A class of their own: exploring the nondeacetylase roles of class IIa HDACs in cardiovascular disease

Lillianne H. Wright1 and Donald R. Menick1,2
1Department of Medicine, Division of Cardiology, Medical University of South Carolina; and 2Ralph Johnson Veteran’s Hospital, Charleston, South Carolina
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Wright LH, Menick DR. A class of their own: exploring the nondeacetylase roles of class IIa HDACs in cardiovascular disease. Am J Physiol Heart Circ Physiol 311: H199–H206, 2016. First published May 20, 2016; doi:10.1152/ajpheart.00271.2016.—Histone deacetylases (HDACs) play integral roles in many cardiovascular biological processes ranging from transcriptional and translational regulation to protein stabilization and localization. There are 18 known HDACs categorized into 4 classes that can differ on the basis of substrate targets, subcellular localization, and regulatory binding partners. HDACs are classically known for their ability to remove acetyl groups from histone and nonhistone proteins that have lysine residues. However, despite their nomenclature and classical functions, discoveries from many research groups over the past decade have suggested that nondeacetylase roles exist for class IIa HDACs. This is not surprising given that class IIa HDACs have, for example, relatively poor deacetylase capabilities and are often shuttled in and out of nuclei upon specific pathological and nonpathological cardiac events. This review aims to consolidate and elucidate putative nondeacetylase roles for class IIa HDACs and, where possible, highlight studies that provide evidence for their noncanonical roles, especially in the context of cardiovascular maladies. There has been great interest recently in exploring the pharmacological regulators of HDACs for use in therapeutic interventions for treating cardiovascular diseases and inflammation. Thus it is of interest to earnestly consider nonenzymatic and or nondeacetylase roles of HDACs that might be key in potentiating or abrogating pathologies. These noncanonical HDAC functions may possibly yield new mechanisms and targets for drug discovery.

cardiovascular disease; class IIa HDACs; epigenetic regulation; HDAC

HISTONE DEACYLASES ARE ENZYMES that remove acetyl groups from proteins that bear lysine residues (9, 10). The 18 known human histone deacetylases (HDACs) are subdivided into 4 classes. Those in class I (HDAC1, HDAC2, HDAC3, and HDAC8) are the most studied and thus well characterized. Class I HDACs are homologous with the yeast reduced potassium deficiency 3 (Rpd3) HDAC (13, 17). These HDACs are constitutively nuclear and have the most robust, classical deacetylase activity of all HDAC classes (17). Class II HDACs are further subdivided into class IIA and class IIB. Class IIA HDACs include HDAC4, HDAC5, HDAC7, and HDAC9 and are homologous to yeast Hda1 and Clr3 histone deacetylase genes. Class IIB HDACs include HDAC6 and HDAC10 (16, 18, 25). Class IIB HDACs do not have any known invertebrate orthologs, however, HDAC6 does share partial homology with zebrafish. Class III HDACs are yeast homologues of silent information regulator 2 (Sir2) and are thus called sirtuins.

Sirtuins are structurally and functionally distinct from class I, II, and IV HDACs in that their deacetylase activity is NAD+ dependent as opposed to Zn2+ dependent (2, 15, 34). HDAC11 is the only known human deacetylase in class IV (19); its physiological role is poorly understood.

HDACs have been widely studied since their discovery and characterization starting in the late 1960s and early 1970s. The roles that HDACs play in pathological and nonpathological events are vast. However, some of their overarching roles include regulating gene expression by direct deacetylation of histones, which often renders genes transcriptionally repressed (10, 17, 75). Direct deacetylation of histones has been largely attributed to class I HDACs because they have the highest affinity and deacetylase activity for histone substrates. Furthermore, class I HDACs have been implicated in regulating signaling and protein stability through deacetylation of nonhistone substrates. The nexus of regulating genes and signaling through acetylation has been largely explored in cardiovascular biology. Additionally, several research groups have independently shown that class IIa HDACs have played enigmatic but seemingly integral roles in cardiovascular biology despite their
poor catalytic ability. The purpose of this review is to address novel, nonenzymatic roles of class IIa HDACs, especially in the context of cardiovascular diseases. We will address consequences of pharmacological inhibition, protein mutation, and gene silencing of class IIa HDACs. We will also aim to propose new concepts in targeting these molecules from the standpoint of their nondeacetylase roles of gene and signaling regulation.

