Differential role of TIMP2 and TIMP3 in cardiac hypertrophy, fibrosis, and diastolic dysfunction

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Aims
Tissue inhibitor of metalloproteinases (TIMPs) can mediate myocardial remodelling, hypertrophy, and fibrosis in heart disease. We investigated the impact of TIMP2 vs. TIMP3 deficiency in angiotensin II (Ang II)-induced myocardial remodelling and cardiac dysfunction.

Methods and results
TIMP2−/−, TIMP3−/−, and wild-type (WT) mice received Ang II/saline (Alzet pump) for 2 weeks. Ang II infusion resulted in enhanced myocardial hypertrophy and lack of fibrosis in TIMP2−/−, and conversely, excess fibrosis without hypertrophy in TIMP3−/− mice. Echocardiographic imaging revealed preserved ejection fraction in all groups; however, exacerbated left ventricular (LV) diastolic dysfunction was detected in Ang II-infused TIMP2−/− and TIMP3−/− mice, despite the suppressed Ang II-induced hypertension in TIMP3−/− mice. Enhanced hypertrophy in TIMP2−/− mice impaired active relaxation, while excess fibrosis in TIMP3−/− mice increased LV passive stiffness. Adult WT cardiomyocytes, only when co-cultured with cardiac fibroblasts, exhibited Ang II-induced hypertrophy which was suppressed in TIMP3−/− cardiomyocytes. In vitro studies on adult cardiofibroblasts (quiescent and cyclically stretched), and in vivo analyses, revealed that the increased fibrosis in TIMP3−/−-Ang II hearts is due to post-translational stabilization and deposition of collagen by matricellular proteins [osteopontin and Secreted Protein Acidic and Rich in Cysteine (SPARC)], which correlated with increased inflammation, rather than increased de novo synthesis. Reduced cross-linking enzymes, LOX and PLOD1, could underlie suppressed collagen deposition in TIMP2−/−-Ang II hearts.

Conclusion
TIMP2 and TIMP3 play fundamental and differential roles in mediating pathological remodelling, independent from their MMP-inhibitory function. TIMP2−/− and TIMP3−/− mice provide a unique opportunity to study myocardial hypertrophy and fibrosis independently, and their impact on cardiac dysfunction.

Keywords
Tissue inhibitor of metalloproteinase ● Hypertrophy ● Fibrosis ● Diastolic dysfunction ● Extracellular matrix ● Matricellular proteins ● Cardiac fibroblast

1. Introduction
Cardiac extracellular matrix (ECM) comprises fibrillar collagens (types I and III), basement membrane, and non-structural proteins.1 Tissue inhibitor of metalloproteinases (TIMPs) are critical in physiological turnover of the ECM by controlling the activity of matrix metalloproteinases (MMPs), while they also play a number of MMP-independent functions.2,3 ECM contributes to transduction of contractile force and structural alignment of cardiomyocytes, while ECM–cardiomyocyte adhesion influences intracellular signalling, cell growth, hypertrophy, and survival.1,4-6 Fibrosis is the outcome of excess ECM accumulation and is a pathological characteristic of a number of cardiomyopathies, including hypertrophic, pressure overload, hypertensive, and inflammatory cardiomyopathies.1,8 Myocardial hypertrophy and fibrosis can result in diastolic and/or systolic dysfunction, ultimately culminating in heart failure.9 Diastolic dysfunction is the result of myocardial remodelling which can result in abnormal left ventricular (LV) filling, reduced LV compliance, and increased diastolic pressure despite sustained systolic function, eventually leading to heart failure with preserved EF (HFrEF).10 Since therapeutic approaches for HFrEF need to be different from those for HF with reduced EF (HFrEF), it is critical to understand the molecular mechanisms underlying diastolic dysfunction in order to develop...
effective therapies. Diastolic dysfunction is often associated with myocardial hypertrophy and fibrosis which have also been associated with LV dyssynchrony in HFrEF.\(^1^\)\(^3\) Since myocardial hypertrophy and fibrosis often co-exist in cardiomyopathies, differentiating between their contributions to disease progression is often challenging.

