Histone Deacetylase 1 (HDAC1) Negatively Regulates Thermogenic Program in Brown Adipocytes via Coordinated Regulation of Histone H3 Lysine 27 (H3K27) Deacetylation and Methylation*

Inhibiting class I histone deacetylases (HDACs) increases energy expenditure, reduces adiposity, and improves insulin sensitivity in obese mice. However, the precise mechanism is poorly understood. Here, we demonstrate that HDAC1 is a negative regulator of the brown adipocyte thermogenic program. The Hdac1 level is lower in mouse brown fat (BAT) than white fat, is suppressed in mouse BAT during cold exposure or β3-adrenergic stimulation, and is down-regulated during brown adipocyte differentiation. Remarkably, overexpressing Hdac1 profoundly blocks, whereas deleting Hdac1 significantly enhances, β-adrenergic activation-induced BAT-specific gene expression in brown adipocytes. β-Adrenergic activation in brown adipocytes results in a dissociation of HDAC1 from promoters of BAT-specific genes, including uncoupling protein 1 (Ucp1) and peroxisome proliferator-activated receptor γ co-activator 1α (Pgc1α), leading to increased acetylation of histone H3 lysine 27 (H3K27), an epigenetic mark of gene activation. This is followed by dissociation of the polycomb repressive complexes, including the H3K27 methyltransferase enhancer of zeste homologue (EZH2), suppressor of zeste 12 (SUZ12), and ring finger protein 2 (RNF2) from (and concomitant recruitment of H3K27 demethylase ubiquitously transcribed tetratricopeptide repeat on chromosome X (UTX) to) Ucp1 and Pgc1α promoters, leading to decreased H3K27 trimethylation, a histone transcriptional repression mark. Thus, HDAC1 negatively regulates the brown adipocyte thermogenic program, and inhibiting Hdac1 promotes BAT-specific gene expression through a coordinated control of increased acetylation and decreased methylation of H3K27, thereby switching the transcriptional repressive state to the active state at the promoters of Ucp1 and Pgc1α. Targeting HDAC1 may be beneficial in prevention and treatment of obesity by enhancing BAT thermogenesis.

Obesity develops when a persistent imbalance between energy intake and energy expenditure occurs (1). Whereas white adipose tissue (WAT)3 is involved in energy storage, the role of brown adipose tissue (BAT) is to dissipate energy as heat due to its unique expression of uncoupling protein 1 (Ucp1) (2–4). In rodents, there exist two types of brown adipocytes. Traditional brown adipocytes are located in discrete areas, whereas “inducible” beige adipocytes are dispersed in WAT (5–8) and can be induced by cold exposure or β3-adrenergic receptor activation (9–13). The ability of brown/beige adipocytes to produce adaptive thermogenesis depends on the unique expression of UCP1 in the inner mitochondrial membrane, which serves to uncouple oxidative phosphorylation from ATP synthesis, thereby profoundly increasing energy expenditure (2–4). Recent reports demonstrate that adult humans also possess metabolically active brown fat; the amount of brown fat is inversely correlated with body weight but positively correlated with energy expenditure (14–16). This important discovery provides new insight into the mechanisms regulating energy homeostasis in adult humans and suggests that increasing functional brown/beige adipocytes in humans is a novel and promising target in treating obesity.

Although the genome is fixed and identical in all cells, the epigenome, the combination of all genome-wide DNA and chromatin modifications, is continuously modified in response to developmental, environmental, physiological, and pathological cues (17–19). Epigenetic modifications, including DNA methylation, histone acetylation, and methylation, result in organization of the chromatin structure on different hierarchical levels, which regulate gene expression (17–19). Recent evidence suggests that epigenetic mechanisms have emerged as an important link between environmental factors and obesity

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3 The abbreviations used are: WAT, white adipose tissue; BAT, brown adipose tissue; HDAC, histone deacetylase; HDAC1, HDAC inhibitor; H3K27, histone H3 lysine 27; H3K27ac, H3K27 acetylation; H3K27me3, H3K27 trimethylation; PPARγ, peroxisome proliferator-activated receptor γ; PGC1α, proliferator-activated receptor γ co-activator 1α; NE, norepinephrine; IP, immunoprecipitation; C/EBPα, CCAAT/enhancer-binding protein α; CRE, cAMP-response element; CREB, CRE-binding protein; DIO, diet-induced obese; TSA, trichostatin A; SAHA, suberoylanilidehydroxamic acid (vorinostat).
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(20–24). For example, *Ucp1* promoter activity is regulated by changes in DNA methylation status (25). The histone H3 Lys-9 demethylase JHDM2a directly regulates *Ucp1* expression, and genetic deletion of *Jhdm2a* in mice results in obesity (26). This is a newly emerging research area; however, much remains to be discovered regarding how epigenetic mechanisms regulate metabolism and energy homeostasis.

