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Original Article

IRAK4 deficiency promotes cardiac remodeling induced by pressure overload

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Abstract: Background: Interluekin1 receptor-associated kinase-4 (IRAK4) plays an essential role in the innate immune system. The aim of this study was to investigate the role of IRAK4 in cardiac remodeling induced by pressure overload and elucidate the underlying mechanisms. Methods: In vivo studies were performed using IRAK4 heterozygous knockout (HET) mice and wild type (WT) mice. Models of cardiac remodeling were induced by aortic banding (AB). Cardiac remodeling was evaluated by Echocardiography and histological analysis. Results: IRAK4 was upregulated in hearts of dilated cardiomyopathy (DCM) patients and also pressure overload-induced mice hearts. IRAK4 HET mice exhibited exacerbated cardiac hypertrophy, dysfunction and fibrosis after 4 weeks of AB compared with that in WT mice. Furthermore, enhanced activation of the MEK-ERK1/2, p38 and NFκB pathways was found in IRAK4 HET mice compared to WT mice. Conclusion: Our results suggest that IRAK4 may play a crucial role in the development of cardiac remodeling via negative regulation of multiple signaling pathways.

Keywords: IRAK4, aortic banding, cardiac remodeling

Introduction

Cardiac remodeling is a cellular response to a variety of pathological stimuli including pressure and volume overload, ischemia, intermittent hypoxia and inherited gene mutations [1-4], characterized by myocyte hypertrophy, hyperplasia of interstitial cell and interstitial fibrosis. It can provide compensatory ejection performance; however, long-term pathological hypertrophy is a common precursor to heart failure, arrhythmia and sudden death, which are increasing in prevalence [5, 6]. Although a series of studies have demonstrated that some signaling pathways, including mitogen activated protein kinases (MAPKs), phosphatidylinositol 3-kinase (PI3K)/Akt and calcineurin/nuclear factor of activated T cells (NFAT), play significant roles in cardiac remodeling [6], mechanisms that antagonize these pathways have not been clearly defined. Therefore, a better understanding of the mechanisms underlying the pathological responses may be needed for finding novel strategies of suppressing cardiac remodeling.

Interluekin1 receptor-associated kinase-4 (IR AK4) is a member of IRAKs family, which is responsible for initiating signaling from Toll-like receptor/Interleukin-1 receptor (TIR) family [7, 8]. After ligand binding, TIRs dimerize and undergo a conformational change required for the recruitment of myeloid differentiation primary response 88 (MyD88). MyD88 then recruits IRAK4 and IRAK. The phosphorylated IRAK mediates the recruitment of TNF receptor-associated factor 6 (Traf6) to the receptor complex. Then the IRAK-TRAF6 complex dissociates from the receptor complex to interact with and activated TGFβ-activated kinase 1 (TAK1), leading to the activation of NF-κB and c-Jun NH2-terminal kinase (JNK), resulting in induction of inflammatory cytokines and chemokines such as IL-1β, IL-6. The significant role of IRAK4 in innate immune system has been reported [7, 8], and nowadays, the connection between innate immune system and cardiovascular diseases has been closer, Maekawa, et al reported that global deletion of IRAK4 had favorable effects on survival and left ventricular remodeling after myocardial ischemia (MI) through mod-

Table 1. A list of the primers used in this study

Gene Name	Forward (5'-3')	Reverse (5'-3')
ANP	ACCTGCACCACCTGGAGGAG	CCTTGGCTGTTATCTTCGGTACCG
BNP	GAGGTCACTCCTATCCTCTGG	GCCATTTCCTCCGACTTTTCTC
SERCA2α	CATTTGCATTGCAGTCTGGAT	CTTTGCCATCCTACGAGTTCC
Acta1	GTGAGATTGTGCGCGACATC	GGCAACGGAAACGCTCATT
Collagen Iα	CCTCAAGGGCTCCAACGAG	TCAATCACTGTCTTGCCCCA
Collagen IIIα	ACGTAGATGAATTGGGATGCAG	GGGTTGGGGCAGTCTAGTC
TGFβ1	ATCCTGTCCAAACTAAGGCTCG	ACCTCTTTAGCATAGTAGTCCGC
TGFβ2	TCGACATGGATCAGTTTATGCG	CCCTGGTACTGTTGTAGATGGA
Fibronectin	CCGGTGGCTGTCAGTCAGA	CCGTTCCCACTGCTGATTTATC
CTGF	TGACCCCTGCGACCCACA	TACACCGACCCACCGAAGACACAG

Acta1 = actin α1 skeletal muscle; ANP = atrial natriuretic peptide; BNP = B-type natriuretic peptide; CTGF = connective tissue growth factor SERCA2α = sarcoendoplasmic reticulum Ca^{2+}-ATPase; TGF-β1 = transforming growth factor-β1; TGF-β2 = transforming growth factor-β2.

ification of the host inflammatory process by blunting the detrimental bone marrow dendritic cells mobilization after MI [9], another study showed that IRAK4-/- mice suffered attenuated viral myocarditis through regulation of interferon production and $CCR5^+$ monocytes/macrophages to the heart [10], what's more, a study using IRAK4 inactive knock-in mice stated that inhibition of IRAK4 might provide an approach in the development of anti-atherosclerosis drugs [11]. However, in our study that focuses on the effect of IRAK4 deficiency on cardiac remodeling, it showed that mice with low expression of IRAK4 have a more serious tendency of cardiac hypertrophy, dysfunction and fibrosis after pressure overload, suggesting an important role of IRAK4 in the hypertrophic response and also the complicated function of IRAK4 in the heart.

