Role of histone deacetylase 2 and its posttranslational modifications in cardiac hypertrophy

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Cardiac hypertrophy is a form of global remodeling, although the initial step seems to be an adaptation to increased hemodynamic demands. The characteristics of cardiac hypertrophy include the functional reactivation of the arrested fetal gene program, where histone deacetylases (HDACs) are closely linked in the development of the process. To date, mammalian HDACs are divided into four classes: I, II, III, and IV. By structural similarities, class II HDACs are then subdivided into IIa and IIb. Among class I and II HDACs, HDAC2, 4, 5, and 9 have been reported to be involved in hypertrophic responses; HDAC4, 5, and 9 are negative regulators, whereas HDAC2 is a pro-hypertrophic mediator. The molecular function and regulation of class IIa HDACs depend largely on the phosphorylation-mediated cytosolic redistribution, whereas those of HDAC2 take place primarily in the nucleus. In response to stresses, posttranslational modification (PTM) processes, dynamic modifications after the translation of proteins, are involved in the regulation of the activities of those hypertrophy-related HDACs. In this article, we briefly review 1) the activation of HDAC2 in the development of cardiac hypertrophy and 2) the PTM of HDAC2 and its implications in the regulation of HDAC2 activity. [BMB Reports 2015; 48(3): 131-138]

INTRODUCTION

Cardiac hypertrophy is an adaptation process to meet the increased hemodynamic demands in peripheral tissues due to underlying diseases, such as hypertension, valve stenosis or regurgitation, or even myocardial infarction (1-3). The characteristics are an increase in cellular burden of individual cardiomyocytes and rebirth of the fetal gene program without an apparent expansion of cardiomyocyte populations. Although the initial hypertrophy is physiological, sustained stimuli without alleviation of the underlying causal disease leads the heart to a pathological state (2). If the underlying disease continues without correction, the function of the hypertrophied heart declines, leading to heart failure. Despite intensive management, heart failure is still one of the most common causes of death (4).

To acquire proper functions, many proteins undergo modification after translation by enzymes, a process referred to as posttranslational modification (PTM). Indeed, diverse PTMs are involved in the regulation of the amount, function, and localization of many proteins. For example, phosphorylation is a critical modification for enzymatic activation (5), binding to DNA (6), forming complexes (7), and subcellular localization (8). Polyubiquitination, a continued link of ubiquitin to the lysine residue of a target protein or ubiquitin itself, affects protein stability, whereas monoubiquitination regulates other functions (9). These kinds of modifications are finely regulated by paired enzymes with opposite functions, such as kinases and phosphatases, methyltransferases and demethylases, acetyltransferases and deacetylases, and ubiquitinylases and deubiquitinylases.

Protein acetylation is also closely related to enzyme activity (10), DNA recruitment (11), and transcription (12). It typically occurs on lysine residues; however, N-terminal acetylation has also been reported (13). This process replaces a hydrogen atom with acetyl moiety. Among acetylation-associated regulation, the biological implications of acetylation of histones H3 and H4 have been established; histone tail acetylation induces loosening of DNA from histones, leading to increased accessibility for the transcriptional machinery. Histone deacetylation, on the other hand, results in transcription silencing (14, 15). Histone acetylation is finely regulated by the balance between histone acetyl transferase and histone deacetylase (HDAC) activities.

HDAC AND DIVERSE DISEASES

Eighteen HDACs have been discovered in mammals and divided into four classes. HDAC1/2/3/8 are class I HDACs. HDAC4/5/6/7/9/10 are members of the class II HDACs. Class
III HDACs (HDAC12-18) are classified as a sirtuin group and they require NAD⁺ for intrinsic activity. HDAC11 is the only class IV HDAC. Class II HDACs are further subcategorized into two subclasses: HDAC4/5/7/9 for class Ila HDACs and HDAC6/10 for class IIb. Molecular structures, tissue distributions, and subcellular localizations are summarized in Fig. 1.

Although class II HDACs have a conserved HDAC domain, it is somewhat controversy whether they actually have deacetylase activity in vivo (16, 17). Indeed, the role of class Ila HDACs is regarded as serving as a scaffold to form a larger complex for transcription repression. Additionally, class II HDACs interfere with binding of transcription activators, such as MEF2 (8, 18). Some reports, including ours, however, showed that the class Ila HDACs possess minute but significant intrinsic deacetylase activity in vivo (19, 20).

