Heart

Matrix Metalloproteinase-2 Mediates a Mechanism of Metabolic Cardioprotection Consisting of Negative Regulation of the Sterol Regulatory Element–Binding Protein-2/3-Hydroxy-3-Methylglutaryl-CoA Reductase Pathway in the Heart

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Abstract—Previously, we reported that cardiac matrix metalloproteinase (MMP)-2 is upregulated in hypertensive mice. How MMP-2 affects the development of cardiac disease is unclear. Here, we report that MMP-2 protects from hypertensive cardiac disease. In mice infused with angiotensin II, the lack of MMP-2 (Mmp2−/−) did not affect the severity of the hypertension but caused cardiac hypertrophy to develop earlier and to a greater extent versus wild-type (Mmp2+/+) mice, as measured by heart weight:body weight ratio and upregulation of hypertrophy and fibrosis markers. We further found numerous metabolic and inflammatory gene expression abnormalities in the left ventricle of Mmp2−/− mice. Interestingly, Mmp2−/− mice expressed greater amounts of sterol regulatory element–binding protein-2 and 3-hydroxy-3-methylglutaryl-coenzyme A reductase (a target of sterol regulatory element–binding protein-2–mediated transcription and rate limiting enzyme in cholesterol and isoprenoids biosynthesis) in addition to markers of inflammation including chemokines of the C-C motif ligand family. We focused on the functionally related genes for sterol regulatory binding protein-2 and 3-hydroxy-3-methylglutaryl-coenzyme A reductase. The 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor, lovastatin, attenuated angiotensin II–induced cardiac hypertrophy and fibrosis in Mmp2−/− and wild-type (Mmp2+/+) mice, with Mmp2−/− mice showing resistance to cardioprotection by lovastatin. MMP-2 deficiency predisposes to cardiac dysfunction as well as metabolic and inflammatory gene expression dysregulation. This complex phenotype is, at least in part, because of the cardiac sterol regulatory element–binding protein-2/3-hydroxy-3-methylglutaryl-coenzyme A reductase pathway being upregulated in MMP-2 deficiency. (Hypertension. 2015;65:882-888. DOI: 10.1161/HYPERTENSIONAHA.114.04989.)

Key Words: cardiomegaly ■ hydroxymethylglutaryl-CoA reductase inhibitors ■ matrix metalloproteinase 2 ■ sterol regulatory element binding proteins

Hypertrophic cardiomyopathy is a major cause of morbidity and mortality in industrialized countries.1 This condition can be caused by sustained hypertrophy as well as metabolic comorbidities, such as diabetes mellitus, hyperlipidemia, and hypercholesterolemia. A common effector mechanism of these detrimental factors is a sustained elevation of the systemic levels of G protein–coupled receptor agonists, including angiotensin II (Ang II). These agonists elicit cardiac remodeling processes (hypertrophy and fibrosis), at least in part, through triggering an excessive transcriptional upregulation and activation of matrix metalloproteinases (MMPs).

Purportedly, MMPs act mainly through the proteolysis of substrates, such as extracellular matrix proteins and growth factors to modulate the development of cardiac hypertrophy and fibrosis. However, the process of cardiac remodeling can eventually progress to cause cardiac dysfunction and, ultimately, heart failure.2–4 Because of their connection with the cardiac remodeling process, MMPs have long been regarded as attractive therapeutic targets to treat hypertrophic cardiomyopathy.

MMP-2 is one of multiple effectors upregulated by prohypertrophic and proinflammatory stimuli.5,6 MMP-2 deficiency
affects cardiac function as suggested by the attenuation of cytokine-induced cardiomyopathy,7 exacerbation of adverse remodeling in experimental myocardial infarction,8 and exacerbation of pressure overload by transverse aortic constriction.9 However, the molecular mechanism(s) of MMP-2 in these models is lacking. Moreover, the purported role of decreased extracellular remodeling because of lack of MMP-2 is both unlikely and unproven to account for the observed phenotypes in these models of disease. The potential contribution of inflammatory pathways regulated by MMP-2 is unclear.

