Regulator of G-Protein Signaling 10 Negatively Regulates Cardiac Remodeling by Blocking Mitogen-Activated Protein Kinase–Extracellular Signal-Regulated Protein Kinase 1/2 Signaling

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Abstract—Regulator of G-protein signaling 10 (RGS10) is an important member of the RGS family and produces biological effects in multiple organs. We used a genetic approach to study the role of RGS10 in the regulation of pathological cardiac hypertrophy and found that RGS10 can negatively influence pressure overload–induced cardiac remodeling. RGS10 expression was markedly decreased in failing human hearts and hypertrophic murine hearts. The extent of aortic banding–induced cardiac hypertrophy, dysfunction, and fibrosis in RGS10-knockout mice was exacerbated, whereas the heart of transgenic mice with cardiac-specific RGS10 overexpression exhibited an alleviated response to pressure overload. Consistently, RGS10 also inhibited an angiotensin II–induced hypertrophic response in isolated cardiomyocytes. Mechanistically, cardiac remodeling improvement elicited by RGS10 was associated with the abrogation of mitogen-activated protein kinase kinase 1/2 transduction abolished RGS10 deletion-induced hypertrophic aggravation. These findings place RGS10 and its downstream signaling mitogen-activated protein kinase kinase 1/2–extracellular signal-regulated protein kinase 1/2 signaling. Furthermore, the inhibition of mitogen-activated protein kinase kinase–extracellular signal-regulated protein kinase 1/2 transduction abolished RGS10 deletion-induced hypertrophic aggravation. These findings place RGS10 and its downstream signaling mitogen-activated protein kinase kinase–extracellular signal-regulated protein kinase 1/2 as crucial regulators of pathological cardiac hypertrophy after pressure overload and identify this pathway as a potential therapeutic target to attenuate the pressure overload–driven cardiac remodeling. (Hypertension. 2016;67:86-98.
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Key Words: angiotensin II ■ cardiomegaly ■ extracellular signal-regulated MAP kinases ■ mitogen-activated protein kinase kinases ■ signal transduction

Cardiac hypertrophy is a common response to various pathological stimuli, such as hypertension, valve disease, myocardial ischemia, and congenital heart disease. Although initial cardiac hypertrophy constitutes an adaptive mechanism to maintain adequate blood flow, sustained overload results in myocardial ischemic burden followed by arrhythmias, heart failure, and sudden death. This pathological process features an increase in myocardial mass, the accumulation of extracellular matrix, the overexpression of fetal genes, and cardiac contractile/diastolic dysfunction. Numerous transduction pathways are implicated in this hypertrophic process, including the phosphatidylinositol 3-kinase/protein kinase B/glycogen synthase kinase 3β pathway, calcium-calmodulin–dependent calcineurin phosphorylation, and mitogen-activated protein kinase signaling. Notably, these key transduction pathways are largely involved in G-protein–coupled receptors (GPCRs)–mediated signaling pathways. Thus, a better understanding of the mechanisms that modulate the function and the signaling of GPCRs in hypertrophic hearts may provide a basic theory and potential targets for the improvement of cardiac hypertrophy and heart failure treatment.

The diverse regulator of G-protein signaling (RGS) protein family was originally discovered to accelerate the termination of G-protein–mediated signaling in response to GPCR stimulation via accelerating the GTPase activity of Gt subunit (Gtq and Gtq/o). An increasing number of studies are demonstrating that RGS proteins are involved in multiple pathophysiological processes in the heart, such as ischemic...
injury\textsuperscript{a} and arrhythmias.\textsuperscript{10,11} On the basis of sequence of RGS domain homology, RGS proteins have been distributed into 8 subfamilies: including A/RZ, B/R4, C/R7, D/R12, E/R4, F/GEF, G/GRK, and H/SNX. As the smallest RGS in the D/R12 subgroup, 21-kDa--sized RGS10 only possesses a single functional domain, which exerts its GTPase-activating function. With this domain, RGS10 specifically contributes to the termination of the G\(\alpha\) subunit (G\(\alpha\)i, G\(\alpha\)o, and G\(\alpha\)z)–mediated signaling pathway.\textsuperscript{12,13} RGS10 is located in the brain, bone marrow, lymph node, and heart atrium.\textsuperscript{14} Accordingly, RGS10 plays essential roles in these tissues, such as neuron-protection,\textsuperscript{15} osteoblast differentiation,\textsuperscript{16,17} inflammatory regulation,\textsuperscript{18,19} and the modulation of atrial G-protein–activated K\textsuperscript{+} channel current.\textsuperscript{20} The mRNA of RGS10 is also expressed in ventricular myocytes and fibroblasts\textsuperscript{a}; however, whether RGS10 is involved in the development of chronic pressure overload–induced cardiac hypertrophy has not been examined to date. Hence, the aims of the present study are as follows: first, to explore whether RGS10 expression is altered in hypertrophic hearts; second, to determine whether RGS10 participates in modulating cardiac hypertrophy; and third, to characterize the potential signal transduction involved in this process.

In the current study, we discovered that RGS10 was downregulated in both hypertrophic hearts and cardiomyocytes. RGS10-knockout (KO) mice exhibited an aggravated hypertrophic phenotype in response to pressure overload, whereas RGS10-transgenic (TG) mice exhibited opposite effects. Moreover, inhibition of mitogen-activated-protein kinase (MEK) 1/2–extracellular signal-regulated kinase (ERK1/2) transduction was required for RGS10-mediated cardioprotection under pressure overload. Our results indicate that RGS10 functions as a novel negative regulator of pathological cardiac hypertrophy via inhibition of the MEK–ERK1/2 signaling pathway.