The subcellular localization of class Ila HDACs is controlled by exogenous signals (8, 21, 22): activated PKC/PKD or CaMKII phosphorylates serine residues that flank the nuclear localization signal domain. Shuttling molecules, such as 14-3-3 or CRM1, bind specifically to phosphorylated class Ila HDACs, which results in the masking of the nuclear localization signal. This enables class Ila HDACs to exit to the cytoplasm. In contrast, information on the regulation of class IIb HDACs is limited; both HDAC6 and HDAC10 are primarily localized in the cytoplasm. HDAC6 specifically regulates acetylation of α-tubulin and thereby microtubule assembly (23); however, the functional implications of HDAC6 remain to be clarified.

Although the molecular sizes of class I HDACs are relatively small, their enzyme activities are greater than those of class Ila HDACs. Class I HDACs are exclusively involved in protein deacetylation. Indeed, class I HDACs contribute most of total HDAC activity measured in vitro in whole cell lysates. HDAC1 and HDAC2 are major deacetylase enzymes of histones for transcriptional repression. Interestingly, HDAC1 redundantly regulates histone acetylation together with HDAC2 (14, 24).

The clinical implications of the roles of HDACs have been suggested based on the effects of HDAC inhibitors (HDACi) in various diseases. For example, vascular calcification (25) may be aggravated by HDAC inhibition. This suggests that loss of HDAC activity may play a key role in the development of the disease. On the other hand, a non-selective HDACi effectively arrests the cell cycle or induces apoptosis (26). For example, trichostatin A (TSA), a pan-HDACi, blocks smooth muscle cell proliferation, suggests that HDACi may have therapeutic potential for atherosclerosis and restenosis after stent insertion in coronary arteries (27). Numerous research groups have tested

| Class | Structure | Subcellular localization | Tissue distribution |
|-------|-----------|--------------------------|--------------------|
| I     | HDAC1     | Nucleus                  | Ubiquitous         |
|       | HDAC2     | Nucleus                  | Ubiquitous         |
|       | HDAC3     | Nucleus/cytoplasm        | Ubiquitous         |
|       | HDAC8     | Nucleus/cytoplasm        | Ubiquitous         |
| IIA    | HDAC4     | Nucleus/cytoplasm        | Skeletal muscle, Heart, Bone |
|        | HDAC5     | Nucleus/cytoplasm        | Skeletal muscle, Heart, Brain |
|        | HDAC7     | Nucleus/cytoplasm        | Smooth muscle, Placenta, Heart |
|        | HDAC9     | Nucleus/cytoplasm        | Skeletal muscle, Heart, Brain |
| IIb    | HDAC6     | Mainly cytoplasm         | Heart, Kidney, Placenta, Liver |
|        | HDAC10    | Mainly cytoplasm         | Liver, Kidney, Spleen |
| IV     | HDAC11    | Nucleus/cytoplasm        | Skeletal muscle. Barin, Kidney |

Fig. 1. Molecular structure, tissue distribution and subcellular localization of histone deacetylase. Color boxes. Red: MEF2C binding domain, Scarlet: PKD phosphorylation sites, Yellow: nuclear localization signal, Blue: nuclear export signal, Black: histone deacetylase domain.
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the anti-neoplastic properties of HDACi and the U.S. Food and Drug Administration has approved suberoylanilide hydroxamic acid and romidepsin for novel regimens against cutaneous T cell lymphoma. Sodium valproate, which has long been used as an anti-epileptic therapeutic, has undergone a phase II clinical trial for treating solid tumors (28).

In addition to anti-neoplastic activity, recent pioneering work has revealed that HDACi are also beneficial for diverse cardiac diseases: arrhythmia (29), myocardial infarction (30-32), hypertension (33), and cardiac hypertrophy (34-38). In the following section, we discuss 1) the role of HDACs and 2) the regulation mechanisms of HDACs in cardiac hypertrophy.

HDACs AND CARDIAC HYPERTROPHY

Class I HDACs are expressed ubiquitously, whereas class II HDACs are not. Among the class II HDACs, HDAC4/5/6/7/9 are expressed in the heart; however, the specific role of each subtype is not yet known. The first understanding of class II HDACs’ roles in the cardiac fields was in relation to HDAC9 (8). Based on the phenotype evaluation of whole-body deletion of HDAC9, it was suggested that HDAC9 may function as a negative regulator of cardiac hypertrophy; cardiac hypertrophy is dramatically exaggerated in HDAC9 knockout (KO) mice.

