Stem Cell Antigen 1 Protects Against Cardiac Hypertrophy and Fibrosis After Pressure Overload

Heng Zhou, Zhou-Yan Bian, Jing Zong, Wei Deng, Ling Yan, Di-Fei Shen, Haipeng Guo, Jia Dai, Yuan Yuan, Rui Zhang, Ya-Fen Lin, Xue Hu, Hongliang Li, Qi-Zhu Tang

Abstract—Stem cell antigen (Sca) 1, a glycosyl phosphatidylinositol-anchored protein localized to lipid rafts, is upregulated in the heart during myocardial infarction and renovascular hypertension-induced cardiac hypertrophy. It has been suggested that Sca-1 plays an important role in myocardial infarction. To investigate the role of Sca-1 in cardiac hypertrophy, we performed aortic banding in Sca-1 cardiac-specific transgenic mice, Sca-1 knockout mice, and their wild-type littermates. Cardiac hypertrophy was evaluated by echocardiographic, hemodynamic, pathological, and molecular analyses. Sca-1 expression was upregulated and detected in cardiomyocytes after aortic banding surgery in wild-type mice. Sca-1 transgenic mice exhibited significantly attenuated cardiac hypertrophy and fibrosis and preserved cardiac function compared with wild-type mice after 4 weeks of aortic banding. Conversely, Sca-1 knockout dramatically worsened cardiac hypertrophy, fibrosis, and dysfunction after pressure overload. Furthermore, aortic banding–induced activation of Src, mitogen-activated protein kinases, and Akt was blunted by Sca-1 overexpression and enhanced by Sca-1 deficiency. Our results suggest that Sca-1 protects against cardiac hypertrophy and fibrosis via regulation of multiple pathways in cardiomyocytes. (Hypertension. 2012;60:00-00.) Online Data Supplement

Key Words: stem cell antigen 1 ★ cardiac hypertrophy ★ fibrosis ★ Src ★ MAPK ★ Akt

Cardiac hypertrophy occurs when the heart experiences elevated workload or injury. Although it is an adaptive response to reduce ventricular wall stress and initially maintain output, sustained hypertrophy leads to ventricular dysfunction and, ultimately, heart failure.1 Left ventricular hypertrophy has been considered to be a maker of increased risk of adverse cardiovascular events.2 The signaling mechanisms involved in the hypertrophic response are complex, including cell surface receptors, signal transduction pathways, and transcriptional and posttranscriptional regulation.3 However, the molecular mechanisms have not been clearly elucidated. A better understanding of the factors that regulate hypertrophic pathways could reveal potential therapeutic targets for treating cardiac hypertrophy and heart failure.4

Stem cell antigen (Sca) 1 is an 18-kDa glycosyl phosphatidylinositol-anchored protein (GPI-AP) in mice that was originally identified as an antigen upregulated in activated lymphocytes and also named lymphocyte activation protein-6A, as a member of the Ly6 gene family.5 GPI-APs are primarily associated with lipid rafts enriched in sphingolipids and cholesterol, which is important for GPI-APs in signal transduction.6 The proteins localized in the lipid raft include GPI-APs, Src family kinases, low molecular weight and heterotrimeric G proteins, and various receptor tyrosine kinases.7,8 Previous studies have shown that inhibition of Sca-1 expression causes the dis inhibition of the Src family kinase Fyn,9 and mutations in Sca-1 and Src family kinases often lead to opposing phenotypes in mice, indicating that Sca-1 serves as a negative regulator of Src family kinase signaling.5

