Histone deacetylase (HDAC) inhibitors attenuate cardiac hypertrophy by suppressing autophagy

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Histone deacetylases (HDACs) regulate cardiac plasticity; however, their molecular targets are unknown. As autophagy contributes to pathological cardiac remodeling, we hypothesized that HDAC inhibitors target autophagy. The prototypical HDAC inhibitor (HDACi), trichostatin A (TSA), attenuated both load- and agonist-induced hypertrophic growth and abolished the associated activation of autophagy. Phenylephrine (PE)-triggered hypertrophy and autophagy in cultured cardiomyocytes were each blocked by a panel of structurally distinct HDAC inhibitors. RNAi-mediated knockdown of either Atg5 or Beclin 1, two essential autophagy effectors, was similarly capable of suppressing ligand-induced autophagy and myocyte growth. RNAi experiments uncovered the class I isoforms of HDAC1 and HDAC2 as required for the autophagic response. To test the functional requirement of autophagic activation, we studied mice that overexpress Beclin 1 in cardiomyocytes. In these animals with a fourfold amplified autophagic response to TAC, TSA abolished TAC-induced increases in autophagy and blunted load-induced hypertrophy. Finally, we subjected animals with preexisting hypertrophy induced by transverse aortic constriction (TAC) to TSA, finding that ventricular mass reverted to near-normal levels and ventricular function normalized completely. Together, these data implicate autophagy as an obligatory element in pathological cardiac remodeling and point to HDAC1/2 as required effectors. Also, these data reveal autophagy as a previously unknown target of HDAC inhibitor therapy.

For some years, heart failure has remained the leading cause of death in industrialized nations, and the epidemic is rapidly expanding to include the developing world. In the United States alone, an estimated 5 million Americans have heart failure, a syndrome with a 5-y mortality of ~50% (1). Accordingly, heart failure, the end result of pathological cardiac remodeling elicited by a variety of stimuli, is responsible for a huge societal burden of morbidity, mortality, and cost.

During pathological cardiac remodeling, both anabolic and catabolic pathways are activated, and complex cascades of protein modifications and protein degradation are triggered. Among the major posttranslational modifications that take place is protein acetylation, a powerful regulator of function that may rival protein phosphorylation in terms of ubiquity and importance. In the case of histone proteins, acetylation of ε-lysine groups leads to decondensation of chromatin structure, enhanced accessibility to DNA-binding proteins, and consequent activation of transcription. This epigenetic mechanism is a powerful regulator of tumor responses to chemotherapy and adaptation to environmental triggers (e.g., hypoxia). Recently, two small molecular inhibitors of histone deacetylases (HDACs) gained Food and Drug Administration approval for cutaneous T-cell lymphoma: vorinostat (Zolinza), a hydroxamic acid derivative also known as suberoylanilide hydroxamic acid (SAHA) and romidepsin (Istodax), a depsipeptide HDAC inhibitor. Currently, there are more than 100 studies exploring the utility of this class of drugs in a variety of malignancies (www.clinicaltrials.gov).

In the case of heart disease, recent work has focused on protein acetylation in the control of cardiac gene expression. Studies in preclinical models suggest that inhibition of HDAC activity—using compounds that show promise in oncology trials—blunts pathological growth of cardiac myocytes (2–6). However, the cellular target(s) of these powerful agents in antagonizing disease progression is unknown.

During cell growth and repair, regulation of proteolysis is critical, especially in long-lived postmitotic cells such as cardiac myocytes, where cell replacement is limited. Long-lived proteins, protein complexes, aggregates of misfolded proteins, and organelles are degraded by lysosomes. Delivery of substrates to the lysosome can occur via several routes; the most common (macropolysome) involves sequestration in double-membrane autophagosomes and subsequent fusion with a lysosome (hereafter termed autophagy) (7–10).