TIMPs have been shown to impact cell growth and hypertrophy.\(^1^\)\(^2^\)\(^3^\)\(^4^\) In vivo animal studies have shown that cardiac pressure overload results in severe cardiac fibrosis and hypertrophy in mice lacking TIMP3\(^1^\)\(^4^\) and TIMP2,\(^5^\) but not in TIMP4\(^1^\)\(^5^\) mice.\(^6\) In this study, we examined the causal role of TIMP2 vs. TIMP3 in agonist-induced cardiac fibrosis and hypertrophy, and subsequently in cardiac remodelling and dysfunction. We report that, in response to angiotensin II (Ang II) infusion, TIMP2 deficiency exacerbates myocardial hypertrophy with suppressed fibrosis, whereas TIMP3 deficiency results in severe myocardial fibrosis in the absence of hypertrophy. This differential myocardial remodelling impacts different aspects of diastolic function as evident by suppressed active relaxation in TIMP3\(^1^)\(^7^)\(^8^)\(^9^)-Ang II mice compared with compromised passive compliance in TIMP3\(^1^)\(^7^)\(^8^)\(^9^)-Ang II mice.

2. Methods

2.1 Experimental animals and protocol

Wild-type (WT, C57Bl/6) and TIMP2\(^-/-\) (C57Bl/L6) mice were purchased from Jackson Laboratory, TIMP3\(^-/-\) mice were generated as described,\(^1^\)\(^6^\) and backcrossed into C57Bl/6 background 12 times. In vivo, Ang II (1.5 mg/kg/day) or saline (control) was delivered by Alzet micro-osmotic pumps (Model 1002, Durect Co.) in anaesthetized (2% isoflurane) 8- to 9-week-old male mice.\(^1^\)\(^7^\)\(^8^\) After 2 weeks, mice were anaesthetized (keta-mine/xylazine, 100 mg/kg; 10 mg/kg), hearts were excised and flash-frozen for molecular analyses, or were fixed in 10% formalin and processed for immunohistological analyses.\(^4^\) All experiments were conducted in accordance with the guidelines of the University of Alberta Animal Care and Use Committee (ACUC) and the Canadian Council of Animal Care.

2.2 Echocardiographic analyses and in vivo haemodynamic measurements

Systolic and diastolic cardiac functions were determined non-invasively by transthoracic echocardiography in anaesthetized mice (0.75% isoflurane) as before.\(^4^\)\(^1^\)\(^9^\)\(^2^\)\(^0^\) Diastolic function was assessed using pulsed-wave Doppler imaging of the transmitial filling pattern as before.\(^4^\)\(^1^\)\(^9^\)\(^2^\)\(^0^\) LV pressure–volume relationships were measured invasively, using 1.2-Fr admittance catheter imaging of the transmitral filling pattern as before.\(^2^\)\(^1^\)\(^9^\)\(^2^\)\(^0^\) Diastolic function was assessed using pulsed-wave Doppler echocardiography in anaesthetized mice (0.75% isoflurane) as before.\(^4^\)\(^1^\)\(^9^\)\(^2^\)\(^0^\) Diastolic function was assessed using pulsed-wave Doppler imaging of the transmitral filling pattern as before.\(^4^\)\(^1^\)\(^9^\)\(^2^\)\(^0^\) Diastolic function was assessed using pulsed-wave Doppler imaging of the transmitral filling pattern as before.\(^4^\)\(^1^\)\(^9^\)\(^2^\)\(^0^\) Diastolic function was assessed using pulsed-wave Doppler imaging of the transmitral filling pattern as before.\(^4^\)\(^1^\)\(^9^\)\(^2^\)\(^0^\) Myocyte cross-sectional area was determined from the WGA-stained sections by using the Metamorph Basic software (version 7.7.0.0) as before.\(^2^\)\(^1^\)\(^2^\)\(^3^\) Paraffin sections were stained with primary antibody for Secreted Protein Acidic and Rich in Cysteine (SPARC), osteopontin (OPN, Abcam), Macrophage (Mac3, Serotec AbD), or Neutrophil (R&D Systems), followed by HRP-conjugated (SPARC and Mac3), FITC-conjugated (OPN), or Cy3-conjugated (Neutrophil) secondary antibodies, and DAPI nuclear staining as before.\(^4^\)\(^2^\)\(^4^\)\(^2^\)\(^5^\)–\(^2^\)\(^6^\)

2.5 Hydroxyproline assay

Collagen content was determined using the hydroxyproline assay as described.\(^2^\)\(^7^\) Collagen content is reported as μg of hydroxyproline per mg of LV tissue.