Sca-1 is expressed by a mixture of stem, progenitor, and differentiated cell types in a wide variety of tissues and organs, and has been used as a marker to identify stem/progenitor cells, and plays an important role in the differentiation and self-renewal of stem cells.5 In the neonatal heart, Sca-1 can be expressed by myocytes and nonmyocytes.10 However, it is mainly expressed by nonmyocytes in the adult heart, and the Sca-1+ nonmyocytes can differentiate into functional cardiomyocytes in vitro.10,11 Transplantation of Sca-1+ cardiac stem cells into infarcted heart has been shown to attenuate infarct size, preserve left ventricular function, and increase myocardial neovascularization.12,13 Sca-1 signaling might be...
an essential component in regulating cardiac stem cell proliferation and survival after transplantation. A previous study showed that Sca-1 was overexpressed in the adult heart during cardiac hypertrophy after renovascular hypertension.10 In our study, we found that Sca-1 could be re-expressed by cardiomyocytes in response to pressure overload. Cardiac-specific transgene of Sca-1 dramatically ameliorated aortic banding (AB)–induced cardiac hypertrophy, whereas Sca-1 deficiency exacerbated the hypertrophic response. Sca-1 may directly affect cardiomyocytes by regulating cell signaling pathways that could mediate cardiac hypertrophy response to pressure overload.

A detailed Methods section is given in the online-only Data Supplement. All of the animal procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and approved by the animal care and use committee of Renmin Hospital of Wuhan University. Sca-1 transgenic mice (C57/BL6 background) were generated with a cardiac-specific promoter system.15 Sca-1 knockout mice (C57/BL6 background) were kindly provided by Prof Peter P. Liu. Mice were genotyped using PCR analysis of toe genomic DNA and underwent AB, as described previously.15,16 Cardiac function was determined by transthoracic echocardiography and hemodynamic analysis. The left ventricle was examined by transthoracic echocardiography and hemodynamic analysis. Excised hearts were arrested in diastole with 10% KCl, weighed, fixed by perfusion with 10% formalin, and embedded in paraffin for histological studies or were frozen for RNA and protein extraction. Sca-1 expression was determined using Western blotting, immunohistochemistry, and immunofluorescence. The effects of Sca-1 on cardiac hypertrophy and fibrosis were studied using hematoxylin-eosin, wheat germ agglutinin, and picrosirius red staining, as well as quantitative real-time RT-PCR. Kinases phosphorylation was examined using Western blotting.

Results

Sca-1 Was Upregulated in Cardiac Tissue in Response to Pressure Overload

We found that the cardiac expression of Sca-1 was dramatically upregulated after 1 day of AB and was maintained at a high level until 8 weeks after AB (Figure 1A). We explored the localization of Sca-1 using immunohistochemistry and immunofluorescence and found that Sca-1 could be detected on the membrane of cardiomyocytes in response to pressure overload, whereas Sca-1 was mainly expressed on cardiac interstitial cells after sham surgery (Figure 1B).

**Generation of Mice With Cardiac-Specific Overexpression of Sca-1**

To examine the function of Sca-1 in the pressure-overloaded mouse heart, transgenic mice with cardiac-specific overexpression of Sca-1 were generated by using an α-myosin heavy chain (MHC) promoter. The levels of cardiac Sca-1 protein in the 3 independent lines were determined by Western blotting. The transgenic (TG) 3 line exhibited a higher Sca-1 protein level, whereas the TG1 and TG2 lines had lower Sca-1 protein levels (Figure S1A, available in the online-only Data Supplement). We selected the TG3 line for further study and examined Sca-1 protein in various tissues, including the heart, lung, liver, spleen, kidney, brain, testis, and muscle. We found that Sca-1 was only overexpressed in the heart (Figure S1B). The results of immunohistochemistry/immunofluorescence showed that Sca-1 could be detected on the membrane of cardiomyocytes in TG mice (Figure S1C).
Effect of Sca-1 on Cardiac Hypertrophy Induced by Pressure Overload

To evaluate the effect of Sca-1 on cardiac hypertrophy, TG mice and wild-type (WT) littermates were subjected to AB surgery or sham operation. After 4 weeks of AB, TG mice showed significant attenuation of chamber dilation, wall thickness, and dysfunction compared with WT mice, as measured by echocardiography and hemodynamics (Table S1, available in the online-only Data Supplement). No obvious differences were observed between the sham-operated TG and WT mice. Sca-1-TG also inhibited cardiac hypertrophy with improvements in heart weight/body weight, heart weight/tibia length, and cardiomyocyte cross-sectional area (Figure 2A). The inhibitory effect of Sca-1 on cardiac hypertrophy was confirmed by the morphology of the gross hearts, hematoxylin-eosin staining, and wheat germ agglutinin staining (Figure 2B). In addition, the induction of hypertrophic makers, including atrial natriuretic peptide, B-type natriuretic peptide, and β-MHC, was strikingly suppressed in TG mice after pressure overload, accompanied by the upregulation of α-MHC and sarcoplasmic reticulum Ca2+-ATPase (Figure 2C).