Materials and methods

Human left ventricular samples

We analyzed the protein level of IRAK4 in myocardial samples of both failing human hearts and healthy controls. Samples of failing hearts were collected from the left ventricles of DCM patients undergoing heart transplants, while control samples were obtained from the left ventricles of healthy donors. The samples were obtained with the approval of the local Ethical Committee (Renmin Hospital of Wuhan University Human Research Ethics Committee, Wuhan, China). The investigation was conducted in accordance with the principles outlined in the Declaration of Helsinki. Informed written consent was obtained from all subjects.

Animal models

Male IRAK4 heterozygous knockout (HET) mice (C57BL/6 background) and their wild-type (WT) littermates aged 8-10 weeks were used in the experiments. Their genotypes were confirmed by PCR (data not shown). Aortic banding (AB) was performed as described previously [12]. All protocols were approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University, and the animal procedures were all performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Echocardiography

Echocardiography was performed in anesthetized (1.5% isoflurane) mice using a Mylab TM30CV (ESAOTE S.p.A) with a 10-MHz linear array ultrasound transducer at 4 weeks after surgery. The left ventricle (LV) dimensions were assessed respectively at end-systole (LVESD) and end-diastole (LVEDD) in parasternal short-axis view. The ejection fraction (EF) and Fractional shortening (FS) were calculated as described previously [13]. After the measurement, hearts and lungs of the euthanized mice were collected and weighed to compare heart weight/body weight (HW/BW, mg/g), lung weight/body weight (LW/BW, mg/g), and heart weight/tibia length (HW/TL, mg/mm) ratios in IRAK4 HET and WT mice.

Histological analysis

Heart samples were randomly assigned for histological and bio-molecular analyses. For histological analysis, the hearts were excised, arrested in diastole with 10% KCl, weighed, fixed in with 10% formalin, and embedded in paraffin. Hearts were cut transversely close to the apex to visualize the left and right ventricles. Several sections of each heart (4-5 μm

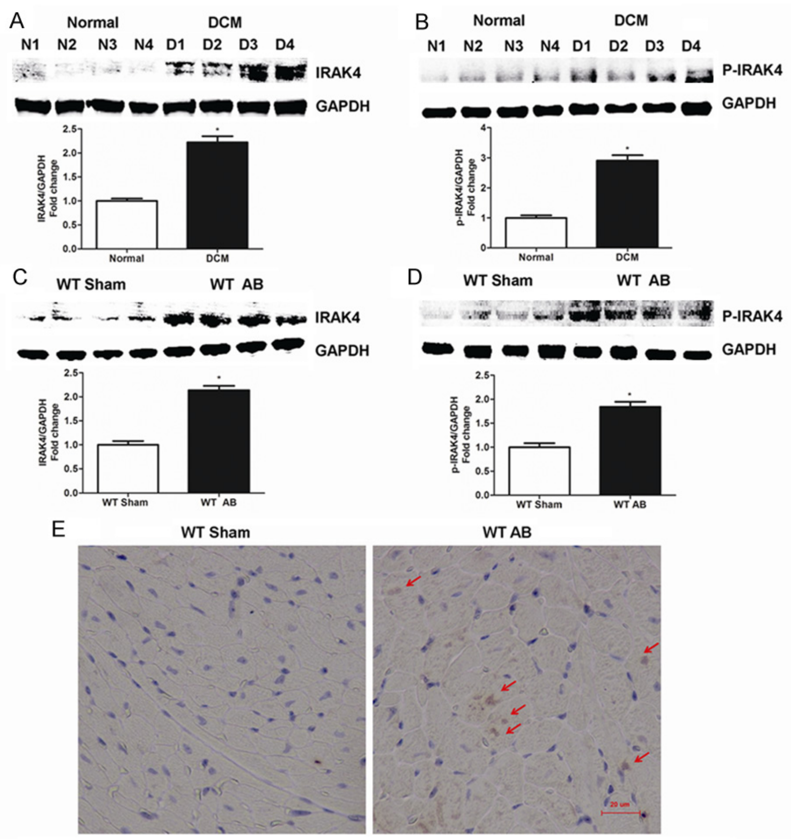


Figure 1. IRAK4's expression in hearts. A. Western blot analysis of cardiac expression of IRKA4 in normal donators and in DCM patients (n=4). B. Representative western blots of p-IRKA4 in normal donators and in DCM patients (n=4). **P*<0.05 vs normal donators. C. Western blot analysis of cardiac expression of IRKA4 in WT mice and their littermates after AB (n=6). D. Representative western blots of p-IRAK4 in WT mice and their littermates after AB (n=6). **P*<0.05 vs WT mice. E. Immunohistochemistry of cardiac IRAK4 protein from WT mice after 4 weeks of AB.

thick) were prepared, and then stained with hematoxylin and eosin (H&E) for histopathology or picrosirius red (PSR) for collagen deposition before visualized by light microscopy. For myocyte cross-sectional area, sections were stained with FITC-conjugated WGA (Invitrogen) for visualize membranes and DAPI for visualize nuclei. For digital measurements of cardiomyocyte cross-sectional areas (CSAs), the outline of 150 myocytes were traced and measured in each group with a quantitative digital image analysis system (Image Pro-Plus, version 6.0). The left ventricle collagen volume fraction was calculated as the area stained by PSR divided by the total area.

