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ORIGINAL RESEARCH ARTICLE

Antihypertrophic Memory After Regression of Exercise-Induced Physiological Myocardial Hypertrophy Is Mediated by the Long Noncoding RNA Mhrt779

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BACKGROUND: Exercise can induce physiological myocardial hypertrophy (PMH), and former athletes can live 5 to 6 years longer than nonathletic controls, suggesting a benefit after regression of PMH. We previously reported that regression of pathological myocardial hypertrophy has antihypertrophic effects. Accordingly, we hypothesized that antihypertrophic memory exists even after PMH has regressed, increasing myocardial resistance to subsequent pathological hypertrophic stress.

METHODS: C57BL/6 mice were submitted to 21 days of swimming training to develop PMH. After termination of exercise, PMH regressed within 1 week. PMH regression mice (exercise hypertrophic preconditioning [EHP] group) and sedentary mice (control group) then underwent transverse aortic constriction or a sham operation for 4 weeks. Cardiac remodeling and function were evaluated with echocardiography, invasive left ventricular hemodynamic measurement, and histological analysis. LncRNA sequencing, chromatin immunoprecipitation assay, and comprehensive identification of RNA-binding proteins by mass spectrometry and Western blot were used to investigate the role of *Mhrt779* involved in the antihypertrophic effect induced by EHP.

RESULTS: At 1 and 4 weeks after transverse aortic constriction, the EHP group showed less increase in myocardial hypertrophy and lower expression of the *Nppa* and *Myh7* genes than the sedentary group. At 4 weeks after transverse aortic constriction, EHP mice had less pulmonary congestion, smaller left ventricular dimensions and end-diastolic pressure, and a larger left ventricular ejection fraction and maximum pressure change rate than sedentary mice. Quantitative polymerase chain reaction revealed that the long noncoding myosin heavy chain–associated RNA transcript *Mhrt779* was one of the markedly upregulated lncRNAs in the EHP group. Silencing of *Mhrt779* attenuated the antihypertrophic effect of EHP in mice with transverse aortic constriction and in cultured cardiomyocytes treated with angiotensin II, and overexpression enhanced the antihypertrophic effect. Using chromatin immunoprecipitation assay and quantitative polymerase chain reaction, we found that EHP increased histone 3 trimethylation (H3K4me3 and H3K36me3) at the a4 promoter of *Mhrt779*. Comprehensive identification of RNA-binding proteins by mass spectrometry and Western blot showed that *Mhrt779* can bind SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 4 (Brg1) to inhibit the activation of the histone deacetylase 2 (Hdac2)/phosphorylated serine/threonine kinase (Akt)/phosphorylated glycogen synthase kinase 3β(p-GSK3β) pathway induced by pressure overload.

CONCLUSIONS: Myocardial hypertrophy preconditioning evoked by exercise increases resistance to pathological stress via an antihypertrophic effect mediated by a signal pathway of *Mhrt779* /Brg1/Hdac2/p-Akt/p-GSK3β

Key Words: exercise ■ heart failure ■ hypertrophy ■ RNA, long noncoding

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Clinical Perspective

What Is New?

- Exercise-induced physiological myocardial hypertrophy can produce a cardioprotective effect, and this cardioprotective effect continues to exist after physiological myocardial hypertrophy subsides (a phenomenon called exercise hypertrophy preconditioning).
- Exercise hypertrophy preconditioning upregulates the expression of the long noncoding RNA *Mhrt779* by increasing the 3-methylation of histone 3 at the a4 promoter of *Mhrt779*.
- Cardiac overexpression or knockdown of *Mhrt779* enhanced or weakened, respectively, the antihypertrophy effect of exercise hypertrophy preconditioning.

What Are the Clinical Implications?

- The present findings should stimulate further research in the mechanisms of exercise hypertrophy preconditioning.
- *Mhrt779* may be a potential therapeutic target for myocardial hypertrophy and heart failure in clinical practice.

Nonstandard Abbreviations and Acronyms

BW	body weight
Smarca4	SWI/SNF–related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4
Bvht	Braveheart
Carmen	cardiac mesoderm enhancer–associated noncoding RNA
Chaer	cardiac hypertrophy–associated epigenetic regulator
Chast	cardiac hypertrophy–associated transcript
Chrf	cardiac hypertrophy related factor
EHP	exercise hypertrophic preconditioning
Fendrr	fetal-lethal noncoding developmental regulatory RNA
GSK3β	glycogen synthase kinase 3β
Hdac2	histone deacetylase 2
HF	heart failure
HW	heart weight
H3K4me3	histone 3 lysine 4 trimethylation
H3K36me3	histone 3 lysine 36 trimethylation
LV	left ventricular
LVPWd	left ventricular diastolic posterior wall thickness
LVPWs	left ventricular systolic posterior wall thickness
Myh7	myosin heavy chain 7
Oe-mhrt	*Mhrt779* overexpresses
p-Akt	phosphorylated Akt
Sh-mhrt	Mhrt779 knockdown
TAC	transverse aortic constriction
TL	tibial length

Despite the availability of pharmacological therapies such as inhibitors of the renin-angiotensin-aldosterone system, sympathetic nervous system, and neprilysin, heart failure (HF) has a high morbidity and mortality. New therapeutic strategies are urgently needed. Exercise is a well-known nonpharmacological intervention capable of improving cardiovascular fitness,[1,2] and exercise training has been recommended as an important component of therapy for patients with HF.[3] However, exercise therapy is not suitable for all patients. Clarifying the underlying molecular mechanisms responsible for the benefits of exercise may provide new therapeutic targets for HF.

Pathological cardiac hypertrophy is a major independent risk factor for the development of HF.[4] In contrast, exercise-induced physiological cardiac hypertrophy is beneficial. Studies found that former athletes had significantly lower systolic blood pressure in later life than their age-matched control subjects,[5] and former elite athletes lived 5 to 6 years longer than control subjects,[6] implying that an elite athlete career during young adulthood is cardioprotective in later life, even after the physiological hypertrophy has regressed. We previously reported a phenomenon called hypertrophic myocardial preconditioning in which short-term pathological hypertrophic stress on the heart has a protective effect against subsequent hypertrophic stress and slows the progression to HF.[7] However, little research has examined the effect of physiological cardiac hypertrophy such as that induced by physical exercise on the resistance to subsequent pathological hypertrophic stress after the hypertrophy has regressed. We hypothesized that an exercise-induced antihypertrophic memory exists even after physiological hypertrophy has regressed after termination of exercise. Thus, we used swimming training in mice to test this hypothesis.

METHODS

The data, methods, and materials related to this study are available to other researchers on reasonable request.

All procedures were performed in accordance with our institutional guidelines for animal research, which conform to *the Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 85-23, revised 1996). This study was approved by the Ethics Committee of Nanfang Hospital, Southern Medical University (Guangzhou, China). The detailed methods and sequences of primers for polymerase

chain reaction (Table I in the Data Supplement) are shown in the Data Supplement.

Statistical Analysis

Quantitative data are reported as mean±SEM. Comparisons of 2 groups were performed by 2-tailed unpaired *t* tests; comparisons between multiple groups, by either 1-way or 2-way (if there were 2 factor levels) ANOVA, followed by Bonferroni correction for post hoc multiple comparisons. The overall survival of sham or transverse aortic constriction (TAC) mice was evaluated with Kaplan-Meier survival analysis. All analyses were performed with GraphPad Prism 7.0 software (GraphPad Software Inc, San Diego, CA), and $P<0.05$ was considered to be statistically significant.

RESULTS

Effects of Exercise Preconditioning on Pathological Myocardial Hypertrophy

During the 21-day swimming training, systolic blood pressure and heart rate in the Exe (exercise, swimming) group were significantly lower than in the sedentary group (Figure IA–IC in the Data Supplement). Along with the body weight (BW) increase (Figure ID in the Data Supplement), sedentary mice had an ≈10–mm Hg increase in systolic blood pressure (Figure IB in the Data Supplement). After 21 days of swimming, the ratios of heart weight (HW) to BW and HW to tibia length (TL) and the cardiomyocyte cross-sectional area (Figure IE–IH in the Data Supplement) had increased by ≈10%, and interventricular septal thickness in diastole and systole, left ventricular (LV) diastolic posterior wall thickness (LVPWd), LV systolic posterior wall thickness (LVPWs), LV ejection fraction, and LV fractional shortening had increased slightly (Table II in the Data Supplement). No changes were found in myocardial fibrosis or in hypertrophic markers of the natriuretic peptide type A (*Nppa*) and myosin heavy chain 7 (*Myh7*) genes (Figure II–IK in the Data Supplement).

