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The nonepigenetic role for small molecule histone deacetylase inhibitors in the regulation of cardiac function

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Eight million US adults are projected to suffer from heart failure (HF) by 2030. Of concern, 5-year mortality rates following HF diagnosis approximate 40%. Small molecule histone deacetylase (HDAC) inhibitors have demonstrated efficacy for the treatment and reversal of HF. Historically, HDACs were studied as regulators of nucleosomal histones, in which lysine deacetylation on histone tails changed DNA–histone protein electrostatic interactions, leading to chromatin condensation and changes in gene expression. However, recent proteomics studies have demonstrated that approximately 4500 proteins can be acetylated in various tissues; the function of most of these remains unknown. This Review will focus on the nonepigenetic role for lysine acetylation in the heart, with a focus on nonepigenetic actions for HDAC inhibitors on cardiac function.

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An estimated 6.5 million US adults were diagnosed with heart failure (HF) in 2014, with a projected increase of 46% or 8 million US adults by 2030 [1]; this increase in HF prevalence is associated with a projected cost of US\$69.7 billion [2]. While standards of care and therapeutics (e.g., β -blockers and angiotensin-converting enzyme inhibitors) improved HF survival rates from 1979 to 2000, current 5-year mortality rates post HF diagnosis remain at approximately 40% [1], stressing the need for improved therapeutics [2].

In response to stress, the myocardium remodels. Myocardial remodeling is characterized by ventricular muscle enlargement, (i.e., hypertrophy) and fibrosis that contributes to diastolic and/or systolic dysfunction and ultimately HF [3]. Current HF therapeutics target signaling cascades, such as β -adrenergic and angiotensin signaling as well as NF- κ B and MAPK, which inhibit cardiac remodeling and pump dysfunction [4]. However, cross-talk and signaling redundancy have been suggested to limit current therapeutics from completely inhibiting or reversing myocardial remodeling and HF. As such, more efficacious therapeutics that target the epigenome have been proposed as global regulators of these shared downstream signaling networks in the treatment of HF.

Chromatin-modifying proteins can be classified as writers, erasers or readers. Writers and erasers add or remove reversible epigenetic marks to chromatin, respectively. Readers, on the other hand, recognize or ‘read’ these epigenetic marks and govern a particular transcriptional outcome. There have been 377 chromatin-modifying enzymes identified in humans, which include histone methyltransferases [5,6] and histone acetyltransferases [7–9] (writers), histone demethylases [10,11] and histone deacetylases [12] (erasers), as well as bromodomain-containing proteins (readers) [13–17]. Changes in chromatin structure play a critical role in cardiac function, as reactivation of fetal genes have been described during the development of cardiac dysfunction [18]. Furthermore, histone deacetylation (HDAC) has been shown to play a critical role in cardiac development and function as genetic deletion of class I HDAC isoforms resulted in embryonic death, while cardiac-specific silencing lead to rapid onset of HF [19,20].

