A disintegrin and metalloprotease-17 regulates pressure overload–induced myocardial hypertrophy and dysfunction through proteolytic processing of integrin β1


Abstract—A disintegrin and metalloprotease-17 (ADAM17) belongs to a family of transmembrane enzymes, and it can mediate ectodomain shedding of several membrane-bound molecules. ADAM17 levels are elevated in patients with hypertrophic and dilated cardiomyopathy; however, its direct role in hypertrophic cardiomyopathy is unknown. Cardiomyocyte-specific ADAM17 knockdown mice (ADAM17fl/fl/αMHC-Cre; ADAM17f/f/Cre) and littermates with intact ADAM17 levels (ADAM17+/+) were subjected to cardiac pressure–overload by transverse aortic constriction. Cardiac function/architecture was assessed by echocardiography at 2 and 5 weeks post transverse aortic constriction. ADAM17 knockdown enhanced myocardial hypertrophy, fibrosis, more severe left ventricular dilation, and systolic dysfunction at 5 weeks post transverse aortic constriction. Pressure overload–induced upregulation of integrin β1 was much greater with ADAM17 knockdown, concomitant with the greater activation of the focal adhesion kinase pathway, suggesting that integrin β1 could be a substrate for ADAM17. ADAM17 knockdown did not alter other cardiomyocyte integrins, integrin α5 or α7, and HB-EGF (heparin-bound epidermal growth factor), another potential substrate for ADAM17, remained unaltered after pressure overload. ADAM17–mediated cleavage of integrin β1 was confirmed by an in vitro assay. Intriguingly, ADAM17 knockdown did not affect the myocardial hypertrophy induced by a subpressor dose of angiotensin II, which occurs independent from the integrin β1–mediated pathway. ADAM17 knockdown enhanced the hypertrophic response to cyclic mechanical stretching in neonatal rat cardiomyocytes. This study reports a novel cardioprotective function for ADAM17 in pressure overload cardiomyopathy, where loss of ADAM17 promotes hypertrophy by reducing the cleavage of cardiac integrin β1, a novel substrate for ADAM17. This function of ADAM17 is selective for pressure overload–induced myocardial hypertrophy and dysfunction, and not agonist–induced hypertrophy. (Hypertension. 2016;68:937-948. DOI: 10.1161/HYPERTENSIONAHA.116.07566.) • Online Data Supplement

Key Words: angiotensin II ■ dilated cardiomyopathy ■ disintegrin ■ hypertrophy ■ upregulation

Myocardial remodeling is a critical process in cardiac response to stress or injury, and it is a major driver of hypertensive heart disease.1,2 Remodeling encompasses changes in shape and architecture of the cardiomyocytes, the extracellular matrix (ECM), and the cell–ECM interactions.3 A disintegrin and metalloproteinases (ADAMs) are membrane-bound enzymes that mediate proteolytic processing of several membrane- and ECM-bound growth factors, and cytokines, and possess a disintegrin domain that can potentially allow membrane- and ECM-bound growth factors, and cytokines, to mediate cardiac hypertrophy through the activation of several membrane-bound molecules. ADAM17 levels are elevated in patients with hypertrophic and dilated cardiomyopathy; however, its direct role in hypertrophic cardiomyopathy is unknown. Cardiomyocyte-specific ADAM17 knockdown mice (ADAM17fl/fl/αMHC-Cre; ADAM17f/f/Cre) and littermates with intact ADAM17 levels (ADAM17+/+) were subjected to cardiac pressure–overload by transverse aortic constriction. Cardiac function/architecture was assessed by echocardiography at 2 and 5 weeks post transverse aortic constriction. ADAM17 knockdown enhanced myocardial hypertrophy, fibrosis, more severe left ventricular dilation, and systolic dysfunction at 5 weeks post transverse aortic constriction. Pressure overload–induced upregulation of integrin β1 was much greater with ADAM17 knockdown, concomitant with the greater activation of the focal adhesion kinase pathway, suggesting that integrin β1 could be a substrate for ADAM17. ADAM17 knockdown did not alter other cardiomyocyte integrins, integrin α5 or α7, and HB-EGF (heparin-bound epidermal growth factor), another potential substrate for ADAM17, remained unaltered after pressure overload. ADAM17–mediated cleavage of integrin β1 was confirmed by an in vitro assay. Intriguingly, ADAM17 knockdown did not affect the myocardial hypertrophy induced by a subpressor dose of angiotensin II, which occurs independent from the integrin β1–mediated pathway. ADAM17 knockdown enhanced the hypertrophic response to cyclic mechanical stretching in neonatal rat cardiomyocytes. This study reports a novel cardioprotective function for ADAM17 in pressure overload cardiomyopathy, where loss of ADAM17 promotes hypertrophy by reducing the cleavage of cardiac integrin β1, a novel substrate for ADAM17. This function of ADAM17 is selective for pressure overload–induced myocardial hypertrophy and dysfunction, and not agonist–induced hypertrophy.
mice\textsuperscript{13} and subjected them to 2 models of myocardial hypertrophy, pressure overload (biomechanical stress), and agonist-induced cardiomyopathy (pressor dose of angiotensin II [Ang II]). Our findings reveal a novel role for ADAM17 in proteolytically cleaving integrin $\beta_1$, and thereby regulating cardiac hypertrophy and function in response to mechanical stress, but not in response to agonist-induced hypertrophy.

