Casein Kinase-2α1 Induces Hypertrophic Response by Phosphorylation of Histone Deacetylase 2 S394 and its Activation in the Heart

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Background—Cardiac hypertrophy is characterized by transcriptional reprogramming of fetal gene expression, and histone deacetylases (HDACs) are tightly linked to the regulation of those genes. We previously demonstrated that activation of HDAC2, 1 of the class I HDACs, mediates hypertrophy. Here, we show that casein kinase-2α1 (CK2α1)–dependent phosphorylation of HDAC2 S394 is required for the development of cardiac hypertrophy.

Methods and Results—Hypertrophic stimuli phosphorylated HDAC2 S394, which was necessary for its enzymatic activation, and therefore the development of hypertrophic phenotypes in rat neonatal cardiomyocytes or in isoproterenol-administered mice hearts. Transgenic mice overexpressing HDAC2 wild type exhibited cardiac hypertrophy, whereas those expressing phosphorylation-resistant HDAC2 S394A did not. Compared with that in age-matched normal human hearts, phosphorylation activity of HDAC2 S394 was dramatically increased in patients with hypertrophic cardiomyopathy. Hypertrophy-induced phosphorylation of HDAC2 S394 and its enzymatic activity were completely blocked either by CK2 blockers or by CK2α1 short interfering RNA. Hypertrophic stimuli led CK2α1 to be activated, and its chemical inhibitors blocked hypertrophy in both phenylephrine-treated cardiomyocytes and isoproterenol-administered mice. CK2α1-transgenic mice developed hypertrophy, which was attenuated by administration of trichostatin A, an HDAC inhibitor. Overexpression of CK2α1 caused hypertrophy in cardiomyocytes, whereas chemical inhibitors of both CK2 and HDAC as well as HDAC2 S394A blunted it. Hypertrophy in CK2α1-transgenic mice was exaggerated by crossing these mice with wild-type-HDAC2-overexpressing mice. By contrast, however, it was blocked when CK2α1-transgenic mice were crossed with HDAC2 S394A-transgenic mice.

Conclusions—We have demonstrated a novel mechanism in the development of cardiac hypertrophy by which CK2 activates HDAC2 via phosphorylating HDAC2 S394. (Circulation. 2011;123:2392-2403.)

Key Words: hypertrophy ■ casein kinase 2 ■ histone deacetylase 2 S394 ■ phosphorylation ■ transgenic mice

Cardiac hypertrophy, an increase in the size of cardiomyocytes, is often caused by diverse pathological conditions such as myocardial infarction, hypertension, aortic stenosis, and valvular dysfunction. Although cardiac hypertrophy itself is an initial adaptive process, uncorrected continuous stimuli often lead the heart to heart failure. Because heart failure is a main cause of human mortality, many researchers are eager to develop interventions to reverse cardiac hypertrophy or to prevent the transition to congestive heart failure.

Clinical Perspective on p 2403

Posttranslational modifications of histones are closely involved in diverse biological processes through the regulation of transcription of downstream target genes. Among these modifications, the acetylation status of the chromatin mediates the epigenetic regulation of gene expression. Two opposing groups of enzymes, histone acetyltransferase and histone deacetylases (HDACs), regulate the acetylation of histone. Recent evidence has demonstrated that HDACs are closely involved in diverse heart diseases, such as arrhythmia, heart failure, and acute coronary syndromes, as well as in cardiac hypertrophy. Histone deacetylases can be divided into 4 families depending on their structure or tissue distributions. Among those families, class II HDACs (HDAC4, HDAC5, HDAC7, and HDAC9) were first highlighted as anti hypertrophic mediators that inhibit hypertrophy-associated genes by phosphorylation-dependent

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shuttling. However, we and others demonstrated that HDAC2, 1 of the class I HDACs, is activated during cardiac hypertrophy and mediates hypertrophy by modulating inositol polyphosphate-5-phosphatase/β-glycogen synthase kinase 3β signals. In addition, we showed that Krüppel-like factor 4 (KLF4) is a downstream target of activated HDAC2 and that it works as a novel antihypertrophic mediator. However, the upstream signal pathways that activate HDAC2 in response to hypertrophic stimuli have not yet been elucidated. In this study, we hypothesized that the phosphorylation of HDAC2 might be important for its enzymatic activation in response to hypertrophic stimuli and observed that casein kinase-2 (CK2), a novel prohypertrophic mediator, is an upstream regulatory molecule that induces the phosphorylation and activation of HDAC2.

Methods

Transgenic Mice
α-Myosin heavy chain (U71441) promoter–driven transgenic mice were generated by a commercial company (Macrogen, Seoul, Korea) on the C57BL/6 background in accordance with National Institutes of Health guidelines. All experimental procedures were approved by the Chonnam National University Medical School Research Institutional Animal Care and Use Committee.

Leucine Incorporation
Incorporation of [3H]-leucine was measured as described with slight modifications.

In Vitro Kinase Assay
The in vitro kinase assay was performed as described with slight modifications.

CK2 Assay
The CK2 assay was done with a commercially available kit (Upstate Biotechnology Inc, Lake Placid, NY).

Human Samples
Autopsied left ventricle specimens were obtained from 6 individuals: 3 hypertrophied and 3 age-matched males. Hypertrophic cardiomyopathy was diagnosed according to total heart weight, left ventricular free wall thickness, and microscopic findings. This work was performed according to the regulations of the institutional review board of Chonnam National University Hospital (No. 2010-10-167).

Statistical Analysis
The data were analyzed by use of either the unpaired Student t test or 1-way ANOVA, which was followed by the Tukey honestly significant difference multiple comparison post hoc test. When the Levene test for unequal variance was significant, Dunnett T3 was used as a post hoc test. If the data were not normally distributed as determined by the Shapiro-Wilk test, the Kruskal-Wallis test and the Mann-Whitney U test with Bonferroni correction were used. Statistical analysis was performed with PASW Statistics 18 (SPSS, an IBM Company, Chicago, IL).

Reagents and all other experimental procedures are described in the online-only Data Supplement.

Results

Histone Deacetylases 2 S394 Is Phosphorylated by Hypertrophic Stresses
Phosphorylation of HDACs is often associated with their activation by increasing their intrinsic enzymatic activity or by promoting their translocation into the nucleus. Thus, we first checked whether HDAC2 is phosphorylated in cardiac hypertrophy. As determined by use of anti-phosphoserine antibody after immunoprecipitation with HDAC2, the amount of phosphorylated serine was increased by endothelin-1 or isoproterenol in both cardiomyocytes and mouse heart (Figure 1A and 1B and Figure I in the online-only Data Supplement). HDAC2 has been reported to have 3 different levels of phosphorylation, and in our study we observed 3 different molecular weights of endogenous HDAC2. Interestingly, only the hyperphosphorylated form of HDAC2 was upregulated in isoproterenol-administered mouse hearts (Figure 1C, top panel).