MTR, an endogenous alternatively spliced form of HDAC9 that lacks the HDAC domain, successfully rescued the absence of HDAC9 in vivo. These observations suggest that the function of HDAC9 in vivo may be as a ‘simple’ transcriptional repressor in a deacetylase enzyme-independent manner. Although the HDAC5 KO phenotype is similar to that of HDAC9 (21, 22), in contrast to HDAC9, interestingly, HDAC5 has the function of an “enzyme” during the development of cardiac hypertrophy. Recently, we reported that HDAC5 deacetylates HDAC2, a pro-hypertrophic mediator, and thereby suppresses its activity (19).

Among the class I HDACs, both HDAC1 and HDAC2 redundantly regulate cardiac development in the embryo (24). Cardiac-specific KO of HDAC1 or HDAC2 did not affect cardiac morphogenesis, whereas double deletion of both HDAC1 and HDAC2 was lethal at postnatal 14 day due to severe dilated cardiomyopathy. HDAC2, however, is the major class I HDAC in the adult heart. At least in the development of cardiac hypertrophy, the enzyme activity of HDAC2 is solely increased in response to hypertrophic stimuli without alteration of its expression level (39). The subcellular localization of HDAC2 was not changed in the development of cardiac hypertrophy. Rather, PTM-dependent activation is a key regulatory mechanism (19, 33, 39).

THE ROLE OF HISTONE DEACETYLASE 2 IN CARDIAC DISEASE

HDAC2’s role in cardiac disease first emerged as a responsible factor for “homeodomain-only protein” (HOPX)-derived hypertrophy (37). Kook et al. reported that cardiac-specific overexpression of atypical HOPX that lacked a DNA binding motif could induce cardiac hypertrophy. Cardiac hypertrophy induced by transgenic overexpression of HOPX was blocked completely by HDACi. Interestingly, HOPX requires HDAC2 to induce cardiac hypertrophy; transgenic mice that overexpress HOPX that do not interact with HDAC2 fail to induce cardiac hypertrophy. This study first reported that class I HDAC might also be involved in cardiac hypertrophy and that it counteracted the function of class IIa HDACs. To delineate the net effect of the simultaneous inhibition of both class I and class II HDACs, non-selective HDACi were used. We (36) and others (38, 40) observed that cardiac hypertrophy provoked by pressure overload or by hypertrophic agonists was inhibited successfully by pan-HDAC. In subsequent studies, our group further showed that class I-selective HDACi also successfully inhibited cardiac hypertrophy, as pan-HDACi did (36, 40), suggesting that class I HDACs are responsible for the anti-hypertrophic action of pan-HDACi. Next, we tried to find which subtype of class I HDAC was responsible for the development of cardiac hypertrophy and found that diverse hypertrophic stresses specifically activated HDAC2. Thus, we concluded that HDAC2 played a central role in cardiac hypertrophy, HDAC2 activation contributes to a common pathway that various hypertrophic stresses merge into and inhibition of HDAC2 may be a therapeutic target to reverse cardiac hypertrophy and concomitant heart failure.

A HDAC2 KO study reinforced the conclusion above. Trivedi et al. (41) reported that whole-body KO of HDAC2 resulted in partial lethality due to failure of myocardial matura- tion and a trabeculation defect in the perinatal period. Surviving HDAC2 KO mice recover growth and cardiac function at 2 months of age. In the adult, genetic disruption of HDAC2 resulted in resistance against hypertrophic stimuli induced by pressure overload, β-adrenergic agonist, or crossing with HOPX transgenic mice. Gain-of-function of HDAC2, however, induced cardiac hypertrophy significantly, whereas transgenic overexpression of HDAC1 or HDAC3 failed to do so.

