Identification of p300-targeted Acetylated Residues in GATA4 during Hypertrophic Responses in Cardiac Myocytes**

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A zinc finger protein, GATA4, is one of the hypertrophy-responsive transcription factors and increases its DNA binding and transcriptional activities in response to hypertrophic stimuli in cardiac myocytes. Activation of GATA4 during this process is mediated, in part, through acetylation by intrinsic histone acetyltransferases such as a transcriptional coactivator p300. However, p300-targeted acetylated sites of GATA4 during myocardial cell hypertrophy have not been identified. By mutational analysis, we showed that 4 lysine residues located between amino acids 311 and 322 are required for synergistic activation of atrial natriuretic factor and endothelin-1 promoters by GATA4 and p300. A tetra-mutant GATA4, in which these 4 lysine residues were simultaneously mutated, retained the ability to localize in nuclei and to interact with cofactors including FOG-2, GATA6, and p300 but lacked p300-induced acetylation, DNA binding, and transcriptional activities. Furthermore, coexpression of the tetra-mutant GATA4 with wild-type GATA4 impaired the p300-induced acetylation, DNA binding, and transcriptional activities of the wild type. When we expressed the tetra-mutant GATA4 in neonatal rat cardiac myocytes using a lentivirus vector, this mutant suppressed phenylephrine-induced increases in cell size, protein synthesis, and expression of hypertrophy-responsive genes. However, its expression did not affect the basal state. Thus, we have identified the most critical lysine residues acting as p300-mediated acetylation targets in GATA4 during hypertrophic responses in cardiac myocytes. The results also demonstrate that GATA4 with simultaneous mutation of these sites specifically suppresses hypertrophic responses as a dominant-negative form, providing further evidence for the acetylation of GATA4 as one of critical nuclear events in myocardial cell hypertrophy.

In response to hormonal and mechanical stimuli, cardiac myocytes undergo hypertrophy, which is closely associated with systolic and diastolic dysfunction of the heart. Myocardial cell hypertrophy is characterized by an increase in cell size, accumulation of contractile proteins, and activation of hypertrophy-responsive transcription factors (1). A zinc finger protein, GATA4, is one such factor and induces expression of genes encoding atrial natriuretic factor (ANF),2 endothelin-1 (ET-1), and β-myosin heavy chain (β-MHC) (2–4). An adenovirus E1A-associated protein, p300, not only serves as a coactivator of GATA4 but also possesses an intrinsic histone acetyltransferase (HAT) activity (5). p300 acetylates histone by its HAT activity to promote an active chromatin configuration (6–8). Furthermore, p300 is able to acetylate GATA4 and to increase its DNA binding and transcriptional activities. A dominant-negative form of p300 inhibits agonist-induced hypertrophy as well as GATA4-dependent transcriptional activity in cardiac myocytes (9). Cardiac overexpression of intact p300 in transgenic mice induces acetylation of GATA4 and myocardial cell hypertrophy and promotes left ventricular remodeling after myocardial infarction in vivo. However, overexpression of mutant p300 lacking HAT activity is unable to achieve these effects (10). These findings demonstrate that the HAT activity of p300 is required for GATA4 acetylation and for myocardial cell hypertrophy. However, the p300-mediated acetylation targets of GATA4 during hypertrophic responses have yet to be identified.

Mice GATA4 has 11 lysine residues that are putative acetylation targets, 9 of which are concentrated at the C-terminal tails of the first and second zinc finger domains. The amino acid sequences of these two lysine-rich motifs and two zinc finger domains in GATA4 are 100% identical between mice and humans. This region includes cofactor/DNA-binding sites and a nuclear localization signal (11, 12). For example, GATA4 interacts with p300 at the second zinc finger (5), with FOG-2 at the first zinc finger (13, 14), and with GATA6 at the region including both zinc fingers and their C-terminal tails (15). The amino acid sequences in this functional region are also well conserved among different members of the GATA family. It has been reported that p300 acetylates these lysine-rich motifs of GATA1, GATA2, and GATA3 and modulates their DNA binding and transcriptional activities (16–18). GATA1 acetylation in these motifs is required for its chromatin occupancy, eryth-

