Published in final edited form as:

Hypertension. 2014 January ; 63(1): 96–104. doi:10.1161/HYPERTENSIONAHA.113.01506.

Signal Regulatory Protein-α Protects Against Cardiac Hypertrophy Via the Disruption of Toll-Like Receptor 4 Signaling

Ding-Sheng Jiang, **Xiao-Fei Zhang**, **Lu Gao**, **Jing Zong**, **Heng Zhou**, **Yu Liu**, **Yan Zhang**, **Zhou-Yan Bian**, **Li-Hua Zhu**, **Guo-Chang Fan**, **Xiao-Dong Zhang***, and **Hongliang Li***

Department of Cardiology, Renmin Hospital of Wuhan University, Wuhan, China (D.-S.J., J.Z., H.Z., Y.L., Y.Z., Z.-Y.B., L.-H.Z., H.L.); Cardiovascular Research Institute of Wuhan University, Wuhan, China (D.-S.J., J.Z., H.Z., Y.L., Y.Z., Z.-Y.B., L.-H.Z., H.L.); College of Life Sciences, Wuhan University, Wuhan, China (X.-F.Z., G.-C.F., X.-D.Z.); Department of Cardiology, Institute of Cardiovascular Disease, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China (L.G.); and Department of Pharmacology and Cell Biophysics, University of Cincinnati, OH (G.-C.F.)

Abstract

Signal regulatory protein-α (SIRPA/SIRPα) is a transmembrane protein that is expressed in various tissues, including the heart. Previous studies have demonstrated that SIRPA is involved in multiple biological processes, including macrophage multinucleation, skeletal muscle differentiation, neuronal survival, protection against diabetes mellitus, and negative regulation of immune cells. However, the role of SIRPA in cardiac hypertrophy remains unknown. To examine the role of SIRPA in pathological cardiac hypertrophy, we used SIRPA knockout mice and transgenic mice that overexpressed mouse SIRPA in the heart. Cardiac hypertrophy was evaluated by echocardiographic, hemodynamic, pathological, and molecular analyses. We observed downregulation of SIRPA expression in dilated cardiomyopathy human hearts and in animal hearts after aortic banding surgery. Accordingly, $SIRPA^{-/-}$ mice displayed augmented cardiac hypertrophy, which was accompanied by increased cardiac fibrosis and reduced contractile function, as compared with $SIRPA^{+/+}$ mice 4 weeks after aortic banding. In contrast, transgenic mice with the cardiac-specific SIRPA overexpression exhibited the opposite phenotype in response to pressure overload. Likewise, SIRPA protected against angiotensin II–induced cardiomyocyte hypertrophy in vitro. Mechanistically, we revealed that SIRPA-mediated protection during cardiac hypertrophy involved inhibition of the Toll-like receptor 4/nuclear factor-κB signaling axis. Furthermore, we demonstrated that the disruption of Toll-like receptor 4 rescued the adverse effects of SIRPA deficiency on pressure overload–triggered cardiac remodeling. Thus,

Correspondence to Hongliang Li, Department of Cardiology, Renmin Hospital of Wuhan University, Jiefang Rd 238, Wuhan 430060, China. lihl@whu.edu.cn.

*H.L. and X.-D.Z. contributed equally to this work.

D.-S.J., X.-F.Z., and L.G are first coauthors.

The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.113.01506/-/DC1.

Disclosures

None.

our results identify that SIRPA plays a protective role in cardiac hypertrophy through negative regulation of the Toll-like receptor 4/nuclear factor-κB pathway.

Keywords

cardiomegaly; fibrosis; SIRPA protein; human; TLR4 receptor

Many clinical and epidemiological studies have shown that sustained cardiac hypertrophy contributes to an increased risk for cardiovascular events and mortality.[1–4] In fact, it is an important independent risk factor for chronic heart failure, coronary heart disease, arrhythmia, and sudden cardiac death.[1–4] Cardiac hypertrophy is a complex dynamic process that occurs when the heart endures injury or an elevated workload.[5] Although cardiac hypertrophy originates as a compensatory response to adverse stress, long-term cardiac overload can lead to maladaptive hypertrophy, which is often characterized by progressive ventricular cavity expansion and wall thinning.[2,4] In addition to increased myocyte size, cardiac hypertrophy is also associated with proliferation and activation of cardiac fibroblasts, which lead to fibrosis, increased myocardial stiffness, and subsequent irreversible deterioration.[6,7] Several studies have implicated multiple signaling pathways in the progression of pathological cardiac hypertrophy; however, effective therapies for antagonizing this process have not yet been developed.

Signal regulatory protein-α (SIRPA/SIRPα), which is also known as Src homology 2 domain containing protein tyrosine phosphatase (SHP) substrate-1, is an ≈115 kDa transmembrane protein that belongs to the SIRP family.[8,9] The SIRPA gene is located on chromosome 20p13 in humans and in chromosome 2 in mice, and the other SIRP family genes are all distributed on the same chromosome.[10] SIRPA expression can be detected in multiple tissues, including the tissues of the heart, brain, spleen, lung, liver, muscle, kidney, and testis.[11,12] Structurally, SIRPA consists of an extracellular region with 3 immunoglobulin-like domains, a transmembrane domain, and a cytoplasmic region that contains 4 tyrosine phosphorylation sites that mediate the binding of SHP-1 and SHP-2.[8,13] In addition, SIRPA contains several potential N-glycosylation sites and multiple serines and threonines.[12,14] The following conclusions have been well established: (1) SIRPA functions as a docking protein to recruit and activate SHP-1 or SHP-2; (2) the tyrosine phosphorylation of SIRPA can be regulated by integrin and by various growth factors/cytokines; and (3) SIRPA is involved in various biological processes, including the suppression of anchorage-independent cell growth, the negative regulation of immune cells, control of macrophage multinucleation, and skeletal muscle differentiation.[13–15] We recently demonstrated that SIRPA protects against the oxidative stress and brain injury that are induced by acute cerebral ischemia.[8] Although SIRPA has been identified as a cell surface marker for cardiomyocytes,[9] it is not known whether SIRPA participates in the development of pathological cardiac hypertrophy. In the present study, we sought to determine the effects of SIRPA deficiency and cardiac-specific SIRPA overexpression on aortic banding (AB)-induced cardiac remodeling.

Our findings have indicated that SIRPA expression was strikingly reduced in human dilated cardiomyopathy (DCM) hearts and in pressure overload–induced hypertrophic mouse hearts. Moreover, we found that disruption of SIRPA accelerated the hypertrophic responses in both in vivo (aortic-banded hearts) and in vitro (angiotensin II [Ang II]-treated neonatal cardiomyocytes) models. In contrast, cardiac-specific overexpression of SIRPA dramatically mitigated pressure overload–induced cardiac hypertrophy. We further revealed that SIRPA-mediated cardioprotection was associated with a downregulation of Toll-like receptor 4 (TLR4) and the inactivation of nuclear factor-κB (NF-κB). Importantly, loss of TLR4 rescued the adverse effects of SIRPA deficiency on cardiac hypertrophy. Taken together, we identify that SIRPA acts as a novel negative modulator of pathological cardiac hypertrophy via regulation of the TLR4/NF-κB signaling axis.

Materials and Methods

An expanded Methods section is available in the online-only Data Supplement.