**Methods**

**Experimental Animals and Protocols**

ADAM17\textsuperscript{fl/fl} (f/f) and $\alpha$-myosin heavy chain (mMHC)-Cre (Cre) mice were purchased from Jackson Laboratories. Cardiomyocyte-specific ADAM17 knockout mice (ADAM17\textsuperscript{f/f}/Cre; f/f/Cre) were generated as described.\textsuperscript{14} Male f/f, f/f/Cre, and Cre mice, at 8 to 9 weeks of age, underwent transverse aortic constriction (TAC) to generate pressure overload as previously described.\textsuperscript{14,15} Briefly, anesthetized mice underwent thoracotomy, a constriction was made on the aortic arch between the left carotid artery and the brachiocephalic trunk, to the diameter of a 27-gauge needle. The incision was closed in layers and the mouse was allowed to recover on a warming pad. Sham animals underwent the same procedure without the constriction of aorta. At 2 or 5 weeks post-TAC, hearts were excised, flash-frozen in OCT medium, or were fixed in 10% formalin and processed for immunohistochemical analyses, or flash-frozen for molecular analyses.

A subpressor dose of Ang II (0.2 mg/kg per day)\textsuperscript{16} or saline (control) was delivered by Alzet micro-osmotic pumps (Model 1002, Alza Corp) and the Canadian Council of Animal Care.

Histological and Immunohistochemical Staining and Imaging

Freshly excised hearts were arrested in diastole, fixed in 10% formalin, paraffin-embedded and processed for trichrome, picrosirius red, and wheat germ agglutinin staining as before.\textsuperscript{14,15} Myocyte cross-sectional area was determined from the wheat germ agglutinin–stained sections by using Metamorph Basic software (version 7.7.0.0) as before.\textsuperscript{15,19} Fibrosis was determined from picrosirius red–stained sections by using Metamorph Basic software as before.\textsuperscript{20} Fluorescent staining for integrin $\beta_1$ and CD31 was performed on OCT-frozen tissue as described.\textsuperscript{13,14,21,22} The number of coronary microvessels in each field was counted from the CD31-stained sections by using Metamorph Basic software.\textsuperscript{13,19}

mRNA Expression and Western Blot

Total RNA was extracted using TRIZol Reagent (Life Technologies) and mRNA expression levels for mouse ADAM9, ADAM10, ADAM12, ADAM17, collagens I and III, brain natriuretic peptide, $\alpha$-skeletal muscle actin, and $\alpha$MHC were detected by TaqMan real-time PCR (polymerase chain reaction) as before.\textsuperscript{13,14,19,21,29} Primer/probe for rat ADAM17 (Rn00571880_m1), brain natriuretic peptide (Rn00580641_m1), ANF (atrial natriuretic factor; Rn00664637_g1), $\beta$-myosin heavy chain (Rn01536269_m1), and the internal control 18S (protocol#: 4308329) were purchased from Invitrogen. Hypoxanthine–guanine phosphoribosyltransferase-1 was used as an internal control. Total protein was extracted using CellLytic Cell Lysis Reagent (Sigma–Aldrich) for Western blot analyses to detect ADAM17 (Santa Cruz Biotechnology), integrin $\beta_1$ (Novus Biologicals), integrin $\alpha_5$ (Abcam), integrin $\alpha_7$ (Novus), and heparin–bound epidermal growth factor (HB-EGF; Abcam) as previously described.\textsuperscript{14,21} Memcode reversible protein stain was used as the loading control for all Western blots. Quantification of band intensity in Western blots was performed after the normalization of each band intensity by its corresponding loading control.