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‡§ The abbreviations used are: ANF, atrial natriuretic factor; ET-1, endothelin-1; MHC, myosin heavy chain; HAT, histone acetyltransferase; FOG, friend of GATA; PE, phenylephrine; CBP, CREB-binding protein; CREB, cAMP-response element-binding protein; GFP, green fluorescent protein; EMSA, electrophoretic mobility shift assay; RT-PCR, reverse transcription-PCR; HA, hemagglutinin.
p300-induced GATA4 Acetylation in Cardiac Hypertrophy

EXPERIMENTAL PROCEDURES

Plasmid Constructs—pcDNAG4 (22, 23), pCMVp300wt (a gift from Dr. Richard Eckner, University of Medicine and Dentistry New Jersey, Newark, NJ) (24), pwtFOG-2 (a gift from Dr. Jeffrey M. Leiden, University of Chicago, IL) (13), phGATA6 (a gift from Dr. Kenneth Walsh, Boston University School of Medicine, Boston, MA) (25), and pCMVβ-gal (Santa Cruz Biotechnology) contain the cytomegalovirus promoter/enhancer fused to cDNA respectively encoding murine GATA4, a full-length human p300, murine FOG-2, human GATA6, and β-galactosidase. pcDNAG4M1 to M7 and M456 were subcloned using overlapping PCR with pcDNAG4 as a template for inserting mutations. pANF-luc and pET-luc consist of the firefly luciferase cDNA driven by a 131-bp rat ANF and a 204-bp rat ET-1 promoter sequence, respectively. pRL-SV40 (TOYO B-Net) contains a sea pansy luciferase cDNA driven by a simian virus 40 promoter. A lentivirus vector, pLenti6/V5-D-TOPO® (Invitrogen), was used as a null expression vector (pLentiNull) for lentiviral infection. pLentiGFP was constructed by inserting enhanced GFP cDNA into pLentiNull. pLentiGFP-G4 and pLentiGFP-G4M56, which express wild-type and M456-GATA4 fused with enhanced GFP, were constructed by inserting GATA4 cDNA from pcDNAG4 and pcDNAG4M56 into pLentiGFP, respectively. pLentiG4 and pLentiG4M56, which express wild-type and M456-GATA4 fused with HA probe, were constructed by inserting GATA4 cDNA from pcDNAG4 and pcDNAG4M56 into pLentiNull, respectively.

COS7 Cells and Transfection—COS7 cells, which lack all GATA factors, were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum. The cells were transfected with DNA in antibiotic-free medium using FuGENE® 6 reagent (Roche Diagnostics). The total DNA content was equalized with pCMVβ-gal in each sample. The cells were lysed and subjected to assays after 48 h of transfection.

Dual-luciferase Assays—Activities of firefly and sea pansy luciferase were measured in the same cell lysate using PicaGene® dual kit (TOYO B-Net). The relative promoter activities were calculated as the ratio of firefly luciferase to sea pansy luciferase.

Immunoprecipitation and Western Blotting—Western blotting were performed as described previously (9) using rabbit polyclonal anti-p300 (Santa Cruz Biotechnology), rabbit and goat polyclonal anti-GATA4 (Santa Cruz Biotechnology), goat polyclonal anti-FOG-2 (Santa Cruz Biotechnology), rabbit and goat polyclonal anti-GATA6 (Santa Cruz Biotechnology), mouse monoclonal anti-β-actin (Sigma), and mouse monoclonal anti-HA (Santa Cruz Biotechnology) antibodies. To detect the acetylated lysine residues in GATA4, nuclear extracts of the cells were immunoprecipitated with goat polyclonal anti-GATA4 antibody or normal goat IgG (Jackson ImmunoResearch). The precipitates were subjected to Western blotting using rabbit polyclonal anti-acetylated lysine antibody (Cell Signaling Technology), which was subsequently detected by rabbit IgG Trueblot™ (eBioscience). To normalize for protein loading, the blots were stripped and reprobed with rabbit polyclonal anti-GATA4 antibody. To detect the physical interaction of GATA4 with p300, FOG-2, or GATA6, nuclear extracts were respectively immunoprecipitated with rabbit polyclonal anti-p300, goat polyclonal anti-FOG-2, or rabbit polyclonal GATA6 antibody as described previously (26, 28). The precipitates were subjected to Western blotting appropriately using goat or rabbit polyclonal anti-GATA4 antibody.