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Antithetical to genetic loss of function, pharmacological inhibition of HDACs has shown efficacy in animal models of HF [2,21–26]. Until recently, HDACs have been examined as regulators of nucleosomal histones, where HDACs remove acetyl groups from the epsilon amino group of lysine residues on histone tails, resulting in gene repression. Conversely, histone acetyltransferases promote transcriptional activation via acetylation of histone proteins [2,27–29]. There have been 18 identified mammalian HDACs, which are grouped into four different classes (I, II, III and IV) based on functionality and orthology to *Saccharomyces cerevisiae* HDAC classes. Gene regulation via histone acetylation was first described in yeast and further supported by the identification of the first mammalian HDAC, HDAC1, by Taunton *et al.* in 1996 [12,30]. HDAC1, and class I HDACs, have been determined as orthologs of the yeast Rpd3 HDAC. Class II HDACs share orthology with the yeast HDA1 protein, and class III HDACs share orthology with the yeast Sir2 protein. HDACs are further divided as zinc-dependent or nicotinamide adenine dinucleotide (NAD⁺)-dependent HDACs. HDACs that require zinc for catalytic activity are classified as class I (HDACs 1, 2, 3 and 8), class II (HDACs 4, 5, 6, 7, 9 and 10) and class IV (HDAC 11). Class II HDACs are subdivided into IIa (HDACs 4, 5, 7 and 9) and IIb (HDACs 6 and 10). Class IV only consists of one HDAC isoform, HDAC11. HDAC11 lacks orthology with yeast Rpd3, HDA1 and Sir2, and little is known regarding its biological function; however, recent studies have shown that HDAC11 associates with BRD2 and suppresses thermogenic gene expression [31]. Class III HDACs, also known as sirtuins, (SIRT1–7) require NAD⁺ as a cofactor for deacetylase activity [28,29]. Sirtuins predominantly localize within the nucleus, cytoplasm and mitochondria, whereas zinc-dependent HDACs localize to the nucleus and cytoplasm [32]. More specifically, class I HDACs mainly consist of nuclear HDACs and class II HDACs have nucleocytoplasmic shuttling capabilities [32,33]. Inhibition of class I and IIb HDACs is efficacious in animal models of HF [2,21–26]. As such, this Review will highlight the role for zinc-dependent HDACs in the nonepigenetic regulation of the heart.

HDAC inhibitors & the heart

HDAC inhibitors

The deacetylase domains of HDACs are highly conserved across the HDAC family, and while the zinc atom is present in the active site, the HDAC binds to the acetylated epsilon-amino group of a lysine residue found on histone tails or nonhistone proteins. During class I HDAC catalysis, a water molecule enters the active site through an internal cavity to be hydrolyzed to produce acetic acid and a free lysine residue. The acetic acid exits the active site through the same internal cavity, which is absent in class II HDACs. Furthermore, the class I-specific internal cavity binds to and accommodates the exodus of acetic acid, suggesting that an inhibitor consisting of an acetate group capable of locating the cavity might demonstrate class I selectivity [34]. Additionally, HDAC6 is the only zinc-dependent HDAC to have a duplicated deacetylase domain [33]. These class-specific characteristics of HDACs could lead to the development of selective inhibitor design and identification.

HDAC enzymes are zinc metal hydrolases, as described above, and HDAC inhibitors (HDACis) are zinc ion chelators. The highly conserved active site of HDACs is comprised of a zinc-binding site pocket and a two Asp-His charge-relay system, which is occupied during inhibition by HDACis such as trichostatin A and suberanilohydroxamic acid (SAHA) [35]. HDACis are comprised of a common pharmacophore model referred to as a ‘cap-linker chelator’ (Figure 1A). The ‘cap’ structure of the HDACi interacts with amino acids located at the external surface and entrance of an *N*-lysine binding channel. The ‘chelator’ refers to the metal-binding functionality of the HDACi to a zinc ion present in the HDAC enzyme active site, which is usually ‘linked’ to the cap structure by a five or six hydrocarbon chain [29,33,36,37]. Refer to Figure 1A for a structural pharmacophore representation of the HDACi SAHA. The cap and chelator have both been shown to direct isoform selectivity. Many HDACis can inhibit more than one HDAC class or isoform, also known as a pan inhibitor; however, inhibiting more than one HDAC can be detrimental as HDACs have specific activities [33,38]. Moreover, benefits of inhibiting specific HDAC isoforms have been reported, for example, HDAC4 inhibition is advantageous in the treatment of Huntington’s disease [33,39] and inhibiting HDAC 8 could fight against schistosomiasis and other human parasite diseases [33,40–42].