The heart provides oxygen and nutrients to the periphery by its rhythmic contraction, which is strictly controlled by an electrical conducting system. Some evidence has been reported that HDAC2 participates in the electrical physiology of the heart. Montgomery et al. performed gene ontology analyses from cardiac-specific disruption of both HDAC1 and HDAC2 and found dramatic upregulation of calcium channels: Cacna1h and Cacna2d2 (24). We also performed cDNA microarray studies to check the mRNAs altered by transgenic overexpression of HDAC2 in the heart and found that several channel subtypes for ion handling were dysregulated: Scn3b, KCne1, Cacnb2, and Scn2a1 (unpublished data). Considering that dysfunction of KCne1 is involved in hereditary familial arrhythmia, by causing long QT syndrome, and that loss of function of Scn3b leads to Burgada syndrome (42), alteration of HDAC2 activity may indeed participate in cardiac arrhythmia.
HDAC2 effectively controls atrial arrhythmia. Ismat et al. (43) performed surface ECG in HOPX-null mice and observed P-wave widening, QRS-complex widening, and prolongation of the QT interval. Connexin40 was also decreased aberrantly in those mice. On the other hand, P-wave duration was prolonged and atrial refractory periods were shortened in HOPX-transgenic mice (29). TSA successfully reversed the conduction defect by normalization of connexin40. On the basis of a physical interaction between HOPX and HDAC2 in vivo, we assume that the anti-arrhythmogenic properties of TSA may be associated with inhibition of HDAC2 recruited by HOPX (37, 44).

**POSTTRANSLATIONAL MODIFICATION OF HISTONE DEACETYLASE 2 IN CARDIAC HYPERTROPHY**

Next, we sought to understand the regulatory mechanism of HDAC2 in cardiac hypertrophy. First, we found that diverse hypertrophic stresses specifically activated HDAC2 (39). By hypertrophic stimuli, heat shock protein (HSP) 70 was induced markedly to maintain HDAC2 activity. The requirement for HDAC2 activation in cardiac hypertrophy was further confirmed by use of an enzymatically inert mutant of HDAC2: HDAC2 H141A that loses HDAC activity due to destruction of the active pocket without altering interactions with other proteins. When HDAC2 H141A was overexpressed, no apparent cardiomyocyte hypertrophy was observed. Based on these observations, we concluded that increased HDAC2 activity was critical for hypertrophy.

Regarding the HDAC2 regulatory mechanism, however, no change in protein amount or mRNA expression was observed, raising the possibility of the involvement of PTM. Indeed, HDAC2 phosphorylation was increased significantly by hypertrophic stimuli. Using bioinformatics and by scrutinizing previous reports, four serine residues were picked as phosphorylation candidates: S394, S411, S424, and S424. By an in vitro kinase assay using synthetic peptides, we found that S394 and S411 phosphorylation were increased significantly in tissue lysates obtained from hypertrophied mouse heart. Interestingly, S422 and S424 were also basally phosphorylated in heart lysate from normal mice; however, we did not observe any further increase in phosphorylation of those residues with hypertrophy. Next, we observed that the intrinsic activity of HDAC2 was decreased significantly when S394, S422, or S424 was mutated, whereas the enzyme activity of HDAC2 was unchanged in the S411A mutant. According to these results, we concluded that basal activity of HDAC2 is maintained by S422 and S424 phosphorylation, whereas hypertrophy-associated phosphorylation and subsequent enzyme activation of HDAC2 were mediated by S394 phosphorylation. Indeed, in primary culture cardiomyocytes and in transgenic mice, overexpression of HDAC2 wild-type successfully induced hypertrophy, whereas HDAC2 S394A failed to do so. HDAC2 S394 phosphorylation and hypertrophic phenotypes were blocked by various casein kinase (CK) 2 inhibitors and by CK2α1 siRNA. Transgenic over-expression of CK2α1 in the heart successfully induced hypertrophy, which was further exacerbated by crossing TgCK2α1 with TgHDAC2 wild-type, but blunted by mating with TgHDAC2 S394A in vivo. Thus, CK2α1-dependent phosphorylation of HDAC2 S394 is the key modification for both HDAC2 activation and cardiac hypertrophy (35).

We also found that HDAC2 itself could be regulated by acetylation dynamics. Various hypertrophic stresses acetylate HDAC2 at lysine 75 and acetylation activates HDAC2. HDAC2 K75R, an acetylation-resistant mutant that loses intrinsic activity, failed to induce hypertrophy. In contrast, the acetylation-mimicking mutant of HDAC2, HDAC2 K75Q, showed an increase in enzyme activity and also possessed pro-hypertrophic properties.

We further examined whether an interplay between acetylation and phosphorylation existed in the regulation of HDAC2 activity; HDAC2 S394 phosphorylation was decreased significantly in HDAC2 K75R. On the other hand, HDAC2 K75 acetylation was unchanged in HDAC2 S394A. The intrinsic activity of HDAC2 K75R was not further downregulated by S394A. These results suggested that 1) HDAC2 K75 acetylation precedes and is mandatory for HDAC2 S394 phosphorylation and 2) the acetylation did not directly activate the intrinsic activity of HDAC2, but indirectly induced activation of HDAC2, by S394 phosphorylation.