Histone acetylation and deacetylation are regulated by the balanced action of histone acetyltransferases and histone deacetylases (HDACs) (17–19). HDACs consist of four major classes: class I (HDAC1, -2, -3, and -8), class II (HDAC4, -5, -6, -7, -9, and -10), class III (SIRT1 to -7), and class IV (HDAC11). The class I HDACs (Sirt1–Sirt7) form a structurally distinct class of NAD-dependent enzymes and can be inhibited by nicotinamide (17, 18). Recent data suggest that HDACs have emerged as important players in the regulation of energy and glucose homeostasis (27). For example, it is reported that class I HDAC inhibitor (HDACi) MS-275 ameliorates obesity and diabetes in animal models through stimulation of oxidative phosphorylation and mitochondrial function in muscle and fat (28). However, the precise mechanism by which class I HDACi exerts these effects is poorly understood.

In the current study, we have identified *Hdac1* as a prominent epigenetic target in regulating the thermogenic program in brown adipocytes. Using loss- and gain-of-function approaches, we demonstrated that *Hdac1* deficiency activated, whereas *Hdac1* overexpression repressed, transcription of brown adipocyte-specific gene expression through regulating the acetylation and methylation status of histone H3 lysine 27 (H3K27) on promoter and enhancer regions of *Ucp1* and peroxisome proliferator-activated receptor γ (Pparγ) co-activator 1-α (Pgc1α). Thus, our data suggest that epigenetics plays an important role in brown adipocyte thermogenesis, and *Hdac1* may be an important regulator during this process.

**Materials and Methods**

*Mice—* C57BL/6J (B6) and A/J mice (Jackson Laboratories, Bar Harbor, ME) were housed with a 12/12-h light/dark cycle in temperature- and humidity-controlled rooms with free access to water and food. To study the role of *Hdac1* in BAT thermogenic function, 7–8-week-old mice were exposed to cold conditions (4 °C) or intraperitoneally injected with genic function, 7–8-week-old mice were exposed to cold conditions (4 °C) or intraperitoneally injected with

**Table 1**

*Sequences of siRNA smart pools for individual HDACs*

| Gene Symbol | Catalog Number | GENE ID | Gene Accession | GI Number | Sequence |
|-------------|----------------|--------|----------------|-----------|----------|
| HDAC1       | L-040287-02-0005 | 4333759 | NM_008228      | 6680192   | AUAACAGCAUAGCGCCGUGA UCAAGGACAGGGCGACAGGU GACAGCAUACACAUC GAA GUCCCUAGUACAA |
| HDAC2       | L-046158-00-0005 | 15182   | NM_008229      | 6680194   | CCAAUGAUGAGCUCCAGAUAA CAAUGUAGUAGUAGACUA GAGGGGAGCUGAGGA AUA GAAAGGAGGGAGG |
| HDAC3       | L-043553-02-0005 | 15183   | NM_010411      | 7110820   | GGGGAGGACUUCCAGAGUA AGGUGAACUAGCGAGAAGU GAGAAGCGGCAAGG |
| HDAC4       | L-043625-00-0005 | 208727  | NM_027225      | 46402200  | GGGUAGGUGUGACUAAGGAA GUGAAGAGAGGAGACAGAAU GAAAAGGCUCAAGG |
| HDAC5       | L-063182-00-0005 | 15184   | NM_001042      | 6996923   | UACGACGCGCCGAGCGGU AGGGAAGAGAGGAGACAGAAU GAAAAGGCUCAAGG |
| HDAC6       | L-043455-02-0005 | 15185   | NM_001043      | 6996925   | GAGGAGCCUAGCGAGUA GAGAAGGGCUCAAGG |
| HDAC7       | L-040703-00-0005 | 56233   | NM_019572      | 40254547  | CUGGUAAGAAGGAGGCAU GUGGCUAGGUUCCUGAAGA GUAGUAAAGCAGG |
| HDAC8       | L-059610-00-0005 | 70315   | NM_027382      | 5637202   | UAGCUAGUAGCAGGAGA GUGAAGAAGCGGCAAGA GAGCCGAGG |
| HDAC9       | L-066143-00-0005 | 79221   | NM_041240      | 70778860  | UAGUCAGAGGAGGCAU GAGAAGCGGCAAGA GAGCCGAGG |
| HDAC10      | L-046437-00-0005 | 170787  | NM_189198      | 40353217  | UAGGUGAGAAGGUGCGA GAGAAGCGGCAAGA GAGCCGAGG |
| HDAC11      | L-051918-00-0005 | 232232  | NM_144919      | 21450316  | UAGGUGAGAAGGUGCGA GAGAAGCGGCAAGA GAGCCGAGG |