Both a previous report and prediction from the phosphosite Web site (http://elm.eu.org/) indicated that the S394, S411, S422, and S424 residues of HDAC2 can be phosphorylated. To determine which serine residues are responsible for the hypertrophy-associated phosphorylation, we next performed an in vitro kinase assay with synthetic peptides, as indicated in Figure 1D. Hypertrophic stress with phenylephrine increased the phosphorylation at S394 and S411 in cardiomyocytes, whereas it failed to do at S422 or S424 (Figure 1D). The results were the same in an experiment with an in vivo isoproterenol infusion pump mouse model (Figure II in the online-only Data Supplement). To rule out other phosphorylation sites in the HDAC2 molecule, we used glutathione-S-transferase (GST) fusion proteins of HDAC2 wild-type (WT) and mutant forms of HDAC2 S394A and HDAC2 S394/411/422/424A for an in vitro kinase assay. No phosphorylation was observed with the HDAC2 S394/411/422/424A mutant, which indicated that no phosphorylation of residues other than those residues takes place (Figure 1E). We also used a phospho-specific antibody against HDAC2 pS394. In isoproterenol-treated heart, a greater amount of phosphorylation was detected (Figure 1C, bottom panel).

Likewise, 20% fetal bovine serum (FBS) stimulation dramatically increased the phosphorylation of HDAC2 S394 (Figure 1F and Figure III in the online-only Data Supplement). Alternate hypertrophic stresses of endothelin-1 (Figure 1G) or phenylephrine (Figure 1H) also significantly increased the pS394 HDAC2 amount. In summary, both the S394 and S411 residues were phosphorylated by hypertrophic stresses both in vivo and in vitro.

Phosphorylation of Histone Deacetylases 2 S394 Is Required for Hypertrophy
We next examined whether the phosphorylation of HDAC2 is essential for cardiac hypertrophy. We first used HDAC2 S394A, S411A, S422/424A, and S394/411/422/424A mutant forms of pcDNA3.1-HDAC2-V5 constructs for an HDAC assay after immunoprecipitation with V5 antibody. Substitution of S394 with alanine significantly reduced the enzymatic activity. In addition, both S422 and S424 residues were critical for the maintenance of enzymatic activity. Interestingly, HDAC2 S411A had enzymatic activity equivalent to the WT (Figure 2A). The observations that hypertrophic stresses induce phosphorylation of S394 and S411 (Figure 1) and that enzymatic activation requires S394, S422, and S424 (Figure 2A) indicated that S394 is the serine residue respon-
sible for the hypertrophy-associated activation of HDAC2, whereas intrinsic basal activity is maintained by S422 and S424. The association of HDAC2 S394 but not S411 with hypertrophy was further confirmed by promoter analysis. Four-fold activation of the promoter of natriuretic peptide precursor type A (Nppa; encoding atrial natriuretic factor), a marker of hypertrophy and failure, was induced by cotransfection with HDAC2wt. However, activation of the Nppa promoter was significantly attenuated by transfection with HDAC2 S394A. Interestingly, substitution of S411 with alanine did not reduce the HDAC2-induced promoter activation (Figure 2B). In addition, HDAC2 S394A failed to increase the cardiomyocyte size (Figure 2C and 2D), indicating that phosphorylation of S394 is required for the hypertrophic response.

We further confirmed the necessity of phosphorylation of HDAC2 S394 in an in vivo animal model. Two independent /H9251-H9251-myosin heavy chain promoter–driven transgenic mouse lines were generated that overexpressed either HDAC2wt-V5 or HDAC2 S394A-V5 in adult hearts. The expression levels of the WT and mutant proteins were evaluated for each transgenic line, and the transgenic lines expressing almost the same level of proteins were selected for further evaluation (Figure 2F, top row).

As evaluated by the ratio of heart weight to tibia length, we observed that transgenic mice overexpressing HDAC2wt showed a dramatic increase in heart size, as reported previously. Interestingly, the hypertrophic response was blunted when HDAC2 S394A was overexpressed in the heart (Figure 2E). Expression of atrial natriuretic factor (Figure 2F, middle row) and the transcript level of skeletal /H9251-actin (Figure IV in the online-only Data Supplement) were not increased in transgenic (Tg) HDAC2 S394A compared with Tg HDAC2wt. The quantitative results for the Nppa and Myh7 transcripts are discussed below. The enzymatic activity of HDAC2 in the heart was also greatly reduced in Tg HDAC2 S394A (Figure 2G).

Phosphorylation of HDAC2 S394 was further confirmed by using the samples obtained from patients with hypertrophic cardiomyopathy. First, we performed immunohistochemistry analysis using HDAC2 pS394-specific antibody. Compared with that in age-matched normal heart, the phosphorylation of HDAC2 was significantly increased in hypertrophic heart (Figure 2H). The level of protein phosphorylation was also examined by Western blot analysis with the samples obtained from 3 individuals, and the phosphorylation of HDAC S394 was significantly increased in the heart samples from all 3 hypertrophic cardiomyopathy patients (Figure 2I).

**CK2 Phosphorylates and Activates Histone Deacetylases 2**

We next searched for the protein kinase responsible for the phosphorylation and activation of HDAC2. First, we per-
formed an in vitro kinase assay with a synthetic peptide of S394 with various protein kinase inhibitors (data not shown). Of the protein kinases tested, a series of CK2 inhibitors such as apigenin and the more selective 4,5,6,7-tetrabromobenzotriazole (TBB) and (E)-3-(2,3,4,5-tetrabromophenyl) acrylic acid (TBCA) successfully inhibited the phosphorylation of S394 induced by infusion of isoproterenol in mice (Figure 3A). The results were also repeated with in vitro cardiomyocyte mod-

Figure 2. Phosphorylation of histone deacetylase (HDAC)2 S394 is required for the development of cardiac hypertrophy. A, HDAC2 enzymatic activity was lost when serine 394 was substituted by alanine. Substitution of serine 411 with alanine did not reduce the enzymatic activity. B, HDAC2 S394 is important for the full activation of natriuretic peptide precursor type A (Nppa) promoter in cardiomyocytes. C and D, HDAC2 S394A failed to increase cardiomyocyte size (C). Cardiomyocytes were transfected with either wild-type (WT) or S394A construct, and the areas of transfected cells were counted (D). E, Cardiac hypertrophy was blunted in HDAC2 S394A-overexpressing mouse hearts compared with HDAC2 WT-overexpressing mice. Horizontal bars indicate the mean of each group, and dots represent heart weight/tibia length ratio values of individual animals. F, Expression of atrial natriuretic factor (ANF) was blunted in transgenic (Tg) HDAC2 S394A compared with Tg HDAC2wt. Expressions of HDAC2WT and S394A were equal in those 2 separate lines. G, HDAC2 enzymatic activity was not increased in Tg HDAC2 S394A hearts. H, The amount of HDAC2 pS394 was markedly increased in the heart sections obtained from hypertrophic cardiomyopathy (HCMP) patients compared with age-matched normal hearts. Immunohistochemistry was performed with anti-HDAC2 pS394 antibody. I, The increase in the level of phosphorylation was further confirmed with immunoblot analysis with the use of anti-HDAC2 pS394 antibody. Autopsy samples of heart from 3 patients and 3 age-matched individuals were prepared for the analysis. *P<0.05; **P<0.01; @P<0.05; @@P<0.01.
Figure 3. Casein kinase (CK)2α1 phosphorylates histone deacetylase (HDAC)2 S394. A, HDAC2 S394 phosphorylation was blocked by CK2 blockers. TBB indicates 4,5,6,7-tetrabromobenzotriazole; TBCA, (E)-3-(2,3,4,5-tetrabromophenyl)acrylic acid. Heart lysates obtained from isoproterenol (ISP)-administered mice were used for the in vitro kinase assay. B, Phosphorylation of S394 induced by either fetal bovine serum (FBS) or phenylephrine (PE) in cardiomyocytes was blocked by apigenin, a CK2 inhibitor. C, Phosphorylation of GST-HDAC2 wild type was completely blocked by either apigenin or TBB. GST indicates glutathione-S-transferase. D, Phosphorylation of HDAC2 induced by either FBS or phenylephrine was significantly attenuated by TBB in cardiomyocytes. E, Hypertrophy-induced HDAC2 activation was completely blocked by CK2 inhibitors. Enzymatic activity of GST-HDAC2 was increased by applying isoproterenol-treated heart lysates, which was completely blocked by TBB, TBCA, apigenin, and 2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole (DMAT) but not by other protein kinase inhibitors. F, FBS-induced activation of HDAC2 was completely blocked by either TBB or CK2α1 short interfering (si)RNA transfection in cardiomyocytes. Note that transfection of CK2α1 activated HDAC2 (column 7). *P<0.05; **P<0.01; @@P<0.01.
els. Phosphorylation by either FBS or phenylephrine was completely blocked by treatment of the cells with apigenin (Figure 3B). CK2 inhibitors also completely abolished the phosphorylation of GST-HDAC2 induced by applying heart lysates from isoproterenol-administered mice (Figure 3C). Blockade of the phosphorylation of HDAC2 S394 by TBB was further repeated in rat neonatal cardiomyocytes; TBB significantly attenuated the amount of pS394 in FBS- or phenylephrine-treated cardiomyocytes, as determined by phospho-specific antibody (Figure 3D).