Autophagy is an adaptive response to nutrient deprivation, as degradation of cytosolic elements provides amino acids and substrates for intermediary metabolism. Autophagy participates in constitutive turnover of mitochondria in highly oxidative tissues, such as cardiac myocytes, and in removal of damaged organelles. Opening of the mitochondrial permeability transition (MPT) and loss of mitochondrial membrane potential (MMP) triggers their autophagic scavenging (11). Conversely, dysregulation of autophagy contributes to the pathogenesis of several diseases, including neurodegenerative disorders, skeletal myopathy, cancer, and microbial infection (12). Recent reports demonstrate that multiple forms of stress, including pressure overload, chronic ischemia, and ischemia–reperfusion provoke an increase in autophagic activity in cardiomyocytes (13–17). In the common clinical scenario of excessive afterload, autophagy is activated and contributes to disease pathogenesis (13, 17). Thus, given that load-induced cardiomyocyte autophagy is maladaptive (13) and that HDACi is capable of blunting adverse remodeling (2–6), we hypothesized that maladaptive autophagy is HDAC dependent. We further posited that suppression of autophagic flux contributes to the salutary effects of HDAC therapy.

Results

Autophagy Triggered by Moderate Pressure Stress Is Abrogated by HDACi. We reported previously that autophagy is activated in afterload-induced cardiac hypertrophy/failure triggered by tight banding of the aorta and that this load-induced autophagic response is maladaptive, contributing to disease pathogenesis (13). To examine further the role of cardiomyocyte autophagy in pathological cardiac remodeling, we used a model of moderate pressure overload induced by transverse aortic constriction (TAC). In our hands, this model induces cardiac hypertrophic growth that reaches steady state at 3 wk and does not manifest systolic dysfunction or clinical heart failure at that time point (18). Male C57BL/6 mice were subjected TAC or sham surgery, and hearts were harvested at 3 wk. Autophagy was evaluated initially by Western blot detection of the faster-migrating, lipiddated isoform of LC3 (LC3-II). Significant increases in LC3-II

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levels, indicative of autophagosome accumulation, were observed (Fig. 1A and B). In a separate experiment, body weight (BW)-normalized heart weight (HW) was measured in individual explanted hearts 3 wk post-TAC, and LC3-II levels were measured in each heart. Interestingly, the level of LC3-II correlated tightly with steady-state cardiac mass \( r^2 = 0.89 \) (Fig. 1C). Activation of autophagy was similarly observed at an earlier time point (1 wk post-TAC), when the growth response is still active (Fig. 1D). Together, these results indicate that autophagy is activated robustly in the setting of modest afterload stress, raising the possibility that this activity participates in the cellular remodeling response. In addition, autophagy remains up-regulated under conditions of steady-state hypertrophy in a fashion that correlates with heart mass.

We have reported previously that HDACi is capable of blunting afterload-induced hypertrophic remodeling (3). To determine the effect of HDACi on load-induced autophagy, C57BL/6 mice were subjected to TAC or sham surgeries and randomized post-surgery to treatment with TSA (1 mg/kg per day). Four treatment arms were evaluated (sham + vehicle, sham + TSA, TAC + vehicle, and TAC + TSA), and hearts were harvested 1 wk postsurgery. TSA treatment was well tolerated in all groups. TSA abrogated the TAC-induced autophagic response, as indicated by normalization of LC3-II levels (Fig. 1D–F). Consistent with prior findings (3), TSA attenuated load-induced increases in heart mass (Fig. 1F).

The myocardium comprises multiple cell types, all of which are capable of eliciting an autophagic response (17). To evaluate autophagy specifically in cardiomyocytes, we studied transgenic mice that express GFP-tagged LC3 under control of the cardiomyocyte-specific promoter α-MHC (13). When cardiomyocyte autophagy is activated in these “autophagy reporter mice,” GFP-tagged LC3 is recruited to autophagosomes, and discrete puncta of GFP fluorescence are readily detected (13). Here, in agreement with findings from the LC3 immunoblots, we detected robust increases in GFP–LC3 puncta 1 wk after TAC (Fig. 1G and H). Importantly, these increases in autophagosome abundance were diminished to baseline levels in animals treated with TSA (Fig. 1G and H).

Activation of a fetal program of gene expression is indicative of maladaptive cardiac remodeling (19). As expected, TAC provoked significant increases in transcripts coding for ANF (atrial natriuretic factor), BNP (brain natriuretic peptide), and αSMA (α-smooth muscle actin) (Fig. 1I). In each case, HDAC inhibition with TSA blunted these increases (Fig. 1I). Together, these data demonstrate that HDAC inhibition with TSA suppresses the induction of autophagy in load-stressed myocardium.