Class IIa Histone Deacetylases—A Broad Look

Several features distinguish the class IIa HDACs from the other HDAC classes. First, class IIa HDACs are not ubiquitously expressed in all mammalian organs and cell types, as most of the class I HDACs are (25, 28). Human HDACs 4, 5, and 6 were first cloned in 1999 based on the yeast Hda1p amino acid sequence (25) and characterized in human T-cell lymphoma cell lines (60, 73). HDAC4, HDAC5, and HDAC9 are expressed primarily in the heart, brain, and skeletal muscle. HDAC7 is expressed in endothelial cells in the vascular system, heart, skeletal muscle, and lung (36, 53). HDAC7 is expressed in endothelial cells in the vascular system, heart, skeletal muscle, and lung (36, 53). Second, in addition to the C-terminal catalytic domain, class IIa HDACs have a large NH2-terminal domain containing highly conserved serine residues (30). These serine residues are subject to signal-dependent phosphorylation in response to stress or injury. Upon phosphorylation these sites interact with the intercellular chaperone protein, 14-3-3, resulting in a conformational change that masks the nuclear localization signal and exposes the conserved nuclear export signal in the COOH-terminal domain (30, 55). Nuclear export of class IIa HDACs in cardiomyocytes results in derepression of a program of genes contributing to pathological cardiac hypertrophy and remodeling. Some of the signal-dependent kinases that have been shown to phosphorylate serine residues on class IIa HDACs (specifically HDAC4 and 5) include Ca2+-calmodulin-dependent kinase II (CaMKII) (1), PKC (27), and G protein-coupled receptor kinase microtubule affinity-regulating kinases (GRK5) (21, 50). Matsushita and colleagues (51) also elucidated that HDAC4 can be oxidized via intranuclear clear sources of reactive oxygen species on conserved, class IIa, cysteine residues (51). Oxidation of class IIa HDACs results in their exportation from the nucleus (51, 74) (Fig. 1). Several previous reviews have provided detailed descriptions of the pathways and kinases that mediate the phosphorylation of class IIa HDACs (52, 53, 74).

Third, the NH2-terminal domain also contains interacting binding sites for transcription factors, DNA binding molecules, and bridging cofactors (52). Specifically, HDACs 4, 5, 7, and 9 have all been reported to play roles in regulating, typically repressing, the expression of cardiotrophic genes that are upregulated by the transcription factor known as myocyte enhancer-binding factor 2 (MEF-2) (58, 79). Additionally, class IIa HDACs are known to repress genes that have been implicated in the control of pathological cardiac hypertrophy by interacting directly or indirectly with other cardiotrophic transcription factors such as serum response factor (SRF), nuclear factor of activated T cells (NFAT), and the natural killer (NK) family of transcription factors. Both HDAC4 and HDAC5 are recruited by Smad3 to the Smad/Runx2 complex at the Runx2 DNA binding sequence (35). HDAC5 interacts with NKx2.5 via binding with CAMTA and with SRF by binding myocardin (77). HDAC4 binds directly with CaMKII and interacts with NFAT by binding to the DnaJ-related factor Mrj (8). The consequence(s) of these interactions are diverse but are underscored with recurring observations that expression of target genes are suppressed when these transcription factors interact with class IIa HDACs. The fourth distinguishing factor and perhaps most relevant to the context of this review is the fact that class IIa HDACs have very poor deacetylase activity. The catalytic activity of class IIa HDACs is much lower than that of class I and IIb HDACs due to the replacement of a conserved catalytic tyrosine residue with a histidine in all vertebrate phyla, and no natural substrate has been conclusively identified (44). In fact, initial studies demonstrate that HDACs 4 and 5, although responsive to the HDAC inhibitor trichostatin A (TSA), have significantly less deacetylase activity on histones compared with HDACs 1 and 6 (25). Because of their extremely low activity against acetyl-lysine moieties, class IIa HDACs have been referred to as pseudoenzymes (44). One example of this pseudo and somewhat enigmatic phenomenon can be observed

**Fig. 1.** Binding domains of class IIa histone deacetylases (HDACs). Class IIa HDACs 4 and 5 share homologous conserved myocyte enhancer-binding factor 2 (MEF2) binding sites as well as nuclear localization sequences. Additionally, class IIa HDACs have several serine residues that can be phosphorylated by specific kinases given physiological conditions. Also, cysteine residues in the catalytic domain are subject to reactive oxygen species-mediated oxidations, which like phosphorylation, leads to the nuclear export of HDAC4 and HDAC5.
in class IIa HDAC9. The HDAC9 splice variant, MEF2-interacting transcription repressor (MITR), which encodes only the NH2-terminal domain with no catalytic domain, is still able to inhibit MEF-dependent transcription in cardiac myocytes (81, 84). Impairment of MEF2-dependent transcription is mediated via the recruitment of corepressors such as CtBP and HP1 to binding domains on the NH2-terminus of HDAC9 (80, 81). Chatterjee et al. (7) demonstrated that the deacetylase domain of HDAC9 was not required for its negative regulatory effect of adipogenic differentiation.

