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Myocardial Hypertrophic Preconditioning Attenuates Cardiomyocyte Hypertrophy and Slows Progression to Heart Failure Through Upregulation of S100A8/A9

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Background—Transient preceding brief ischemia provides potent cardioprotection against subsequent long ischemia, termed ischemic preconditioning. Here, we hypothesized that transient short-term hypertrophic stimulation would induce the expression of hypertrophy regression genes and render the heart resistant to subsequent hypertrophic stress, and slow the progression to heart failure, as well.

Methods and Results—Cardiomyocyte hypertrophy was induced in mice by either transverse aortic constriction or an infusion of phenylephrine, and in neonatal rat ventricular cardiomyocytes by norepinephrine exposures. In the preconditioning groups, hypertrophic stimulation was provided for 1 to 7 days and then withdrawn for several days by either aortic debanding or discontinuing phenylephrine or norepinephrine treatment, followed by subsequent reexposure to the hypertrophic stimulus for the same period as in the control group. One or 6 weeks after transverse aortic constriction, the heart weight/body weight ratio was lower in the preconditioning group than in the control group, whereas the lung weight/body weight ratio was significantly decreased 6 weeks after transverse aortic constriction. Similar results were obtained in mice receiving phenylephrine infusion and neonatal rat ventricular cardiomyocytes stimulated with norepinephrine. Both mRNA and protein expression of S100A8 and S100A9 showed significant upregulation after the removal of hypertrophic stimulation and persisted for 6 weeks in response to reimposition of transverse aortic constriction. The treatment with recombinant S100A8/A9 inhibited norepinephrine-induced myocyte hypertrophy and reduced the expression of calcineurin and NFATc3, but the silencing of S100A8/A9 prevented such changes.

Conclusions—Preconditioning with prohypertrophic factors exerts an antihypertrophic effect and slows the progression of heart failure, indicating the existence of the phenomenon for hypertrophic preconditioning. (***Circulation*. 2015;131:1506-1517. DOI: 10.1161/CIRCULATIONAHA.114.013789.**)

Key Words: cardiomegaly ■ heart failure ■ myocardial preconditioning ■ S100A8 protein ■ S100A9 protein

Myocardial hypertrophy is characterized by an increase of cardiomyocyte protein synthesis and cell volume, and it is crucial for the transition from adaptive to maladaptive cardiac function with the progression to irreversible changes. Although some extent of cardiac hypertrophy serves to reduce wall stress and compensate for an increased load on the myocardium,[1] the effect of sustained prohypertrophic signaling on cardiomyocytes is detrimental and makes a major contribution to eventual progression to heart failure.[2,3] Clinical and experimental studies have shown that the withdrawal of pressure overload, such as aortic debanding in animals and aortic valve replacement in patients with aortic stenosis, leads to the regression of myocardial hypertrophy[4–6] and various beneficial molecular changes.[4,7] It has been reported that intermittent systolic overload promotes the improvement of myocardial performance in adult animals,[8] producing both a mild hypertrophic response and a favorable fetal gene expression profile.[9] However, it is completely unknown whether the removal of short-term or long-term pressure overload renders

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the heart resistant to subsequent prolonged prohypertrophic stimulation.

The phenomenon of ischemic preconditioning whereby brief episodes of ischemia increase cardiac resistance to subsequent prolonged ischemia has received considerable attention since it was first reported by Murry et al in 1986.[10] In addition to ischemia, pretreatment with hypoxia, hyperbaric oxygen, or certain drugs can induce this protective effect of preconditioning.[11–15] Similarly, it would be plausible that short-term hypertrophic stimulation makes the heart resistant to subsequent hypertrophic stress. Indeed, an animal study has shown that a short-term antihypertensive therapy has a prolonged antihypertrophic effect on the myocardium and can protect the heart.[16] In addition, it was reported that relief from cardiac pressure overload significantly alters the gene expression profile, including some of the known antihypertrophic genes.[17] Thus, it appears that antihypertensive therapy or the removal of prohypertrophic stimulation creates an antihypertrophic memory, but it is unclear how long such an effect persists.

It is well known that a similar level of pressure overload (eg, hypertension) can cause different degrees of myocardial hypertrophy. Also, the prevalence of myocardial hypertrophy is <50% in patients with essential hypertension,[18] suggesting that factors that resist prohypertrophic stimulation exist in many patients. Experimental studies have demonstrated that some factors can prevent cardiac hypertrophy independent of an antihypertensive effect,[19] but it remains unclear how to induce such antihypertrophic factors for therapeutic purposes. Based on the points mentioned here, we propose a new concept termed myocardial hypertrophic preconditioning. Our hypothesis is that short-term hypertrophic stimulation can render the heart resistant to subsequent hypertrophic stress and slow the progression to heart failure. In this study, we attempted to demonstrate the phenomenon of hypertrophic preconditioning and investigate the mechanisms involved.

Methods

All procedures were performed in accordance with our Institutional Guidelines for Animal Research and the investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised in 1996).

Cell Culture

The neonatal rats were euthanized by 2% isoflurane inhalation and cervical dislocation. The isolation and culturing of neonatal rat ventricular cardiomyocytes (NRVCs) and fibroblasts were performed as described previously.[20,21] Three groups were designed: (1) the norepinephrine (NE) group: 1 μmol/L NE (dissolved in Dulbecco's modification of Eagle's medium) for 48 hours; (2) the preconditioning (Pre)+NE group: after stimulation for 12 hours, NE was removed for another 12 hours, and then NE was added again to stimulate for 48 hours; (3) the control group: Dulbecco's modification of Eagle's medium treatment for 48 hours (Figure 1A). Cardiomyocytes were harvested and analyzed for cell surface and expression of atrial natriuretic peptide (ANP) and β-myosin heavy chains (β-MHCs).

The effects of recombinant murine S100A8 (also called myeloid-related protein [MRP] 8, Abcam), S100A9 (mouse MRP-14, Abcam) on NRVCs were examined. Five groups were designed as follows: (1) NE group: 1 μmol/L NE treatment for 48 hours; (2) NE+S100A8 group: treatment with 1 μmol/L NE and S100A8 (1 μg/mL) for 48 hours; (3) NE+S100A9 group: treatment with 1 μmol/L NE and S100A9 (1 μg/mL) for 48 hours; (4) NE+S100A8/A9 group: treatment with 1 μmol/L NE and S100A8/A9 (1 μg/mL) for 48 hours; and (5) control group: treatment with Dulbecco's modification of Eagle's medium for 48 hours. Cell surface area and the expression of ANP, β-MHC, calcineurin, and nuclear factor of activated T cells (NFAT) in cardiomyocytes, procollagen I, and procollagen III mRNA in fibroblasts were analyzed.

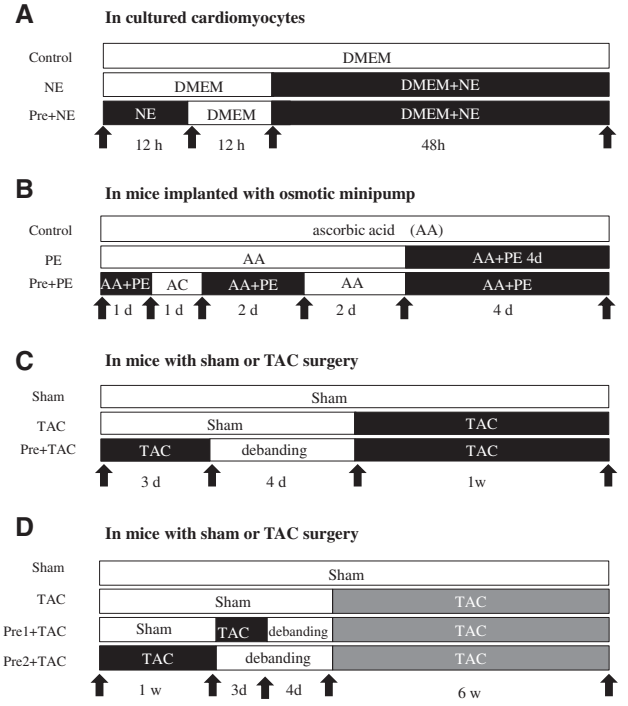

Figure 1. Experimental protocols for the detection of myocardial hypertrophic preconditioning (Pre). **A**, Experiment 1: Pre in cultured cardiomyocytes: norepinephrine (NE)-induced myocardial hypertrophy. **B**, Experiment 2: Hypertrophic preconditioning in phenylephrine (PE) infusion–induced myocardial hypertrophy in mice. **C**, Experiment 3: Short-term effect of Pre on myocardial hypertrophy in mice with transverse aortic constriction (TAC). **D**, Experiment 4: Long-term effect of Pre on myocardial hypertrophy and heart failure in mice with TAC. DMEM indicates Dulbecco's modification of Eagle's medium.

Animal Models and Experimental Protocols

Creation of Drug-Induced Myocardial Hypertrophy Model

C57BL/6 male mice (8–10 weeks, 20–25 g) were anesthetized with a mixture of xylazine (5 mg/kg IP) and ketamine (100 mg/kg IP). After anesthesia, the mice were subjected to subcutaneous pump implantation in the back with an osmotic minipump (Alzet) filled with phenylephrine (PE, 30 $mg \cdot kg^{-1} \cdot d^{-1}$, dissolved in 0.5 mmol/L ascorbic acid) or vehicle (0.5 mmol/L ascorbic acid), the incision was closed with 2 wound clips. For the preconditiong group, changing from PE to vehicle or vice versa was performed by removing the old pump and implanting a new one. Animals in the control group and PE group received a similar procedure for replacing of pump (filled with vehicle alone) during the preconditioning time window. These mice were euthanized by overdose anesthesia (pentobarbital sodium 150 mg/kg IP) and cervical dislocation at 4 to 8 days to obtain heart and calculate heart weight/body weight ratio. The mice were divided into 3 groups, with 6 to 7 mice in each group, as follows: (1) PE group: PE infusion (in ascorbic acid) for 4 days; (2) Pre+PE group: PE infusion for 1 day and then stop for 1 day, followed by PE infusion for 2 days and stop for 2 days, and finally recovered PE infusion for 4 days; (3) control group: ascorbic acid infusion for 4 days (Figure 1B).