**Small Interfering RNA (siRNA) and Plasmid DNA Transfection**—The MGC fully sequenced mouse cDNA expression plasmids for *Hdac1* (Clone ID 4976514), enhancer of zeste homologue (Ezh2) (Clone ID 3586689), suppressor of zeste 12 (Suz12) (Clone ID 6821922), ring finger protein 2 (Rnf2) (Clone ID 4021046), the ON-TARGET plus mouse *Hdac1* siRNA-SMART pool (L-040287-02-0005), and *Hdac1–Hdac11* siRNAs for our initial screening were purchased from GE Healthcare. The sequences for *Hdac1–Hdac11* siRNAs are listed in Table 1. All other plasmids were in mammalian expression vector pSPORT6 except for Suz12, which was in a non-expressing vector, pXY-Asc. To clone Suz12 into pSPORT6 vector, Pacl and Sall were used to release the Suz12 cDNA insert from pXY-Asc and subcloned into pSPORT6. The overexpressing plasmids or siRNAs were transfected into BAT1 or HIB-1B brown adipocytes by Amaza Nucleofector II Electro-porator (Lonza) using Amaza cell line nucleofector kit L according to the manufacturer’s instructions (Lonza). Briefly, at
cell samples were homogenized in a modified radioimmune precipitation assay lysis buffer containing 50 mM Tris-HCl, 1 mM EDTA, 1 mM NaCl, 1 mM sodium fluoride, 1 mM phenylmethylsulfonyl fluoride, 1% protease inhibitor mixture, 1% Nonidet P-40, supplemented with protease inhibitors, pH 8.0) using a Dounce homogenizer to isolate nuclei. The nuclei were resuspended in nuclei lysis buffer (50 mM Tris-HCl, 10 mM EDTA, and 1% SDS, supplemented with protease inhibitors, pH 8.1) and sonicated to shear genomic DNA to an average fragment length of 200–1,000 bp with a Diagenode Bioruptor (Diagenode, Denville, NJ). Lysates were centrifuged, and the supernatants were collected. Fifty μl of each sample was removed as the input control. The supernatants underwent overnight immunoprecipitation, elution, reverse cross-linking, and protease K digestion, according to the manufacturer’s manual. A mock immunoprecipitation without antibody was also included for each sample. Eluted DNA was analyzed by real-time PCR using SYBR Green quantitative PCR (Life Technologies). Primer sequences used in this study were as follows: Ucp1 proximal promoter, 5′-CCCCATAGCAGCTTT-GGA-3′ and 5′-CTTGGAGACGCTCAAGAGGT; Ucp1 enhancer region, 5′-CTCTTACAGCGTACAAGAGG-3′ and 5′-AGTCTGAGAAGGTGTT-GA-3′; Pgc1α cAMP-response element (CRE) region, 5′-CAAAAGCTGGCTTCAG-TCAACA-3′ and 5′-AAAAGTGAGCTGGGCTGTCA-3′.

**Statistical Analysis**—Data were expressed as mean ± S.E. Statistical tests were performed using SPSS software (version 16.0, SPSS Inc., Chicago, IL). One-way analysis of variance followed by the Student-Newman-Keuls test was used to determine multiple comparisons. Statistical significance was accepted at p < 0.05.

**Results**

Hdac1 Is Enriched in WAT Versus BAT and Is Down-regulated in BAT during β-Adrenergic Stimulation and during Brown Adipocyte Differentiation—Recent studies reported that class I but not class II HDACs enhanced whole body energy expenditure and attenuated high fat diet-induced insulin resistance through increased mitochondrial biogenesis in skeletal muscle and adipose tissues (28). However, little is known about whether these effects are exerted directly through activating brown fat thermogenesis and which HDAC is responsible for these beneficial effects. Thus, we first knocked down individual HDACs (Hdac1–Hdac11) in brown adipocyte cell line HIB-1B cells using an siRNA approach. Interestingly, although knockdown of other HDACs exerted minimal effects, reducing the expression of the class I HDAC family member Hdac1 by siRNA knockdown significantly enhanced NE-stimulated Ucp1 expression (Fig. 1A). This prompted us to investigate the role of HDAC1 in regulating the brown adipocyte thermogenic program.

We first measured the HDAC1 expression pattern in brown and white adipose tissues. As expected, UCP1 protein was enriched in BAT but not detectable in epididymal WAT (Epi (WAT)) in adult mice housed at ambient temperature.
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TABLE 3
Primer/probe sequences for gene expression experiments

| Gene Symbol | Company | Catalog no. |
|-------------|---------|-------------|
| Hdac1       | Applied Biosystems | Mm02391771-g1 |
| Pdmd16     | Applied Biosystems | Mm00712554-m1 |
| Dio2        | Applied Biosystems | Mm00515664-m1 |
| Acox1       | Applied Biosystems | Mm01246834-m1 |
| Cox1        | Applied Biosystems | Mm00225345-g1 |
| Eva1        | Applied Biosystems | Mm0068397-m1 |
| Otop1       | Applied Biosystems | Mm0054705-m1 |
| Fg21        | Applied Biosystems | Mm00840165-g1 |
| Cpt1b       | Applied Biosystems | Mm00487191-g1 |
| Cidea       | Applied Biosystems | Mm00432554-m1 |
| Ppar        | Applied Biosystems | Mm00440945-m1 |