While there are at least four different chemical classes of HDACis comprised of the pharmacophore model; hydroxamic acids (SAHA); short chain fatty acids, also known as carboxylic acids, (valproic acid); benzamides (MS-275); and cyclic peptides (depsipeptide); recent reports have demonstrated a fifth HDACi group ortho-amino aniline HDACis [43]. Selectivity and potency vary among HDACis (Figure 1B), where hydroxamic acids exhibit strong chelating properties that allow for HDAC inhibition at nanomolar concentrations. Short-chain fatty acids are less potent, in which HDAC inhibition requires millimolar concentrations. Short-chain fatty acids are readily

Figure 1. Histone deacetylase inhibitors, from structure to selectivity. (A) Structural pharmacophore representation of the HDAC inhibitor suberanilohydroxamic acid, including the cap, linker and chelator. (B) Chemical classes of zinc-dependent HDAC inhibitors; hydroxamic acids, cyclic peptides, benzamides and carboxylic acids. Potency is indicated from high (red arrow) to low (green arrow), and class HDAC selectivity is noted for each class.

HDAC: Histone deacetylase; SAHA: Suberanilohydroxamic acid; TSA: Trichostatin A.

taken up and transported in the cell, and have modest selectivity for class I HDACs [28,29,44]. Both, benzamide and cyclic peptide HDACis are selective for class I HDACs, specifically HDACs 1, 2 and 3. Cyclic peptides are usually characterized by their alkyl- and chelation- binding properties that allow for high potency [28,29,44]. Last, ortho-aminoanilide HDACis have a similar structure to benzamide HDACis and selectivity toward HDACs 1 and 2 [43].

HDACis have been investigated for the treatment of cancers, as well as many other diseases, and to date, four HDACis have been approved by the US FDA for use in hematologic cancers; FDA-approved HDACis include SAHA (PubChem Compound Database CID = 5311; $C_{14}H_{20}N_2O_3$), also known as vorinostat, romidepsin (PubChem Compound Database CID = 5352062; $C_{24}H_{36}N_4O_6S_2$), panobinostat (PubChem Compound Database CID = 6918837; $C_{21}H_{23}N_3O_2$), and belinostat (PubChem Compound Database CID = 6918638; $C_{15}H_{14}N_2O_4S$) (www.clinicaltrials.gov; Drugs@FDA). Although studies have shown chemo-therapeutic efficacy for HDACis, benefits to the heart are at odds with standard detrimental impacts for chemotherapeutics; HDACi chemotherapy demonstrated improved cardiovascular function for myocardial infarction [45,46], atherosclerosis [47,48] and HF [49–51], extending HDACis as a therapeutic for cardiovascular diseases.

Research & animal models

HDACs have been implicated in pathological cardiac events such as hypertrophy [52,53] and fibrosis [22,54–56] as well as regulate cardiac contractility [57–60] and energy metabolism [19,61]. While early examination of HDACis demonstrated efficacy in HF prevention models [3,4], recent reports have shown that treatment with HDACis not only blocks but can also reverse remodeling and improve heart function [24,60]. These recent reports demonstrate the potential for small-molecule HDACis for the treatment of cardiac disease.

SAHA, for instance, was shown to decrease infarct size and improved systolic function in response to ischemia/reperfusion injury; improvement was seen for rabbits treated with SAHA presurgery or treated at time of reperfusion. Moreover, plasma concentrations for SAHA were similar to that of cancer patients [24]. In this report, it was noted that treatment with SAHA increased autophagy, which was previously shown to play a role in reperfusion injury. It has been postulated that increased autophagy during reperfusion injury is necessary to remove

damaged mitochondria to maintain energy balance in cardiomyocytes and thus protect against cell death [62]. Indeed, SAHA-induced autophagy was necessary for cardioprotection [24].

In the second report, Givinostat (ITF2357), which is currently in Phase III clinical trials for Duchenne muscular dystrophy, was shown to inhibit diastolic dysfunction in the hypertension Dahl salt-sensitive rat; no reductions in blood pressure were noted. In addition, age-dependent diastolic dysfunction was blocked in 9-month old mice fed a normal chow diet with ITF2357 for 11 months compared with age-matched controls. Echocardiography demonstrated maintained cardiac relaxation with ITF2357 fed mice compared with control littermates. Of importance, inhibition of HDAC activity did not inhibit cardiac fibrosis or hypertrophy in these models but instead directly improved myofibril relaxation [60].