Transverse Aortic Constriction Model

C57BL/6 male mice (8–10 weeks, 18–25 g) were subjected to transverse aortic constriction (TAC) or debanding or sham operation as described elsewhere.[4,22] In brief, after a left-sided thoracotomy in the second intercostal space, a 7-0 silk ligature was tied around the transverse aorta and a 27-gauge blunted needle that was subsequently removed (Movie I in the online-only Data Supplement), whereas, in sham-operated animals, the ligature was tied loosely around the aorta. At the indicated time, a debanding operation was performed by carefully removing the ligature (Movie II in the online-only Data Supplement). To avoid possible confounding effects of the repeated surgical injury, sham or nonpreconditioning animals were also subjected to a similar open-chest operation. Two experimental protocols were designed for this model (Figure 1C): (1) short-term effect of hypertrophic preconditioning. Three groups were included: the sham group and the TAC group, observation for 7 days; and the Pre+TAC group, debanding the aorta after 3 days of TAC (TAC for 3 days in mice is sufficient to induce significant cardiac hypertrophy; see Figure I in the online-only Data Supplement), and banded again 4 days later followed by observation for 7 days. (2) Long-term effect of preconditioning (Figure 1D). Four groups were designed: the sham group and the TAC group, observation for 6 weeks; the Pre1+TAC group, debanding the aorta after 3 days of TAC, and banded again 4 days later followed by observation for 6 weeks; and the Pre2+TAC group, debanding the aorta after 1 week of TAC, and banded again 1 week later followed by observation for 6 weeks. These mice and sham-operated mice were euthanized by overdose anesthesia (pentobarbital sodium 150 mg/kg IP) at 1 to 8 weeks after the operation. Preconditioning with TAC for 3 days or 1 week induced significant myocardial hypertrophy (Figure I in the online-only Data Supplement), which may be assured as compensatory hypertrophy.

Left ventricular (LV) hemodynamics was evaluated by using a Millar catheter and Blood Pressure Module software in some mice before euthanization as we reported elsewhere.[23]

Echocardiography, Western Blot, Polymerase Chain Reaction Immunofluorescence, Construction of Lentivirus Carrying Overexpressed or Short-Hairpin RNA for S100A8 or A9, Cell Viability Assay, and Histological Examinations

See details in Materials in the online-only Data Supplement. Infection efficiency and the expression levels of targeted genes S100A8 or A9 are shown in Figure II in the online-only Data Supplement. Sequences of primers for routine polymerase chain reaction, quantitative real-time polymerase chain reaction, and synthesis of S100A8/A9 cDNA are shown in Tables I through III in the online-only Data Supplement, respectively.

Statistical Analysis

Quantitative data are expressed as mean±standard error of the mean. For all statistical tests, multiple comparisons were performed by 1-way analysis of variance with the Bonferroni multiple comparison test (SPSS 16.0). The least-squares method was used to assess linear correlations between selected variables. The overall survival of TAC mice for 10 days was evaluated by using Kaplan-Meier survival analysis, and groups were compared by the log-rank test. P values of <0.05 were considered to be statistically significant.

Results

Antihypertrophic Effect of Hypertrophic Preconditioning In Vitro

Using our database of TAC or sham mice, we analyzed cardiac hypertrophy, pulmonary congestion, and left ventricle hemodynamics in 74 C57 male mice subjected to either TAC or sham operation for 4 to 8 weeks (Figure IIIA through IIIC in the online-only Data Supplement), and found that some animals displayed antihypertrophic phenomena even under a similar high-pressure overload, suggesting that antihypertrophic factors are inducible to render the heart resistant to the persistent pressure overload. Then we used hypertrophic Pre treatments that were designed with modification according to the ischemic preconditioning protocol to test whether hypertrophic preconditioning affords cardiac protection. In the cultured cardiomyocytes, we noted that NRVCs showed a significant increase of cell size in response to NE stimulation, whereas preconditioning treatment suppressed this increase (Figure 2A). Meanwhile, the increased expression of fetal genes (ANP and β-MHC) in the preconditioning group was significantly attenuated (Figure 2B). These results indicate that hypertrophic preconditioning renders an antihypertrophic role in cardiomyocytes.

Antihypertrophic Effect of Hypertrophic Preconditioning In Vivo

In mice with induction of myocardial hypertrophy by persistent infusion of PE for a short term of 4 days, the heart weight/body weight ratio and expression levels of hypertrophic markers ANP and β-MHC were significantly smaller in the preconditioning group than in the PE group (Figure 2C and 2D, $P<0.05$), but no detectable difference was noted on myocardial fibrosis assessed with Masson trichrome staining (Figure 2E).

Using mouse TAC model, we noted that 1 week after TAC, the heart weight/body weight ratio was smaller in the preconditioning group than in the TAC group (5.35±0.17 mg/g versus 5.99±0.22 mg/g, $P=0.014$; Figure 3A). Our previous study showed that TAC mice may die of acute heart failure[23]; thus, we here examined whether hypertrophic preconditioning exerts influence on survival. As shown in Figure 3B, the survival rate for the first 10 days after TAC was significantly lower in mice receiving preconditioning for 3 days than in mice with TAC alone, suggesting the acute cardioprotection of hypertrophic preconditioning. We further investigated the long-term effect of hypertrophic preconditioning on hypertrophy. At 6 weeks after TAC, heart weight/body weight ratio was significantly smaller in the 2 preconditioning groups than in the TAC group (7.16±0.33 mg/g for TAC, 5.32±0.14 mg/g for Pre1+TAC, and 5.43±0.11 mg/g for Pre2+TAC, $P<0.01$; Figure 3C and 3D), whereas the cardiomyocyte area was significantly smaller in the Pre1+TAC and Pre2+TAC groups than in the TAC group (Figure 3E). In addition, the increase of fetal gene expression (ANP and β-MHC) was significantly attenuated in the 2 preconditioning groups (Figure 3F). The above findings indicate that hypertrophic preconditioning in vivo improves acute-phase survival and attenuates myocardial hypertrophy.

Hypertrophic Preconditioning Slows Progression of Cardiac Remodeling

In TAC mice, serial echocardiography showed a time-dependent increase of LV end-diastolic dimension and LV end-systolic dimension (Figure 4A and 4B), and of diastolic and systolic LV wall thickness (Figure 4C and 4D), as well, whereas LV fractional shortening decreased over time (Figure 4E). In contrast, hypertrophic preconditioning significantly slowed the increase of LV wall thickness (Figure 4C and 4D), the enlargement of LV dimensions (Figure 4A and 4B), and the decline of LV fractional shortening (Figure 4E). No significant differences were noted between the 2 preconditioning groups (Figure 4).