**Materials and Methods**

**Animal Models**

The animal protocol was approved by the Animal Care and Use Committee of the Third Xiangya Hospital, Central South University, China. The investigation complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Generation of Cardiac-Specific Transgenic Mice**

Cardiac-specific transgenic mice were created as previously described.\textsuperscript{21,22} Briefly, the transgene vector of pCAG-loxP-CAT-loxP-RGS10 was constructed by replacing the lacZ gene in pCAG-loxP-CAT-loxP-lacZ with the cDNA of full-length mouse RGS10. The construct was linearized and purified using the QiAquick Gel Extraction Kit (Qiagen, 28704), and then was used for pronuclear microinjection. Founder transgenic mice were identified by tail DNA amplification and then bred with C57BL/6j mice. Tail genomic DNA was identified using polymerase chain reaction (PCR). Primers for PCR include CAG gene promoter: forward primer 5’-CCCCCTGAACTCTGGAAACA-3’ and reverse primer 5’-TGAATCTCCGACCCCCCTCT-3’. The expected size for the amplification product was 509 bp. Cardiac-specific RGS10-TG mice were generated by mating α-myosin heavy chain (MHC)-Cre transgenic mice (Jackson Laboratory, 005650). Four independent transgenic lines were established. To induce RGS10 expression specifically in the heart, these double-TG mice (6 weeks of age) were intraperitoneally injected with tamoxifen (80 mg/kg per day; Sigma, T-5648) for 5 consecutive days to induce Cre-mediated CAT gene excision. The CAG-CAT-RGS10/MHC-Cre mice without tamoxifen administration (CRMC) served as the control group.

**Generation of RGS10-KO Mice**

To generate RGS10-KO mice, guide sequences for the RGS10 gene target site (Figure 3A) in the mouse genome were predicted using an online CRISPR design tool (clustered regularly interspaced short palindromic repeats; http://crispr.mit.edu). A pair of oligomers (oligo1: TAGGCAGATATCCATGACGGAGAT and oligo2: AAACATCTCCGTCATGATAATCCTG) was annealed and cloned into the Bsal restriction site of the PacI7–sgRNA expression vector (Addgene, 51132). DNA was amplified by PCR with primers spanning the T7 promoter and sgRNA regions (forward primer: 5’-GATCCCTAATACGACTCACTATAG-3’; reverse primer: 5’-AAAAAAGACCCAGCTCGGT-3’). After purification, sgRNA was transcribed using the MEGASHortscript Kit (Ambion, AM1354) and purified using the miRNeasy Micro Kit (Qiagen, 217084). The Cas9 expression plasmid (Addgene 44758) was linearized with Pmel and used as the template for in vitro transcription using the T7 Ultra Kit (Ambion, AM1345). Cas9 and sgRNA mRNA injections of 1-cell embryos were performed using the FemtoJet 5247 microinjection system under standard conditions. Genomic DNA from mouse tail was extracted by phenol-chloroform and alcohol precipitation. A 371-bp DNA fragment that overlaps the sgRNA target site was amplified with PCR using the following primers: forward primer 5’-ATGGTGTTGTTGGACCTTACG-3’ and reverse primer 5’-CCGCACATCTCCCATACA-3’. The purified PCR product was denatured and reannealed in NEB Buffer 2 (NEB) to form heteroduplex DNA, which was subsequently digested with T7EN (NEB, M0302L) for 45 minutes and analyzed on a 3.0% agarose gel (Figure 3B). These mice were sequenced to select for frameshift mutations (Figure 3C). The RGS10 forward primer 5’-ACAGAATCCTTGGTGGGTGA-3’ and reverse primer 5’-CCACTTGGTGCTGCTCTTA-3’ were used to screening F1 and F2 offspring. The PCR products were analyzed using 3.0% agarose gel electrophoresis. The wild-type (WT) allele yielded an amplicon of 132 bp, whereas the mutant allele yielded an amplicon of 115 bp (Figure 3D).

**Echocardiography Evaluation**

Echocardiography measurements evaluating the cardiac function of the mice before harvesting the heart were performed as previously described.\textsuperscript{26}

**Histological Analysis and Immunofluorescence Staining**

The histological staining of hematoxylin and eosin, picrosirius red, and immunofluorescence staining of wheat germ agglutinin using heart section were performed as previously described.\textsuperscript{23–25}

**Quantitative Real-Time PCR and Western Blotting**

The methods of quantitative real-time PCR and Western blotting using cardiac tissue and cultured cardiomyocytes were performed in accordance with previously described methods.\textsuperscript{27}

**Cardiomyocyte Culture and Infection With Recombinant Adenoviral Vectors**

Primary neonatal rat cardiomyocytes (NRCMs) and cardiac fibroblasts were isolated and cultured as previously described.\textsuperscript{28,29} We infected cardiac myocytes with adenoviral RGS10 (AdRGS10), adenoviral green fluorescent protein (AdGFP), adenoviral short hairpin RGS10 (AdshRGS10), or adenoviral short hairpin RNA (AdshRNA) for 12 hours. The culture medium was then replaced with serum-free medium overnight, followed by medium containing 1-μmol/L angiotensin II (Ang II) for 48 hours.
Human Heart Samples
All the procedures involving human samples complied with the principles outlined in the Declaration of Helsinki and were approved by the Ethics Committee at the Third Xiangya Hospital, Central South University. Failing human hearts samples were collected from the patients with heart failure and dilated left ventricles (LVs) and undergoing heart transplant surgery. Control samples were collected from normal heart donors who had died of accidental injuries and whose hearts were not suitable for transplantation. Informed consent was obtained from the families of the prospective heart donors.

Statistical Analysis
Data are presented as the mean±SD. Comparisons between the 2 groups were performed using a 2-tailed Student t test. One-way ANOVA with least significant difference (equal variances assumed) test or Tamhane’s T2 (equal variances not assumed) test was used to determine the differences in >2 groups. P <0.05 was considered significant.