To further understand the role of Sca-1 in cardiac hypertrophy, we performed the AB surgery or sham operation on Sca-1 knockout (KO) mice and WT littermates. The cumulative survival rate until 4 weeks after AB was strikingly decreased by Sca-1 deficiency (Figure 2D). Sca-1-KO mice exhibited worse cardiac hypertrophy and dysfunction compared with WT mice, as measured by left ventricular end-diastolic diameter, left ventricular end-systolic diameter, interventricular septal thickness at end diastole, left ventricular posterior wall thickness at end diastole, and fractional shortening after 4 weeks of AB (Table S2). Pressure-volume loop analysis further revealed that Sca-1 deficiency worsened hemodynamic dysfunction in response to AB (Table S2). Sca-1-KO mice also showed increased heart weight/body weight, lung weight/body weight, heart weight/tibia length, and cardiomyocyte cross-sectional area compared with WT mice under pressure overload (Figure 2E), according with the morphology of the gross hearts, hematoxylin-eosin staining, and wheat germ agglutinin staining (Figure 2F). AB-induced upregulation of atrial natriuretic peptide, B-type natriuretic peptide, and β-MHC was enhanced in Sca-1-KO mice, accompanied by the decrease in α-MHC and sarcoplasmic reticulum Ca2+-ATPase (Figure 2G).

Effect of Sca-1 on Cardiac Fibrosis After Pressure Overload

Left ventricular interstitial fibrosis was evaluated by picrosirius red performed on paraffin-embedded sections. Evidently, perivascular and interstitial fibrosis were observed in the WT mice in response to pressure overload and were remarkably rescued by Sca-1 overexpression (Figure 3A and 3B). The induction of transforming growth factor (TGF)-β and connective tissue growth factor, which are responsible for cardiac fibrosis, were dramatically blunted in Sca-1-TG mice after 4 weeks of AB (Figure 3C). Sca-1 overexpression also resulted in AB-induced collagen synthesis, as measured by the mRNA expression of collagen Iα and collagen IIIα (Figure 3C). Conversely, the extent of cardiac fibrosis and the mRNA expression of TGF-β, connective tissue growth factor, collagen Iα, and collagen IIIα were remarkably increased in the KO mice compared with the WT mice in response to AB (Figure 3D through 3F).

Effect of Sca-1 on Src, Mitogen-Activated Protein Kinase, and Akt Signaling

To investigate the underlying mechanism of the antihypertrophic actions of Sca-1, Src phosphorylation was assessed by Western blot. Src phosphorylation at Tyr418 was increased in response to AB, which was inhibited in Sca-1-TG mice and increased in Sca-1-KO mice (Figure S2A).

Sca-1-TG mice displayed decreased phosphorylation of mitogen-activated protein kinase 1/2, extracellular signal-regulated kinase (ERK) 1/2, c-Jun N-terminal kinase, p38, and p90 under conditions of AB, whereas Sca-1 deficiency enhanced the activation of mitogen-activated protein kinase (MAPK) signaling (Figure S2B). Akt activation induced by pressure overload was also blunted by Sca-1, and the phosphorylation of FOXO3a, a downstream target of Akt, was downregulated by Sca-1; however, the phosphorylation of mammalian target of rapamycin and glycogen synthase kinase 3β was not affected by Sca-1 (Figure S2C).

Discussion

Cardiac Sca-1 plays an important role in cardiomyocyte differentiation and repair after ischemic injury.11–14 In this study, we used mice with a cardiac-specific transgene of Sca-1 and Sca-1-KO mice to examine the role of Sca-1 in the hypertrophic response. Cardiac overexpression of Sca-1 significantly attenuated cardiac hypertrophy and fibrosis under conditions of pressure overload, whereas the cardiac function was saved. Conversely, Sca-1 disruption aggravated cardiac hypertrophy, fibrosis, and dysfunction after AB injury. These results suggest that Sca-1 has antihypertrophic activity and plays a protective role in the cardiac response to biomechanical stress.