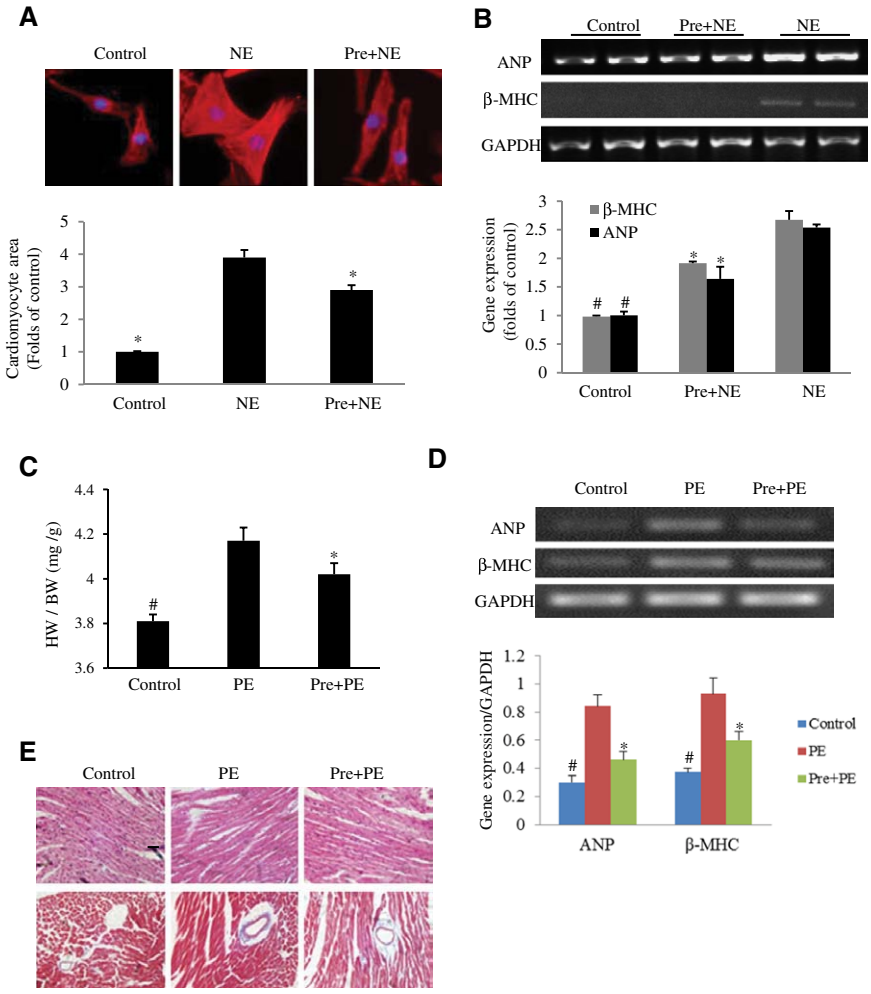

Figure 2. Effect of hypertrophic preconditioning (Pre) on myocardial hypertrophy in cultured cardiomyocytes and phenylephrine (PE) infusion mouse model. **A**, Representative confocal microscopic images of cultured neonatal rat cardiomyocytes stained with α-actin plus DAPI staining of the nucleus and semiquantitative analysis of cardiomyocyte area in response to NE stimulation with/without preconditioning or vehicle treatment. **P*<0.01 vs NE. **B**, Results of PCR for ANP and β-MHC in cultured cardiomyocytes. #*P*<0.01, **P*<0.05 vs NE. **C**, Effect of PE infusion-induced preconditioning on heart weight/body weight ratio (HW/BW), n=7, 7, and 6 in vehicle, PE, and Pre group, respectively. **D**, PCR results of myocardial ANP and β-MHC, n=5 in each group, #*P*<0.01, **P*<0.05 vs PE. **E**, Representative pictures of H&E (**Top**) and Masson (**Bottom**) stained myocardial tissues. Scale bar, 20 μm. ANP indicates atrial natriuretic peptide; DAPI, 4′,6-diamidino-2-phenylindole; H&E, hematoxylin and eosin; β-MHC, β-myosin heavy chain; NE, norepinephrine; and PCR, polymerase chain reaction.

Hypertrophic Preconditioning Improves the Pathophysiology of Heart Failure

TAC induced congestive heart failure (HF) with an increase of the LW/BW. Six weeks after TAC, the LW/BW was markedly smaller in the Pre1+TAC and Pre2+TAC groups than in the TAC group (9.88±1.00 mg/g for TAC, 5.98±0.12 mg/g for Pre1+TAC (*P*=0.008), and 6.15±0.11 for Pre2+TAC [*P*=0.046]; Figure 5A and 5B). In addition, histological examination showed that both myocardial fibrosis and perivascular fibrosis were significantly attenuated in both preconditioning groups in comparison with the TAC group (Figure 5C through 5E). Echocardiographic LV dimensions (Figure 5F) were smaller, LV fractional shortening was larger (Figure 5G), LV end-diastolic pressure was lower (Figure 5H), and LV contractility (Figure 5I) was higher in the preconditioning groups than in TAC alone group (all *P*<0.05). No significant differences were noted between them on LV posterior wall thickness (LV cavity enlargement in TAC group would decrease wall thickness), LV systolic pressure (suggesting similar pressure overload), heart rate, and LV pressure change rate (Figure IV in the online-only Data Supplement). These findings indicated that hypertrophic preconditioning has an inhibitory effect on cardiac hypertrophy and HF. We subsequently investigated the possible mechanisms involved.

Upregulation of S100A8/A9 After Withdrawal of Prohypertrophic Stimulation

S100A9 was reported to be one of the genes that is specifically induced during the regression of cardiac hypertrophy,[17] so we examined the expression of S100A8 and S100A9 after the removal of stimulation. We found that expression of S100A8 and S100A9 mRNA and their corresponding proteins in cultured NRVCs was similar between control cells and NE-stimulated cells, but was markedly upregulated at 12 hours after the withdrawal of NE (Figure 6A and 6B). Consistent with these findings, myocardial gene and protein expression

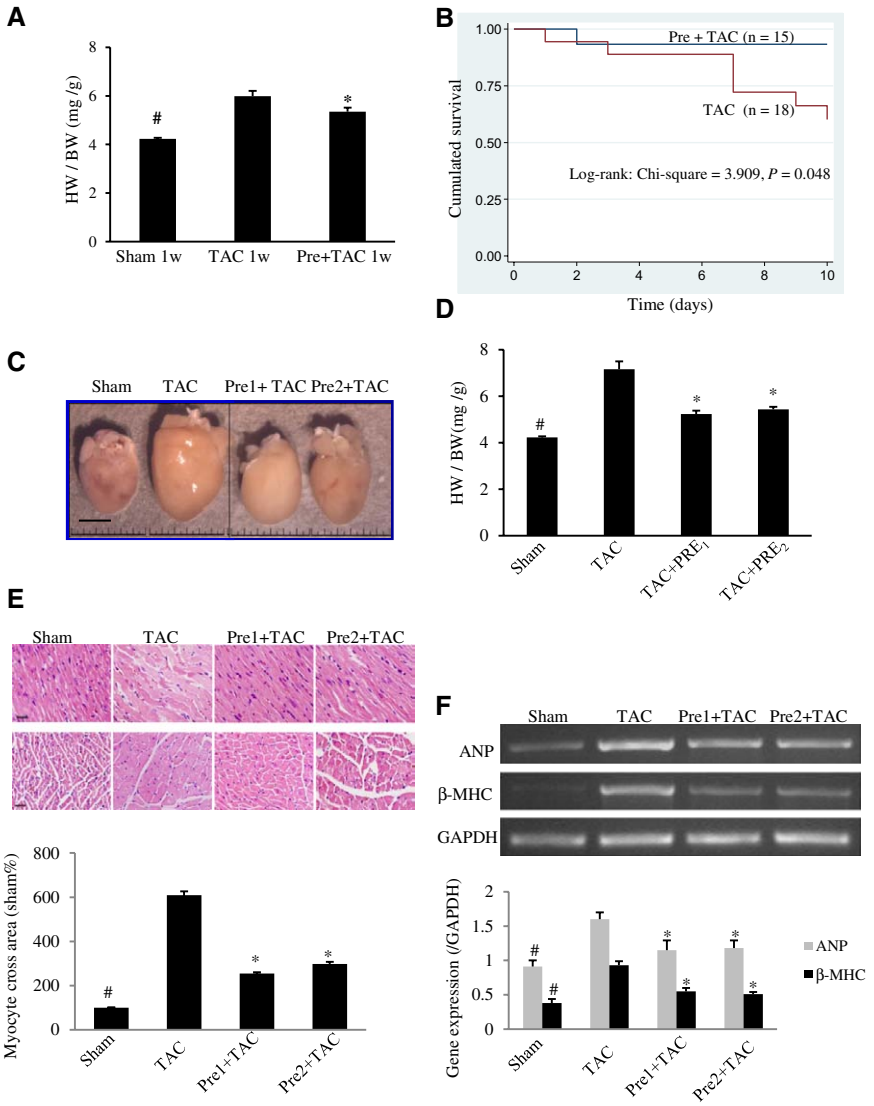

Figure 3. Effect of hypertrophic preconditioning (Pre) on myocardial hypertrophy. **A**, HW/BW at 1 week after transverse aortic constriction (TAC) or sham operation, n=6 in each group. **B**, Survival rate in the first 10 days after TAC in TAC group and Pre1+TAC group. **C**, Representative pictures of whole heart at 6 weeks after TAC or sham operation, scale bar=2 mm. **D**, HW/BW at 6 weeks after TAC or sham operation, n=6 or 7 in each group. **E**, Cardiomyocyte cross area in different groups (sham %) at 6 weeks after TAC or sham operation. Scale bar, 50 μm. **F**, Semiquantitative analysis of myocardial ANP and β-MHC at 6 weeks after TAC or sham operation, n=4 in each group. #P<0.01, *P<0.05 vs PE or TAC. (Protocols for Pre1 and Pre2 are shown in Figure 1D). ANP indicates atrial natriuretic peptide; β-MHC, β-myosin heavy chain; HW/BW, heart weight/body weight ratio; and PE, phenylephrine.

of S100A8 and S100A9 was also significantly increased in mice 1 day after debanding that had been preceded by 3 days or 1 week of TAC (Figure 6C through 6E).

We further checked how long the upregulation of S100A8/A9 would persist after reimposition of pressure overload followed by debanding for 4 days. As shown in Figure 6D and 6E, S100A8 or A9 was significantly increased in response to debanding, which was continued until 1 week and 6 weeks after reimposition of hypertrophic stimuli.

Recombinant S100A8/A9 Attenuates Hypertrophy and Fibrosis In Vitro

We further investigated whether recombinant S100A8 and S100A9 proteins had antihypertrophic effects in cultured NRVCs and fibroblasts. As shown in Figure 7A and 7B, the treatment with either S100A8 or S100A9 (or both proteins) significantly suppressed the NE-induced increase in the surface area of cardiomyocytes. In comparison with control cells, exposure to NE for 48 hours increased the expression of ANP and β-MHC mRNA in NRVCs (Figure 7C and 7D), and the expression of procollagen I and III mRNA in fibroblasts, as well (Figure 7E), whereas treatment with S100A8, S100A9, or both of these proteins prevented such changes (Figure 7C through E). These findings suggested that S100A8 and S100A9 could attenuate NE-induced hypertrophy and fibrosis in cultured cardiac cells.