TABLE 4
Primer/probe sets for gene expression experiments

| Gene Symbol | Company | Catalog no. |
|-------------|---------|-------------|
| Hdac1       | Applied Biosystems | Mm02391771-g1 |
| Pdmd16     | Applied Biosystems | Mm00712554-m1 |
| Dio2        | Applied Biosystems | Mm00515664-m1 |
| Acox1       | Applied Biosystems | Mm01246834-m1 |
| Cox1        | Applied Biosystems | Mm00225345-g1 |
| Eva1        | Applied Biosystems | Mm0068397-m1 |
| Otop1       | Applied Biosystems | Mm0054705-m1 |
| Fg21        | Applied Biosystems | Mm00840165-g1 |
| Cpt1b       | Applied Biosystems | Mm00487191-g1 |
| Cidea       | Applied Biosystems | Mm00432554-m1 |
| Ppar        | Applied Biosystems | Mm00440945-m1 |

(Fig. 1B). Interestingly, in contrast to UCP1 expression, HDAC1 protein level was enriched in WAT but much lower in BAT (Fig. 1B). BAT1 and HIB-1B brown adipocyte differentiation was marked with significantly increased UCP1 mRNA and protein levels (Fig. 1, C and D), which were accompanied by decreased mRNA and protein levels of HDAC1 (Fig. 1, C and D).

It is well documented that cold temperature triggers sympathetic discharge, leading to the release of NE in BAT and WAT (3, 38). Thus, we tested whether Hdac1 expression in BAT was regulated by sympathetic stimulation. A/J mice were exposed to cold (4°C) or intraperitoneally injected with β2-adrenergic agonist (CL-316,243) for up to 7 days. Hdac1 expression was profoundly reduced in BAT after 6 h of cold exposure and tended to stay reduced up to 7 days of cold exposure (Fig. 1E). A similar reduction of Hdac1 expression was also observed in BAT after CL-316,243 injection for 7 days (Fig. 1F). These data indicate that decreased Hdac1 may be a marker of mature brown adipocytes and that Hdac1 may negatively regulate the BAT thermogenic program.

Hdac1 Regulates Brown Adipocyte Thermogenic Gene Expression—To investigate the role of Hdac1 in the regulation of brown fat-specific gene expression, we performed gain- or loss-of-function experiments in differentiated BAT1 cells. It has been reported that Hdac1 regulates the early steps of adipocyte differentiation (39). Thus, to avoid the confounding effects of Hdac1 on differentiation, we have focused on the role of Hdac1 in mature brown adipocyte gene expression by knocking down or overexpressing Hdac1 in BAT1 cells after 4–6 days of differentiation. Hdac1 mRNA in knockdown cells was reduced by 80%, as measured by real-time RT-PCR, and HDAC1 protein expression was also significantly decreased, as assessed by immunoblotting (Fig. 2A). As expected, knocking down Hdac1 in mature BAT1 cells did not affect the mRNA levels of general adipocyte differentiation markers, including Ppar, CCAAT/enhancer-binding protein α (C/ebpα), sterol regulatory element-binding protein 1C (Srebp1c), adipocyte protein 2 (aP2), and adiponectin (AdipoQ) (Fig. 2B). Remarkably, Hdac1 knockdown in BAT1 cells significantly increased basal and isoproterenol-stimulated expression of brown adipocyte-specific genes, including Ucp1, Pgc1α (Fig. 2C), Pgc1β, PR domain-containing protein 16 (Prdm16), Ppara, type 2 deiodinase (Dio2), acyl-CoA oxidase 1 (Acox1), cytochrome c oxidase 1 (Cox1), epithelial V-like antigen 1 (Eva1), and otopetin 1 (Otop1) (Fig. 2D). In addition, isoproterenol significantly stimulated UCP1 protein expression in BAT1 cells, which was further enhanced by Hdac1 knockdown (Fig. 2E).

We then overexpressed Hdac1 in BAT1 brown adipocytes, as shown by increased HDAC1 protein expression (Fig. 3A). Overexpression of Hdac1 in BAT1 brown adipocytes significantly suppressed basal and/or isoproterenol-stimulated brown adipocyte-specific gene expression, including Ucp1, Pgc1α, Pgc1β, Prdm16, C/ebpβ, fibroblast growth factor 21 (Fg21), Ppara, carnitine palmitoyltransferase 1B (Cpt1b), Acox1, Cox1, Otop1, and cell death-inducing DNA fragmentation factor α subunit-like effector A (Cidea) (Fig. 3, B and C).

Similar results were observed in HIB-1B brown adipocyte cell line. In HIB-1B cells with Hdac1 siRNA knockdown, Hdac1 mRNA was reduced by more than 80% (Fig. 4A), and knocking down Hdac1 significantly increased basal and/or NE-stimulated brown adipocyte-specific gene expression, including Ucp1 (Fig. 4B), Pgc1α, Pgc1β, Cox1, Acox1, Cidea, Prdm16, Cpt1b, and Ppara (Fig. 4, C and D). Similarly, Hdac1 overexpression significantly reduced NE-stimulated Ucp1 expression in HIB-1B cells (Fig. 4E). These data demonstrate that Hdac1 is a negative regulator of the thermogenic program in mature brown adipocytes.