We next tested whether CK2 is required for the activation of HDAC2 by use of an HDAC2 activity analysis in the presence of CK2 inhibitor (Figure 3E) or CK2 short interfering (si)RNA (Figure 3F). As we reported previously,10 the intrinsic activity of the chimeric protein of GST-HDAC2 was greatly increased when isoproterenol-treated heart lysates were applied to the proteins (column 4 in Figure 3E). The activation was completely blocked when TBB, TBCA, apigenin, and 2-dimethylamino-4,5,6,7-tetramethyl-1H-benozimidazole (DMAT), an alternate CK2 inhibitor, were applied. However, other protein kinase inhibitors failed to attenuate the HDAC2 activation (columns 9 through 13). These results were also confirmed in the cardiomyocytes (Figure 3F). The FBS-induced activation of HDAC2 was significantly blocked by TBB (column 9). More specifically, downregulation of CK2α1 by utilization of siRNA dramatically reduced FBS-stimulated (column 11) HDAC2 activity. In contrast, transfection of pCMV-HA-CK2α1 mimicked the hypertrophic stress–induced activation of HDAC2 (column 7). Interestingly, however, CK2α2 siRNA failed to inhibit the FBS-stimulated activation of HDAC2 (column 12).

**Hypertrophic Stimuli Activate and Translocate CK2α1**

Next, we questioned whether CK2 activity is increased in response to hypertrophic stresses. We modified an in vitro kinase assay with specific peptides to measure CK2 enzyme activity. Phenylephrine, FBS, and isoproterenol significantly increased the CK2 activity in cardiomyocytes (Figure 4A) and in heart (Figure 4B). Previous reports have demonstrated that shuttling between the nucleus and the cytoplasm is also an important regulatory mechanism of CK2 activity in diverse biological responses.21,22 In contrast, Hauck et al23 recently reported that CK2α2 does not shuttle but phosphorylates p27 in the cytoplasm in response to hypertrophic stresses in cardiomyocytes. Thus, we checked whether our CK2α1 follows the case of CK2α2 by observing its intracellular distribution after treatment with the hypertrophic stimulus of FBS (Figure 4C and 4D). Although total amounts of CK2α1 were not altered (top 2 panels), the amount of CK2α1 in the nuclear fraction was increased (middle 2 panels), whereas that in the cytosolic fraction was reduced (bottom 2 panels). Immunocytochemical analysis showed increased nuclear localization after FBS stimulation (Figure 4D). These results suggest that in addition to its intrinsic activity, the translocation of CK2α1 participates in the activation of HDAC2.

**CK2α1 Induces Cardiac Hypertrophy**

We examined whether activation of CK2α1 is responsible for relaying the hypertrophic phenotypes in cardiomyocytes and mouse heart. Transfection of pCMV-HA-CK2α1 to cardiomyocytes significantly increased leucine incorporation. Furthermore, phenylephrine- or FBS-induced increases were markedly blocked by either CK2 inhibitors or transfection of CK2α1 siRNA (Figure 5A). Both basal and phenylephrine-stimulated Nppa promoter activity was inhibited by TBB in a dose-dependent fashion (Figure 5B).

We next investigated whether HDAC2 is an ultimate downstream of CK2α1 to induce hypertrophic phenotypes. Both HDAC2 siRNA and trichostatin A completely blocked the CK2α1-induced increase in leucine incorporation (Figure 5C). We reported previously that KLF4, a novel antihypertrophic mediator, is a downstream target of HDAC2 in the development of cardiac hypertrophy.11 Thus, by utilizing
Figure 5. Casein kinase (CK2) inhibitors block cardiac hypertrophy. A, Transfection of CK2α1 or administration of phenylephrine (PE) or fetal bovine serum (FBS) significantly increased [³H]-leucine incorporation in cardiomyocytes, which was blocked by CK2 inhibitors. B, Natriuretic peptide precursor type A (Nppa) promoter activity was blocked by CK2 inhibitor. Either basal or phenylephrine-stimulated Nppa minimal promoter activity was dose-dependently decreased by TBB. C, CK2α1-induced increase in [³H]-leucine incorporation was completely blocked by either HDAC2 short interfering (si)RNA or trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor. D, 4,5,6,7-Tetrabromobenzotriazole (TBB) and (E)-3-(2,3,4,5-tetrabromophenyl)acrylic acid (TBCA) failed to inhibit the -638 Krüppel-like factor 4 (KLF4)–binding element disrupted Nppa promoter activity. E, CK2α1 siRNA decreased Nppa promoter activity in either vehicle- or phenylephrine-treated cardiomyocytes. F, Isoproterenol (ISP)-induced cardiac hypertrophy was blunted by apigenin in mice. G, Isoproterenol-induced HDAC2 activation in mouse heart was significantly blocked by coadministration of apigenin. *P<0.05, **P<0.01; @@P<0.01.
KLF4-binding element disrupted Nppa promoter, we investigated whether the CK2 inhibitor–induced effect is also KLF4 dependent. Treatment with either TBB or TBCA failed to reduce the mutant promoter activity (Figure 5D). These results suggest that CK2/H9251-induced hypertrophic events are dependent on both HDAC2 and KLF4.

Transfection of CK2a1 siRNA completely blocked the Nppa promoter activity compared with that in scramble-treated cardiomyocytes (Figure 5E). The effect of CK2 inhibitor was also reiterated in vivo. Continuous infusion of isoproterenol induced cardiac hypertrophy, which was blunted by simultaneous intraperitoneal administration of apigenin (Figure 5F). Again, HDAC2 enzymatic activity in the mouse heart was significantly lowered in apigenin-treated mice (Figure 5G).

