Loss of Endogenous HMGB2 Promotes Cardiac Dysfunction and Pressure Overload-Induced Heart Failure in Mice

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Background: The rapid increase in the number of heart failure (HF) patients in parallel with the increase in the number of older people is receiving attention worldwide. HF not only increases mortality but decreases quality of life, creating medical and social problems. Thus, it is necessary to define molecular mechanisms underlying HF development and progression. HMGB2 is a member of the high-mobility group superfamily characterized as nuclear proteins that bind DNA to stabilize nucleosomes and promote transcription. A recent in vitro study revealed that HMGB2 loss in cardiomyocytes causes hypertrophy and increases HF-associated gene expression. However, it's in vivo function in the heart has not been assessed.

Methods and Results: Western blotting analysis revealed increased HMGB2 expression in heart tissues undergoing pressure overload by transverse aorta constriction (TAC) in mice. Hmgb2 homozygous knockout (Hmgb2−/−) mice showed cardiac dysfunction due to AKT inactivation and decreased sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA)2a activity. Compared to wild-type mice, Hmgb2−/− mice had worsened cardiac dysfunction after TAC surgery, predisposing mice to HF development and progression.

Conclusions: This study demonstrates that upregulation of cardiac HMGB2 is an adaptive response to cardiac stress, and that loss of this response could accelerate cardiac dysfunction, suggesting that HMGB2 plays a cardioprotective role.

Key Words: Aging; Heart failure; HMGB2; Transverse aorta constriction (TAC) model
Here, we assessed HMGB2 effects on cardiac pathophysiology following loss of endogenous HMGB2 in mice. First, we found that in wild-type mice, HMGB2 expression increased in stressed heart tissues following pressure overload induced by transverse aorta constriction subsequent HF development in mice. Moreover, in vitro analysis indicates that HMGB2 loss causes cardiomyocyte hypertrophy and increased expression of HF-associated genes. Thus far, there are no reports regarding the in vivo role of HMGB2 in the heart.

Figure 1. HMGB2 is upregulated in a stressed heart following transverse aorta constriction (TAC) surgery. (A) Hmgb2 mRNA expression in indicated tissues and organs (n=4 per group). (B) Comparison Myh6 (Left) and Hmgb2 (Right) mRNA levels in cardiomyocytes and non-cardiomyocytes (n=6 per group). (C,D) Changes in fractional shortening (%FS) and heart weight/body weight (HW/BW) (mg/g) in Sham-operated or TAC groups after surgery in wild-type (WT) mice (n=8–10 per group). (E) Representative western blots of HMGB2 protein in the heart before TAC surgery (Pre) and at 2, 4, and 8 weeks (W) after TAC in WT mice (Left) and quantitation of HMGB2 protein levels (Right) (n=6 per each group). Hsc70 serves as a loading control. Protein intensity values in the Pre-control were set to 1. (F) Representative western blot (Upper) and quantitation (Lower) of HMGB2 protein levels in nuclear extracts made from whole heart tissue of WT mice, 4 weeks after TAC. Histone H3 serves as a loading control. Values in Sham were set to 1. (G) Serum HMGB2 concentration before TAC surgery (Pre) and at 2, 4, and 8 weeks (W) after TAC in WT mice (n=6 per group). Data are presented as means±SEM. Statistical significance was determined by an unpaired Student’s t-test (B) or one-way ANOVA (C–G). *P<0.05, **P<0.01, †P<0.001 between groups. n.s., not significant.
(TAC). Second, Hmgb2 homozygous knockout (Hmgb2−/−) mice showed cardiac dysfunction caused by AKT inactivation and decreased sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA)2a activity. Third, in the TAC-induced HF model, Hmgb2−/− mice exhibited exacerbated cardiac dysfunction and accelerated HF development and progression. To the best of our knowledge, this is the first report showing that loss of endogenous HMGB2 promotes cardiac dysfunction, and that increases in cardiac HMGB2 protein in heart tissue stressed by pressure overload might be cardioprotective.