2.6 Isolation and culture of adult cardiac fibroblasts and myocytes

Cardiac fibroblast (CFB) and cardiac myocyte (cMyo) were isolated from adult mice of different genotypes as previously described.\(^2^\)\(^8^\)\(^2^\)\(^9^\) All CFBs were used at the second passage. Fibroblasts were cultured on collagen-coated BioFlex under quiescent conditions, or subjected to cyclic mechanical stretching (5% elongation 0.33 Hz) using the Flexcell FX-5000\(^2^\)\(^0^\) Tension System. CFBs were serum-deprived for 24 h prior to the experimental protocol. For cMyo – CFB co-cultures, cardiac fibroblasts were added to the cultured cMyo.\(^2^\)\(^8^\)\(^2^\)\(^9^\) Cultures and co-cultures were treated with 1 μM Ang II or saline for 24 h in FBS-free medium. At the end of the protocol, cells were harvested and RNA was extracted using Trizol as before.\(^3^\)\(^2^\)

2.7 Fibroblast proliferation and migration assays

Migration of CFB was assessed using in vitro scratch assay as described in serum-starved CFBs in the presence of Ang II (1 μM) or saline. Images were captured at 0, 24, and 36 h. Proliferation of CFB was assessed by determining the rate of 5-bromo-2-deoxyuridine (BrDU) incorporation in serum-starved CFBs treated with saline or Ang II. The number of positively staining cells is reported as the percentage of total number of cells in the same field.\(^3^\)\(^4^\)

2.8 Statistical analysis

All analyses were performed using the IBM SPSS Statistics 19 software. Averaged data are presented as mean ± SEM. Two-way ANOVA was used to compare each TIMP-deficient group with the WT. Student’s t-test was used to compare the Ang II-treated group with the saline group for each genotype. Statistical significance was recognized at P < 0.05.

3. Results

3.1 Ang II infusion enhanced hypertrophy in TIMP2\(^-/-\) and fibrosis in TIMP3\(^-/-\) mice

Two weeks of Ang II infusion resulted in myocardial hypertrophy in WT mice as evident by the cross-sectional images of the heart (Figure 1A), elevated heart weight-to-tibial length ratio (Figure 1B), and increased myocyte cross-sectional area (Figure 1Ci and ii). TIMP2 deficiency augmented the Ang II-induced myocardial hypertrophy resulting in a more gross enlargement of the heart, significantly greater heart weight-to-tibial length ratio (Figure 1A and B) and myocyte cross-sectional area (Figure 1G and ii). In TIMP3\(^-/-\) mice, however, the hypertrophy response was completely blunted (Figure 1A–C).

Assessment of myocardial fibrosis revealed that Ang II infusion resulted in interstitial and perivascular fibrosis in WT mice (Figure 2A and B), increased hydroxyproline content (Figure 2C), and increased de novo mRNA synthesis of collagen types I and III (Figure 2D and E). TIMP2\(^-/-\)-Ang II mice exhibited reduced myocardial fibrosis.
consistent with unchanged hydroxyproline content (Figure 2C), although collagen type I mRNA levels were elevated (Figure 2D). TIMP32/2 mice, however, showed striking interstitial and perivascular fibrotic lesions (Figure 2A and B) and elevated hydroxyproline content (Figure 2C), but no increase in de novo synthesis of collagen type I (Figure 2D), although collagen type III levels were elevated compared with the parallel saline group (Figure 1E). Therefore, collagen synthesis and protein deposition are uncoupled in TIMP22/2 and TIMP32/2 hearts.