Hdac1 Deficiency Mediates the Beneficial Effects of Class I HDACi on Improving Metabolic Phenotypes—It is reported that the pan-HDACi SAHA and class I HDACi MS-275, but not class II HDACi MC-1568, ameliorate obesity and diabetes in animal models, possibly through stimulation of oxidative phosphorylation and mitochondrial function in muscle and fat (28). To our surprise, treating BAT1 brown adipocytes with the pan-HDACi TSA and SAHA resulted in complex effects on brown fat-specific gene expression. Whereas TSA and SAHA enhanced isopropenol-stimulated expression of genes involved in mitochondria oxidative activity, such as Pgc1α and Acox1, they significantly suppressed isopropenol-stimulated expression of other BAT-specific genes, including Ucp1, Prdm16, Ppara, and Otop1 (Fig. 5, A and B). In contrast, the
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A reducing Hdac1 expression by siRNA knockdown in HIB-1B brown adipocytes up-regulates Ucp1 expression. HIB-1B cells were transfected with scramble siRNA or siRNAs targeting individual HDACs. After 48 h, cells were treated with or without 1 μM norepinephrine for 4 h, and RNA was isolated for gene expression measurements. Class I HDACs are shown in blue. UCP1 and HDAC1 protein levels in BAT and epididymal (Epi) WAT from C57BL/6J (B6) mice. C and D, UCP1 and HDAC1 RNA (C) and protein (D) levels in BAT1 and HIB-1B brown adipocytes during differentiation. E and F, Hdac1 expression in A/J mouse BAT tissues exposed to 4 °C at the indicated time (E) or treated with β3-agonist CL-316,243 for 7 days. Data are expressed as mean ± S.E. (error bars), * p < 0.05 versus undifferentiated samples (C), time 0 (E), or control (Ctrl) (F).

class I HDACi MS-275 significantly enhanced isoproterenol-stimulated BAT-specific gene expression, including Ucp1, Elovl3, and Pgc1α, whereas it had minimal effects on Prdm16 and Pparγ expression (Fig. 5C).

We also tested whether Hdac1 might be the molecular target of class I HDACi MS-275 in regulating BAT-specific gene expression in BAT1 cells. As expected, MS275 or Hdac1 siRNA knockdown enhanced isoproterenol-stimulated gene expression of Ucp1 and Pgc1α in BAT1 cells to a similar extent when treated individually; however, they did not exert any additive effects on these gene expressions when treated in combination in BAT1 cells (Fig. 5D). Our data suggest that Hdac1 may be the molecular target of the class I HDACi MS-275 in stimulating brown fat function.

Sympathetic Activation in Brown Adipocytes Reduces HDAC1 Binding and Increases H3K27 Acetylation at Ucp1 and Pgc1α Promoters—HDACs repress gene expression by removing acetyl groups from lysine residues in histone proteins at specific gene promoters (18). We reasoned that if HDAC1 indeed negatively regulates brown fat functions, sympathetic activation in BAT may trigger HDAC1 dissociation from promoter regions of key genes regulating brown fat thermogenesis. This may facilitate subsequent chromatin modifications and DNA unwinding, which can eventually lead to increased accessibility to transcription factor binding and gene activation (17–19).

Acetylation at histone H3 lysine 27 (H3K27ac) results in transcriptional activation (48). We found that isoproterenol treatment in BAT1 cells significantly increased H3K27ac levels at the enhancer and proximal promoter regions of Ucp1 and the CRE region at the Pgc1α promoter (Fig. 6A) upon sympathetic activation. As expected, in BAT of mice treated with β3-adrenergic agonist CL-243,316, binding of HDAC1 was significantly reduced at the enhancer and proximal promoter regions of Ucp1 and the CRE cis-element region at the Pgc1α promoter (Fig. 6D). In addition, isoproterenol treatment also significantly reduced HDAC1 binding to these promoter/enhancer regions in differentiated BAT1 brown adipocytes (Fig. 6E). These data indicate that β3-adrenergic stimulation in brown adipocytes dissociates HDAC1 from promoters of key genes regulating brown adipocyte thermogenesis, including Ucp1 and Pgc1α. This may facilitate subsequent chromatin modifications and DNA unwinding, which can eventually lead to increased accessibility to transcription factor binding and gene activation (17–19).
On the other hand, the acetylation levels at histone H3 lysine 14 (H3K14ac) and histone H3 lysine 9 (H3K9ac), two other lysine residues that can be modified by acetylation (18, 49), did not exhibit similar responsiveness to Hdac1 knock-down and/or isoproterenol stimulation in BAT1 cells (Fig. 6H). Thus, our data suggest that H3K27 may be the major target of HDAC1 in brown adipocytes regulating brown-specific gene expression.