We further tried to find the enzymes involved in HDAC2 acetylation. We observed that HDAC2 acetylation was finely balanced by pCAF and HDAC5, a class IIa HDAC. Both HDAC2 acetylation and phosphorylation were upregulated significantly in HDAC5 null mice. Phosphorylated HDAC5 underwent cytoplasmic redistribution after recognition by CRM1. Leptomycin B, a CRM1 inhibitor, interfered with the shuttling of HDAC5 to the cytoplasm and thereby showed anti-hypertrophic features by tethering HDAC5 in the nucleus. Adenoviral expression of HDAC2 K75Q, a constitutively acetylated form of HDAC2, overcame the anti-hypertrophic activity of leptomycin B. Agonist-induced cardiac hypertrophy in HDAC5 KO mice was alleviated by HDAC2 K75R delivery to the heart, delivered via the tail vein (19). To summarize, hypertrophic stresses activate both CK2α1 and PKD. PKD phosphorylates class IIa HDACs and phospho-class IIa HDACs shuttle out to the cytoplasm. HDAC2 undergoes acetylation by pCAF and then phosphorylation by activated-CK2α1. Alternatively, hypertrophic stresses also induce the expression of HSP70. HSP70 recognizes and binds to phospho-HDAC2, which results in protection of HDAC2 phosphorylation against phosphatases (Fig. 2).

**DEACETYLATION TARGET OF ACTIVATED HDAC2 IN THE DEVELOPMENT OF CARDIAC HYPERTROPHY**

So, which signal cascade relays activation of HDAC2 to hypertrophic phenotypes in the cardiomyocytes? Trivedi et al. (41)
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Fig. 2. Working hypothesis. In the basal condition, HDAC2 interacts with HDAC5 and protein phosphatase. Both HDAC5 and protein phosphatase keep HDAC2 inactivated. Loss of intrinsic activity of HDAC2 leads to de-repression of anti-hypertrophic gene activation, such as INPP5F or KLF4. The cardiac fetal gene program remains arrested by the inhibition of INPP5F or KLF4 (left). When the myocardium is stimulated by hypertrophic stresses, numerous signaling cascades are activated. For example, PKC/PKD is activated and phosphorylates class IIa HDACs. HSP70 is induced dramatically by hypertrophic stress. HDAC5 and p300/CBP-associated factor may bind to and deacetylate HDAC2, allowing CK2α1 to phosphorylate HDAC2 at serine 394. HSP70 specifically binds to phosphorylated HDAC2. Functionally activated HDAC2 suppresses the expression of negative regulators of hypertrophy, which induces reactivation of the fetal gene program. Red stars indicate phosphorylation. Abbreviations. Ac: acetylation, CK2α1: casein kinase 2α, HDAC: histone deacetylase, HSP70: heat shock protein 70, INPP5F: inositol polyphosphate-5-phosphatase F, KLF4: Krüppel-like factor 4, pCAF: p300/CBP-associated factor, PP: protein phosphatase.

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DISCUSSION

We have clarified the role of HDAC2 and its regulatory mechanism in cardiac hypertrophy (Fig. 2). When the myocardium is stimulated by various exogenous stresses, numerous signaling cascades are activated. For example, PKC/PKD is activated and phosphorylates class IIa HDACs. HSP70 is induced dramatically by hypertrophic stress. HDAC5 and p300/CBP-associated factor may bind to and deacetylate HDAC2, allowing CK2α1 to phosphorylate HDAC2 at serine 394. HSP70 specifically binds to phosphorylated HDAC2. Functionally activated HDAC2 suppresses the expression of negative regulators of hypertrophy, which induces reactivation of the fetal gene program. Red stars indicate phosphorylation. Abbreviations. Ac: acetylation, CK2α1: casein kinase 2α, HDAC: histone deacetylase, HSP70: heat shock protein 70, INPP5F: inositol polyphosphate-5-phosphatase F, KLF4: Krüppel-like factor 4, pCAF: p300/CBP-associated factor, PP: protein phosphatase.
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Cytosolically in the cytoplasm and pCAF is also activated in a PTM-dependent manner in the nucleus. Phosphorylated class IIa HDACs are captured by shuttling molecules and redistributed into the cytoplasm, whereas HSP70 shuttles into the nucleus. pCAF acetylates HDAC2 K75, which then allows CK2α1 to phosphorylate HDAC2 S394. Activated HDAC2 is then recruited on promoters that drive the expression of anti-hypertrophic mediators, such as INPP5F or KLF4, which, in turn, downregulates the expression of those mediators by deacetylation of histones associated with their promoters. Downregulation of anti-hypertrophic regulators results in reactivation of an arrested fetal gene program, thereby resulting in cardiac hypertrophy.