Results
Downregulation of RGS10 in Failing Human Hearts and Hypertrophic Murine Hearts
We first sought to determine whether RGS10 expression was altered during the process of pathological hypertrophy. According to Western blotting results, RGS10 protein levels in human failing hearts were reduced to one quarter that of normal donor hearts. Meanwhile, this decrease was accompanied by an enhancement of fetal gene profile of atrial natriuretic peptide (ANP) and β-MHC (Figure 1A). Information on patients with dilated cardiomyopathy is available in Table S1 in the online-only Data Supplement. Furthermore, the AB experiment revealed that the RGS10 protein levels were reduced over time, by 68% and 92% at 4 and 8 weeks after AB, respectively. Similarly, the expression levels of ANP and β-MHC were dramatically increased at week 4 and more pronounced at week 8 (Figure 1B). Moreover, compared with the content in the PBS-treated NRCMs, endogenous expression of RGS10 was reduced by 67% or 88%, respectively, after stimulation of Ang II for 24 or 48 hours, and the protein levels of ANP and β-MHC significantly increased after stimulation (Figure 1C). However, the protein levels of RGS10 remained unchanged when stimulated the isolated cardiac fibroblast with Ang II for 24 or 48 hours (Figure S1).

RGS10 Negatively Regulates of Ang II–Induced Cardiomyocyte Hypertrophy In Vitro
To explore the actual contribution of RGS10 to cardiomyocyte hypertrophy, we infected cultured NRCMs with AdRGS10 to

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**Figure 1.** Regulator of G-protein signaling 10 (RGS10) is expressed in the heart and is downregulated under hypertrophic stimuli. A, Western blot analysis of atrial natriuretic peptide (ANP), β-myosin heavy chain (β-MHC), and RGS10 protein expression in normal donor hearts (n=4) and failing hearts from patients with dilated cardiomyopathy (DCM; n=4, *P<0.05 vs donor hearts). B, Western blot analysis of ANP, β-MHC, and RGS10 protein expression in hypertrophic hearts from experimental mice 4 or 8 wk after aortic banding (AB; n=4, *P<0.05 vs sham). C, Western blot analysis of ANP, β-MHC, and RGS10 protein expression in neonatal rat cardiomyocytes stimulated by angiotensin II (Ang II; 1 μmol/L) for 24 or 48 h, PBS was control group (n=4, *P<0.05 vs PBS). Protein expression was quantified and normalized to GAPDH. The data are presented as the mean±SD.
overexpress RGS10 or AdshRGS10 to knockdown RGS10 expression, AdGFP and AdshRNA were used as controls. The resulting protein expression levels of RGS10 were analyzed (Figure 2A and 2B). We conducted an experiment using in vitro NRCMs stimulated by Ang II (1 μmol/L) for 48 hours, followed by α-actinin immunostaining for size measurement.

**Figure 2.** Regulator of G-protein signaling 10 (RGS10) regulates angiotensin II (Ang II)–induced cardiomyocyte hypertrophic phenotype. A, RGS10 protein expression in neonatal rat cardiomyocytes (NRCMs) infected with adenoviral RGS10 (AdRGS10), adenoviral short hairpin RGS10 (AdshRGS10), and controls (n=4 per group). Protein expression was quantified and normalized to GAPDH. B, Representative microscopic images of NRCMs infected with indicated adenovirus, stimulated with Ang II or PBS, and stained with an anti-α-actinin antibody (blue, nucleus; green, α-actinin; scale bar, 20 μm). C, Statistical results for the cell surface area (CSA) of NRCMs infected with AdshRGS10 compared with adenoviral short hairpin RNA (AdshRNA) in response to Ang II or PBS (n=50 cells per group; *P < 0.05 vs AdshRNA/PBS; #P < 0.05 vs AdshRNA/Ang II). D, Quantification of the CSA of NRCMs infected with AdRGS10 compared with adenoviral green fluorescent protein (AdGFP) group in response to Ang II (n=50 cells per group; *P < 0.05 vs AdGFP/PBS; #P < 0.05 vs AdGFP/Ang II). E, Atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) mRNA levels in PBS- or Ang II–treated NRCMs infected with AdshRGS10 or AdshRNA (n=4; *P < 0.05 vs AdshRNA/PBS; #P < 0.05 vs AdshRNA/Ang II). F, ANP and BNP in PBS- or Ang II–treated NRCMs infected with AdRGS10 or AdGFP (n=4; *P < 0.05 vs AdGFP/PBS; #P < 0.05 vs AdGFP/Ang II). The data are presented as mean±SD.
Under basal conditions, neither the NRCMs infected with AdRGS10 nor those with AdshRGS10 exhibited altered size when compared with the NRCMs infected with AdGFP or AdshRNA. Nevertheless, when triggered with Ang II, the surface area of RGS10-deficient NRCMs was 1.4-fold larger than AdshRNA-infected controls, whereas cells with RGS10 overexpression exhibited significantly reduced cell surface areas (≈45%) compared with AdGFP-infected cells (Figure 2B–2D). Accordingly, myocardial hypertrophic markers indicated that the mRNA expression levels of ANP and brain natriuretic peptide were sharply enhanced in AdshRGS10-infected cells, but suppressed in AdRGS10-infected cells (Figure 2E and 2F).

**RGS10 Deletion Exacerbated AB-Induced Cardiac Hypertrophy**

To explore the potential regulation of RGS10 deficiency on cardiac hypertrophy in response to AB in vivo, RGS10-KO mice were generated using CRISPR/Cas9 methods by coexpressing a sgRNA specific to the targeted RGS10 gene and endonuclease Cas9 in cells (Figure 3A–3D). Representative Western blots of RGS10 expression in heart tissue from RGS10-KO and littermate-control mice were examined (Figure 3E). It was emphasized that RGS10-KO mice displayed no pathological alterations at baseline. More severe hypertrophy was observed in the RGS10-KO mice under pressure overload than in the WT groups, as manifested by the increased ratios of heart weight:body weight (HW/BW), lung weight:BW, and HW:tibia length (Figure 4A), as well as the enlarged size of the heart and cardiomyocytes (Figure 4B and 4C). Subsequently, we evaluated the effects of RGS10 deletion on cardiac fibrosis in pressure-overloaded hearts. Fibrosis was determined by visualizing the extent of collagen staining and calculating the total collagen volume. Perivascular and interstitial fibrosis were markedly noted in groups subjected to AB, in which, fibrosis was triggered more when RGS10 was deleted (Figure 4B and 4D). Furthermore, hypertrophic and fibrotic differences among the groups were reconfirmed by detecting the mRNA of hypertrophic markers (ANP, brain natriuretic peptide, and β-MHC) and fibrotic markers (collagen I, collagen III, and fibronectin; Figure 4E). We also measured echocardiography measurements to dynamically detect cardiac function. The parameters of cardiac function, such as left ventricular end-diastolic diameter, left ventricular end-systolic diameter, and fractional shortening, indicated that myocardial contraction in AB-treated RGS10-KO mice was reduced when compared with the WT group subjected to AB (Figure 4F; Table S2). Summarily, these findings reveal that mice lacking RGS10 synthesis are more sensitive to hypertrophic stimuli and manifest more severe cardiac remodeling.