Mouse Models

All the experimental procedures involving animals were approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University. SIRPA knockout (SIRPA$^{-/-}$) mice (B6.129P2-Sirpa<tm1Nog>; C57BL/6 background) were kindly provided by Takashi Matozaki (Gunma University) and shipped by RIKEN (RBRC01544).[16] Cardiac-specific SIRPA transgenic (TG) mice were generated by cloning full-length mouse SIRPA cDNA (OriGene; MC218099) downstream of the α-myosin heavy chain (α-MHC) promoter. TLR4 knockout (TLR4$^{-/-}$) mice (B6.B10ScN-*Tlr4*$^{lps\text{-}del}$/JthJ) were ordered from Jackson Laboratory (stock number 007227). The SIRPA$^{-/-}$ mice were crossed with TLR4$^{-/-}$ mice to generate SIRPA/TLR4 double knockout mice.

Aortic Banding

We established a model of pressure overload–induced cardiac hypertrophy via AB as previously described.[4,17,18]

Echocardiography and Hemodynamic Measurements

Echocardiography and invasive hemodynamic measurements were performed on mice as described previously.[4,17–20]

Recombinant Adenoviral Vectors

Replication-defective adenoviral vectors were used to overexpress rat SIRPA (AdSIRPA) under the control of the cytomegalovirus promoter, and a similar adenoviral vector expressing green fluorescent protein (GFP) was used as a control. And shSIRPA adenoviral constructs were purchased from SABiosciences (KR50731G).

Cultured Neonatal Rat Cardiac Myocytes

Neonatal rat cardiomyocytes (NRCMs) were prepared as previously described.[17]

Immunoprecipitation and Confocal Microscopy

Immunoprecipitation and coimmunostaining were performed as previously described.[21]

Statistical Analysis

Data are represented as means±SD. Two-tailed Student *t* tests were used to compare the means of 2 sample groups, and 1-way ANOVA tests with least significant difference (equal variances assumed) or Tamhane T2 (equal variances not assumed) were applied to multiple groups. Statistical significance was set at $P<0.05$.

Results

SIRPA Expression Is Downregulated in DCM Human Hearts and AB-Operated Mouse Hearts

To investigate the correlation between SIRPA and cardiac hypertrophy, we first examined SIRPA expression in heart samples collected from human patients with DCM and from mice that had been subjected to AB. Our data revealed that the protein levels of various inducers of cardiac hypertrophy, including atrial natriuretic peptide and β-MHC, P-p65, and P-IκBα, were dramatically increased in DCM hearts compared with normal hearts (Figure 1A and 1B; Figure S1A and S1B in the online-only Data Supplement); moreover, relative to normal hearts, the DCM hearts demonstrated decreased SIRPA expression (Figure 1A and 1B; Figure S1C). Information on DCM patients is available in Table S1. In particular, at 4 and 8 weeks after AB surgery, AB-treated mice exhibited cardiac SIRPA levels that were also reduced by ≈36% and 74%, respectively (Figure 1C and 1D; Figure S1D; $P<0.05$ versus sham). Similarly, the expression of endogenous SIRPA was strikingly downregulated in Ang II- or phenylephrine-induced hypertrophic cardiomyocytes (Figure 1E and 1F; Figure S1E). Also, SIRPA luciferase activity was significantly reduced in H9C2 cells treated with 1 μmol/L of Ang II compared with PBS control (Figure S1F). Moreover, the phosphorylation level of SIRPA was increased in response to Ang II stimulation in NRCMs (Figure S1G). These results suggest that SIRPA may be involved in the development of cardiac hypertrophy.

SIRPA Prevents Cardiomyocyte Hypertrophy In Vitro

To identify whether SIRPA regulated the progression of cardiac hypertrophy, we infected NRCMs with either AdSIRPA to overexpress SIRPA or AdshSIRPA to knockdown SIRPA (Figure S2A). Subsequently, these adenovirus-infected myocytes were treated with Ang II (1 μmol/L) for 48 hours to induce hypertrophy. Cell sectional area was then measured by immunostaining with α-actinin. Importantly, under control conditions (PBS treatment), neither the overexpression (by AdSIRPA) nor the knockdown (by shSIRPA) of SIRPA affected the size of neonatal cardiomyocytes compared with appropriate control cells (AdGFP- and AdshRNA-infected cells, respectively; Figure S2B–S2D). However, following Ang II stimulation, there was significantly greater cell sectional area in cells with SIRPA knockdowns (by 1.4-fold) compared with AdshRNA-infected controls (Figure S2B and S2C). In addition, Ang II–induced cardiomyocyte hypertrophy was remarkably attenuated in AdSIRPA-infected cells compared with GFP controls (Figure S2B and S2D). In line with

these findings, Ang II–induced expression of the hypertrophic hallmarks atrial natriuretic peptide and β-MHC and the ratio of protein/DNA were profoundly activated in shSIRPA cells (Figure S2E and S2G) and were significantly suppressed in SIRPA-overexpressing cardiomyocytes (Figure S2F and S2H), compared with the respective controls. Overall, these data suggest that increased expression of SIRPA could produce an antihypertrophic effect, whereas reduced levels of SIRPA may contribute to the progression of cardiac hypertrophy.

SIRPA Deficiency Augments Pressure Overload–Induced Cardiac Hypertrophy and Fibrosis

We then performed AB surgery on SIRPA$^{-/-}$ mice to further define the role of SIRPA in cardiac hypertrophy. The ablation of SIRPA in SIRPA$^{-/-}$ mice hearts was confirmed by Western blotting (Figure S3A). Although SIRPA$^{-/-}$ mice exhibited a normal cardiac phenotype at baseline (Table S2), these mice demonstrated aggravated cardiac hypertrophy after 4 weeks of AB, as evidenced by higher ratios of heart weight (HW)/body weight (BW), HW/tibia length (TL), and lung weight (LW)/BW, compared with SIRPA$^{+/+}$ mice (Figure 2C–2E). In parallel, both H&E and WGA staining consistently demonstrated that cardiomyocyte cell sectional area was significantly increased in SIRPA$^{-/-}$ hearts in relation to SIRPA$^{+/+}$ samples (Figure 2A and 2B). Accordingly, the expression levels of examined hypertrophy markers (atrial natriuretic peptide, type B natriuretic peptide, and β-MHC) were greatly increased in AB-treated SIRPA$^{-/-}$ hearts compared with control hearts (Figure S3B). Furthermore, SIRPA deficiency was also associated with accelerated left ventricular (LV) dilation and dysfunction, as evaluated by LV end-diastolic diameter, LV end-systolic diameter, and fractional shortening (Table S3). Hemodynamic analyses also revealed worse systolic and diastolic LV function in SIRPA$^{-/-}$ mice than in control mice, as determined by the maximum and minimum rates of pressure increase during LV isovolumic contractions (dp/dt max and dp/dt min, respectively; Table S3).

Given that fibrosis is an integral hallmark of cardiac hypertrophy,[22,23] we next assessed cardiac fibrosis in AB-operated SIRPA$^{-/-}$ and SIRPA$^{+/+}$ mice. Heart sections were stained with picrosirius red to evaluate their degrees of fibrosis. Our results revealed that the extent of cardiac fibrosis was more prominent in SIRPA$^{-/-}$ mice compared with control SIRPA$^{+/+}$ mice on 4-week AB (Figure 2F and 2G). It is well known that the most abundant heart collagens are type I and III, which contribute to pressure overload–induced collagen deposition,[24] and that connective tissue growth factor is a major extracellular signal that promotes fibrosis in the hypertrophic heart.[7,22,24] Therefore, we next aimed to determine whether mRNA levels of connective tissue growth factor, collagen Iα, and collagen III were altered in these hypertrophic hearts relative to normal hearts. RT-PCR results demonstrated that pressure overload triggered much greater induction of connective tissue growth factor, collagen Iα, and collagen III in SIRPA$^{-/-}$ hearts than in SIRPA$^{+/+}$ samples (Figure S3C). Collectively, these data indicate that loss of cardiac SIRPA leads to exaggerated hypertrophy and fibrosis on chronic pressure overload.