Pulse-labeling Analysis—The detection of acetylated GATA4 was also performed by pulse labeling as described previously (9, 10). Briefly, COS7 cells were resuspended in the medium containing 200 μCi/ml [1-14C]acetic acid sodium salt (GE Healthcare) and incubated for 12 h. Nuclear extracts from these cells were precipitated with anti-GATA4 antibody as described above, resolved by SDS-PAGE, fixed, and autoradiographed using a bioimaging analyzer, BAS 3000 (FUJIX).

Electrophoretic Mobility Shift Assays (EMSAs)—EMSAs using the GATA site in the rat ET-1 promoter as a probe were carried out as described previously (9, 10). EMSAs using immunoprecipitates were performed as described previously (29).

Cardiac Myocytes and Lentiviral Infection—Primary neonatal rat cardiac myocytes were prepared as described previously (30). Recombinant lentiviral stocks were produced in 293FT packaging cells (Invitrogen). Virus-containing medium was collected after 48 h of transfection and filtered through a 0.45-μm filter. Myocytes were infected with lentivirus by replacing the medium with virus-containing medium (containing 8 μg/ml Polybrene®) for 24 h. Then myocytes were stimulated with saline or 30 μmol/liter phenylephrine (PE) in serum-free medium for 48 h.

Immunohistochemistry and Measurements of Cell Surface Area—Cardiac myocytes were fixed and stained for β-MHC as described previously (31). Surface areas of myocytes were semi-automatically measured using ImageJ software (National Institutes of Health).

Protein Synthesis Measurements—Cardiac myocytes cultured on 6-well plates were incubated with 2.5 μCi/ml 14C-leucine (GE Healthcare) for 48 h in the serum-free medium with saline or PE. The cells were precipitated by 10% trichloroacetic acid and resuspended in 0.15 N NaOH, as described previously (32, 33). Radioactivity was measured by a scintillation counter.

Real-time RT-PCR—Total RNAs from cardiac myocytes were isolated, reverse-transcribed, and amplified as described previously (34). Real-time quantitative PCRs were performed as described previously (35). Primer sequences from 5’ to 3’ of ANF are TTC CTC GTC TGT TTG GCC TTT TG (sense) and CCT CAT CTT CCA CCG GCA TCT TC (antisense). Primer sequences of β-MHC (36) and GAPDH (27) were described previously.

Statistical Analysis—Results are presented as means ± S.E. Statistical comparisons were performed using unpaired two-tailed Student’s t tests or analysis of variance with Scheffe’s test.
two motifs, we prepared seven GATA4 mutants in which lysines were substituted by alanines. Mutations M1 (K245A) and M2 (K255A) are located in the N-motif, and mutations M3 (K299A), M4 (K311A), M5 (K318A/K320A), M6 (K322A), and M7 (K326A/K328A) are located in the C-motif. We examined the p300-induced transcriptional activation of ANF and ET-1 promoters by these GATA4 mutants using the PicaGene dual-luciferase assay system produced by TOYO B-Net. Wild-type or mutant GATA4 was expressed with or without p300 in COS7 cells. As shown in Fig. 1, B and C, coexpression of wild-type GATA4 and p300 resulted in 6- and 22-fold activations of ANF and ET-1 promoter activities, respectively. However, the M4, M5, and M6 mutations within the C-motif of GATA4 markedly reduced the transcriptional activities and responsiveness to p300. The expression of M4-, M5-, or M6-GATA4 with p300 resulted in only slightly higher activation than the expression of p300 alone. In contrast, the M1 and M2 mutations within the N-motif of GATA4 did not affect the transcriptional activity or responsiveness to p300 of either the ANF or the ET-1 promoters. As shown in Fig. 1D, the expression levels of wild-type and mutant GATA4 were similar in the absence and presence of p300. These data demonstrate that the lysine residues within the C-motif of GATA4 are required for p300-induced transcriptional activation.

**Simultaneous Mutation of 4 Lysines in GATA4 Results in Complete Loss of Its Transcriptional Activation Synergism with p300**—Although the M4, M5, and M6 mutations markedly reduced the transcriptional activity of GATA4, these mutants still exhibited residual activities. To obtain a mutant that had completely lost its transcriptional activity, we prepared M456-GATA4, in which 4 lysines (Lys-311, Lys-318, Lys-320, and Lys-322) in the C-motif are simultaneously mutated. The effects of M456-GATA4 on the p300-dependent transcriptional activation of the ANF and ET-1 promoters were examined by luciferase assays. In the presence of wild-type GATA4, p300 activated the ANF (Fig. 2A) and ET-1 (Fig. 2B) promoters in a dose-dependent manner. However, p300 did not activate these promoters at all in the pres-
ence of M456-GATA4. As shown in Fig. 2C, wild-type and M456-GATA4 levels were similar in the presence of different doses of p300. Thus, M456-GATA4 exhibited a complete lack of transcriptional activation synergism with p300.