Exercise has been reported to induce physiological cardiac growth and protect the heart against pressure overload,[8] but it is unclear whether this antihypertrophic effect still exists after physiological myocardial hypertrophy has regressed (exercise hypertrophic preconditioning [EHP]). At 7 days after TAC, the EHP group showed less increase in interventricular septal thickness in diastole and systole, LVPWd, LVPWs, and LV mass than the sedentary group (Figure 1A and 1B and Table III in the Data Supplement). As shown in Figure 1C through 1H, the increase in the HW:BW and HW:TL ratios, myocardial cell cross-sectional area, myocardial fibrosis, and expression of embryonic genes *Nppa* and myosin heavy chain 7 (*Myh7*) was significantly lower in TAC mice from the EHP group than in those from the sedentary group.

Effects of Exercise Preconditioning on HF Progression

We extended the post-TAC observation period to 4 weeks to test the effect of EHP on HF. We set the peak flow velocity at the aortic banding site ≥3.5 m/s as an indicator of successful aortic constriction (Figure II in the Data Supplement). In addition, the BW was associated with the progression of HF. One week after surgery, no significant difference was found in BW between sedentary and exercise TAC mice, whereas it decreased significantly at 4 weeks after surgery in sedentary TAC group (Figure III in the Data Supplement). Four weeks after TAC, 52% and 62% of mice in the sedentary group and EHP group, respectively, survived ($P<0.01$). About half of mice died of acute HF within 7 days after TAC surgery (Figure IV in the Data Supplement). Four weeks after TAC or the sham operation, echocardiography showed a significant increase in the LV wall thickness (LVPWd and LVPWs) and LV dimensions and a significant decrease in the systolic function parameters LV ejection fraction and LV fractional shortening in the TAC group compared with the sham group. Furthermore, LVPWd, LVPWs, LV end-diastolic diameter, and LV end-systolic diameter were significantly smaller and systolic function was better in the EHP TAC group than in the TAC sedentary mice (Figure 2A–2G). The LV systolic pressure, LV end-diastolic pressure, and the exponential time constant of LV relaxation (τ) were also higher in the TAC groups than in the corresponding sham groups, and maximum rates of change of LV pressure (LV dP/dtmax), minimum rates of change of LV pressure LV (dP/dtmin), and LV contractility were lower (Figure 2I–2N). LV dP/dtmax, LV dP/dtmin, and LV contractility were significantly larger in the EHP TAC mice than in the sedentary TAC mice, and LV end-diastolic pressure and τ were significantly smaller (Figure 2H–2N).

Cardiac remodeling was significantly attenuated in the EHP TAC group, as evidenced by a significantly smaller HW:BW ratio, HW:TL ratio, and cell cross-sectional area and lower mRNA levels of *Nppa* and *Myh7* and percentage of myocardial fibrosis than in the sedentary TAC group (Figure 3A–3E). The EHP group had significantly less pulmonary congestion, as evidenced by a lower lung weight:BW ratio and lung weight:TL ratio in the EHP TAC group than in the sedentary TAC group (Figure 3F and 3G).

To observe how long the antihypertrophic role of EHP can persist, we extended the start of post-TAC stress to 4 weeks after termination of exercise. We found that the blood pressure and heart rate of the mice in the EHP group were not significantly different from those in the sedentary group after 4 weeks of detraining (Figure VA–VC in the Data Supplement). At 4 weeks after TAC, the HW:BW and HW:TL ratios were still moderately smaller in the EHP group than in the sedentary

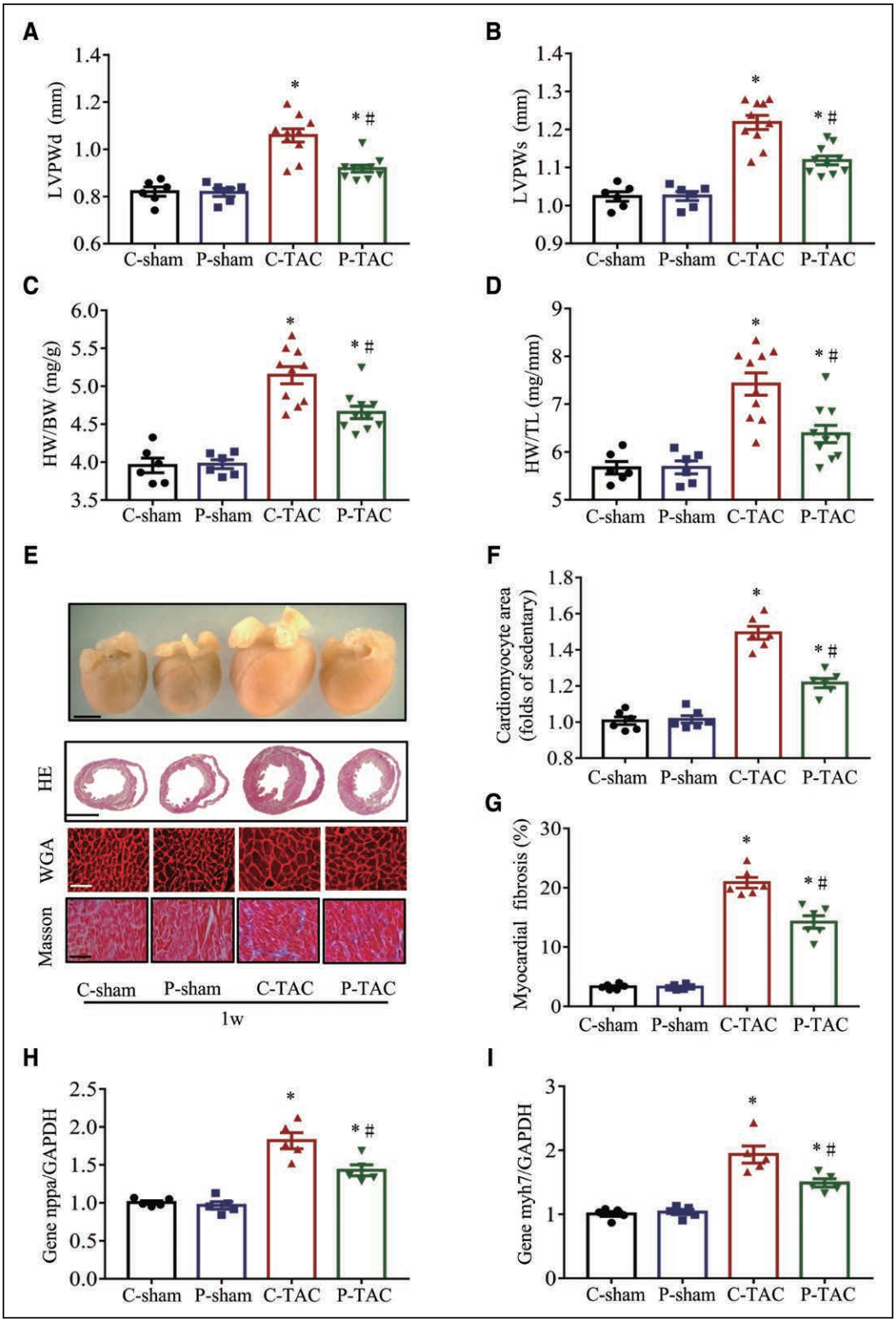

Figure 1. Exercise hypertrophic preconditioning (through swimming) attenuates pathological myocardial hypertrophy in mice at 1 week after transverse aortic constriction (TAC).

A, Echocardiographic left ventricular (LV) diastolic posterior wall thickness (LVPWd). **B**, LV systolic posterior wall thickness (LVPWs). **C**, Heart weight (HW; milligrams) to body weight (BW; grams) ratio. **D**, HW (milligrams) to tibial length (TL; millimeters) ratio. **E**, Representative macroscopic photographs of hearts (scale bar, 2 mm), hematoxylin-eosin (HE)–stained sections of hearts (scale bar, 2 mm), wheat germ agglutinin (WGA)–stained myocardial sections (scale bar, 40 μm), and Azan-Masson–stained myocardial fibrosis (scale bar, 40 μm). **F**, Quantitative analysis of cardiomyocyte cross-sectional area. **G**, Quantitative analysis of myocardial fibrosis. Quantitative polymerase chain reaction for myocardial *Nppa* (**H**) and myosin heavy chain 7 (*Myh7*; **I**). P indicates exercise preconditioning. **P*<0.01 vs corresponding sham group. #*P*<0.05 vs control (C) TAC group.