SIRPA Overexpression Mitigates AB-Induced Cardiac Remodeling

We subsequently sought to determine whether SIRPA overexpression could prevent the progression of cardiac hypertrophy in vivo. To achieve this objective, we used an α-MHC promoter to create a TG mouse model with cardiac-specific overexpression of SIRPA (Figure S3D). In total, 4 lines of TG mice were created (Figure S3E). Notably, all these TG lines were viable and fertile, and there were no abnormalities in cardiac size and structure at 16 weeks of age (Table S2). Importantly, our preliminary studies demonstrated that these established lines exhibited no obvious differences in their responses to hypertrophic stress (data not shown). Hence, for our subsequent experiments, we selected Tg9, which would moderately express SIRPA. After 8 weeks of AB, the SIRPA TG hearts revealed an attenuated hypertrophic response compared with AB-treated nontransgenic (NTG) hearts (Figure 3A–3E). Moreover, histological results showed that cardiomyocyte size was dramatically decreased in TG mice compared with NTG mice after 8 weeks of AB (Figure 3A and 3B). In addition, the HW/BW, LW/BW, and HW/TL ratios were significantly lower in AB-treated TG hearts than in AB-treated NTG hearts (Figure 3C–3E). In accordance with these results, after 8 weeks of AB, myocardial contractile function, as measured by echocardiography and hemodynamic analyses, was greatly improved in TG mice compared with NTG mice (Table S4).

We also evaluated the effect of SIRPA overexpression on AB-induced cardiac fibrosis. Picrosirius red staining revealed a remarkable decrease in perivascular and interstitial fibrosis in TG mice compared with NTG mice in response to AB (Figure 3F and 3G). In combination, these gain-of-function data support the notion that elevated SIRPA levels could retard the progression of pressure overload–induced cardiac hypertrophy.

SIRPA Inhibits the TLR4 Signaling Pathway

The present results demonstrated that SIRPA plays a protective role in cardiac hypertrophy. However, the potential mechanism through which SIRPA prevents cardiac hypertrophy and diminishes predisposition to heart failure remains unknown. To examine this mechanism, we used microarray hybridization to identify the differentially expressed genes in the hearts of wild-type (WT) and SIRPA$^{-/-}$ mice at 2 weeks after AB surgery. We found that, at the indicated times after AB surgery, the expression levels of hypertrophy-related genes, fibrosis-related genes, and inflammation-related genes were significantly increased in SIRPA$^{-/-}$ mice relative to WT mice, whereas the expression levels of certain other genes were decreased in the knockout mice relative to WT controls (Figure S4A). Unexpectedly, TLR4 is rather distinct from other changed genes because TLR4 demonstrates an ≈6.5-fold increase in SIRPA$^{-/-}$ mice compared with WT controls; these results were confirmed through RT-PCR and Western blotting in cultured cardiomyocytes and in SIRPA$^{-/-}$ and TG mice at 2 weeks after AB (Figure S4B–S4D; Figure 4A and 4B). In particular, RT-PCR and Western blot data revealed that the loss of SIRPA significantly increased the expression of TLR4 (Figure S4B; Figure 4A), whereas the overexpression of SIRPA attenuated AB-triggered TLR4 expression compared with the appropriate controls (Figure S4B; Figure 4B). Furthermore, TLR4 expression was induced in cultured cardiomyocytes that had been exposed to 1 μmol/L of Ang II; this induced expression was enhanced by SIRPA knockdown (Figure S4C) and blocked by SIRPA overexpression (Figure S4D). We then

performed coimmunostaining and coimmunoprecipitation experiments to validate whether SIRPA physically interacts with TLR4. Our results consistently indicated that TLR4 colocalized with SIRPA (Figure 4C) and that TLR4 and SIRPA coimmunoprecipitated each other (Figure 4D), which is consistent with the results of coimmunoprecipitation for endogenous proteins in NRCMs (Figure S4E and S4F). Collectively, these results demonstrate that SIRPA inhibits TLR4 expression and directly interacts with TLR4.

Given that NF-κB is a signaling molecule that lies downstream of TLR4,[25] we tested whether NF-κB activation is altered in SIRPA-overexpressing and SIRPA-deficient hearts after AB. At present, it is well established that dimeric NF-κB family members (primarily p50/p65) are inactivated by becoming bound to IκBs in cells at rest.[26] The stimulation of cells by stress signals causes the phosphorylation of IκB, which releases IκB from binding to the NF-κB complex, resulting in the translocation of p65/p50 from the cytosol to the nucleus.[26] Subsequently, phosphorylated IκB will be degraded.[26] Therefore, we evaluated the levels of total and phosphorylated IκBα in AB-treated SIRPA$^{-/-}$ and SIRPA TG hearts. Western blotting results demonstrated that the levels of phosphorylated IκBα were significantly increased and the total levels of IκBα were greatly reduced in SIRPA$^{-/-}$ hearts compared with WT hearts after 2 weeks of AB (Figure 4E). In contrast, SIRPA overexpression reduced AB-triggered activation of IκBα, as evidenced by remarkably decreased phosphorylation of IκBα and increased total levels of IκBα in SIRPA TG hearts compared with control hearts (Figure 4F). In accordance with these results, the nuclear translocation of NF-κB was restricted by the overexpression of SIRPA, as evidenced by the presence of lower levels of phosphorylated P65 in AB-treated SIRPA TG hearts than in AB-treated NTG hearts (Figure 4F). Conversely, SIRPA$^{-/-}$ hearts demonstrated significantly increased AB-triggered phosphorylation of P65 compared with control hearts (Figure 4E).

In addition, NF-κB activation has been implicated in the upregulation of inflammation in many cell types, including cardiac myocytes.[27] Thus, we next measured the expression profiles of NF-κB–dependent inflammatory factors of tumor necrosis factor-α, monocyte chemoattractant protein-1, and interleukin-1β in animal hearts after chronic AB. Both RT-PCR analysis (Figure S4H) and Western blotting (Figure S4J) revealed that the overexpression of SIRPA remarkably blunted AB-induced increases in the expression levels of tumor necrosis factor-α, monocyte chemoattractant protein-1, and interleukin-1β. By contrast, the loss of SIRPA produced the opposite effect (Figure S4G and S4I). These results further support the notion that SIRPA protects against cardiac hypertrophy by inactivating the TLR4/NF-κB signaling pathway.