In addition, overexpression of class IIa HDACs can regulate cellular functions independently of acetylation. The physical presence of class IIa HDACs can indirectly regulate transcription by possible recruitment of sumoyl-conjugating enzymes to promoters and inhibiting transcription by the sumoylation of MEF2 (23, 24). HDAC4 inhibits androgen receptor activity by facilitating androgen receptor sumoylation (78). Overexpression of HDAC7 in cultured neurons blocks low potassium-induced cell death. Interestingly, deletion of the HDAC7 catalytic domain has no effect on HDAC7's neuroprotective effect (48), again giving support to noncanonical/nonenzymatic roles for class IIa HDACs.

The Class IIb HDACs 6 and 10 are less well characterized than those in class IIa; however, only a few studies have addressed the roles they play in cardiovascular pathologies. HDAC6 was first discovered and characterized as “microtubule” HDAC and was shown to play a key role in tubulin stabilization and destabilization through acetylation (25). This class IIb HDAC is constitutively found in the cytoplasm (it does not have a nuclear localization sequence), although some studies have suggested the possibility that it has subcellular localization in nonnuclear organelles such as mitochondria (19, 20). Interestingly, HDAC6 possesses two catalytic domains, yet the known substrate targets outside of tubule proteins are limited (20, 33, 39). HDAC10 was characterized as having similar homology to HDAC6, with the major differences being that it can be found in both cytosolic and nuclear fraction and it also possess only one catalytic deacetylase domain that is essentially nonfunctional (36, 37). However, as has been observed in certain instances, HDAC10 is known to interact with HDAC3—a catalytically efficient class I HDAC—and is believed to repress transcription through a “nondeacetylase” mechanism (69). Given the relatively abundant studies and evidence of the existence of class IIa-mediated regulation of cardiovascular disease, the remainder of this review will focus primarily on class IIa HDACs and, where appropriate, address class IIb HDACs as well.

Class II HDACs in Cardiovascular Disease

Pressure overload hypertrophy. Pressure overload is a type of cardiovascular pathology that often leads to the gross remodeling of the heart through hypertrophic signaling and enlargement/hypertrophy of myocytes. Enzymatically active HDACs, particularly HDACs 1, 2, and 3, remove acetyl groups from histone tails, which often counterbalance the activity of histone acetyltransferases (HATs) (10). The antagonizing ratio between acetylation and deacetylation of histone tails confers whether gene expression is increased or remains at a basal/quiescent level. Under quiescent or nonpathological conditions, class IIa HDACs are found in the nuclei of myocytes, often in complexes containing corepressors, and class I HDACs. Additionally, Cao et al. (3) elucidated that HDACs are important regulators of agonist- and pressure overload-induced autophagy. Expounding upon their own previous studies showing that autophagy is a maladaptive response to cardiac injuries, Zhu et al. (85) later found that HDAC inhibitors abrogate adverse autophagic responses to cardiac injury. Furthermore, in vitro studies whereby HDACs 1 and 2 were independently silenced through small interfering RNA (siRNA) determined that the deacetylase activity of HDAC2 is more relevant in regulating autophagy in cardiomyocytes (3).

Class IIb HDAC6 has also been shown to play a key role in pressure overload-related fibrosis and aberrant cardiac function. Specifically, studies by Demos-Davies et al. (11) demonstrated that both pharmacological inhibition and gene-specific deletion of HDAC6 yields some degree of cardioprotection in pressure overload models of pressure overload. They also observed that the effects of chronic exposure to angiotensin, a well-characterized agonist of hypertrophy/hypertension, are blunted when HDAC6 is either directly inhibited with HDAC6-specific tubastatin A or in the HDAC6-null mouse model. The loss of either HDAC6 activity and/or HDAC6 knockout prevents the loss of muscle wasting that is often associated with chronic hypertrophy signaling.