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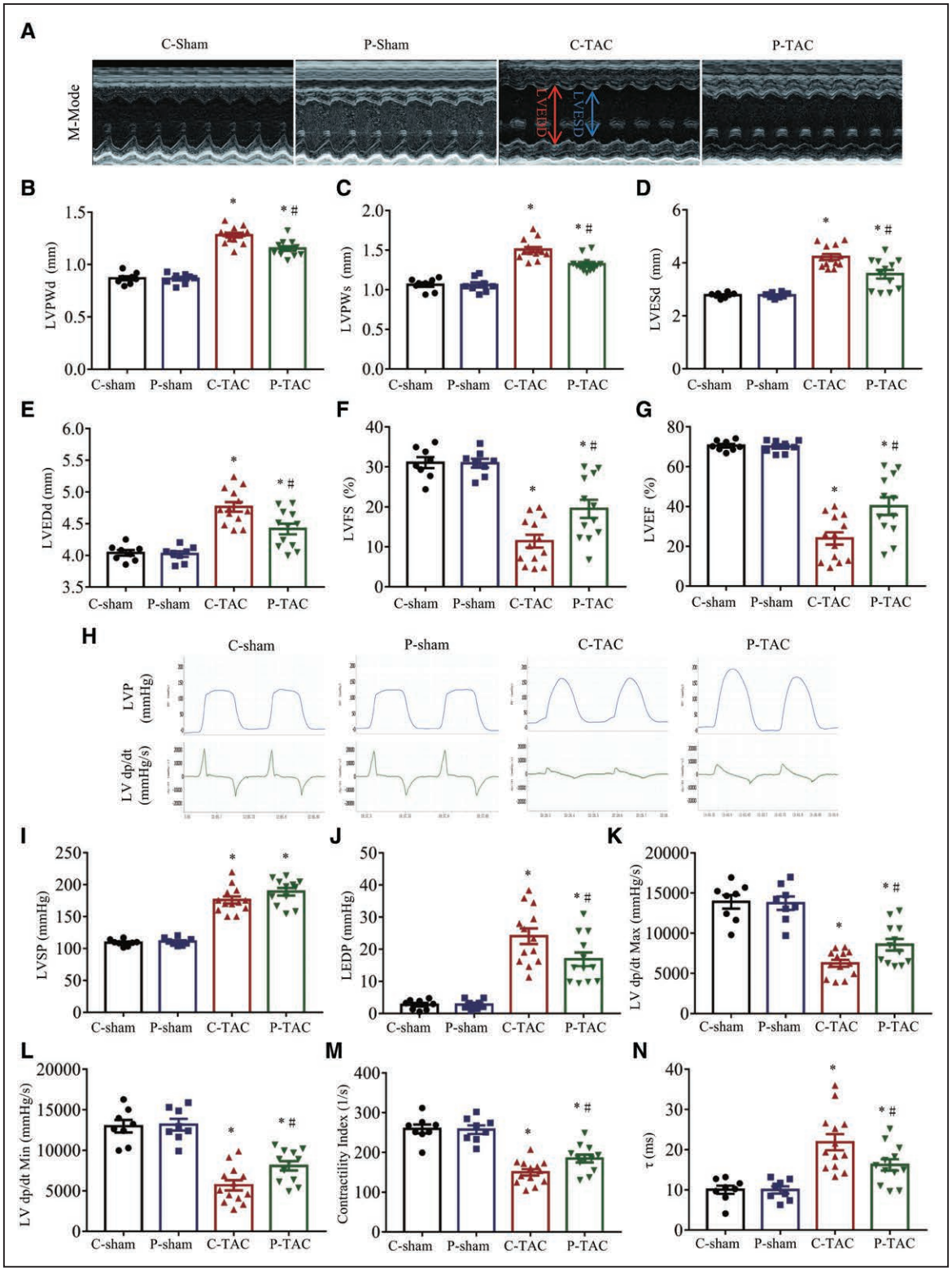

Figure 2. Effect of hypertrophic preconditioning through exercise (swimming) on cardiac remodeling and hemodynamic in mice at 4 weeks after transverse aortic constriction (TAC).

A, Representative photographs of M-mode echocardiography of left ventricle (LV). **B**, LV posterior wall diastolic thickness (LVPWd). **C**, LV posterior wall systolic thickness (LVPWs). **D**, LV end-systolic diameter (LVESd). **E**, LV end-diastolic diameter (LVEDd). **F**, LV fractional shortening (LVFS). **G**, LV ejection fraction (LVEF). **H**, Representative pressure curves obtained with a Millar pressure catheter. **I**, LV systolic pressure (LVSP). **J**, LV end-diastolic pressure (LVEDP). **K**, Maximum rising rate of LV pressure (dP/dtmax). **L**, Maximum descending rate of LV pressure (dP/dtmin). **M**, LV contractility. **N**, The exponential time constant of LV relaxation (τ). P indicates exercise preconditioning. **P*<0.01 vs corresponding sham group. #*P*<0.05 vs control (C) TAC group.

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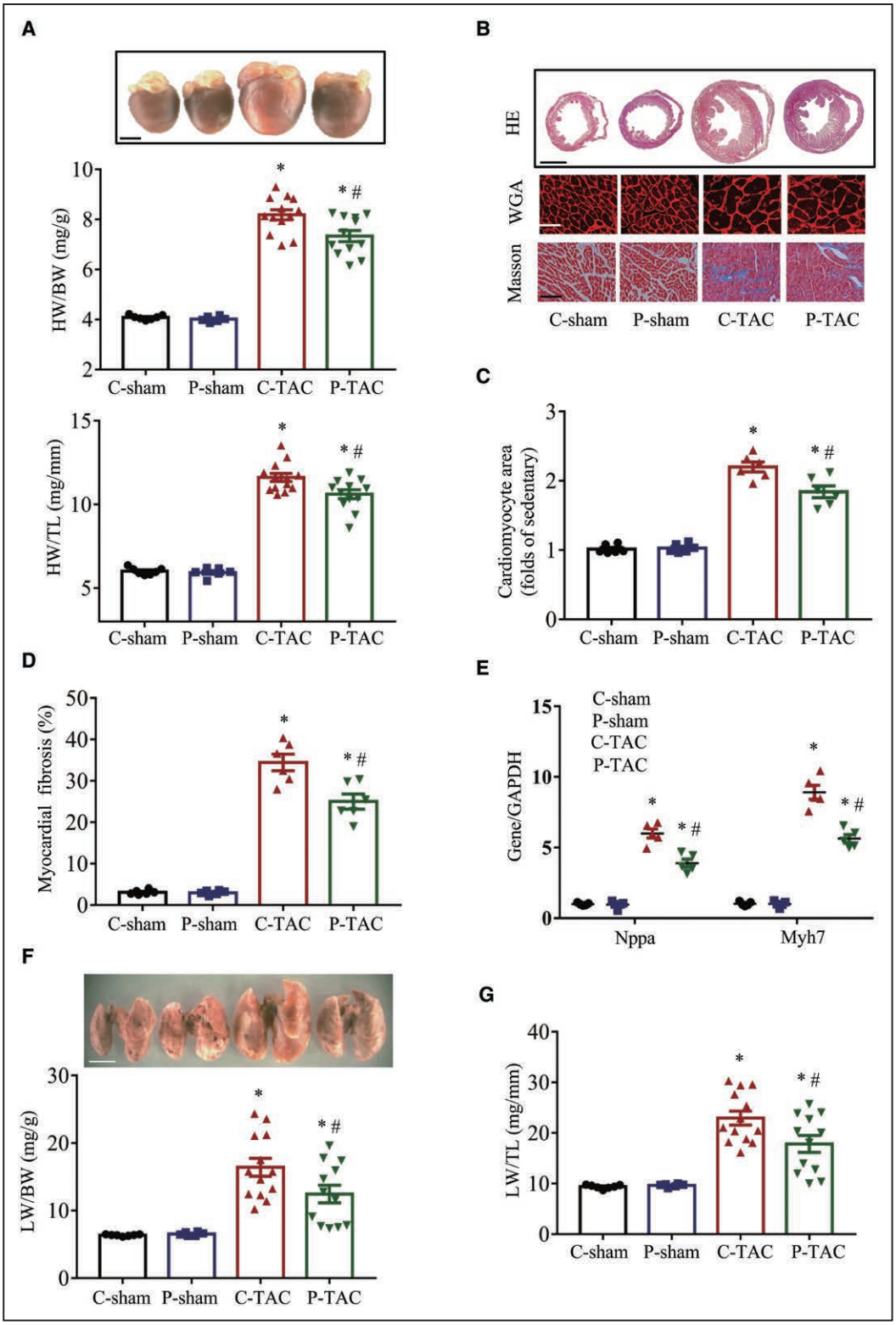

Figure 3. Effect of hypertrophic preconditioning through exercise (swimming) on myocardial hypertrophy and heart failure in mice at 4 weeks after transverse aortic constriction (TAC).