The exposure of NRVCs to NE resulted in increased expression of calcineurin, but this was abrogated by the treatment with either S100A8 or S100A9, or both of these proteins (Figure 7F and 7H). When the subcellular localization of NFATc3 was assessed by Western blotting, it was primarily localized in the cytoplasm of control cells and underwent

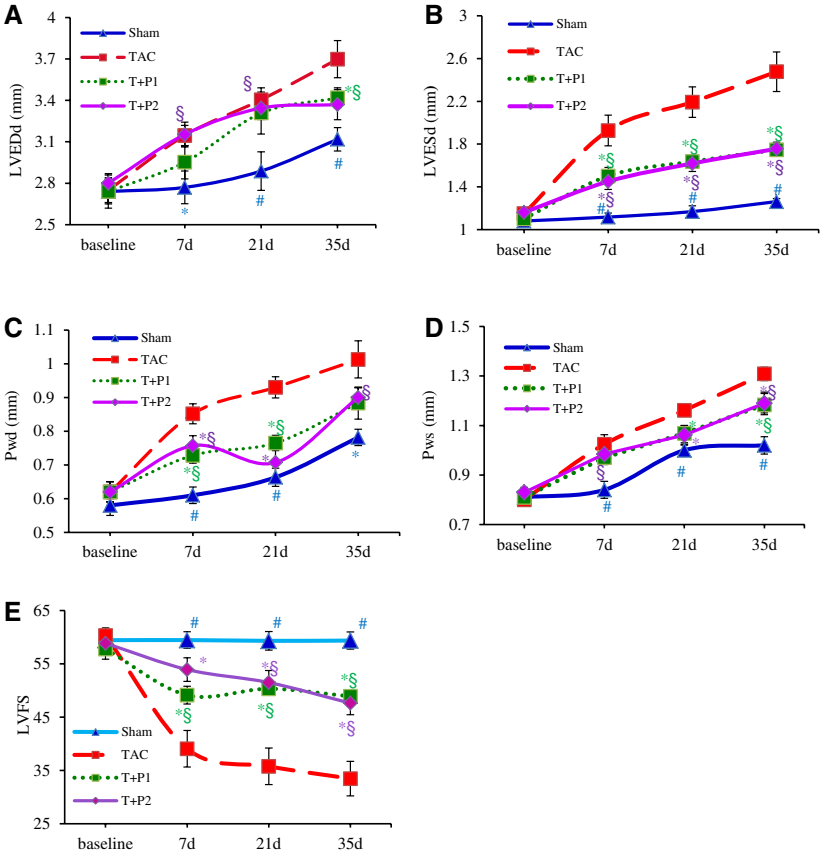

Figure 4. Effect of hypertrophic preconditioning (P) on cardiac remodeling after transverse aortic constriction (TAC or T) over time. **A**, Left ventricular end-diastolic dimension (LVEDd). **B**, Left ventricular end-systolic dimension (LVESd). **C**, Left ventricular diastolic posterior wall thickness (Pwd). **D**, Left ventricular systolic posterior wall thickness (Pws). **E**, Left ventricular fractional shortening (LVFS). **P*<0.05, #*P*<0.01 vs TAC group, §*P*<0.05 vs Sham, n=7 per group. Protocols for P1 and P2 are shown in Figure 1D.

translocation to the nucleus in response to NE stimulation, whereas treatment with S100A8 or S100A9, or both of these proteins, inhibited NE-induced nuclear translocation of NFATc3 (Figure 7G and 7H).

Silencing of S100A8/A9 Attenuates the Antihypertrophic Effects of Hypertrophic Preconditioning

The aforementioned results suggest the important role of S100A8/A9 in myocardial hypertrophy. We then used approaches for gain and loss of function to further address this issue. Infection of cardiomyocytes with lentivirus-S100A8 or A9 upregulated S100A8 or A9 by >200-fold (Figure II in the online-only Data Supplement), which was much higher than the upregulation amplitude in response to debanding (about 6-fold).[17] The cell viability test showed that high-dose overexpression of endogenous S100A8 or A9 and a high dose of exogenous S100A8 or A9 increased cardiomyocyte death (Figure V in the online-only Data Supplement), suggesting that the role of S100A8/A9 is dose dependent. Accordingly, we chose the S100A8/A9-silencing approach. Lentivirus (Lv) carrying short-hairpin (Sh) RNA for S100A8/A9 led to a significant silencing effect (Figure 8A). In comparison with the preconditioning group, both Lv-Sh-S100A8 and A9 significantly reduced the hypertrophic preconditioning effects manifested by an increase of cardiomyocyte cell surface area (Figure 8B), the upregulation of ANP and β-MHC in cardiomyocytes (Figure 8C), and the upregulation of procollagens in fibroblasts (Figure 8D), increase of calcineurin protein levels (Figure 8E), and nuclear translocation of NFAT3 (Figure 8F). In cardiomyocytes infected with both Lv-Sh-S100A8 and A9, a low dose of exogenous S100A8 or A9 still significantly inhibited NE-induced upregulation of calcineurin protein (Figure 8G), suggesting that exogenous S100A8 or A9 can work as nonheterodimer.

Discussion

This study provided evidence for a new concept termed myocardial hypertrophic preconditioning. We demonstrated that preconditioning by prohypertrophic factors increases the resistance of the heart to subsequent hypertrophic stress and delays the progression from hypertrophy to HF, indicating the existence of the hypertrophic preconditioning phenomenon. We further showed that upregulation of S100A8/A9 following removal of transient hypertrophic stimulus contributes to the antihypertrophic and anti-HF effect of hypertrophic preconditioning, at least in part by suppressing the calcineurin/NFAT pathway.

Cardiac protection by hypertrophic preconditioning has already been reported in the setting of congenital heart disease. In patients with transposition of the great vessels, the LV does not develop properly because it is pumping against low resistance and needs to be strengthened by applying a pulmonary artery band in preparation for corrective surgery.[24–26] Traditional banding procedures quickly reach the target level of stenosis for ventricular retraining, but cause the abrupt onset of fixed systolic overload that can result in LV failure.[24,26] Experimental studies have shown that myocardial edema and necrosis occur in hearts with abrupt systolic overload, usually followed by the development of ventricular failure.[23,27] In contrast, Sekarski et

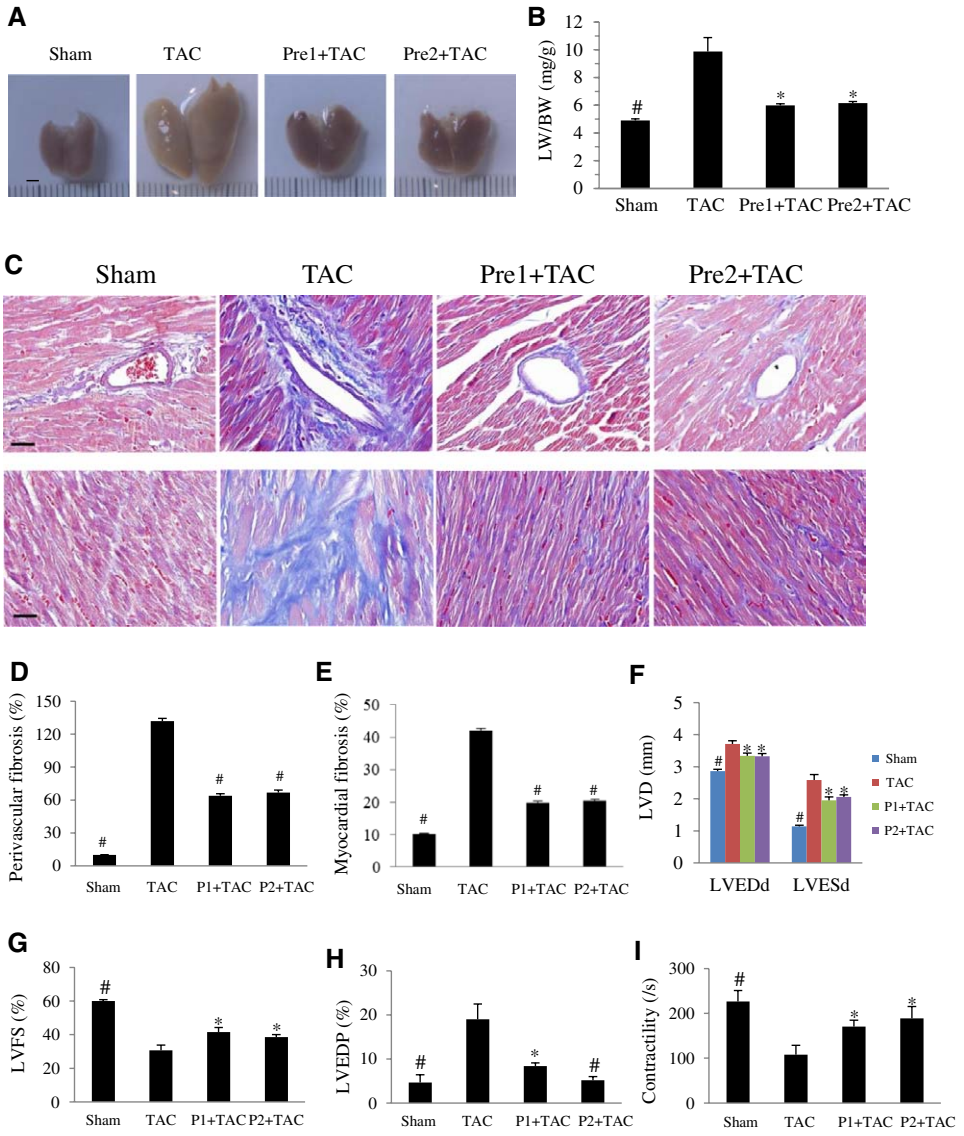

Figure 5. Effects of hypertrophic preconditioning (Pre or P) on cardiac function and fibrosis. **A**, Representative macroscopic appearance of the lungs in each group. Scale bar, 2 mm. **B**, Lung weight/body weight (LW/BW) ratio at 6 weeks after TAC, n=6 or 7 in each group. **C**, Representative Masson-stain pictures of perivascular and myocardial fibrosis from each group. Scale bar, 50 μm. **D**, Quantitative analysis of perivascular fibrosis, n=4 per group. **E**, Quantitative analysis of myocardial fibrosis, n=4 per group. For **B**, **D** and **E**, **P*<0.05, #*P*<0.01 vs TAC group. Before euthanization, left ventricular dimensions (LVD; **F**) and LV fractional shortening (LVFS; **G**) were measured by using echocardiography, whereas LV end-diastolic pressure (LVEDP; **H**) and LV contractility (**I**) were measured by using a Millar catheter. For **F** through **I**, n=5 to 7, **P*<0.05, #*P*<0.01 vs TAC group. LV indicates left ventricular; LVEDd, left ventricular end-diastolic dimension; LVESd, left ventricular end-systolic dimension; and TAC, transverse aortic constriction.

al demonstrated that ventricular retraining with an adjustable banding device (the target stenosis was gradually reached by the telemetric control system) led to better survival.[26] In addition, Miana et al[8] reported that intermittent systolic overload by pulmonary banding promoted better myocardial performance in goats, mimicking the physiological hypertrophy achieved by exercise in athletes. These results support our finding that hypertrophic preconditioning improves HF.