Sca-1 is expressed by a mixture of stem, progenitor, and differentiated cell types in a wide variety of tissues and organs, including the heart.5 It can be detected on both...
myocytes and nonmyocytes in the neonatal heart but is mainly expressed by nonmyocytes in the adult heart.\(^\text{10}\) However, Sca-1 expression in the adult heart is upregulated in response to various stresses, such as myocardial infarction and renovascular hypertension.\(^\text{10,13}\) Because the induction of the fetal gene program is a common response of the heart to altered hemodynamic situations,\(^\text{18}\) Sca-1 may activate as a fetal gene in the adult heart under pressure overload. Our data showed that cardiac Sca-1 increased after AB surgery and was re-expressed on the cardiomyocyte membrane. It has been suggested that the upregulated Sca-1 is involved in AB-induced hypertrophy, and, as a GPI-AP localized to lipid

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**Figure 3.** Effect of stem cell antigen (Sca) 1 on cardiac fibrosis. **A** through **C**, Gain of Sca-1 function attenuates aortic banding (AB)-induced cardiac fibrosis. **D** through **F**, Loss of Sca-1 promotes AB-induced cardiac fibrosis. **A** and **D**, Histological sections of the left ventricle were stained using picrosirius red for the indicated groups. **B** and **E**, Fibrotic areas from histological sections were quantified using an image-analyzing system. **C** and **F**, The mRNA expressions of transforming growth factor (TGF)-β, connective tissue growth factor (CTGF), collagen I, and collagen III in the myocardium were obtained from the indicated groups using RT-PCR analysis (n=6). *P*<0.05 vs wild-type (WT)/sham. #P<0.05 vs WT/AB.
rafts, Sca-1 may have a direct effect on cardiomyocytes through interaction with other proteins. We demonstrated that a cardiac-specific transgene of Sca-1 significantly attenuates cardiac hypertrophy and dysfunction in mice that endured pressure overload, whereas disruption of Sca-1 aggravated the hypertrophic response. These data indicated that Sca-1 plays a protective role in cardiac hypertrophy, and the upregulation of Sca-1 serves as an adaptive response under conditions of pressure overload. Coincidently, a study published when our article was in preparation showed related results on the role of Sca-1 deficiency in cardiac hypertrophy. The study suggested that Sca-1 deficiency promoted cardiomyocyte dysfunction in response to pressure overload involving uncontrolled precursor recruitment and exhaustion of the precursor pool. Although the differentiation of the cardiac stem cell was not affected, a reduction of the amount of cardiac Nkx2.5+ cells, which represent a population of cardiac precursor cells, was detected in mice lacking Sca-1. Sca-1 progenitor cells may also contribute to cardiac homeostasis through paracrine action on cardiac cells and angiogenesis stimulation. However, our result suggested that Sca-1 may directly affect cardiomyocytes independent of stem/progenitor cells. In our study, Sca-1 was induced in cardiomyocytes by pressure overload, indicating the direct interaction between Sca-1 and cardiomyocytes. Furthermore, attenuated cardiac hypertrophy in mice with cardiomyocyte-specific Sca-1 overexpression confirmed the direct cardioprotective effects of Sca-1. Therefore, Sca-1 progenitor cells may be involved in cardiac hypertrophy, but our results suggested that the antihypertrophic effect of Sca-1 is mediated at least partly through the direct affection on cardiomyocytes.