Transgenic Overexpression of CK2a1 Induces Cardiac Hypertrophy

CK2a1-overexpressing transgenic mice were generated to study whether CK2a1 is prohypertrophic in vivo. Cardiac hypertrophy was evaluated by echocardiography; both the interventricular septum (Figure 6A) and the left ventricular free wall thickness (Figure 6B) were increased in TgCK2a1 hearts. WT indicates wild type. C. Hypertrophic markers as well as phosphorylation of HDAC2 S394 were increased in TgCK2a1 hearts. D, CK2a1-mediated hypertrophy is histone deacetylase (HDAC) dependent. Overexpression of CK2α1 in mouse heart induced cardiac hypertrophy, which was completely blocked by trichostatin A (TSA), an HDAC inhibitor. **P<0.01. E, Real-time reverse transcription polymerase chain reaction results showing the changes in natriuretic peptide precursor type A (Nppa) expression in TgCK2a1+TSA mice. Because the data of E were not normally distributed as determined by the Shapiro-Wilk test, P value was obtained by the Mann-Whitney U test with Bonferroni correction. ¶,§P<0.0125.

CK2α1-Induced Cardiac Hypertrophy Is Attenuated by Histone Deacetylases 2 S394A

Given that CK2α1 induces HDAC2 phosphorylation and activation, a phosphorylation-resistant HDAC2 mutant may block CK2-induced hypertrophic phenotypes. We used a leucine incorporation study to check this hypothesis. As expected, cotransfection of CK2α1 and HDAC2wt further increased the incorporation. However, transfection of HDAC2 S394A blocked CK2α1-induced incorporation (Fig-
Blockade of the CK2 effect by HDAC2 S394A was also observed by Nppa- or Myh7-promoter analysis (Figure 7B and 7C). We also evaluated cardiac hypertrophy by crossing TgCK2α1 mice with TgHDAC2 S394A mice. We found that the cardiac enlargement was greatly exaggerated when TgCK2α1 mice were crossed with TgHDAC2wt. Interestingly, however, no exaggeration of cardiac hypertrophy was observed when TgCK2α1 mice were crossed with TgHDAC2 S394A (Figure 7D and Figure VIA in the online-only Data Supplement). The changes in the Nppa (Figure 7E) and Myh7 (Figure VIB in the online-only Data Supplement) transcripts were also compared by real-time reverse transcription polymerase chain reaction analysis. F, Cross sections of double transgenic hearts. Note that enlargement of individual cardiomyocytes in TgCK2α1 was enhanced in double transgenic hearts of TgCK2α1+/−;HDAC2 S394A+/−. However, no increase in cardiomyocytes was observed in TgCK2α1+/−;HDAC2wt S394A+/− hearts. WT indicates wild type littermate. *P<0.05; **P<0.01; @P<0.05; @@P<0.01.
also supported the results. Figure 7F shows that CK2α1-induced enlargement of individual cardiomyocyte area was further increased in TgCK2α1/HDAC2wt mice but not in TgCK2α1/HDAC2 S394A. We checked whether transgenic overexpression of CK2α1 or HDAC2 causes hyperplasia rather than hypertrophy by counting proliferating cell nuclear antigen; however, we found no significant changes in the animal groups (Figure VII in the online-only Data Supplement).

**Discussion**

We have established the existence of a previously undescribed signal pathway in the development of cardiac hypertrophy: that of CK2α1-mediated phosphorylation of HDAC2 S394 and subsequent activation of HDAC2. When hypertrophic stimuli act on cardiomyocytes, the stimuli activate CK2, which then phosphorylates HDAC2 S394, and the resulting enzymatic activation of HDAC2 mediates the hypertrophic phenotype (Figure 8).

All mammalian HDACs have potential phosphorylation sites, and phosphorylation is a key mechanism of regulating activity by modifying substrate specificity or by regulating stability with other corepressor complexes, such as RbAp48, MTA-2, mSin3A, and CoREST complex. Although the phosphorylation of HDAC2 is known to be required for its association with mSin3 and Mi2 corepressors, the biological significance of the phosphorylation of HDAC2 and its activation is largely unknown. A recent study reported that phosphorylation of HDAC2 regulates corepressor formation and activates enzymatic activity in association with cigarette smoking. In addition, the phosphorylation and activation of HDAC2 are closely associated with tumor cell progression and metastasis. Previous findings reported that phosphorylation on S394 is tightly related to complex formation and that deacetylation substrate is dissociated from HDAC2 after S424 phosphorylation.

We and others have shown the involvement of HDAC2 in cardiac hypertrophy. In the present study, we have shown for the first time that the hypertrophic stimuli–induced phosphorylation of HDAC2 is required for HDAC2 activation, and therefore for the development of cardiac hypertrophy. The enzymatic activity of HDAC2 was regulated by the S394, S422, and S424 residues, whereas hypertrophic stresses phosphorylated HDAC2 S394 and S411. These results suggest that only the phosphorylation of S394 is responsible for the enzymatic activation in association with hypertrophic stimuli, and that S422 and S424 are related to basal enzymatic activity but not hypertrophy–induced activity. Thus, hypertrophic stress–induced phosphorylation of HDAC2 S394 is a key regulatory mechanism in relaying hypertrophic phenotypes. Indeed, we also observed that phosphorylation of HDAC2 S394 takes place in heart samples obtained from hypertrophic cardiomyopathy patients. Although the biological significance of the phosphorylation of HDAC2 S411 should be investigated further, it can be assumed that this phosphorylation is related either to intracellular localization or to binding affinity for other transcription cofactors.

The implication of the biological roles of CK2 was first highlighted in cancer biology. CK2 levels are elevated in cancer tissues, and inhibition of CK2 by selective blockers attenuates cell proliferation. CK2 enhances the transforming potential of oncogenes, generates abnormal prosurvival and antiapoptotic signals, and supports neovascularization. CK2 has multiple target molecules in diverse tissues because the substrate consensus protein sequence is ubiquitous in diverse cellular types. Growing numbers of reports have appeared dealing with the functional roles of CK2 in cardiac diseases. For example, some subtypes of CK2 are closely related to ischemia/reperfusion injury and embryonic heart development.

Recently, CK2α2 was shown to induce cardiac hypertrophy, which is mediated by the CK2α2-dependent degradation of p27, an important antihypertrophic cell cycle regulator. That report clearly demonstrated that CK2α2 is also a prohypertrophic mediator. However, the working mechanism was quite different from ours. In addition to the different target, those authors did not observe the shuttling of CK2α2 in response to cardiac hypertrophy. Because many proteins could be substrates of CK2, it is likely that targets other than HDAC2 may participate in relaying the hypertrophic phenotypes. Indeed, we also observed that p27 was slightly down-regulated in hypertrophic stresses in cardiomyocytes (data not shown). However, in our study, phosphorylation-resistant HDAC2 S394A blunted the CK2α1-mediated cardiac hypertrophy, which strongly suggests that HDAC2 phosphorylation is a key regulatory mechanism of CK2α1–induced cardiac hypertrophy.

It would be interesting to determine whether phosphorylated HDAC2 is associated with other hypertrophy–associated proteins that can be altered by diverse stresses to cardiomyocytes. Indeed, some hypertrophy–related factors preferentially bind to hyperphosphorylated HDAC2 (data not shown). We also observed that heat shock protein 70, which was reported as an HDAC2 activator by our group, synergistically induced hypertrophic phenotypes together with phosphorylated HDAC2 (data not shown). These results suggest that HDAC2 phosphorylation and activation may be a complex process in the development of cardiac hypertrophy, although...
detailed mechanisms should be elucidated. In addition, the manner in which CK2α1 is activated by hypertrophic stimuli should be clarified. It is known that signals such as Wnt,\textsuperscript{36} UV irradiation,\textsuperscript{37} or dysinhibition of p53\textsuperscript{38} induce the activity of CK2α1 in cancer. However, it is still unclear whether this signal cascade takes place in tumorigenesis in the same fashion as in the development of cardiac hypertrophy.

