Molecular Screen Identifies Cardiac Myosin–Binding Protein-C as a Protein Kinase G-Iα Substrate

Robrecht Thoonen, PhD; Shewit Giovanni, MD; Suresh Govindan, PhD; Dong I. Lee, PhD; Guang-Rong Wang, MD; Timothy D. Calamaras, PhD; Eiki Takimoto, MD, PhD; David A. Kass, MD; Sakthivel Sadayappan, PhD, MBA; Robert M. Blanton, MA, MD

Background—Pharmacological activation of cGMP-dependent protein kinase G I (PKG) has emerged as a therapeutic strategy for humans with heart failure. However, PKG-activating drugs have been limited by hypotension arising from PKG-induced vasodilation. PKG-Iα antiremodeling substrates specific to the myocardium might provide targets to circumvent this limitation, but currently remain poorly understood.

Methods and Results—We performed a screen for myocardial proteins interacting with the PKG-Iα leucine zipper (LZ)–binding domain to identify myocardial-specific PKG antiremodeling substrates. Our screen identified cardiac myosin–binding protein-C (cMyBP-C), a cardiac myocyte–specific protein, which has been demonstrated to inhibit cardiac remodeling in the phosphorylated state, and when mutated leads to hypertrophic cardiomyopathy in humans. GST pulldowns and precipitations with cGMP-conjugated beads confirmed the PKG-Iα–cMyBP-C interaction in myocardial lysates. In vitro studies demonstrated that purified PKG-Iα phosphorylates the cMyBP-C M-domain at Ser-273, Ser-282, and Ser-302. cGMP induced cMyBP-C phosphorylation at these residues in COS cells transfected with PKG-Iα, but not in cells transfected with LZ mutant PKG-Iα, containing mutations to disrupt LZ substrate binding. In mice subjected to left ventricular pressure overload, PKG activation with sildenafil increased cMyBP-C phosphorylation at Ser-273 compared with untreated mice. cGMP also induced cMyBP-C phosphorylation in isolated cardiac myocytes.

Conclusions—Taken together, these data support that PKG-Iα and cMyBP-C interact in the heart and that cMyBP-C is an antiremodeling PKG-Iα kinase substrate. This study provides the first identification of a myocardial-specific PKG-Iα-LZ-dependent antiremodeling substrate and supports further exploration of PKG-Iα myocardial LZ substrates as potential therapeutic targets for heart failure. (Circ Heart Fail. 2015;8:1115-1122. DOI: 10.1161/CIRCHEARTFAILURE.115.002308.)

Key Words: cyclic GMP-dependent protein kinase type I ■ heart failure ■ leucine zippers ■ myosin-binding protein C ■ ventricular remodeling

The cGMP-dependent protein kinase G I (PKG) regulates functions in multiple cardiovascular tissues, including the systemic vasculature, where it induces vasorelaxation, and the myocardium,1 where its role remains less completely understood. Preclinical studies have revealed that PKG signaling inhibits the process of pathological cardiac remodeling in vivo.2,3 Pharmacological strategies to activate PKGI, including phosphodiesterase inhibition (sildenafil),4–7 natriuretic peptide administration (nesiritide and carperitide),8,9 and nitrate therapy,10 have emerged as potential antiremodeling treatments for patients with heart failure (HF).11 Recently, the Prospective Comparison of ARNI with ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) trial of the combined angiotensin receptor–neprilysin inhibitor LCZ696 demonstrated reduced death from cardiovascular causes or first HF hospitalization in patients with HF with reduced ejection fraction.11 LCZ696 activates PKGI by preventing neprilysin enzymatic breakdown of extracellular natriuretic peptides, further supporting the clinical efficacy of PKG activation in HF.11

Clinical Perspective on p 1122

Importantly, LCZ696 and other pharmacological PKGI activators have been limited to date by significant hypotension arising from PKGI-induced vasodilation.12,13 Identifying downstream PKGI substrates specific to the myocardium might therefore reveal targets to inhibit remodeling yet avoid excessive hypotension. However, PKGI antiremodeling substrates in the myocardium remain poorly understood. PKG-Iα, the predominant PKGI isoform in the heart, contains a unique N-terminal leucine zipper (LZ) domain which mediates binding with critical kinase substrates.12 Global mutation of
the PKGlα LZ domain leads to accelerated HF and cardiac remodeling in response to LV pressure overload by transaortic constriction (TAC), supporting an important role of PKGlα LZ substrates in attenuating pathological remodeling. However, these PKGlα LZ–binding substrates specific to the myocardium have not been explored.