**HDAC Inhibitors Block Hypertrophic Agonist-Induced Autophagy.** Next, we set out to determine whether the ability of HDACi to suppress pathological cardiac remodeling is cell autonomous. Also, we asked whether structurally distinct HDAC inhibitors are effective at suppressing autophagy and whether HDACi suppression of myocyte autophagy extends to other activators of myocyte growth. To answer these questions, neonatal rat ventricular myocytes (NRVMs) were treated with either phenylephrine (PE) (Fig. 2A) or endothelin-1 (ET-1) (Fig. 2B) in the presence or absence of TSA, and LC3-II levels were quantified (Fig. 2C). As expected, treatment of NRVMs with these agonists elicited cell growth and activation of the fetal gene program (Fig. 2F), and it elicited autophagosome accumulation as evidenced by increased levels of LC3-II (Fig. 2A–C). Treatment with TSA blocked agonist-induced LC3-II accumulation in each case (Fig. 2A–C). To corroborate this observation, we tracked GFP–LC3 delivered via GFP–LC3 fusion protein-expressing adenovirus. Infected cardiomyocytes displayed lower levels of autophagosome puncta in the setting of TSA treatment (Fig. 2D). Interestingly, baseline levels of autophagy assessed by both of these assays were diminished by TSA (Fig. 2A–D) consistent with the notion that NRVMs maintained in culture manifest activation of autophagy. Similar efficacy at blunting autophagy was observed with multiple HDAC inhibitors with molecular structures distinct from TSA (Fig. 2E–H). TSA blunted PE-induced activation of the fetal gene program (Fig. 2F).

HDACi-dependent decreases in LC3-II protein or aggregation of GFP–LC3 could stem from decreased autophagic flux or increased downstream lysosomal activity. To decipher the specific step in the autophagic process affected by HDAC inhibition, we treated cardiomyocytes with TSA in the presence or absence of lysosomal inhibitors E64D and pepstatin A. As expected, these agents provoked increases in LC3-II by blocking downstream degradative events (Fig. 2A and B). Importantly, however, TSA was able to block the accumulation of LC3-II even when downstream lysosomal processes were inhibited (Fig. 2A and B). On the basis of these observations, we conclude that HDAC inhibition interferes with autophagic flux upstream of LC3-II formation, rather than by activating lysosomal degradative events.

HDACi can provoke cell death in tumors (20). To test for activation of apoptosis in HDACi-treated hearts, we probed ventricular lysates for activated caspase 3, 6, and 9. Here, we failed to detect evidence of apoptosis in HDACi-treated hearts exposed to sham operation or TAC conditions in both wild-type (WT) or Beclin 1 TG genotypes (Fig. S2).
HDAC Inhibition Blocks the Exaggerated Growth Response in Beclin 1 Transgenic Mice. To determine whether activation of HDAC-dependent cardiomyocyte autophagy contributes to the load-induced remodeling response, we studied a transgenic mouse line that manifests an exaggerated autophagic response to pressure overload. We reasoned that any potential contribution of autophagy to load-induced growth will be most apparent in this model. Further, by amplifying the autophagic flux response, we are better able to discern the role of HDAC in targeting that response. We have reported previously that cardiomyocyte overexpression of the essential autophagy effector Beclin 1 results in an increased autophagic response to stress and an exacerbated growth response to moderate pressure overload (13). To test the effects of HDAC inhibition in these mice, we subjected them to TAC or sham surgery, randomizing them subsequently to TSA or vehicle injections. Animals were tested at 1 wk and 3 wk post-surgery. As expected, moderate pressure overload triggered an amplified growth response in Beclin 1 transgenics: TAC elicited an 80% (±5.0, n = 7–11) increase in HW/BW ratio in Beclin 1 Tg mice, compared with a 37% (±10.1, n = 7) increase in wild-type animals (Fig. 3A). Treatment with TSA, however, abolished the exacerbated cardiac growth (Fig. 3B and C). Interestingly, the amount of TAC-induced hypertrophy remaining in TSA-treated mice was similar in WT and Beclin 1 Tg animals (Fig. 3C), consistent with a model in which TSA blunts hypertrophy through suppression of autophagy. Indeed, the antihypertrophic effect of TSA in Beclin 1 mice was significantly greater than in WT (Fig. 3D), suggesting strongly that suppression of the amplified autophagic response contributed to the attenuated growth response.