In summary, hypertrophic stress–induced activation of CK2α1 phosphorylates HDAC2 S394, which results in the activation of HDAC2 enzymatic function to repress the expression of antihypertrophic genes. Note, however, that the CK2/HDAC2 signal is not the only mechanism in the development of cardiac hypertrophy. For instance, diverse traditional pathways like G-protein or calcium-dependent signals are well established in cardiac hypertrophy. However, when one considers that subtype-selective HDAC inhibitors and CK2 blockers are being investigated intensively as potential therapeutics for various diseases such as cancer\textsuperscript{39} or inflammation,\textsuperscript{40} our findings may provide valuable targets for the development of novel drugs for cardiac hypertrophy and heart failure.

Cardiac hypertrophy is a response to diverse forms of heart disease, including myocardial infarction, hypertension, and valvular dysfunctions. Although the initial hypertrophic responses are an adaptation to those stimuli, the sustained stress may lead to cardiomyopathy and heart failure, a major cause of human morbidity and mortality. Few interventions have proven effective in blocking hypertrophy or in preventing the transition to heart failure. Recently, transcriptional reprogramming of fetal gene expression by histone deacetylases was shown to be tightly linked to cardiac hypertrophy. We showed previously that inhibition of class I HDACs prevents cardiac hypertrophy and that activation of HDAC2, 1 of the class I HDACs, in association with heat shock protein 70, is essential for hypertrophy. In addition, we have reported that KLF4, a novel antihypertrophic mediator, relays the HDAC2 effects. Here we show that CK2α1-dependent phosphorylation of HDAC2 S394 induces enzymatic activation of HDAC2 and thereby mediates hypertrophy. Interestingly, the phosphorylation of HDAC2 S394 was dramatically increased in hearts from hypertrophic cardiomyopathy patients. Hypertrophic stimuli led CK2α1 to be activated, and overexpression of CK2α1 caused cardiac hypertrophy both in vivo and in vitro, whereas chemical inhibitors of CK2α1 blunted this response. Modulators of the activity of both CK2 and HDAC are under extensive investigation for widespread clinical use for noncardiac diseases, including neoplasia. Our work implicates CK2α1/HDAC2 signal cascades as novel therapeutic targets for the treatment or prevention of cardiac hypertrophy and heart failure worthy of further validation and investigation.

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Disclosures

None.

References

Cardiac hypertrophy is a response to diverse forms of heart disease, including myocardial infarction, hypertension, and valvular dysfunctions. Although the initial hypertrophic responses are an adaptation to those stimuli, the sustained stress may lead to cardiomyopathy and heart failure, a major cause of human morbidity and mortality. Few interventions have proven effective in blocking hypertrophy or in preventing the transition to heart failure. Recently, transcriptional reprogramming of fetal gene expression by histone deacetylases (HDACs) was shown to be tightly linked to cardiac hypertrophy. We showed previously that inhibition of class I HDACs prevents cardiac hypertrophy, and that activation of HDAC2, 1 of the class I HDACs, in association with heat shock protein 70, is essential for hypertrophy. In addition, we have reported that Kriippel-like factor 4, a novel antihypertrophic mediator, relays the HDAC2 effects. Here, we show that casein kinase-2 (CK2α1)–dependent phosphorylation of HDAC2 S394 induces enzymatic activation of HDAC2 and thereby mediates hypertrophy. Interestingly, the phosphorylation of HDAC2 S394 was dramatically increased in hearts from hypertrophic cardiomyopathy patients. Hypertrophic stimuli led CK2α1 to be activated, and overexpression of CK2α1 caused cardiac hypertrophy both in vivo and in vitro, whereas chemical inhibitors of CK2α1 blunted this response. Modulators of the activity of both CK2 and HDAC2 are under extensive investigation for widespread clinical use for the treatment or prevention of cardiac hypertrophy and heart failure worthy of further validation and investigation.

**CLINICAL PERSPECTIVE**

Cardiac hypertrophy is a response to diverse forms of heart disease, including myocardial infarction, hypertension, and valvular dysfunctions. Although the initial hypertrophic responses are an adaptation to those stimuli, the sustained stress may lead to cardiomyopathy and heart failure, a major cause of human morbidity and mortality. Few interventions have proven effective in blocking hypertrophy or in preventing the transition to heart failure. Recently, transcriptional reprogramming of fetal gene expression by histone deacetylases (HDACs) was shown to be tightly linked to cardiac hypertrophy. We showed previously that inhibition of class I HDACs prevents cardiac hypertrophy, and that activation of HDAC2, 1 of the class I HDACs, in association with heat shock protein 70, is essential for hypertrophy. In addition, we have reported that Kriippel-like factor 4, a novel antihypertrophic mediator, relays the HDAC2 effects. Here, we show that casein kinase-2α1 (CK2α1)–dependent phosphorylation of HDAC2 S394 induces enzymatic activation of HDAC2 and thereby mediates hypertrophy. Interestingly, the phosphorylation of HDAC2 S394 was dramatically increased in hearts from hypertrophic cardiomyopathy patients. Hypertrophic stimuli led CK2α1 to be activated, and overexpression of CK2α1 caused cardiac hypertrophy both in vivo and in vitro, whereas chemical inhibitors of CK2α1 blunted this response. Modulators of the activity of both CK2 and HDAC2 are under extensive investigation for widespread clinical use for noncardiac diseases, including neoplasia. Our work implicates CK2α1/HDAC2 signal cascades as novel therapeutic targets for the treatment or prevention of cardiac hypertrophy and heart failure worthy of further validation and investigation.
Casein Kinase-2α1 Induces Hypertrophic Response by Phosphorylation of Histone Deacetylase 2 S394 and its Activation in the Heart

Gwang Hyeon Eom, Young Kuk Cho, Jeong-Hyeon Ko, Sera Shin, Nakwon Choe, Yoojung Kim, Hosouk Joung, Hyung-Seok Kim, Kwang-II Nam, Hae Jin Kee and Hyun Kook

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In the article by Eom et al, “Casein Kinase-2α1 Induces Hypertrophic Response by Phosphorylation of Histone Deacetylase 2 S394 and its Activation in the Heart,” which was published in the May 31, 2011 issue of the journal (Circulation. 2011;123:2392–2403), one of the grant numbers in the Sources of Funding section on page 2402 was incorrect and should have read, “…National Research Foundation of Korea grant funded by the Korean government (MEST, No. 2010-0015012).”

DOI: 10.1161/CIR.0b013e3182323d4d
Supplemental Material

Reagents - Anti-HDAC2, HDAC2 pS394, PCNA, and CK2α1 were from Abcam (Abcam, Cambridge, UK); phosphorserine and β-actin were from Sigma (Sigma-Aldrich Corp., St. Louis, MO, USA); skeletal α-actin, α-tubulin, and Gapdh were from Santa Cruz Biotechnology (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA); pGSK3β was from Cell Signaling (Cell Signaling Technology, Inc., Danver, MA, USA); and atrial natriuretic factor was from Meridian (Meridian Life Science, Inc., Saco, ME, USA).