We therefore undertook the present study to identify PKGlα LZ–binding partners as candidate antiremodeling substrates in the myocardium. To this end, we designed a molecular screen for myocardial PKGlα LZ–binding molecules. Here, we describe the identification of cardiac myosin–binding protein-C (cMyBP-C), a CM-specific protein and critical inhibitor of cardiac remodeling, as a PKGlα LZ–binding partner and kinase substrate.

**Methods**

**Preparation of GST Fusion Proteins**

pGEX plasmids containing the N-terminal amino acids 1 to 59 of the wild-type (WT) PKGlα LZ domain (PKGlαLZWT), mutated LZ domain (PKGlαLZmut), and glutathione S-transferase (GST) alone were transformed into BL21, as described. LV lysates were obtained from adult male Sprague–Dawley rats (Harlan Laboratories, 175–200 g) and were plated at a nonconfluent density (25–50 cells/mm2) on laminin-coated dishes in DMEM low-glucose media (Invitrogen, Woburn, MA) was incubated with 100 ng of His-tagged cMyBP-C for 30 minutes at 37°C. Reactions were incubated with either vehicle or with 100 μmol/L 8Br-cGMP. Reactions were stopped with 2× loading dye and heat denaturation. Proteins were resolved on 4% to 15% SDS-PAGE gels, followed by Western blot analyses against anti-C0 domain–specific antibodies for cMyBP-C, and anti-phospho-specific antibodies against Ser-273, Ser-282, and Ser-302. The C0-C1-M-C2 domain was also detected by silver stain. In the phosphorylation assay, a specific PKGlα inhibitor Rp-8-pCPT-cGMP (MBL International Corporation, Woburn, MA) was used as described in the manufacturer protocol.

**Western Blotting**

GST pulldowns and PKGI precipitation with cGMP and RP-8AE7-cGMPs fusion beads were performed as described. Western blotting for PKGlα and cMyBP-C was performed with anti-PKGlα and anti-cMyBP-C (Santa Cruz) antibodies at 1:1000 and 1:100, respectively. For in vivo phosphorylation studies, antibodies to cMyBP-C was performed with anti-PKGlα (4 U; Cat no. CY-E1161-2, MBL International Corporation, Woburn, MA) was incubated with 100 ng of His-tagged cMyBP-C for 30 minutes at 37°C. Reactions were incubated with either vehicle or with 100 μmol/L 8Br-cGMP. Reactions were stopped with 2× loading dye and heat denaturation. Proteins were resolved on 4% to 15% SDS-PAGE gels, followed by Western blot analyses against anti-C0 domain–specific antibodies for cMyBP-C, and anti-phospho-specific antibodies against Ser-273, Ser-282, and Ser-302. The C0-C1-M-C2 domain was also detected by silver stain. In the phosphorylation assay, a specific PKGlα inhibitor Rp-8-pCPT-cGMP (MBL International Corporation, Woburn, MA) was used as described in the manufacturer protocol.

**Cardiac Myocyte Culture**

Adult rat ventricular myocytes were isolated as previously described with the following modifications. Adult rat ventricular myocytes were obtained from adult male Sprague–Dawley rats (Harlan Laboratories, 175–200 g) and were plated at a nonconfluent density (25–50 cells/mm²) on laminin-coated dishes in DMEM low-glucose media (Invitrogen, 12320-032) supplemented with 0.1% penicillin–streptomycin. After 1 hour of plating, the media were replaced, and the cells were used for experimentation the following day as indicated in the figure legends.

**Animal Models**

All studies were performed under institutional approval by the Tufts Institutional Animal Care and Use Committee. TAC was performed as described for a duration of 5 weeks. Sildenafil was administered orally at a dose of 200 mg/kg per day as described, beginning 1 week after TAC.

**Statistical Analysis**

Quantitative data are presented as dot plots with the sample mean denoted by a horizontal bar. Two-sample comparisons were analyzed by the Mann–Whitney rank sum test. Multiple groups were compared...
using Kruskal–Wallis 1-way ANOVA on ranks, followed by multiple comparison testing with the Tukey method (or Dunn method if group sizes differed). Multiple comparisons were performed if the initial Kruskal–Wallis test was significant or near significant, as described in the figure legends. Data were analyzed with SigmaPlot version 12.5.