In vitro Integrin Cleavage Assay

The ectodomain of recombinant integrin $\alpha_5\beta_1$ heterodimer (6 g; R&D) was incubated with increasing concentrations of recombinant ADAM17 (R&D Systems) at a substrate:enzyme ratio of 60:1, 6:1, and 3:1 for 24 hours at 37°C in activity assay buffer (for ADAM17) according to the manufacturer’s instructions.\textsuperscript{15} Subsequently, the incubation products were separated on an SDS-PAGE gel. The gel was stained with 0.25% Coomassie Brilliant Blue R-250 (Bio-Rad) and destained until the background was clear. The gel was imaged using ImageQuant LAS 4000 (GE Healthcare).

**Statistical Analysis**

All analyses were performed using IBM SPSS Statistics 19 software. Averaged values represent mean±SEM. The tests for normality and homogeneity of variance were done for all data. Two-way ANOVA was used to compare the groups between different genotypes. Multiple comparisons within the same genotype involved one-way ANOVA followed by Bonferroni test. Statistical significance was recognized at $P<0.05$.

**Results**

Exacerbated Myocardial Hypertrophy and Fibrosis in Cardiomyocyte ADAM17 Knockdown Mice After Pressure Overload

Cardiac pressure overload triggers myocardial hypertrophy and fibrosis. We assessed whether reduced cardiomyocyte ADAM17 levels could alter these responses. ADAM17\textsuperscript{fl/fl}-Cre mice exhibited greater hypertrophy at 2 weeks and 5 weeks post-TAC because evident from the transverse cross-sectional macroimages of the heart (Figure 1A), the higher heart weight/tibial length ratio (Figure 1B), expression of hypertrophy markers, brain natriuretic peptide and $\alpha$-skeletal actin (Figure 1C), and myocyte cross-sectional area measured in wheat germ agglutinin–stained hearts (Figure 1Di and 1Dii), in ADAM17\textsuperscript{fl/fl}-Cre compared with ADAM17\textsuperscript{f/f} mice post-TAC. To ensure that the enhanced hypertrophy response is because...
of reduced ADAM17 levels and not related to the expression of αMHC-Cre, we also assessed hypertrophy in αMHC-Cre mice post-sham/TAC and found that the hypertrophy in αMHC-Cre mice was comparable to the control ADAM17f/f mice post-TAC (Figure 1Ai and 1B).

ADAM17f/f/Cre hearts exhibited 50% and 40% of reduction in ADAM17 mRNA and protein levels, respectively (Figure S1Ai and S1Aii in the online-only Data Supplement). After pressure overload (TAC), ADAM17 protein increased in ADAM17f/f mice but remained significantly lower in ADAM17f/f/Cre mice (Figure S1Aii). Assessment of other ADAMs expressed in the heart showed similar baseline levels in the 2 genotypes; after pressure overload, ADAM9 increased similarly in both genotypes (Figure S1Bii). ADAM10 remained comparable and unaltered (Figure S1Bii), whereas ADAM12 increased to a greater degree in ADAM17f/f/Cre hearts (Figure S1Bii).

Figure 1. Enhanced myocardial hypertrophy in a disintegrin and metalloprotease-17 (ADAM17) knockdown mice after pressure overload. A, Macroscopic images of heart cross section from ADAM17flox/flox and ADAM17flox/flox/Cre mice after sham, and 2 and 5 weeks of transverse aortic constriction (TAC; i). Heart cross-section images of α-myosin heavy chain (αMHC)-Cre mice after sham or 5 weeks post-TAC (ii). B, Heart weight/tibial length (HW/TL) ratio for indicated genotypes after sham, 2 weeks or 5 weeks of TAC. n=10 to 20/group per genotype (n=4/Cre). C, mRNA levels of markers of heart disease, brain natriuretic peptide (BNP), and α-skeletal muscle actin (αSKA) at 5-week postsham or TAC. n=6 to 9/group per genotype. D, Representative wheat germ agglutinin (WGA)–stained images (i) and averaged cardiomyocyte cross-sectional area in the indicated groups. n=200 to 220 cells/group; 3 hearts/group per genotype. f/f: ADAM17flox/flox, Cre: αMHC-Cre, f/f/Cre: ADAM17flox/flox/αMHC-Cre. Averaged data represent mean±SEM. HPRT indicates hypoxanthine–guanine phosphoribosyltransferase. *P<0.05 compared with corresponding sham group, #P<0.05 compared with corresponding f/f group.
LV Dilation and Dysfunction Worsened in ADAM17\textsuperscript{fl/fl}/Cre Mice Post-TAC