**RGS10 Overexpression Suppresses AB-Induced Cardiac Hypertrophy**

We further explored whether RGS10-TG mice exhibited attenuated cardiac hypertrophy induced by pressure overload. To confirm this hypothesis, we established 4 germ lines of transgenic mice with the cardiac-specific overexpression of mouse RGS10 using the CAG promoter (Figure 5A and 5B). TG line 4, which expressed the highest levels of RGS10 according to...
A statistical analysis of heart weight (HW)/body weight (BW), lung weight (LW)/BW, and HW/tibia length (TL) of wild-type (WT) and RGS10-KO mice 4 wk after sham or aortic banding (AB) surgery (n=12 per group). B. Hematoxylin and eosin (H&E) staining (first and second row: scale bars, 2000 and 50 μm), wheat germ agglutinin (WGA) staining (third row: scale bars, 50 μm), and picrosirius red staining (fourth and fifth row: scale bars, 50 μm) of WT and RGS10-KO mice 4 wk after sham or AB surgery (n=5 per group). C. Statistical results for the cardiomyocyte cross-sectional area in WT and RGS10-KO mice 4 wk after sham or AB surgery (n=5 per group). D. Quantification of the fibrosis areas in WT and RGS10-KO mice 4 wk after sham or AB surgery (n=5 per group). E. mRNA levels of hypertrophic and fibrotic markers in the hearts of WT and RGS10-KO mice 4 wk after sham or AB surgery (n=4 per group). F. Echocardiographic parameters for WT and RGS10-KO mice 4 wk after sham or AB surgery (n=6–7 per group). The data are presented as mean±SD. *P<0.05 vs WT/sham. #P<0.05 vs WT/AB. ANP indicates atrial natriuretic peptide; BNP, brain natriuretic peptide; FS, fractional shortening; β-MHC, β-myosin heavy chain; LVEDd, left ventricular end-diastolic diameter; and LVESd, left ventricular end-systolic diameter.

the Western blotting analysis, was selected for further experimentation. We ascertained that all RGS10-TG mice and their CRMC littermates were healthy, without any apparent cardiac structural or functional abnormalities under basal conditions. However, the morphological disparity occurred when comparing RGS10-TG with CRMC mice at 4 weeks after AB:
Figure 5. Cardiac hypertrophy is alleviated in regulator of G-protein signaling 10-transgenic (RGS10-TG) mice subjected to aortic banding (AB).

A, Schematic diagram for the construction of TG mice with cardiac-specific expression of RGS10. B, Western blot analysis of the quantification of RGS10 levels in hearts from TG mice and their CAG-CAT-RGS10/MHC-Cre littermates without tamoxifen administration (CRMC). Protein expression was quantified and normalized to GAPDH. (continued)
compared with the CRMC mice, the RGS10-TG mice exhibited improved inhibition in AB-induced cardiac deterioration, as evidenced by lower ratios of HW/BW, HW/tibia length, and lung weight/BW (Figure 5C), smaller heart and cardiomyocyte sizes (Figure 5D and 5E), and reduced fibrosis volumes (Figure 5D and 5F). Accordingly, the mRNA levels of ANP, brain natriuretic peptide, β-MHC and collagen I, collagen III, fibronectin were also reduced in RGS10-TG mice under AB (Figure 5G). Parameters indicating cardiac function (left ventricular end-diastolic diameter, left ventricular end-systolic diameter, and fractional shortening) suggested that RGS10 overexpression markedly retarded pressure overload–induced cardiac dysfunction (Figure 5H; Table S3). In total, these data suggest that elevated RGS10 expression may mitigate the progression of pressure overload–induced cardiac hypertrophy.

RGS10 Suppressed Cardiac Hypertrophy Via the MEK–ERK1/2 Signaling Pathway

The results presented above clearly demonstrate that RGS10 alleviates the development of cardiac remodeling. To determine the mechanism of this antihypertrophic effect, the expression and activity of mitogen-activated protein kinase signaling molecules were detected. Our data suggested that the phosphorylation levels of MEK1/2, ERK1/2, c-Jun N-terminal kinase 1/2 (JNK1/2), and P38 were significantly elevated under the trigger of AB, whereas total protein expression remained unchanged. However, only the levels of phosphorylated MEK1/2, ERK1/2, and downstream transcription factor GATA-binding protein 4 were notably increased 4.3–4.5- and 2-fold, respectively, when RGS10 was deleted when compared with the WT mouse hearts subjected to AB (Figure 6A; Figure S2A). Meanwhile, the upregulation of RGS10 sharply abrogated the AB-induced phosphorylation of MEK1/2, ERK1/2, and GATA-binding protein 4 (Figure 6B; Figure S2B). Neither P38 nor c-Jun N-terminal kinase phosphorylation was altered in AB-induced RGS10-TG and RGS10-KO hearts.

To further verify the alteration of MEK1/2 and ERK1/2, we infected NRCMs with either AdshRGS10 or AdRGS10 to decrease or increase the basal expression of RGS10. Western blot analysis showed that under Ang II treatment, RGS10 removal from the NRCMs prominently enhanced the expression of phosphorylated MEK1/2 and ERK1/2 when compared with AdshRNA-infected cells (Figure 6C), whereas RGS10 upregulation strongly suppressed the levels of MEK1/2 and ERK1/2 phosphorylation in AdRGS10-infected NRCMs compared with the AdGFP-infected group (Figure 6D).

