Original Research *Highlight article*

Reduced expression of transmembrane protein 43 during cardiac hypertrophy leads to worsening heart failure in mice

Yang Gu, Yi-Ren Yao, Ying Ding and Xi-Wen Zhang

Department of Cardiology, The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University, Huai'an 223300, P.R. China
Corresponding author: Xi-Wen Zhang. Email: hayyzhxw@njmu.edu.cn

Impact Statement

Heart failure is a global health problem that affects approximately 1% to 2% of the adult population worldwide. Cardiac hypertrophy is an early pathological event in heart failure. However, methods for protecting against the progression of cardiac hypertrophy to heart failure are currently limited, and the mechanism underlying the cardioprotective effect of transmembrane protein 43 remains unclear. This study demonstrates that TMEM43 deficiency increases nuclear factor kappa B (NF-κB) activation in mouse hearts post-aortic banding (AB), while TMEM43 over-expression reduces NF-κB activation in cardiomyocytes upon AngII stimulation. This suggests that targeting TMEM43 may protect against pressure overload–induced cardiac hypertrophy and may aid the prevention and treatment of heart failure.

Abstract

Transmembrane protein 43 (TMEM43), a member of the transmembrane protein subfamily, was found to be associated with arrhythmogenic right ventricular cardiomyopathy. However, its role in cardiac hypertrophy has not been elucidated. Here, we used a pressure overload–induced cardiac hypertrophy model to explore the role of TMEM43 in heart failure. Mice were subjected to aortic banding (AB) to induce cardiac hypertrophy. The mice were also randomly selected to receive injection of adeno-associated virus 9 (AAV9)-shTMEM43 to knockdown TMEM43 in cardiomyocytes or control AAV9 (ScRNA). Four weeks after AB, the mice were subjected to echocardiography to evaluate cardiac function. Neonatal rat cardiomyocytes (NRCMs) were stimulated with angiotensin II (AngII, 1 μM) and transfected with an adenovirus to over-express TMEM43. We found that TMEM43 was downregulated in mouse hearts and cardiomyocytes poststimulation. Mice with TMEM43 knockdown showed worsening heart failure accompanied by deteriorating cardiac function and exacerbated cardiac hypertrophy and fibrosis at 4 weeks post-AB. NRCMs over-expressing TMEM43 exhibited an ameliorated hypertrophic response. Moreover, we found that TMEM43 deficiency increased nuclear factor kappa B (NF-κB) activation in mouse hearts post-AB, while TMEM43 over-expression reduced NF-κB activation in cardiomyocytes upon AngII stimulation. Thus, we conclude that reduced expression of TMEM43 during cardiac hypertrophy leads to worsening heart failure in mice.

Keywords: Transmembrane protein 43, cardiac hypertrophy, heart failure, NF-κB

Experimental Biology and Medicine **2023; 248: 1437–1445. DOI: 10.1177/15353702231191111**

Introduction

Heart failure is a global health problem that affects approximately 1% to 2% of the adult population worldwide.[1] It is a systemic disease and the terminal stage of cardiovascular disease after heart injury.[2] Cardiac hypertrophy is an early pathological event in heart failure. During this process, neurohumoral dysfunction, dysregulation between cardiac cells, and the activation of molecules in cardiac cells lead to cardiac volume overload, increased sympathetic activity, circulatory redistribution, and clinical signs and symptoms of cardiac exhaustion.[3] It has been confirmed that a variety of inflammatory signaling pathways play an important role in the process of myocardial hypertrophy.[4] Among them, the nuclear factor kappa B (NF-κB) pathway is activated during the progression of various cardiovascular diseases to heart failure, leading to myocarditis, promoting myocardial injury and accelerating the progression of heart failure.[5] Therefore, targeting the NF-κB pathway is expected to be a new therapeutic approach for heart failure.

Transmembrane protein 43 (TMEM43), a member of the transmembrane protein subfamily, was initially found to promote cancer progression in many cancer cells.[6,7] In human pancreatic cancer, the expression of TMEM43 is increased, and TMEM43 is associated with poor overall survival.[7] TMEM43 was found to be a crucial component of the epidermal growth factor receptor signaling pathway in inducing NF-κB activation, thus promoting cancer cell proliferation, survival, migration, and epithelial-to-mesenchymal transition.[8] Recently, TMEM43 was found to be associated with arrhythmogenic right ventricular cardiomyopathy. Haywood AF *et al*.[9] found that a TMEM43 missense

mutation that causes arrhythmogenic cardiomyopathy in patients from the UK and Canada. Zheng G also found that the TMEM43-S358L mutation leads to increased activation of NF-κB signaling in heart tissues.[10] Thus, we hypothesized that TMEM43 may play a role in cardiac hypertrophy and heart failure. In this study, we used aortic banding to induce cardiac hypertrophy and heart failure in mice to explore the role and mechanism of TMEM43 in heart failure.

Materials and methods

Animals and animal model

C57BL6J mice were obtained from and raised at the SPF Laboratory Animal Center of Nanjing Medical University. All animal experiments were approved by the Institutional Animal Care and Use Committee of Nanjing Medical University (Nanjing, China).

To construct the mouse aortic banding (AB) model, mice (aged 8–10 weeks, 23.5–27.5 g) were subjected to aortic banding as previously described.[11] Briefly, the mice were anesthetized with sodium pentobarbital and placed in the left lateral decubitus position after skin preparation. The mouse skin was cut, and the mouse aorta was exposed at the third to fourth costal margins. A 27G needle was placed parallel to the aorta, and the aorta was ligated. After the ligation was successful, the needle was removed. In the sham operation group, the aorta was exposed but not ligated. The mouse skin was sutured layer by layer, and analgesics were administered 3 days after the operation. To knockdown TMEM43, mice were injected with adeno-associated virus 9 (AAV9)-shTMEM43 (from Vigene Bio-Tek, Shanghai, China) or control AAV9 (ScRNA) 1 week before AB.

AAV vector

Recombinant AAV9-shTMEM43 and control AAV9-scRNA, which contained a shortened version of the Troponin T (TnT) promoter to allow delivery to cardiomyocytes, were constructed by Vigene Bioscience Company (Shanghai, China).[12] Mice were injected with AAV9-shTMEM43 or AAV9-scRNA (5.0–6.5 × 1013 VG/mL, 60–80 μL in total) through the tail vein 1 week before AB or sham surgery.