Methods

Animal Studies

All experimental procedures were approved by the Kumamoto University Ethics Review Committee for Animal Experimentation. All animals were fed a normal diet (CE-2, CLEA, Tokyo, Japan), bred in a mouse house with automatically controlled lighting (12 h on, 12 h off), and maintained at a stable temperature of 23°C. Genetically engineered mice used in this study were: Tg mice overexpressing enhanced green fluorescent protein (EGFP) driven by the murine αMHC promoter (αMHC-EGFP)17 and Hmgb2 knockout (KO) mice,18 kindly provided by Prof. Marco E. Bianchi (San Raffaele University, Italy). Hmgb2 KO mice were backcrossed to C57BL/6NJcl (CLEA, Tokyo, Japan) for more than 5 generations and maintained at a stable temperature of 23°C. Genetically engineered mice used in this study were: Tg mice overexpressing enhanced green fluorescent protein (EGFP) driven by the murine αMHC promoter (αMHC-EGFP)17 and Hmgb2 knockout (KO) mice,18 kindly provided by Prof. Marco E. Bianchi (San Raffaele University, Italy). Hmgb2 KO mice were backcrossed to C57BL/6NJcl (CLEA, Tokyo, Japan) for more than 5 generations and maintained by heterozygous breeders. αMHC-EGFP (10-week-old), Hmgb2−/− (12-week-old) male mice were used in this study. We measured mouse systolic blood pressure and heart rate at room temperature using a tail cuff method and a MK2000 blood pressure monitor (Muromachi Kikai, Tokyo, Japan).

Transverse Aortic Constriction (TAC) HF Model

Male mice approximately 12 weeks old (body weight of 28–32 g) were subjected to pressure overload using a TAC protocol previously described.17,19 After surgery, mice underwent ultrasonic echocardiographic and histological analyses, as previously described.17,19

Additional details about the Supplementary Methods are available in the Supplementary File.

Results

HMGB2 Protein Levels in a Mouse Heart Significantly Increase Following Pressure Overload

To assess Hmgb2 mRNA expression in mouse tissues, we performed quantitative reverse-transcription polymerase chain reaction (RT-PCR) analysis in wild-type male mice. Hmgb2 was expressed ubiquitously, with highest expression in the heart, spleen, lung, thymus and cerebellum (Figure 1A). Next, to determine which cells in the heart expressed Hmgb2 mRNA, we separated cardiomyocytes from non-cardiomyocytes in transgenic mice expressing EGFP under control of the cardiomyocyte-specific promoter, αMHC (αMHC-EGFP Tg mice), by flow cytometry. Quantitative RT-PCR analysis revealed that Myh6 mRNA, a cardiomyocyte specific marker, was expressed only in GFP-positive cardiomyocytes. In contrast, Hmgb2 mRNA was expressed in both GFP-positive cardiomyocytes and GFP-negative non-cardiomyocytes, but expression levels in cardiomyocytes were significantly greater than in non-cardiomyocytes (Figure 1B). Next, we asked whether expression levels and/or location of HMGB2 protein in wild-type mice changed in patho-physiological stressed heart tissues following pressure overload following TAC. We confirmed that TAC surgery reduced the percentage of fractional shortening (FS) and increased heart tissue weight per body weight compared to values before TAC surgery (Figure 1C, D). Western blotting analysis of whole heart tissues revealed that HMGB2 protein levels in the stressed heart induced by pressure overload were significantly greater than those in control mice before TAC surgery (Figure 1E). Interestingly, Western blotting analysis revealed that 4 weeks after TAC surgery, levels of nuclear-localized HMGB2 protein in hearts of TAC-operated mice increased significantly, whereas these changes were not observed in sham-operated mice (Figure 1F). In contrast, levels of HMGB2 protein in the circulation of TAC-operated mice were comparable to those in sham-operated mice (Figure 1G). Taken together, we conclude that levels of nuclear-localized HMGB2 protein increase in stressed hearts of TAC-operated mice.