A third report investigated the effects of the novel pan-HDACi MPT0E014 on HF using isoproterenol-induced dilated cardiomyopathy [63]. Rats treated with MPT0E014 had improved fractional shortening, reduced left ventricle end-diastolic and systolic diameters, as well as reduced expression of ANP, fibrosis, AT1R and TGF- β . These findings demonstrated a decrease in cardiac fibrosis, which contributed to an attenuation in myocardial remodeling and improved cardiac contractility function in HF rats. This group also investigated MPT0E014 in diabetic cardiomyopathy and its effects on cardiac PPARs- α , γ , δ and fatty acid metabolism [64]. This study utilized diabetes mellitus rats treated with MPT0E014, and healthy rats (used as a control) to evaluate expression levels of pAKT, myocardial inflammatory cytokines (TNF α and IL-6), the fatty acid transporter protein (CD36) and PPARs. In short, MPT0E014 improved insulin signaling and metabolic homeostasis, and reversed hyperglycemic effects on cardiac PPARs in diabetes mellitus rats.

Combined, these reports suggest that pan-HDACis regulate cardiac function. There are numerous HDACis, in addition to the four FDA-approved inhibitors, that are currently in clinical trials for various cancers; however, there are no current HDACis in human clinical trials for cardiovascular diseases (www.clinicaltrials.gov) [8,65]. Furthermore, pan-HDACis may cause adverse side effects given the pleiotropic activities involving HDACs [38,66]. Therefore, investigating specific HDAC isoforms in the development of cardiomyopathies as well as selective isoform inhibitors will be more advantageous in the treatment of HF.

Isoform-specific HDAC inhibition & the heart

Currently, there are several isoform-specific HDACis in Phase I or II clinical trials for hematological cancers and neurological disease. The benzamide HDACi entinostat (MS-275) is being tested in Phase I and II clinical trials as anticancer drugs and has shown relative selectivity for HDAC1, as well as HDAC9 [67]. MS-275 was tested in isolated rat hearts prior to ischemia reperfusion (IR) and contractile function was restored as well as significant reduction of infarct area, compared with IR rat hearts treated with the pan-HDACi, trichostatin A, and the class IIb HDACi, tubastatin A [68]. Thus, class I, or HDAC1, seems to be cardioprotective against IR. The benzamide HDAC1 and 2 inhibitor, Mocetinostat (MGCD0103), is in Phase II clinical trials for several different types of lymphomas, including Hodgkin lymphoma, as it inhibits various cancer cell growth [67]. MGCD0103 has been suggested as a potential therapeutic for the treatment of cardiac fibrosis [56]. A reduction of bone marrow-derived fibrocyte differentiation into active myofibroblasts was observed in the hearts of mice treated with Angiotensin II (AngII) and MGCD0103, suggesting a class I, or more specifically, HDAC1 or 2, enzyme is involved in AngII-induced cardiac fibrosis [56]. Furthermore, the cyclic peptide, depsipeptide (FK228), inhibits HDAC1 and 2 deacetylase activity and is being tested in Phase II clinical trials as an anticancer therapy [38,65,69]. Although, electrocardiogram abnormalities were observed in preclinical and Phase I testing, cardiac studies on Phase II participants were conducted and concluded that FK228 did not contribute to any impaired cardiac functionality and was not associated with myocardial damage [70].

Studies have suggested class I HDACs promote epigenetic myocardial remodeling, while class IIa HDACs have been shown to be cardioprotective. Little is known about class IIb HDAC function in the heart; however, studies investigating HDAC6 inhibition demonstrated attenuation of cardiomyopathies. HDAC6 null mice, as well as HDAC6 inhibition, improved cardiac function in both AngII-induced cardiac hypertrophy and pressure overload mediated by transverse aortic constriction [59]. HDAC6 is a known tubulin deacetylase [71]; inhibiting HDAC6-dependent tubulin deacetylation was shown to protect against protein aggregation in cardiomyocytes and inhibit cardiac protein toxicity [72]. Last, tubulin hyperacetylation via HDAC6 inhibition protected rodents against doxorubicin-induced cardiac injury [73].