Echocardiographic evaluation

Transthoracic echocardiography was performed as previously described.[13,14] Mice were anesthetized by 1.5% isoflurane. To obtain M-mode echocardiography data, we performed echocardiography with a 10 MHz linear-array ultrasound transducer. The following parameters were evaluated to assess cardiac function: left ventricle (LV) end-diastolic dimension (LVIDd), end-systolic dimension (LVISd), LV ejection fraction (LVEF), and fractional shortening (LVFS). There were 10 mice in each group.

Hematoxylin and eosin and picrosirius red staining

To observe cardiac hypotrophy and fibrosis, histological sections were subjected to hematoxylin and eosin (HE) staining and picrosirius red (PSR) staining as previously described.[15] For cross-sectional area (CSA) quantification, 10 sections per heart were analyzed. PSR staining was used for visualization. For LV collagen volume calculation, six sections per heart were analyzed. All the data were evaluated in a blinded manner in Image-Pro Plus 6.0 software, and there were six mice in each group.

Cardiomyocyte isolation and culture

Neonatal rat cardiomyocytes (NRCMs) were isolated and cultured as previously described.[13,14] Briefly, the hearts of Sprague–Dawley rats (1–3 days old) were quickly removed and separated, and the ventricles were collected. The ventricular tissues were digested with 0.125% trypsin-EDTA (Gibco, Waltham, MA, USA) four times for 15 min each time. DMEM-F12 supplemented with 15% fetal bovine serum (FBS; Gibco) was used to stop the digestion. Afterward, the cells were collected and incubated with DMEM-F12 supplemented with 15% FBS. To exclude the non-cardiomyocyte cells adhered to the bottom of the 100-mm dish, the supernatant was collected after 90 min, and the upper layer of the supernatant, which contained NRCMs, was collected and seeded in a six-well plate. α-Actin staining was used to determine the purity of NRCMs.

Cells were transfected with an adenovirus over-expressing TMEM43 (Ad-TMEM43, multiplicity of infection (MOI) = 50, Vigene Biosciences, Jinan, China). Then, the cells were stimulated with 1-μM angiotensin II (AngII) for 48 h to induce the cardiomyocyte hypertrophic response.

Western blotting and quantitative polymerase chain reaction

Total protein was isolated from heart tissues, and the protein concentrations were determined by the bovine serum albumin (BSA) assay. An equal amount of protein (50 μg) from each sample was loaded onto a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel. After the proteins were transferred onto immobilon membranes (Millipore, Billerica, MA, USA), the membranes were incubated overnight at 4°C with primary antibodies against total NF-κB and phosphorylated (P)-NF-κB (1:1000 dilution) from Abcam (Waltham, MA, USA) and an antibody against GAPDH (1:1000 dilution) from Cell Signaling Technology (Danvers, MA, USA). The blots were developed with enhanced chemiluminescence (ECL) reagents (Bio-Rad, Hercules, CA, USA) and imaged by a ChemiDoc MP Imaging System (Bio-Rad). GAPDH served as an internal reference protein.

Total RNA (2 μg per sample) from frozen mouse heart tissue and cardiomyocytes was reverse transcribed into complementary DNA (cDNA) using oligonucleotide (DT) primers and a first-strand cDNA synthesis kit (Roche, Shanghai, China). Then, a LightCycler 480 instrument (software version 1.5; Roche) and SYBR Green PCR master mix (Roche) were used to perform reverse transcription polymerase chain reaction (RT-PCR). All data were normalized to GAPDH expression.

Immunofluorescence staining

Cells were seeded on cell slides, and the treated cells were fixed with 4% formalin, permeabilized with 0.2% Triton