An integral hallmark of cardiac hypertrophy is fibrosis, which is characterized by a disproportionate accumulation of fibrillar collagen and other extracellular matrix components. The most abundant collagen types in the heart are type I and III, which together account for >90% of the total collagen and which are the main contributors to pressure-overload-induced collagen deposition. Uncontrolled fibrosis could affect myocardial compliance leading to an increase in myocardial stiffness. Sca-1 has been considered necessary in muscle regeneration, and a lack of Sca-1 can increase fibrosis in injured muscle. As shown here, Sca-1 ablation significantly accelerated cardiac fibrosis after pressure overload; interestingly, cardiomyocyte-specific overexpression of Sca-1 attenuated the fibrotic response, indicating that Sca-1 may indirectly affect fibroblasts through cardiomyocytes. Previous studies suggested that cardiomyocytes can regulate the fibroblast phenotype through paracrine hormonal pathways, such as secreting TGF-β and connective tissue growth factor, which are 2 major extracellular signals that promote fibrosis in the hypertrophic heart. Here, we show that Sca-1 serves as a negative regulator of cardiac TGF-β and connective tissue growth factor production in response to pressure overload. Interestingly, Sca-1 expression in myogenic cells could be downregulated by TGF-β in a previous study; conversely, Sca-1 could disrupt TGF-β signaling by targeting TGF-β ligand growth differentiation factor 10. These data suggest that a negative feedback loop may exist between Sca-1 and TGF-β, which may mediate the antifibrosis effect of Sca-1 in the hypertrophic heart.

Sca-1 is a GPI-AP localized to lipid rafts, which are sphingolipid and cholesterol-rich domains of the plasma membrane and are able to recruit a variety of signaling proteins, such as Src kinases, MAPKs, and phosphatidylinositol 3-kinase/Akt. Src is a nonreceptor protein tyrosine kinase, is characterized by Src homology domains, and is involved in cardiac hypertrophy induced by various stresses. Mutations in Sca-1 and Src family kinases often lead to opposing phenotypes in mice and downregulation of Sca-1 in C2C12 myoblasts result in activation of Fyn, a member of the Src family. These results suggest that Sca-1 is a negative regulator of Src family kinases. As shown in our study, Sca-1 blunted Src activation in response to AB surgery, which may contribute to the antihypertrophic effect of Sca-1.

MAPKs is a 3-tiered kinase cascade that consists of 3 primary subfamilies, including ERK, c-Jun N-terminal kinase, and p38. Recent evidence has suggested that MAPKs can be activated in cardiomyocytes by various stress stimuli and subsequently phosphorylate a wide array of substrates, including numerous transcription factors, resulting in the reprogramming of cardiac gene expression. In granulopoietic precursors, Sca-1 expression positively correlated with ERK activation, and a lack of Sca-1 led to ERK inactivation. However, here we showed that Sca-1 negatively regulated ERK activation in a hypertrophic heart, which seems to be inconsistent with previous data. A potential reason for the discrepancy is that the effect of Sca-1 on the ERK pathway depends on the cell, tissue, and disease background. c-Jun N-terminal kinase and p38, the other MAPKs members, were also inhibited by Sca-1 under the condition of pressure overload in our present results.

Akt is a critical pathway in heart development. However, the long-term activation of Akt causes pathological hypertrophy and heart failure. Akt-mediated cardiac hypertrophy may occur via regulation of its downstream targets, including glycogen synthase kinase 3β, mammalian target of rapamycin, and forkhead box O-class 3a. We showed that Sca-1 disruption enhanced Akt activation induced by AB, whereas Sca-1 overexpression inhibited phosphorylation of cardiac Akt, indicating that Sca-1 is a negative regulator of Akt. Forkhead box O-class 3a is inactivated when phosphorylated by Akt, exhibits a higher phosphorylation level under pressure-overload conditions, and has the ability to protect against cardiac hypertrophy. The inhibition of forkhead box O-class 3a after AB surgery was strikingly rescued in Sca-1-TG mice in our study. However, the activation of glycogen synthase kinase 3β and mammalian target of rapamycin was not affected by Sca-1. Interestingly, the MAPKs and Akt pathways could also be activated by Src. Therefore, the effect of Sca-1 on hypertrophic pathways is complex, and the exact mechanism by which Sca-1 regulates Src, MAPKs, and Akt/forkhead box O-class 3a needs further exploration. Our data revealed that Sca-1 could protect against cardiac hypertrophy via the regulation of multiple signaling pathways.
Perspectives
Cardiac hypertrophy response to stress, such as pressure overload, often culminates in heart failure and is associated with adverse cardiovascular events. However, the molecular mechanisms involved in hypertrophic progression have not been clearly elucidated, and classic pharmacological treatment strategies to heart failure are ineffective in a number of patients. Here we demonstrated that Sca-1, a GPI-AP localized in lipid rafts, is induced in cardiomyocytes by pressure overload. Cardiac-specific transgene of Sca-1 dramatically ameliorated AB-induced cardiac hypertrophy, whereas Sca-1 deficiency exacerbated the hypertrophic response. The cardioprotective role of Sca-1 may be mediated via the regulation of multiple signaling pathways in cardiomyocytes. Although a human ortholog of Sca-1 has not yet been identified, these observations of Sca-1 in mice are still crucial to further understanding of the effect of Ly6 family proteins and GPI-APs on cardiac hypertrophy and to provide implications for the development of strategies for the treatment of cardiac hypertrophy and heart failure.