A, Representative heart pictures (scale bar=2 mm) and heart weight (HW; milligrams) to body weight (BW; grams) ratio and HW (milligrams) to tibial length (TL; millimeters) ratio. **B**, Representative pictures of cardiac cross-sections stained with hematoxylin-eosin (HE; scale bar, 2 mm), wheat germ agglutinin (WGA; scale bar, 40 μm), or Azan-Masson (scale bar, 50 μm). **C**, Quantification of cardiomyocyte cross-sectional area. **D**, Quantification of myocardial fibrosis. **E**, Real-time polymerase chain reaction for myocardial natriuretic peptide type A (*Nppa*) and myosin heavy chain 7 (*Myh7*) expression. **F**, Representative lung pictures (scale bar, 5 mm) and lung weight (LW; milligrams) to BW (grams) ratio. **G**, LW (milligrams) to TL (millimeters) ratio. P indicates exercise preconditioning. *P<0.01 vs corresponding sham group. #P<0.05 vs control (C) TAC.

group, but the difference was not statistically significant (P=0.08 and P=0.07, respectively; Figure VD and VE in the Data Supplement).

Changes in *Mhrt779* Expression After Regression of Physiological Hypertrophy

To screen the potentially involved lncRNAs in EHP, lncRNA sequencing was performed in 3 EHP mouse hearts and 3 sedentary mouse hearts (raw data are available in the Gene Expression Omnibus database with an accession number GSE161030). Heat map, volcano plot, and principal-component analysis plot are shown in Figure 4A and 4B and Figure VIA in the Data Supplement, respectively. There were 2162 lncRNAs differentially expressed between the EHP and sedentary groups (1482 and 680 lncRNAs were significantly higher and lower, respectively, in the EHP group than the sedentary group, |log2 [fold change]| >1 (P<0.05; Figure 4A and 4B). The function of lncRNA is achieved mainly by acting on the protein-encoding target gene in cis or trans. According to the principle of cis action, the function of lncRNA is related to the function of its source gene or its adjacent genes. We predicted the biological process of the lncRNAs in heat map by gene ontology analysis, and the lncRNAs involved in muscle tissue development were selected as candidate targets (Figure VIB in the Data Supplement). We found that among the candidate genes (Table IV in the Data Supplement), the lncRNA *Mhrt779*, an antisense RNA of *Myh7*, has been confirmed to have an antihypertrophic effect by Han et al.[9] The screening for lncRNAs with real-time polymerase chain reaction showed that *Mhrt779* expression was significantly upregulated in EHP mice at 1 week after termination of exercise and had returned to the baseline level at 4 weeks after termination of exercise (Figure VII in the Data Supplement and Figure 4C and 4D). However, *Mhrt779* expression was markedly downregulated in TAC mice at 1 and 4 weeks (Figure 4E). Preconditioning significantly inhibited the pressure overload–induced downregulation of *Mhrt779* (Figure 4E), suggesting a potential role of *Mhrt779* in EHP. *Mhrt779* was specifically expressed in cardiomyocytes and enriched in the nucleus (Figure VIII in the Data Supplement). To study how *Mhrt779* is upregulated during the period of EHP, we tested the histone methylation and acetylation at promoter of the *Mhrt779* in sedentary, swimming 3 weeks, swimming 3 weeks+sedentary 1 week groups (Figures IX and X in the Data Supplement). We found that histone3 acetylation (H3ac27) at the a3/4 (a3 and a4) promoters and histone3 methylation (histone 3 lysine 4 trimethylation [H3K4me3]; histone 3 lysine 36 trimethylation [H3K36me3]) at the a3 promoter were not significantly different among the 3 groups. At the a4 promoter, histone methylation levels (H3K4me3, H3K36me3) were significantly higher in the swimming 3 weeks group than in the sedentary group and were higher in the swimming 3 weeks+sedentary 1 week (EHP) group than in the swimming 3 weeks group (Figure 4F and 4G). These results were also confirmed by DNA pull down plus Western blot experiments (Figure 4H).

Effects of *Mhrt779* on the Antihypertrophic Effect of EHP

Four weeks after local injection of adeno-associated virus that overexpresses (Oe-*Mhrt779*) or knocks down (Sh-*Mhrt779*) *Mhrt779* in the myocardium, we found that *Mhrt779* was significantly upregulated or downregulated, respectively (Figure XI in the Data Supplement). In addition, efficiency of *Mhrt779* overexpression and knockdown was further confirmed by real-time polymerase chain reaction in Oe-*Mhrt779*+TAC group and Sh-*Mhrt779*+TAC group, respectively (Figure XII in the Data Supplement). Compared with the Scramble+TAC mice, the Oe-*Mhrt779*+TAC–treated mice had significantly smaller LVPWd and LVPWs (P<0.05; Figure 5A and 5B). Furthermore, LV ejection fraction and LV fractional shortening were larger (Figure 5C and 5D) and LV end-systolic diameter and LV end-diastolic diameter were slightly smaller in the Oe-*Mhrt779*–treated mice than in the Scramble+TAC mice (Figure XIII in the Data Supplement), but the differences were not statistically significant (P>0.05). The HW:BW and HW:TL ratios and cardiomyocyte cross-sectional area were smaller in the Oe-*Mhrt779*+TAC group than in the Scramble+TAC group (Figure 5E–5H). The opposite results were obtained on all echocardiography and histology parameters in Sh-*Mhrt779*+TAC mice (all P<0.05; Figure 5A–5H). Compared with the Scramble+TAC mice, the Sh-*Mhrt779*+TAC mice had significantly larger LVPWd and LVPWs (Figure 5A and 5B), significantly smaller LV ejection fraction and LV fractional shortening (Figure 5C and 5D), significantly larger LV end-systolic diameter and LV end-diastolic diameter (Figure XIII in the Data Supplement), and significantly larger HW:BW and HW:TL ratios and cross-sectional area of cardiomyocytes (Figure 5E–5H).

Mhrt779 Binding to Brg1 and Effects on the Histone Deacetylase 2/Akt/Glycogen Synthase Kinase 3β Pathway

The results of the comprehensive identification of RNA-binding proteins by mass spectrometry experiments are presented in Figure XIVA in the Data Supplement and Table V in the Data Supplement. The quality control report confirmed that the products of the probes could be used for subsequent mass spectrometry (Figure XIVB in the Data Supplement). Comprehensive identification of RNA-binding proteins by mass spectrometry identified 31 and 21 proteins specifically enriched in