To determine whether MEK–ERK1/2 plays an essential role in the RGS10-mediated inhibition of cardiac hypertrophy, U0126 was applied to WT and RGS10-KO mice. Western blot analysis confirmed that the levels of phosphorylated MEK1/2 and ERK1/2 were suppressed in RGS10-KO mice treated with U0126 when compared with dimethyl sulfoxide–treated controls (Figure 7A). Compared with the RGS10-KO group treated with dimethyl sulfoxide, U0126 treatment in the RGS10-KO group significantly attenuated AB-triggered cardiac hypertrophy, fibrosis, and dysfunction as manifested by decreased HW/BW, lung weight/BW, and HW/tibia length ratios (Figure 7B); reduced cardiomyocytes size and collagen volume (Figure 7C–7E); and improved cardiac function (Figure 7F; Table S4). These parameters were equal in the U0126-treated RGS10-KO mice and the U0126-treated WT mice (Figures 7B–7F). These data imply that the cardioprotective effects of RGS10 may occur via the suppression of MEK–ERK1/2 signaling.

Discussion

In the present study, we identified RGS10 as an important negative regulator of cardiac remodeling. The data demonstrated that RGS10 protein expression significantly decreased in hypertrophic hearts. Furthermore, RGS10 deletion accelerated the hypertrophic response to AB in mice hearts, whereas the overexpression of RGS10 alleviated AB-induced cardiac hypertrophy. Consistently, in vitro experiments indicated the role of RGS10 in inhibiting NRCMs hypertrophy. Importantly, RGS10-mediated cardioprotection was attributed to the inhibition of the MEK–ERK1/2 signaling axis, which was confirmed by use of MEK inhibitor in vivo. To our knowledge, this study provides the first evidence of an essential function of RGS10 in cardiac remodeling, and moreover, the unexplored transcription mediated by RGS10 in this pathophysiologic process.

RGS10 negatively regulates proinflammatory factors in microglia to exert neuroprotection; however, RGS10 expression decreases at old age, thus increase neurons vulnerability to inflammation-induced degeneration. This suggests that RGS10 expression can be regulated under certain conditions. The current study exhibited that RGS10 markedly decreased in failing human hearts, AB-induced mice hearts, and Ang II–stimulated cardiomyocytes. These results preliminarily imply that RGS10 may be a susceptible factor in response to hypertrophic stimuli and may function as a protectant against the development of cardiac hypertrophy. Previous studies have demonstrated that RGS10 gene transcription was suppressed due to epigenetic regulation by DNA methyltransferase–mediated hypermethylation in the gene promoter and the histone deacetylase 1–mediated reduction of histone acetylation in cancer cells. In failing human hearts, the GpC island in RGS10 promoter is hypomethylated compared with normal hearts; in this case, the low expression of RGS10 in failing heart was less likely to be attributed to DNA methylation. However, we acknowledge
that the association between histone deacetylase 1 and the RGS10 gene may be a potential mechanism for RGS10 reduction in hypertrophic hearts because class I HDACs (including histone deacetylase 1) are prominently prohypertrophic factors that induce cardiac hypertrophic response to AB or Ang II.44–47 Aberrant signal transductions mediated by GPCRs are major contributors to multiple pathophysiological processes in the heart, thus the balance between RGS proteins determining the amplitude and duration of the G-protein–mediated signaling cascades versus the ligand-triggered activity of GPCRs is essential in maintaining the physiological function of heart. We hypothesized that under chronic pressure overload, hypertrophy-related triggers prompt the overwhelmed transductions initiated by GPCRs and simultaneously suppress negatively regulatory factors of RGS10 to promote cardiac vulnerability and pathophysiological consequences. To regain a balance of

Figure 6. Effects of regulator of G-protein signaling 10 (RGS10) on the mitogen-activated protein kinase signaling pathway in cardiomyocytes and hearts of experimental mice. A and B, Representative Western blots and quantitative results indicate the phosphorylation and total protein levels of mitogen-activated-protein kinase 1/2 (MEK1/2), extracellular signal-regulated kinase 1/2 (ERK1/2), c-JUN N-terminal kinase 1/2 (JNK1/2), and P38 in the hearts of wild-type (WT) and RGS10-knockout mice (A; n=4 mice per group; *P<0.05 vs WT/sham; #P<0.05 vs WT/aortic banding [AB]; n.s. no significance vs WT/AB); and in CAG-GAT-RGS10/MHC-Cre littermates without tamoxifen administration (CRMC) and RGS10-transgenic mice (B; n=4 mice per group; *P<0.05 vs CRMC/sham; #P<0.05 vs CRMC/AB; n.s. no significance vs CRMC/AB) 4 wk after sham or aortic banding. Protein expression was quantified and normalized to GAPDH. C and D, Representative Western blots and quantitative results indicates the phosphorylation and total protein levels of MEK1/2 and ERK1/2 in neonatal rat cardiomyocytes infected with shRGS10 (C) or AdRGS10 (D) in response to angiotensin II (Ang II) or PBS (n=4; *P<0.05 vs shRNA/PBS or adenoviral green fluorescent protein AdGFP/PBS; #P<0.05 vs shRNA/Ang II or adenoviral green fluorescent protein AdGFP/Ang II). The data are presented as mean±SD. KO indicates knockout; and TG, transgenic.
the 2 factors above, additional studies are required to validate the mechanism of RGS10 expression suppression.

Previous studies revealed the positive contributions of RGS10 to deactivation of atrial G-protein–activated K+ channel current and osteoclast differentiation, which could also be made by RGS proteins such as RGS6 and RGS12; however, RGS2 had no significant effect on G-protein–activated K+ channel current, and RGS18 acted as a negative regulator of osteoclastogenesis. Hence, the effects of RGS10 and other RGS proteins on phenotypes may overlap or diverge in tissues.
a cardioprotection effect against hypertrophy under the stimuli of AB or isoproterenol. About the function of RGS10 in the heart, whether RGS10 serves as a component that inhibits the development of cardiac hypertrophy or exhibits unanticipated influence, remains unknown. The present study demonstrated that RGS10 exhibited a significant improvement in pathological cardiac hypertrophy, and we think that RGS10 and B/R4 members work collaboratively under this situation.