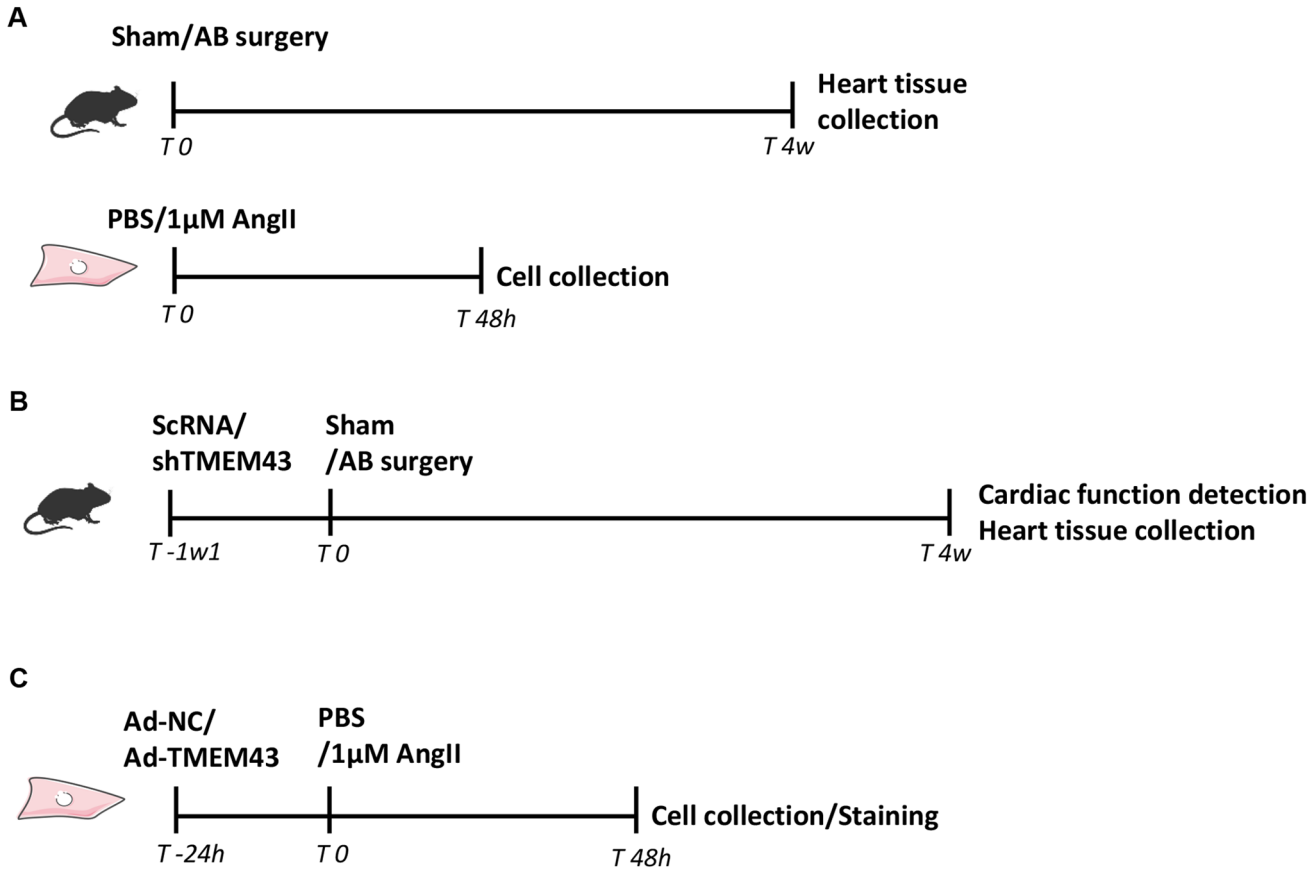

Figure 1. Experimental protocols. (A) Mice were subjected to sham surgery or aortic banding (AB), and heart tissues were collected 4 weeks after surgery for measurement of TMEM43 protein expression. NRCMs were incubated with PBS or 1-μM AngII for 48 h and then collected for measurement of TMEM43 protein expression. (B) Mice were injected with SCRNA or shTMEM43, and 1 week later, they were subjected to sham surgery or aortic banding (AB). Cardiac function was evaluated, and heart tissues were collected for measurement of hypertrophic parameters 4 weeks after surgery. (C) NRCMs were transfected with Ad-NC or Ad-TMEM43 for 24 h and then incubated with PBS or 1-μM AngII for 48 h. The cells were collected and stained to estimate hypertrophic parameters.

X-100, blocked with 8% goat serum, and incubated with the appropriate primary antibody, such as an α-actin or P-NF-κB antibody (1:100 dilution), from Abcam. The cells were subsequently incubated with fluorescent secondary antibodies, and nuclei were stained with DAPI. Then, the cells were photographed with a fluorescence microscope (Olympus DX51; Olympus, Tokyo, Japan).

Statistical analysis

The data are presented as the mean ± SD. Comparisons between two groups were analyzed by unpaired Student's *t*-test. Differences among more than two groups were analyzed by two-way analysis of variance followed by Tukey's post hoc test. *P* values less than 0.05 indicated statistical significance.

Results

The expression level of TMEM43 in the heart and cardiomyocytes

Procedures for our experiments were shown in Figure 1. We explored the expression level of TMEM43 during cardiac hypertrophy. We found that the protein expression of TMEM43 was downregulated in the heart tissues of mice subjected to AB compared with those of sham mice at 4 weeks post-AB (Figure 2(A)). We also measure the protein level of TMEM43 in cardiomyocytes stimulated with AngII. A decreased expression level of TMEM43 was observed in cardiomyocytes exposed to AngII (Figure 2(B)).

TMEM43 knockout exacerbates cardiac hypertrophy

We knocked down TMEM43 by using AAV9-shTMEM43 carrying the TnT promoter. As shown in Figure 3(A), the expression level of TMEM43 was reduced in AAV9-shTMEM43-injected mice in both the sham and AB groups compared with ScRNA-injected mice in the corresponding groups. Four weeks after AB, the hearts were removed. As shown in Figure 3(B) and (C), the heart weight (HW) to body weight (BW) ratio (HW/BW), HW to tibia length (TL) ratio (HW/TL), lung weight (LW) to BW ratio (LW/BW), and LW to TL ratio (LW/TL) were increased in the AB group. The HW/BW, HW/TL, LW/BW, and LW/TL were increased in TMEM43 knockout mice in the AB group compared with ScRNA-injected mice in the AB group. H&E staining was used to measure the CSA of cardiomyocytes. As shown in Figure 3(D), the CSA was increased in the AB group, while TMEM43 deficiency further increased this change in mice subjected to AB. The cardiac collagen volume was evaluated by PSR staining. AB increased the left ventricular (LF) collagen volume, while TMEM43 deficiency further accelerated collagen deposition (Figure 3(E)). We also measured the