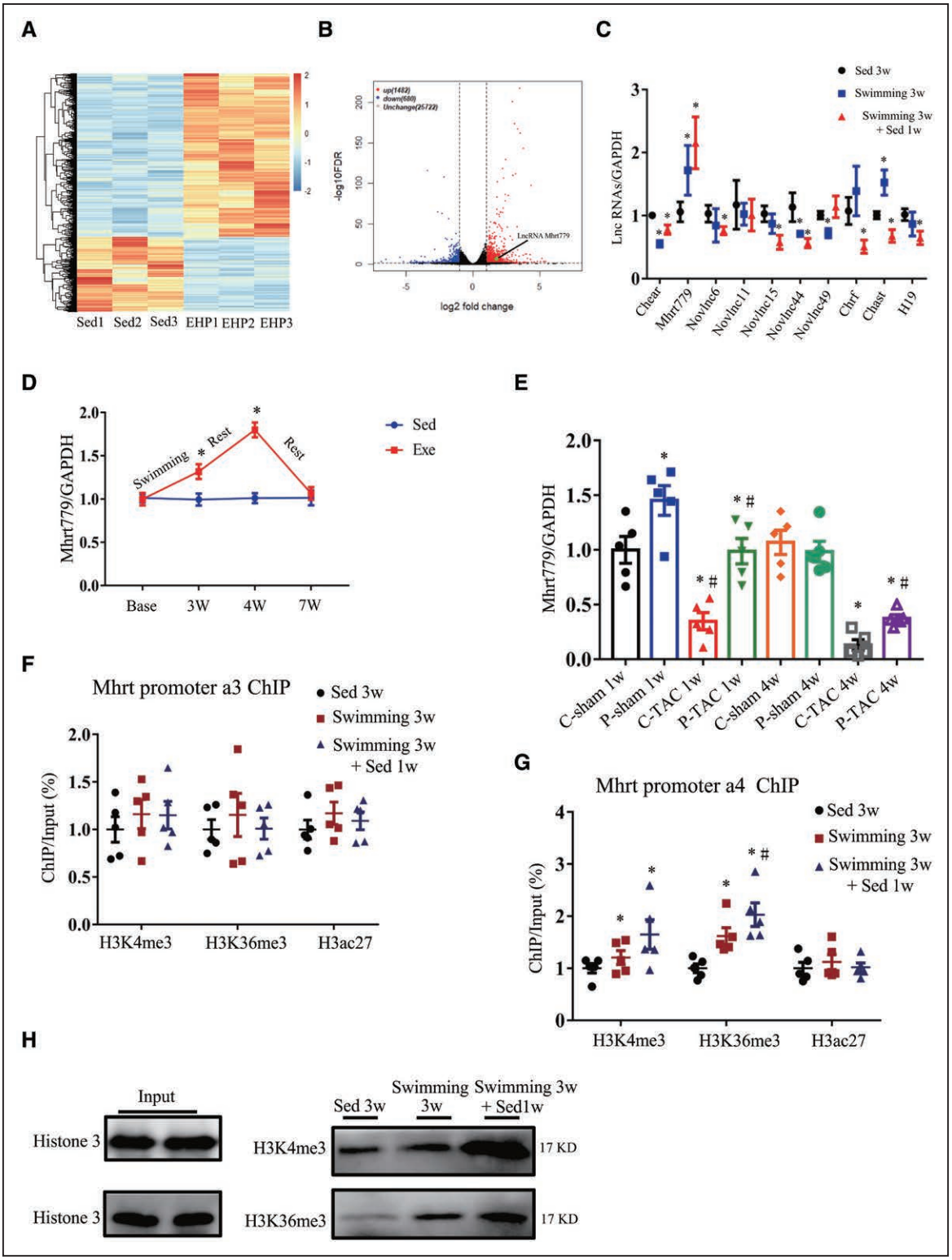

Figure 4. Effect of hypertrophic preconditioning through exercise (swimming) on the myocardial expression of Mhrt779 in adult mice.

A, Heat map of the differentially expressed lncRNAs in mouse heart tissues from sedentary (Sed) and exercise hypertrophic preconditioning (EHP) groups (n=3 in each group), Scale is $\log_2$. Statistical criteria from DESeq analysis were ≥2-fold change and $P<0.05$. Top half of each heat map is the cluster of genes that are upregulated with EHP. Bottom half of each heat map shows the genes that are downregulated with EHP. **B**, Volcano plot demonstrating magnitude and significance of lncRNAs in both the EHP and Sed groups. lncRNAs upregulated by EHP vs sedentary are plotted in red. lncRNAs downregulated by EHP vs sedentary are plotted in blue. *Mhrt779* was called out (green point) in the volcano plot. **C**, Real-time polymerase chain reaction results showing effect of exercise on 10 lncRNAs in mouse heart. *$P<0.05$ vs Sed 3 weeks group; n=6 in each group. **D**, Time course of *Mhrt779* expression in response to exercise and after exercise. *$P<0.05$ vs Sed group; n=12 in each group. (*Continued*)

Figure 4 Continued. E, Influence of exercise preconditioning on myocardial *Mhrt779* expression in mice subjected to transverse aortic constriction (TAC) at 1 or 4 weeks; n=5 in each group, **P*<0.05 vs control (C) sham at 1 week. #*P*<0.05 vs C-TAC at 4 weeks. **F** and **G**, Chromatin immunoprecipitation assay (ChIP)–quantitative polymerase chain reaction analysis of *Mhrt779* promoter using antibodies against histone 3 lysine 4 trimethylation (H3K4me3), histone 3 lysine 36 trimethylation (H3K36me3), and acetyl-histone 3 lysine 27 (H3ac27) in adult mouse heart tissue. **P*<0.05 vs sedentary group. #*P*<0.05 vs swimming 3 weeks+sedentary 1 week group. **H**, Western blotting analysis of histone methylase activity specific for histone 3 at *Mhrt779* promoter a4 after DNA pull down. EHP indicates exercise 3 weeks+sedentary 1 week; Exe, swimming for 21 days; and Sed, sedentary for 21 days.

the CHIRP lysate from EHP and TAC mice, respectively, but only 2 proteins enriched in the lysate from sedentary mice (Figure XIVC and Tables VI–VIII in the Data Supplement). Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis showed that most of these proteins are involved in hypertrophic and dilated cardiomyopathy (Figure XIVD and XIVE in the Data Supplement). Fourteen proteins were coincidently enriched in the CHIRP lysate of both EHP and TAC mouse hearts but not enriched in sedentary mouse hearts. On the basis of the responding genes, we predicted the biological process by gene ontology analysis (Figure 6A). In this study, we focused on the hypertrophic pathway related to the Brg1 protein (gene: SWI/SNF–related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4 [*Smarca4*]). Using CHIRP-Western blot, we validated that *Mhrt779* could bind to Brg1 (Figure 6B) and that Brg1 could be coimmunoprecipitated by Hdac2 (histone deacetylase 2) protein in the TAC hearts but not in the sham hearts. In addition, Oe-*Mhrt779* suppressed myocardial Brg1 protein expression in TAC mice but did not affect the binding of Brg1 and Hdac2 in the hearts of the sham and TAC mice (Figure 6D). Using Western blot, we further noted that TAC treatment significantly increased myocardial protein expression of Hdac2, phosphorylated Akt (p-Akt), and phosphorylated glycogen synthase kinase 3β (p-GSK3β) and that these changes were markedly blocked in EHP mice (Figure 6E). Similarly, in EHP mice, the overexpression of *Mhrt779* exerted a significant inhibitory effect on TAC-induced activation of the Hdac2/Akt/GSK3β pathway (Figure 6F), whereas silencing of *Mhrt779* enhanced activation of the Hdac2/Akt/GSK3β pathway (Figure 6G).

Effects of *Mhrt779* on Angiotensin II–Induced Cardiomyocyte Hypertrophy

We confirmed that Ad-*Mhrt779* was successfully transfected into neonatal rat cardiomyocytes, which hardly expressed *Mhrt779* at baseline (Figure XVA and XVB in the Data Supplement). *Mhrt779* overexpression significantly suppressed myocyte hypertrophy and hypertrophic markers (*Nppa*, *Myh7*) caused by angiotensin II in neonatal rat cardiomyocytes (Figure 7A and 7B). Western blot results showed that the angiotensin II–activated Hdac2/Akt/GSK3β pathway was blocked by *Mhrt779* overexpression (Figure 7C). Neonatal mouse cardiomyocytes natively express *Mhrt779*, and we noted that *Mhrt779* was reduced by 61% in Ad-sh*Mhrt779*–transfected neonatal mouse cardiomyocytes (Figure XVC and XVD in the Data Supplement). In angiotensin II–stimulated neonatal mouse cardiomyocytes, treatment with Ad-sh*Mhrt779* significantly increased myocyte cross-sectional area, expression of the genes *Nppa* and *Myh7*, and expression of the proteins Hdac2, p-Akt, and p-GSK3β (Figure 7D–7F).

DISCUSSION

Previous studies have demonstrated that exercise has an inhibitory effect on pathological myocardial hypertrophy in the presence of physiological hypertrophy,[8,10,11] but it is unclear whether an antihypertrophic memory exists after the regression of physiological hypertrophy attributable to termination of exercise. This study provides the first evidence of an antihypertrophic memory induced by EHP, a phenomenon we called exercise-induced myocardial hypertrophy preconditioning. We found that the antihypertrophic memory of EHP in mice was still present 4 weeks after termination of exercise. By using a biological screening approach, we noted that the cardiac-specific lncRNA *Mhrt779* made important contributions to the antihypertrophic effect of EHP by influencing a signal pathway of Hdac2/p-Akt/p-GSK3β (Figure 8).