Cardiac function and structure measurements by echocardiography revealed more LV dilation and dysfunction in ADAM17\textsuperscript{fl/fl}/Cre mice post-TAC (Figure 3Ai and 3Aii; Table S1). ADAM17\textsuperscript{fl/fl} developed compensatory hypertrophy with preserved ejection fraction at 2 weeks post-TAC, but reduced ejection fraction was detected in ADAM17\textsuperscript{fl/fl}/Cre mice at 2 weeks, which exacerbated by 5 weeks post-TAC (Figure 3Aiii). ADAM17\textsuperscript{fl/fl}/Cre mice also exhibited more severe LV dilation at 5 weeks post-TAC (Figure 3Aiv), whereas LV wall thickness increased similarly in both genotypes (Figure 3Av). Consistent with the more severe LV dysfunction, left atrium/body weight ratio was significantly elevated in ADAM17\textsuperscript{fl/fl}/Cre mice post-TAC compared with ADAM17\textsuperscript{fl/fl}-TAC and the sham groups (Figure 3Avi). Heart rates were comparable between ADAM17\textsuperscript{fl/fl} and ADAM17\textsuperscript{fl/fl}/Cre mice in the sham groups (440±11 bpm versus 433±23 bpm), and 5 weeks post-TAC (448±10 bpm versus 439±11 bpm), and 5 weeks post-TAC (448±10 bpm versus 423±10 bpm). The extent of diastolic dysfunction was comparable in ADAM17\textsuperscript{fl/fl} and ADAM17\textsuperscript{fl/fl}/Cre mice post-TAC (Table S1).

Cardiomyocyte ADAM17 Knockdown Is Associated With Reduced Coronary Density Post-TAC

Transition from compensated hypertrophy to decompensated hypertrophy and LV dilation is associated with impaired angiogenesis in the hypertrophied heart.\textsuperscript{30,31} We previously reported that cardiomyocyte ADAM17 knockdown resulted in reduced angiogenesis in ischemic injury.\textsuperscript{13} Therefore, we investigated whether the more severe LV dilation in ADAM17 knockdown mice post-TAC could be because reduced vascular density. Staining for CD31, an endothelial cell marker (Figure 3Bi), and quantification of coronary density (Figure 3Bii) showed comparable coronary density after sham or 2 weeks of TAC between the 2 genotypes. However, by 5 weeks post-TAC, coronary density was significantly lower in ADAM17\textsuperscript{fl/fl}/Cre-TAC compared with ADAM17\textsuperscript{fl/fl}-TAC hearts (Figure 3Bi and 3Biii).

ADAM17\textsuperscript{fl/fl}/Cre-TAC Hearts Exhibit Higher Integrin β1 Levels, but No Difference in Other Integrins or HB-EGF Compared With ADAM17\textsuperscript{fl/fl}-TAC Mice

The integrin complex that mediates cell–ECM interaction is a key contributor to biomechanical stress-mediated myocardial hypertrophy.\textsuperscript{32} Immunohistochemical staining and quantification (Figure 4Ai and 4Aii) and Western blotting (Figure 4Bi and 4Bii) showed increased integrin β1 levels after pressure overload in both genotypes. However, a significantly higher level of integrin β1 was detected in hearts with reduced ADAM17 (ADAM17\textsuperscript{fl/fl}/Cre) compared with ADAM17\textsuperscript{fl/fl} hearts post-TAC (Figure 4Bi and 4Bii). Consistent with the higher integrin β1 levels, the phosphorylation/activation of focal adhesion kinase, a downstream kinase of integrin β1, was also elevated in ADAM17\textsuperscript{fl/fl}/Cre-TAC hearts (Figure 4Ci through 4Civ). The rise in integrin β1 and focal adhesion kinase activation was suppressed by 5 weeks post-TAC in both genotypes (data not shown). We tested the hypothesis that ADAM17 cleaves integrin β1, and therefore integrin β1 is elevated in ADAM17 knockdown hearts.