SIRPA-Mediated Antihypertrophic Effects Are Largely Dependent on the Inactivation of TLR4

To determine whether SIRPA-elicited antihypertrophic effects are directly or indirectly associated with the inhibition of TLR4, we induced cellular hypertrophy in SIRPA-overexpressing NRCMs by treating these cells with 1 μmol/L of Ang II, in the presence or absence of lipopolysaccharide (LPS) (an agonist of TLR4) for 48 hours. The results of cell size analysis revealed that Ang II–induced myocyte hypertrophy was more pronounced in LPS-treated GFP-infected cells than in PBS-treated GFP-infected cells (Figure S5A); these

findings are consistent with previous reports that reveal that LPS promotes myocyte hypertrophy.[28] However, we observed that significantly larger cross-sectional cell area was observed in the LPS plus Ang II plus AdSIRPA treated group than in the PBS plus Ang II plus AdSIRPA control group (Figure S5A). This suggests that the treatment of myocytes with LPS relieved the antihypertrophic effects of SIRPA. Simultaneously, we treated myocytes that exhibited reduced SIRPA levels (AdshSIRPA cells) with eritoran, an antagonist for TLR4, in the presence of Ang II and culture medium. Similarly to previous observations,[29] the inhibition of TLR4 with eritoran limited Ang II–induced cell hypertrophy (Figure S5B). Interestingly, the prohypertrophic effects that were induced by SIRPA knockdown were dramatically mitigated by eritoran (Figure S5B). Collectively, these data suggest that the negative effects of SIRPA on pathological cardiac hypertrophy are at least partially dependent on the inhibition of TLR4.

Disruption of TLR4 Rescued the Adverse Effect of SIRPA Deficiency on Cardiac Hypertrophy

We know that (1) SIRPA is downregulated in DCM human hearts and hypertrophic animal hearts (this study; Figure 1); (2) increased TLR4 expression has been observed in animal hypertrophic hearts (this study; Figure S4A and S4B; Figure 4A), failing human hearts, and ischemic hearts[30]; and (3) TLR4-deficient mice showed attenuated cardiac hypertrophy after AB.[31] Therefore, we next assessed whether the disruption of TLR4 could rescue the detrimental effects on cardiac hypertrophy that were observed in SIRPA knock down cardiomyocytes/knockout hearts. To accomplish this objective, we generated TLR4 and SIRPA double knockout mice by crossing $TLR4^{-/-}$ mice with $SIRPA^{-/-}$ mice (Figure S6A and S6B). Double knockout mice, $TLR4^{-/-}$ mice, $SIRPA^{-/-}$ mice, andWT mice ($SIRPA^{+/+}$ mice) were subjected to AB surgery. We could observe that the loss of TLR4 suppressed AB-induced cardiac hypertrophy (Figure 5A–5E); this finding was consistent with previous reports.[31] Remarkably, prohypertrophic effects induced by SIRPA deficiency were also blocked by TLR4 ablation, as determined by histological analyses; myocyte cross-sectional areas; and the ratios of HW/BW, LW/BW, and HW/TL (Figure 5A–5E). In accordance with our results, myocardial dysfunction and fibrosis that were caused by SIRPA deficiency after AB were significantly eliminated in the double knockout mice (Table S5 and Figure S6C and S6D). Mechanistically, the increased phosphorylation of IκBα and p65 in $SIRPA^{-/-}$ hearts as a result of pressure overload was abolished by the disruption of TLR4 (Figure 5F and 5G). In combination, these results suggest that TLR4 targeting may limit the prohypertrophic effects of SIRPA decreases in hearts.

Discussion

Our novel results have demonstrated that SIRPA plays a protective role in cardiac hypertrophy and fibrosis through the inhibition of the TLR4 signaling pathway. Major findings of our study include: (1) SIRPA expression was strikingly decreased in DCM human hearts and in pressure overload–induced hypertrophic animal hearts; (2) SIRPA deficiency accelerated the hypertrophic response of mouse hearts on AB, whereas the overexpression of SIRPA protected against pressure overload–triggered cardiac hypertrophy; (3) SIRPA-mediated cardioprotection was associated with the inhibition of

TLR4/NF-κB signaling axis; and (4) TLR4 disruption rescued the adverse effects of SIRPA deficiency on cardiac hypertrophy. Altogether, these data suggest that SIRPA elevation may serve as a novel approach to the treatment of pathological cardiac hypertrophy.

Currently, SIRPA has been implicated in various biological processes, including the suppression of anchorage-independent cell growth, the negative regulation of immune cells, controlling of macrophage multinucleation, skeletal muscle differentiation, neuronal survival, and the development of contact hypersensitivity.[8,14,32] In addition, SIRPA plays important roles in negatively regulating both TLR3 and cytoplasmic pathways in type I interferon induction,[33] promoting insulin secretion, and protecting against diabetes mellitus.[34] Notably, SIRPA is expressed in heart tissue and has been identified as a cell surface marker of cardiomyocytes.[9,12] In this study, we found that cardiac SIRPA expression was suppressed not only in AB-treated mice hearts but also in the hearts of human DCM patients. This suggests that SIRPA may be involved in the development of pathological cardiac hypertrophy. We observed that loss of SIRPA augmented AB-induced cardiac hypertrophy, indicating that the reduction of SIRPA levels that normally occurs in hypertrophic hearts may promote the progression of cardiac hypertrophy and failure. Meanwhile, our results also found that phosphorylation level of SIRPA was increased in response to Ang II stimulation in NRCMs (Figure S1G). Many studies have showed SIRPA to be tyrosine-phosphorylated by binding to cluster of differentiation 47 (CD47)/integrin-associated protein, or various growth factor stimulants (eg, brain-derived neurotrophic factor, epidermal growth factor, insulin).[12,35–38] Moreover, TLR4- and LPS-induced SIRPA phosphoyrlation and degradation play an essential role in activation of mitogen-activated protein kinases and NF-κB pathways in macrophage.[39] However, further study is needed to clarify whether phosphorylation of SIRPA plays a role in the degradation of SIRPA or in the development of cardiac hypertrophy.

A key finding in the current study is that SIRPA regulates cardiac hypertrophy and fibrosis by targeting TLR4 signaling. TLR4 is a pattern recognition receptor expressed mainly on myeloid cells, but has also been found on nonprofessional immunocytes, such as cardiomyocytes and microvascular endothelial cells.[25,30] Moreover, recent evidence has suggested that TLR4 is involved in cardiovascular diseases. For example, increased TLR4 expression has been observed in failing human hearts and in ischemic hearts.[30] Furthermore, TLR4-deficient mice displayed reduced cardiac hypertrophy and fibrosis relative to WT mice in response to AB.[31] In our study, we found that the expression of TLR4 was downregulated by SIRPA after pressure overload and that SIRPA can directly interact with TLR4. These data suggest that SIRPA-mediated antihypertrophic effects may be associated with the inhibition of TLR4 signaling axis. In support of this, NF-κB, a critical downstream transcription factor of the TLR4-mediated signaling pathway,[25] was inhibited in SIRPA-overexpressing hearts. Hamid et al[40] revealed that cardiomyocyte NF-κB promotes adverse remodeling (fibrosis and hypertrophy) in heart failure. In addition, we showed that TLR4 disruption rescued the adverse effects of SIRPA deficiency on cardiac hypertrophy, which suggested that TLR4 activation may be required for the generation of the prohypertrophic effects of SIRPA deficiency. Both loss-of-function and gain-of-function data consistently support the notion that SIRPA negatively regulates pathological cardiac remodeling by targeting the TLR4/NF-κB signaling pathway.