Increasing evidence has demonstrated multiple benefits of physical exercise, making it a therapeutic modality for patients with a variety of chronic diseases such as HF,[12] fatty liver,[13] and stroke.[14] It is well established that regular exercise improves quality of life and increases the life span.[15] Furthermore, aerobic exercise is recognized as a central component of cardiac rehabilitation because it reduces morbidity and mortality in patients with cardiovascular disease.[16,17] Thijssen et al[18] reported that exercise induces a cardioprotective preconditioning, providing early cardioprotection for 2 to 3 hours and a more robust and longer period of protection after 24 hours that persists for several days. In the Lennon et al[19] study, short-term exercised animals kept a higher cardiac work under global ischemia/reperfusion at 1, 3, and 9 days after exercise termination, and the exercise-induced cardioprotection vanished at 18 days after exercise cessation. Furthermore, Calvert et al[20] showed that voluntary exercise reduces myocardial injury in mice after a 4-week training period and that these protective

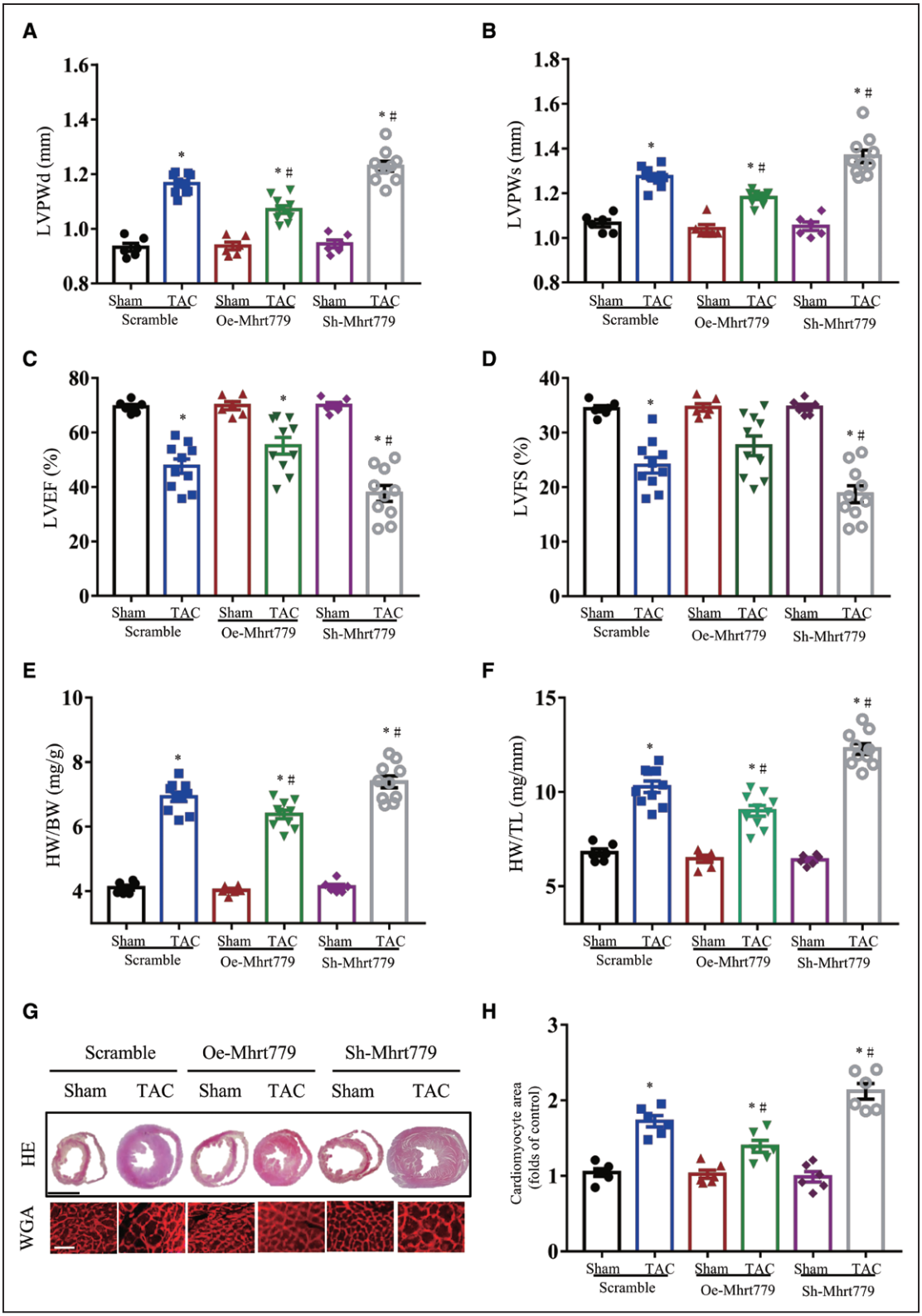

Figure 5. Effect of overexpression or silencing of Mhrt779 on myocardial hypertrophy induced by transverse aortic constriction (TAC).

A and **B**, Echocardiographic left ventricular (LV) posterior wall thickness at systole (LVPWs) and diastole (LVPWd). **C**, Echocardiographic LV ejection fraction (LVEF). **D**, Echocardiographic LV shortening fraction (LVFS). **E**, Heart weight (HW; milligrams) to body weight (BW; grams) ratio. **F**, HW (milligrams) to tibial length (TL) ratio. **G**, Representative pictures of cardiac cross-sections stained with hematoxylin-eosin (HE; first row, scale=2 mm) or wheat germ agglutinin (WGA; second row, scale, 40 μm), **H**, Quantitative analysis of cardiomyocyte cross-sectional area. *P<0.01 vs corresponding sham group. #P<0.05 vs adeno-associated virus (AAV)–*Mhrt779* overexpression (Oe-*Mhrt779*)+TAC group. Sh-*Mhrt* indicates *Mhrt779* knockdown.

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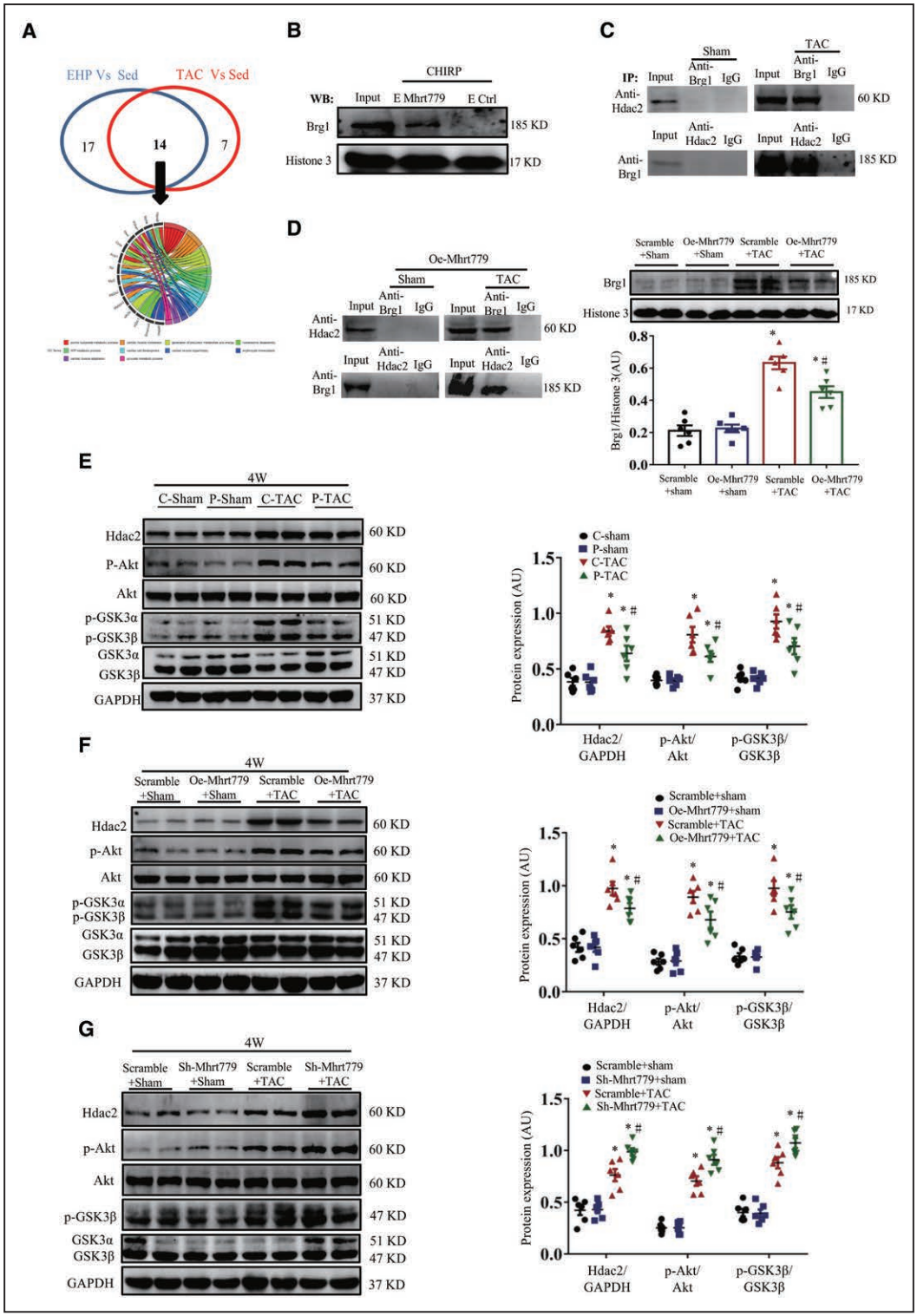

Figure 6. Identification of proteins bound by Mhrt779 and the effect of Mhrt779 on activation of the Hdac2 (histone deacetylase 2)/Akt/glycogen synthase kinase 3β (GSK3β) signal pathway in mice.