A mechanism of hypertrophic cardiac disease relevant to this study depends on the activity of a ubiquitous enzyme, 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR).10 HMGCR catalyzes the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonate,11 which is the rate-limiting step in a series of enzymatic conversions that transform 3-hydroxy-3-methylglutaryl-coenzyme A to 5-carbon, 15-carbon, and eventually 20-carbon activated isoprenoids, such as isopentenyl pyrophosphate, farnesyl pyrophosphate and geranylgeranyl pyrophosphate. Notably, isoprenylation of small GTPases of the Ras superfamily switch on or off intracellular signaling of hypertrophy, fibrosis, and oxidative stress in response to G protein–coupled receptor agonists.12

HMGCR is strongly regulated at the transcriptional level by sterol regulatory element–binding protein (SREBP)-2.11 Here, we show that MMP-2 deficiency predisposes to cardiac dysfunction as well as metabolic and inflammatory gene expression dysregulation. This complex phenotype is, at least in part, a consequence of the cardiac SREBP-2/HMGCR pathway being upregulated in the absence of MMP-2.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Animal Models

Animal protocols were conducted in accordance with institutional guidelines issued by the Canadian Council on Animal Care and US National Institutes of Health. All animals were fed regular chow and housed at the University of Alberta. Male C57BL/6 mice were purchased from Charles River (Wilmington, MA). Male Mmp2−/− mice were bred and housed at the University of Alberta. The mice used in the studies were 11 to 14 weeks old. ALZET osmotic minipumps (DURECT Corporation, Cupertino, CA) delivering either PBS or Ang II (1.4 or 2.0 mg/kg/day; EMD Millipore, Billerica, MA) were implanted subcutaneously on the posterior midsection of mice anesthetized by isoflurane.

Statistical Analysis

Results were analyzed using 1-way ANOVA (between multiple groups) or t test (between 2 groups; Systat SigmaPlot 11 software). All data are reported as mean±SEM.

Results

MMP-2 Deficiency Predisposes to Ang II–Induced Cardiac Hypertrophy

Ang II infusion induced systemic hypertension in wild-type (WT), as expected (Figure S1) in the online-only Data Supplement. We observed no significant differences in either time course (data not shown) or magnitude of maximal systolic blood pressure in Mmp2−/− mice versus WT mice (Figure S1). Within 2 weeks of Ang II infusion, mice of either genotype developed maximal cardiac hypertrophy, as indicated by the relative increase in the heart weight to either body weight or tibia length (Figure 1A; Figure S2).

Interestingly, in Mmp2−/− mice, hypertrophy developed earlier and to a greater extent than WT mice (Figure 1A). Fibrosis in the Ang II model was both interstitial and perivascular (Figure 1B), with a significant overexpression of marker genes of cardiac fibrosis and hypertrophy as assessed by quantitative reverse transcription polymerase chain reaction (Figure 1C–1F). No spontaneous cardiac hypertrophy was observed. These data suggested that in agonist-induced hypertrophic heart disease, MMP-2 may be cardioprotective.

Deficiency of MMP-2 Affects Cardiac Expression of Metabolic and Inflammatory Genes

We next explored whether the MMP-2 deficiency predisposed to hypertensive cardiac disease by way of altering the cardiac gene expression. Based on our hypothesis13 that alterations in inflammatory or lipid metabolic genes may underlie the development of heart disease, which was being pursued by independent lines of research in our laboratory, we examined the cardiac expression of 56 genes comprising inflammatory and lipid metabolic transcription factors, enzymes, and mediators. The analysis revealed a large elevation of Srebf2 and Hmgcr (genes encoding SREBP-2 and HMGCR, respectively), as well as a decreased expression of Nr1h3 (which encodes a liver X receptor-α, a major lipid metabolic transcription factor) in Mmp2−/− relative to WT mice (Figure 2A). Markers of inflammation, including chemokines of the C-C motif ligand family: Ccl5, Ccl2, and Cce6, were among the most upregulated of the genes measured. Similar to whole hearts, isolated cardiomyocytes demonstrated an upregulation of Srebf2, Hmgcr, and Ldlr (Figure S3). These data show the role of MMP-2 in modulating cardiac inflammation and lipid metabolic gene expression.