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Disclosures
None.

References

**Novelty and Significance**

**What Is New?**
- Sca-1 is re-expressed on the cardiomyocyte membrane in response to pressure overload and may activate as a fetal gene in the hypertrophic heart.
- Sca-1 plays a protective role in cardiac hypertrophy and fibrosis after pressure overload.
- Sca-1 protects against cardiac hypertrophy through regulation on multiple hypertrophic pathways.

**What Is Relevant?**
- Cardiac hypertrophy induced by pressure overload is similar to what occurs in hypertension.
- The molecular mechanisms of hypertension-induced cardiac hypertrophy are not elucidated.

**Summary**

This study provides new information for further understanding the effect of Ly6 family proteins and GPI-APs on cardiac hypertrophy and implications for the development of strategies for the treatment of cardiac hypertrophy and heart failure.

Sca-1 is induced by pressure overload in cardiomyocytes, and Sca-1 could protect against cardiac hypertrophy via the regulation of multiple signaling pathways. The observations are crucial to further understanding of the effect of Ly6 family proteins and GPI-APs on cardiac hypertrophy and to provide implications for the development of strategies for the treatment of cardiac hypertrophy and heart failure.
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**Short title:** Sca-1 protects against cardiac hypertrophy  

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Supplemental Materials and Methods

Animals and animal models
All animal procedures were 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) and approved by the Animal Care and Use Committee of Renmin Hospital of Wuhan University. A murine Sca-1 cDNA was cloned downstream of the cardiac α-myosin heavy chain (α-MHC) promoter. Transgenic mice were produced by microinjecting the α-MHC-Sca-1 construct into fertilized mouse embryos (C57/BL6 background). Transgenic mice were identified by PCR analysis of toe genomic DNA. The expression levels were analyzed as pairs of α-MHC-Sca-1 (TG) and littermate nontransgenic wild-type (WT) male mice ranging in age from 7 to 8 weeks. Male Sca-1 knockout (KO) mice (C57BL/6 background) and their WT littermates aged 8 to 10 weeks were used in this study. Sca-1 KO mice were kindly provided by Professor Peter P. Liu at Division of Cardiology, Heart and Stroke/Richard Lewar Centre of Excellence, University Health Network, University of Toronto. Aortic banding (AB) was performed as described previously 1, 2. Four weeks after surgery, the hearts and lungs of the sacrificed mice were harvested and weighed to compare the 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 KO and WT mice or TG and WT mice.

Echocardiography and hemodynamics
Cardiac function was determined by transthoracic echocardiography and hemodynamic analysis as described previously 3. Echocardiography was performed in mice anesthetized with 1.5% isoflurane, using a Mylab 30CV (ESAOTE S. P. A) with a 10-MHz linear-array ultrasound transducer. The left ventricle (LV) dimensions were assessed in the parasternal short-axis view. End-systole or end-diastole was defined as the phase in which the smallest or largest area of the LV was obtained, respectively. For hemodynamic measurements, a microtip catheter transducer (SPR-839, Millar Instruments, Houston, TX, USA) was inserted into the right carotid artery and advanced into the left ventricle of mice anesthetized with 1.5% isoflurane. The signals were continuously recorded using a Millar Pressure-Volume System (MPVS-400, Millar Instruments, Houston, TX, USA), and the data were processed by PVAN data analysis software.