Isoproterenol (ISP), phenylephrine (PE), endothelin-1, 2,2,2-tribromoethanol, trichostatin A (TSA), 4,5,6,7-tetrabromobenzotriazole (TBB), 2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole (DMAT), 2-(4-Morpholinyl)-8-phenyl-1(4H)-benzopyran-4-one hydrochloride (LY294002), (9S,10S,12R)-2,3,9,10,11,12-hexahydro-10-hydroxy-9-methyl-1-oxo-9,12-epoxy-1H-diindolo[1,2,3-fg:3′,2′,1′-kl]pyrrolo[3,4-i][1,6]benzodiazone-10-carboxylic acid hexyl ester (KT5720), N-[2-(p-bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride (H-89), 3-[1-(dimethylaminopropyl)indol-3-yl]-4-(indol-3-yl)maleimide hydrochloride, bisindolylimaleimide I hydrochloride (GF109203X), and 3-[1-[3-(dimethylamino)propyl]-5-methoxy-1H-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione (Gö6983) were purchased from Sigma (Sigma-Aldrich Corp., St. Louis, MO, USA), and (E)-3-(2,3,4,5-tetrabromophenyl) acrylic acid (TBCA) was from Merck (Merck, Darmstadt, Germany). CK2α1 siRNA, siCK2α2 siRNA, HDAC2 siRNA, and scramble were purchased from Dharmacon (Dharmacon, Lafayette, CO, USA).

The pcDNA3.1-HDAC2-V5 and pGEX4T-HDAC2 constructs were described previously1.
Phosphorylation-resistant mutants, S394A, S411A, S422/424A, and S394/411/422/424A, were generated by using the QuickChange kit (Stratagene Inc., La Jolla, CA, USA). pCMV-HA-CK2α1 was a gift from Dr. Kunhong Kim (Yonsei University College of Medicine, Seoul, Korea). pGL3-638 Nppa-promoter luciferase plasmids were kindly provided by Dr. Youngsook Lee (University of Wisconsin Medical School, Madison, WI, USA). GST-HDAC2 chimeric proteins were obtained by use of a bacterial overexpression system. Briefly, pGEX4T-HDAC2 transformed BL-21 was pre-inoculated in 6 ml overnight with vigorous shaking at 37 °C and re-inoculated in 250 ml. When O.D.600 reached 0.5, isopropyl β-D-1-thiogalactopyranoside (IPTG) (Sigma-Aldrich Corp., St. Louis, MO, USA) was applied at a concentration of 200 µM. Forced bacterial induction was performed at 20 °C for 4 hours.

Transgenic mice – The α-MHC promoter cloned vector (U71441) was used for transgenic overexpression. α-MHC-CK2α1 was subcloned from pCMV-CK2α1 and both α-MHC-HDAC2wt-V5 and α-MHC-HDAC2 S394A-V5 were derived from pcDNA3.1-based constructs. Germ-line transmission was checked by PCR amplification of genomic DNA, and mice were backcrossed six generations. The level of protein expression in transgenic mouse heart was examined by immunoblot with specific antibodies.

Hypertrophy models and evaluation of cardiac hypertrophy - Six- to 8-week-old adult male CD1 mice, 7-week-old adult female C57BL/6 mice, and neonatal Sprague-Dawley rats were purchased from Daehan Biolink (Daejeon, Korea) and were housed in plastic cages individually in a temperature-, humidity-, and daytime-controlled room. Osmotic pump insertion and echocardiography were performed as described previously under anesthesia with 2,2,2-tribromoethanol (300 mg/kg, IP). For the induction of cardiac
hypertrophy, ISP (30 mg/kg/day) was infused with an osmotic pump (Alzet®, Durect Corp., Cupertino, CA, USA). The hearts were harvested after euthanasia. In vitro hypertrophy was introduced by treatment of neonatal cardiomyocytes with 100 μM PE. The preparation of primary cultured rat neonatal cardiomyocytes and cell culture of H9c2 cardiomyoblast cell lines were described previously1. All experimental procedures were approved by the Chonnam National University Medical School Research Institutional Animal Care and Use Committee.

Cardiac hypertrophy was evaluated by heart weight to tibia length (HW/TL) or heart weight to body weight (HW/BW) ratios and by echocardiogram. Echocardiographic studies were performed as described previously2,3. In brief, the two-dimensional (2-D)-guided M-mode of the left ventricle was measured from the parasternal view. The left ventricular cavity dimension and thickness of the interventricular septum and left ventricular free walls were checked.

Cell cultures and cell size measurement - Rat neonatal cardiomyocytes were prepared as described1 with slight modifications. After atria and large arteries were completely removed, ventricles were minced and treated with 0.1% type 2 collagenase/ADS buffer for 30 min at room temperature. The dissociation process was stopped by the addition of 10% FBS containing DMEM. Fibroblasts were removed twice by 1-hour preplating. The cardiomyocytes were counted and plated on collagen-coated culture dishes and were maintained in 10% FBS in DMEM without antibiotics.

Cardiomyocyte hypertrophy was measured by use of the i solution program (Image & Microscope Technology Inc., Rochester, NY, USA) under fluorescent microscopy after
visualization with Texas-Red-conjugated phalloidin actin. More than 40 transfected cells were measured and each experiment was repeated three times.

**HDAC activity and leucine incorporation** - HDAC activity was measured as described previously. GST-HDAC2 protein activity was measured in the presence of heart lysates.

Forty-eight hours after the isolation of cardiomyocytes, cells were incubated in serum-free DMEM overnight. In the small interfering RNA group, cardiomyocytes were transfected 48 hours after seeding. Cardiomyocytes were stimulated with PE or fetal bovine serum (FBS) and were incubated with 1.0 μCi/ml [3H]-leucine overnight. After treating with 10% trichloroacetic acid, cardiomyocytes were solubilized in 0.25 N NaOH, and incorporation was measured in a liquid scintillation counter (Wallac, Gaithersburg, MD, USA) after retrieval.

**In vitro kinase assay** - Ten microliters of heart lysates from ISP-treated or control mice, 1 μl of [γ-32p]ATP, 5 μl of recombinant protein as substrate, 7 μl of reaction buffer (20 mM HEPES pH7.4, 10 mM MgCl₂, 1 mM Na₃VO₄, 1 mM DTT, 5 mM NaF, 10 μM ATP), and 2 μl of various kinase inhibitors were mixed and kept at 30 °C for 30 min. Reactants were electrophoresed by the standard SDS-PAGE method. After separation, the SDS-PAGE gel was dried with a gel dryer. X-ray film (Eastman Kodak Co., Rochester, NY, USA) was exposed to the dried gel and kept at -80 °C overnight and then developed (Eastman Kodak Co., Rochester, NY, USA).

**Casein kinase 2 assay** - Five microliters of CK2α1-immunoprecipitates, 1 μl of [γ-32p]ATP, 5 μl of substrate and 9 μl of reaction buffer were mixed and kept 30 °C for 10 min. Reaction
was stopped by the addition of 40% trichloroacetate (TCA), and the mixture was then promptly spun down. Ten microliters of supernatant was dripped to filter paper. The papers were washed with 0.4% phosphoric acid 5 times and were then washed with acetone. After the filter papers were completely dried, radioactivity was counted by use of a scintillation counter (Wallac, Gaithersburg, MD, USA).