Quantitative real-time RT-PCR

The mRNA expression levels of hypertrophic and fibrotic markers were detected by Real-time PCR. Total RNA was extracted from frozen mice heart samples using TRIzol (Invitrogen, 15596-026), and the yield and purity of the samples were spectrophotomereicallyestimated using the A260/A280 and A230/260 ratios viaaSmartSpecPlusSpectrophotometer (Bio-Radc.DNA was synthesized from 2 μg RNA of each sample using the Transcripto First Strand cDNA Synthesis Kit (Roche, 04896966001). The PCR amplifications were quantified using LightCycler 480 SYBR Green 1 Master Mix (Roche, 04707516001) and the results were normalized to those of glyceraldehyde-3-phosphate dehydrogenase(GAPDH) gene expression. The primer sequences used for RT-PCR are shown in **Table 1**.

Western blotting

Heart samples were lysed in RIPA lysis buffer, and the total protein concentration was detected using BCA protein assay kit (Themo, 23227) by ELISA (Synergy HT, Bio-tek). The cell lysate (50 μg) was used for SDS/transfer membranes (Millipore, IPFL00010). The primary anti-bodies included antibodies specific for GAPDH (Cell Signaling Technology, 2118), p-IRAK4 (Cell Signaling Technology, 11927), IRAK4 (Cell Signaling Technology, 4363), p-TAK1 (Cell Signaling Technology, 9339), T-TAK1 (Cell Signaling Technology, 4505), p-MEK1/2 (Cell Signaling Technology, 9154), T-MEK1/2 (Cell Signaling Technology, 9122), p-ERK1/2 (Cell Signaling Technology, 4370), T-ERK1/2 (Cell Signaling Technology, 4695), p-p38 (Cell Signaling Technology, 4511), T-p38 (Cell Signaling Technology, 9212), p-JNK (Cell Signaling Technology, 4668), T-JNK (Cell Signaling Technology, 9258), p-GATA4 (Santa Cruz, 32823), T-GATA4 (Santa Cruz, 9053), p-AKT (Cell Signaling Technology, 4060), T-AKT

Table 2. Echocardiographic and anatomic data in mice after 4 weeks of surgery

Parameter	Sham		AB	
	WT (n=8)	KO (n=8)	WT (n=8)	KO (n=8)
HR (min-1)	517.3±15.6	506.4±10.6	491.6±20.2	487.2±9.4
IVSd (mm)	0.68±0.02	0.70±0.01	0.76±0.02*	0.81±0.01#
LVEDd (mm)	3.65±0.03	3.78±0.05	4.11±0.11*	4.64±0.07#
LVPWd (mm)	0.71±0.01	0.71±0.01	0.76±0.01*	0.79±0.02*
LVESd (mm)	2.10±0.04	2.32±0.05	2.68±0.09*	3.33±0.08#
EF (%)	79.00±1.13	75.23±0.77	71.17±0.80*	61.28±1.32#
FS (%)	41.33±0.95	38.40±0.67	35.11±0.70*	28.22±0.87#
BW (g)	27.53±0.67	30.34±0.36	28.53±0.33	29.78±0.39
HW (mg)	119.1±1.5	148.3±2.8	183.0±1.9*	209.6±17.4#
LW (mg)	143.75±3.27	153.50±4.50	146.50±4.44	158.50±5.52
TL (mm)	18.25±0.23	17.75±0.53	18.31±0.13	17.81±0.09
HW/BW (mg/g)	4.33±0.05	4.89±0.05	6.42±0.07*	7.04±0.16#
LW/BW (mg/g)	5.22±0.10	5.07±0.18	5.29±0.15	5.32±0.16
HW/TL (mg/mm)	186.86±3.36	202.83±2.82	330.91±5.17*	368.56±4.96#

*P<0.05 vs WT/sham. #P<0.05 vs WT/AB after AB. BW = body weight; EF = ejection fraction; FS = fractional shortening; HR = heart rate; HW = heart weight; IVSd = interventricular septal thickness at end-diastole; LVEDd = left ventricular end-diastolic diameter; LVESd = left ventricular end-systolic diameter; LVPWD = left ventricular posterior wall thickness at end-diastole; LW = lung weight. All values are mean ± SEM.

(Cell Signaling Technology, 4691), p-NFκBp65 (Bioworld, BS4135), NFκBp65 (Cell signaling Technology, 8242) and IL-1β (R&D, AF-401-NA). The secondary antibody was goat anti-rabbit (LI-COR, 926-32211) IgG. The blot was scanned by a two-color infrared imaging system (Odyssey, LICOR). Specific protein expression levels were normalized to GAPDH protein for total cell lysates and cytosolic proteins.