Hmgb2−/− Mice Develop Enlarged Cardiomyocytes and Decreased Cardiac Contractile Ability

We next investigated whether HMGB2 loss altered the cardiac physiology using Hmgb2−/− mice. We confirmed that Hmgb2−/− mice show grossly normal phenotypes (data not shown), as reported elsewhere.18 Interestingly, ultrasonic echocardiographic analysis revealed decreased cardiac systolic contractile ability in 1-year-old Hmgb2−/− mice compared to wild-type control mice (Figure 2A–C). Histological analysis revealed cardiomyocytes from 1-year-old Hmgb2−/− mice were enlarged relative to controls (Figure 2D–E), and that fibrotic areas in hearts of 1-year-old Hmgb2−/− mice increased in size compared to controls (Figure 2D–F). In contrast, we observed no difference in systolic blood pressure or heart rate between genotypes (Supplementary Figure 1). Quantitative RT-PCR analysis of heart tissues revealed that 1-year-old Hmgb2−/− mice showed increased expression of the HF markers, Bnp and Myh7, and the fibrosis markers, Collagen1 and Ctgf, compared to wild-type controls (Figure 2G), indicating that HMGB2 loss induces phenotypes commonly seen in hearts of older subjects. We next asked whether HMGB2 loss promoted progression of cardiac aging by comparing various cardiac phenotypes between young Hmgb2−/− mice and littermate wild-type controls. Expectedly, ultrasonic echocardiographic analysis revealed relatively decreased cardiac systolic contractile ability in 12-week-old Hmgb2−/− mice (Figure 3A–C). Histological analysis revealed enlargement of cardiomyocytes from 12-week-old Hmgb2−/− mice relative to controls (Figure 3D, E), and that fibrotic areas in the heart of 12-week-old Hmgb2−/− mice were more extensive than those in controls (Figure 3D, F). Quantitative RT-PCR of heart tissue of 12-week-old Hmgb2−/− mice revealed increased expression of the HF marker, Bnp, and the fibrosis marker, Collagen1, compared to wild-type controls (Figure 3G). In both Hmgb2−/− mice and littermate controls, the percentage of FS in 1-year-old mice was significantly decreased compared to that seen in corresponding 12-week-old mice. However, that percentage decrease was greater in Hmgb2−/− mice compared to wild-type control mice (Figure 3H). Overall, these findings indicate that HMGB2 loss accelerates cardiac aging and are consistent with reports that HMGB2 expression in dermal fibroblasts decreases in human subjects with aging.
**Figure 2.** HMGB2 loss predisposes 1-year-old mice to cardiac dysfunction. (A) Shown are representative M-mode echocardiography recordings (top row), hematoxylin-eosin (HE)-stained sections of heart mid-portion (second row, Scale bar, 1 mm), gross appearance of whole heart (third row, Scale bar, 5 mm) and lung (fourth row, Scale bar, 5 mm) and sections of Masson’s Trichrome (MT)-stained heart tissue (bottom row, Scale bar, 100 μm) from 1-year-old Hmgb2<sup>−/−</sup> (−/−) and wild-type (WT) littermate mouse. (B) Body weight (BW)(g), heart weight per body weight (HW/BW) ratio (mg/g) and lung weight per body weight (LW/BW) ratio (mg/g) in 1-year-old Hmgb2<sup>−/−</sup> and WT mice (n=6 per group). (C) Left ventricular end-diastolic diameter (LVDd), left ventricular end-systolic diameter (LVDs) and percent fractional shortening (%FS). (D) Shown are representative left ventricle sections from 1-year-old Hmgb2<sup>−/−</sup> and WT littermate mice stained with wheat germ agglutinin (WGA; as an indicator of cardiomyocyte size) (Upper, Scale bar, 100 μm) and 4',6-diamidino-2-phenylindole (DAPI) (Lower, Scale bar, 100 μm). (E) Distribution of myocardial cell size (μm², Left) and changes in relative cardiomyocyte size (Right). (F) Percentage of fibrosis area (%). (G) Relative expression of genes associated with heart failure and fibrosis in hearts of 1-year-old Hmgb2<sup>−/−</sup> mice relative to littermate mice. WT values were set to 1 (n=6–8 per group). Data are presented as means±SEM. Statistical significance was determined by using an unpaired Student’s t-test. *P<0.05, **P<0.01, †P<0.001 between groups. n.s., not significant.