To determine the ability of ADAM17 to directly cleave integrin β1, we first used the BLAST protein database search program\textsuperscript{13} to detect all the identified cleavage sequences by ADAM17\textsuperscript{fl/fl} within mouse integrin β1 protein and found 2 possible sites in the ectodomain region of integrin β1 sequence (Figure 4D). We then used an in vitro assay where the ectodomain of integrin α5β1 dimer (cardiomyocyte isoform) was incubated with increasing concentrations of recombinant ADAM17. We used integrin α5β1 dimer rather than integrin β1 monomer to ensure that its interaction with ADAM17 in this in vitro experiment simulates the physiological condition, where integrins are present as heterodimers. After 24 hours of incubation, bands at 100, 30, and <25 kDa appeared with increasing concentrations of rADAM17, representing ADAM17-mediated degradation products (Figure 4E). Intriguingly, other potential substrates of ADAM17 such as integrin α5, integrin α7, and HB-EGF were not differentially altered in ADAM17 knockdown hearts after sham or TAC (Figure S2).

Agonist-Induced Hypertrophy Is Not Altered by ADAM17 Downregulation

Next, we investigated whether the contribution of ADAM17 to myocardial hypertrophy is specific to cardiac pressure overload or whether it also applies to other models of hypertrophy. We chose the Ang II–mediated hypertrophy model, and to differentiate between the direct hypertrophic effects of Ang II versus the hypertrophy secondary to the Ang II–associated hypertension, we used a subpressor dose of Ang II (0.2 mg/kg per day), which has been shown not to elevate the blood pressure.\textsuperscript{16} Two weeks of Ang II infusion at this subpressor dose resulted in similar cardiac hypertrophy in ADAM17\textsuperscript{fl/fl} and ADAM17\textsuperscript{fl/fl}/Cre mice (Figure 5A). Unlike cardiac pressure overload, Ang II did not trigger a rise in integrin β1 levels (Figure 5B), but elevated HB-EGF levels equally in both genotypes (Figure 5C).

ADAM17 Knockdown In Vitro Augmented Stretch-Induced Hypertrophy

We next investigated the role of ADAM17 in stretch-induced hypertrophy using neonatal rat ventricular myocytes cultured under static conditions or subjected to cyclic stretching. This simulates the mechanical stress that the pressure-overloaded heart is subjected to in vivo. Although cyclic stretching resulted in a small but significant hypertrophy in the control (scrambled siRNA) cells, the hypertrophy was markedly greater with ADAM17 downregulation (siRNA #1 or #2;
Figure 2. Excess myocardial fibrosis in a disintegrin and metalloprotease-17 (ADAM17)$^{flox/flox}$/Cre mice at 5-week post transverse aortic constriction (TAC). Representative trichrome (A), picrosirius red (PSR) staining (Bii), and averaged quantification of interstitial (Bii) and perivascular fibrosis (Biii); n=15 to 59 fields/heart, 3 hearts/group per genotype. C, mRNA levels for procollagen type Ia1 chain (i) and procollagen type III a1 chain (n=5–7/group per genotype), t/f: ADAM17$^{flox/flox}$, t/f/Cre: ADAM17$^{flox/flox}$/α-myosin heavy chain-Cre. Averaged data represent mean±SEM. HPRT indicates hypoxanthine–guanine phosphoribosyltransferase. *$P<0.05$ vs parallel sham group, #$P<0.05$ vs parallel t/f group.
Figure 3. Exacerbated left ventricular (LV) dilation and dysfunction and reduced coronary density in a disintegrin and metalloprotease-17 (ADAM17)$^{flox/flox}$/Cre mice post transverse aortic constriction (TAC). A, Representative M-mode images from f/f (i) and f/f/Cre (ii) mice at 5-week postsham/TAC. Averaged ejection fraction (iii), LV internal diameter during diastole (LVIDd, iv), LV posterior wall thickness (LVPWd, v), and left atrial size normalized to body weight (vi); n=10/sham per genotype; n=10 to 17/TAC per genotype per time point. B, (i) Representative images stained for CD31 in sham, and after 2 or 5 weeks of TAC. Scale bar, 50 µm (inset, 20 µm). ii, Averaged vascular density (per field) for the indicated groups; n=12 fields/sham per genotype; n=25 to 35/TAC per genotype. Graphs represent mean±SEM. *P<0.05 compared with corresponding sham group, #P<0.05 compared with corresponding f/f group. f/f: ADAM17$^{flox/flox}$, f/f/Cre: ADAM17$^{flox/flox}$/α-myosin heavy chain (αMHC)-Cre. LVEDD indicates left ventricular end-diastolic diameter; and LVESD, left ventricular end-systolic diameter.
Figure 4. Reduced cardiomyocyte a disintegrin and metalloprotease-17 (ADAM17) is associated with higher levels of integrin β1 and focal adhesion kinase (FAK) phosphorylation at 2 weeks of post transverse aortic constriction (TAC). A, Representative integrin β1 staining (i) and averaged quantification (ii) in ADAM17f/f and ADAM17f/f/Cre after sham and 2 weeks of TAC. B, Representative Western blot (i) and averaged protein quantification (ii) for integrin β1 in the indicated groups (n=3–4/group). C, Representative Western blot (i) and averaged protein quantification for phospho-FAK (ii), total FAK (iii) and pFAK-to-total FAK ratio (iv) in the indicated groups (n=3–4/group). D, Predicted ADAM17-mediated cleavage sites in integrin β1. E, In vitro cleavage of integrin β1 by rADAM17. "Degradation products of integrin β1 (because integrin α5 is not cleaved by ADAM17)." Averaged data represent mean±SEM. Memcode membrane staining was used as loading control, and band intensities were normalized to the corresponding lane in loading control. *P<0.05 compared with corresponding sham. #P<0.05 compared with corresponding f/f group. sh, sham; t/f, ADAM17flox/flox; t/f/Cre, ADAM17flox/flox/α-myosin heavy chain (αMHC)-Cre.
Figure 6Ai and 6Aii). The similar effects of the 2 ADAM17 siRNA confirm the lack of off-target effects. Cyclic stretching increased ADAM17 mRNA, which was significantly reduced by ADAM17-siRNA treatment (Figure 6B). In addition, the stretch-induced rise in the expression of hypertrophy markers, βMHC, atrial natriuretic peptide, and brain natriuretic peptide were significantly greater with ADAM17 knockdown (Figure 6Ci through 6Ciii). ADAM17 knockdown in static culture conditions did not trigger a significant hypertrophy (Figure 6).