Together, these data highlight the HDAC isoforms and class/isoform-specific HDACis that are efficacious for the treatment of cardiomyopathies. In addition to selectively inhibiting HDAC isoforms to treat certain cardiac

conditions and diseases, isoform-specific inhibition would be predicted to have limited side effects compared with pan inhibitors, which in the clinic contribute to hyponatremia, hypokalemia, fatigue, nausea and diarrhea [74].

HDACis in the human heart

Less is known about the functions of HDACs in the development of cardiomyopathies in the human heart due to the poor availability of human heart tissue samples at disease onset. The best human cardiac cell model to date may be human-induced pluripotent stem cell-derived cardiomyocytes; however, HDAC inhibition studies within this cell model are limited [75]. A recent study obtained human heart samples from patients with HF with preserved ejection fraction (HFpEF) at the time of orthotopic cardiac transplantation and examined for myofibril function [60]. The heart samples were obtained under certain criteria, such as presence of diastolic dysfunction as well as no known genetic predispositions to HFpEF to determine that myofibril relaxation impairment does in fact contribute to diastolic dysfunction in humans. Although, no HDACi studies were performed on these tissue samples, the report provided a model for HDAC inhibition to improve diastolic function in the treatment of HFpEF. This model was supported by their investigation of HDAC2 inhibition in hypertensive Dahl salt-sensitive rats resulted in improved myofibril relaxation. Historically, HFpEF patients have not been referred for cardiac transplantation; therefore, to overcome the obstacle of the lack in human heart availability, researchers can consider using larger animal models for future translational investigations, such as feline or canine models of HF.

To date, observational studies involving HDACis in cancer patients or antiepileptic patients taking the short-chain fatty acid HDACi, sodium valproate (SVA), have provided our best understanding for HDACi therapy on the heart. For instance, Brookes *et al.* [76], pooled three prospective studies that examined stroke risk in patients who were prescribed SVA compared with non-SVA-prescribed patients or patients receiving antiepileptic drugs (AED) other than SVA. At 1 year post-treatment, 100% of patients prescribed SVA remained stroke free, whereas 95% of patients that received non-SVA and 88% of patients on AED therapy remained stroke free. However, by year 15, 88% of patients that received SVA remained stroke free, while 79% of patients taking non-SVA medication and 74% of patients receiving AED therapy remained stroke free. Moreover, recurrent stroke rate was lower for SVA (17 or 168) patients compared with other AED (105 of 530) [76]. It should be noted, however, that this study did not determine if SVA inhibited HDAC activity or increased histone acetylation in these patients. While these findings demonstrate benefit, a recent systematic review of pan-HDACis in cancer patients showed some concern for mild cardiac side effects that included ST-segment and T wave (ST/T) abnormalities and QT prolongation [77], which is of concern for lethal ventricular arrhythmias. Based on this Review of 62 papers, the authors ultimately concluded global cardiovascular safety for HDACis in cancer patients. Of note, dosing time and concentration potentially play a role in these cardiac side effects and may be altered with human HF patients.

Lysine acetylation

Reversible post-translational modifications (PTMs) regulate the function of many proteins. Several PTMs have been identified, however, few have been extensively investigated. The most prominent PTMs include methylation, phosphorylation, glycosylation, ubiquitination and acetylation [78,79]. PTMs can occur on several different amino acid residues, for example, serine, threonine and tyrosine are common sites for protein phosphorylation. The most modified amino acid residue is arguably the lysine side chain. Lysine residues have been shown to be methylated, ubiquitinated, sumoylated, glycosylated and acetylated, among many other PTMs [80].