Assessment of MMPs and their activity showed the absence of cleaved MMP2 in TIMP22/2 hearts, consistent with the lack of TIMP2,4,35 while total collagenase activity was not significantly altered in either TIMP-deficient group compared with WT-Ang II group (see Supplementary material online, Figure S1Ai–ii and B). At mRNA level, expression of MMP2 and MT1-MMP were increased in all Ang II-infused groups; however, these increases were less in TIMP22/2-Ang II hearts along with a reduction in MMP9 and MMP13 expression (see Supplementary material online, Figure S1Ci, iv, and v). The increase in MMP8 reached statistical significance only in TIMP32/2-Ang II hearts (see Supplementary material online, Figure S1Ci and ii). TIMP1 expression was elevated in all Ang II-infused hearts, whereas other TIMPs were not altered by Ang II infusion (see Supplementary material online, Figure S1D). These data indicate that the observed impact of TIMP2 or TIMP3 deficiency on myocardial hypertrophy and fibrosis is likely not through their MMP-inhibitory function.

### 3.2 Diastolic dysfunction with distinct characteristics in TIMP22/2-Ang II vs. TIMP32/2-Ang II mice

Myocardial hypertrophy and fibrosis have been associated with diastolic dysfunction.36,37 We investigated the differential impact of enhanced hypertrophy in TIMP22/2-Ang II vs. fibrosis in TIMP32/2-Ang II on myocardial dysfunction, using non-invasive echocardiographic measurements followed by invasive haemodynamic pressure–volume measurements. All three genotypes showed preserved systolic function while TIMP22/2-Ang II and TIMP32/2-Ang II mice exhibited diastolic dysfunction compared with WT-Ang II mice (see Supplementary material online, Table S2 and Figure S3). Interestingly, different aspects of LV diastolic function were altered in TIMP22/2-Ang II compared with TIMP32/2-Ang II infusion. Based on echocardiography (Figure 3A and see Supplementary material online, Table S2), reduced peak E-wave (i), E′-wave (ii), E-wave deceleration rate (iii), and increased left atrium-to-body weight ratio (iv) were observed in Ang II-infused TIMP22/2 and TIMP32/2 mice; however, prolonged isovolumic relaxation time (v) and deceleration time (vi) were only detected in TIMP22/2-Ang II, but not in TIMP32/2-Ang II, mice compared.
with their saline and WT-Ang II groups. TIMP3\(^{-/-}\)-Ang II mice additionally exhibited elevated \(E/E'\) ratio (Figure 3Avii), a measure of LV filling pressure. Invasive haemodynamic measurements revealed that the slope of the EDPVR, obtained by occlusion of IVC, was increased in WT-Ang II and TIMP3\(^{-/-}\)-Ang II, but not in TIMP2\(^{-/-}\)-Ang II, mice (Figure 3B). Averaged EDPVR, an indirect measure of myocardial stiffness, was markedly greater in TIMP3\(^{-/-}\)-Ang II, but not in TIMP2\(^{-/-}\)-Ang II, mice (Figure 3C). Consistent with the elevated filling pressure in TIMP3\(^{-/-}\)-Ang II mice, end-diastolic pressure (EDP) was significantly elevated in these mice, but not in TIMP2\(^{-/-}\)-Ang II mice (Figure 3D). These data collectively demonstrate that the excess hypertrophy in TIMP2\(^{-/-}\)-Ang II mice prolonged the active phase of LV relaxation, whereas fibrosis in the absence of hypertrophy in TIMP3\(^{-/-}\)-Ang II mice increased the passive LV stiffness.

### 3.3 Hypertrophic response in TIMP2\(^{-/-}\) and TIMP3\(^{-/-}\) cMyo in vitro

Cardiomyocyte hypertrophy can result from mechanical stress (increased afterload) and/or direct hormonal effects. Ang II, in addition to being a hypertrophic agent, is a strong hypertensive agent which can trigger marked significant hypertension. We previously reported that