Cardiac SREBP-2 Transcriptional Pathway Is a Target of Ang II in Cardiac Hypertrophy

We next examined the transcriptional regulation of cardiac HMGCR in Ang II–induced hypertensive cardiac disease. In WT mice, reverse transcription polymerase chain reaction analysis indicated an increase in HMGCR mRNA within the first week of Ang II infusion, followed by an eventual normalization (Figure 3A). In contrast to WT mice, the baseline expression of HMGCR in the Mmp2−/− mice was upregulated. This upregulation persisted for the first week of Ang II infusion, but by week 2 decreased to WT levels. In both WT and Mmp2−/− mice, SREBP-2 (transcriptional regulator of HMGCR and low-density lipoprotein receptor) and low-density lipoprotein receptor transcription followed a similar pattern of expression as HMGCR over the course of Ang II infusion (Figure 3B and 3C).

Interestingly, HMGCR protein immunoreactivity in Mmp2−/− hearts was elevated at both baseline and after 2 weeks of Ang II (Figure 3D). Because HMGCR mediates pathways of cardiac hypertrophy,10,14 we hypothesized that the baseline elevation of HMGCR in Mmp2−/− mice could contribute to cardiac hypertrophy.
MMP-2–Dependent Negative Regulation of HMGCR Protects Against Pathological Cardiac Hypertrophy and Fibrosis

To examine the role of HMGCR in the pathology of hypertrophic heart disease, we administered lovastatin to mice infused with Ang II. Treatment with lovastatin dose dependently prevented Ang II–induced cardiac hypertrophy in Mmp2−/− mice, as well as in WT mice, confirming the key contribution of HMGCR to Ang II–induced cardiac disease in both genotypes (Figure 4A). When lovastatin was given at a dose of 54 mg/kg/day, hypertrophy and fibrosis marker gene expression were significantly reduced in the hearts of both WT and Mmp2−/− mice required a 2-fold higher dose of lovastatin to prevent the development of cardiac hypertrophy, which was consistent with their higher HMGCR levels. The elevated expression of inflammatory markers in Mmp2−/− mice was, in part, reduced by administration of lovastatin (Figure 4B).

Discussion

We have found that lack of MMP-2 results in significant alteration of cardiac gene expression. Among the most upregulated genes in the left ventricle of mice are SREBP-2 and targets thereof, such as HMGCR (rate-limiting enzyme in the...
In addition, we found that MMP-2 deficiency upregulates proinflammatory genes, as well as predisposes to cardiac hypertrophy. Indeed, a major finding of this study is that, during the development of hypertensive cardiac disease, MMP-2 is cardioprotective through a novel mechanism involving negative regulation of the SREBP-2/HMGCR axis of cardiac hypertrophy (Figure 158). As a consequence of their upregulated cardiac SREBP-2/HMGCR pathway, Mmp2−/− mice were less susceptible to cardioprotection by lovastatin.

MMPs have long been implicated in the modulation of cardiac remodeling, with most studies centered on the proteolytic cleavage of substrates, such as extracellular matrix proteins, growth factors, and receptors. Here, we show that MMP-2 negatively regulates a fundamental metabolic mechanism.
and may thus affect the development and severity of cardiac disease. Indeed, \textit{Mmp2}−/− mice had surprisingly high baseline mRNA levels of cardiac SREBP-2, a major transcription factor for HMGCR and low-density lipoprotein receptor genes. We have previously observed that MMP-2 is elevated in the heart during the early stages of Ang II–induced hypertensive heart disease, but the function of this augmentation has been unclear.17 Elevated MMP-2 activity has been proposed to be detrimental after myocardial infarction. 8,9 Here, we observed that MMP-2 deficiency predisposes to cardiac hypertrophy, suggesting that cardiac MMP-2 overexpression in the hypertensive heart may be cardioprotective, at least in part, by preventing excessive expression of cardiac HMGCR. However, cardioprotection by MMP-2 is eventually overcome by Ang II because the characteristically high baseline mRNA levels of SREBP-2 and HMGCR in \textit{Mmp2}−/− mice declined to WT levels once cardiac hypertrophy was established (around week 2 on Ang II). Therefore, MMP-2 cardioprotection has a limited time window.