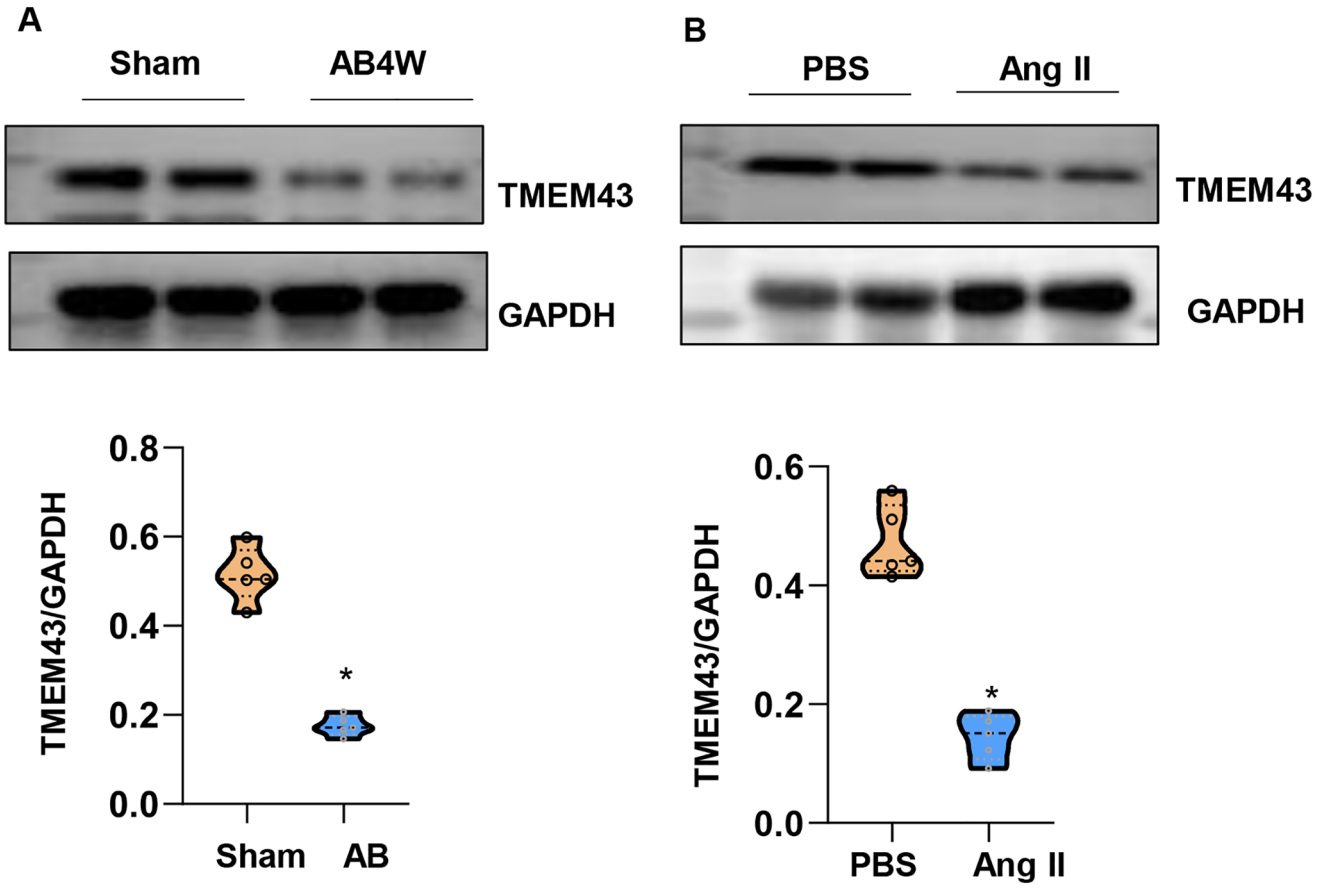

Figure 2. The expression level of TMEM43 in the heart and cardiomyocytes. (A) Protein level of TMEM43 in mouse hearts 4 weeks post-aortic banding (AB) ($n = 5$). (B) Protein level of TMEM43 in cardiomyocytes stimulated with AngII ($n = 5$).
*$P < 0.05$ versus the sham/PBS group.

transcription levels of the heart failure markers atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP), which were proven to be further increased in the hearts of TMEM43 knockout mice post-AB (Figure 3(F)).

TMEM43 deficiency aggravates AB-induced cardiac dysfunction

Cardiac function was assessed by echocardiography 4 weeks after AB, and there were no significant differences in heart rate among the four groups (Figure 4(A)). However, the LVIDd and LVISd were increased in the two AB groups; the systolic internal septal thickness (IVSd) and systolic left ventricular posterior wall thickness (LVPWd) were also increased in the AB group. The LVEF and LVFS were decreased in the AB group compared with the control group. These results suggested that our heart failure model was successfully established and exhibited impaired cardiac systolic and diastolic function. TMEM43 knockout induced increased the LVIDd, LVISd, thicker IVSd, and LVPWd and decreased the LVEF and LVFS, indicating exacerbated cardiac dysfunction (Figure 4(B-D)).

TMEM43 affects NF-κB activation

A previous study found that TMEM43 mutation leads to increased activation of NF-κB signaling in heart tissues.[10] We assessed the level of NF-κB in the heart tissues of mice with heart failure as well as cardiomyocytes. As shown in Figure 4(A), the total level of NF-κB was unchanged in the hearts of mice in the two AB groups. However, phosphorylated NF-κB was upregulated in the hearts of mice in the AB group, while TMEM43 knockout further increased the phosphorylation of NF-κB (Figure 5(A) and (B)). We also assessed the downstream inflammatory response in heart tissue, as NF-κB is a crucial inflammatory signaling molecule. As expected, the mRNA expression of tumor necrosis factor α (TNFα), interleukin-1 (IL-1), and interleukin-6 (IL-6) was upregulated in the hearts of mice in the AB group and further increased in the hearts of TMEM43 knockout mice (Figure 5(C)). These data indicate that TMEM43 deficiency promotes the activation of NF-κB in failing mouse hearts.

TMEM43 over-expression protects cardiomyocytes against AngII-induced hypertrophy

To confirm the protective effect of TMEM43 on hearts, cardiomyocytes were transfected with Ad-TMEM43 to overexpress TMEM43 (Figure 6(A)). The cells were then exposed to AngII to establish a cardiomyocyte failure model. AngII induced a remarkable hypertrophic response, as evidenced by an increased cell surface area and increased transcription