Hdac1 Regulates Ucp1 and Pgc1α Expression by Further Modifying H3K27 Methylation—Except for acetylation, histone lysine residues can also be mono-, di-, and trimethylated and are associated with either gene repression or activation, depending on the lysine residues that are methylated and the degree of methylation. Trimethylation of H3K27 (H3K27me3) is a hallmark of gene repression, whereas trimethylation of histone H3 Lys-4 marks transcriptional activation (17–19). Importantly, histone acetylation and methylation mutually affect each other in the regulation of transcriptional process (49). We thus tested whether modulation of histone acetylation by HDAC1 led to further alterations in histone methylation. ChIP assay analysis demonstrated that isoproterenol treatment in BAT1 brown adipocytes resulted in a significant decrease in H3K27me3 levels at Ucp1 and Pgc1α promoters (Fig. 7, A and B). Reducing Hdac1 expression by siRNA knockdown mimicked isoproterenol’s effect by reducing H3K27me3 levels at these promoter/enhancer regions to a similar extent as isoproterenol and did not further decrease isoproterenol-suppressed H3K27me3 levels (Fig. 7A). In contrast, Hdac1 overexpression significantly blocked isoproterenol-suppressed H3K27me3 levels at these promoter/enhancer regions (Fig. 7B).

H3K27 methylation is dynamically regulated by both histone methyltransferases and demethylases (18, 50, 51). EZH2 is a
methyltransferase that specifically di- and trimethylates H3K27 (18, 50, 51), whereas lysine-specific demethylase 6A (KDM6A)/ubiquitously transcribed tetratricopeptide repeat on chromosome X (UTX) is a di- and trimethyl-H3K27 demethylase (52).

A ChIP assay demonstrated that isoproterenol treatment in BAT1 brown adipocytes resulted in a significant decrease in EZH2 binding at Ucp1 and Pgc1α promoters (Fig. 7, C and D). Reducing Hdac1 expression by siRNA knockdown mimicked isoproterenol’s effect by reducing EZH2 binding at these promoter/enhancer regions to a similar extent as isoproterenol and did not further decrease isoproterenol-suppressed EZH2 binding (Fig. 7C), whereas Hdac1 overexpression completely
reversed isoproterenol-induced suppression of EZH2 binding (Fig. 7D).

In contrast, isoproterenol treatment in BAT1 brown adipocytes resulted in a significant increase in UTX binding at Ucp1 and Pgc1α promoters (Fig. 7, E and F). Reducing Hdad1 expression by siRNA knockdown also induced a similar increase in UTX binding to the Ucp1 enhancer region and further enhanced isoproterenol-induced binding of UTX to the Ucp1 proximal promoter and Pgc1α CRE region (Fig. 7E), whereas HDAC overexpression completely prevented the isoproterenol-induced increase in UTX binding to these promoter/enhancer regions (Fig. 7F). Overall, our data demonstrated that Hdad1 may coordinately regulate H3K27ac and H3K27me3 levels at Ucp1 and Pgc1α promoters through differential recruitment of the H3K27 methyltransferase EZH2 and the H3K27 demethylase UTX, thus regulating the expression of these genes.

EZH2 is a component of the polycomb group proteins, which are known to mediate gene silencing by regulating chromatin structure (51). Two major polycomb group proteins exist in mammals, namely polycomb-repressive complex 1 and 2 (PRC1 and PRC2). PRC1 comprises three main components: ring finger protein 1 (RING1), RNF2, and BMI1 polycomb ring finger oncogene (BMI1). PRC2 comprises three major components: EZH2, SUZ12, and EED (embryonic ectoderm development). PRC2 promotes H3K27 methylation through the methyltransferase activity of EZH2 and also facilitates the recruitment of PRC1 onto methylated H3K27, which in turn leads to further gene repression (51). It has been shown that HDAC1 may be associated with PRC2 (51). We thus investigated whether HDAC1 regulates the recruitment of components of PRC1/2 to Ucp1 and Pgc1α promoters. The ChIP assay demonstrated that isoproterenol treatment in BAT1 cells decreased binding of SUZ12 (Fig. 8, A and C) and RNF2 (Fig. 8, B and D) to
Ucp1 and Pgc1α promoters. Importantly, reducing HDAC1 expression by siRNA knockdown mimicked isoproterenol's effect by inducing a similar decrease in SUZ12 and RNF2 binding and did not further reduce isoproterenol-suppressed SUZ12 and RNF2 binding at these promoters (Fig. 8, A and B), whereas Hdac1 overexpression completely prevented isoproterenol-suppressed SUZ12 and RNF2 binding to these promoters (Fig. 8, C and D).

We further performed co-IP experiments to test whether HDAC1 physically interacts with components of PRC1 and PRC2 complexes. Our data demonstrated that HDAC1 interacts with the PRC2 complex components Ezh2 and SUZ12 and the PRC1 component RNF2 in HEK293T cells overexpressing Hdac1, Ezh2, Suz12, and Rnf2 (Fig. 8E) and, most importantly, in endogenous BAT1 brown adipocytes (Fig. 8F).