**M456-GATA4 Loses Its Acetylation and DNA Binding Activity**—To examine the levels of p300-induced acetylation of wild-type and M456-GATA4, wild-type or M456-GATA4 was expressed with or without p300 in COS7 cells (Fig. 3A). Nuclear extracts from these cells were subjected to immunoprecipitation with anti-GATA4 antibody followed by Western blotting using anti-acetylated lysine antibody. As shown in Fig. 3B, whereas p300 markedly induced acetylation of wild-type GATA4, the acetylation of M456-GATA4 was completely lacking even in the presence of p300. Acetylated state of wild-type and M456-GATA4 were also confirmed by pulse-labeling experiment (Fig. 3C). In the presence of p300, incorporation of sodium $[^{14}C]$acetate was clearly detected in wild-type GATA4 but not in M456-GATA4. We next examined whether M456-GATA4 will still be able to interact with p300. The extracts were immunoprecipitated with anti-p300 antibody and subjected to Western blotting for GATA4. As shown in Fig. 3D, M456-GATA4 as well as wild-type GATA4 formed complex with p300. These results demonstrate that M456-GATA4 retains the ability to interact with p300 but completely lacks p300-induced acetylation.

To examine the effect of M456 mutation on the p300-induced DNA binding activity of GATA4, EMSAs were performed. The same nuclear extracts used to detect the acetylation signals were probed with a radiolabeled double-strand oligonucleotide containing the ET-1 GATA site. As shown in Fig. 3E, a retarded band represented specific binding (lanes 2–4) and was immunoreactive for GATA4 (lanes 5 and 6). These findings demonstrate that this band contains a complex of GATA4 with ET-1 GATA oligonucleotide. As shown in Fig. 3F, p300 markedly increased the amount of the wild-type GATA4 complex (compare lanes 1 and 2). However, the amount of the M456-GATA4 complex was very low even in the presence of p300 (lane 3). These data demonstrate that mutation of the 4 lysine residues in the C-motif of GATA4 resulted in the loss of its p300-induced acetylation and its DNA binding activity.

**M456-GATA4 Impairs p300-induced Acetylation and DNA Binding of Wild-type GATA4**—M456-GATA4 almost completely lost its p300-induced acetylation but retained the ability to interact with p300. We examined whether M456-GATA4 binds to other GATA4-associating proteins such as FOG-2 and GATA6. Wild-type or M456-GATA4 was expressed with FOG-2 or GATA6 in COS7 cells. Nuclear extracts from these cells were subjected to immunoprecipitation with anti-FOG-2 or anti-GATA6 antibody. In each experiment, p300 was coexpressed to enhance the acetylation state of the wild-type but not that of the mutant. As shown in Fig. 4A, similar to wild-type GATA4, M456-GATA4 was able to interact with FOG-2. Concomitantly, M456-GATA4, to an extent similar with wild-type GATA4, interacted with GATA6 as well (Fig. 4B). These data indicate that the acetylation is not essential for GATA4 to bind to its associating proteins such as FOG-2 and GATA6.
We subsequently examined the effects of M456-GATA4 coexpression on the p300-induced acetylation and DNA binding of wild-type GATA4. Nuclear extracts from COS7 cells expressing wild-type GATA4 and p300 with or without M456-GATA4 were immunoprecipitated with anti-GATA4 antibody. As shown in Fig. 4C, coexpression of M456-GATA4 repressed the p300-induced acetylation of GATA4. Since M456-GATA4 is almost completely lacking p300-induced acetylation, the detected acetylation signal may be mainly composed of acetylated wild-type GATA4. In addition, coexpression of M456-GATA4 resulted in the reduction of GATA4/DNA binding (Fig. 4D). These results suggest that M456-GATA4 impairs the p300-induced acetylation and DNA binding of wild-type GATA4.