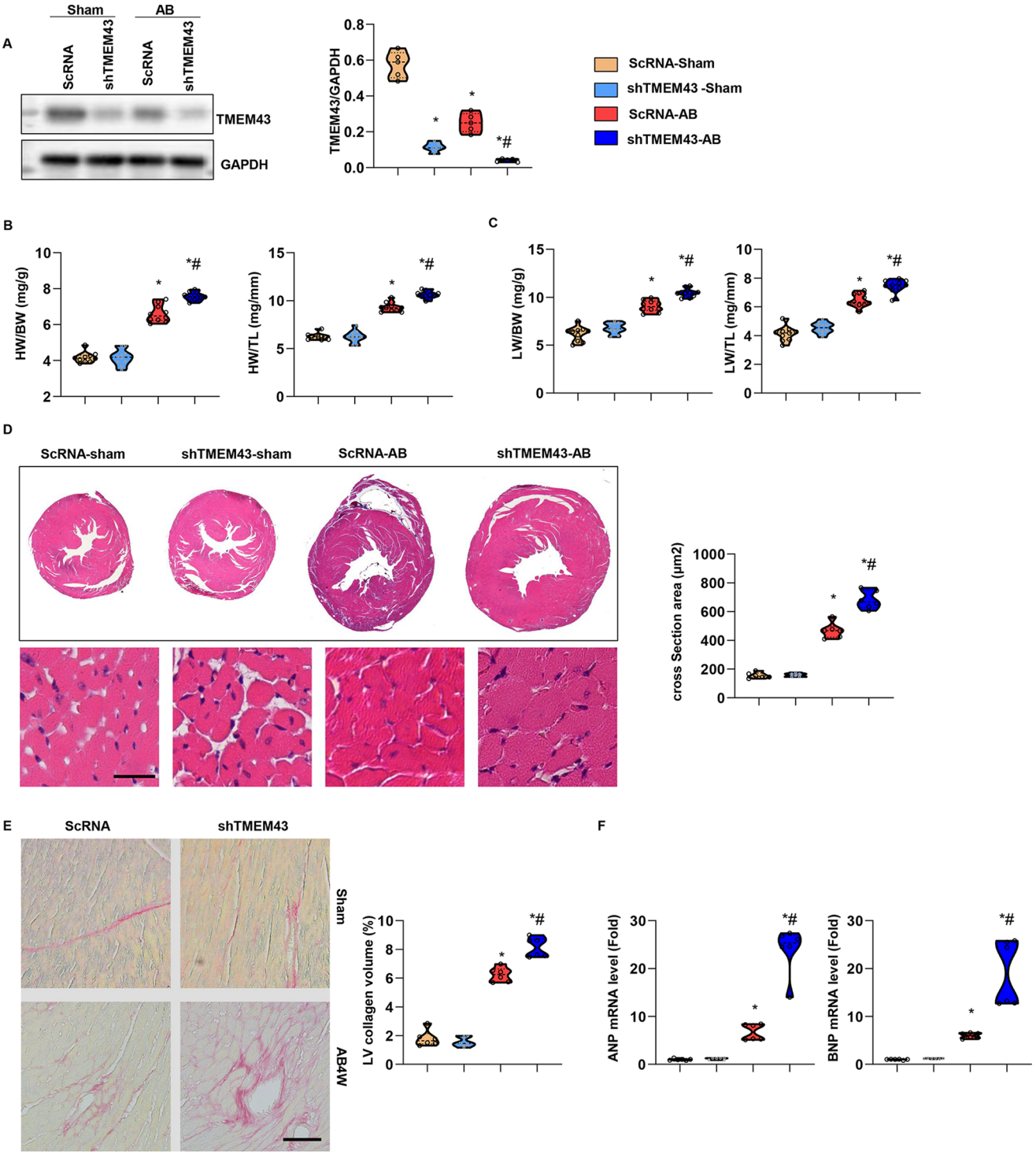

Figure 3. TMEM43 knockout exacerbates cardiac hypertrophy. (A) Protein level of TMEM43 in mouse hearts 4 weeks post-aortic banding (AB) ($n=5$). (B) and (C) The heart weight (HW) to body weight (BW) ratio (HW/BW), HW to TL ratio (HW/TL), lung weight (LW) to BW ratio (LW/BW), and LW to TL ratio (LW/TL) of mice ($n=10$). (D) Image of H&E staining and quantification of the CSA of mouse cardiomyocytes (scale bar, 50 μm) ($n=6$). (E) Image of PSR staining and quantification of the LV collagen volume in mouse hearts (scale bar, 100 μm) ($n=6$). (F) Transcription levels of ANP and BNP in mouse hearts ($n=6$).
*$P<0.05$ versus the ScRNA-sham group.
#$P<0.05$ versus the ScRNA-AB group.

of ANP and BNP (Figure 6(B-C)). TMEM43 over-expression ameliorated the hypertrophic response (Figure 6(B-C)). Moreover, cells transfected with Ad-TMEM43 showed reduced NF-κB activation and nuclear translocation (Figure 6(D)). These data indicate that by targeting NF-κB, TMEM43 exerts protective effects on cardiomyocytes.

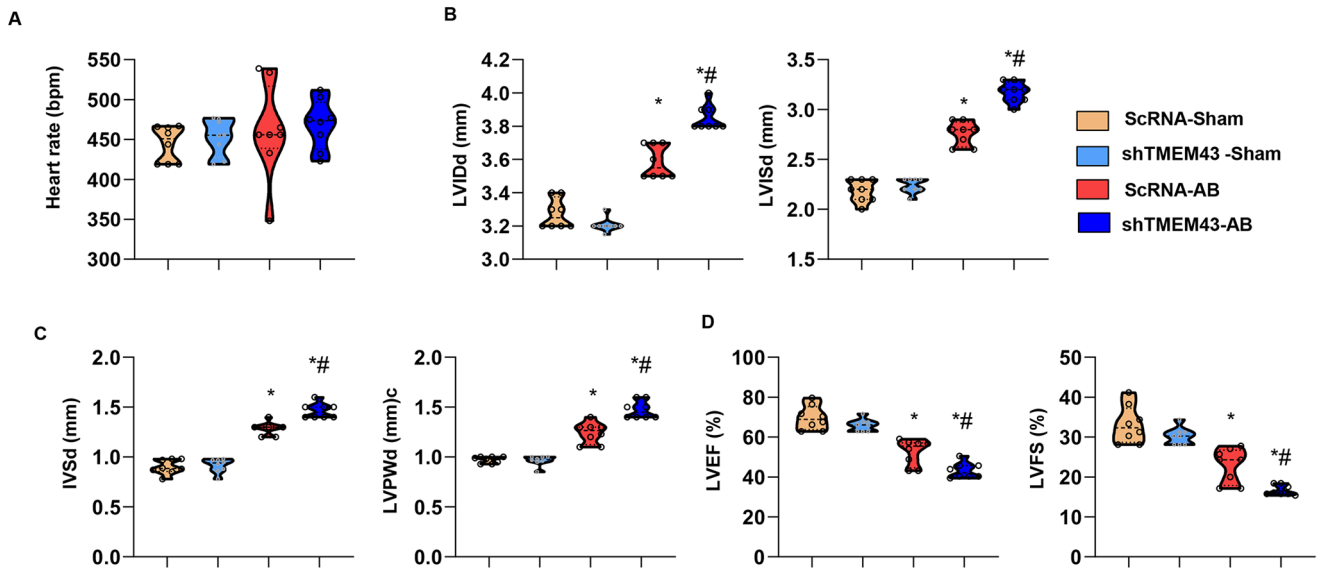

Figure 4. TMEM43 deficiency aggravates AB-induced heart failure. (A–D) Echocardiographic data of mouse hearts 4 weeks post-aortic banding (AB) ($n=8$).
*$P<0.05$ versus the ScRNA-sham group.
#$P<0.05$ versus the ScRNA-AB group.