In cardiovascular systems, G-protein-coupled GPCRs induce the activation of mitogen-activated protein kinase cascades consisting of P38, c-Jun N-terminal kinase, and ERK1/2; the activation of ERK1/2 is particularly prominent. In AB-treated mice with altered RGS10 expression, we did not observe significant alterations in the phosphorylation of c-Jun N-terminal kinase or P38. However, both ERK1/2 and its upstream molecule MEK1/2 exhibited altered phosphorylation levels. Constitutively, ERK1/2 activation could promote physiological cardiac hypertrophy in vivo under normal condition. Under pathological circumstances, direct inhibition of MEK–ERK1/2 signaling did improve pressure overload–induced pathological cardiac hypertrophy, as evidenced by our current study using U0126. Moreover, the study using ERK1/2-KO mice identified that ERK1/2 might be a necessary component to drive hypertrophic response with cell widening under hypertrophic stimuli. Clinically, the occurrence of hypertrophic cardiomyopathy in patients with Noonan syndrome might arise from increased ERK1/2 signaling activity. GATA-binding protein 4, acting as a known transcriptional factor downstream from the ERK1/2 signaling, could be a crucial effector inducing this hypertrophic phenotype via directly regulating expression of α-MHC, ANP, brain natriuretic peptide. Therefore, RGS10-attenuated pathological cardiac hypertrophy was potentially mediated by inhibition of activated MEK–ERK1/2 signaling cascade induced by pressure overload.

Cardiac fibrosis is another feature of pathological hypertrophy. Under circulatory overloaded pressure, increasing collagens and other components generated by fibroblast accumulate in the myocardium. The present study showed that the expression of RGS10 remained unchanged in fibroblast in response to profibrotic stimuli, suggesting that profibrotic signaling in fibroblast might not be linked directly to RGS10 in pathological process. However, the antifibrotic effect of myocyte-restricted upregulation of RGS10 supported the notion that activated cardiomyocytes could also contribute to fibrosis in vivo. Mice with ERK2 excision specifically from cardiac cells also had markedly reduced fibrosis in response to pressure overload, suggesting that ERK1/2 might be the key molecule mediating RGS10-induced antifibrosis.

In conclusion, our present work provides in vitro and in vivo evidence to support the notion that increased RGS10 expression attenuates pressure overload–induced cardiac hypertrophy, fibrosis, and dysfunction via the negative regulation of the MEK–ERK1/2 signaling axis. These observations implicate RGS10 as a novel therapeutic target for the treatment of pathological cardiac hypertrophy.

**Perspectives**

For the first time, this study provides the evidence that RGS10 serves a critical negative regulator of pathological cardiac hypertrophy by inhibiting the MEK–ERK1/2 signaling pathway. Targeting RGS10 could be useful for the treatment of cardiac remodeling.

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**Disclosures**

None.

**References**


15. Lee JK, Chung J, Dreyu KM, Tansey MG. RGS10 exerts a neuroprotective role through the PKA–AMP response-element (CREB) pathway in...


**Novelty and Significance**

**What Is New?**
- The level of regulator of G-protein signaling 10 decreases in human failing human hearts and murine hypertrophic hearts.
- Regulator of G-protein signaling 10 (RGS10) negatively regulates aortic banding–induced cardiac remodeling.
- The mechanism by which RGS10 alleviates cardiac remodeling is via inhibition of the mitogen-activated protein kinase kinase–extracellular signal-regulated protein kinase 1/2 pathway.

**What Is Relevant?**
- Many hypertrophic activators have been investigated, but relatively little is known about the negative modulators of cardiac remodeling.
- The effects and molecular mechanisms of RGS10 on the development of cardiac remodeling have not been elucidated.
- This study advances our understanding of the important role of endogenous inhibitor that modulates undesired transduction in the process of cardiac remodeling, and provides a basis for searching for therapeutic targets to prevent cardiac hypertrophy and heart failure.

**Summary**

The findings of this study are that RGS10 deficiency profoundly exacerbates pressure overload–induced cardiac hypertrophy, fibrosis, and dysfunction via activation of the mitogen-activated protein kinase kinase–extracellular signal-regulated protein kinase 1/2 signaling pathway, whereas RGS10 overexpression in heart blunts the pathological hypertrophic response. These observations suggest that RGS10 may be an effective therapeutic target for treatment of pathological cardiac remodeling.
Regulator of G-Protein Signaling 10 Negatively Regulates Cardiac Remodeling by Blocking Mitogen-Activated Protein Kinase–Extracellular Signal-Regulated Protein Kinase 1/2 Signaling
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Regulator of G-protein signaling 10 Negatively Regulates Cardiac Remodeling

By Blocking MEK-ERK1/2 Signaling

Rujia Miao¹, Yao Lu², Xiaowei Xing², Ying Li², Zhijun Huang², Hua Zhong¹, Yun Huang², Alex F. Chen¹, Xiaohong Tang¹, Hongliang Li³,⁴, Jingjing Cai¹,²* and Hong Yuan¹,²*

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Supplemental Materials and Methods

Reagents
Antibodies and their commercial sources are listed below: Cell Signaling Technology: anti-phospho-MEK1/2 (#9154), anti-MEK1/2 (#9122), anti-phospho-ERK1/2 (#4370), anti-ERK1/2 (#4695), anti-phospho-JNK1/2 (#4668), anti-JNK1/2 (#9258), anti-phospho-P38 (#4511), and anti-P38 (#9212); Santa Cruz Biotechnology: anti-ANP (#sc20158), and anti-β-MHC (#sc53090); Aviva Systems Biology: anti-RGS10 (#ARP42856_P050); and Bioworld Technology: anti-GAPDH (#MB001); Abcam: anti-GATA4 (ab84593), anti-GATA4 (phosphor S105, ab5245). The bicinchoninic acid (BCA) protein assay kit was obtained from Pierce (Rockford, IL, USA). Fetal calf serum (FCS) was obtained from HyClone. All other reagents including cell culture reagents were purchased from Sigma.