Figure 3. HMGB2 loss predisposes 12-week-old mice to cardiac dysfunction. (A) Shown are representative M-mode echocardiography recordings (top row), hematoxylin-eosin (HE)-stained sections of heart mid-portion (second row, Scale bar, 1mm), gross appearance of a whole heart (third row, Scale bar, 5mm) and lung (fourth row, Scale bar, 5mm) and sections of Masson’s Trichrome (MT)-stained heart tissue (bottom row, Scale bar, 100μm) from Hmgb2<sup>−/−</sup> (−/−) and wild-type (WT) littermate 12-week-old mice. (B) BW (g), heart weight per body weight (HW/BW) ratio (mg/g) and lung weight per body weight (LW/BW) ratio (mg/g) in 12-week-old Hmgb2<sup>−/−</sup> and WT mice (n=6 per group). (C) Left ventricular end-diastolic diameter (LVDd) and percent fractional shortening (%FS). (D) Shown are representative left ventricle sections from 12-week-old Hmgb2<sup>−/−</sup> and WT littermate mice stained with wheat germ agglutinin (WGA; as an indicator of cardiomyocyte size) (Upper, Scale bar, 100μm) and 4',6-diamidino-2-phenylindole (DAPI) (Lower, Scale bar, 100μm). (E) Distribution of myocardial cell size (μm<sup>2</sup>, Left) and changes in relative cardiomyocyte size (Right). (F) Percentage of fibrosis area (%). (G) Relative expression of genes associated with heart failure and fibrosis in hearts of 12-week-old Hmgb2<sup>−/−</sup> mice relative to littermate mice. WT values were set to 1 (n=6 per group). (H) Comparison of percent reduction of FS in WT and Hmgb2<sup>−/−</sup> mice at 12 weeks or 1 year of age (n=6 per group). Data are presented as means±SEM. Statistical significance was determined by using an unpaired Student’s t-test. *P<0.05, **P<0.01, †P<0.001 between groups. n.s., not significant.
Figure 4. Hmgb2−/− mice show enhanced development of transverse aorta constriction (TAC)-induced heart failure. (A) Shown are representative M-mode echocardiography recordings (top row), hematoxylin-eosin (HE)-stained sections of heart mid-portion (second row, Scale bar, 1 mm), gross appearance of a whole heart (third row, Scale bar, 5 mm) and lung (fourth row, Scale bar, 5 mm) and sections of Masson’s Trichrome (MT)-stained heart tissue (bottom row, Scale bar, 100 μm) from 12-week-old Hmgb2−/− (−/−) and wild-type (WT) littermate controls 4 weeks after TAC surgery (n=5–6 per group). (B) BW (g), heart weight per body weight (HW/BW) ratio (mg/g) and lung weight per body weight (LW/BW) ratio (mg/g). (C) Left ventricular end-diastolic diameter (LVDd), left ventricular end-systolic diameter (LVDs) and percent fractional shortening (%FS). (D) Shown are representative left ventricle sections stained with wheat germ agglutinin (WGA; as an indicator of cardiomyocyte size) (Upper, Scale bar, 100 μm) and 4',6-diamidino-2-phenylindole (DAPI) (Lower, Scale bar, 100 μm). (E) Distribution of myocardial cell size (μm², Left) and changes in relative cardiomyocyte size (Right). (F) Percentage of fibrosis area (%). (G) Relative expression of genes associated with heart failure and fibrosis in hearts of 12-week-old Hmgb2−/− mice relative to littermate mice. WT values were set to 1 (n=5–6 per group). Data are presented as means±SEM. Statistical significance was determined by using an unpaired Student’s t-test. *P<0.05, **P<0.01, †P<0.001 between groups. n.s., not significant.
HMGB2 Loss Impairs Cardiomyocyte Contractility