A, Gene ontology analysis of 14 functional proteins bound by *Mhrt779*. **B**, CHIRP-Western blot confirmation of Brg1 binding to *Mhrt779* in the nucleus. **C**, Coimmunoprecipitation of Brg1 and Hdac2 in hearts from sham or transverse aortic constriction (TAC) mice. **D**, Effects of *Mhrt779* overexpression (Oe-*Mhrt779*) on the expression of Brg1 protein and on the binding between Hdac2 and Brg1 in hearts from sham or TAC mice. **E**, At 4 weeks, the TAC group showed increased myocardial protein expression of Hdac2, phosphorylated Akt (p-Akt), and phosphorylated GSK3β (p-GSK3β), but expression of these proteins was blocked in mice with exercise hypertrophic preconditioning and TAC (P-TAC group). **P*<0.01 vs corresponding sham group. #*P*<0.05 vs control TAC group (C-TAC). **F**, Effect of adeno-associated virus (AAV) with Oe-*Mhrt* on Western blot results of Hdac2, p-Akt, and p-GSK3β. **P*<0.01 vs corresponding sham group. #*P*<0.05 vs control virus (Scramble)+TAC group. **G**, Effect of *Mhrt779* silencing with AAV (Sh-*Mhrt*) on Western blot results of Hdac2, p-Akt, and p-GSK3β. **P*<0.01 vs corresponding sham group. #*P*<0.05 vs Scramble+TAC group. H3 indicates histone 3.

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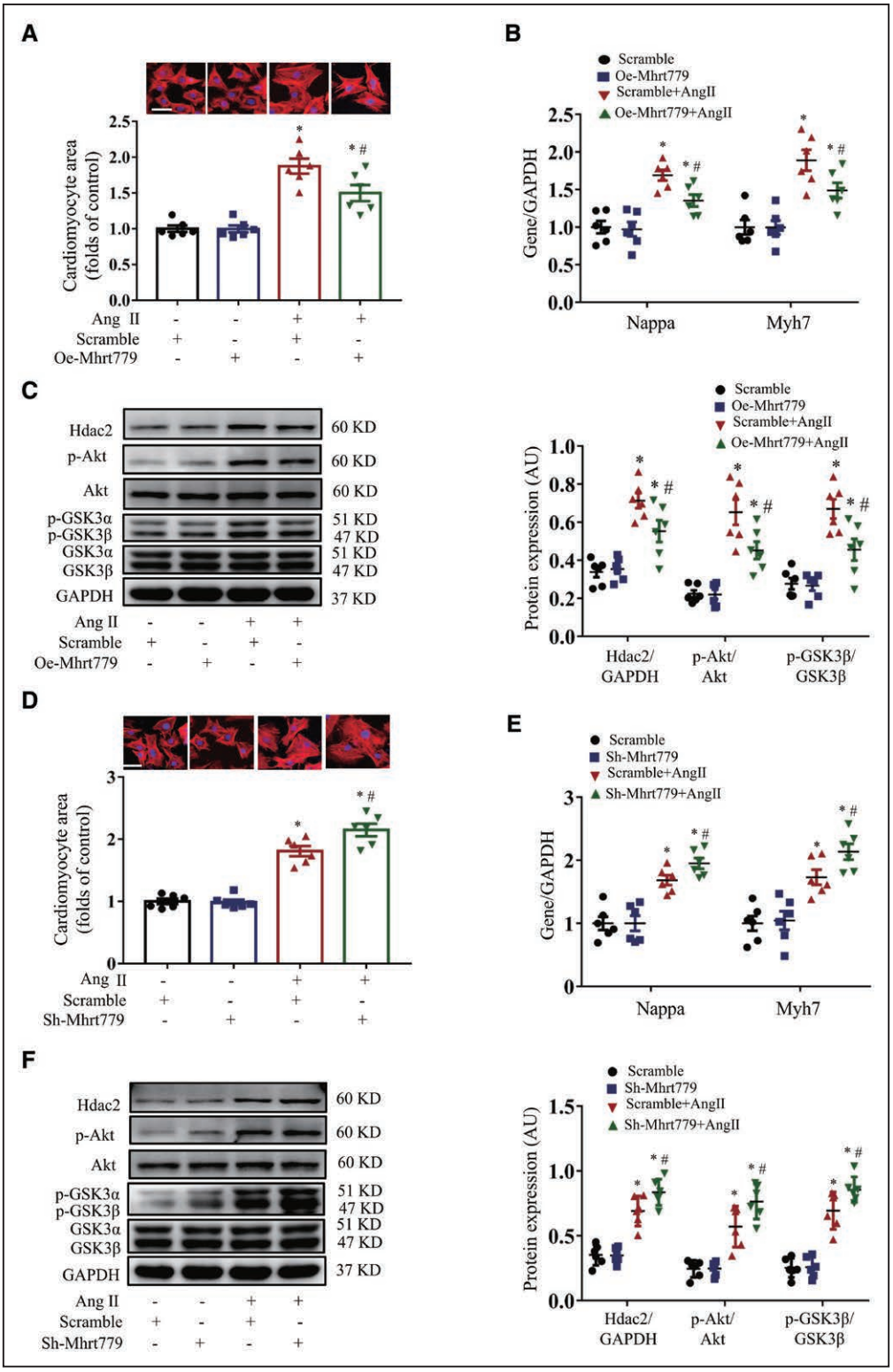

Figure 7. Effects of silencing or overexpression of Mhrt779 on angiotensin II (Ang II)–induced cardiomyocyte hypertrophy in neonatal rat cardiomyocytes (NRCMs) or neonatal mouse cardiomyocytes (NMCMs).

A, Surface area of Ang II–stimulated NRCMs in the presence or absence of *Mhrt779* overexpression with adenovirus (Oe-*Mhrt*). **B**, Effect of Oe-*Mhrt* on Ang II–induced expression of *Nppa* and *Myh7*. **C**, Oe-*Mhrt* suppressed Ang II–induced activation of the Hdac2 (histone deacetylase 2)/Akt/glycogen synthase kinase 3β (GSK3β) signal pathway. **D**, Surface area of Ang II–stimulated NMCMs in the presence or absence of *Mhrt779* silencing with adenovirus (Sh-*Mhrt*). **E**, Effect of Sh-*Mhrt* on Ang II–induced expression of *Nppa* and *Myh7*. **F**, Sh-*Mhrt* augmented Ang II–induced activation of the Hdac2/Akt/GSK3β signal pathway; n=6 in each group. **P*<0.01 vs corresponding sham group. #*P*<0.05 vs Ang II group.