In patients with aortic stenosis, the regression of cardiac hypertrophy occurs after aortic valve replacement.[28,29] Mechanical unloading can cause the regression of hypertrophy and functional improvement,[30–32] during which process certain genes may be specifically upregulated to either block hypertrophic signaling pathways or trigger atrophic signaling pathways.[7,17] Yang et al[7] identified a set of genes specifically induced during the regression of hypertrophy, and confirmed that eyes absent 2 homolog (eya2) blocks the development of cardiomyocyte hypertrophy. Among those genes induced during the regression process, S100A9 was upregulated by ≈6-fold, but its role in myocardial hypertrophy and HF remains elusive. S100A8 (calgranulin A or migration inhibitory factor–related protein 8 [MRP-8]) and its binding partner S100A9 (calgranulin B, or MRP-14) are members of the S100 calcium-binding family of proteins, which have anti-inflammatory and immunoregulatory actions.[33–35] Although the expression of both S100A8 and S100A9 was reported to be increased in acute coronary syndromes,[36] atherosclerosis,[37,38] and endotoxin-induced cardiac dysfunction,[39] their exact roles

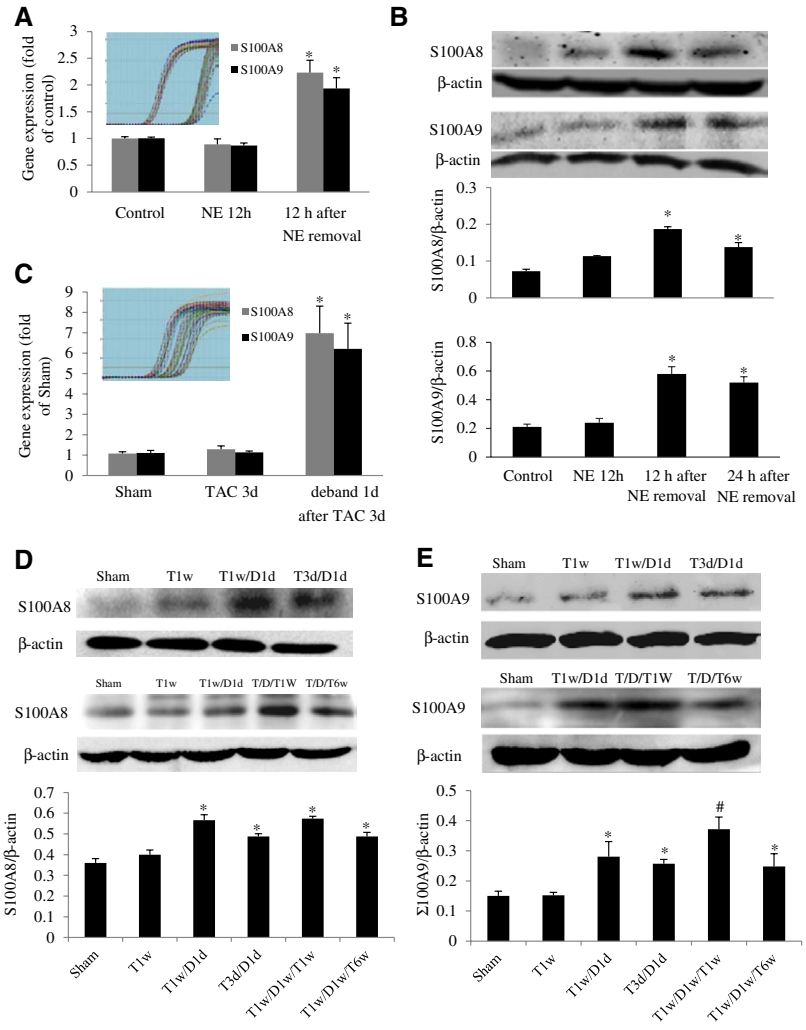

Figure 6. S100A8/A9 mRNA and protein expression after the removal of prohypertrophic stimulation. **A**, Real-time quantitative polymerase chain reaction (PCR) for expression of S100A8 and S100A9 in cultured neonatal rat ventricular cardiomyocytes (NRVCs). **B**, Western blot analysis of S100A8 and S100A9 expression in cultured NRVCs. **C**, Real-time quantitative PCR for myocardial expression of S100A8 and S100A9 in mice. For **A** through **C**, **P*<0.05 vs norepinephrine (NE) or transverse aortic constriction (TAC) group, n=3 to 5 per group. Insets in **A** and **C** are amplification curves of real-time PCR. **D** and **E**, Western blot analysis of myocardial S100A8 (**D**) and S100A9 (**E**) expression in mice in response to TAC (T) or sham, or debanding or reimposition of pressure overload. **P*<0.05, #*P*<0.01 vs TAC 1w group, n=5 in each group. D indicates debanding; T, TAC; T/D/T1w, TAC for 1 week, then debanding for 1 week followed by re-TAC for 1 week; and T/D/T6w, TAC for 1 week, then debanding for 1 week followed by re-TAC for 6 weeks.

have not been clarified. However, cardiac overexpression of S100A8 and S100A9 was reported to decrease calcium flux,[39] suggesting that these genes may exert an antihypertrophic effect. In the present study, we demonstrated that treatment with S100A8 and S100A9 inhibited NE-induced cardiomyocyte hypertrophy. Fibrosis is well known to play a critical role in chronic HF. Degradation and accumulation of extracellular matrix are important in the process of LV remodeling, and it has been proposed that matrix metalloproteinases can be used as markers of inflammation and fibrosis.[40] In agreement with a recent report[41] that stimulation of chondrocytes with S100A8 and S100A9 caused marked downregulation of type II collagen, we found that treatment with S100A8 and S100A9 downregulated procollagens I and III in cultured fibroblasts receiving NE stimulation, which may be a potential mechanism for slowing the progression of HF by hypertrophic Pre.

Although S100A8/S100A9 are generally viewed as proinflammatory, accumulated evidence suggests that S100A8/A9 can exert pleiotropic roles such as anti-inflammatory and immune regulatory actions in a context-dependent and cell type–specific manner. S100A8 is essential for life because S100A8 knockout mice died during embryonic development.[42] It seems that their doses, posttranslational modifications, their binding to the different receptors may lead to distinct functional outcomes. Our supplementary experiments also showed that a high dose (10 μg/mL) or overexpression of S100A8/A9 reduced the viability of cardiomyocytes, whereas a low dose (1 μg/mL) exerted no harmful effect, which is in agreement with previous studies showing that a high dose of S100A8 (30 μg/mouse) aggravates lung injury,[43] whereas its low dose (10 μg/mouse) protected from acute lung injury.[44] Several lines of recent evidence support the idea that S100A8/A9 may exert cell-protective roles. S100A8 was reported to promote angiogenesis,[45] which should be beneficial for the improvement of cardiac dysfunction by alleviating the relative ischemia of hypertrophied myocardium. S100A9 knockout mice increased renal damage and fibrosis in response to ischemia/reperfusion,[46] and S100A8/A9 also showed the ability to inhibit the cell growth of cancer,[47] lending support to our finding that hypertrophic Pre attenuates myocardial fibrosis and myocyte hypertrophy, in part, by the upregulation of S100A9. Similar to ischemia preconditioning, it is plausible that the mechanisms of hypertrophic preconditioning may be far more complicated than the involvement S100A8/9, which needs to be clarified in future studies.

Ca^{2+} is essential for transcriptional activation during cardiac hypertrophy. Among the Ca^{2+}-dependent signaling pathways implicated in cardiac hypertrophy, the activation of calcineurin and subsequent nuclear translocation of NFAT are particularly