Histological analysis
Excised hearts were arrested in diastole with 10% KCl, weighed, fixed by perfusion with 10% formalin, and embedded in paraffin. The hearts were cut transversely close to the apex to visualize the left and right ventricles. Several sections of each heart (4-5 μm thick) were prepared, stained with hematoxylin-eosin (HE), WGA and picrosirius red (PSR) to determine the myocyte cross-sectional area (CSA) and collagen deposition, and were measured using a quantitative digital image analysis system (Image Pro-Plus, version 6.0).

**Immunohistochemistry and immunofluorescence**

The procedures were described previously. Heart sections were heated using the pressure cooker method for antigen retrieval. For immunohistochemistry (IHC), the sections were blocked with 3% H₂O₂, incubated with anti-Sca-1 (Epitomics, 3121-1, rabbit) overnight at 4°C, then incubated with an anti-rabbit EnVisionTM+/HRP reagent, and stained using a DAB detection kit. For immunofluorescence (IF), the sections were blocked with 10% goat serum, incubated with anti-Sca-1 and anti-α-actinin (Millipore, 05-384, mouse) overnight at 4°C, incubated with Alexa Fluor® 568 goat anti-rabbit IgG (Invitrogen, A11011) and Alexa Fluor® 488 goat anti-mouse IgG (Invitrogen, A11001), and mounted with coverslips using SlowFade Gold antifade reagent with DAPI (Invitrogen, S36939).

**Quantitative real-time RT-PCR**

Quantitative real-time RT-PCR was performed as described previously. Total RNA was extracted from frozen mouse cardiac tissue using TRIzol (Invitrogen, 15596-026). Two micrograms RNA of each sample was reverse-transcribed into cDNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche, 04896866001). A LightCycler 480 SYBR Green 1 Master Mix (Roche, 04707516001) was used to quantify the PCR amplifications. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal control.

**Western blotting**

Methods of Western blotting have been described previously. Cardiac tissue was lysed in RIPA lysis buffer, and the protein concentration was measured with the BCA protein assay kit (Themo, 23227) by ELISA (Synergy HT, Bio-tek). Protein lysates were electrophoresed on 10% SDS-PAGE gels, transferred onto Immobilon-FL transfer membranes (Millipore, IPFL00010), and blocked with 5% non-fat milk for 2 hours. The following primary antibodies were used: p-Src (Bio world, BS4176), T-Src (Bio world, BS1331), 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-p90RSK (Cell Signaling Technology, 9335), T-p90RSK (Cell Signaling Technology, 9347), p-AKT (Cell Signaling Technology, 4060), T-AKT (Cell Signaling Technology, 4691), p-mTOR (Cell Signaling Technology, 2971), T-mTOR (Cell Signaling Technology, 2983), p-GSK3β (Cell Signaling Technology, 9322), T-GSK3β (Cell Signaling Technology, 9315), p-FOXO3a (Cell Signaling Technology, 9465), T-FOXO3a (Cell Signaling Technology, 2497), GAPDH (Cell Signaling Technology, 2118) and Sca-1 (Epitomics, 3121-1). The secondary antibody was goat anti-rabbit (LI-COR, 926-32211) IgG. The blots were scanned and analyzed using a two-color infrared imaging system (Odyssey, LI-COR).

Statistical analysis
Data are presented as means ± SEM. Differences among the groups were determined by two-way ANOVA followed by a post hoc Tukey test. Comparisons between the two groups were performed using the unpaired Student’s t-test. Survival rates were analyzed using the Kaplan–Meier method and compared using the log-rank test. P<0.05 was considered to be significantly different.