**Discussion**

Hypertension is by far the most common antecedent condition in patients with heart failure and drives adverse myocardial remodeling and pathological hypertrophy.2,35 In this study, we report a unique function for ADAM17 in regulating myocardial hypertrophy in response to mechanical stress by proteolytic cleavage of integrin β1, but not integrin α5, integrin α7, or HB-EGF. Integrin β1 levels post-TAC were greater in ADAM17f/f/Cre hearts concomitant with the increased activation of focal adhesion kinase, a key signaling molecule in pressure overload–induced hypertrophy downstream of integrin β1.36 Although the integrin β1–focal adhesion kinase signaling is essential for optimal cardiac function32 and the compensatory response to injuries,37–39 uncontrolled activity of this pathway can lead to excess hypertrophy and accelerated disease progression. The increased ADAM17 levels in patients with dilated cardiomyopathy10 could trigger pathological pathways, such as activation of inflammatory cytokines. Our findings suggest that this rise in ADAM17 may indeed serve as a regulatory mechanism to keep the hypertrophic response under control because reduced ADAM17 levels (in ADAM17f/f/Cre mice) resulted in worsening of myocardial hypertrophy, LV dilation, and dysfunction. Consistent with these in vivo observations, ADAM17 knockdown enhanced hypertrophy triggered by cyclic stretching in neonatal rat ventricular myocyte. Intriguingly, this function of ADAM17 does not apply to agonist-induced hypertrophy, which occurs through an
Figure 6. A disintegrin and metalloprotease-17 (ADAM17) knockdown in neonatal rat ventricular myocytes (NRVMs) increased the stretch-mediated hypertrophy. A, (i) Representative images of NRVM cultured under static or cyclic-stretching conditions, treated with ADAM17-siRNA#1 or ADAM17-siRNA#2, or scrambled siRNA (control). (ii) Averaged cell surface area for the indicated groups (n=110 cells/group; 2 independent isolations, 40 rat pups (4 separate litters). B, ADAM17 mRNA levels in control (scrambled siRNA) and ADAM17-siRNA (#1 and #2)-treated NRVM. C, mRNA expression for β-myosin heavy chain (βMHC, i), atrial natriuretic peptide (ANP, ii), and brain natriuretic peptide (BNP, iii; n=6 plates/group). *P<0.05 compared with the corresponding scrambled siRNA group. #P<0.05 compared with the corresponding static group.
integrin β1–independent mechanism. It has been reported that prolonged αMHC-Cre expression can lead to cardiotoxicity and hypertrophy with age. However, in this study, in a parallel αMHC-Cre group that underwent aortic constriction at 8 weeks of age, the resulting cardiac hypertrophy was similar to that in the control ADAM17 Δ/Δ mice. Therefore, the enhanced hypertrophy observed in ADAM17 Δ/Δ/Cre mice post-TAC is because of the reduced cardiomyocyte ADAM17 levels and not because of the expression of Cre recombinase.