Further evidence implicating the MMP-2/HMGCR axis in the cardiac disease development came from pharmacological studies where we used lovastatin to inhibit HMGCR. Lovastatin attenuated the induction of hypertrophy and fibrosis marker genes in both \textit{Mmp2}−/− and WT mice. However, compared with WT mice, \textit{Mmp2}−/− mice had reduced responsiveness to lovastatin. These results indicate that higher baseline levels of HMGCR predispose to cardiac disease in \textit{Mmp2}−/− mice, and further implicate HMGCR in pathological cardiac remodeling.10,14 Although this study advances our understanding of the significance of MMP-2 for the development of cardiac disease, the question as to how MMP-2 negatively regulates SREBP-2 transcription remains and is being actively investigated by our laboratory.

Previous studies have established that one of the mechanisms whereby HMGCR, activity contributes to cardiac hypertrophy is through the synthesis of mevalonate—a common precursor of cholesterol and isoprenoids.

Figure 4. Matrix metalloproteinase (MMP)-2–dependent negative regulation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) protects against pathological cardiac hypertrophy and fibrosis. A, Heart weight normalized to body weight (HW/BW) as an indicator of cardiac hypertrophy in wild-type (WT; left) and \textit{Mmp2}−/− (right) mice treated with or without angiotensin II (Ang II; 2 weeks) and lovastatin (beginning 3 days before Ang II) at the indicated doses. Data for baseline (Ang II=0) and Ang II (1.4 mg/kg/day) were pooled from multiple experiments. B, Quantitative reverse transcription polymerase chain reaction analysis of hypertrophy, fibrosis and inflammatory marker gene expression in the hearts of WT or \textit{Mmp2}−/− mice treated with Ang II (2 weeks of 2.0 mg/kg/day) and lovastatin (54 mg/kg/day). n=3 for \textit{Mmp2}−/−+lovastatin, n=4 for all other groups. C, Cardiac MMP-2 elicits a novel metabolic cardioprotective mechanism whereby MMP-2 negatively regulates the sterol regulatory element–binding protein (SREBP)-2/HMGCR pathway. Results are means±SEM. *P≤0.05 versus corresponding WT. ‡P≤0.05 versus no Ang II. †P≤0.05 versus no lovastatin. ND indicates not detected.
Synthesis of isoprenoids is necessary for the signaling activity of small GTPases, such as Rac1 and Rho, and previous studies have demonstrated that Ang II induces the development of cardiac hypertrophy through isoprenylation of these GTPases, indicating the prohypertrophic potential of isoprenoids synthesized downstream of HMGCR.\textsuperscript{10,14} Isoprenylation of Rho is required for cardiac hypertrophy signaling through mitogen-activated protein kinases, whereas isoprenylation of Rac1 contributes through activation of nicotinamide adenine dinucleotide phosphate oxidase and superoxide production. Synthesis of cholesterol does not seem to be a major contributor to cardiac hypertrophy signaling at least in normcholesterolemic mice. In line with this notion, statins have been shown to protect from cardiac hypertrophy without causing any detectable alteration in low-density lipoprotein-cholesterol levels.\textsuperscript{10,14} In rat cardiomyocytes, HMGCR activity has been suggested to account for the intracellular cholesterol levels,\textsuperscript{15,19} although cardiomyocytes may acquire cholesterol from the circulation.\textsuperscript{20} Recent metabolites profile studies using mass spectrometry–based analysis combined with high-temperature gas chromatography show a 7-fold increase in cholesterol levels in hypertrophic cardiac tissues.\textsuperscript{21} However, in our studies, mice fed chow supplemented with \( \leq 1.5\% \) cholesterol for 15 days did not exhibit significant differences in the magnitude of cardiac hypertrophy induced by Ang II (unpublished observations). Although the role of cardiac cholesterol in the pathogenesis of hypertensive heart disease remains unclear, high levels of cholesterol in the circulation do correlate with increased risk of atherosclerosis and coronary artery disease.

**Clinical Significances**

Our findings suggest that therapeutic agents targeting MMPs in the context of cardiovascular and noncardiovascular conditions could be detrimental for heart function by decreasing cardiac MMP-2 levels. This is important because MMPs remain attractive therapeutic targets in the context of cardiovascular conditions (eg, atherosclerosis, ischemia reperfusion, hypertrophic heart disease, and postmyocardial infarction), as well as in noncardiovascular disorders (cancer, rheumatoid arthritis, and inflammation).