Statistical analysis

Data are presented as the means ± SEM. Differences among the groups were determined by two-way ANOVA followed by Tukey's multiple-comparison test. Student's t-tests were used to compare means between the two groups. P<0.05 was considered to be significantly different.

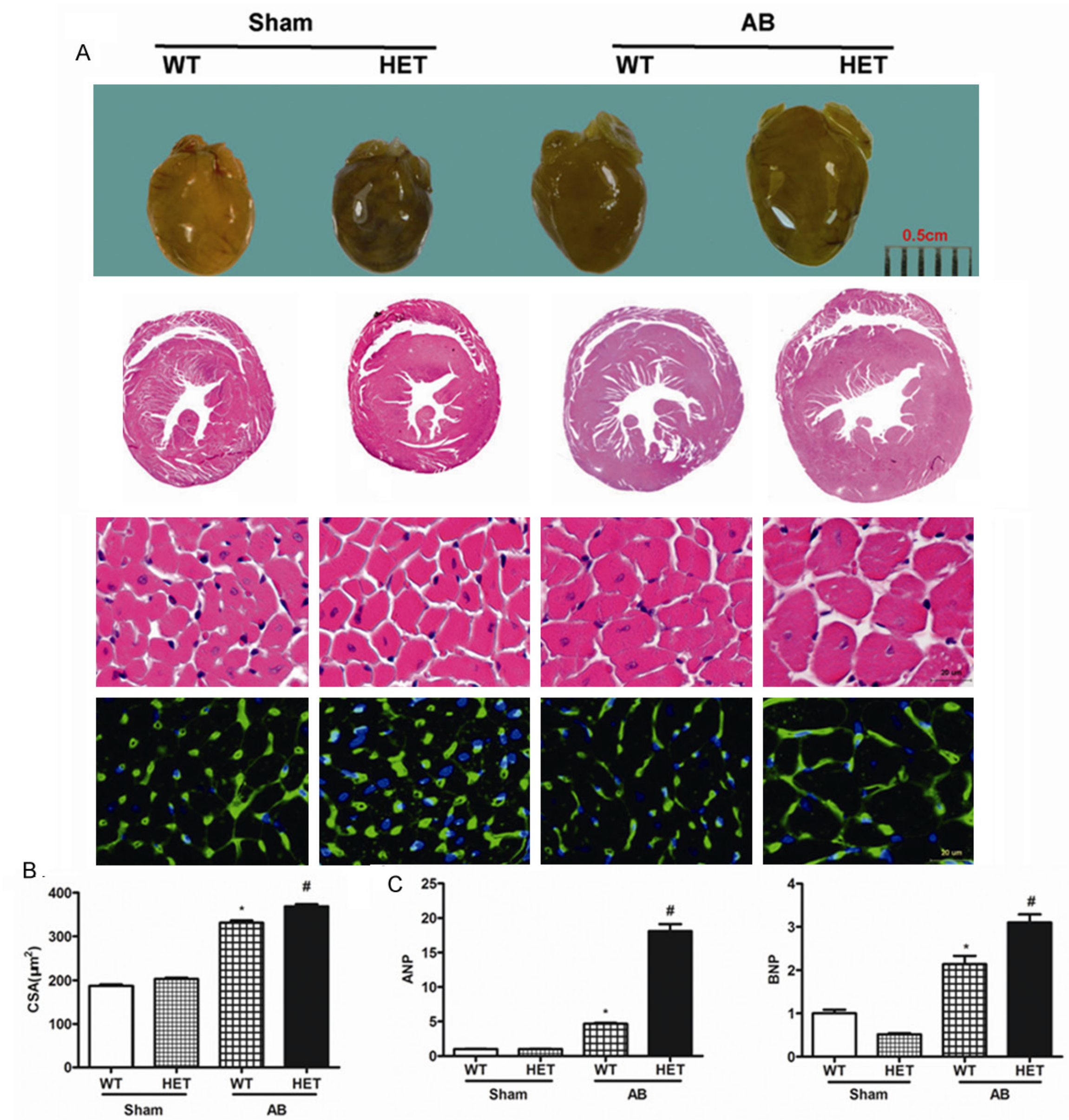
Results

IRAK4 protein levels increased in the ventricles of DCM patients and mice suffering cardiac remodeling

We examined the IRAK4 expression in human ventricular samples from both DCM patients and healthy donators, the human data from DCM and healthy patients were the same with that in our previous study [14]. The cardiac IRAK4 expression of DCM patients exhibited a 2 fold increase relative to those of normal donors (**Figure 1A**), as shown in **Figure 1B**, the expression of p-IRAK4 in the hearts of DCM patients was also significantly up-regulated in all DCM hearts. So, IRAK4 may involve in cardiovascular diseases with the similar pathophysiological changes to DCM. To explore the potential role of IRAK4 in cardiac remodeling, we then examined p-IRAK4 and IRAK4 expression in pressure overload-induced mice hearts. Then we found that the levels of p-IRAK4 and IRAK4 protein both increased in mice hearts at 4 weeks after AB (**Figure 1C-E**). These results strengthen the possible involvement of IRAK4 in cardiac remodeling.