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**Figure 2** Distinct myocardial fibrosis response in TIMP2\(^{-/-}\) vs. TIMP3\(^{-/-}\) mice following Ang II infusion. Representative images from trichrome-stained (A) and PSR-stained hearts (B) after 2 weeks of Ang II or saline infusion in indicated groups. Scale bar = 50 μm. (C) Hydroxyproline levels in indicated groups. TaqMan mRNA levels of collagen type I (D) and collagen type III (E) normalized to HPRT. *\(n = 5\)/group/genotype. RE, relative expression. *\(P < 0.05\) compared with the corresponding saline group.
Ang II-induced hypertension in TIMP2 \(^{-/-}\) mice is comparable to WT mice, but reduced in TIMP3 \(^{-/-}\) mice. To examine whether the differential Ang II-induced myocardial hypertrophy in TIMP2 \(^{-/-}\) vs. TIMP3 \(^{-/-}\) mice is due to the different degrees of hypertension, or inherent to the cardiomyocyte of each genotype, we tested the hypertrophic response of isolated adult cMyo in vitro. When cultured alone, cMyo of neither genotype showed an increase in the expression of hypertrophy markers, \(\beta\)MHC, \(\alpha\)-skeletal actin, and BNP, in response to Ang II (1 \(\mu M\), Figure 4Ai–iii). Meanwhile, when co-cultured with adult cFB, Ang II treatment triggered marked elevations in expression of hypertrophy markers in WT and TIMP2 \(^{-/-}\), but not in TIMP3 \(^{-/-}\), co-cultures (Figure 4Biv–vi). These data indicate that TIMP3 deficiency suppresses the Ang II-induced hypertrophic capacity of the cardiomyocytes.

3.4 Fibrogenic properties of adult cFB lacking TIMP2 or TIMP3 in vitro

Next, we investigated if the function of TIMP2- and TIMP3-deficient cFBs in vitro could explain the differential Ang II-induced fibrogenic response in these mice. Second-passage adult cFB from each genotype was cultured under quiescent conditions or subjected to cyclic mechanical stretching, and treated with 1 \(\mu M\) Ang II or saline. Under quiescent conditions (Figure 5Ai–C), WT fibroblasts exhibited no increase in the expression of collagen type I, while collagen type III expression increased following 24 h of Ang II treatment (Figure 5Ai and ii); however, Ang II treatment did not alter the expression of collagen type I (i) or type III (ii) in TIMP2 \(^{-/-}\) or TIMP3 \(^{-/-}\) cFBs (Figure 5B and C). Similar
observations were made with different concentrations of Ang II (0.1 and 10 μM) or longer treatment of cFBs (48 h) (data not shown).

To simulate the *in vivo* condition where the cFBs are subjected to a rhythmic stretch and strain as a result of cardiac contraction and relaxation, we subjected the cultured cFB to a cyclic stretch protocol, 5% elongation at 0.33 Hz, as reported by others. This protocol of mechanical stretching was selected as it did not increase collagen production. The combination of cyclic mechanical stretch and 1 μM Ang II significantly increased the expression of collagen types I and III in WT cFB (*Figure 5D* and *ii*) and collagen type I synthesis in TIMP2−/− cFB (*Figure 5E*). These data closely resemble the pattern of Ang II-induced expression of collagens in vivo, demonstrating that the TIMP3−/− cFBs in fact have reduced Ang II-induced *de novo* collagen production.

We further examined whether the more severe myocardial fibrosis in TIMP3−/−-Ang II hearts could be due to enhanced migration or proliferation of the cFB. We compared the rate of migration (scratch assay) and proliferation (BrdU incorporation) of cultured cFB among genotypes. The rate of cFB migration was comparable among genotypes treated with saline or Ang II (see Supplementary material online, *Figure S2A*). BrdU incorporation increased with Ang II treatment in WT, but not in TIMP2−/− or TIMP3−/− cFB (see Supplementary material online, *Figure S2Bi and ii*). Ang II did not trigger cell death in any genotype as assessed by lactate dehydrogenase levels in the culture media (data not shown). Therefore, the exacerbated myocardial fibrosis in TIMP3−/−-Ang II hearts is not due to increased collagen production capacity, migration, or proliferation of TIMP3-deficient cFB.