Discussion

Recent data suggest that HDACs have emerged as important players in the regulation of energy and glucose homeostasis (27). The pan-HDAC inhibitors sodium butyrate and trichostatin A increase energy expenditure, reduce adiposity, and improve insulin sensitivity in diet-induced obese (DIO) mice (53). This is possibly exerted through the inhibition of class I HDACs, because the specific class I HDACi exerts similar effects in DIO mice, whereas class II HDACi has no effects (28). However, the precise mechanism by which class I HDACi exerts these beneficial effects is poorly understood. Here, we demonstrate that the class I HDAC1 negatively regulates the brown adipocyte thermogenic program. This is based on the following observations. First, in contrast to Ucp1 expression, which is usually enriched in BAT, Hdac1 is enriched in WAT, but its expression is much lower in BAT. Second, brown adi-
pocyte differentiation is associated with significantly up-regulated Ucp1 expression, which is concomitantly associated with reduction of Hdac1 RNA and protein levels. Third, sympathetic activation of BAT is associated with down-regulation of Hdac1 expression. These data suggest that Hdac1 may be a negative regulator of brown adipocyte thermogenic program. Indeed, knocking down Hdac1 in brown adipocytes was transfected with scramble or Hdac1 siRNA (A, C, and E) or pSPORT6- or pSPORT6-HDAC1-overexpressing plasmids (B, D, and F). Two days later, cells were treated with or without isoproterenol (Isop; 1 μM) for 3 h, and a ChIP assay was performed as described under "Materials and Methods." Data are expressed as mean ± S.E. (error bars). n = 4–6. *, p < 0.05. n.s., not statistically significant.

Interestingly, we found that whereas both the pan-HDACi TSA and SAHA and the class I-specific HDACi MS-275 (57) stimulate the expression of genes involved in mitochondrial function and oxidative activity, such as Pgc1α, the effect of these different classes of HDACis on the expression of other genes that are important for brown adipocyte differentiation and determination is different. For example, the class I HDACi MS-275 potently stimulates, whereas the pan-HDACis TSA and SAHA significantly suppress, isoproterenol-stimulated Ucp1 expression in brown adipocytes. In addition, TSA also significantly inhibited Prdm16 and Ppar expression, whereas MS-275 had minimal effects on these gene expressions. These TSA-suppressed genes are either key players in brown adipocyte differentiation and determination (Ppar and Prdm16) or a marker of brown adipocyte (Ucp1). Because TSA and SAHA inhibit both class I and II HDACs (57), and because it has expression of Hdac1 will be needed to study the role of HDAC1 in energy homeostasis and metabolism in whole animals.

FIGURE 7. HDAC1 regulates trimethylation of H3K27me3 at Ucp1 and Pgc1α promoters through coordinated regulation of EZH2 and UTX binding to BAT-specific promoters. A–F, H3K27me3 levels (A and B), EZH2 (C and D), and UTX binding (E and F) at Ucp1 and Pgc1α promoters in BAR1 cells. BAT1 brown adipocytes were transfected with scramble or Hdac1 siRNA (A, C, and E) or pSPORT6- or pSPORT6-HDAC1-overexpressing plasmids (B, D, and F). Two days later, cells were treated with or without isoproterenol (Isop; 1 μM) for 3 h, and a ChIP assay was performed as described under "Materials and Methods." Data are expressed as mean ± S.E. (error bars). n = 4–6. *, p < 0.05. n.s., not statistically significant.

Thus, our data suggest that HDAC1 may be important in regulating energy homeostasis in animal models, and inhibiting HDAC1 in brown adipocytes may contribute to the beneficial effects of class I HDACi in DIO mice (28). Further studies using genetic animal models with brown fat-specific deletion or overexpression of Hdac1 will be needed to study the role of HDAC1 in energy homeostasis and metabolism in whole animals.
been shown that class II HDACs are mainly involved in the regulation of cellular differentiation (58), the difference in the gene regulation between the pan-HDACi TSA and the class I HDACi MS-275 may be mainly due to the different functions of class I and class II HDACs. Thus, further studies are warranted to elucidate the differential effects of class I and II HDACIs on brown adipocyte function. Nonetheless, our data suggest that the class I HDACi MS-275 may contribute to its beneficial effect on improving metabolic phenotypes in DIO mice (as reported by Galmozzi et al. (28)) at least partly by directly stimulating brown adipocyte function, and HDAC1 may mediate this effect. Thus, class I HDACis, such as MS-275, and, more specifically, Hdac1, may serve as promising therapeutic targets in treating obesity and associated metabolic syndrome.