The excess hypertrophy and fibrosis in ADAM17 Δ/Δ/Cre-TAC mice were associated with early transition from compensatory hypertrophy to decompensated dilated cardiomyopathy. Compromised angiogenesis has been shown to underlie transition from compensatory hypertrophy to decompensation, LV dilation, and dysfunction. ADAM17 Δ/Δ/Cre hearts exhibit suppressed angiogenesis in response to ischemic injury because of the reduced expression and activation of VEGFR2 (vascular epidermal growth factor receptor-2). Similarly, after pressure overload, coronary density in ADAM17 Δ/Δ/Cre mice was significantly reduced compared with parallel ADAM17 Δ/Δ mice, and this suppressed angiogenesis could contribute to the accelerated transition into decompensation (LV dilation and dysfunction) in these mice.

The increased fibrosis in ADAM17 Δ/Δ/Cre-TAC mice could result from the higher integrin β1 levels in these hearts. Cardiomyocyte integrin complex can trigger fibrosis by transmitting cell traction forces to the latent transforming growth factor-β1 (TGFβ1) complex. TGFβ is secreted as a large latency complex consisting of the TGFβ molecule noncovalently bound to latency–associated peptide and bound to latent TGFβ1–binding protein 1 via a disulphide bond. Latency–associated peptide contains the amino acid sequence motif RGD (Arg–Gly–Asp), which serves as a recognition site for several integrins including integrinβ1. As such, cardiomyocyte integrins can bind to the RGD motif in latency–associated peptide, which cause conformational changes, release active TGFβ, trigger collagen expression (by fibroblasts) and subsequently myocardial fibrosis. This is consistent with our observation that the increased fibrosis in ADAM17 Δ/Δ/Cre-TAC hearts was concurrent with the elevated mRNA expression of collagens type I and III, the predomi-
nant fibrillar collagens in the heart.

We report a novel role for cardiomyocyte ADAM17 in pro-
teolytic processing of integrin β1 in response to biomechanical stress, and therefore serving as a key regulator of myocardial hypertrophy ensuing pressure overload. Although ADAM12 levels were elevated in ADAM17 Δ/Δ/Cre-TAC mice, this likely did not contribute to the hypertrophy response because ADAM12-mediated hypertrophy is through the shedding of HB-EGF and activation of EGFβ, and we found comparable HB-EGF levels in both genotypes post-TAC. Moreover, ADAM12 likely does not participate in shedding of integrin β1 because integrin β1 levels were elevated in ADAM17 Δ/Δ/Cre-TAC hearts, despite the rise in ADAM12. An interaction between ADAM17 and integrin β1 in the heart had been speculated, but this interaction remained unexplored perhaps because of the lethality of ADAM17–deficient mice. A non-proteolytic interaction between ADAM17 and integrins has been reported in HeLa cells (in vitro), whereas integrin-mediated ADAM17 inactivation has also been reported in kidney mesangial cells. This study is the first to provide evidence on proteolytic processing of integrin β1 by ADAM17 after pressure overload, thereby suppressing myocardial hypertrophy and fibrosis. However, additional studies will be required to demonstrate a direct interaction between ADAM17 and integrin β1 in vivo, and subsequent degradation of integrin β1.

In summary, the rise in ADAM17 in hypertrophic and dilated cardiomyopathy hearts in patients and in mice could be a compensatory mechanism to prevent excess hypertrophy after biomechanical stress. The findings from this study identify integrin β1 as a novel substrate for ADAM17, and reveal a beneficial function for ADAM17 in biomechanical stress-induced cardiomyopathy. However, as ADAM17 is a multifunctional enzyme with several downstream substrates, the contribution of other signaling pathways, in addition to integrin β1, to the hypertrophy response after biomechanical stress cannot be ruled out.