### 3.5 Elevated matricellular proteins co-localize with fibrotic myocardium in TIMP3−/−-Ang II hearts

Matricellular proteins such as SPARC and OPN mediate post-translational modification, deposition, and organization of ECM proteins independent from their synthesis. Immunostaining for SPARC revealed greater elevations in Ang II-infused TIMP3−/−, but not TIMP2−/− compared with WT hearts (*Figure 6A*), whereas mRNA expression of SPARC was increased following Ang II infusion in all genotypes (*Figure 6Aii*). Immunostaining and fluorescent microscopy (*Figure 6Bi and ii*) and expression analysis for OPN (*Figure 6Bii*) showed increased protein and mRNA levels in WT-Ang II and to a significantly greater extent in TIMP3−/−-Ang II hearts, whereas no increase
Figure 5  Ang II-increased collagen expression in adult mouse cardiac fibroblast (cFB) in vitro. (A–C) TaqMan mRNA levels of collagen type I (i) and collagen type III (ii) in cFB isolated from WT (A), TIMP2−/− (B), and TIMP3−/− hearts (C) cultured under quiescent conditions and treated with saline or Ang II. (D–F) TaqMan mRNA levels of collagen type I (i) and collagen type III (ii) in cFB isolated from WT (A), TIMP2−/− (B), and TIMP3−/− hearts (C) cultured with cyclic mechanical stretching (5\% elongation, 0.33 Hz) and treated with saline or Ang II. n = 4 hearts/genotype, 4 culture plates/group/genotype. *P < 0.05 compared with the corresponding saline.
was detected in Ang II-infused TIMP2−/− hearts. The elevated OPN and SPARC levels co-localized predominantly with the fibrotic regions within the myocardium highlighting their region-specific function.

To determine whether the elevated matricellular proteins in TIMP3−/−-Ang II hearts are the direct result of TIMP3 deficiency in the fibroblasts, which have been reported to be the main source of matricellular proteins, we measured the expression of SPARC and OPN in cultured cFB (quiescent and cyclically stretched). However, we found that Ang II treatment did not increase the expression of SPARC or OPN in cultured cFBs (see Supplementary material online, Figure S3). Inflammatory cells could be another source of matricellular proteins. We assessed if Ang II infusion triggered an inflammatory response in any genotype. Infiltration of macrophages and neutrophils was negligible in WT and TIMP2−/− hearts, but greatly elevated in TIMP3−/−-Ang II hearts, primarily in the fibrotic areas (Figure 7A and B). Consistently, expression of inflammatory cytokine, interleukin-1β, was increased in TIMP3−/−-Ang II hearts while the increase in interleukin-6, tumor necrosis factor, and monocyte chemoattractant protein-1 did not reach statistical significance (Figure 7C).

We further assessed if the lack of TIMP2 or TIMP3 could alter transport of pro-collagen to the extracellular space, conversion of pro-collagen to collagen and finally cross-linking of the triple helices and formation of collagen fibres. coat protein complex II (COPII) vesicles, surrounded by a coat consisting of a number of genes including SEC23-24 and SEC13-31, mediate intracellular transport of numerous molecules. Given the large size of pro-collagens, Cul3-KLHL12 are required to make the COPII vesicles large enough to accommodate pro-collagen molecules. Expression of the COPII-related genes (Sec23A, Sec31A, KLHL1, and Cullin 3) were not different among genotypes (see Supplementary material online, Figure S4A). Among
pro-collagen endopeptidase and enhancers reported to contribute to cardiac fibrosis (BMP-1, PCPE1, and PCPE2\(^{44,45}\)). BMP1 and PCPE1 increased in WT-Ang II, but not in TIMP2\(^{-/-}\) or TIMP3\(^{-/-}\)-Ang II, hearts (see Supplementary material online, Figure S4Bi and ii). Lysyl hydroxylase (PLOD1) and lysyl oxidase (LOX) are responsible for hydroxylation and oxidative deamination of collagens leading to maturation and stability of collagen fibres.\(^ {46,47}\) Ang II infusion increased expression of PLOD1 in WT and TIMP3\(^{-/-}\), but not in TIMP2\(^{-/-}\) hearts, while LOX expression increased with Ang II infusion but was lower in TIMP2\(^{-/-}\)-Ang II compared with other groups (see Supplementary material online, Figure S4Gi and ii).