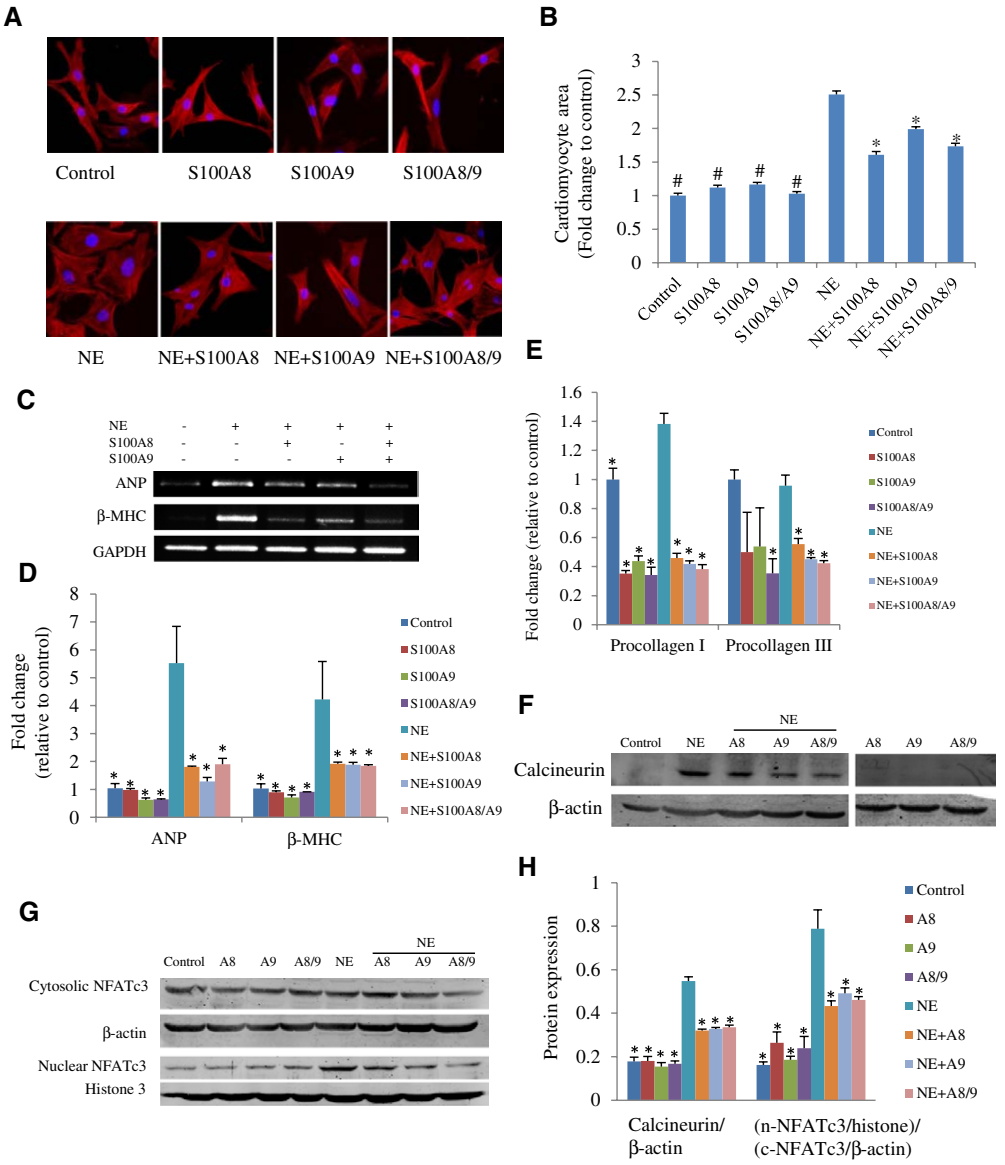

Figure 7. Effect of S100A8/A9 treatment on myocyte hypertrophy in cultured neonatal rat ventricular cardiomyocytes (NRVCs) and fibroblasts. **A**, Cardiomyocytes stained with α-actin and DAPI. **B**, Semiquantitative analysis of cardiomyocyte area. **C**, PCR for ANP and β-MHC expression in NRVCs. Real-time quantitative PCR for expression of ANP and β-MHC in NRVCs (**D**) and for expression of procollagen I and procollagen III in fibroblasts (**E**). **F**, Representative Western blot of calcineurin in NRVCs treated with recombinant S100A8/A9. **G**, Detection of nuclear factor of activated T cells (NFAT) in subcellular fractions by Western blotting. Histone 3 was the loading control for nuclear extracts, and β-actin was the loading control for cytosolic extracts. **H**, Semiquantitation of calcineurin and NFATc3 Western blotting results in NRVCs (n = nuclear, c = cytosolic). **P*<0.05, #*P*<0.01 vs NE group, experiments were repeated for 3 to 5 times. ANP indicates atrial natriuretic peptide; DAPI, 4′,6-diamidino-2-phenylindole; β-MHC, β-myosin heavy chain; NE, norepinephrine; and PCR, polymerase chain reaction; A8, S100A8; A9, S100A9; A8/9, S100A8/9.

important.[48,49] As members of the S100 calcium-binding family of proteins, S100A8 and S100A9 are likely to exert their intracellular regulatory activities by interacting with specific targets in a Ca^{2+}-dependent manner.[50,51] Using cultured cardiomyocytes, we showed that NE increased the expression of calcineurin and nuclear translocation of NFAT, whereas treatment with S100A8 and S100A9 prevented these changes, indicating that S100A8 and S100A9 attenuate cardiac hypertrophy by inhibiting the calcineurin/NFAT–signaling pathway.

It is likely that other factors induced after withdrawal or attenuation of hypertrophic stimulation may contribute to protection of the heart in addition to S100A8/A9. A clinical investigation has shown that periodic intravenous infusion (5 consecutive days every 6 weeks) of iloprost (a prostacyclin analog) protects against the onset or exacerbation of pulmonary artery hypertension and decreases the serum level of N-terminal pro B-type natriuretic peptide,[52] effects similar to those of hypertrophic Pre. Because iloprost has a short half-life of 20 to 30 minutes, its pharmacological antihypertensive effect would not persist for as long as 6 weeks, suggesting that 5 days of treatment with iloprost induces the production of antihypertensive factors that prevent the development of pulmonary hypertension after its withdrawal. Similarly, several experimental studies have demonstrated that antihypertensive

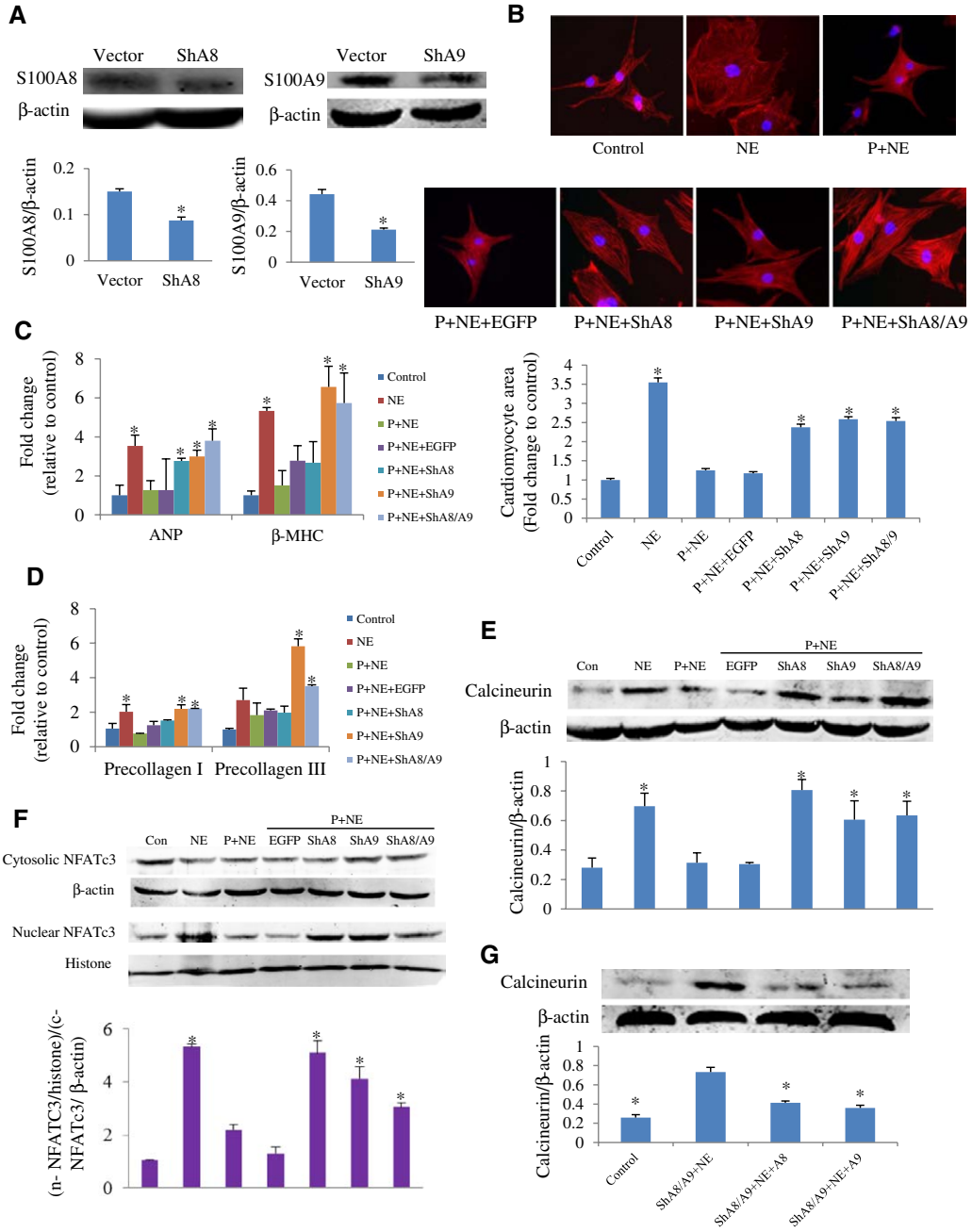

Figure 8. Silencing of S100A8/A9 antagonizes the effect of hypertrophic preconditioning in neonatal rat ventricular cardiomyocytes (NRVCs) and fibroblasts. S100A8 and S100A9 were knockdown by using short hairpin of RNA for S100A8 (ShA8) and S100A9 (ShA9) and then constructed into lentivirus. **A**, Silencing effect of ShA8 and ShA9. *P<0.05 vs vector, n=3. **B**, Representative pictures of cardiomyocytes stained with α-actin and DAPI and semiquantitative analysis of cardiomyocyte area. Real-time quantitative PCR for expression of ANP and β-MHC in NRVCs (**C**) and for expression of procollagen I and procollagen III in fibroblasts (**D**). **E**, Western blot analysis of calcineurin expression in cardiomyocytes. *P<0.05 vs P+NE group. **F**, Western blot analysis of nuclear factor of activated T cells (NFAT) in subcellular fractions by Western blotting in cardiomyocytes. Histone 3 and β-actin were the loading control for nuclear (n) extracts and cytosolic (c) extracts, respectively. *P<0.05 vs P+NE group. **G**, Western blotting of calcineurin in cardiomyocytes with S100A8/A9 silencing and NE stimulation (1 μmol/L) in the presence/absence of exogenous S100A8 or S100A9 (1 μg/mL). *P<0.05 vs ShA8/9+NE group. All experiments were repeated 3 to 5 times. ShA8/9 = short hairpin RNA of S100A8/9, multiplicity of infection (MOI)=5 for ShA8 or ShA9. ANP indicates atrial natriuretic peptide; β-MHC, β-myosin heavy chain; NE, norepinephrine; P, preconditioning; and PCR, polymerase chain reaction.

and organ protective effects can persist for some time after the discontinuation of antihypertensive therapy.[53–55]

Inhibition of the compensatory hypertrophy is traditionally believed to be detrimental for cardiac function. However substantial evidence from experimental studies, especially from gene-targeted animals, call into question the necessity of hypertrophic growth of the heart as a compensatory response to hemodynamic stress.[56] In the present study, it is plausible to postulate that preconditioning stimuli (short-term TAC) per se should induce a compensatory hypertrophy and then inhibit the progression of hypertrophy (maybe pathological or decompensatory phase) in response to reimposition of hypertrophic stimuli.