Animal surgery
Mice were anesthetized via an intraperitoneal injection of sodium pentobarbital (50 mg/kg, Sigma). After blunting dissection at the second intercostal space, the left chest was opened, and the thoracic aorta was identified. We tied the thoracic aorta to a 27-G (for body weights of 24-25 g) or 26-G (for body weights of 26-27 g) needle with a 7-0 silk suture. Immediately after ligation, the needle was removed quickly, and the thoracic cavity was closed. Finally, the adequacy of aortic constriction was determined by Doppler analysis. Sham-treated groups had an untied suture in the same AB location.

Treatment with U0126 in mice
The mice were injected intraperitoneally with U0126 (an inhibitor of MEK1/2, Cell Signaling Technology, Beverly, MA) dissolved in dimethyl sulfoxide (DMSO) at a volume of 1 ml per 100 g of body weight every three days (1 mg/kg/3 days) after AB. The control group was injected with a similar volume of DMSO.

Echocardiography evaluation
A Mylab30CV (ESAOTE) ultrasound system switched to M-mode with a 15-MHz probe was used to determine echocardiography. Left ventricle (LV) end-diastolic dimension (LVEDd), LV end-systolic dimension (LVESd) and LV fractional
shortening (FS, \text{FS}(\%)=(LVEDd-LVESd)/LVEDd \times 100\%) were measured from the short axis of the LV at the level of the papillary muscles. The measurements were obtained from three beats and averaged.

**Histological analysis and immunofluorescence staining**

Hearts were harvested, arrested in diastole with 10% potassium chloride solution, fixed with 10% formalin, dehydrated and embedded in paraffin. Next, the hearts were transversely sectioned every 5μm. Sections at the mid-papillary muscle level were stained with haematoxylin and eosin (H&E) and picrosirius red (PSR) to calculate the cardiomyocyte cross-sectional area (CSA) and collagen deposition volume using software of Image-Pro Plus 6.0, respectively. Fluorescein isothiocyanate-conjugated wheat germ agglutinin (WGA, Invitrogen) was also used to visualize the size of cardiomyocytes.

**Quantitative real-time PCR and western blotting**

Total mRNA was extracted from primary cardiac cells and ventricles with TRIzol reagent (Invitrogen). cDNA was synthesized using the Transcriptor First Strand cDNA Synthesis Kit (Roche). Quantitative real-time PCR was performed using the SYBR Green (Roche) to determine the expression levels of the genes of interest, and the results were normalized to the expression of GAPDH.

Cardiac tissue and cultured cardiac myocytes were lysed in RIPA lysis buffer, and the protein concentration was determined with the BCA protein assay kit. A total of 50 μg protein was loaded, separated using SDS-PAGE (Invitrogen), and transferred to polyvinylidene fluoride membranes (Millipore) that were subsequently blocked and incubated with primary antibodies. The membranes were washed thrice and incubated with a secondary antibody for one hour at room temperature. Finally, enhanced chemiluminescence-treated membranes were visualised using a Bio-Rad ChemiDoc™ XRS+(Bio-Rad). The results were normalized to the GAPDH signal.

**Cardiomyocyte culture and infection with recombinant adenoviral vectors**

Primary neonatal rat cardiomyocytes (NRCMs) were prepared from the hearts of one- to two-day-old Sprague-Dawley rats. First, neonatal hearts were excised from the thoracic cavities, and the heart tissue was finely minced and digested in a
phosphate-buffered saline (PBS) solution containing 0.03% trypsin and 0.04% collagenase type II. After removing fibroblasts using a differential attachment technique, the NRCMs were planted in six-well plates and cultivated in DMEM/F12 medium containing 20% FCS, bromodeoxyuridine (0.1 mM, to inhibit fibroblast proliferation), and penicillin/streptomycin.

The heart obtained from neonatal rates were digested as described above for myocytes. The adherent fibroblasts fractions during pre-plating were obtained and grown in DMEM containing 10% FCS until confluent and passaged. The first three passages were used for further experiment.

The prepared cells were replaced with serum-free medium overnight, followed by medium containing 1-μM angiotensin II (Ang II) for 24 or 48 hours.
## Supplemental Tables

### Table S1: Information of donor hearts and human DCM hearts

<table>
<thead>
<tr>
<th>n</th>
<th>Type</th>
<th>Age</th>
<th>Sex</th>
<th>LVEF(%)</th>
<th>LVEDD(mm)</th>
<th>Therapy before transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ACEI</td>
</tr>
<tr>
<td>1</td>
<td>Donor</td>
<td>37</td>
<td>F</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>Donor</td>
<td>30</td>
<td>F</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>3</td>
<td>Donor</td>
<td>28</td>
<td>M</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>4</td>
<td>Donor</td>
<td>43</td>
<td>M</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>7</td>
<td>DCM</td>
<td>65</td>
<td>M</td>
<td>23</td>
<td>74</td>
<td>Y</td>
</tr>
<tr>
<td>8</td>
<td>DCM</td>
<td>39</td>
<td>F</td>
<td>17</td>
<td>80</td>
<td>Y</td>
</tr>
<tr>
<td>9</td>
<td>DCM</td>
<td>41</td>
<td>M</td>
<td>9</td>
<td>84</td>
<td>Y</td>
</tr>
<tr>
<td>10</td>
<td>DCM</td>
<td>44</td>
<td>F</td>
<td>22</td>
<td>76</td>
<td>Y</td>
</tr>
</tbody>
</table>