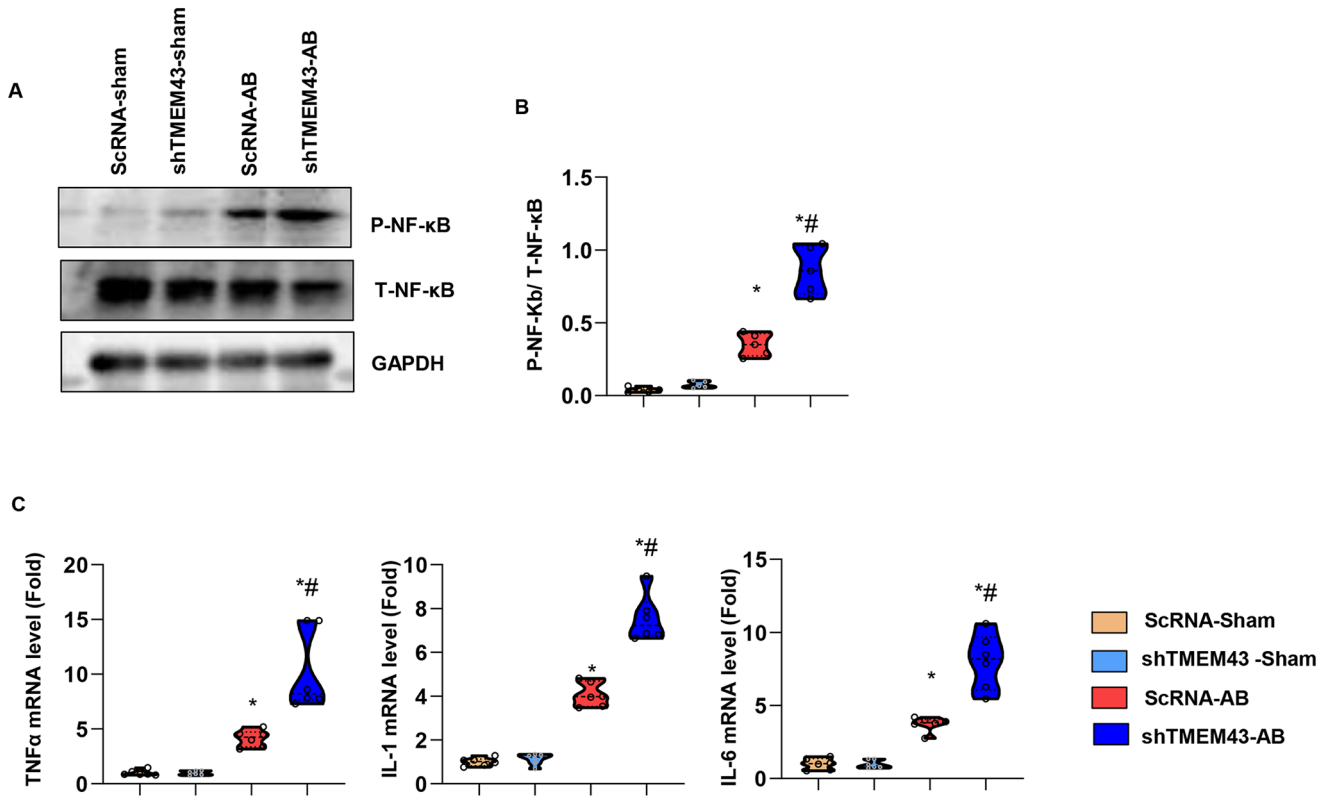

Figure 5. TMEM43 affects HSP72 phosphorylation. (A) and (B) Protein levels of total NF-κB and P-NF-κB in mouse hearts 4 weeks post-aortic banding (AB) ($n=5$). (C) Transcription levels of TNFα, IL-1, and IL-6 in mouse hearts ($n=6$).
*$P<0.05$ versus the ScRNA-sham group.
#$P<0.05$ versus the ScRNA-AB group.

Discussion

TMEM43 is a member of the TMEM subfamily. This gene is highly conserved and expressed in most species, including bacteria, animals, and humans.[16] The TMEM43 protein is an endoplasmic reticulum membrane protein. TMEM43 gene mutation can cause right ventricular arrhythmia cardiomyopathy.[17] However, its role in heart failure has not been reported. This study found for the first time that TMEM43 is downregulated in the cardiac hypertrophy stage of heart