**Perspectives**

Integrins mediate cell–ECM connections and are central to transmitting the extracellular stimuli (such as mechanical stress) to the intracellular milieu. After pressure overload, levels of integrin β1 are elevated to strengthen the connection between the cardiomyocytes and the ECM, and to trigger compensatory hypertrophy. Although this initial rise in integrin β1 and the resulting compensatory hypertrophy are essential for optimal cardiac response to pressure overload, a regulatory mechanism is required to prevent uncontrolled and excess hypertrophy. Our findings indicate that ADAM17 plays this role. The rise in ADAM17 levels after pressure overload mediates ectodomain shedding of the upregulated integrin β1, thereby disrupting the integrin–ECM connection and limiting the hypertrophic signaling. This function of ADAM17 is of particular importance after sustained pressure overload, but not when hypertrophy is triggered through an integrin-independent pathway, nor under control conditions. Although reduced ADAM17 levels exacerbate myocardial hypertrophy, overexpression of ADAM17 in mechanically overloaded hearts may not be an ideal therapeutic approach given the multifunctionality of ADAM17 and its numerous target substrates. Hence, additional studies are required to determine how exactly ADAM17 needs to be manipulated as a therapeutic option for patients with heart failure.

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**Disclosures**

None.
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Novelty and Significance

What Is New?
- This study provides evidence for a novel substrate for a disintegrin and metalloprotease-17 (ADAM17) in the heart, integrin β1.
- ADAM17 can cleave integrin β1 that is upregulated in response to cardiac pressure overload, thereby it can modify the resulting myocardial hypertrophy.
- This function of ADAM17 is specific to cardiac hypertrophy induced by pressure overload, but not by a hypertrophic agonist.

What Is Relevant?
- Findings in this study demonstrate that (1) elevated ADAM17 after cardiac pressure overload plays a partially protective role in keeping cardiac hypertrophy under control; (2) the differential function of ADAM17 in the 2 different models of hypertrophy further supports the importance of personalized therapy for patients with heart disease.

Summary

ADAM17 can proteolytically process/release several membrane- and extracellular matrix-bound molecules. This study reports that ADAM17 can mediate proteolytic cleavage of integrin β1 in heart disease secondary to cardiac pressure overload, thereby it can play a protective role in regulating the severity of myocardial hypertrophy.
A Disintegrin and Metalloprotease-17 Regulates Pressure Overload–Induced Myocardial Hypertrophy and Dysfunction Through Proteolytic Processing of Integrin β1


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ADAM17 regulates pressure overload-induced myocardial hypertrophy and
dysfunction through proteolytic processing of integrin beta1

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### Table S1- Echocardiographic assessment of systolic and diastolic function in TACE^flx/flx^ and TACE^flx/flx/^α-MHC-cre mice after 2 or 5 weeks of TAC, and corresponding sham groups. n=number of mice per group; HR=Heart rate; LVEF=Left ventricular ejection fraction; LVIDd=Left ventricular internal diameter at the end of diastole; LVIDs=Left ventricular internal diameter at the end of Systole; LA=Left atrial diameter; IVRT=Isovolumic relaxation time of LV; E-wave= early transmitral inflow velocity; A-wave=transmitral inflow velocity due to atrial contraction; E’=Early tissue Doppler velocity; A’=Tissue Doppler velocity due to atrial contraction; E’/A’=Ratio of early Doppler velocity to the tissue Doppler velocity due to atrial contraction. *p<0.05 vs. corresponding sham; †p<0.05 vs. corresponding WT group.
Figure S1. Alterations in ADAM17 and other cardiac ADAMs following pressure overload.

A) ADAM17 mRNA (i), representative Western blot and averaged protein levels (ii) in ADAM17(f/f) and ADAM17(f/f)/Cre hearts following sham or 5 week TAC.

B) mRNA levels for ADAM9, ADAM10 and ADAM12 in ADAM17(f/f) and ADAM17(f/f)/Cre hearts post-sham or -TAC. n=7-12/group/genotype. HPRT was used as internal control.

f/f: ADAM17flox/flox; f/f/Cre: ADAM17(flox/flox)/αMHC-Cre; TAC: transverse aortic constriction; R.E.: relative expression.

Bar graphs represent mean±SEM; *p<0.05 compared to corresponding sham, #p<0.05 compared to corresponding f/f group.
**Figure S2.** Protein levels of integrin α5, α7, and HBEGF were comparable between ADAM17(f/f) and ADAM17(f/f)/Cre at 5 weeks post-sham or TAC. 

f/f: ADAM17floxflox, f/f/Cre: ADAM17floxflox/αMHC-Cre. Averaged data represent mean±SEM. *p<0.05 compared to corresponding sham group, #p<0.05 compared to corresponding f/f group