IRAK4 deficiency facilitates cardiac hypertrophy and dysfunction in response to pressure overload

We performed the AB surgery or a sham operation on IRAK4 HET mice and WT littermates to estimate the effect of IRAK4 on cardiac hypertrophy. Echocardiography was performed 28 days after the operation to evaluate the structural and functional changes of the left ventricle. There were no significant differences between the sham-operated IRAK4 HET and WT mice; however, IRAK4 HET mice exhibited aggravated cardiac hypertrophy and



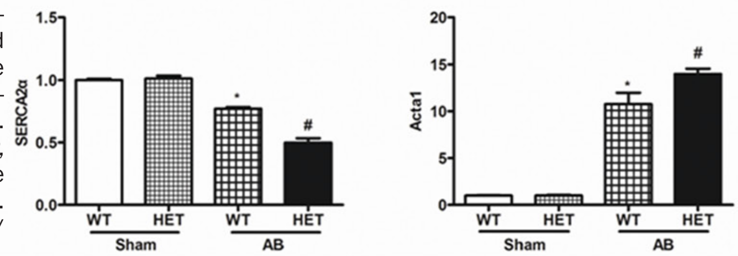


Figure 2. Effects of IRAK4 on cardiac remodeling. A. Cross heart, HE staining and WGA-FITC staining of sham and AB mice at 4 weeks post surgery. B. Statistical results of myocte CSAs of indicated groups. C. Expression of transcripts of ANP, BNP, SERC2α and Acta1 induced by AB were determined by RT-PCR analysis (n=6). $*P<0.05$ vs WT/sham. $\#P<0.05$ vs WT/AB after AB.

dysfunction compared to WT mice, as measured by HW/BW ratio, HW/TL ratio, LVEDd, LVESd, interventricular septal thickness at end-diastole (IVSd), left ventricular posterior wall thickness at end-diastole (LVPWd), EF and FS 4 weeks after AB (**Table 2**). Histological analyses including gross hearts, H&E and WGA staining confirmed the adverse effect of IRAK4 deficiency in response to pressure overload (**Figure 2A**). The CSAs also strikingly increased in the pressure-overloaded IRAK4 HET mice compared to WT mice (**Figure 2B**), while no significant differences were observed in LW/BW ratios (**Table 2**). We next used real-time