Another interesting finding of this study is that SIRPA causes the reduction of TLR4 in hypertrophic hearts. Both SIRPA and TLR4 are known to be membrane-resident proteins,[8,31] and we verified their localization pattern in this study (Figure 4C). Thus, it may be hard to explain how SIRPA causes the reduction of TLR4 in hypertrophic hearts. The studies of Husebye et al[41] and Zanoni et al[42] may provide an explanation for this finding. Husebye et al[41] revealed that in nonstimulated human monocytes TLR4 was found in the endoplasmic reticulum, the plasma membrane, and the Golgi of cells and was sporadically detected in endosomal structures. After LPS stimulation, TLR4 accumulated in the endosomes and was subsequently transported to the lysosomes for degradation. Therefore, LPS-mediated TLR4 reduction occurs through the endocytic pathway. Zanoni et al[42] reported that CD14 controls the LPS-induced endocytosis of TLR4. In addition, CD47 is the ligand of SIRPA, and CD47/SIRPA interaction is the major determinant of escape from phagocyte-mediated cell clearance.[43] In our study, we demonstrated that SIRPA physically interacted with TLR4; therefore, SIRPA may behave similarly to CD14,[42] which promotes the endosome/lysosome-mediated degradation of TLR4. Future studies will be required to clarify how SIRPA modulates the TLR4 degradation pathway in hypertrophic hearts.

In conclusion, our present work provides in vitro and in vivo evidence to support the notion that increased SIRPA expression attenuates pressure overload–induced cardiac hypertrophy and failure via negative regulation of TLR4/NF-κB signaling axis. Our observations implicate SIRPA as a novel therapeutic target for the treatment of pathological cardiac hypertrophy.

Perspectives

Our in vitro and in vivo studies demonstrated that SIRPA, a transmembrane protein, was downregualted in hypertrophic heart and plays a protective role during cardiac hypertrophy by inhibition of the TLR4/NF-κB signaling axis. These findings will provide potential therapeutic targets for cardiac hypertrophy and heart failure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dr Takashi Matozaki (Gunma University) for kindly providing the SIRPA knockout mice. Rui Zhang, Li-Hua Gan, Ya-Fen Lin, Xue-Yong Zhu, and Xin Zhang are acknowledged for providing experimental technology help.

Sources of Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 81100230, No. 81070089, and No. 81200071), National Science and Technology Support Project (No. 2011BAI15B02 and No. 2012BAI39B05), the National Basic Research Program of China (No. 2011CB503902), and the key project of the National Natural Science Foundation (No. 81330005).

References

1. Zile MR, Gottdiener JS, Hetzel SJ, McMurray JJ, Komajda M, McKelvie R, Baicu CF, Massie BM, Carson PE. I-PRESERVE Investigators. Prevalence and significance of alterations in cardiac

structure and function in patients with heart failure and a preserved ejection fraction. Circulation. 2011; 124:2491–2501. [PubMed: 22064591]

2. Selvetella G, Hirsch E, Notte A, Tarone G, Lembo G. Adaptive and mal-adaptive hypertrophic pathways: points of convergence and divergence. Cardiovasc Res. 2004; 63:373–380. [PubMed: 15276462]
3. Roger VL, Go AS, Lloyd-Jones DM, et al. American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics–2012 update: a report from the American Heart Association. Circulation. 2012; 125:e2–e220. [PubMed: 22179539]
4. Lu J, Bian ZY, Zhang R, et al. Interferon regulatory factor 3 is a negative regulator of pathological cardiac hypertrophy. Basic Res Cardiol. 2013; 108:326. [PubMed: 23307144]
5. Kehat I, Molkentin JD. Molecular pathways underlying cardiac remodeling during pathophysiological stimulation. Circulation. 2010; 122:2727–2735. [PubMed: 21173361]
6. Zeisberg EM, Kalluri R. Origins of cardiac fibroblasts. Circ Res. 2010; 107:1304–1312. [PubMed: 21106947]
7. Snider P, Standley KN, Wang J, Azhar M, Doetschman T, Conway SJ. Origin of cardiac fibroblasts and the role of periostin. Circ Res. 2009; 105:934–947. [PubMed: 19893021]
8. Wang L, Lu Y, Deng S, Zhang Y, Yang L, Guan Y, Matozaki T, Ohnishi H, Jiang H, Li H. SHPS-1 deficiency induces robust neuroprotection against experimental stroke by attenuating oxidative stress. J Neurochem. 2012; 122:834–843. [PubMed: 22671569]
9. Dubois NC, Craft AM, Sharma P, Elliott DA, Stanley EG, Elefanty AG, Gramolini A, Keller G. SIRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells. Nat Biotechnol. 2011; 29:1011–1018. [PubMed: 22020386]
10. Brooke G, Holbrook JD, Brown MH, Barclay AN. Human lymphocytes interact directly with CD47 through a novel member of the signal regulatory protein (SIRP) family. J Immunol. 2004; 173:2562–2570. [PubMed: 15294972]
11. Adams S, van der Laan LJ, Vernon-Wilson E, Renardel de Lavalette C, Döpp EA, Dijkstra CD, Simmons DL, van den Berg TK. Signal-regulatory protein is selectively expressed by myeloid and neuronal cells. J Immunol. 1998; 161:1853–1859. [PubMed: 9712053]
12. Kharitonenkov A, Chen Z, Sures I, Wang H, Schilling J, Ullrich A. A family of proteins that inhibit signalling through tyrosine kinase receptors. Nature. 1997; 386:181–186. [PubMed: 9062191]
13. Okajo J, Kaneko Y, Murata Y, Tomizawa T, Okuzawa C, Saito Y, Kaneko Y, Ishikawa-Sekigami T, Okazawa H, Ohnishi H, Matozaki T, Nojima Y. Regulation by Src homology 2 domain-containing protein tyrosine phosphatase substrate-1 of alpha-galactosylceramide-induced antimetastatic activity and Th1 and Th2 responses of NKT cells. J Immunol. 2007; 178:6164–6172. [PubMed: 17475843]
14. Oshima K, Ruhul Amin AR, Suzuki A, Hamaguchi M, Matsuda S. SHPS-1, a multifunctional transmembrane glycoprotein. FEBS Lett. 2002; 519:1–7. [PubMed: 12023008]
15. Shen X, Xi G, Radhakrishnan Y, Clemmons DR. Identification of novel SHPS-1-associated proteins and their roles in regulation of insulin-like growth factor-dependent responses in vascular smooth muscle cells. Mol Cell Proteomics. 2009; 8:1539–1551. [PubMed: 19299420]
16. Inagaki K, Yamao T, Noguchi T, Matozaki T, Fukunaga K, Takada T, Hosooka T, Akira S, Kasuga M. SHPS-1 regulates integrin-mediated cytoskeletal reorganization and cell motility. EMBO J. 2000; 19:6721–6731. [PubMed: 11118207]
17. Li H, He C, Feng J, Zhang Y, Tang Q, Bian Z, Bai X, Zhou H, Jiang H, Heximer SP, Qin M, Huang H, Liu PP, Huang C. Regulator of G protein signaling 5 protects against cardiac hypertrophy and fibrosis during biomechanical stress of pressure overload. Proc Natl Acad Sci U S A. 2010; 107:13818–13823. [PubMed: 20643937]
18. Huang H, Tang QZ, Wang AB, Chen M, Yan L, Liu C, Jiang H, Yang Q, Bian ZY, Bai X, Zhu LH, Wang L, Li H. Tumor suppressor A20 protects against cardiac hypertrophy and fibrosis by blocking transforming growth factor-beta-activated kinase 1-dependent signaling. Hypertension. 2010; 56:232–239. [PubMed: 20585109]
19. Li HL, Zhuo ML, Wang D, Wang AB, Cai H, Sun LH, Yang Q, Huang Y, Wei YS, Liu PP, Liu DP, Liang CC. Targeted cardiac overexpression of A20 improves left ventricular performance and

reduces compensatory hypertrophy after myocardial infarction. Circulation. 2007; 115:1885–1894. [PubMed: 17389268]