Our findings can also help explain the results of human studies showing that MMP-2 expression is negatively correlated with the susceptibility to hypertensive heart disease. Two striking examples are (1) functional genetic polymorphisms, which increase MMP-2 gene expression, reportedly protects against cardiac remodeling including increases in end-diatolic diameter and left ventricular mass index in hypertensive subjects and (2) a rare panethnic genetic disease of deficiency in human MMP-2 enzyme activity affects some Saudi Arabian, Indian, and Turkish family with congenital heart disease, including atrial and ventricular septal defects.\textsuperscript{22} Our findings suggest a metabolic basis for the presentation of deleterious proinflammatory cardiac phenotypes in MMP-2-deficient humans. Future research may reveal to what extent MMP-2 upregulation by genetic or pharmacological means can be exploited to either prevent or ameliorate hypertensive heart disease.

**Perspectives**

MMP-2 mediates a novel metabolic mechanism of cardio- protection involving negative regulation of the SREBP-2/HMGCR pathway in the heart and, thereby, inhibition of pathological cardiac remodeling. Importantly, our data suggest that caution should be exercised before implementing therapeutic strategies targeting MMP-2 because MMP-2 deficiency could predispose to cardiac dysfunction.

**Sources of Funding**

This work was funded by studentships from Alberta Innovates Health Solutions (X. Wang) and the Queen Elizabeth II and 75th anniversary graduate studentships from Faculty of Medicine and Dentistry University of Alberta (E. Berry), by operating grants from the Canadian Institutes of Health Research (Z. Kassiri and C.F. Patron) and a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada (C.F. Patron).

**Disclosures**

None.

**References**


What Is New?

• Previous research showed that the development of hypertension is accompanied by an increase in cardiac matrix metalloproteinase (MMP)-2 levels, which is generally presumed to be detrimental. We found that cardiac hypertrophy and fibrosis developed earlier and to a greater extent when hypertensive mice lacked MMP-2. These data exposes a previously unknown cardioprotective action of MMP-2 in the hypertensive heart.

• We further observed abnormal mRNA expression of lipid metabolic genes in the heart, including 3-hydroxy-3-methylglutaryl-coenzyme A reductase. Targeted expression/function studies indicated that MMP-2 negatively regulates 3-hydroxy-3-methylglutaryl-coenzyme A reductase and, thereby, 3-hydroxy-3-methylglutaryl-coenzyme A reductase–dependent cardiac hypertrophy.

What Is Relevant?

• MMPs are attractive therapeutic targets in cardiovascular and noncardiovascular conditions. Our study indicates that therapeutic strategies that inhibit MMP-2 could predispose to hypertensive heart disease.

• Humans affected by the MMP-2 gene deficiency may also be predisposed to hypertensive heart disease.

Summary

We describe a novel mechanism of metabolic cardioprotection consistent of MMP-2–mediated negative regulation of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase and, thereby, cardiac hypertrophy. These findings should advance the understanding and treatment of hypertensive heart disease.
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_Hypertension_. 2015;65:882-888; originally published online February 2, 2015; doi: 10.1161/HYPERTENSIONAHA.114.04989

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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MMP-2 mediates a mechanism of metabolic cardioprotection consisting of negative regulation of the SREBP-2/HMGCR pathway in the heart

Running Title: MMP-2/HMGCR cardioprotective pathway

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MATERIALS AND METHODS

Animal models Animal protocols were conducted in accordance with institutional guidelines issued by the Canada Council on Animal Care and US National Institutes of Health. All animals were fed regular chow and housed at the University of Alberta. Male C57BL/6 mice were purchased from Charles River (Wilmington, MA, USA). Mmp2^-/- mice were bred and housed at the University of Alberta. Limited availability of Mmp2^-/- mice during these studies was due to high in utero demise, an issue recently addressed. The mice (11-14 week old) were anesthetised by 2.0% isoflurane inhalation and ALZET osmotic minipumps (DURECT Corporation, Cupertino, CA, USA) delivering either PBS or Ang II (1.4 or 2.0 mg/kg/d, EMD Millipore, Billerica, MA, USA) were implanted subcutaneously on the posterior midsection of mice anaesthetized by Isofluorane. All mice were euthanized using sodium pentobarbital (65 mg/kg).