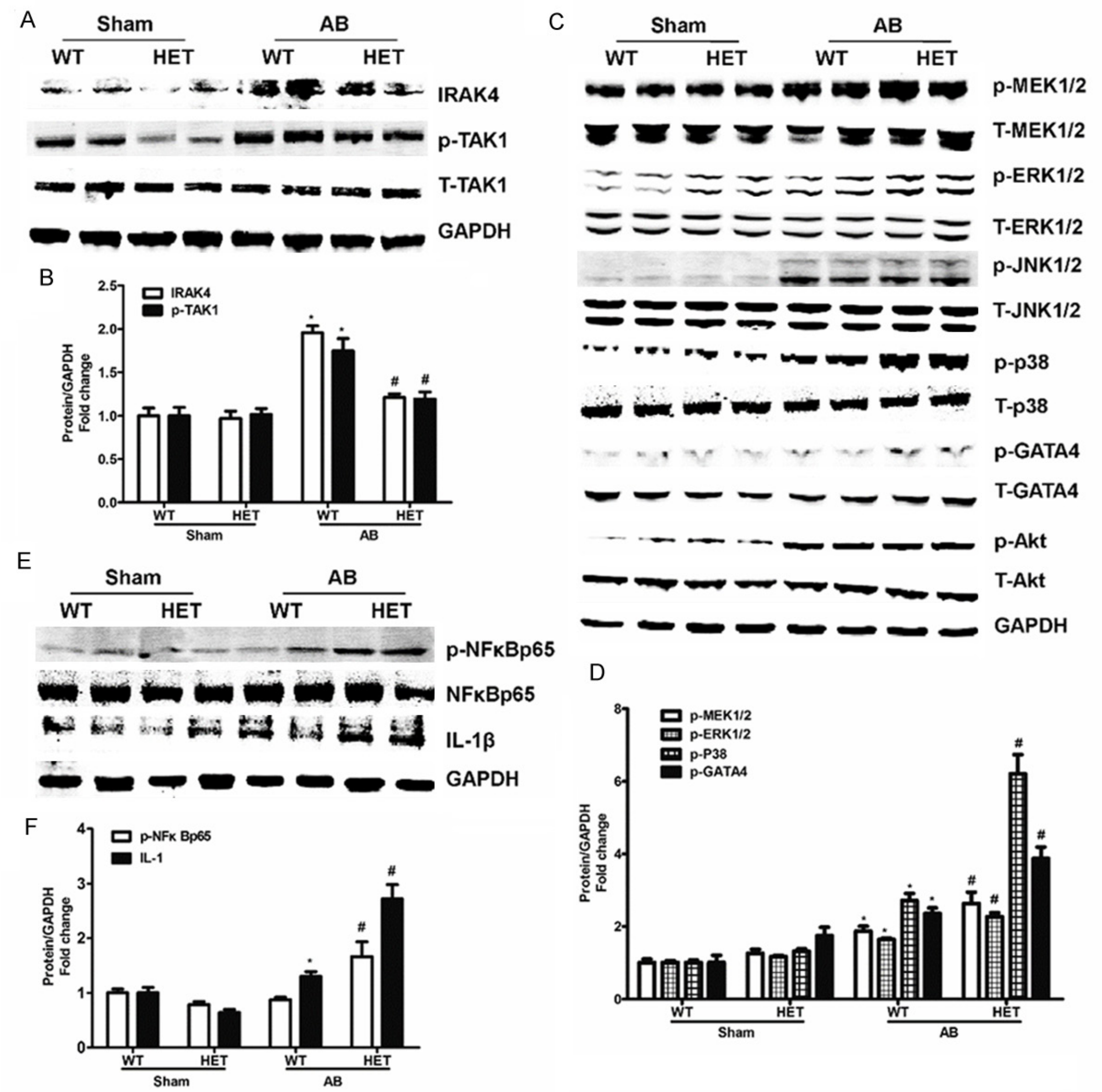


Figure 3. Effects of IRAK4 on the MAPKs, Akt and NFκB signaling pathways. A and B. Representative blots and quantitative results for IRAK4, p-TAK1 and TAK1 in mice hearts in the indicated groups (n=6). C and D. Representative blots and quantitative results for MEK1/2, ERK1/2, JNK, P38, GATA4 and Akt phosphorylation and their total protein expression in the heart tissues of mice in the indicated groups (n=6). E and F. Representative blots and quantitative results for p-NFκB p65, NFκB p65 and IL-1β in the heart tissues of mice in the indicated groups (n=6). *$P<0.05$ vs WT/sham. #$P<0.05$ vs WT/AB after AB.

PCR analysis to examine the mRNA expression of hypertrophic genes, including atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), sarcoendoplasmic reticulum Ca2+-ATPase (SERCA2α) and actin α1 skeletal muscle (Acta1). The levels of these cardiac foetal genes including ANP, BNP, and Acta1 were strongly up-regulated in IRAK4 HET mice, while SERCA2α expression level exhibited a significant down-regulation (**Figure 2C**). These results suggested that partial IRAK4 deficiency promoted cardiac hypertrophy and deteriorated impaired cardiac function after pressure overload.

Effects of IRAK4 on MAPKs, AKT and NFκB signaling

We first examine the classic signaling pathway involving IRAK4. Less expression of IRAK4 attenuated the phosphorylation of TAK1 (**Figure 3A**, **3B**). Accumulating evidence suggests that MAPKs and Akt are among the most