*Immunocytochemistry* - Cardiomyocytes at a density of 1×10^5 per well were seeded in an eight-well chamber slide precoated with poly-D-lysine (Nalge Nunc international Corp., Rochester, NY, USA). Cells were fixed with 3.7% paraformaldehyde for 10 minutes and washed with PBS containing 0.5% bovine serum albumin (BSA). Blocking was carried out at room temperature for 30 minutes by use of normal goat serum. After permeabilization with 0.2% Triton X-100 in 0.5% BSA/PBS, cells were incubated overnight with primary antibodies in 1% BSA and for 40 min with the appropriate fluorescent Alexa-conjugated secondary antibodies (Molecular Probes, Eugene, OR, USA). Before mounting the slide, 4',6-diamidino-2-phenylindole (DAPI) (Molecular Probes, Eugene, OR, USA) was added to make 300 nmol/L. Phycoerythrin-conjugated IgG2A (R&D Systems, Inc., Minneapolis, MN, USA) was used as an isotype-matched control. The sections were photographed by fluorescent microscopy (Carl Zeiss, Jena, Germany).

*Human samples* – Paraffin-embedded left ventricle specimens were provided from the Department of Forensic Medicine, Chonnam National University Medical School, which were autopsied from six individuals: three hypertrophied patients and three age-matched males. Hypertrophic cardiomyopathy was diagnosed according to total heart weight, left ventricular free wall thickness, and microscopic findings. Protein extraction was done as described previously^4^. This work was performed according to the regulations of the
Institutional Review Board of Chonnam National University Hospital (#2010-10-167). For immunohistochemistry, phosphorylated HDAC2 was probed by phosphor-S394 HDAC2-specific antibody (1:400) after antigen-retrieval by microwave. Phosphorylation of HDAC2 was visualized with a commercially available kit (LSAB®2 System-HRP, Dako North America, Inc., Carpinteria, CA, USA). Phosphorylation of HDAC2 S394 was further quantified by immunoblots.

**Histology** – Mouse heart histology was done as described previously\(^1\). Simple hematoxylin-eosin staining was performed to check the wall thickness and the cardiac muscle bundle in transgenic mice or wild-type mice. Mouse hearts were cross-sectioned at the level of the papillary muscle.

**Quantitative real-time polymerase chain reaction** – To examine the transcript amount of ANF, Myh7, and Acta1 in the transgenic mice or wild-type mice, qRT-PCR was done as described previously\(^1\). Quantification of the mRNA was confirmed with the SYBR Green PCR kit (Qiagen, Hilden, Germany). Relative expression levels of each transcripts were compared with those of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).
### Supplemental table. Echocardiographic parameters in wild type and transgenic mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Genotype</th>
<th>Wild type (n=5)</th>
<th>CK2α1 HDAC2wt (n=7)</th>
<th>CK2α1/HDAC2wt (n=6)</th>
<th>HDAC2 S394A (n=8)</th>
<th>CK2α1/HDAC2 S394A (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVSd, mm</td>
<td>0.57 ± 0.04 †</td>
<td>0.69 ± 0.02 *</td>
<td>0.71 ± 0.03 *</td>
<td>0.83 ± 0.02 **††</td>
<td>0.59 ± 0.01 ††</td>
<td>0.61 ± 0.03 †</td>
</tr>
<tr>
<td>LVDd, mm</td>
<td>3.50 ± 0.11</td>
<td>3.47 ± 0.07</td>
<td>3.36 ± 0.05</td>
<td>3.41 ± 0.06</td>
<td>3.23 ± 0.10</td>
<td>3.19 ± 0.08</td>
</tr>
<tr>
<td>LVFWd, mm</td>
<td>0.65 ± 0.05 ††</td>
<td>0.80 ± 0.03 **</td>
<td>0.85 ± 0.02 **</td>
<td>0.93 ± 0.01 **††</td>
<td>0.67 ± 0.02 ††</td>
<td>0.70 ± 0.04 †</td>
</tr>
<tr>
<td>IVSs, mm</td>
<td>0.74 ± 0.04 ††</td>
<td>0.93 ± 0.02 **</td>
<td>0.88 ± 0.01 *†</td>
<td>1.04 ± 0.01 **††</td>
<td>0.77 ± 0.02 ††</td>
<td>0.78 ± 0.30 ††</td>
</tr>
<tr>
<td>LVDs, mm</td>
<td>2.70 ± 0.10</td>
<td>2.64 ± 0.07</td>
<td>2.55 ± 0.11</td>
<td>2.58 ± 0.06</td>
<td>2.46 ± 0.11</td>
<td>2.54 ± 0.07</td>
</tr>
<tr>
<td>LVFWs, mm</td>
<td>0.78 ± 0.03 ††</td>
<td>1.04 ± 0.03 **</td>
<td>0.99 ± 0.02 **</td>
<td>1.17 ± 0.02 **††</td>
<td>0.83 ± 0.01 ††</td>
<td>0.85 ± 0.02 ††</td>
</tr>
<tr>
<td>FS, %</td>
<td>22.8 ± 0.78</td>
<td>23.8 ± 0.97</td>
<td>24.1 ± 2.86</td>
<td>24.3 ± 1.53</td>
<td>23.6 ± 3.16</td>
<td>20.5 ± 1.20</td>
</tr>
</tbody>
</table>

p value was calculated by non-parametric Mann-Whitney U-test
* p<0.05 with wild type group, ** p<0.01 with wild type group
† p<0.05 with TgCK2α1 group, †† p<0.01 with TgCK2α1 group

Data are mean ± SEM of 5 to 9 mice. IVS indicates interventricular wall thickness; LVD, LV dimension; LVFW, LV free wall thickness; d, end-diastolic phase; s, end-systolic phase; FS, fractional shortening
Supplemental Figure 1

A

pHDAC2 / total HDAC2 amount ratio (arbitrary unit)

ET - +

p < 0.01

B

pHDAC2 / total HDAC2 amount ratio (arbitrary unit)

ISP - +

p < 0.05
Supplemental Figure 2

S394  CHERGDED
S411  SIRASDKRI
S422/424  EFSDSEDEG

CPM (arbitrary unit)

ISP -  +  -  +  -  +
S394  S411  S422/424
Supplemental Figure 3

1% FBS  
20% FBS  

α-pS394 HDAC2
Supplemental Figure 4

![Bar graph showing skeletal α-actin transcript amount (fold change) for WT, Tg HDAC2 wt, and Tg HDAC2 S394A. The graph indicates that Tg HDAC2 wt has a significantly higher transcript amount compared to WT and Tg HDAC2 S394A, with p-values of <0.05 for both comparisons.](image-url)
Supplemental figure legends

**Supplemental Figure 1.** Quantification of phosphorylated HDAC2 compared with total HDAC2 after treatment with ET-1 (A) in cardiomyocytes or ISP (B) in mice. HDAC2 was immunoprecipitated and phosphor-serine antibody was used to detect phosphorylation of HDAC2.

**Supplemental Figure 2.** Hypertrophic stimuli phosphorylate S394 and S411 in the hearts. Lysates obtained from hearts of ISP-administered mice were utilized for the *in vitro* kinase assay. Synthetic peptides used for the assay are shown on the left.

**Supplemental Figure 3.** pS394 HDAC2 is increased in cardiomyocytes after treatment with 20% FBS. Fluorescent immunocytochemistry was performed with pS394 HDAC2-specific antibody.