**Results**

**Identification of Cardiac Myosin Protein-C Through a Screen for PKG1α LZ Interacting Proteins**

To screen for specific PKG1α LZ interacting proteins in the myocardium, we incubated LV lysates with GST fused to: the WT PKG1α LZ domain (PKG1–59); the PKG1α LZ mutant domain (PKG1ZM); or with GST alone, followed by separation by SDS-PAGE and Coomassie staining. Figure 1A outlines the screening strategy. The Coomassie stain identified a band at =150 kDa, detected only in the PKG1–59 pulldowns (Figure 1). Mass spectroscopy revealed cMyBP-C as the predominant protein in the band. cMyBP-C is a CM-specific protein, which is required for normal CM and LV diastolic and systolic function, although its regulation by PKG1α has not been described. Of note, in other PKG1–59–specific bands, we also detected proteins previously shown to bind the PKG1α LZ domain, including troponin I and PKG1α, which is known to form a homodimer through LZ–LZ interactions. We therefore explored this potential PKG1α–cMyBP-C binding further. To confirm that the PKG1α LZ domain precipitates cMyBP-C, we performed, on the same GST pulldown samples, Western blotting for cMyBP-C. cMyBP-C was detected with the PKG1–59, PKG1ZM, or GST alone (Figure 2A), supporting a specific interaction with the WT LZ domain. We next tested whether full-length PKG1α also complexes with cMyBP-C in the LV. We incubated LV lysates with agarose-conjugated cGMP and RP-8AET-cGMP beads, which each bind PKG1α with high affinity and activate or inhibit PKG1α kinase activity, respectively. Precipitation of PKG1α with either of the cGMP-linked beads, but not with control agarose beads, coprecipitated cMyBP-C (Figure 2B), supporting cointeraction of native PKG1α and cMyBP-C in the myocardium.

**Requirement of PKG1α LZ Domain for Phosphorylation of Full-Length cMyBP-C**

cMyBP-C contains multiple phosphorylation sites, including 3 serine residues on the M-domain (Ser-273, Ser-282, and Ser-302). Prior studies identified that phosphorylation of cMyBP-C on these 3 M-domain sites by protein kinase A improves cardiac myocyte (CM) and LV systolic and diastolic function. In addition, phosphorylation status of these sites inhibits pathological remodeling in vivo. Group-based phosphorylation scoring analysis of cMyBP-C strongly predicted PKG1α phosphorylation at each of these 3 sites (Figure 3A). We therefore tested the requirement of the PKG1α LZ domain for the phosphorylation of full-length cMyBP-C. We cotransfected COS cells with full-length cMyBP-C and either WT PKG1α or LZ mutant PKG1α. The membrane-permeable cGMP analogue 8Br-cGMP (100 μmol/L) induced cMyBP-C phosphorylation at Ser-273, Ser-282, and Ser-302 in WT PKG1α transfected cells (Figure 3B, supporting the specificity of this reaction. These findings support that cGMP induces PKG1α phosphorylation of cMyBP-C, and moreover that the PKG1α LZ domain is required for this cGMP-induced phosphorylation.

**Direct PKG1α Phosphorylation of cMyBP-C**

We next tested whether PKG1α directly phosphorylates cMyBP-C in vitro, by incubating purified PKG1α with recombinant His-tagged cMyBP-C spanning the C0-C1-M-C2 domains (Figure 4A). PKG1α induced cMyBP-C phosphorylation of: 10.42±0.81 ADUs on Ser-273; 2.37±0.63 ADUs on Ser-282; and 2.34±0.14 ADUs on Ser-302. PKG1α activation with the cGMP analogue 8Br-cGMP (100 μmol/L) further increased cMyBP-C phosphorylation at these sites to 20.34±3.5 ADUs on Ser-273 (P<0.05 versus control); 4.50±1.02 ADUs on Ser-282 (P<0.05 versus control); and 3.07±0.42 ADUs on Ser-302 (P<0.05 versus control; Figure 4). Furthermore, the PKG1α–specific inhibitor Rp-8pCPT-cGMP decreased PKG1α-induced phosphorylation of these sites (Figure 4), supporting the specificity of this reaction.