20. Xiao J, Moon M, Yan L, Nian M, Zhang Y, Liu C, Lu J, Guan H, Chen M, Jiang D, Jiang H, Liu PP, Li H. Cellular FLICE-inhibitory protein protects against cardiac remodelling after myocardial infarction. Basic Res Cardiol. 2012; 107:239. [PubMed: 22202974]
21. Wang XA, Zhang R, Jiang D, et al. Interferon regulatory factor 9 protects against hepatic insulin resistance and steatosis in male mice. Hepatology. 2013; 58:603–616. [PubMed: 23471885]
22. Weber KT, Sun Y, Bhattacharya SK, Ahokas RA, Gerling IC. Myofibroblast-mediated mechanisms of pathological remodelling of the heart. Nat Rev Cardiol. 2013; 10:15–26. [PubMed: 23207731]
23. Jiang DS, Bian ZY, Zhang Y, Zhang SM, Liu Y, Zhang R, Chen Y, Yang Q, Zhang XD, Fan GC, Li H. Role of interferon regulatory factor 4 in the regulation of pathological cardiac hypertrophy. Hypertension. 2013; 61:1193–1202. [PubMed: 23589561]
24. Creemers EE, Pinto YM. Molecular mechanisms that control interstitial fibrosis in the pressure-overloaded heart. Cardiovasc Res. 2011; 89:265–272. [PubMed: 20880837]
25. Colasanti T, Alessandri C, Capozzi A, Sorice M, Delunardo F, Longo A, Pierdominici M, Conti F, Truglia S, Siracusano A, Valesini G, Ortona E, Margutti P. Autoantibodies specific to a peptide of β2-glycoprotein I cross-react with TLR4, inducing a proinflammatory phenotype in endothelial cells and monocytes. Blood. 2012; 120:3360–3370. [PubMed: 22932793]
26. Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. Cell. 2008; 132:344–362. [PubMed: 18267068]
27. Maier HJ, Schips TG, Wietelmann A, Krüger M, Brunner C, Sauter M, Klingel K, Böttger T, Braun T, Wirth T. Cardiomyocyte-specific IκB kinase (IKK)/NF-κB activation induces reversible inflammatory cardiomyopathy and heart failure. Proc Natl Acad Sci U S A. 2012; 109:11794–11799. [PubMed: 22753500]
28. Liu CJ, Cheng YC, Lee KW, Hsu HH, Chu CH, Tsai FJ, Tsai CH, Chu CY, Liu JY, Kuo WW, Huang CY. Lipopolysaccharide induces cellular hypertrophy through calcineurin/NFAT-3 signaling pathway in H9c2 myocardiac cells. Mol Cell Biochem. 2008; 313:167–178. [PubMed: 18398669]
29. Ehrentraut H, Weber C, Ehrentraut S, Schwederski M, Boehm O, Knuefermann P, Meyer R, Baumgarten G. The toll-like receptor 4-antagonist eritoran reduces murine cardiac hypertrophy. Eur J Heart Fail. 2011; 13:602–610. [PubMed: 21613426]
30. Kaya Z, Leib C, Katus HA. Autoantibodies in heart failure and cardiac dysfunction. Circ Res. 2012; 110:145–158. [PubMed: 22223211]
31. Ha T, Li Y, Hua F, Ma J, Gao X, Kelley J, Zhao A, Haddad GE, Williams DL, William Browder I, Kao RL, Li C. Reduced cardiac hypertrophy in toll-like receptor 4-deficient mice following pressure overload. Cardiovasc Res. 2005; 68:224–234. [PubMed: 15967420]
32. Motegi S, Okazawa H, Murata Y, Kanazawa Y, Saito Y, Kobayashi H, Ohnishi H, Oldenborg PA, Ishikawa O, Matozaki T. Essential roles of SHPS-1 in induction of contact hypersensitivity of skin. Immunol Lett. 2008; 121:52–60. [PubMed: 18809435]
33. Kapoor GS, O'Rourke DM. SIRPalpha1 receptors interfere with the EGFRvIII signalosome to inhibit glioblastoma cell transformation and migration. Oncogene. 2010; 29:4130–4144. [PubMed: 20473329]
34. Kobayashi M, Ohnishi H, Okazawa H, Murata Y, Hayashi Y, Kobayashi H, Kitamura T, Matozaki T. Expression of Src homology 2 domain-containing protein tyrosine phosphatase substrate-1 in pancreatic beta-Cells and its role in promotion of insulin secretion and protection against diabetes. Endocrinology. 2008; 149:5662–5669. [PubMed: 18635660]
35. Takada T, Matozaki T, Takeda H, Fukunaga K, Noguchi T, Fujioka Y, Okazaki I, Tsuda M, Yamao T, Ochi F, Kasuga M. Roles of the complex formation of SHPS-1 with SHP-2 in insulin-stimulated mitogen-activated protein kinase activation. J Biol Chem. 1998; 273:9234–9242. [PubMed: 9535915]
36. Araki T, Yamada M, Ohnishi H, Sano SI, Hatanaka H. BIT/SHPS-1 enhances brain-derived neurotrophic factor-promoted neuronal survival in cultured cerebral cortical neurons. J Neurochem. 2000; 75:1502–1510. [PubMed: 10987830]

37. Matozaki T, Murata Y, Okazawa H, Ohnishi H. Functions and molecular mechanisms of the CD47-SIRPalpha signalling pathway. Trends Cell Biol. 2009; 19:72–80. [PubMed: 19144521]

38. Lindberg FP, Gresham HD, Schwarz E, Brown EJ. Molecular cloning of integrin-associated protein: an immunoglobulin family member with multiple membrane-spanning domains implicated in alpha v beta 3-dependent ligand binding. J Cell Biol. 1993; 123:485–496. [PubMed: 7691831]

39. Kong XN, Yan HX, Chen L, Dong LW, Yang W, Liu Q, Yu LX, Huang DD, Liu SQ, Liu H, Wu MC, Wang HY. LPS-induced down-regulation of signal regulatory protein {alpha} contributes to innate immune activation in macrophages. J Exp Med. 2007; 204:2719–2731. [PubMed: 17954568]

40. Hamid T, Guo SZ, Kingery JR, Xiang X, Dawn B, Prabhu SD. Cardiomyocyte NF-κB p65 promotes adverse remodelling, apoptosis, and endoplasmic reticulum stress in heart failure. Cardiovasc Res. 2011; 89:129–138. [PubMed: 20797985]

41. Husebye H, Halaas Ø , Stenmark H, Tunheim G, Sandanger Ø , Bogen B, Brech A, Latz E, Espevik T. Endocytic pathways regulate Toll-like receptor 4 signaling and link innate and adaptive immunity. EMBO J. 2006; 25:683–692. [PubMed: 16467847]

42. Zanoni I, Ostuni R, Marek LR, Barresi S, Barbalat R, Barton GM, Granucci F, Kagan JC. CD14 controls the LPS-induced endocytosis of Toll-like receptor 4. Cell. 2011; 147:868–880. [PubMed: 22078883]

43. Legrand N, Huntington ND, Nagasawa M, Bakker AQ, Schotte R, Strick-Marchand H, de Geus SJ, Pouw SM, Böhne M, Voordouw A, Weijer K, Di Santo JP, Spits H. Functional CD47/signal regulatory protein alpha (SIRP(alpha)) interaction is required for optimal human T- and natural killer-(NK) cell homeostasis in vivo. Proc Natl Acad Sci U S A. 2011; 108:13224–13229. [PubMed: 21788504]

Novelty and Significance

What Is New?