In conclusion, this study provided the first evidence for the phenomenon of myocardial hypertrophy preconditioning. We demonstrated that preconditioning by prohypertrophic factors exerts an antihypertrophic effect and slows the progression of HF, indicating the existence of hypertrophic preconditioning. Suppression of the calcineurin/NFAT pathway by S100A8/A9 partially explains the cardiac protection of hypertrophic preconditioning.

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Disclosures

None.

References

1. Meerson FZ. Compensatory hyperfunction of the heart and cardiac insufficiency. *Circ Res*. 1962;10:250–258.
2. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N Engl J Med*. 1990;322:1561–1566. doi: 10.1056/NEJM199005313222203.
3. Hill JA, Olson EN. Cardiac plasticity. *N Engl J Med*. 2008;358:1370–1380. doi: 10.1056/NEJMra072139.
4. Bjørnstad JL, Sjaastad I, Nygård S, Hasic A, Ahmed MS, Attramadal H, Finsen AV, Christensen G, Tønnessen T. Collagen isoform shift during the early phase of reverse left ventricular remodelling after relief of pressure overload. *Eur Heart J*. 2011;32:236–245. doi: 10.1093/eurheartj/ehq166.
5. Lund O, Emmertsen K, Dørup I, Jensen FT, Fløe C. Regression of left ventricular hypertrophy during 10 years after valve replacement for aortic stenosis is related to the preoperative risk profile. *Eur Heart J*. 2003;24:1437–1446.
6. Chen J, Chemaly ER, Liang LF, LaRocca TJ, Yaniz-Galende E, Hajjar RJ. A new model of congestive heart failure in rats. *Am J Physiol Heart Circ Physiol*. 2011;301:H994–1003. doi: 10.1152/ajpheart.00245.2011.
7. Yang DK, Choi BY, Lee YH, Kim YG, Cho MC, Hong SE, Kim do H, Hajjar RJ, Park WJ. Gene profiling during regression of pressure overload-induced cardiac hypertrophy. *Physiol Genomics*. 2007;30:1–7. doi: 10.1152/physiolgenomics.00246.2006.
8. Miana LA, Assad RS, Abduch MC, Gomes GS, Nogueira AR, Oliveira FS, Telles BL, Souto MT, Silva GJ, Stolf NA. Intermittent systolic overload promotes better myocardial performance in adult animals. *Arq Bras Cardiol*. 2010;95:364–372.
9. Perrino C, Naga Prasad SV, Mao L, Noma T, Yan Z, Kim HS, Smithies O, Rockman HA. Intermittent pressure overload triggers hypertrophy-independent cardiac dysfunction and vascular rarefaction. *J Clin Invest*. 2006;116:1547–1560. doi: 10.1172/JCI25397.
10. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986;74:1124–1136.
11. Shizukuda Y, Mallet RT, Lee SC, Downey HF. Hypoxic preconditioning of ischaemic canine myocardium. *Cardiovasc Res*. 1992;26:534–542.
12. Kim CH, Choi H, Chun YS, Kim GT, Park JW, Kim MS. Hyperbaric oxygenation pretreatment induces catalase and reduces infarct size in ischemic rat myocardium. *Pflugers Arch*. 2001;442:519–525.
13. Liu Y, Tsuchida A, Cohen MV, Downey JM. Pretreatment with angiotensin II activates protein kinase C and limits myocardial infarction in isolated rabbit hearts. *J Mol Cell Cardiol*. 1995;27:883–892.
14. Gourine AV, Molosh AI, Poputnikov D, Bulhak A, Sjöquist PO, Pernow J. Endothelin-1 exerts a preconditioning-like cardioprotective effect against ischaemia/reperfusion injury via the ET(A) receptor and the mitochondrial K(ATP) channel in the rat in vivo. *Br J Pharmacol*. 2005;144:331–337. doi: 10.1038/sj.bjp.0706050.
15. Elliott GT. Monophosphoryl lipid A: a novel agent for inducing pharmacologic myocardial preconditioning. *J Thromb Thrombolysis*. 1996;3:225–237.
16. Baumann M, Sollinger D, Roos M, Lutz J, Heemann U. Prehypertensive preconditioning improves adult antihypertensive and cardioprotective treatment. *J Pharmacol Exp Ther*. 2010;332:1121–1126. doi: 10.1124/jpet.109.161075.
17. Stansfield WE, Charles PC, Tang RH, Rojas M, Bhati R, Moss NC, Patterson C, Selzman CH. Regression of pressure-induced left ventricular hypertrophy is characterized by a distinct gene expression profile. *J Thorac Cardiovasc Surg*. 2009;137:232–8, 238e1. doi: 10.1016/j.jtcvs.2008.08.019.
18. Cuspidi C, Sala C, Negri F, Mancia G, Morganti A; Italian Society of Hypertension. Prevalence of left-ventricular hypertrophy in hypertension: an updated review of echocardiographic studies. *J Hum Hypertens*. 2012;26:343–349. doi: 10.1038/jhh.2011.104.
19. Cacciapuoti F. Molecular mechanisms of left ventricular hypertrophy (LVH) in systemic hypertension (SH)-possible therapeutic perspectives. *J Am Soc Hypertens*. 2011;5:449–455. doi: 10.1016/j.jash.2011.08.006.
20. Korhonen T, Hänninen SL, Tavi P. Model of excitation-contraction coupling of rat neonatal ventricular myocytes. *Biophys J*. 2009;96:1189–1209. doi: 10.1016/j.bpj.2008.10.026.
21. Tiede K, Melchior-Becker A, Fischer JW. Transcriptional and posttranscriptional regulators of biglycan in cardiac fibroblasts. *Basic Res Cardiol*. 2010;105:99–108. doi: 10.1007/s00395-009-0049-8.
22. Liao Y, Ishikura F, Beppu S, Asakura M, Takashima S, Asanuma H, Sanada S, Kim J, Ogita H, Kuzuya T, Node K, Kitakaze M, Hori M. Echocardiographic assessment of LV hypertrophy and function in aortic-banded mice: necropsy validation. *Am J Physiol Heart Circ Physiol*. 2002;282:H1703–H1708. doi: 10.1152/ajpheart.00238.2001.
23. Liao Y, Bin J, Asakura M, Xuan W, Chen B, Huang Q, Xu D, Ledent C, Takashima S, Kitakaze M. Deficiency of type 1 cannabinoid receptors worsens acute heart failure induced by pressure overload in mice. *Eur Heart J*. 2012;33:3124–3133. doi: 10.1093/eurheartj/ehr246.
24. Mavroudis C, Backer CL. Arterial switch after failed atrial baffle procedures for transposition of the great arteries. *Ann Thorac Surg*. 2000;69:851–857.
25. Poirier NC, Mee RB. Left ventricular reconditioning and anatomical correction for systemic right ventricular dysfunction. *Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu*. 2000;3:198–215.
26. Sekarski N, Hurni M, von Segesser LK, Meijboom EJ, Di Bernardo S. Adaptable pulmonary artery band for late arterial switch procedure in transposition of the great arteries. *Ann Thorac Surg*. 2012;94:1311–1316. doi: 10.1016/j.athoracsur.2012.04.095.
27. Bishop SP, Melsen LR. Myocardial necrosis, fibrosis, and DNA synthesis in experimental cardiac hypertrophy induced by sudden pressure overload. *Circ Res*. 1976;39:238–245.
28. Christakis GT, Joyner CD, Morgan CD, Fremes SE, Buth KJ, Sever JY, Rao V, Panagiotopoulos KP, Murphy PM, Goldman BS. Left ventricular mass regression early after aortic valve replacement. *Ann Thorac Surg*. 1996;62:1084–1089. doi: 10.1016/0003-4975(96)00533-4.
29. Walther T, Falk V, Langebartels G, Kruger M, Bernhardt U, Diegeler A, Gummert J, Autschbach R, Mohr FW. Prospectively randomized evaluation of stentless versus conventional biological aortic valves: Impact on early regression of left ventricular hypertrophy. *Circulation*. 1999;100:II6–10.
30. Dipla K, Mattiello JA, Jeevanandam V, Houser SR, Margulies KB. Myocyte recovery after mechanical circulatory support in humans with end-stage heart failure. *Circulation*. 1998;97:2316–2322.
31. Müller J, Wallukat G, Weng YG, Dandel M, Spiegelsberger S, Semrau S, Brandes K, Theodoridis V, Loebe M, Meyer R, Hetzer R. Weaning from mechanical cardiac support in patients with idiopathic dilated cardiomyopathy. *Circulation*. 1997;96:542–549.
32. Rodrigue-Way A, Burkhoff D, Geesaman BJ, Golden S, Xu J, Pollman MJ, Donoghue M, Jeyaseelan R, Houser S, Breitbart RE, Marks A, Acton S. Sarcomeric genes involved in reverse remodeling of the heart during left ventricular assist device support. *J Heart Lung Transplant*. 2005;24:73–80. doi: 10.1016/j.healun.2003.10.016.
33. Perera C, McNeil HP, Geczy CL. S100 Calgranulins in inflammatory arthritis. *Immunol Cell Biol*. 2010;88:41–49. doi: 10.1038/icb.2009.88.
34. Lim SY, Raftery MJ, Geczy CL. Oxidative modifications of DAMPs suppress inflammation: the case for S100A8 and S100A9. *Antioxid Redox Signal*. 2011;15:2235–2248. doi: 10.1089/ars.2010.3641.
35. Goyette J, Geczy CL. Inflammation-associated S100 proteins: new mechanisms that regulate function. *Amino Acids*. 2011;41:821–842. doi: 10.1007/s00726-010-0528-0.
36. Altwegg LA, Neidhart M, Hersberger M, Müller S, Eberli FR, Corti R, Roffi M, Sütsch G, Gay S, von Eckardstein A, Wischnewsky MB, Lüscher TF, Maier W. Myeloid-related protein 8/14 complex is released