**M456-GATA4 Acts as a Dominant-negative Form**—To clarify whether M456-GATA4 acts as a dominant-negative form, we examined the effects of this mutant on the transcriptional activities induced by wild-type GATA4 and p300. M456-GATA4 was coexpressed with wild-type GATA4 and p300 in COS7 cells, and the promoter activities of ANF and ET-1 were measured by luciferase assays. As shown in Fig. 5, the expressions of wild-type GATA4 with p300 resulted in 12- and 16-fold activations of ANF and ET-1 promoter activities, respectively. These activations were dose-dependently attenuated by the coexpression of M456-GATA4. Coexpression of M456-GATA4 at the maximal dose resulted in almost no activation. Thus, M456-GATA4 acted as a dominant-negative form and repressed the transcriptional activation of the ANF and ET-1 promoters mediated by wild-type GATA4 and p300.

**M456-GATA4 Represses Hypertrophic Responses in Cardiac Myocytes**—Since the M456 mutation is located at the nuclear localization signal, we examined the subcellular localization of M456-GATA4 in cultured neonatal rat cardiac myocytes. GFP-fused wild-type or M456-GATA4 was introduced into myocytes by lentiviral infection. As shown in Fig. 6, over 90% of infected myocytes expressed lentivirus-derived GFP proteins. GFP-M456-GATA4 was localized at the nuclei,
as was the wild-type GATA4 in cardiac myocytes. The nuclear localization of GFP-M456-GATA4 was confirmed in COS7 cells as well (data not shown). The M456 mutation did not affect the nuclear localization of GATA4.

Next, we evaluated the effect of M456-GATA4 on hypertrophic responses induced by an α1-adrenergic agonist, PE, in cardiac myocytes. M456-GATA4 expression plasmid was introduced into cultured neonatal rat cardiac myocytes by lentiviral infection. As a control, a null or wild-type GATA4 plasmid was introduced into cultured neonatal rat cardiac myocytes. M456-GATA4 expression plasmid was stably expressed in both saline-stimulated and PE-stimulated cardiac myocytes. M456-GATA4 was expressed in both saline-stimulated and PE-stimulated cardiac myocytes. M456-GATA4 expression plasmid was stably expressed in both saline-stimulated and PE-stimulated cardiac myocytes.

FIGURE 4. M456-GATA4 competes with wild-type (wt) GATA4. A, COS7 cells were transfected with 1 μg of pCDNA4 or pCDNA4/M456, 2 μg of pwtFOG-2, and 4 μg of pCMVp300. Nuclear extracts from these cells were immunoprecipitated (IP) with anti-FOG-2 antibody followed by sequential Western blotting with anti-FOG-2 and anti-GATA4 antibodies. We performed three independent experiments and obtained similar results. B, COS7 cells were transfected as in panel A; 1 μg of phGATA6 was transfected instead of pwtFOG-2. Nuclear extracts from these cells were immunoprecipitated with anti-GATA6 antibody followed by sequential Western blotting with anti-GATA6 and anti-GATA4 antibodies. We performed three independent experiments and obtained similar results. C, COS7 cells were transfected with 1 μg of pCDNA4, 1 μg of pCMVβ-gal or pLentiG4M456, and 4 μg of pCMVp300. Nuclear extracts from these cells were immunoprecipitated with anti-GATA4 antibody followed by sequential Western blotting with anti-GATA4, (Ac-Lys), anti-GATA4, and anti-HA antibodies. A photograph shows a representative result of three independent experiments. Arrows, wild-type GATA4; arrowheads, M456-GATA4 fused with HA-probe. D, immunoprecipitants used for panel C were subjected to EMSAs as performed in Fig. 3. A photograph shows a representative result of four independent experiments. Arrow, GATA4/oligonucleotide complex.

surface areas (Fig. 7C). On the other hand, such PE-induced changes were minimal in myocytes expressing M456-GATA4. In the PE-stimulated condition, the surface area of myocytes expressing M456-GATA4 was significantly smaller than that of myocytes with a null or wild-type GATA4 infection. However, in the saline-treated condition, the cell surface areas were similar among any myocytes. As shown in Fig. 7D, protein synthesis rates were increased by PE in myocytes with a null or wild-type GATA4 infection. However, such a PE-induced increase was suppressed in myocytes expressing M456-GATA4. These data demonstrate that exogenous M456-GATA4 specifically represses PE-induced myocardial cell growth.