- Signal regulatory protein-α (SIRPA) expression levels were markedly decreased in the left ventricles in response to pressure overload.
- SIRPA plays a protective role in cardiac remodeling after pressure overload by negative regulation of the Toll-like receptor 4 (TLR4)/nuclear factor-κB (NF-κB) pathway.

What Is Relevant?

- Multiple signaling pathways have been elucidated to contribute to the progression of pathological cardiac hypertrophy.
- The effects and molecular mechanisms of SIRPA on hypertension-induced cardiac remodeling are not elucidated.
- This finding provides additional information for further understanding the effect of SIRPA on cardiac remodeling and implications for the development of strategies for the treatment of cardiac remodeling and heart failure.

Summary

Our results demonstrate that SIRPA expression levels were down-regulated in pathological human and animal hearts. SIRPA knockout mice displayed augmented cardiac hypertrophy, fibrosis, and cardiac dysfunction, whereas overexpression of myocardial SIRPA protects hearts against chronic pressure overload–induced cardiac hypertrophy via inhibition of TLR4/NF-κB signaling axis. These observations suggest that SIRPA could be considered a therapeutic target for the prevention of cardiac remodeling, although warranting additional validation and investigation.

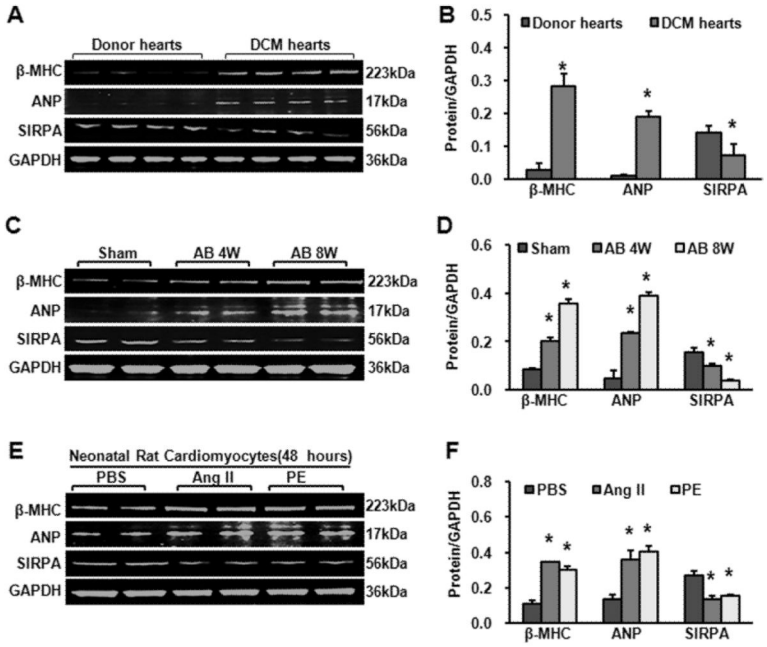

Figure 1.

Signal regulatory protein-α (SIRPA) expression is downregulated in dilated cardiomyopathy (DCM) human hearts and aortic banding (AB)-operated mouse hearts. **A** and **B**, Protein levels of β-myosin heavy chain (β-MHC), atrial natriuretic peptide (ANP), and SIRPA in samples from donor hearts and DCM hearts (n=3; **P*<0.05 vs donor hearts). **C** and **D**, Protein levels of β-MHC, ANP, and SIRPA in samples from wild-type mice at the indicated times after sham or AB surgery (n=3; **P*<0.05 vs sham). **E** and **F**, Protein levels of β-MHC, ANP, and SIRPA in samples from neonatal rat cardiomyocytes treated with angiotensin II (Ang II; 1 μmol/L) or phenylephrine (PE; 100 μmol/L) for 48 h (n=3; **P*<0.05 vs PBS). Representative blots (**A**, **C**, and **E**) and quantitative results (**B**, **D**, and **F**). n indicates number of independent experiments.

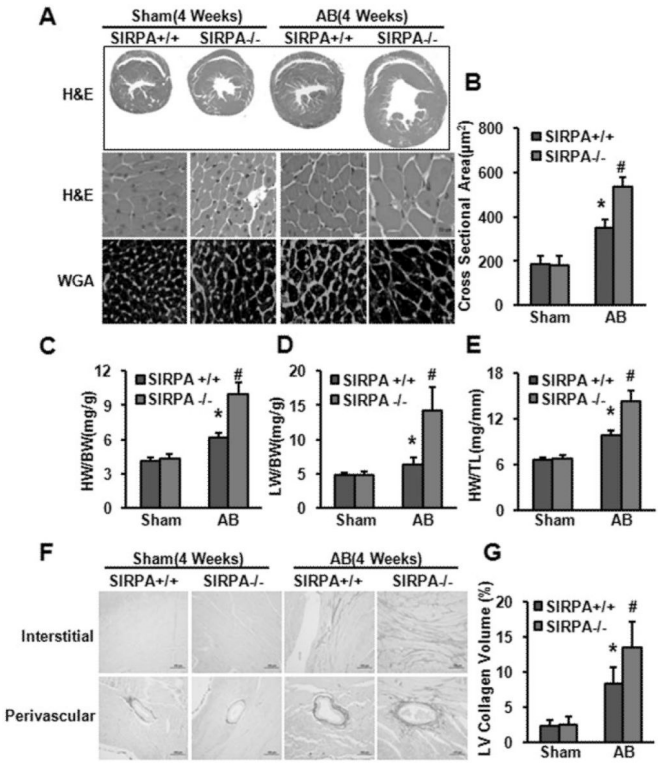

Figure 2.

Signal regulatory protein-α (SIRPA) deficiency augments pressure overload–induced cardiac hypertrophy and fibrosis. **A**, Images of heart sections stained with H&E and WGA from wild-type (WT) and knockout mice 4 weeks after sham or aortic banding (AB) surgery (n=6–10 mice per experimental group). **B**, Statistical results for the cell sectional area (n=>100 cells). **C**–**E**, Statistical results for the ratios of (**C**) heart weight (HW)/body weight (BW), (**D**) lung weight (LW)/BW, and (**E**) HW/tibia length (TL) in the indicated groups (n=13–15 mice per experimental group). **F**, Images of heart sections stained with picrosirius red from WT and knockout mice 4 weeks after sham or AB surgery (n=6–10 mice per experimental group). **G**, Statistical results for the left ventricular (LV) collagen volume (%; n=>40 fields). **P*<0.05 vs WT/sham; #*P*<0.05 vs WT/AB.

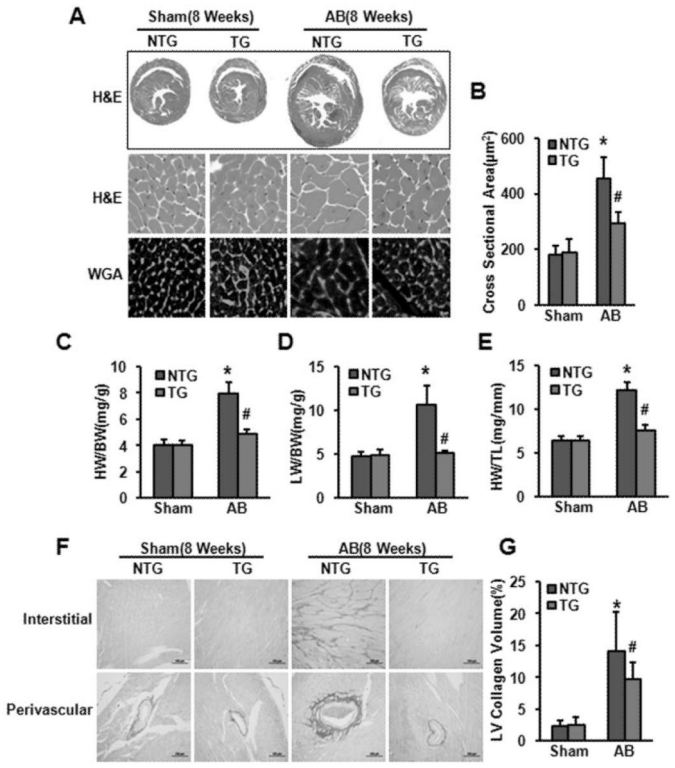

Figure 3.

