Linear mixed model analysis demonstrated that significant predictors of MT1-MMP activity included aortic diameter (F = 16.85, P = 0.0004), time (F = 6.09, P = 0.0205), and the interaction between aortic diameter and time (F = 7.62, P = 0.0104).
between mRNA and protein levels suggest that a posttranscriptional regulatory mechanism may play a significant role in mediating MMP-2 activation (25, 30, 41). For example, the TIMP-2 mRNA half-life may be very short, and, upon mRNA structural modification, stability may be significantly increased, allowing for rapid protein production under the appropriate conditions. In similar fashion, translational regulatory mechanisms may inhibit MT1-MMP protein production at 2 wk despite elevated mRNA levels. This may also suggest that the baseline levels of MT1-MMP are sufficient to activate pro-MMP-2. Moreover, it may indicate that MT1-MMP naturally localizes to cellular sites where pro-MMP-2 is processed and is perhaps preloaded in the activational complex. Together, these data suggest that the formation of the pro-MMP-2 activational complex is the likely source of increased active MMP-2 observed in the initial weeks after TAA induction, thereby implicating MT1-MMP in early thoracic aneurysm development.

At later time points (4–16 wk post-TAA induction), MT1-MMP protein levels were elevated from baseline and continued to increase through 16 wk post-TAA induction. Although some MT1-MMP activity was likely required for the continued activation of pro-MMP-2, the temporal association of elevated MT1-MMP with continued aneurysm growth between 8 and 16 wk post-TAA induction would suggest that MT1-MMP contributes to TAA progression through alternate activities. These may include the activation of other MMPs, such as pro-MMP-13 (22, 33), the degradation of the local ECM, and the release of ECM-bound growth factors. This is further supported by the observation that the relative ratio of active to latent MMP-2 decreases at the later time points, when MT1-MMP protein levels are elevated. Together, this suggests that
has revealed a role for increased transforming growth factor-
encoded MMP activity. Interestingly, a recent study (23) indi-
cated that human recombinant MT1-MMP could be
phosphorylated by PKC-θ, consistent with the findings of Moss et al. (26) and suggests
that the posttranslational modification of MT1-MMP may play
an important role in the regulation of its activity and/or cellular
localization. Second, the temporal changes in MT1-MMP activity
observed in the present study were not dissimilar to the
time-dependent change in aortic diameter. Aortic diameter was
increased at 2 wk post-TAA induction, was further dilated at 4
wk post-TAA induction, plateaued at 8 wk post-TAA induc-
tion, and increased further at 16 wk post-TAA induction.
Accordingly, regression analysis revealed a significant corre-
lation between MT1-MMP activity and the time-dependent
change in aortic diameter. To verify the relationship among
time, aortic diameter, and MT1-MMP activity, a linear mixed
model analysis was used and confirmed the significant predic-
 tors of MT1-MMP activity. Thus, these results suggest that
elevated MT1-MMP activity may be essential during aortic
dilatation.

To establish the functional significance of MT1-MMP in
TAA development, MT1-MMP abundance was quantitated in
the descending thoracic aorta in live TAA-induced mice using
a novel imaging approach. High-resolution micro-ultrasound
was used to quantitate the specific binding of an MT1-MMP
antibody-targeted microbubble contrast agent at the site of
TAA induction. Streptavidin-conjugated lipid vesicles (micro-
bubbles) were incubated in the presence of biotinylated MT1-
MMP rabbit polyclonal antibody. The subsequent MT1-MMP-
targeted contrast agent was then injected into the tail vein of
normal and TAA-induced mice. Cine-loops of micro-ultrasound
images were analyzed by digital subtraction to reveal areas of
MT1-MMP microbubble binding within the aortic wall. The
results demonstrated increased MT1-MMP-targeted contrast
binding within the CaCl2-treated region of the aorta in TAA-
induced animals, whereas minimal binding was observed in the
same region in control animals. These unique data demon-
strate, for the first time, that elevated MT1-MMP protein levels
coincide with increased aortic dilatation in developing aneu-
rysms in live mice and further support the hypothesis that TAA
induction results in elevated MT1-MMP expression and abun-
dance. This noninvasive imaging modality could be further
developed to track MT1-MMP-dependent TAA progression
while simultaneously providing high-resolution aortic struc-
tural information.