**Supplemental Figure 4.** Comparison of skeletal α-actin transcript amounts in either TgHDAC2wt or TgHDAC2 S394A by real-time qRT-PCR. Four hearts were harvested for each transgenic line and used for evaluation.

**Supplemental Figure 5.** (A) Cardiac hypertrophy induced by overexpression of *CK2α1* was blocked by TSA. Heart weight to body weight ratio was measured. (B) Transcript levels of *skeletal α-actin (Acta1)* transcript in TgCK2α1 or TgCK2α1 + TSA mice. qRT-PCR was performed for the evaluation.
Supplemental Figure 6. (A) Comparison of heart weight to body weight ratios between Tg^{CK2α1;HDAC2wt} and Tg^{CK2α1;HDAC2 S394A}. (B) Comparison of Myh7 transcript amounts in double transgenic mice. Increase in Myh7 transcript amount in Tg^{CK2α1} was further potentiated when Tg^{CK2α1} was crossed with Tg^{HDAC2wt}, whereas it was not when crossed with Tg^{HDAC2 S394A}.

Supplemental Figure 7. Enlargement in heart size was caused mainly by hypertrophy and not hyperplasia. (A) Cellular proliferation was detected by proliferating cellular nuclear antigen (PCNA) staining in transverse sections of heart samples. (B) PCNA-positive cells were counted.
Supplemental References


Correction

In the article by Eom et al, “Casein Kinase-2α1 Induces Hypertrophic Response by Phosphorylation of Histone Deacetylase 2 S394 and its Activation in the Heart,” which was published in the May 31, 2011 issue of the journal (*Circulation*. 2011;123:2392-2403), one of the grant numbers in the Sources of Funding section on page 2402 was incorrect and should have read, “…National Research Foundation of Korea grant funded by the Korean government (MEST, No. 2010-0015012).”
Casein Kinase-2α1 활성화에 의한 Histone Deacetylase 2의 S394 위치 인산화는 심근비후를 유도한다: Histone의 acetylation을 통한 외성적(epigenic) 유전자 조절로도 심근비후 예방이 가능하다

최 동 주 교수 봉양서울대학교병음순환기내과

Summary

배경
심근비후는 테이 유전자 발현의 재프로그래밍이 중요한 특징이며, 이를 위해서는 HDACs(histone deacetylase)가 이런 유전자 조절에 필요한 인산화를 가지고 있음이 잘 알려져 있다. 본 연구에서는 HDAC5의 하나인 HDAC2의 활성화가 심근비후 유도에 중요한 역할을 할 것으로 보였다. 이에 본 연구에서는 CKα2(Casein Kinase-2α1)에 의존한 HDAC2 S394의 인산화가 심근비후 발생에 필요함을 보였다.

방법 및 결과
HDAC2 인산화에 의한 심근비후 저그 염산소가 활성화를 동반하며, 심장 죽은 심근세포 혹은 isoproterenol이 투여된 죽은 심장에서 심근비후를 유도하였다. HDAC2가 과발현한 죽에는 심근비후가 발생하였으나, HDAC2 S394A 변이 유전자가 과발현되어 인산화가 되지 않는 죽은 심근비후가 발생하지 않았다. 비교적 정상인과 비교하였을 때, HDAC2 S394의 인산화는 비후성 심근병증 환자에서 현저하게 증가되어 있었다. CK2 차단제나 CKα2 siRNA(short interfering RNA)를 사용하여 HDAC2 S394의 심근비후 유도 안산화와

결론
본 연구에서는 CK2가 HDAC2 S394의 인산화에 의한 HDAC2를 활성화하여 심근비후를 유발하는 새로운 기전을 밝혔다.
심근비후는 심근경색, 고혈압, 판막질환 등의 여러 가지 심장-혈관질환에 의해 유발되는 심장의 병리학적 자극에 대한 적응기전으로 알려져 있다. 심근비후를 유전자 발현 단계에서 조절할 수 있는 물질 중 class II HDACs(HDAC4, HDAC5, HDAC7, HDAC9)가 심근비후를 억제할 수 있는 것으로 알려져 있다. 그러나 최근 연구에 의하면 class I HDAC1, HDAC2도 심근비후와 연관되어 있는 것으로 알려지고 있으며, 이런 단계 신호전달 물질인 KLF4(Kruppel-like factor 4)가 항상 심근비후의 새로운 치료 목표로 관심을 모으고 있다.

Histone의 작용을 발현 후(posttranslational) 단계에서 조절하는 것이 그 외 유전자 유전자 전사(transcription)를 조절할 수 있다. 이러한 histone 조절 작용 중 chromatin의 acetylation 상태는 유전자 발현의 외생적(epigenic) 조절을 만드는다. 두 가지의 상태인 변호된 histone acetyltransferase와 HDAC가 histone의 acetylation을 조절한다. 최근 연구들에 의하면 HDAC는 부정연, 심부전, 관통막질환, 심근비후 등 여러 심장-혈관질환 발생에 연관되어 있다.

심장 혹은 심근비후는 처음에는 외부의 병적 자극에 대한 적응 반응으로 시작될 수 있으나, 지속적으로 계속되는 경우 심근병증이나 심부전으로 발전하게 된다. 이때 심근비후가 발생하는 것을 자단하거나 심부전으로 이행하는 것을 예방하는 확실한 치료법은 없다. 최근 HDAC에 의한 유전자 발현의 재설정이 심근비후 발생과 밀접한 관계를 갖고 있음을 밝히게 되었다. Class I HDAC의 억제가 심근 비후를 약화시키고, 이들의 활성화는 heat shock protein 70을 활성화하여 심근비후를 유발할 가능성이 있다는 데가 있다. 심근비후 억제 물질인 KLF4는 HDAC2와 관련된다.

본 연구에서는 CK2α1에 의한 HDAC2 S394의 인산화가 심근비후를 억제시키며, 심근비후 환자의 심장에서 HDAC2 S394의 인산화는 현저히 증가되어 있다. 심근비후 발생을 유도하는 자극은 CK2α1을 활성화하고, CK2α1의 과발현은 심근비후를 유발시키는 반면, CK2α1의 억제는 이러한 심근비후 반응을 억제시킨다. CK2나 HDAC의 작용을 조절하는 물질은 증양학 등의 심장 외적인 분야에서 임상적용을 위하여 적극적으로 연구 중이다. 본 연구로 CK2α1/HDAC2 신호전달계는 심근비후 및 심부전의 치료와 예방을 위한 치료 목표로서 제안될 수 있으며, 적극적인 실험과 연구에 힘써야 한다.

Epigenic regulation
DNA 유전자 염기 서열이 변하거나, 돌연변이 없이 유전자 발현이 변화하는 현상. 대표적인 예로는 1) DNA metylation과 2) histone acetylation을 들 수 있는데, 유전자 발현을 억제하는데 있어 모든 sliced gene의 유전자 서열(sequence) 변화를 동반하지 않는다.

Histone acetylation을 조절하는 효소는 Histone deacetylase(HAT)와 HDAC의 반반되는 작용의 두 가지 효소에 의해 조절되는데, HAT는 Acetyl-Coenzyme A로부터 유신전기를 갖는 acetyl기를 histone N-terminal에 붙여, 양성한 histone을 중화시키고, 이러한 전하 상태는 유신된 DNA에 협력해 붙도록 한다. 반대로 HDAC는 histone과 DNA가 강하게 붙게 하여 단백질 전사를 억제하며, 이는 심비거리 등을 억제할 수 있다 (heterochromatin).