To further examine the effect of M456-GATA4 on myocardial cell hypertrophy, we quantified the mRNA levels of ANF and β-MHC genes by real-time RT-PCR (Fig. 7, E and F). PE stimulation increased the ANF and β-MHC mRNA levels in myocytes with a null or wild-type GATA4 infection. However,
such PE-induced increases were suppressed by expression of M456-GATA4. These results indicate that exogenous M456-GATA4 inhibits PE-induced expression of hypertrophy-responsive genes in cardiac myocytes.

**DISCUSSION**

The present study demonstrated that mutations of lysine residues in the C-motif (M4, M5, and M6) of GATA4 reduced p300-induced transcriptional activities, whereas mutations in the N-motif (M1 and M2) did not. M456-GATA4, a mutant in which 4 lysines (Lys-311, Lys-318, Lys-320, and Lys-322) in the C-motif are simultaneously mutated, completely lost p300-induced acetylation, DNA binding, and transcriptional activities. These findings suggest that these 4 lysines are the main acetylation targets in GATA4. Although the simultaneous substitution of 4 lysines in GATA4 was sufficient to ablate the p300-induced acetylation, the exact number and positions of acetylated lysines remain to be determined. To clarify the acetylation status of each lysine residue in GATA4, analysis by mass spectrometry will be needed. Interestingly, Lys-318 and Lys-322 within acetylation targets of GATA4 do not exist in GATA1, GATA2, and GATA3. Conversely, a major acetylation target of GATA3, Lys-305, in the N-motif does not exist in GATA4 (18). Although most lysine residues are conserved among different members of the GATA family, acetylation targets may be distinct among different members of this family.

The present study identified p300-acetylated lysine residues of GATA4. In addition to p300, CREB-binding protein (CBP) possesses HAT activity and is able to acetylate GATA factors as well as to function as a coactivator of these factors (6, 7, 19, 20). Although p300 acetylates predominantly the C-motif of GATA1 and increases its DNA binding activity (16), CBP acetylates both the N-motifs and the C-motifs without affecting its DNA binding (20). These facts suggest that p300 and CBP have non-redundant roles for modulating the function of GATA factors. Cofactor-specific acetylation sites remain to be identified. Mutant mouse embryos lacking p300 exhibit a reduction in the thickness of the myocardium and suffer embryonic death due to heart failure (38). In contrast, knock-out mice of CBP do not exhibit apparent heart defects and die of brain hemorrhage during the embryonic stage (39). These findings suggest that p300 plays a more important role than CBP in heart development. However, the precise roles of p300 and CBP in myocardial cell hypertrophy should be clarified by further studies.

The GATA4 mutant, M456-GATA4, not only lost p300-induced activities by itself but also inhibited transcriptional activities mediated by wild-type GATA4 and p300. M456-GATA4 retained the ability to interact with its cofactors such as FOG-2,
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GATA6, and p300 but lost p300-induced acetylation and DNA binding. These findings suggest that M456-GATA4 competes with wild-type GATA4 for binding to p300 and acts as a dominant-negative form. Exogenous M456-GATA4 impaired p300-induced acetylation and DNA binding of wild-type GATA4 and inhibited hypertrophic responses in cardiac myocytes. Not only GATA4 but also multiple DNA-binding transcription factors are involved in myocardial hypertrophic responses. GATA6, another member of the GATA family, is expressed in cardiac tissue during development (40, 41), physically interacts with p300 (42), and activates various GATA-dependent cardiac genes with potency similar to GATA4 (43). GATA4 and GATA6 functionally cooperate with each other to synergistically activate transcription at a single GATA element of the ANF promoter in cardiac myocytes (15, 43). Forced expression of GATA6 in cardiac myocytes induces a striking hypertrophic response (45). Therefore, it is possible that exogenous M456-GATA4 competes with endogenous GATA6 as well as GATA4 and inhibits p300-induced GATA6 acetylation during myocardial cell hypertrophy. Other hypertrophy-responsive transcription factors, such as serum-response factor and myocyte enhancer factor-2, also interact with p300 (37, 44). Since the amount of p300 in cardiac myocytes is limited, competition with these factors for p300 might also be a mechanism of M456-GATA4-mediated inhibition of hypertrophy. In any event, the data obtained in this study provide further evidence for the role of p300-induced GATA4 acetylation in myocardial cell hypertrophy. These findings may be helpful for establishing novel heart failure therapy that targets nuclear events in cardiac myocytes.

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