To determine the cellular origin of the autophagic signal, we next examined autophagic activity in Beclin 1 Tg mice crossed into the αMHC–GFP–LC3 autophagy reporter line. As expected, we observed a substantially increased abundance of GFP–LC3 puncta in TAC-treated Beclin 1 Tg mice (Fig. S3A and B). And consistent with our model, TSA dramatically reduced the GFP–LC3 signal (Fig. S3A and B). Similar changes were observed in adult myocytes isolated from αMHC–Beclin 1:αMHC–GFP–LC3 compound transgenic mice (Fig. S3C). We also calculated the percentage of myocytes positive for GFP–LC3 puncta, finding similar results (Fig. S3D).

**Autophagic Activation Is Required for Cardiomyocyte Hypertrophy.** To test the functional necessity of cardiomyocyte autophagy in myocyte growth, we used siRNA technology to suppress the autophagy-essential gene Atg5 and Beclin 1. First, we tested the autophagy blocking agent chloroquine (CQ), which inhibits autophagosome–lysosome fusion (21), and hence functions distinctly from TSA. CQ blunted PE-induced protein synthesis to an extent comparable to that of TSA (Fig. S4A). Next, we used siRNA to evaluate the role of specific molecular elements. Efficiency of RNAi knockdown of Atg5 and Beclin 1 was confirmed using two independent constructs in each case to ensure specificity (Fig. S4B and C). We then stimulated NRVMs with the hypertrophic agonist PE, which induced stress fiber formation and an increase in cross-sectional area in cells exposed to scrambled siRNA (siRNA NC) (Fig. S4D and E). Suppression of autophagy by Atg5 knockdown diminished the hypertrophic response to PE (Fig. S4D and E). Similar effects were observed when Beclin 1, another protein essential to autophagic activation,
was down-regulated by RNAi (Fig. S4 F and G). The hypertrophic markers, ANF and αSMA, were similarly increased with PE treatment, and these increases were blunted by knockdown of either Atg5 or Beclin 1 (Fig. S4 H and I).

**PE-Induced Cardiomyocyte Autophagy Is Mediated by HDAC2.** Having identified autophagy as a specific target of HDAC inhibitor therapy, and HDACi-dependent suppression of autophagy as a requisite element of their antiremodeling effects, we next set out to identify the specific HDAC isoforms involved. Recent work has implicated class II HDACs (HDACs 4–7, 9, and 10) as suppressing heart growth and class I HDACs (HDACs 1–3, and 8) as promoting it (22). This fact, along with the fact that class IIa HDACs (HDACs 4, 5, 7, and 9) have minimal catalytic activity, led us to propose that relevant HDAC targets fell within the categories of class I and class IIb.

First, SB and VPA did not induce tubulin acetylation (Fig. S5 A and B) at concentrations that inhibited autophagy (Fig. 2 E–H). This suggests that HDAC6, a class IIb HDAC known to target tubulin, does not mediate the HDACi-inhibitory effect on autophagy. In contrast, VPA, a selective class I HDAC inhibitor (23) abolished PE-induced cardiomyocyte hypertrophy as measured by [3H]-leucine incorporation (Fig. S5C). This observation is consistent with the notions that class I HDACs promote cardiac remodeling and that their suppression mitigates it.

To pursue this further, we individually and selectively targeted HDAC1 and HDAC2 in cardiomyocytes. First, suppression of HDAC1 or HDAC2 diminished the autophagic response induced by PE (Fig. S5 D and E). Interestingly, even though HDAC1 and HDAC2 were suppressed similarly by RNAi, knockdown of HDAC2 was more effective at inhibiting autophagy. A second RNAi of independent sequence targeting HDAC2 revealed a similar degree of autophagy suppression (Fig. S5F). TSA was capable of suppressing autophagy even further, likely stemming from the fact that RNAi-dependent protein knockdown was not 100% (Fig. S5G). Aggregate data are shown in Fig. S5H.