MT1-MMP localization to fibroblasts. The wall of an aortic
aneurysm is populated by multiple cell types, including fibro-
blasts, smooth muscle cells, and inflammatory cells. Because
each of these cell types is capable of producing MT1-MMP
posttranslational regulatory mechanisms may alter the activity
and/or cellular localization of MT1-MMP and thereby alter its
substrate access or specificity. Recent work by Moss et al. (26)
demonstrated that human recombinant MT1-MMP could be
phosphorylated by PKC-θ within its short cytoplas-
ic domain. MT1-MMP phosphorylation has been shown to
induce tumor cell migration and proliferation, possibly due to
enhanced proteolytic activity. Interestingly, a recent study (23)
has revealed a role for increased transforming growth factor
(TGF)-β signaling in genetic aneurysm syndromes. Bound in a
large latent complex, TGF-β is typically found associated with
fibrillin-1 (17), a vital component of elastin microfibrils and a
known substrate for MT1-MMP (2). Enhanced MT1-MMP
production during continued aneurysm growth, may therefore
function to release latent TGF-β, which could then further
drive TAA progression. Elucidating the role of MT1-MMP
throughout TAA development may therefore identify multiple
unique therapeutic strategies focused on this single protease.

To examine the temporal changes in MT1-MMP activity
during TAA development, an MT1-MMP-specific activity assay
was performed. The results revealed two primary implica-
tions. First, discordance between MT1-MMP activity and pro-
tein content suggest that MT1-MMP activity is regulated inde-
dependently of its protein abundance. This observation is
consistent with the findings of Moss et al. (26) and suggests
that the posttranslational modification of MT1-MMP may play
an important role in the regulation of its activity and/or cellular
localization. Second, the temporal changes in MT1-MMP activ-
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increased at 2 wk post-TAA induction, was further dilated at 4
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Accordingly, regression analysis revealed a significant corre-
lation between MT1-MMP activity and the time-dependent
change in aortic diameter. To verify the relationship among
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Fig. 6. Immunohistochemical localization of discoidin domain receptor 2
(DDR2) and MT1-MMP. Immunostaining of 3-μm aortic tissue sections from
control and TAA-induced mice (16 wk post-TAA induction) demonstrated the
presence of DDR2-positive cells (top) and MT1-MMP-positive cells (middle
top and middle bottom) within the aortic wall. Bottom left: quantification
of MT1-MMP immunostaining revealed an increased number MT1-MMP-posi-
tive cells in the 16-wk TAA-induced sections (33.3% increase vs. control,
*P < 0.05). Bottom right: dual sequential immunostaining demonstrated the
colocalization of MT1-MMP (blue) with DDR2 (brown) in aortic fibroblasts.
Representative images are shown. Bars = 20 μm.
than in the abdominal aorta. For example, CaCl2 may induce aortic dilation in the thoracic aorta via a different mechanism. Moreover, upon isolation of aortic fibroblasts from control and TAA-induced mice, it was determined that the TAA-induced fibroblasts had undergone a significant phenotypic change during TAA formation, resulting in an enhanced proteolytic gene expression profile, including a twofold increase in MT1-MMP expression. Accordingly, the present study assessed the localization of MT1-MMP in aortic sections from control and TAA-induced mice and determined whether MT1-MMP, like MMP-9, would localize to mesenchymal fibroblasts. Indeed, MT1-MMP demonstrated significant colocalization with DDR2-positive cells (fibroblasts) within the thoracic aortic wall. In a previous study by Nollendorfs et al., MT1-MMP was shown to localize to both mesenchymal cells as well as infiltrating monocytes/macrophages during aneurysm development in the abdominal aorta. Moreover, in an elegant study by Xiong and coworkers, AAA formation was found to be highly dependent on macrophage-derived MT1-MMP. The authors clearly demonstrated that lethally irradiated wild-type mice reconstituted with bone marrow from MT1-MMP-deficient animals failed to form AAAs in response to periadventitial CaCl2 exposure. While the results of the present study are consistent with those of Nollendorfs et al. in part, suggesting that MT1-MMP was expressed in mesenchymal cells during aneurysm development, previous studies from this laboratory have failed to show a significant accumulation of monocytes/macrophages in the aortic media during TAA formation. Taken together, these data suggest that exposure of the thoracic aorta to CaCl2 instigates aortic dilation in the thoracic aorta via a different mechanism than in the abdominal aorta. For example, CaCl2 may induce changes in gene expression in native mesenchymal cells, leading to enhanced MMP production and the initiation of aortic dilatation. These results further substantiate the hypothesis of regional heterogeneity between the thoracic and abdominal aorta, extending the evidence to include differential mechanisms regulating MT1-MMP expression and abundance. Interestingly, the present results, demonstrating a progressive elevation of MT1-MMP over the time course of TAA development, combined with recent results reporting the emergence of a fibroblast-derived myofibroblast population, suggest that the elevated MT1-MMP levels observed may be due to an increased number of fibroblasts/myofibroblasts expressing MT1-MMP in the aorta. Accordingly, these results may also explain the discordance between MT1-MMP mRNA levels and protein abundance over the time course of TAA formation. When MT1-MMP was localized in control and 16-wk TAA-induced aortic tissue sections, a 33% increase in the number of cells staining positive for MT1-MMP was observed in 16-wk TAA-induced sections. While this technique does not account for changes in staining intensity, the measured increase in MT1-MMP protein abundance at 16 wk post-TAA induction (Fig. 2A) would suggest that not only is there an increase in the number of cell producing MT1-MMP but also an increase in the amount of MT1-MMP being produced by each cell.