The scenario for class IIa HDACs in the context of pressure overload has been quite different. As opposed to class I and their IIb counterparts, class IIa HDACs have been largely accepted as repressors of maladaptive responses to pressure overload. McKinsey (52) and Zhang et al. (80) first elucidated that class IIa HDACs were biological repressors of MEF-2-mediated transcriptional activation and that class I HDACs, particularly HDACs 1 and 2, are biological activators of MEF-2. This repression is alleviated upon hypertrophic signaling in pressure-overload models in which class IIa HDACs become phosphorylated and shuttled out of myocyte nuclei (23, 27). Overexpression of HDACs 4, 5, and 9 in cultured neonatal cardiac myocytes results in the suppression of MEF-2-dependent transcription and blocked agonist-stimulated cardiac hypertrophy (1, 71, 82). In fact, models of class IIa HDAC knockout mice have hearts that become hypertrophic and hypersensitive to surgically induced pressure overload because of the loss of these endogenous hypertrophy repressors (5). When pathological pressure overload occurs, there is in increase in activity of several class I and class II HDACs; however, class I HDACs are typically found associated with MEF2 transcription factors and thus support upregulation of several hypertrophic and procell survival genes (53). Thus it has often been reported that class I and class IIa HDACs are endogenous cardiotoxic activators and repressors, respectively (41, 79). In this context, it is not surprising therefore that several research groups have reported that pan-HDAC inhibition that targets both class I and class IIa HDACs ameliorates adverse hypertrophic responses in pressure overload models (53).

However, what has been largely unaddressed in many studies is that despite the seemingly antagonistic nature between class I and class IIa HDACs in pressure overload research studies, often both class I and class IIa HDACs are found to coimmunoprecipitate together in complexes on proximal promoter regions of cardiotoxic genes.
Myocardial infarction. Myocardial infarctions (MIs) are a type of pathological incident whereby part or all of a major coronary artery becomes occluded. MIs, like pressure overload hypertrophy, result in compensatory tissue and heart remodeling as a consequence of the injury. However, the tissue surrounding the occluded regions of the artery often becomes necrotic with the loss of blood supply. MI injuries are replete with acute and chronic proinflammatory wound healing and cell death/prosurvival signaling cascades. HDACs have long been implicated in playing a significant role in the dysregulation of genes leading to aberrant tissue remodeling, myocyte cell death, and ineffective wound healing events post-MI (4, 49, 53, 54). Class I and class IIa HDAC inhibitors have shown promise in ameliorating the effects of adverse tissue remodeling post-MI. Several comprehensive in vivo studies have shown that pan-HDAC inhibitors such as suberoylanilide acid, TSA, and valproic acid not only reduce infarct scar area but also improve cardiac function after an MI (22, 49, 72, 76).

The effects of pharmacological HDAC IIa inhibition vs. loss of HDAC IIa protein expression in MI studies are complex. In fact, gender differences alone can affect the functionality and roles that class IIa HDACs may play in potentiating adverse MI-related outcomes. Specifically, female mice that lack HDAC5 or HDAC9 have a beneficial estrogen-mediated, proangiogenic outcome after an MI, thus suggesting a degree of cardioprotection in females (63). Interestingly, work by Zhang et al. (83) shows that pharmacological inhibition of class I and class IIa HDACs by TSA of male-only cohorts in mice that are subjected to an acute MI injury is also beneficial through a proangiogenic, pro-AKT/survival fashion. Together, these data suggest that there may be sex-specific and temporal considerations for targeting HDACs in post-MI treatments.

Perhaps one of the most compelling observations of nonhistone acetylation-mediated regulation was the discovery that cardiac contractile sarcomeres, specifically myosin chains, can be reversibly acetylated and colocalize with both class I HDAC3 and class IIa HDAC4, albeit on different regions of the myosin chains (26). Upon acetylation, myosin chains have higher sliding velocities (64) and, thus increased, indirect inotropic potential. This may explain why broad inhibition of deacetylation during pressure overload, MI injuries, and other cardiac maladies helps to restore some capacity of cardiac function. However, here again, the deacetylase-deficient HDAC4 is found in association with class I HDAC3. It is pertinent to this review that although both HDAC3 and HDAC4 are associated with the myofilaments, only HDAC3 is capable of deacetylating myosin (64). Other reports have shown that HDAC3 is capable of binding in regulatory complexes containing nuclear receptor corepressor (NCoR)-HDAC4. Given the fact that class IIa HDACs are shuttled in and out of nuclei upon proper stimuli, perhaps in the aforementioned scenario the integral role of HDAC4 may not be to antagonize acetylation of myosin heavy chains but to simply shuttle HDAC3 to its target protein.