Blood pressure measurement Systolic blood pressure was measured using a computerized tail cuff plethysmography system (Kent Scientific Corporation, Torrington, CT, USA). Conscious mice were maintained at 32-35ºC using a heating pad and restrained during measurements. Averages of 10 inflation/deflation cycles were conducted to obtain mean systolic blood pressure for each mouse.

HMGCR inhibition using lovastatin Lovastatin (54 or 108 mg/kg/d, BioVision, San Francisco, CA, USA) or vehicle (soybean oil) was delivered daily by gavage feeding. PBS- or Ang II (2.0 mg/kg/d)-delivering minipumps were implanted 3 days after initiation of lovastatin administration. The mice were euthanized 2 weeks after minipump implantation for endpoint analysis.

Tissue homogenization for protein analysis Heart were washed in isotonic saline buffer, rinsed and weighed. Protein was extracted in 20 mmol/L Tris (pH 8.0), 150 mmol/L NaCl, 10% glycerol, 1% SDS, 0.1% Triton X-100 and protease inhibitor cocktail (Roche, Mannheim, Germany). To visualize protein content, homogenates were separated by 10% SDS-PAGE followed by densitometric analysis of Coomassie Brilliant Blue-stained bands. Equal protein quantities were loaded for subsequent immunoblotting.

Protein immunoblotting The expression of specific proteins was determined by immunoblotting. Homogenates were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was probed with primary antibodies against HMGCR (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and corresponding secondary antibodies (GE Healthcare, Buckinghamshire, UK), before being detected using ECL western blotting detection reagent (GE Healthcare).

RNA expression analysis by TaqMan qRT-PCR Total RNA was extracted from tissue using TRIzol reagent (Invitrogen, Burlington, ON, Canada) and cDNA was generated from 2 µg RNA using random hexamers (Invitrogen). Expression analysis of the reported genes was performed by TaqMan qRT-PCR using ABI 7900 sequence detection system (Applied Biosystems, Carlsbad, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal standard.
Isolation of cardiomyocytes Cardiomyocytes were isolated using a standard collagenase method. Briefly, hearts were dissected, minced, and washed in PBS. The heart pieces were next incubated at 37°C with a mixture of 0.1% trypsin and 0.1% collagenase. The resultant cell suspension was filtered and centrifuged, then the cell pellet was suspended in MEM supplemented with 10% FBS for immediate use for qRT-PCR analysis.

Histological analysis Mice hearts were fixed in 10% neutral-buffered formalin overnight and embedded in paraffin. 4 µm-thick sections were cut and stained with a modified Lillie’s variant of Masson’s trichrome stain. Briefly, sections were deparaffinised, mordanted overnight in Bouin’s solution (Sigma-Aldrich), stained successively with fresh Weigert’s hematoxylin, Biebrich scarlet-acid fuchsin (0.9% Biebrich scarlet, 0.1% acid fuchsin, 1% acetic acid), 2.5% phosphomolybdic – 2.5% phosphotungstic acid and aniline blue (2.4% aniline blue, 2% acetic acid ) solutions (all from Sigma-Aldrich). Each staining step was performed for 5 minutes and followed by washes in running tap water and/or distilled water. After brief rinsing in 1% acetic acid (3 minutes), sections were dehydrated to xylene, mounted using Permount (Thermo Fisher Scientific) and visualized using a Leica microscope (Leica Microsystems Inc., Concord, ON).

Statistical analysis Results were analyzed using one-way ANOVA (between multiple groups) or t-test (between two groups) (Systat SigmaPlot 11 software). All data are reported as means +/- SEM.

Supplemental References

Supplemental Figure S1 Systolic blood pressure of WT and Mmp2^{-/-} treated with minipumps containing PBS (Control) or Ang II for 2 weeks (1.4 mg/kg/d). Results are means ± sem. ‡: \( P \leq 0.05 \) vs. no Ang II.
Supplemental Figure S2 Body weight and tibia length data related to Figure 1. Mice were subjected to Ang II (1.4 mg/kg/d) or PBS infusion for 1, 2 or 4 weeks. Results are means ± sem.
Supplemental Figure S3 qRT-PCR analysis of cardiomyocytes from WT and Mmp2<sup>-/-</sup> mice. Results are means ± sem. n=3 replicate cultures per genotype. *: P≤0.05 vs. WT. ND = not detected.