Signal regulatory protein-α overexpression mitigates aortic banding (AB)-induced cardiac remodeling. **A**, Images of heart sections stained with H&E and WGA from nontransgenic (NTG) and transgenic (TG) mice 8 weeks after sham or AB surgery (n=6 mice per experimental group). **B**, Statistical results for the cell sectional area (n=>100 cells). **C–E**, Statistical results for the ratios of (**C**) heart weight (HW)/body weight (BW), (**D**) lung weight (LW)/BW, and (**E**) HW/tibia length (TL) in the indicated groups (n=14–15 mice per experimental group). **F**, Images of heart sections stained with PSR from NTG and TG mice 8 weeks after sham or AB surgery (n=6 mice per experimental group). **G**, Statistical results for the left ventricular collagen volume (%; n=>40 fields). **P*<0.05 vs NTG/sham; #*P*<0.05 vs NTG/AB.

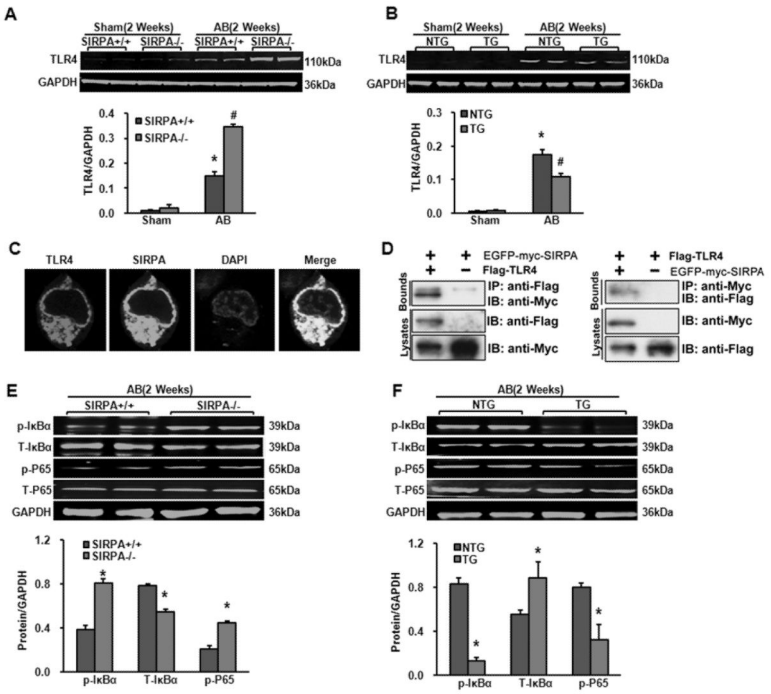

Figure 4.

Signal regulatory protein-α (SIRPA) inhibits the Toll-like receptor 4 (TLR4) signaling pathway. **A** and **B**, Protein expression levels of TLR4 are upregulated in the SIRPA knockout mice but downregulated in the SIRPA transgenic mice 2 weeks after aortic banding (AB) surgery (n=3; **P*<0.05 vs wild-type [WT] or nontransgenic (NTG)/sham; #*P*<0.05 vs WT or NTG/AB). Representative blots (**top**); quantitative results (**bottom**). **C**, Representative colocalization images of SIRPA and TLR4 in HEK293T cells. Red, TLR4; green, SIRPA; blue, nucleus. **D**, SIRPA interacts with TLR4. Western blot with the Flag or Myc antibody after the coimmunoprecipitation of TLR4 from HEK293T whole-cell lysates using the Flag antibody (**left**). Western blot with the Flag or Myc antibody after the coimmunoprecipitation of SIRPA from HEK293T whole-cell lysates using the Myc antibody (**right**). **E** and **F**, Phosphorylation and total protein levels of IκBα and P65 in samples from (**E**) WT and SIRPA knockout mice and (**F**) NTG and SIRPA transgenic mice 2 weeks after AB surgery (n=3; **P*<0.05 vs WT or NTG/AB). Representative blots (**top**); quantitative results (**bottom**). n indicates number of independent experiments.

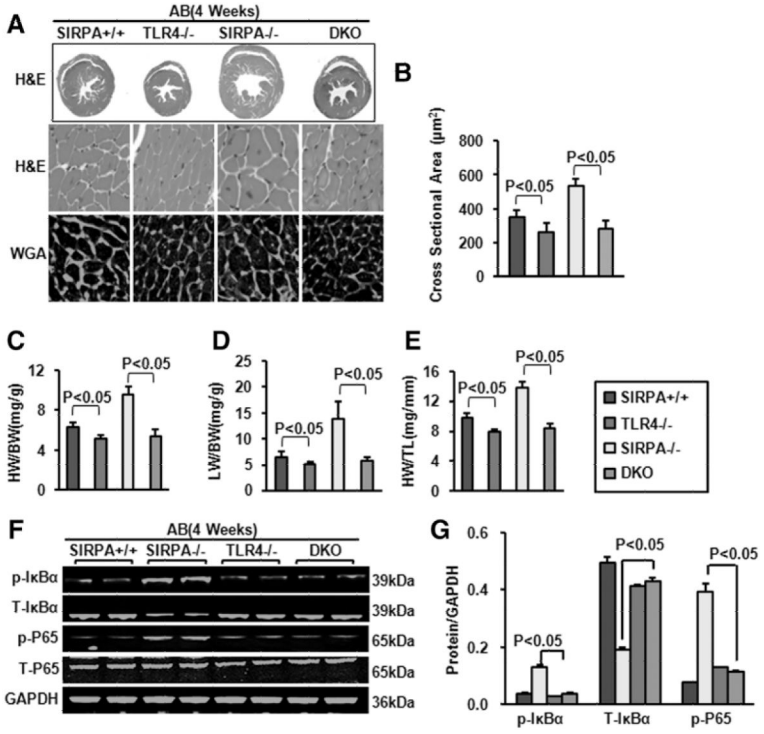

Figure 5.

Disruption of Toll-like receptor 4 (TLR4) rescued the adverse effect of signal regulatory protein-α (SIRPA) deficiency on cardiac hypertrophy. **A**, Images of heart sections stained with H&E and WGA from the indicated groups (n=5 mice per experimental group). **B**, Statistical results for the cell sectional area (n=>100 cells). **C**–**E**, Statistical results for the ratios of (**C**) heart weight (HW)/body weight (BW), (**D**) lung weight (LW)/BW, and (**E**) HW/tibia length (TL) in the indicated groups (n=11 mice per experimental group). **F** and **G**, Phosphorylation and total protein levels of IκBα and P65 in samples from the indicated groups 4 weeks after aortic banding(AB) surgery (n=3 independent experiments). Representative blots (**F**); quantitative results (**G**). DKO indicates double knockout.