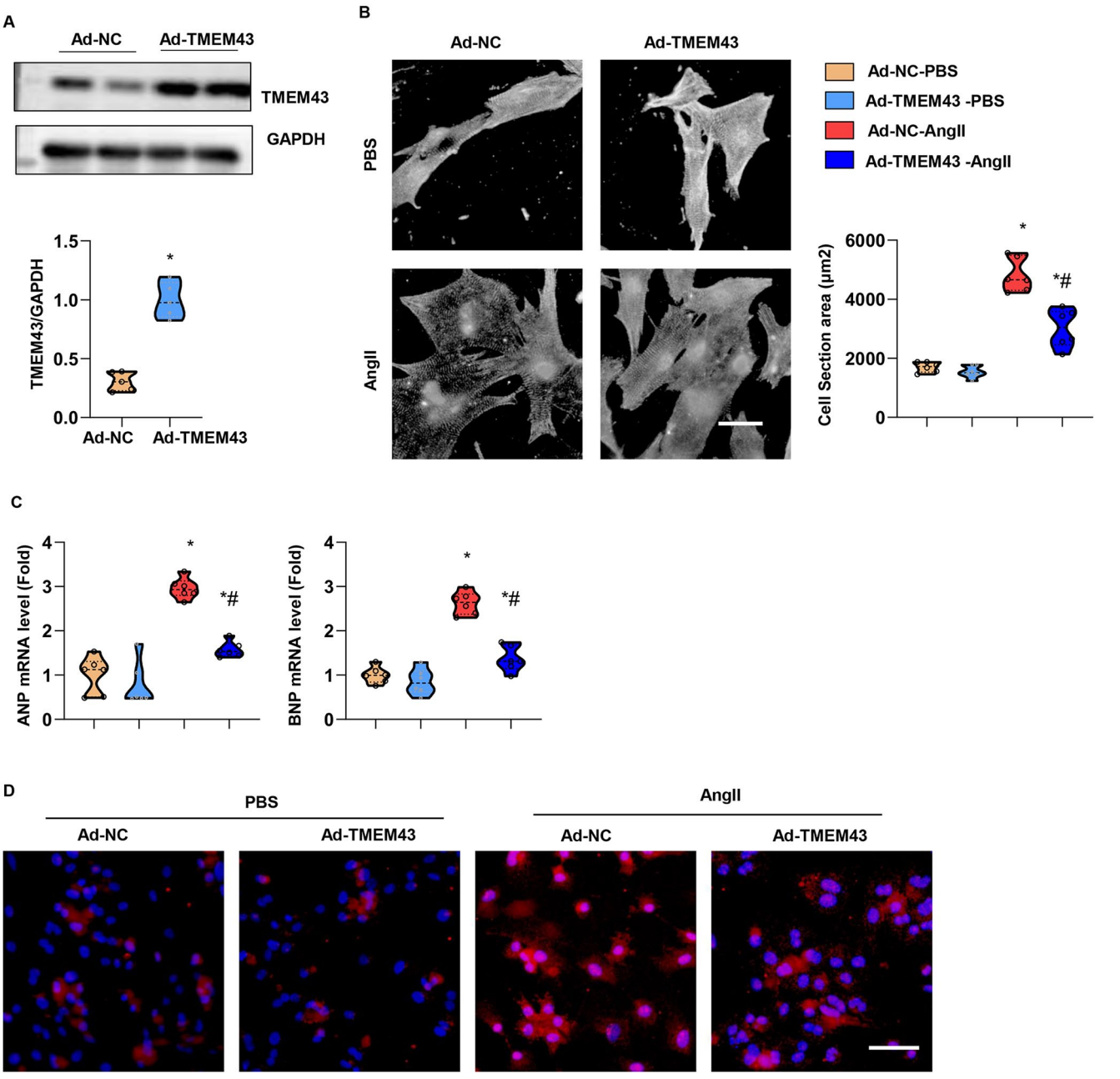

Figure 6. TMEM43 over-expression protects cardiomyocytes against AngII-induced hypertrophy.
(A) Protein level of TMEM43 in cardiomyocytes transfected with Ad-TMEM43 ($n=5$). (B) Image of α-actin staining and quantification of the SCA of cardiomyocytes treated with AngII (scale bar, 50 μm) ($n=6$). (C) Transcription levels of ANP and BNP in cardiomyocytes treated with AngII ($n=6$). (D) Image of P-NF-κB staining (scale bar, 100 μm).
*$P<0.05$ versus the Ad-NC-PBS group.
#$P<0.05$ versus the Ad-NC-AngII group.

failure. Deletion of TMEM43 in cardiomyocytes aggravated cardiac hypertrophy and fibrosis induced by pressure overload and exacerbated cardiac dysfunction. However, over-expression of the TMEM43 protein in cardiomyocytes delayed the AngII-induced cardiomyocyte hypertrophic response. This suggests that during the process of heart failure, reduced expression of TMEM43 may accelerate the pathological progression of heart failure.

Inflammation is a biological response of the immune system that can be triggered by a variety of factors, including pathogens, damaged cells, and toxic compounds.[18,19] Aseptic inflammation plays an important role in the progression of a variety of cardiovascular diseases to heart failure.[20,21] After myocardial injury, the exposed organelles and nuclei of cardiomyocytes encounter danger-associated molecular patterns (DAMPs), such as Toll-like receptors (TLRs), on the surface of inflammatory cells or the cardiomyocyte membrane.[18,22] These receptors activate the downstream classical inflammatory pathway, the NF-κB pathway. NF-κB transcription factors play an important role in inflammation, the immune

response, survival, and apoptosis.[18] Under physiological conditions, NF-κB stays in the cytoplasm with the IκB protein, which inhibits NF-κB activation.[18] TLR activates NF-κB by phosphorylating NF-κB. Subsequently, NF-κB translocates to the nucleus to promote the transcription of inflammation-related genes. This signaling causes an inflammatory cascade.[3,18] Studies have confirmed that activation of the NF-κB pathway plays a key role in heart failure induced by various etiologies.[20,21] A previous study found that haploinsufficiency of TMEM43 in cardiomyocytes leads to increased DNA damage and causes activation of the smad pathway and cardiac fibrosis.[23] Moreover, TMEM43 mutation was reported to be associated with a high risk of sudden cardiac death in arrhythmogenic right ventricular cardiomyopathy patients.[17] Takemoto *et al*.[24] also reported that TMEM43 is associated with TGFβ signaling, which leads to fibrosis. Here, we found that TMEM43 deficiency increased the activation of NF-κB signaling and that TMEM43 over-expression reduced NF-κB activation in cardiomyocytes. Our finding is consistent with that of Zheng *et al.*'s[10] study, which found that in arrhythmogenic right ventricular cardiomyopathy, the TMEM43-S358L mutation enhances NF-κB signaling, leading to TGFβ activation. Thus, by targeting NF-κB signaling, TMEM43 promotes the progression of heart failure.

Conclusions

Our study found for the first time that TMEM43 is downregulated in the mouse heart and cardiomyocytes during cardiac hypertrophy. Reduced expression of TMEM43 accelerates the progression of heart failure in mice. TMEM43 deficiency may increase the activation of NF-κB signaling in heart failure, leading to an inflammatory cascade.

AUTHORS' CONTRIBUTIONS

YG and X-WZ contributed to the conception and design of the experiments; YG, Y-RY, and YD carried out the experiments; Y-RY and YD analyzed the experimental results and revised the manuscript; X-WZ and YG wrote and revised the manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by the China Heart House—Chinese Cardiovascular Association HX fund (2022-CCA-HX-008) the Natural Science Research Program of Huai'an (HAB202025) and Innovative Key Talents' Project of The Affiliated Huaian No. 1 People's Hospital of Nanjing Medical University (ZC202203).

DATA AVAILABILITY STATEMENT

The raw data are available from the corresponding author upon request.