To test the sufficiency of HDAC1 and HDAC2 in cardiomyocyte autophagy, we studied cardiomyocytes that overexpress HDAC1 or HDAC2. First, cardiomyocytes coinfected with GFP–LC3 adenovirus and HDAC1- (or HDAC2-) expressing lentivirus demonstrated increased autophagosome formation (Fig. S5 J–K). Also, LC3-II levels were increased in cardiomyocytes overexpressing HDAC2, whereas overexpression of HDAC1 had little effect (Fig. S5 I and J). (Interestingly, overexpression of HDAC1 suppressed levels of endogenous HDAC2). Overexpression of HDAC2 induced autophagy (Fig. S5K). Together, these data support the notion that HDAC2 plays a dominant role in mediating PE-induced autophagy in cardiomyocytes.

**HDAC Inhibitor Therapy Reverses Existing Cardiac Hypertrophy and Restores Cardiac Function.** Often, patients present to medical attention at the time of symptom development. As such, reversal of existing structural changes is a typical objective of therapeutic intervention. To investigate whether HDAC inhibitor therapy was capable of reversing existing cardiac hypertrophy, we subjected Beclin 1 transgenic mice to long-term pressure overload stress. To test the sufficiency of HDAC1 and HDAC2 in cardiomyocyte autophagy, we studied cardiomyocytes that overexpress HDAC1 or HDAC2. First, cardiomyocytes coinfected with GFP–LC3 adenovirus and HDAC1- (or HDAC2-) expressing lentivirus demonstrated increased autophagosome formation (Fig. S5 J–K). Also, LC3-II levels were increased in cardiomyocytes overexpressing HDAC2, whereas overexpression of HDAC1 had little effect (Fig. S5 I and J). (Interestingly, overexpression of HDAC1 suppressed levels of endogenous HDAC2). Overexpression of HDAC2 induced autophagy (Fig. S5K). Together, these data support the notion that HDAC2 plays a dominant role in mediating PE-induced autophagy in cardiomyocytes.

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Discussion

Protein acetylation has emerged as a powerful mechanism governing function, regulating both transcriptional and nontranscriptional events. Pharmacologic inhibition of enzymes that deacetylate proteins (HDACs) leads to chromatin relaxation and consequent transcriptional activation of many genes (24, 25); as numerous cytosolic proteins are governed by acetylation, HDACs have nongenomic actions, as well. Given this complexity, mechanisms underlying the benefit conferred by this promising strategy in the treatment of malignancy (26) remain obscure. As recent work has uncovered salutary effects of HDAC inhibition in models of heart disease (3, 6), we set out to decipher mechanisms of HDAC-dependent cardiac plasticity. First, we report that HDAC activity is required for hypertrophic growth of the myocardium, such that inhibition of HDAC activity is capable of preventing or reversing pathological cardiac remodeling elicited by pressure-overload stress. Second, as cardiomyocyte autophagy is a hallmark feature of pathological remodeling, we tested the effects of HDACi on autophagy, finding that multiple structurally distinct HDAC inhibitors are capable of blocking this pathway of protein and organelle degradation. Third, our findings go on to uncover cardiomyocyte autophagy as a required element in the pathogenesis of load-induced cardiac hypertrophy. Fourth, we report an interesting correlation between the extent of cardiac hypertrophic growth in vivo and the steady-state level of cardiomyocyte autophagic activity. Finally, we identify two class I HDAC isoforms, HDAC1 and HDAC2, as specifically involved in the cardiomyocyte autophagic response and the critical point of action of HDACi therapy.

Class I HDAC Activity Is Required for Pathological Cardiac Growth. HDACs are a family of enzymes which have been divided into four classes. Before this study, the HDAC inhibitors reported to have heart mass correlated with echocardiography-based estimates, corroborating that HDACi therapy was capable of eliciting hypertrophy reversal despite the persistence of TAC-induced increases in afterload.
antiremodeling capacity in the stressed heart inhibit both class I and II HDACs collectively. However, it is important to differentiate the functions of these two classes of HDACs, as they accomplish distinct functions in the process of cardiac growth. One difference is that class I HDACs share substantial sequence homology, and genetic studies in mice have revealed both redundant and isoform-specific functions (22). Another difference is that class I HDACs are prohypertrophic (27), and genetic inactivation of HDAC1 results in early embryonic lethality (28). Conversely, HDAC2 deficiency provokes lethal cardiomyopathy (29) or viable animals with reduced body weight (27, 30, 31), depending on experimental context and genetic strain.