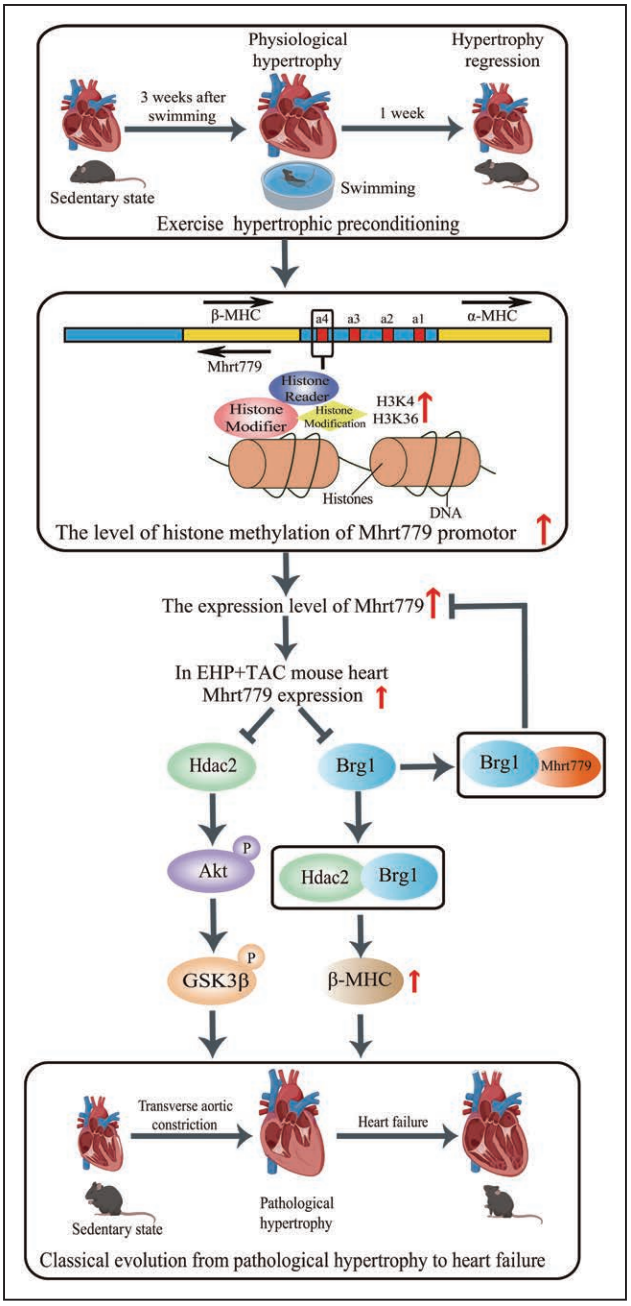

Figure 8. Illustration of exercise-induced myocardial hypertrophic preconditioning (EHP).
After swimming training for 3 weeks, the mice developed physiological myocardial hypertrophy (PMH), which had regressed 1 week after stopping swimming. EHP upregulated the expression of lncRNA *Mhrt779* by increasing the trimethylation of histone 3 at a4 promoter of *Mhrt779* even after PMH has regressed. By binding to Brg1/Hdac2 in transverse aortic constriction (TAC) mouse heart, *Mhrt779* downregulated Brg1 and inhibited the activation of the Hdac2 (histone deacetylase 2)/Akt/ glycogen synthase kinase 3β (GSK3β) signaling pathway and consequently attenuated pathological myocardial hypertrophy. Brg1 could bind Hdac2 at *Mhrt779* promoter to induce β-myosin heavy chain (β-MHC) and could inhibit *Mhrt779* via feedback H3K4me3 , histone 3 lysine 4 trimethylation; H3K36me3, histone 3 lysine 36 trimethylation; *Smarca4*, SWI/SNF–related, matrix-associated, actin-dependent regulator of chromatin, subfamily a, member 4; ↑, promotion or upregulation; and ⊥, inhibition.

effects can be sustained for at least 1 week after the cessation of the training. Kettunen et al[6] investigated life expectancy among former elite athletes and control subjects with a median follow-up time of 50 years. They reported that the hazard risk for ischemic heart disease and stroke was ≈30% to 50% lower and median life expectancy was 6.2 years higher in the former endurance sports athletes than in the controls. Although there are no reports to clarify whether EHP exerts cardioprotection to myocardial ischemia or ischemia/reperfusion injury, mortality from ischemic heart disease was lower in former athletes than in control subjects.[21] These findings indicate the existence of long-lasting beneficial effects of exercise on the cardiovascular system. However, it is unclear whether this beneficial exercise "memory" contributes to inhibiting pathological myocardial hypertrophy. Our study revealed that in mice the antihypertrophic effect of EHP induced by swimming, an endurance sport, may persist for 4 weeks after the end of exercise (Figure VD and VE in the Data Supplement).

Physical exercise is not a feasible treatment modality for patients with loss of locomotion. Thus, understanding the key molecular mechanisms responsible for the beneficial memory of EHP is of significant importance to identify novel therapeutic targets. Although exercise-induced physiological cardiac hypertrophy is believed to be cardioprotective, the underlying mechanisms are not well understood. In the presence of exercise-induced myocardial hypertrophy, exercise was reported to protect against myocardial ischemia/reperfusion injury by mediating miR-222 and miR-17-3p[22,23] and against pressure overload–induced myocardial hypertrophy by mediating PI3K-p110α,[10] CCAAT/enhancer binding protein,[8] and heat shock factor 1 and nuclear factor-κB p65.[11] The mechanisms of antihypertrophic memory after the regression of physiological cardiac hypertrophy are not yet known. The lncRNA sequencing analysis could provide sufficient clues, and in this study, we focused on the role of lncRNA *Mhrt779* in exercise preconditioning.

Recent studies highlighted that lncRNAs play an important role in cardiac remodeling. As we reviewed elsewhere,[24] the lncRNAs *Mhrt779*, Braveheart (*Bvht*), cardiac mesoderm enhancer–associated noncoding RNA (*Carmen*), fetal-lethal noncoding developmental regulatory RNA (*Fendrr*), cardiac hypertrophy–associated transcript (*Chast*),[25] cardiac hypertrophy–associated epigenetic regulator (*Chaer*),[26] cardiac hypertrophy related factor (*Chrf*),[27] *H19*,[28] and the heart-specific lncRNAs of Novlnc6, 11, 15, 44, and 49[29] were reported to be involved in myocardial hypertrophy. However, studies are needed to clarify whether these lncRNAs play a role in the inhibitory effect of exercise preconditioning on pathological cardiac hypertrophy. We found that 1 week after swimming was terminated the prohypertrophic lncRNAs *Chaer*, *Chrf*, and *Chast* were significantly downregulated and antihypertrophic lncRNA *Mhrt779* was significantly

upregulated, and these changes persisted for several weeks. Although the lncRNAs *Chaer*, *Chrf*, and *Chast* may contribute to the antihypertrophic effect of exercise preconditioning, in this study, we focused on the role of *Mhrt779* in swimming-induced antihypertrophy. Mhrt779 is highly enriched in the nuclear fraction of cardiomyocytes and is downregulated by pressure overload,[9] as validated in our study.

Overexpression of *Mhrt779* has been reported to attenuate pressure overload–induced myocardial hypertrophy,[9] but it is unclear whether *Mhrt779* is associated with exercise preconditioning. In this study, by performing lncRNA sequencing analysis and quantitative polymerase chain reaction verification, we found that lncRNA *Mhrt779* was significantly upregulated in the EHP group. Chromatin immunoprecipitation assay–quantitative polymerase chain reaction and DNA pull-down experiments indicated that EHP significantly increased the histone methylation (H3K4me3 and H3K36me3) levels at the a4 promoter of *Mhrt779*. This epigenetic change should have contributed to the upregulation of *Mhrt779* expression in EHP mice. We also noted that overexpression or silencing of *Mhrt779* enhanced or attenuated, respectively, the antihypertrophic effect of exercise preconditioning.

To clarify the potential mechanisms of *Mhrt779* mediation of antihypertrophy, we screened for proteins capable of binding *Mhrt779*. We identified 40 such proteins, most of which are associated with energy metabolism and cardiomyopathy. Brg1, an ATPase subunit of the SWI/SNF chromatin remodeling complex, was 1 of 14 proteins with a higher binding rate to *Mhrt779* in the EHP group than in the sedentary or TAC group and in the TAC group than in the sedentary group. By binding to the helicase domain of Brg1, *Mhrt779* is able to prevent Brg1 from recognizing its genomic DNA targets,[9] It was reported that Brg1 protein and *Mhrt779* could directly coimmunoprecipitate without involving other factors,[9] and Brg1 coimmunoprecipitated with Hdac2 was detectable in TAC rather than sham-treated hearts,[30] consistent with our results. Han et al[9] reported that Brg1 transfection caused a 50% reduction of *Mhrt779* promoter activity, and such a reduction was abolished by trichostatin-A, an Hdac inhibitor. In the pressure-overload heart, we also confirmed the formation of Brg1/*Mhrt779* and Brg1/Hdac2 complexes and found that Oe-*Mhrt779* downregulated Brg1/Hdac2/p-Akt/GSK3β but did not affect the formation of Brg1/Hdac2 complex. Previous studies have provided firm evidence that Hdac2, Akt, and GSK3β are components of a regulatory pathway in myocardial hypertrophy.[31–34] Brg1 can bind Hdac2 at its promoter of *Myh6* or *Myh7* to activate the fetal gene *Myh7*,[30] whereas the *Myh6*-to-*Myh7* isoform shift is a maladaptive response under the situation of myocardial hypertrophy and HF.[35] Taken together, Brg1/Hdac2/Akt/GSK3β are downstream of *Mhrt779*, whereas Brg1 exerts a feedback inhibition on *Mhrt779* and activates Hdac2 through complex formation.

In this study, we found that the antihypertrophic effect tends to disappear after 4 weeks of detraining when the exercise-induced lower blood pressure and heart rate were restored (Figure VA–VC in the Data Supplement). It would be interesting to clarify the beneficial molecular memory induced by different degrees of exercise (with/without physiological LV hypertrophy) and whether regression of a larger physiological LV hypertrophy would induce a longer memory of antihypertrophy. Considering that exercise preconditioning memory is not permanent, regular or intermittent physical exercise should be recommended to obtain persistent cardioprotection. In addition to the effects of *Mhrt779*, some myokines or alteration of hematopoietic progenitor cells induced by exercises could also lead to cardioprotection.[36] Therefore, identifying other mechanisms of exercise preconditioning would provide additional new therapeutic targets to treat cardiac hypertrophy and HF.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Materials

Expanded Methods
Data Supplement Figures I–XV
Data Supplement Tables I–VIII
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