ORCID ID

Xi-Wen Zhang https://orcid.org/0000-0001-5644-2279

REFERENCES

1. Slivnick J, Lampert BC. Hypertension and heart failure. *Heart Fail Clin* 2019;**15**:531–41
2. Savarese G, Stolfo D, Sinagra G, Lund LH. Heart failure with mid-range or mildly reduced ejection fraction. *Nat Rev Cardiol* 2022;**19**:100–16
3. Nakamura M, Sadoshima J. Mechanisms of physiological and pathological cardiac hypertrophy. *Nat Rev Cardiol* 2018;**15**:387–407
4. Xu L, Brink M. MTOR, cardiomyocytes and inflammation in cardiac hypertrophy. *Biochim Biophys Acta* 2016;**1863**:1894–903
5. Sun J, Niu C, Ye W, An N, Chen G, Huang X, Wang J, Chen X, Shen Y, Huang S, Wang Y, Wang X, Wang Y, Jin L, Cong W, Li X. FGF13 is a novel regulator of NF-kappaB and potentiates pathological cardiac hypertrophy. *Iscience* 2020;**23**:101627
6. Gowrikumar S, Primeaux M, Pravoverov K, Wu C, Szeglin BC, Sauve CG, Thapa I, Bastola D, Chen XS, Smith JJ, Singh AB, Dhawan P. A claudin-based molecular signature identifies high-risk, chemoresistant colorectal cancer patients. *Cells* 2021;**10**:2211
7. Li J, Song Y, Zhang C, Wang R, Hua L, Guo Y, Gan D, Zhu L, Li S, Ma P, Yang C, Li H, Yang J, Shi J, Liu X, Su H. TMEM43 promotes pancreatic cancer progression by stabilizing PRPF3 and regulating RAP2B/ERK axis. *Cell Mol Biol Lett* 2022;**27**:24
8. Jiang C, Zhu Y, Zhou Z, Gumin J, Bengtsson L, Wu W, Songyang Z, Lang FF, Lin X. TMEM43/LUMA is a key signaling component mediating EGFR-induced NF-kappaB activation and tumor progression. *Oncogene* 2017;**36**:2813–23
9. Haywood AF, Merner ND, Hodgkinson KA, Houston J, Syrris P, Booth V, Connors S, Pantazis A, Quarta G, Elliott P, McKenna W, Young TL. Recurrent missense mutations in TMEM43 (ARVD5) due to founder effects cause arrhythmogenic cardiomyopathies in the UK and Canada. *Eur Heart J* 2013;**34**:1002–11
10. Zheng G, Jiang C, Li Y, Yang D, Ma Y, Zhang B, Li X, Zhang P, Hu X, Zhao X, Du J, Lin X. TMEM43-S358L mutation enhances NF-kappaB-TGFbeta signal cascade in arrhythmogenic right ventricular dysplasia/cardiomyopathy. *Protein Cell* 2019;**10**:104–19
11. Wu QQ, Xiao Y, Duan MX, Yuan Y, Jiang XH, Yang Z, Liao HH, Deng W, Tang QZ. Aucubin protects against pressure overload-induced cardiac remodelling via the beta3-adrenoceptor-neuronal NOS cascades. *Br J Pharmacol* 2018;**175**:1548–66
12. Zhang X, Hu C, Kong CY, Song P, Wu HM, Xu SC, Yuan YP, Deng W, Ma ZG, Tang QZ. FNDC5 alleviates oxidative stress and cardiomyocyte apoptosis in doxorubicin-induced cardiotoxicity via activating AKT. *Cell Death Differ* 2020;**27**:540–55
13. Zong J, Li FF, Liang K, Dai R, Zhang H, Yan L, Liu JL, Xu LH, Qian WH. Nuclear localization leucine-rich-repeat protein 1 deficiency protects against cardiac hypertrophy by pressure overload. *Cell Physiol Biochem* 2018;**48**:75–86
14. Gu Y, Hu X, Ge PB, Chen Y, Wu S, Zhang XW. CTRP1 aggravates cardiac dysfunction post myocardial infarction by modulating TLR4 in macrophages. *Front Immunol* 2021;**12**:635267
15. Bian Z, Liao H, Zhang Y, Wu Q, Zhou H, Yang Z, Fu J, Wang T, Yan L, Shen D, Li H, Tang Q. Never in mitosis gene A related kinase-6 attenuates pressure overload-induced activation of the protein kinase B pathway and cardiac hypertrophy. *PLoS ONE* 2014;**9**:e96095
16. Jang MW, Kim TY, Sharma K, Kwon J, Yi E, Lee CJ. A deafness associated protein TMEM43 Interacts with KCNK3 (TASK-1) two-pore domain K^+ (K2P) channel in the cochlea. *Exp Neurobiol* 2021;**30**:319–28
17. Dominguez F, Zorio E, Jimenez-Jaimez J, Salguero-Bodes R, Zwart R, Gonzalez-Lopez E, Molina P, Bermúdez-Jiménez F, Delgado JF, Braza-Boïls A, Bornstein B, Toquero J, Segovia J, Van Tintelen JP, Lara-Pezzi E, Garcia-Pavia P. Clinical characteristics and determinants of the phenotype in TMEM43 arrhythmogenic right ventricular cardiomyopathy type 5. *Heart Rhythm* 2020;**17**:945–54

18. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget* 2018;**9**:7204–18
19. Xiang K, Yang JF, Wu X, Peng J, Guo JJ, Fan CM. Advances in traditional Chinese medicine for cardiovascular disease therapy in 2020. *Tradit Med Res* 2021;**6**:27.
20. Zhang DX, Ma DY, Yao ZQ, Fu CY, Shi YX, Wang QL, Tang QQ. ERK1/2/p53 and NF-kappaB dependent-PUMA activation involves in doxorubicin-induced cardiomyocyte apoptosis. *Eur Rev Med Pharmacol Sci* 2016;**20**:2435–42
21. Chen X, Li X, Zhang W, He J, Xu B, Lei B, Wang Z, Cates C, Rousselle T, Li J. Activation of AMPK inhibits inflammatory response during hypoxia and reoxygenation through modulating JNK-mediated NF-kappaB pathway. *Metabolism* 2018;**83**:256–70
22. Su Q, Li L, Sun Y, Yang H, Ye Z, Zhao J. Effects of the TLR4/Myd88/NF-kappaB signaling pathway on NLRP3 inflammasome in coronary microembolization-induced myocardial injury. *Cell Physiol Biochem* 2018;**47**:1497–508
23. Rouhi L, Cheedipudi SM, Chen SN, Fan S, Lombardi R, Chen X, Coarfa C, Robertson MJ, Gurha P, Marian AJ. Haploinsufficiency of Tmem43 in cardiac myocytes activates the DNA damage response pathway leading to a late-onset senescence-associated pro-fibrotic cardiomyopathy. *Cardiovasc Res* 2021;**117**:2377–94
24. Takemoto Y, Kadota S, Minami I, Otsuka S, Okuda S, Abo M, Punzalan LL, Shen Y, Shiba Y, Uesugi M. Chemical genetics reveals a role of squalene synthase in TGFbeta signaling and cardiomyogenesis. *Angew Chem Int Ed Engl* 2021;**60**:21824–31

(Received December 29, 2022, Accepted April 25, 2023)