Cardiac hypertrophy is a double-edged sword. Hypertrophy, a form of cardiac remodeling, however, is also an essential adaptation for increased hemodynamic demands, at least at the beginning of a cardiac compensation period. The transition mechanism from physiological to pathological hypertrophy remains unclear. We observed the enzyme activity of HDAC2 peaked prior to the apparent hypertrophy and returned to basal levels in the heart failure phase (39). Considering that increased HDAC2 activity is a key step in cardiac hypertrophy, the abrupt loss of HDAC2 activity might be a cue signal to transit to heart failure. A study using HDAC2 activators would be necessary to assess this.

We found a novel crosstalk between class I and class II HDACs in vivo. Fischle et al. (16) also reported the formation of a functional complex including class I and class II HDACs. The class II HDAC recruits and uses the class I HDAC to deacetylate promoters. In that model, however, the class II HDAC does not serve as an enzyme but as a scaffold. Moreover, it is also believed that diverse signaling cascades converge into the two classes HDACs and both classes of HDACs regulate cardiac hypertrophy independently. We observed, however, that HDAC5 does have intrinsic activity and the enzyme activity of HDAC5 is indispensable for the deacetylation of HDAC2 and that the HDACs-mediated signal cascade, considered as two independent axes, can merge into a single stream.

FUTURE ASPECTS

Other types of PTM of HDAC2 beyond phosphorylation and acetylation have also been reported in various disease models (54-57). Colussi et al. (55) reported that HDAC2 was significantly upregulated in Duchenne muscular dystrophy and blockade by HDAC1 or cysteine-S-nitrosylation was beneficial to improve dystrophy symptoms. Nott et al. (54) observed in neuronal development that S-nitrosylation of HDAC2 induced its dissociation from DNA, which then resulted in histone acetylation and then transcription activation. Thus, it is plausible that the S-nitrosylation modification of HDAC2 is a potent modification to suppress its activity. Recently, HDAC2 sumoylation has also been reported (57), which causes enzymatic activation.

We assume that dephosphorylation may participate in the inactivation of HDAC2 (19, 35, 39). Though a hypertrophy-associated phosphatase of HDAC2 has not been identified, in other disease conditions, some phosphatases have been suggested as HDAC2 phosphatases (58-60). The total phosphorylation level of HDAC2 is increased significantly by simultaneous treatment with okadaic acid, a protein phosphatase 2A (PP2A) inhibitor. In our models, we also observed that the catalytic subunit of PP2A interacted physically with HDAC2. PP2A reduced both phosphorylation and the intrinsic activity of HDAC2 significantly. Overexpression of PP2A blocked cardiomyocyte hypertrophy induced by HDAC2 (Min et al., unpublished observations).

We (19, 27, 34-37, 39, 46) and others (38, 40, 41, 45) have demonstrated the functional relevance of HDAC2 and cardiac remodeling and HDAC2 may be a promising target for cardiac remodeling. One may argue, however, that unwanted effect would be expected given the ubiquitous distribution of HDAC2, which may restrict its therapeutic use. However, as shown in the previous report (41), HDAC2 is exclusively expressed in the developing heart. Moreover, considering that HDACs work in a combinatorial fashion with diverse transcription factors that determine the downstream target and that these combinations would vary greatly depending on cellular context and/or disease processes, tissue specificity would be achieved by simultaneous modulation of these factors as well as their cellular contexts. Thus, understanding these complex pathways and the functions of the associated transcription factors would be of great importance for the specificity of HDAC modulation. In addition, it is important to understand the PTM of HDAC and their regulators. On the basis of knowledge that intrinsic of HDACs can be regulated by dynamics of diverse PTMs, modifiers of HDACs could also provide notable therapeutic targets by minimizing the adverse effects of HDAC. Indeed, we have already shown that anti-hypertrophic properties of CK2 inhibitors, which interfered with HDAC2 phosphorylation, was as effective as HDACi in vivo (35). Like CK2 inhibitors, certain chemicals targeted downstream of HDAC2 could be important for the treatment of cardiac remodeling.

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