Abnormal Ca²⁺ handling in cardiomyocytes causes cardiac contractile dysfunction. To investigate excitation-contraction (E-C) coupling in Hmgb2⁻/⁻ cardiomyocytes, we analyzed contractility and Ca²⁺ transients induced by electrical stimulation at 1 Hz in single cells isolated from Hmgb2⁻/⁻ or littermate wild-type control mice. FS was significantly reduced in Hmgb2⁻/⁻ cardiomyocytes relative to controls (Figure 5A). In addition, in Hmgb2⁻/⁻ cardiomyocytes, the magnitude of electrically evoked Ca²⁺ transients was 73.4% that of wild-type values (Figure 5B, C). In contrast, there were no differences in the time to peak [Ca²⁺]i and the time constant of Ca²⁺ transient decay (Figure 5D, E). Moreover, sarcoplasmic reticulum (SR) Ca²⁺ content in cardiomyocytes was comparable in both groups (Figure 5F). These findings indicate that HMGB2 loss in cardiomyocytes impairs cardiac contractile ability and Ca²⁺ cycling.

HMGB2 Loss Inactivates AKT-SERCA2a Signaling in Cardiomyocytes

AKT-SERCA2a signaling plays an important role in maintaining cardiac contractility. For example, transgenic mice overexpressing AKT in cardiomyocytes exhibit or in cases of progeria.

Hmgb2⁻/⁻ Mice Exhibit Enhanced TAC-Induced HF Development

Next, we asked whether HMGB2 deficiency would alter TAC-induced cardiac dysfunction using Hmgb2⁻/⁻ mice. Four weeks after TAC, wild-type mice developed adaptive cardiac hypertrophy without left ventricular dilatation (Figure 4A–C). By contrast, Hmgb2⁻/⁻ mice developed marked left ventricular dilatation with a significant decrease in percentage of FS, resulting in HF development and accompanying lung congestion (Figure 4A–C). Four weeks after surgery, the size of cardiomyocytes from Hmgb2⁻/⁻ mice, as estimated by histological analysis of heart tissue, was significantly greater, as was the size of the fibrotic area relative to control TAC mice (Figure 4D–F). Moreover, expression levels of HF and fibrosis markers, as estimated by quantitative RT-PCR of heart tissue, significantly increased in Hmgb2⁻/⁻ relative to control mice (Figure 4G). These results suggest that increased HMGB2 levels seen in cells from a stressed heart may be protective against HF development and progression.
Loss of HMGB2 Activity Causes Cardiac Dysfunction

Figure 6. Transplantation with wild-type bone marrow does not alter transverse aorta constriction (TAC)-induced heart failure in Hmgb2−/− mice. (A) Diagram showing time course of bone marrow transfer (Upper) and relationships of donors or recipients (Lower). BMT, bone marrow transfer; TEE, transthoracic echocardiography. (B) BW (g), heart weight per body weight (HW/BW) ratio (mg/g) and lung weight per body weight (LW/BW) ratio (mg/g). (C) Left ventricular end-diastolic diameter (LVDd), left ventricular end-systolic diameter (LVDs) and percent fractional shortening (%FS). (D) Relative expression of genes associated with heart failure and fibrosis in hearts. Data are presented as means±SEM. n=5–6 per each group. Statistical significance was determined by using the Kruskal-Wallis test (more than 3 groups with unequal distribution) with Dunn’s multiple comparisons post-test. *P<0.05, **P<0.01, †P<0.001 between groups. n.s., not significant.
enhanced left ventricular function associated with increased expression of SERCA2a. To identify mechanisms underlying the effect of HMGB2 on cardiac contractility, we used western blotting analysis to assess potential changes in AKT-SERCA2a signaling in *Hmgb2*+/− cardiomyocytes. SERCA2a levels were significantly decreased and AKT phosphorylation at both Ser473 and Thr308 was attenuated in hearts of *Hmgb2*+/− mice relative to littermate wild-type control mice (Figure 5G, H), indicating that HMGB2 loss inactivates the AKT-SERCA2a cascade. Mitochondrial energy metabolism also plays an important role in maintaining cardiac contractility. Therefore, we asked whether HMGB2 deficiency in cardiomyocytes alters mitochondrial energy metabolism. Quantitative RT-PCR revealed no differences in expression of genes associated with energy metabolism, β-lactam acid oxidation or mitochondrial biogenesis in hearts of 12-week-old *Hmgb2*−/− mice vs. littermate wild-type control mice (Supplementary Figures 2, 3A, 3B). Moreover, western blotting analysis revealed no differences in protein levels of components of the mitochondrial complex in heart tissues from either genotype (Supplementary Figure 3C). Taken together, these observations suggest that HMGB2 loss primarily impairs cardiac contractility and Ca++ cycling by inactivating AKT-SERCA2a signaling.