In contrast, class IIa HDACs, such as HDAC5 and HDAC9, function as signal-responsive repressors of cardiac hypertrophy by inactivating MEF2 (32–34), an action mediated independently of enzymatic deacetylase activity (32). Interestingly, recent studies have elucidated the biochemical basis for the different enzymatic activities of class I and class IIa HDACs. A highly conserved tyrosine residue located in the catalytic pocket of vertebrate class I HDACs is replaced by histidine in the class IIa isoforms. As this tyrosine residue functions as a transition state stabilizer in the deacetylation reaction, this alteration is suggested to account for the more than 1,000-fold reduction in the catalytic activity of class IIa HDACs compared with class I (35).

Given these results, we focused on class I and IIb HDACs as candidates responsible for the augmented autophagic activity induced by stress in cardiac myocytes. We eliminated class IIb HDACs on the basis of the fact that these HDACs are resistant to sodium butyrate and valproic acid (23, 36), each of which is a histone deacetylase (HDAC) inhibitor. Therefore, we assayed evidence implicating HDAC1 and HDAC2 as the major mediators of increased autophagy during neurohumoral agonist-induced myocyte growth in vitro.

Activation of Autophagic Flux Is Required for Cardiomyocyte Hypertrophic Growth. Data presented here demonstrate that elevated autophagy is a required element in hypertrophic growth of the myocardium. First, amplification of autophagy by Beclin 1 overexpression greatly increased the pathological remodeling elicited by pressure stress. In this instance, as well as in banded wild-type mice, HDAC1 prevented hypertrophic growth. Second, in the case of the Beclin 1 transgensics, HDAC1 reverted the growth response to that seen in wild type; in other words, it abrogated the increment in growth associated with increased autophagy, suggesting that HDAC activity is uniquely involved in the autophagic response.

Our earlier studies have shown that Beclin 1 haploinsufficiency is associated with blunting of the autophagic response and diminished growth in the setting of pressure overload (13). Overexpression of Beclin 1, conversely, leads to increased induction of autophagy and exaggerated, maladaptive hypertrophic growth (13). These findings suggest that autophagy is an important element in cardiac hypertrophy and pathological remodeling. However, they do not address the critical question of whether stress-induced autophagy is required for hypertrophic growth. In the current study, we sought to answer this question by selectively targeting autophagy-required genes by RNAi. We found that knocking down Beclin 1 and ATG5 diminished PE-induced cardiomyocyte hypertrophic growth and blunted expression of hypertrophic markers. In aggregate, these data lend support to the idea that autophagy is a necessary component of cardiac hypertrophy and a rate-limiting step for cardiomyocyte growth under stress conditions.

HDAC1 Targets Load-Induced Cardiomyocyte Autophagy. Our findings go on to uncover a robust and specific effect of HDAC1 to suppress cardiomyocyte autophagy. Modulation of autophagy has been suggested previously as a possible mechanism of HDAC inhibition action in the context of cancer chemotherapy. However, depending on the cell type, HDAC1 can promote or suppress tumor cell autophagy. For example, Shao et al. showed initially that suberoylanilide hydroxamic acid (SAHA)-induced cell death in human cancer cell lines with features of autophagosome formation (37). This type of cell death was not prevented by abrogation of apoptosis by Apaf-1 knockdown, Bcl-XL overexpression, or caspase inhibition (37). Reports from two more recent studies, however, demonstrate that inhibiting autophagy dramatically enhanced the anticancer effect of SAHA (38, 39). In fact, the autophagic inhibitor chloroquine has entered clinical trials for treating breast cancer and small cell lung cancer (www.clinicaltrials.gov). Thus, HDAC inhibitors, an emerging and promising strategy in oncology, induce or inhibit autophagy depending on the cellular context.