DCM: Dilated cardiomyopathy; LVEF: Left ventricular ejection fraction; LVEDD: Left ventricular end-diastolic diameter. F: Female; M: Male. N/A: not available. Y: Yes; N: No.
### Table S2. Echocardiographic evaluation in wild-type (WT) and RGS10-knockout (RGS10-KO) mice at 4 weeks after sham or aortic banding (AB)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>AB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>RGS10-KO</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>575±16</td>
<td>546±27</td>
</tr>
<tr>
<td>IVSD (mm)</td>
<td>0.65±0.04</td>
<td>0.63±0.05</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>3.31±0.23</td>
<td>3.30±0.24</td>
</tr>
<tr>
<td>LVPWD (mm)</td>
<td>0.62±0.04</td>
<td>0.63±0.05</td>
</tr>
<tr>
<td>IVSS (mm)</td>
<td>1.01±0.04</td>
<td>0.97±0.04</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>1.83±0.13</td>
<td>1.83±0.20</td>
</tr>
<tr>
<td>LVPWS (mm)</td>
<td>1.01±0.04</td>
<td>1.00±0.00</td>
</tr>
<tr>
<td>FS (%)</td>
<td>44.9±1.9</td>
<td>44.6±2.6</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>82.4±2.4</td>
<td>82.7±3.1</td>
</tr>
</tbody>
</table>

HR=heart rate; IVSD=left ventricular wall thickness at end diastole; LVEDD=left ventricular end-diastolic dimension; LVPWD=left ventricular posterior wall thickness at end diastole; IVSS=ventricular septum wall thickness at end systole; LVESD=left ventricular end-systolic dimension; LVPWS=left ventricular posterior wall thickness at end systole; FS=fractional shortening; FS=fractional shortening; LVEF=left ventricular ejection fraction.

*P < 0.05 versus WT sham; #P < 0.05 versus WT AB.

All values are presented as means ± SD.
Table S3. Echocardiographic evaluation in control (CRMC) and cardiac-specific RGS10-transgenic (RGS10-TG) mice at 4 weeks after sham or aortic banding (AB)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th></th>
<th>CRMC</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CRMC</td>
<td>RGS10-TG</td>
<td>CRMC</td>
<td>RGS10-TG</td>
</tr>
<tr>
<td>HR(beats/min)</td>
<td>546±17</td>
<td>547±18</td>
<td>552±30</td>
<td>572±17</td>
</tr>
<tr>
<td>IVSD(mm)</td>
<td>0.69±0.03</td>
<td>0.66±0.04</td>
<td>0.77±0.07*</td>
<td>0.69±0.06#</td>
</tr>
<tr>
<td>IVEDD(mm)</td>
<td>3.3±0.14</td>
<td>3.1±0.08</td>
<td>4.2±0.29*</td>
<td>3.8±0.12#</td>
</tr>
<tr>
<td>LVPWD(mm)</td>
<td>0.68±0.05</td>
<td>0.66±0.04</td>
<td>0.75±0.03*</td>
<td>0.68±0.05#</td>
</tr>
<tr>
<td>IVSS(mm)</td>
<td>1.03±0.08</td>
<td>0.99±0.03</td>
<td>1.15±0.09*</td>
<td>1.08±0.07#</td>
</tr>
<tr>
<td>LVESD(mm)</td>
<td>1.85±0.10</td>
<td>1.72±0.08</td>
<td>2.85±0.26*</td>
<td>2.20±0.14#</td>
</tr>
<tr>
<td>LVPWS(mm)</td>
<td>1.03±0.05</td>
<td>1.00±0.00</td>
<td>1.16±0.07*</td>
<td>1.08±0.07#</td>
</tr>
<tr>
<td>FS(%)</td>
<td>43.8±1.9</td>
<td>45.8±1.6</td>
<td>32.3±2.4*</td>
<td>41.4±2.1#</td>
</tr>
<tr>
<td>LVEF(%)</td>
<td>82.0±2.5</td>
<td>83.2±1.3</td>
<td>59.9±2.9*</td>
<td>79.0±3.9#</td>
</tr>
</tbody>
</table>

*P < 0.05 versus CRMC sham; #P < 0.05 versus CRMC AB.

All values are presented as means ± SD.
Table S4. Echocardiographic evaluation in wild-type (WT) and RGS10-knockout (RGS10-KO) mice treated with DMSO or U0126 at 4 weeks after aortic banding (AB).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DMSO</th>
<th>U0126</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>RGS10-KO</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>497±28</td>
<td>489±34</td>
</tr>
<tr>
<td>IVSD (mm)</td>
<td>0.78±0.06</td>
<td>0.88±0.06</td>
</tr>
<tr>
<td>LVEDD (mm)</td>
<td>4.63±0.13</td>
<td>5.11±0.15</td>
</tr>
<tr>
<td>LVPWD (mm)</td>
<td>0.78±0.06</td>
<td>0.84±0.06</td>
</tr>
<tr>
<td>IVSS (mm)</td>
<td>1.23±0.09</td>
<td>1.28±0.10</td>
</tr>
<tr>
<td>LVESD (mm)</td>
<td>3.20±0.10</td>
<td>4.03±0.11</td>
</tr>
<tr>
<td>LVPWS (mm)</td>
<td>1.26±0.06</td>
<td>1.22±0.07</td>
</tr>
<tr>
<td>FS (%)</td>
<td>30.8±2.53</td>
<td>21.29±2.43</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>63.1±3.87</td>
<td>44.43±3.05</td>
</tr>
</tbody>
</table>

*P < 0.05 versus KO/DMSO; #P < 0.05 versus WT/U0126.

All values are presented as means ± SD.
Figure S1. The relative RGS10 protein levels in neonatal cardiac fibroblasts after stimulation with Ang II for 24 or 48 hours. n.s. no significance.
Figure S2. A and B, Representative western blots and quantitative results indicate the phosphorylation and total protein levels of GATA4 in wild-type (WT) and RGS10-knockout mice (A) (n=4 mice per group; *P<0.05 vs. WT/sham; #P<0.05 vs. WT/AB), and in CAG-CAT-RGS10/MHC-Cre littermates without tamoxifen administration (CRMC) and RGS10-transgenic mice (B) (n=4 mice per group; *P<0.05 vs. CRMC/sham; #P<0.05 vs. CRMC/AB) four weeks after sham or aortic banding.