VG Allfrey *et al.* [7] discovered lysine acetylation of histone protein tails >50 years ago. Twenty years later, L'Hernault and Rosenbaum demonstrated that nonhistone proteins, in this case α -tubulin, can also be acetylated on lysine residues [71]. It took another 10 years to show that tumor suppressor p53 [81] and HIV transcriptional regulator Tat [82] are also acetylated. While the historical role for acetylation has examined gene regulation [28,32], improvements in proteomics have shown that the acetylome rivals the kinome in prevalence as a PTM [83]. Recent proteomic studies have identified approximately 15,000 lysine acetylation marks associated to roughly 4500 nonhistone proteins [83,84]. Lysine acetylation has also been observed in various organisms from humans to bacteria [32,85,86], suggesting diversity and conservation of regulatory functions for protein acetylation. Furthermore, acetylation is the most prevalent mitochondrial PTM in bacteria, more prevalent than phosphorylation [87–90]. Not surprising then, it has been noted that over 60% of mitochondrial proteins contain acetylation sites [91]. Of these, the long-chain acyl-CoA dehydrogenase (LCAD), which catalyzes the first reaction of fatty-acid oxidation, is regulated by acetylation. Acetylation of LCAD reduced its activity, while HDAC (SIRT3)-dependent deacetylation increased LCAD activity and fatty-acid oxidation [92].

Nonhistone protein lysine acetylation usually occurs on sites within structured domains (α -helices and β -sheets) and highly conserved proteins (ribosomes, chaperones and metabolic enzymes) [32,84,93]. However, recent proteomic studies have revealed that acetylation targets proteins within major pathways specific to tissue type, as well as subcellular compartments [83,84,94–96]. These studies observed abundant levels of lysine acetylation within the mitochondria [83,94,96], this may in part be due to high levels of acetyl-CoA needed for mitochondrial respiration; acetyl-CoA serves as a donor for lysine acetylation. As cardiac muscle is highly enriched in mitochondria, it has been postulated that nonhistone protein acetylation for mitochondrial and nonmitochondrial proteins imparts important physiological functions in the heart.

Lysine acetylation & the heart

Our understanding of the acetylome in cardiac biology remains fragmentary. However, recent proteomic findings demonstrate significant changes in nonhistone protein acetylation in the failing heart; in particular, mitochondrial protein hyperacetylation was reported in human and rodent HF [94]. As sirtuins, which localize to the mitochondria, are downregulated under conditions of pathological stress [97–99], these findings suggest that increased mitochondrial protein acetylation contributes to mitochondrial respiratory dysfunction. Indeed, mitochondrial protein hyperacetylation was reported to be associated with decreased NAD^+ and respiration. In addition, pseudo-acetylation of succinate dehydrogenase led to reductions in oxygen consumption rates [94].

While early reports focused on a role for sirtuins in the regulation of mitochondrial protein deacetylation [94,97–99], recent evidence also suggests that zinc-dependent HDACs participate in mitochondrial protein deacetylation [100]. It has been shown for instance that HDAC1 and potentially HDAC2 localize to the mitochondria in cardiac tissue. Moreover, inhibition with the class I-selective HDACi, MS-275, or mitochondrial-selective inhibition of HDAC1 was shown to attenuate ischemia/reperfusion injury [100]. Investigators further showed that HDACi-mediated improvements in myocardial injury were, in part, dependent on mitochondrial-HDAC1 inhibition that subsequently attenuated succinate dehydrogenase activity, which led to reductions in reactive oxygen species [100]. Together, these reports implicate HDACs in the nonepigenetic regulation of mitochondrial protein function and further highlight the nonepigenetic potential for small-molecule HDACis in HF.

Unlike sirtuins, zinc-dependent HDAC activity, in particular, class I and II, is elevated in multiple models of cardiac remodeling [101,102]. In addition, class I and II HDACs, HDACs 2, 3 and 6, have been shown to localize to the sarcomere [58–60]. Together, this would suggest decreased lysine acetylation. Consistent with this postulate, unpublished proteomics data from our lab demonstrated overall decreased acetylation of sarcomere proteins, contrasting hyperacetylation reported for many of the mitochondrial proteins [94]. This is of interest, as accumulating evidence suggests that sarcomere protein acetylation is critical for muscle contractility [57–59,103].