by monocytes and granulocytes at the site of coronary occlusion: a novel, early, and sensitive marker of acute coronary syndromes. *Eur Heart J*. 2007;28:941–948. doi: 10.1093/eurheartj/ehm078.
37. Miyamoto S, Ueda M, Ikemoto M, Naruko T, Itoh A, Tamaki S, Nohara R, Terasaki F, Sasayama S, Fujita M. Increased serum levels and expression of S100A8/A9 complex in infiltrated neutrophils in atherosclerotic plaque of unstable angina. *Heart*. 2008;94:1002–1007. doi: 10.1136/hrt.2007.121640.
38. van Leeuwen M, Gijbels MJ, Duijvestijn A, Smook M, van de Gaar MJ, Heeringa P, de Winther MP, Tervaert JW. Accumulation of myeloperoxidase-positive neutrophils in atherosclerotic lesions in LDLR-/- mice. *Arterioscler Thromb Vasc Biol*. 2008;28:84–89. doi: 10.1161/ATVBAHA.107.154807.
39. Boyd JH, Kan B, Roberts H, Wang Y, Walley KR. S100A8 and S100A9 mediate endotoxin-induced cardiomyocyte dysfunction via the receptor for advanced glycation end products. *Circ Res*. 2008;102:1239–1246. doi: 10.1161/CIRCRESAHA.107.167544.
40. Consolo M, Amoroso A, Spandidos DA, Mazzarino MC. Matrix metalloproteinases and their inhibitors as markers of inflammation and fibrosis in chronic liver disease (Review). *Int J Mol Med*. 2009;24:143–152.
41. Schelbergen RF, Blom AB, van den Bosch MH, Slöetjes A, Abdollahi-Roodsaz S, Schreurs BW, Mort JS, Vogl T, Roth J, van den Berg WB, van Lent PL. Alarmins S100A8 and S100A9 elicit a catabolic effect in human osteoarthritic chondrocytes that is dependent on Toll-like receptor 4. *Arthritis Rheum*. 2012;64:1477–1487. doi: 10.1002/art.33495.
42. Passey RJ, Williams E, Lichanska AM, Wells C, Hu S, Geczy CL, Little MH, Hume DA. A null mutation in the inflammation-associated S100 protein S100A8 causes early resorption of the mouse embryo. *J Immunol*. 1999;163:2209–2216.
43. Kuipers MT, Vogl T, Aslami H, Jongsma G, van den Berg E, Vlaar AP, Roelofs JJ, Juffermans NP, Schultz MJ, van der Poll T, Roth J, Wieland CW. High levels of S100A8/A9 proteins aggravate ventilator-induced lung injury via TLR4 signaling. *PLoS One*. 2013;8:e68694. doi: 10.1371/journal.pone.0068694.
44. Hiroshima Y, Hsu K, Tedla N, Chung YM, Chow S, Herbert C, Geczy CL. S100A8 induces IL-10 and protects against acute lung injury. *J Immunol*. 2014;192:2800–2811. doi: 10.4049/jimmunol.1302556.
45. Ahn GO, Seita J, Hong BJ, Kim YE, Bok S, Lee CJ, Kim KS, Lee JC, Leeper NJ, Cooke JP, Kim HJ, Kim IH, Weissman IL, Brown JM. Transcriptional activation of hypoxia-inducible factor-1 (HIF-1) in myeloid cells promotes angiogenesis through VEGF and S100A8. *Proc Natl Acad Sci U S A*. 2014;111:2698–2703. doi: 10.1073/pnas.1320243111.
46. Dessing MC, Tammaro A, Pulskens WP, Teske GJ, Butter LM, Claessen N, van Eijk M, van der Poll T, Vogl T, Roth J, Florquin S, Leemans JC. The calcium-binding protein complex S100A8/A9 has a crucial role in controlling macrophage-mediated renal repair following ischemia/reperfusion. *Kidney Int*. 2015;87:85–94. doi: 10.1038/ki.2014.216.
47. Khammanivong A, Wang C, Sorenson BS, Ross KF, Herzberg MC. S100A8/A9 (calprotectin) negatively regulates G2/M cell cycle progression and growth of squamous cell carcinoma. *PLoS One*. 2013;8:e69395. doi: 10.1371/journal.pone.0069395.
48. Vega RB, Rothermel BA, Weinheimer CJ, Kovacs A, Naseem RH, Bassel-Duby R, Williams RS, Olson EN. Dual roles of modulatory calcineurin-interacting protein 1 in cardiac hypertrophy. *Proc Natl Acad Sci U S A*. 2003;100:669–674. doi: 10.1073/pnas.0237225100.
49. Bueno OF, Wilkins BJ, Tymitz KM, Glascock BJ, Kimball TF, Lorenz JN, Molkentin JD. Impaired cardiac hypertrophic response in Calcineurin Abeta -deficient mice. *Proc Natl Acad Sci U S A*. 2002;99:4586–4591. doi: 10.1073/pnas.072647999.
50. Donato R. Intracellular and extracellular roles of S100 proteins. *Microsc Res Tech*. 2003;60:540–551. doi: 10.1002/jemt.10296.
51. Bhattacharya S, Bunick CG, Chazin WJ. Target selectivity in EF-hand calcium binding proteins. *Biochim Biophys Acta*. 2004;1742:69–79. doi: 10.1016/j.bbamcr.2004.09.002.
52. Caravita S, Wu SC, Secchi MB, Dadone V, Bencini C, Pierini S. Long-term effects of intermittent Iloprost infusion on pulmonary arterial pressure in connective tissue disease. *Eur J Intern Med*. 2011;22:518–521. doi: 10.1016/j.ejim.2011.02.005.
53. Naelten G, Liu KL, Lo M. Durable improvement of renal function after perindopril withdrawal in Lyon hypertensive rats. *J Cardiovasc Pharmacol*. 2011;57:240–245. doi: 10.1097/FJC.0b013e318204bb7b.
54. Rakusan D, Kujal P, Kramer HJ, Husková Z, Vanourková Z, Vernerová Z, Mrázová I, Thumová M, Cervenka L, Vanecková I. Persistent antihypertensive effect of aliskiren is accompanied by reduced proteinuria and normalization of glomerular area in Ren-2 transgenic rats. *Am J Physiol Renal Physiol*. 2010;299:F758–F766. doi: 10.1152/ajprenal.00259.2010.
55. Guerrero EI, Ardanaz N, Sevilla MA, Arévalo MA, Montero MJ. Cardiovascular effects of nebivolol in spontaneously hypertensive rats persist after treatment withdrawal. *J Hypertens*. 2006;24:151–158.
56. Frey N, Katus HA, Olson EN, Hill JA. Hypertrophy of the heart: a new therapeutic target? *Circulation*. 2004;109:1580–1589. doi: 10.1161/01.CIR.0000120390.68287.BB.

CLINICAL PERSPECTIVE

Pathological myocardial hypertrophy is detrimental and contributes to the eventual progression to heart failure. Although myocardial ischemic preconditioning is known to be cardioprotective and is now applicable in clinical practice, it is unknown whether a similar phenomenon for hypertrophic stress and myocardial hypertrophy/heart failure, which we termed myocardial hypertrophic preconditioning, exists. In this study, we demonstrated that preconditioning by prohypertrophic factors increases the resistance of the heart to subsequent hypertrophic stress and delays progression from hypertrophy to heart failure, indicating the existence of the hypertrophic preconditioning phenomenon. We further showed that the upregulation of S100A8/A9 following the removal of transient hypertrophic stimulus contributes to the antihypertrophic and anti–heart failure effect of hypertrophic preconditioning, at least in part by suppressing the calcineurin/nuclear factor of the activated T cells pathway. These findings suggest that the induction of hypertrophic preconditioning has the potential to become a new approach to providing cardioprotection for patients with pressure overload. There are clinical clues for the phenomenon of hypertrophic preconditioning. Physical exercise can induce physiological myocardial hypertrophy and also benefit patients with heart failure, whereas, in patients with transposition of the great vessels, ventricular retraining with an adjustable banding device may lead to better survival. Further study is warranted to optimize hypertrophic preconditioning for the clinical prevention and therapy of heart failure.