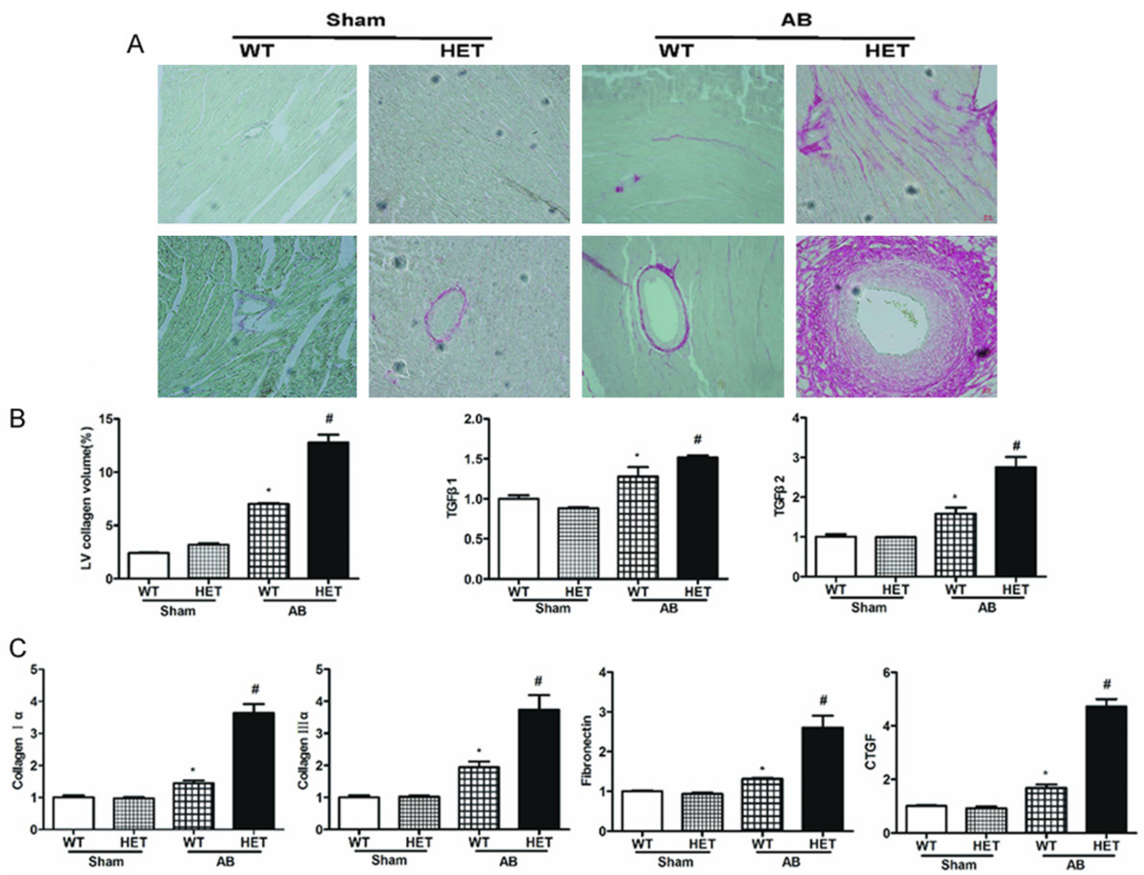


Figure 4. IRAK4 deficiency exacerbates the fibrotic response induced by pressure overload. A. Histological sections of the left ventricle were stained for picrosirius red for the indicated groups. B. Fibrotic areas from histological sections were quantified using an image-analyzing system. C. The mRNA expression of collagen Iα, collagen IIIα, TGF-β1, TGF-β2, fibronectin and CTGF in the myocardium were obtained from indicated groups using RT-PCR analysis. **P*<0.05 vs WT/sham. #*P*<0.05 vs WT/AB after AB.

characterized signaling pathways activated by pressure overload-induced remodeling [15, 16]. We therefore focused our analysis on these two pathways to investigate how IRAK4 affected the hypertrophic response. The results strongly indicated that the phosphorylation of MEK-ERK1/2, JNK and p38 were significantly promoted in WT mice after AB and that IRAK4 deficiency reinforced the phosphorylation of MEK-ERK1/2 and p38. On the other hand, there was no difference in phosphorylated JNK levels between IRAK4 HET and WT mice. We then examined the activation of GATA4, a downstream effector of ERK1/2 and P38 [17, 18], and found the phosphorylation of GATA4 after AB showed a similar tendency to that of ERK1/2 and p38. Notably, the phosphorylation of Akt was not affected by the deficiency of IRIK4 (**Figure 3C**, **3D**). Previous studies suggest that the NFκB signaling plays an important role in the pathogenesis of cardiac remodeling and heart failure [19], and IRAK4is linked closely with NFκB signaling [20], therefore, we examined NFκB signaling in the mice hearts. The activation of NFκBp65 was dramatically enhanced in IRAK4 HET mice after AB compared with that in WT mice (**Figure 3E**, **3F**). We also examined the expression of a kind of inflammatory factor IL-1β, and found that the protein level of IL-1β increased in IRAK4 HET mice compared to WT mice (**Figure 3E**, **3F**). These results indicate that partial IRAK4-induced aggravated cardiac remodeling induced by pressure overload may be related to these multiple signaling pathways.

IRAK4 deficiency exacerbates the fibrotic response induced by pressure overload

Fibrosis is an essential part of the pathological process of cardiac hypertrophy characterized by the accumulation of collagen and mediated by various cytokines [21]. After PSR staining, remarkable perivascular and interstitial fibrosis was revealed in the WT mice in response to AB, and the extent of cardiac fibrosis was even more severe in the IRAK4 HET mice (**Figure 4A**, **4B**).

In order to elucidate the mechanisms of collagen synthesis, we examined the mRNA expression of collagen Iα, collagen IIIα, transforming growth factor-β1 (TGF-β1), TGF-β2, fibronectin, and connective tissue growth factor (CTGF), which are all known mediators of fibrosis. Our data shows that partial IRAK4 deficiency enhanced the increase of CTGF, TGF-β1, TGF-β2, collagen Iα, collagen IIIα, and fibronectin expression in response to AB (**Figure 4C**).

Discussion

A variety of cardiac disease can induce cardiac hypertrophy and lead to heart failure. A plethora of signal transduction events may be involved in the complex disease states, including some in the innate immunity system [22]. In this study, we demonstrated the role of IRAK4, which is a ubiquitously expressed kinase involved in the regulation of innate immunity [23], in cardiac hypertrophy induced by pressure overload in vivo using IRAK4 HET mice. We found that (i) the expression of phosphorylated IRAK4 and total IRAK4 upregulated in hearts of DCM patients and also hearts of mice after 4 weeks of pressure overload. (ii) IRAK4 HET mice exhibited exacerbated cardiac hypertrophy, dysfunction and fibrosis after 4 weeks of aortic banding (AB) compared with that in WT mice. Furthermore, enhanced activation of the MEK-ERK1/2, p38 and NFκB pathways was found in IRAK4 HET mice compared to WT mice. Together, we demonstrated that the regulation of IRAK4 expression in the heart might affect the responses of heart to pressure overload.

Phosphorylated IRAK4 and total IRAK4 expression were both up-regulated in human DCM hearts and also in mice hearts after 4 weeks of AB.