**TAC-Induced HF Development in Hmgb2−/− Mice Is Not Ameliorated by Transplant of Wild-Type Bone Marrow (BM)**

As noted above, *Hmgb2* is expressed in both cardiomyocytes and non-cardiomyocytes in the heart (Figure 1B). To determine which cell type is critical for HMGB2’s cardioprotective function, we assessed severity of TAC-induced HF development in wild-type recipients that had undergone transplantation with either wild-type or *Hmgb2*−/− BM cells (Figure 6A). All recipients were irradiated before BM transplantation. Four weeks after TAC surgery, we observed no differences in cardiac function or in expression of HF or fibrosis markers between the two groups (Figure 6B-D). We next analyzed the severity of TAC-induced HF development in *Hmgb2*−/− recipients that had undergone transplantation with either wild-type or *Hmgb2*−/− BM cells (Figure 6A). Four weeks after TAC surgery, we observed no differences in cardiac function or expression of HF and fibrosis markers between groups (Figure 6B-D). Thus, neither transplant of wild-type nor of *Hmgb2*−/− BM cells could ameliorate TAC-induced HF development and progression in *Hmgb2*−/− or WT mice, suggesting that the cardioprotective effect of HMGB2 expressed in BM-derived non-cardiomyocytes against HF development is minimal.

**Discussion**

The key findings of this study are as follows: (1) HMGB2 is expressed in both cardiomyocytes and non-cardiomyocytes; (2) HMGB2 expression increases in heart tissues, especially in the nucleus of cardiomyocytes, following pressure overload by TAC in wild-type mice; (3) HMGB2 deficiency in cardiomyocytes attenuates left ventricular contractile ability, predisposing mice to HF development after pressure overload; and (4) activation of AKT as well as SERCA2a protein levels are significantly suppressed in *Hmgb2*-deficient heart tissues. These findings suggest that HMGB2 activates the AKT-SERCA2a cascade, and that increases in endogenous HMGB2 protein in the stressed heart play an adaptive and protective role against HF development and progression.

Interestingly, a previous study reported that HMGB2 expression in H9C2 rat embryonic cardiomyocytes increases under hypoxic conditions. Moreover, in rat myocardial infarction models, HMGB2 expression in infarcted heart also increased. We found that that HMGB2 expression increases in stressed heart tissues following pressure overload using TAC in wild-type mice, and that HMGB2 loss in the heart triggers cardiac dysfunction and HF development. These studies overall suggest that increased HMGB2 protein levels in the stressed heart are cardioprotective. However, others report that injection of HMGB2 recombinant protein into the peri-infarct zone of heart tissue worsened ischemic injury by increasing production of reactive oxygen species (ROS) in rat myocardial infarction models. Accordingly, in addition to increased HMGB2 protein (Figure 1E-G), we also observed increased ROS production in a mouse heart after TAC, based on assessment of 8-hydroxy-2-deoxyguanosine (8-OHdG) and 4-hydroxynonenal (4-HNE) levels (Supplementary Figure 4). However, ROS production in the heart after TAC was comparable in *Hmgb2*−/− and wild-type control mice (Supplementary Figure 4).

Although our findings in the present study suggest that increases in endogenous HMGB2 protein in the stressed heart play an adaptive and protective role against HF development and progression, it is possible that non-physiological levels of cardiac HMGB2 are harmful in some contexts. To address this possibility, we attempted to generate transgenic mice overexpressing *Hmgb2* in cardiomyocytes under control of the aMHC promoter (aMHC- *Hmgb2* Tg mice). We obtained 6 independent founder males but could not achieve germline transmission using any of the 6 (data not shown), suggesting that very high levels of cardiac HMGB2 are indeed toxic. Further investigation is needed to evaluate this possibility. We also asked whether changing the dose of HMGB2 expression would alter cardiac phenotypes seen following TAC by assessing *Hmgb2*−/− mice. *Hmgb2*−/− mice developed a significant decrease in percentage of FS compared to wild-type mice (Supplementary Figure 5). The severity of cardiac dysfunction seen in *Hmgb2*−/− mice tended to be milder than that seen in *Hmgb2*+/− mice, but it was not significant (Supplementary Figure 5). Thus, even in heterozygotes, decreases in HMGB2 protein levels predispose mice to HF development after pressure overload. We conclude that proper HMGB2 protein levels in cardiomyocytes are critical to maintain cardiac function.