Early reports demonstrated that myofibrils treated with trichostatin A, a class I and II HDACi, had increased contractile activity [57], suggesting nonepigenetic actions for HDACis in cardiomyocyte sarcomere function. These investigators further reported that HDAC3 localized to the A-band of cardiac sarcomeres and deacetylated myosin heavy chain. Whereas acetylation of myosin heavy chain was shown to increase contractile function, deacetylation by HDAC3 decreased contractile performance [58]. More recently, HDAC3 was shown to interact with CapZ β 1, a capping protein known to regulate myofibril assembly. Further, inhibition of class I HDACs increased CapZ β 1 acetylation on lysine residue 199 (K199). Acetylation of CapZ β 1 increased CapZ dynamics important for capping of the actin filaments [104]. HDAC6, a known tubulin deacetylase, has also been reported to localize to the myofibril [59]. Loss of HDAC6 function, via knockout or tubastatin A-selective HDAC6 inhibition, was shown to protect mice from angiotensin II-mediated systolic failure. Consistent with finding above, HDAC6 inhibition directly improved myofibril contractility [59] and thus improved systolic function. Additional reports have demonstrated that HDAC6 inhibition attenuated atrial fibrillation [103] via nonepigenetic regulation of tubulin acetylation. Last, HDAC2 was reported to localize to the sarcomere. In these studies, genetic or pharmacological inhibition of HDAC2 improved myofibril relaxation; this led to attenuation of age-associated and hypertension-mediated diastolic dysfunction, or HFpEF [60]. Collectively, these reports suggest that the cardiac acetylome is critical for cardiac function and that regulation of mitochondrial or sarcomere function by HDACis likely involves nonepigenetic regulation of protein acetylation and not just traditional regulation attributed to gene expression.

Conclusion

Four FDA-approved HDACis currently exist for cancer treatment (vorinostat, romidepsin, belinostat and panobinostat). As current research demonstrates efficacy for HDACis in small and large animal models of HF, it is our

Figure 2. Schematic demonstrating the impact for sarcomere and mitochondrial protein acetylation in the heart. (A) In response to pathological stress, HDAC activity is increased in the myocardium. Increased HDAC activity within the sarcomere contributes to sarcomere protein deacetylation that impacts contractile function. **(B)** In the mitochondria, increased pathological stress attenuates nicotinamide adenine dinucleotide (NAD⁺) levels as well as sirtuin, in particular, SIRT3 expression. This, in combination with increased acetyl CoA contributes to mitochondrial protein hyperacetylation of SIRT3-dependent targets. In addition, HDAC1 is localized to the mitochondria in response to stress and HDAC1 activity is increased, thus deacetylating HDAC1-specific mitochondrial proteins. Mitochondrial acetylation and deacetylation contribute to metabolic function. HDAC: Histone deacetylase.

opinion that repurposing of HDACis for the treatment of human HF is feasible. It should be noted, however, that there are no current clinical trials in the USA for HDACis in HF. However, this Review highlights the relevance of small molecule HDACis as epigenetic and nonepigenetic modifiers that can target shared downstream mediators for the treatment of cardiac diseases, and in particular HF.