4. Discussion

Myocardial hypertrophy and fibrosis are two characteristics of a number of cardiomyopathies which can lead to cardiac dysfunction culminating in...
Interaction between cMyo and fibroblasts are essential for the heart. Disruption of ECM can adversely impact the cell–cell and ECM–cardiomyocyte interactions triggering pathological signalling and hypertrophy, while excessive accumulation of fibrillar ECM proteins, primarily collagen types I and III, results in fibrosis. In this study, we report that the lack of TIMP2 and TIMP3 distinctly and differentially influences cardiac hypertrophy and fibrosis, thereby impacting different aspects of diastolic function. Diastolic dysfunction, if left untreated, can lead to HFpEF which is a common and increasingly prevalent health problem affecting up to 50% of all patients with chronic heart failure.

The lack of TIMP2 or TIMP3 did not trigger compensatory up-regulation of other TIMPs, while TIMP1 expression increased similarly in all genotypes with Ang II infusion. An increase in TIMP1 levels is frequently linked to fibrosis, and therefore, its elevation in TIMP2/+/Ang II hearts, which exhibited that no fibrosis is intriguing and could be linked to the elevated collagen mRNA. We found that the lack of myocardial fibrosis despite elevated collagen mRNA in TIMP2/−/−Ang II hearts, compared with the excess fibrotic lesions in TIMP3/−/− hearts without increased de novo synthesis of collagen, is not due to inherent response of cFB of either genotype to Ang II. Moreover, quiescently cultured cFBs did not exhibit the Ang II-induced collagen production observed in vivo. This is not surprising since quiescently cultured cFBs has been compared with the status in a healthy heart where the intact matrix shields cFB from mechanical stretching, sustaining their quiescent phenotype with basal production of ECM proteins. As such, it is plausible that fibrogenic capacity of quiescent FB is compromised. An earlier study by Lovelock et al. reported that overexpression of human TIMPs in quiescently cultured mouse cFB increased proliferation, while TIMP2 further induced apoptosis and TIMP2 increased collagen production by these cells. These findings are different from ours most likely due to differences in experimental settings. Lovelock et al. utilized quiescently cultured cFB at passages 4–5, whereas we limited our studies to cFB in passage 2 since myofibroblast characteristics are detected beyond the second passage.

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OPN levels in TIMP3−/−–Ang II hearts. The marked up-regulation of these matricellular proteins, together with the lack of de novo expression of collagens in the TIMP3−/−–Ang II myocardium, indicates that the post-translational processing and deposition of collagen fibres underlie the severe perivascular and interstitial myocardial fibrosis in these mice.

In summary, our findings challenge the canonical concepts regarding TIMP biology by establishing that reduced TIMP levels do not invariably result in reduced fibrosis due to increased ECM-degrading function of MMPs. This highlights the complex function of TIMPs in cardiac pathology beyond their MMP-inhibitory function. TIMP2−/− and TIMP3−/− mice provide the unique opportunity to decipher the impact of myocardial hypertrophy vs. fibrosis in diastolic dysfunction. Our studies demonstrate that myocardial hypertrophy impairs active relaxation, while fibrosis enhances passive stiffness of the myocardium. Our in vitro studies further demonstrate the complexity of cellular events involved in these processes, highlighting the significance of post-translational regulation of matrix proteins, independent from their de novo synthesis, in mediating myocardial fibrosis.

5. Clinical relevance

Diastolic dysfunction can occur in patients with normal LV EF and can lead to diastolic heart failure (HFpEF). The hospital re-admission rates of patients with HFpEF are similar to those with HFrEF, and worsening of diastolic dysfunction has been shown to be an independent predictor of mortality. In this study, we demonstrate that, in a model of diastolic dysfunction with sustained EF, TIMP2 and TIMP3 have divergent effects on cardiac remodelling and can impact different aspects of diastolic dysfunction with preserved systolic function.

Supplementary material

Supplementary material is available at Cardiovascular Research online.

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