**In Vivo Regulation of cMyBP-C by PKG1α in the Pressure Overloaded LV**

The phosphodiesterase 5 inhibitor, sildenafil, activates PKG in the myocardium and inhibits pressure overload–induced remodeling and HF. We tested whether PKG1α activation with sildenafil increased cMyBP-C phosphorylation in mice subjected to LV pressure overload by TAC for 5 weeks. In these previously reported mice, sildenafil improved LV fractional shortening and reduced LV mass compared with vehicle-treated mice, as well as increasing PKG1α kinase activity. Using cardiac tissues from these previously reported mice, we measured the phosphorylation state of cMyBP-C. Compared with vehicle-treated mice, sildenafil treatment induced a 2.5±0.26-fold increase in cMyBP-C phosphorylation of...
Ser-273 in mice subjected to TAC (P<0.05; Figure 5). Sildenafil treatment did not affect cMyBP-C phosphorylation at Ser-282 and Ser-302 (data not shown). These findings indicate that pharmacological activation of PKGI induces myocardial phosphorylation of cMyBP-C at Ser 273 in the setting of pressure overload and suggests that this may mediate the antiremodeling effect of PKGI in this model.

To examine whether cGMP augmentation directly regulated cMyBP-C phosphorylation in the CM, as opposed to a secondary effect, we tested the effect of 8-Br-cGMP on cMyBP-C phosphorylation in isolated adult rat ventricular myocytes. 8-Br-cGMP induced a dose-dependent increase in cMyBP-C phosphorylation at Ser-273 and Ser-282 compared with vehicle-treated CMs (100 μmol/L 8-Br-cGMP induced 44.1±7.8-fold increase on Ser-273, P<0.05 versus vehicle; and 100 μmol/L 8-Br-cGMP induced 5.7±0.6-fold increase on Ser-282, P<0.05 versus vehicle; Figure 5C and 5D).

**Discussion**

In this study, we performed a molecular screen for myocardial PKGIα substrates, based on the growing clinical development of PKG activation strategies to treat HF. We observed (1) selective precipitation of cMyBP-C with the PKGIα LZ–binding domain in LV lysates; (2) cointeraction of PKGIα and cMyBP-C in the heart; (3) phosphorylation of cMyBP-C by PKGIα in vitro and in cell culture in an LZ-dependent fashion, on sites previously shown to inhibit remodeling when phosphorylated; and (4) increased cMyBP-C phosphorylation on Ser-273 in response to PKGI activation with sildenafil in hearts of mice subjected to LV pressure overload. Taken together, our findings support a model in which PKGIα inhibits cardiac remodeling through phosphorylation of cMyBP-C in the myocardium, through a process mediated by LZ domain binding. We interpret these findings to reveal cMyBP-C as a novel, myocardial-specific PKGIα kinase substrate in the heart.

**cMyBP-C as a Myocardial PKGIα-Interacting Protein**

Our identification of cMyBP-C as a PKGIα-binding molecule supports an important role of PKGIα in regulating myocardial-specific substrates. Pharmacological activation of PKGIα attenuates LV remodeling in vivo, and this requires an intact
remodeling. Furthermore, cMyBP-C attenuates LV remodeling and diastolic dysfunction and subsequent pathological LV expansion. Conditional deletion of cMyBP-C in mice leads to systolic dysfunction, although the role of these interactions in the chronic remodeling process has not been established. PKGIα LZ domain. However, specific PKGIα substrates in the myocardium, which may mediate these effects, remain poorly understood. Previous work has identified ubiquitously expressed PKGIα substrates, such as the regulator of G protein signaling 2, as regulating the cardiac remodeling response in vivo. The role, if any, of PKGIα in the myocardium has been less well appreciated, and its role in the CM in regulating remodeling remains controversial. More recently, CM-specific PKGI substrates have been identified, such as troponin I and titin. Importantly, our screen also confirmed phosphorylation of troponin I with the PKGIα LZ domain. PKG phosphorylation of these proteins may acutely regulate CM function, although the role of these interactions in the chronic remodeling process has not been established.

To our knowledge, our study identifies cMyBP-C as the first myocardial-specific antiremodeling molecule regulated by PKGIα. cMyBP-C functions in the sarcomere to regulate actin–myosin interaction and dynamics. Moreover, cMyBP-C is required for normal LV structure and function in vivo. In humans, structural mutations in cMyBP-C in mice leads to systolic and diastolic dysfunction and subsequent pathological LV remodeling. Furthermore, cMyBP-C attenuates LV remodeling in response to pressure overload. In humans, structural mutations in cMyBP-C represent the most common genetic cause of hypertrophic cardiomyopathy and can also lead to dilated cardiomyopathy.