Future perspective

The work highlighted in this Review illustrates an emerging topic of interest focused on the nonepigenetic function for lysine acetylation in the heart. Specifically, this Review focused on the nonepigenetic impact for HDACis in the regulation of mitochondrial and sarcomere protein deacetylation (Figure 2). While our current understanding suggests that deacetylation of sarcomere proteins (Figure 2A) contributes to cardiac dysfunction, less is known regarding the implications for mitochondrial protein acetylation. Reports have shown that a majority of the proteins within the mitochondria are hyperacetylated in response to HF [94]. As acetyl-CoA is abundant in the mitochondria and Sirt3 expression is attenuated, these data would suggest that mitochondrial hyperacetylation disrupts energy balance contributing to mitochondrial dysfunction and cardiac disease (Figure 2B). However, recent reports demonstrating that HDAC1 can localize to the mitochondria, imply that this hypothesis is not straightforward. Moreover, not all proteins in the mitochondria were hyperacetylated in HF patients, several proteins were deacetylated, which suggests a potential role for increased HDAC1 activity. Combined, this would suggest that decreased Sirt3 contributes to hyperacetylation of target proteins in the mitochondria, while increased HDAC1 deacetylates mitochondrial HDAC1-specific proteins in the regulation of cardiometabolic dysfunction (Figure 2B). Future examination for sarcomere and mitochondrial protein acetylation in the regulation of cardiac disease will be a topic of immense interest as investigators continue to explore the epigenetic and nonepigenetic actions for HDACs in the heart.

This Review specifically examined a role for zinc-dependent HDACs in the nonepigenetic regulation of mitochondrial and sarcomere protein dysfunction, yet important functions of HDACs in compartment-specific protein deacetylation require further investigation. For instance, this Review did not focus on the role for HDACs or HDACis in the direct interactions and regulation of endoplasmic reticulum stress, in which HDACs 1, 2 and 3 localize to the endoplasmic reticulum and regulate the unfolded protein response [105]. These studies were not

performed in the heart and thus examination on improved heart function would be warranted. Likewise, we did not discuss emerging data to support the role for HDAC inhibition on increased autophagy in the heart. While previous reports have shown that inhibition of HDACs can improve cardiac function in ischemia/reperfusion injury as well as protein toxicity via improvements in autophagy, these reports failed to assess whether increased autophagy was dependent on changes in autophagy-mediated genes or direct actions related to formation of the autophagolysosome [24,72]. It should be noted, however, that HDAC6 inhibition, a known tubulin deacetylase, has been suggested to increase tubulin acetylation and thus regulate microtubule stability. Further acetylated tubulin has the potential to mediate assembly of autophagic cargo necessary for increased degradation [72,106]. Together, these data would suggest that classical examination for HDACs and HDACis in the epigenetic regulation of cardiac disease must be broadened in order to better understand the role for the cardiac acetylome in the heart.

Executive summary

Lysine acetylation

- Lysine acetylation has historically been studied in the context of histone acetylation and the regulation of gene expression.
- Advances in proteomics technologies have allowed investigators to pursue protein modifications. From these studies, 4500 proteins have been identified that can be acetylated on approximately 15,000 acetylation sites.
- The function of many of these acetylated proteins, identified from these proteomics approaches, remains unclear.

Histone deacetylases & histone deacetylase inhibitors

- Histone deacetylases (HDACs) remove acetyl groups from lysine residues.
- HDACs are categorized as zinc-dependent or nicotinamide adenine dinucleotide (NAD⁺)-dependent HDACs.
- In the heart, zinc-dependent HDAC inhibitors block and potentially reverse myocardial remodeling.
- Early investigations of zinc-dependent HDAC inhibitors examined their impact on cardiac gene regulation.

Nonepigenetic implications for HDAC inhibitors

- Zinc-dependent HDACs can localize to the nucleus, cytosol, mitochondria, sarcomere and endoplasmic reticulum.
- HDAC1 localizes to the mitochondria where it deacetylates mitochondrial proteins. Inhibition of mitochondrial HDAC1 decreased mitochondrial reactive oxygen species generation.
- HDAC2 localizes to the sarcomere. Genetic or pharmacological inhibition of HDAC2 improves myofibril relaxation and thereby attenuates diastolic heart failure.
- HDAC3 localizes to the sarcomere and regulates myosin heavy-chain deacetylation. Acetylation of myosin heavy chain improves cardiac contractility, whereas deacetylation worsens cardiac contractile performance.
- HDAC6 localizes to the sarcomere. Genetic or pharmacological inhibition of HDAC6 improves myofibril contractility and attenuates systolic failure.

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