**Limitations.** While this study clearly demonstrated that the expression, abundance, and activity of MT1-MMP were temporally regulated throughout TAA development, this study is not without limitations. First, the mRNA and protein levels measured at each time point in this study were compared relative to baseline values; no absolute quantification was performed. Therefore, the baseline levels of MT1-MMP were assumed to be sufficient for pro-MMP-2 activation. Additional studies will be required to define the dynamic cellular localization and association with pro-MMP-2 activation.

**Characterizing potential targets for nonsurgical interventions to impede thoracic aortic aneurysm growth.** May significantly reduce the morbidity and mortality associated with this disease. Despite limitations, the novel findings of the present study are threefold. First, the results demonstrate that MT1-MMP protein levels are progressively elevated during TAA development. This was established through immunoblot analysis of aortic homogenates from control mice and TAA-induced mice as well as by high-resolution microultrasound, demonstrating, for the first time, elevated MT1-MMP protein levels in live 8-wk TAA-induced mice through enhanced MT1-MMP antibody-targeted microbubble contrast binding to the descending thoracic aorta at the site of TAA induction. Second, the present study demonstrated that MT1-MMP activity was correlated with the time-dependent change in aortic diameter, suggesting that MT1-MMP may be required for aortic dilatation. Finally, the present study demonstrated that MT1-MMP localizes to DDR2-positive cells within the aortic wall, suggesting that the structural enhancements that occur with TAA development may be mediated in...
part by MT1-MMP produced within endogenous fibroblasts or fibroblast-derived cells. Together, these novel results suggest a multifunctional role for MT1-MMP in TAA development that is defined through two phases of expression and activity. The first phase defines a role for MT1-MMP early in aneurysm development, when its primary function involves the activation of MMP-2. The second phase defines a role for MT1-MMP at later time points, when the activation of other MMPs, pericellular degradation of matrix components, and potential release of latent bound growth factors are likely prominent functions of MT1-MMP. Thus, these results suggest that MT1-MMP may be a turnkey mediator of TAA formation and suggest that targeting this protease may have significant therapeutic implications.

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DISCLOSURES

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