Our observations both in vitro and in cell culture that PKGIα directly phosphorylates the cMyBP-C M-domain on Ser-273, Ser-282, and Ser-302 support an important mechanism through which PKGIα may attenuate pathological remodeling. Accumulating evidence demonstrates that M-domain phosphorylation increases cMyBP-C interaction with the myosin head and improves CM systolic and diastolic function. Furthermore, genetic disruption of cMyBP-C M-domain phosphorylation leads to chronic remodeling, whereas genetic substitution of aspartate at these sites to mimic constitutive phosphorylation limits the chronic cardiac remodeling process in vivo. In human hypertrophied and failing hearts, cMyBP-C phosphorylation is decreased compared with hearts of normal donors. cMyBP-C M-domain phosphorylation increases rapidly after LV pressure overload but is reduced in the remodeled LV. Despite the apparent antiremodeling effect of cMyBP-C M-domain phosphorylation, kinases previously demonstrated to phosphorylate these sites, including protein kinase A, calmodulin kinase II, protein kinase C, and AKT, generally promote rather than inhibit pathological remodeling. Our observations therefore identify PKGIα phosphorylation on cMyBP-C M-domain sites as a novel upstream antiremodeling mechanism of cMyBP-C regulation.

Our finding that sildenafil increases cMyBP-C phosphorylation at Ser-273 in an in vivo model of cardiac remodeling, and HF further supports that PKGIα inhibits cardiac remodeling through regulation of cMyBP-C. Sildenafil inhibits cGMP catalysis by the cGMP-selective phosphodiesterase 5, thus increasing intracellular cGMP and activating PKGI. In the pressure overloaded LV, where phosphodiesterase 5 activity increases significantly, phosphodiesterase 5 inhibition...
prevents cGMP breakdown and promotes PKGI kinase activity, leading to attenuated LV remodeling. Our study demonstrates that, in addition to previously reported antiremodeling effects, sildenafil treatment also increases cMyBP-C phosphorylation in pressure-overloaded hearts. This finding therefore provides evidence that pharmacological PKGI activation increases cMyBP-C phosphorylation on antiremodeling residues. Because cMyBP-C phosphorylation at Ser-273 inhibits cardiac remodeling,15,23,24 we interpret our findings to support that PKGI may normally inhibit pressure overload–induced remodeling through phosphorylation of cMyBP-C.

Significance of cMyBP-C as a Cardiac Myocyte–Specific Antiremodeling Substrate of PKGI

Although PKGI inhibits cardiac remodeling in vivo, the downstream myocardial LZ-dependent substrates mediating this effect had not been systematically studied. Our findings that cMyBP-C functions as a CM-specific PKGIα substrate support an antiremodeling function of PKGIα within the CM. These findings also support the broader hypothesis that PKGIα separately regulates vasodilation and myocardial remodeling at least in part through independent sets of substrates. This is of substantial clinical relevance because trials of PKGIα-activating drugs, such as nesiritide, cinaciguat, and sildenafil, have been limited by vasodilating side effects arising from systemic PKGI activation.1 More recently, the compound LCZ696, which activates PKGI through inhibition of upstream natriuretic peptide degradation, has been shown to improve outcomes in HF, but also to induce excess hypotension.11 These clinical trial results further highlight the importance of understanding CM-specific PKGIα effectors, such as cMyBP-C, which might serve as therapeutic targets to selectively inhibit remodeling, but avoid excess hypotension arising from systemic PKGI activation.

Limitations

Our study has several limitations. First, although we demonstrate that PKGIα phosphorylates cMyBP-C on 3 critical antiremodeling sites, this study did not explore additional sites on cMyBP-C, which may also be regulated by PKGIα. Second, although we identified PKGI augmentation with sildenafil or cGMP as increasing cMyBP-C phosphorylation in vivo or in the cultured CM, we did not determine the degree to which inhibition of remodeling correlated with phosphorylation on a cell-by-cell or individual mouse level. Therefore, the degree to which the phosphorylation of cMyBP-C correlates with cellular remodeling remains untested. Third, it should be noted that in our initial screen, we determined the molecular species only in bands precipitating with the PKG1-59 LZ domain, but did not perform mass spectroscopy analysis on the corresponding region of negative control lanes that had no detectable bands. However, we circumvented this limitation by comparing phosphorylation levels between treatment groups and control groups.