Thoracic aortic aneurysm. Thoracic aortic aneurysms (TAAs) are an often fatal type of cardiovascular event in which the aortic wall becomes weakened, which is accompanied by structural changes and, in some instances, lethal rupturing. Improper connective tissue assembly/formation and/or deformed bicuspid valves are the leading culprits of this malady in younger patients. However, in older patients, atherosclerotic plaques are often to blame for the gross remodeling of the aortic wall. There have been a few indirect implications of class IIa HDACs playing a role in pre- and post-TAA vascular injuries, particularly those caused by atherosclerotic mechanisms. A single nucleotide polymorphism in HDAC4 that was identified in a statistically significant number of ethnic patients was strongly associated with carotid intima-media thickness—a clinically relevant marker of atherosclerosis (45). Several groups of researchers also report that broad HDAC inhibition actually prevents or attenuates the spread of neointimal proliferations (66, 67, 70) that are so often associated with sclerotic plaque formation. Kee and Kook (40) reported that HDAC inhibition through TSA in vascular smooth muscle cells increases the activity and expression of the transcription factor KLF4; this activation in turn activates and upregulates p21 and p27, which are key cell proliferation suppressors. However, it is unclear which specific HDACs might be involved in this specific signaling cascade.

Atrial fibrillation. Atrial fibrillation (AF), a common pathological subtype of arrhythmia or irregular heartbeat, has been largely associated with improper protein stability, turnover, and subcellular localization (6, 12, 46). Specifically, AF is associated with improper heartbeats and/or improper rates of contractility needed to effectively fill or remove blood from appropriate heart chambers. It is not surprising therefore that HDACs are implicated in playing key roles in this type of cardiac event. HDAC6 has been shown to directly affect the stabilization of tubulin in AF events. HDAC6 enzymatic activity is elevated in patients with AF, and specific inhibition attenuates hypoacetylation during AF and subsequently causes an improvement in contractility (11, 46). Interestingly, as has been previously noted, HDAC3 (class I) and HDAC4 (class II) can also colocalize to sarcomeres and tubulin structures (26), suggesting that perhaps class I and class IIb HDACs play distinct roles in acetylation regulation. The colocalization of multiple classes of HDACs to the same myosin or tubulin structures may also suggest that class I and class IIa HDACs possess some degree of substrate specificity/preference for discreet protein-protein interactions.

Writers, Erasers, and Readers

Writers are enzymes that add unique epigenetic markers on histone tails and specific regions of chromatin (14). Canonical writers include histone methyltransferases, which add methyl groups to lysines; phosphorylases, which add a phosphate group to an acceptor; and HATs, which add acetyl groups to lysine residues (38, 68). Erasers, often considered to be endogenous antagonists to writers, include demethyltransferases, phosphatases, and HDACs (14, 53). Readers are enzymes that possess unique binding regions that can alter the activity, accessibility, and/or efficiency of writers and erasers; one can almost consider readers to be an additional level of epigenetic regulation in that they can regulate epigenetic regulators. Classic readers include bromodomains, chromodomains, plant homology domains, and tudor domains (68). What is interesting, however, is the recurrent observations by many groups of researchers showing the enzymatically deficient class IIa HDACs, which bind tightly to acetyl-lysine ε, are often found in proximity and/or in complex with methyltransferases and
class I HDACs. Therefore, one might speculate that one of the putative biological roles for class IIa HDACs may indeed be that of a reader. Our work (outlined below) suggests that class IIa HDACs can serve as a scaffold for class I HDACs to help facilitate deacetylase-mediated signaling of cardiotoxic genes (29).