In this study, we also found that HMGB2 loss decreased cardiac contractile ability and SERCA2a protein levels in heart tissues (Figure 5). Previous in vitro analysis by others reported that HMGB2 knockdown downregulated SERCA2a mRNA expression in neonatal rat myocardium, which is in agreement with our observation of decreased SERCA2a protein levels in the heart. Relevant to mechanisms, several studies report that AKT activity significantly decreases in *Hmgb2* knockdown mouse embryonic stem cells and in *Hmgb2* knockdown human pancreatic cancer cell lines. Conversely, He et al reported AKT activation in human aortic smooth muscle cells after treatment with recombinant HMGB2. We conclude that HMGB2 activates the AKT cascade, which is required for SERCA2a expression, thereby maintaining cardiac contractile ability.
Recently, a role for the DNA damage response (DDR) in cardiomyocytes has received attention as a mechanism underlying HF development.\(^6\) Relevant to this, we also found that DDR occurs in TAC-induced HF development in mice based on our analysis of levels of p53 protein, a key transcription factor activating various DDR-associate genes (Supplementary Figure 6). Interestingly, we found that p53 protein levels in the TAC-induced stressed heart of Hmgb2\(^{-/-}\) mice significantly increased compared with wild-type mice (Supplementary Figure 6), suggesting that activated DDR might be one of causal molecular mechanisms of acceleration of HF development that induced HMGB2 loss. Further investigations of this outcome are necessary to confirm this possibility.

In vitro analysis by other research indicates that HMGB1 expression is reciprocally upregulated in HMGB2 knockdown neonatal rat cardiomyocytes.\(^6\) It is also reported that cardiac HMGB1 attenuates pressure overload-induced cardiac hypertrophy and subsequent HF development in vivo.\(^8\) In short, these papers suggest that HMGB1 protein can counteract cardiac hypertrophy and contractile abnormality seen in Hmgb2\(^{-/-}\) mice. However, cardiac hypertrophy and contractile abnormality are phenotypes observed in Hmgb2\(^{-/-}\) mice, and we observed no differences in HMGB1 protein levels in whole heart tissues between Hmgb2\(^{-/-}\) and wild-type control mice (Supplementary Figure 7). Moreover, we revealed that HMGB2 deficiency in mice predisposes their hearts to HF development due to declining left ventricular contractile ability following pressure-overload induced by TAC. Interestingly, some researchers report that maintenance of stable nuclear HMGB1 levels in cardiomyocytes has a protective effect on HF,\(^8\) while others report that in the mouse heart after TAC surgery, nuclear HMGB1 protein levels significantly decrease while cytosolic HMGB1 protein levels significantly increase.\(^6\) Conversely, we observed a significant increase in nuclear-localized HMGB2 protein levels in the mouse heart after TAC surgery (Figure 1F). These findings indicate that HMGB1 function may decline in the stressed heart following pressure-overload, thereby minimizing potential compensatory HMGB1 activity.

Hmgb2 is expressed in both cardiomyocytes and non-cardiomyocytes in the heart (Figure 1B). Non-cardiomyocytes in TAC-operated heart tissue are either resident cells or non-resident infiltrated cells, the latter derived primarily from BM. Our BM transplantation experiments suggest that the cardioprotective effect of HMGB2 expressed in non-cardiomyocytes is minimal, and thus that HMGB2 loss in cardiomyocytes or resident non-cardiomyocytes is what underlies cardioprotection. We could not assess whether HMGB2 in resident non-cardiomyocytes functions in cardioprotective activities. However, our in vitro single cell analysis clearly revealed that HMGB2 loss in cardiomyocytes impairs cardiac contractility and Ca\(^{2+}\) cycling by inactivating AKT-SERCA2a signaling (Figure 5). Future studies are needed to address whether HMGB2 expressed in resident non-cardiomyocytes also has a cardioprotective effect.

In summary, this study provides the first in vivo evidence that upregulation of endogenous cardiac HMGB2 is an adaptive response to cardiac stress. Increased HMGB2 expression in the heart could play a crucial role in preventing cardiac contractile abnormalities and subsequent HF development.

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Disclosures
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Supplementary Files