Figure 5. In vivo regulation of cardiac myosin–binding protein-C (cMyBP-C) by protein kinase G Iα (PKGIα). A, Western blot for cMyBP-C Ser-273 phosphorylation and total cMyBP-C in hearts of mice subjected to 5 weeks of transaortic constriction and treated with either vehicle or 200 mg/kg per day sildenafil. B, Quantification of Ser-273 phosphorylation to total cMyBP-C. *P<0.05 by Mann–Whitney rank sum test; n=4 per group. C, Western blot for cMyBP-C Ser-273 and Ser-282 phosphorylation in adult rat ventricular myocytes treated for 1 h with 8-Br-cGMP (0, 10, and 100 μmol/L). Representative of 4 independent experiments. D, Quantification of Ser-273 and Ser-282 phosphorylation to total cMyBP-C from C. Kruskal–Wallis testing revealed global P<0.05 for each Ser-273 and Ser-282 phosphorylation sites. P values for multiple comparison by Dunn test are shown. Sil indicates sildenafil; and TAC, transaortic constriction. †P<0.05 vs 0 μmol/L control; n=3 to 4 per group.
by performing Western blot for cMyBP-C on identical pull-down experiments. These findings (Figure 2A) strongly support that cMyBP-C binds specifically with the WT PKGIα LZ domain and not with the LZ mutant domain. Finally, although cMyBP-C phosphorylation decreases in the human failing heart,35 our study only examined the PKGIα regulation of cMyBP-C in animal and in vitro experiments. Therefore, although we speculate that PKGI-activating drugs, such as nesiritide, nitrates, sildenafil, and LCZ696, inhibit remodeling by inducing cMyBP-C phosphorylation, this remains untested in humans.

In summary, our study provides the first identification of a myocardial-specific PKGIα anti remodeling substrate. Our findings provide the rationale for broader investigation of PKGIα LZ substrates in the heart as a strategy to identify novel anti remodeling therapies.

Sources of Funding
Dr Sadayappan was supported by National Institutes of Health grants (R01HL105826 and K02HL114749). Dr Govindan was supported by American Heart Association Midwest Fellowships (13POST14720024). Dr Blanton was supported by National Institutes of Health (1R03AG042367 and 1KL2TR001063-01), the American Heart Association (10SDG2630161), and a T. Franklin Williams Scholarship Award. Funding for the Williams Award was provided by Atlantic Philanthropies, Inc., the John A. Hartford Foundation, the Association of Specialty Professors, and the American College of Cardiology.

Disclosures
None.

References


**CLINICAL PERSPECTIVE**

Over the past decade, preclinical studies have identified that protein kinase G I (PKGI) inhibits the process of pathological cardiac remodeling. PKGI-activating drugs, including phosphodiesterase 5 inhibitors (sildenafil and tadalafil), nitric oxide donors (nitrates), guanylate cyclase stimulators/activators (riociguat), natriuretic peptides (nesiritide), and most recently neprilysin inhibitors (LCZ-696), have garnered interest as potential antiremodeling therapies for patients with heart failure. However, because PKG also induces vascular relaxation, these PKGI-activating agents have been limited significantly by hypotension. Understanding PKGI molecular substrates specific to the myocardium might therefore identify new therapeutic strategies to inhibit pathological remodeling yet avoid excess vasodilation. For this reason, we performed a molecular screen for novel myocardial PKGIα kinase substrates. Our screen revealed a cardiac myocyte-specific protein, cardiac myosin-binding protein-C (cMyBP-C), as binding to PKGIαt. We found that PKGIαt binds with cMyBP-C in heart tissue and also phosphorylates cMyBP-C on 3 specific amino acids which, when phosphorylated, are known to limit pathological remodeling. In mice subjected to left ventricular pressure overload, sildenafil administration, which inhibits adverse remodeling, augmented myocardial cMyBP-C phosphorylation. From the clinical perspective, these findings suggest that promoting cMyBP-C phosphorylation might represent a way to target the PKGI antiremodeling effect but avoid excess hypotension. These findings also support the emerging clinical paradigm that PKGI-activating drugs inhibit remodeling through signaling mechanisms specific to the cardiac myocyte rather than solely through reduction of afterload. This suggests that understanding novel cardiac myocyte–specific PKGIα substrates, including cMyBP-C and possibly others, may provide new therapeutic targets for heart failure.
Molecular Screen Identifies Cardiac Myosin–Binding Protein-C as a Protein Kinase G-1α Substrate

Robrecht Thoonen, Shewit Giovanni, Suresh Govindan, Dong I. Lee, Guang-Rong Wang, Timothy D. Calamaras, Eiki Takimoto, David A. Kass, Sakthivel Sadayappan and Robert M. Blanton

Circ Heart Fail. 2015;8:1115-1122; originally published online October 18, 2015;
doi: 10.1161/CIRCHEARTFAILURE.115.002308
Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/8/6/1115

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Heart Failure can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Heart Failure is online at:
http://circheartfailure.ahajournals.org//subscriptions/