Complexity of Complexes

As tools and reagents have become more sophisticated, specific, and accessible, so has it become more prevalent to observe both class I HDACs and class IIa HDACs communoprecipitating and/or colocalizing with corepressor complex molecules. What has been equally observed but less understood throughout the past few years is that both class I and class IIa HDACs seem to simultaneously localize with regulatory complexes as well. Even class I HDACs do not function autonomously, but are the catalytic core of large corepressor complexes. HDAC3 is a part of a distinct complex that contains either nuclear receptor corepressor (NCoR) or its homolog, silencing mediator of retinoic and thyroid receptors (SMRT) (59). Importantly, pioneering research has elucidated that HDACs 4 and 5 can associate with HDAC3 in vivo (13). Other early studies demonstrated that class IIa HDACs are associated with HDAC3 and interact with the SMRT/NCoR corepressor complex (16). Eric Verdin’s group (16) demonstrated that when class IIa HDACs were associated with HDAC3/SMT/NCOR corepressor complex, they did not contribute to its enzymatic activity. Although these studies show that class IIa HDACs could interact with the HDAC3/SMRT/NCOR corepressor complex, further studies were necessary to directly determine whether class IIa HDACs might still play a functionally relevant role in regulating gene expression while within these complexes. To this end, Mihahlova and colleagues (56) showed HDAC4 and HDAC5 play a role in recruiting HDAC3 to regulate the activation of the transcription factor FOXO via deacetylation, but their study did not show whether this also included NCoR or SMRT complex members.

Mammalian HDAC7 was first described in 2000 by Kao et al. (36) as being a member of the SMRT/NCoR and mSin3A corepressor complexes. The studies by Kao et al. also denoted that both HDAC5 and HDAC7 possess multiple repressor domains. The crystal structure of human HDAC7 confirmed that these repressor domains have intricate protein-protein interactions that could modulate its relatively weak deacetylase activity (65). Like HDAC7, full-length HDAC9 is known to colocalize and communoprecipitate with NCoR repressor sub-units (61).

Class IIa HDACs are found associated with other corepressors and corepressor complexes. Eric Olson’s laboratory demonstrated that HDACs 4, 5, and 9 associated with heterochromatin protein 1 (HP1), which recognizes methylated lysines on histone tails (82). They further demonstrated that HDACs 4, 5, and 9 (MTR) form a complex with the histone methyltransferase SUV39H1. A recent study followed up on this work and, using human left ventricular myocardium, demonstrated that HDAC4 was associated with SUV39H1 in the healthy heart. Importantly, its presence correlates with an increase in repressive dimethylation and trimethylation of H3K9 in the adjacent chromatin and the resulting increased binding the transcriptional repressor HP1. Hypertrophic stimuli result in the export HDAC4 from the nucleus and disruption of the association of the HDAC4 with SUV39H1, and the resulting demethylation of H3K9 marks the loss of HP1 binding (32) (Fig. 2). Additionally, Rafiei et al. (62) used chromatin immunoprecipitation (ChIP) in parallel with ChIP sequencing and also DNA methylation sequencing to identify and quantitate posttranslational modifications on histones relevant to HDAC-mediated signaling. Their work also characterizes differential acetylation in human aortic endothelial cells that are stimulated with TSA, suggesting an important role for HDACs in regulating cardiac

![Diagram of protein interactions](image-url)

Fig. 2. Summary of recent studies supporting a role for class IIa HDACs as scaffolds that recruit repressor complexes to specific promoters to regulate prohypertrophic genes. HDAC4 recruits the histone methyltransferase SUV39H1 and the methyl histone binding protein (HP1) via binding to MEF1 to repress prohypertrophic gene expression (32, 80). The recruitment of the complex results in an increase in repressive H3K9 dimethylation and trimethylation marks. HDAC5 has been shown to be required for the recruitment of the Sin3a/HDAC1/HDAC2 complex to the prohypertrophic gene Ncx1 and Bnp via interaction with the Ncx2.5 and YY1 transcription factors, respectively (29). In response to hypertrophic stimuli, HDAC4 and HDAC5 are phosphorylated, release SUV39H1/HP1 and Sin3a/HDAC1/HDAC2, respectively, and are exported from the nucleus. Prohypertrophic gene expression is stimulated by the recruitment of histone acetyltransferases to the free MEF2, Ncx2.5, and YY1 transcription factors.

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vascular integrity, inflammation, and function (62). Importantly, they show that changes in histone acetylation alone do not necessarily correlate with differential gene expression. It will be important, moving forward toward better HDAC interventions, to characterize both global impact and specificity of HDAC inhibitors on multiple gene targets.