As autophagy is critical to protein and organelle quality control, beneficial effects from its inhibition might seem counterintuitive. However, emerging evidence points to a requirement that autophagic activation remain fixed within a zone where its adaptive effects can take place (16, 17); when autophagic flux is activated to excessive levels, or when it drops below a certain threshold, then pathological effects emerge (a phenomenon we have termed the “goldilocks” zone of autophagy—not too much, not too little). Therefore, it is important to recognize that HDACi likely does not completely eliminate cardiomyocyte autophagy; rather, it suppresses autophagic flux without altering basal, constitutive levels of activation. Thus, when autophagy is activated to excessive levels—triggering maladaptive effects within the myocyte—HDACi reverts the response back toward basal levels, apparently without undermining the requirement of basal, constitutive autophagy in protein quality control.

Blunting Catabolic Pathways as a Therapeutic Strategy. Increases in anabolic activity during hypertrophic growth have been widely described, and numerous pathways, including calcineurin/NFAT, MEF2/HDAC, G protein-coupled receptors, receptor tyrosine kinases, PI3 kinase/Akt, and MAPK are implicated (40). The role(s) of catabolic processes, critical to the growing cell’s increased demand for protein quality control, are less well characterized. It has been known for some time that protein degradation by the ubiquitin-proteasome system (UPS) is activated in animal models of cardiac hypertrophy and in human cardiomyopathy (41, 42). This study uniquely examines the functional necessity of autophagic flux to myocyte growth.

At first glance, it seems paradoxical that blunting a mechanism of protein degradation suppresses cell growth. Indeed, one might imagine that activation of autophagy in isolation would lead to myocyte shrinkage, and such a finding has been reported previously (43). However, we and others have reported unequivocal growth-associated increases in autophagic flux. As noted above, the UPS, another major catabolic process, is well known to be activated during cell hypertrophy (41, 42). Thus, we favor a model where cell growth is accompanied by activation of mechanisms of protein degradation, which serve two functions: (i) these pathways perform critical actions in the restructuring of a growing myocyte and in eliminating misfolded proteins; (ii) excessive activation of autophagic flux targets critical cellular elements required for cell viability, thereby contributing to disease progression. Further, precedent exists for catabolic events being required for cell growth. For example, proteasome inhibition can prevent, and even reverse, cardiac hypertrophy induced by pressure overload or neuroendocrine agonists (41, 44, 45). Here, we show that inhibiting the other major catabolic process in cardiac myocytes, i.e., autophagy, likewise confers an antihypertrophic response.

One might speculate that suppressing overactive autophagy in the setting of myocyte remodeling would lead to accumulation of cellular debris (e.g., protein aggregates, dysfunctional organelles) that escape the now-suppressed autophagic response. Our experimental evidence, however, does not support this contention. Pharmacological suppression of the UPS is well tolerated in models of cardiac hypertrophy (41, 44, 45), and HDACi therapy was well tolerated here and previously (3), even during long-term trials involving up to 9 wk of treatment.
In addition to the therapeutic promise of HDACi in cardiac hypertrophy, Danon disease is likely another myopathy that can be targeted by HDACi. LAMP-2 deficiency in these patients results in lysosomal dysfunction, accumulation of autophagic vacuoles, and consequent skeletal and cardiac myopathies (46). Currently, there is no specific treatment. It is conceivable that decreasing autophagic flux by HDACi might diminish autophagosome accumulation and lessen phenotypic severity.

Together, these facts suggest that targeting catabolic processes activated by stress may be a unique strategy in the treatment of heart disease. Critically, it will be important to titrate this suppression; complete abrogation of autophagy through the deletion of Atg5, a gene required for autophagy (7–10), results in rapid and spontaneous development of end-stage heart failure (14). Similarly, recent work demonstrates that basal levels of autophagy are required for maintenance of skeletal muscle mass (47).

**Conclusion.** Data presented here demonstrate that HDAC activity is required for stress-induced cardiomyocyte autophagy and show that the class 1 HDACs, HDAC1 and HDAC2, are critically involved. This augmented autophagic response is required for the load-induced hypertrophic growth process. We report that the antihypertrophic action of HDAC inhibitors is (due, at least in part, to the unique action of inhibiting augmented autophagic flux.

**Materials and Methods**

**Primary Culture of NRVMs.** Cardiomyocytes were isolated and plated as described previously (48).

Additional details regarding materials and methods are provided in *SI Materials and Methods.*

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