To investigate the molecular mechanism by which IRAK4 mediate its beneficial effect on cardiomyocytes, we examined MAPK, AKT and NF-κB signaling, which were all pivotal contributors to the development of cardiac hypertrophy. ERK, p38 and NF-κB phosphorylation in response to hypertrophic stimuli significantly upregulated in IRAK4 HET mice. However, in a septic shock model induced by LPS, mice lack of IRAK4 were shown to have decreased JNK activation and NF-κB activation. Furthermore, IL-1-induced NF-κB, JNK and p38 activation were all severely defective in cells lacking IRAK4[8]. The role of IRAK4 in the signaling network seemed confusing, especially in different cell types. Another study demonstrated that IL-1 stimulation could lead to similar level of phosphorylation of JNK, IκB, and p38 and NF-κB activation in BM-derived macrophages from wild-type and IRAK4 kinase-inactive knock-in mice, but these signaling events were greatly diminished in BM-derived macrophages from IRAK4-deficient mice [24]. It suggested that partial loss of IRAK4 kinase function and complete IRAK4 deficiency leaded to different consequences. In our study, partial loss of IRAK4 kinase function leads to significantly higher level of phosphorylation of ERK, p38 and NF-κB in mice hearts. It indicated that while there was a certain amount expression of IRAK4, IRAK4 kinase might act as a protector of heart from pressure overload via regulation of ERK, p38 and NF-κB signaling pathways.

The mechanism by which IRAK4 specifically blocks ERK, p38 and NFκB pathways remains unknown. IRAK4 may modulate them directly or modulate a specific molecular or molecular complex that specifically regulates these signaling pathways. One study suggested that complete loss of IRAK4 protected mice heart from viral myocarditis through upregulating interferon-α and interferon-γ production and CCR5+ monocytes/macrophages recruitment to the heart [10], indicating that the importance of cell migration regulated by complete IRAK4 loss in the heart. In the situation of pressure overload, there may be cell migration that may be involved in the process of cardiac hypertrophy. Another study showed that in IRAK4-/-mice, cardiac DCs had lower expression of CD80 and CD86 genes after myocardial infarction and BMDCs had less ability to proliferate CD4+ T lymphocytes, indicating that decreased inflammation in the infracted myocardium of IRAK4-/-mice may be associated with decreased T-lymphocyte activation, but the extent of autoimmune response contributes to the inflammatory process after myocardial infarction remains unclear [9]. In cardiac hypertrophy model, autoimmune response may also contribute to the inflammatory process. However, it may be totally different in IRAK4 HET mice from that in IRAK4-/-mice. Further studies are called to establish a more complete theory system for the anti-hypertrophic effect of IRAK4 in the presence of original IRAK4 expression in heart.

Cardiac fibrosis, characterized by an increase in collagens and other extracellular matrix (ECM) components in the interstitial and perivascular regions of myocardium, is an important feature of pathological hypertrophy [25]. The most abundant collagen types in the heart are the fibrillar collagens, type I and III, accounting together for over 90% of the total collagen [21]. We found excessive collagen deposition in IRAK4 HET mice, as well as the up-regulated mRNA levels of collagen I and III after pressure overload, and both results revealed that partial IRAK4 deficiency facilitates collagen synthesis. In addition, we demonstrated the enhanced mRNA expression of TGFβ and CTGF, two major extracellular signals that promote fibrosis in the hypertrophy. These all suggested IRAK4 might have a significant role in protecting cardiac remodeling after AB.

In conclusion, the findings in our study uncover an essential role of IRAK4 in regulating pathological cardiac hypertrophy, cardiac dysfunction and fibrosis via the negative feedback to the ERK, p38 and NFκB signaling cascades.

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Disclosure of conflict of interest

None.

Abbreviations

AB, aortic banding; Acta1, actin α1 skeletal muscle; AngII, Angiotension II; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; BW, body weight; CSAs, cardiomyocyte cross-sectional areas; CTGF, connective tissue growth factor; DCM, dilated cardiomyopathy; ECM, extracellular matrix; EF, ejection fraction; FS, Fractional shortening; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; H&E, hematoxylin and eosin; HET, heterozygous knockout; HR, heart rate; HW, heart weight; HW/BW, heart weight/body weight; HW/TL, heart weight/tibia length; IRAK4, Interluekin1 receptor-associated kinase-4; IVSd, interventricular septal thickness at end-diastole; JNK, c-Jun NH2-terminal kinase; LVEDd, left ventricular end-diastolic diameter; LVESd, left ventricular end-systolic diameter; LVPWD, left ventricular posterior wall thickness at end-diastole; LW, lung weight; LW/BW, lung weight/body weight; MAPKs, mitogen activated protein kinases; MI, myocardial ischemia; MyD88, myeloid differentiation primary response 88; NFAT, nuclear factor of activated T cells; PI3K, phosphatidylinositol 3-kinase; PSR, picrosirius red; SERCA2α, sarcoendoplasmic reticulum Ca^{2+}-ATPase; TAK1, TGFβ-activated kinase 1; TGF-β1, transforming growth factor-β1; TGF-β2, transforming growth factor-β2; TIR, Interleukin-1 receptor; Traf6, TNF receptor-associated factor 6; WT, wild type.

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