Recently, we have observed that HDAC5 can interact with additional class I HDACs such as HDAC1 and/or HDAC2 (29). Furthermore, double communoprecipitation assays assessing male mouse heart homogenates show that HDAC5 can interact simultaneously with HDAC1 and mSin3A (29). Specifically, our work elucidates that HDAC5 is indeed found in a Sin3/HDAC1/2 corepressor complex in nonpathological conditions (29). However, in HDAC5-null mice, it is no longer possible to recruit the Sin3a/HDAC1/2 corepressor complex to the proximal promoters of the procollagen IIIA and Bp genes. These observations suggest that HDAC5 is perhaps responsible, in part, for recruiting the enzymatically active Sin3/HDAC1/2 complex to the promoter of these prohypertrophic genes that are upregulated in HDAC5-null mice. These findings may also explain why murine class IIa HDAC knock-out models experience age-related cardiac hypertrophy. The physical presence, not necessarily the enzymatic activity, is what confers the regulation of genes by class IIa HDACs.

Concluding Thoughts: Think Beyond Zn$^{+}$

Some of the most recent and perhaps most promising efforts to target class IIa HDACs come from Lobera et al. (47) who have identified trifluoromethoxyadiazole (TFMO)-based metal binding group inhibitors that target the acetyl-lysine sites of class IIa HDACs. These TFMO class IIa selective inhibitors have the potential to help us better understand how the catalytic or “reader” domains of class IIa HDACs participate in the regulation of gene expression. Unlike what is known concerning class I and IIb HDAC inhibition, inhibiting class IIa activity had a much lower effect on the number of genes up- or downregulated with inhibition in monocytes (47). Class IIa inhibitors had their greatest effect on expression of chemokines and the highly expressed cell-surface genes that are much more abundant in monocytes than in lymphocytes and T cells. This may explain why the TMFO inhibitors had even less of an effect on gene expression in lymphocytes and T cells (42, 47). TMFO inhibitors also blocked the upregulation of CCL2 in monocytes treated with macrophage colony-stimulating factor. Although these studies are exciting and can have indirect correlations to the inflammatory responses in cardiovascular disease, additional cardiovascular-specific studies with these compounds will be essential to understanding their potential in cardiovascular disease.

However, the majority of studies over the past 15 years have shown that class IIa HDACs are integral in regulating cardiovascular diseases despite their poor deacetylase ability. This intriguing observation may point to the fact that there are great untapped opportunities for therapeutic interventions that are going largely unaddressed. Perhaps pharmacological interventions targeting epigenetic regulators such as class IIa HDACs in cardiovascular disease should include drugs that target their protein-protein interactions, scaffolding abilities, or nonenzymatic functions, which may be important in disease progression. For example, because class IIa HDACs (endogenous suppressors or pressure overload-induced hypertrophy) lose suppressive functionality upon being phosphorylated and shuttle out of nuclei, perhaps pharmacological intervention should block their specific phosphorylation sites. In vitro characterization of compounds that regulate class IIa export show promise (31, 57), but they will need to be further studied in vivo models. Another example of a putative class IIa HDAC-based therapeutic concept may involve targeted gene delivery such as epicardial painting, a gene transfer technique whereby adenoviral vectors are “painted” directly onto myocardium (43). Specifically, in classic in vivo models of ligated coronary arteries to induce acute MI injury, it may be advantageous to simultaneously administer a vector that contains siRNA that targets a specific class IIa HDAC. This might be an advantageous strategy for treating women because in female mice but not in male mice, the lack of HDAC5 and/or HDAC9 is helpful in ischemic injury remodeling. These data may in turn provide a foundational basis for combining gene therapy with traditional therapeutic interventions such as coronary bypass or angioplasty.

Much attention has been placed on the possible therapeutic potential of HDAC inhibitors in cardiovascular disease. Many of these pharmacologically based interventions focus on targeting the Zn$^{+}$ binding domains and/or substrate interactions. The new generation of TMFO class II selective inhibitors will be useful for identifying class IIa substrates and binding sites. These inhibitors will possibly also help us to discriminate between class IIa catalytic vs. reader activity. However, we must be ever cognizant of class IIa HDACs having critical, nondeacetylase-mediated roles in signaling. Thus it will be potentially most valuable and efficacious to think beyond their catalytic binding domains and instead focus on their protein-protein interactions, binding affinities for acetyl-lysine targets, and their own posttranslational modifications.

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AUTHOR CONTRIBUTIONS

L.H.W. and D.R.M. conception and design of research; L.H.W. and D.R.M. drafting manuscript; L.H.W. and D.R.M. editing and revising manuscript; L.H.W. and D.R.M. approved final version of manuscript.

REFERENCES

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