References
Table S1  Echocardiographic and Hemodynamic parameters in Sca-1-TG and WT mice after 4 weeks of surgery

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<td>23.7±1.65*</td>
<td>13.8±1.31#</td>
</tr>
<tr>
<td>EDV (µl)</td>
<td>28.9±0.92</td>
<td>27.4±1.38</td>
<td>33.9±1.80*</td>
<td>27.1±1.70#</td>
</tr>
<tr>
<td>EF(%)</td>
<td>64.6±2.83</td>
<td>67.9±3.97</td>
<td>37.6±1.73*</td>
<td>52.8±2.30#</td>
</tr>
<tr>
<td>dP/dt max (mmHg/s)</td>
<td>10414±509</td>
<td>9860±669</td>
<td>7711±362*</td>
<td>9644±458#</td>
</tr>
<tr>
<td>dP/dt min (mmHg/s)</td>
<td>-8975±338</td>
<td>-9063±510</td>
<td>-7583±460*</td>
<td>-8013±450</td>
</tr>
</tbody>
</table>

HR, heart rate; IVSD, interventricular septal thickness at end-diastole; LVPWD, left ventricular posterior wall thickness at end-diastole; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; FS, fractional shortening; ESP, end-systolic pressure; EDP, end-diastolic pressure; ESV, end-systolic volume; EDV, end-diastolic volume; EF, ejection fraction; dp/dtmax, maximal rate of pressure development; dp/dtmin, maximal rate of pressure decay.

*P<0.05 vs WT/sham. # P<0.05 vs WT/AB.
Table S2  Echocardiographic and Hemodynamic parameters in Sca-1-KO and WT mice after 4 weeks of surgery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>AB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT(n=8)</td>
<td>KO(n=8)</td>
</tr>
<tr>
<td>HR (min⁻¹)</td>
<td>517±18</td>
<td>504±13</td>
</tr>
<tr>
<td>IVSD(mm)</td>
<td>0.68±0.01</td>
<td>0.70±0.01</td>
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<tr>
<td>LVPWD(mm)</td>
<td>0.70±0.01</td>
<td>0.70±0.01</td>
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<tr>
<td>LVEDD(mm)</td>
<td>3.66±0.02</td>
<td>3.69±0.04</td>
</tr>
<tr>
<td>LVESD(mm)</td>
<td>2.14±0.05</td>
<td>2.22±0.03</td>
</tr>
<tr>
<td>FS(%)</td>
<td>40.5±1.13</td>
<td>39.3±0.61</td>
</tr>
<tr>
<td>ESP (mmHg)</td>
<td>108±1.87</td>
<td>101±2.13</td>
</tr>
<tr>
<td>EDP (mmHg)</td>
<td>14.9±1.55</td>
<td>12.1±1.22</td>
</tr>
<tr>
<td>ESV (µl)</td>
<td>10.6±0.41</td>
<td>11.0±0.96</td>
</tr>
<tr>
<td>EDV (µl)</td>
<td>27.9±0.36</td>
<td>26.2±1.52</td>
</tr>
<tr>
<td>EF(%)</td>
<td>65.6±1.20</td>
<td>62.7±2.82</td>
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<tr>
<td>dP/dt max (mmHg/s)</td>
<td>9972±351</td>
<td>10845±528</td>
</tr>
<tr>
<td>dP/dt min (mmHg/s)</td>
<td>-9223±345</td>
<td>-9351±542</td>
</tr>
</tbody>
</table>

*P<0.05 vs WT/sham. # P<0.05 vs WT/AB.
Figure S1. Characterization of the cardiac-specific Sca-1 transgenic mice. A, Representative western blots of cardiac Sca-1 from 3 lines of both WT and TG mice. B, Representative western blots of Sca-1 from different tissues as indicated in TG mice. C, Detection of cardiac Sca-1 from WT and TG mice using immunohistochemistry (left) and immunofluorescence (right).
Figure S2. Effect of Sca-1 on Src, MAPK and Akt signaling after 4 weeks of AB. A-C, Representative blots and quantitative results for Src, MEK-ERK1/2, JNK1/2/3, p38, p90, Akt, FOXO3a, GSK3β and mTOR phosphorylation and their total protein expression in the heart tissue from mice in the indicated groups. D, Proposed model of the anti-hypertrophic process involving Sca-1. Sca-1 expression is induced in cardiomyocyte in response to AB. Multiple pathways involved in cardiac hypertrophy are inhibited by Sca-1, which mediates attenuated hypertrophic phenotype, reduced production of TGF-β and CTGF, and consequently blunted interstitial fibrosis under the condition of pressure overload. *P<0.05 vs. WT/sham. # P<0.05 vs. WT/AB.