Histone acetylation and methylation are epigenetic mechanisms that regulate gene expression by remodeling chromatin structure (17–19). Whereas H3K27ac is a histone transcriptional activation mark, H3K27me3 serves as a histone transcriptional repression mark (17–19, 48). Histone acetylation is regulated by balanced action of histone acetyltransferases and HDACs, whereas histone methylation is regulated by histone methyltransferases and demethylases (17–19). Specifically, the di- and trimethylation of H3K27 are catalyzed by the PRC2 complex. Within PRC2, EZH2 is the catalytic subunit that possesses methyltransferase activity toward H3K27, and its activity also requires binding to the two other PRC2 protein components, SUZ12 and EED (51). The di- and trimethylated H3K27 further recruits PRC1 to the target genes, which, through the E3 ubiquitin ligase activity of the PRC1 components RING1 and RNF2, induces further chromatin compaction through histone 2A ubiquitination (51). On the other hand, UTX is a histone demethylase that specifically demethylates di- and trimethylated H3K27 (52). Interestingly, we find that HDAC1 physically interacts with the PRC2 components EZH2 and SUZ12 and the
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PRC1 catalytic subunit RNF2, suggesting a role of HDAC1 in connecting H3K27 deacetylation to methylation and possibly further chromatin compaction by histone ubiquitination. Indeed, we find that knocking down Haad1 in brown adipocytes not only results in an increase in H3K27ac; it also leads to reduced H3K27me3 in promoters of BAT-specific genes, including Ucp1 and Pgc1α. This is exerted through the dissociation of the PRC2 components EZH2 and SUZ12 and PRC1 catalytic subunit RNF2 from these promoters and a reciprocal recruitment of the H3K27 demethylase UTX to these promoters. Thus, our data demonstrate that HDAC1 negatively regulates the brown adipocyte thermogenic program through interaction with PRC1/2 complexes, thereby promoting H3K27 deacetylation and methylation at promoters of BAT-specific genes, such as Ucp1 and Pgc1α. In addition, our data also demonstrate that β-adrenergic activation induces dissociation of HDAC1 along with PRC complexes from Ucp1 and Pgc1α promoters, which in turn leads to gene activation.

Sympathetic signaling is essential in brown/beige adipocyte activation (9–13). We find that β-adrenergic activation in BAT1 adipocytes is associated with increased H3K27ac and decreased H3K27me3 through dissociation of HDAC1 and PRC complexes and concomitant recruitment of UTX on BAT-specific gene promoters, including Ucp1 and Pgc1α. These data indicate that HDAC1 may be one of the epigenetic modulations triggered by sympathetic signaling in brown adipocytes, which eventually leads to thermogenic activation. It would be interesting to study how β-adrenergic stimulation triggers the dissociation of HDAC1 from BAT-specific gene promoters in brown adipocytes. HDAC1 is a part of the catalytic core of several multimeric corepressor complexes, including SIN3A, NuRD (nucleosome remodeling deacetylase), and CoREST (corepressor of RE1-silencing transcription factor) (59–61), and is also a part of the PRC2 complex (51). The recruitment of these multiprotein complexes is usually triggered by cell-specific transcriptional factors or the histone recognition motifs found within the complex components (59–61). In this context, pRB (retinoblastoma protein) and RIP140 (receptor-interacting protein 140) are potent negative regulators of BAT-specific gene expression (62). It has been reported that pRB and RIP140 silence promoter activity and gene expression through recruitment of HDACs, including HDAC1 (63–65). Thus, it is possible that HDAC1 may be recruited to BAT-specific promoters through association with negative transcriptional regulators of BAT, such as pRB and RIP140.

In addition, HDAC1 itself is subjected to various post-transcriptional modifications. For example, phosphorylation of HDAC1 by casein kinase II up-regulates its activity, whereas acetylation of HDAC1 by the acetyltransferase p300 suppresses its activity (61). Interestingly, recent studies using phosphoproteomics have identified casein kinase II as a negative regulator of BAT function through phosphorylating and regulating HDAC1 activity (66). Thus, our data fall in line with the results from Shinoda et al. (66) and further demonstrate the importance of HDAC1 in the regulation of BAT function.

Moreover, HDAC1 activity or protein levels can also be regulated by ubiquitination, SUMOylation, nitrosylation, and carbonylation (61). Further study is required to decipher the cellular signaling cascades and mechanisms that regulate HDAC1 activity and recruitment to BAT-specific promoters in response to sympathetic and other stimuli in brown adipocytes.

In the present study, we have focused on the epigenetic regulation of Pgc1α by Haad1, because it is well established that PGC1α plays a central role in regulating important pathways involved in mitochondrial biogenesis and thermogenesis (40, 41). However, we have found that HDAC1 regulates H3K27 acetylation and methylation at the promoter and enhancer regions of Ucp1, a brown adipocyte terminal differentiation marker. Thus, it would be interesting to know whether HDAC1 also regulates H3K27 deacetylation and methylation at other genes’ promoters to regulate their transcription. Unbiased approaches, such as ChIP sequencing, will be required to explore the gene profile that HDAC1 regulates through H3K27 deacetylation.

Although both express UCP1 and share striking similarities in morphological and biological properties, traditional brown fat and inducible beige adipocytes are derived from distinct cell origins during embryonic development (7, 8, 67). In rodents, traditional brown adipocytes are originated from the skeletal muscle lineage (7), whereas at least a subset of beige cells arise from the smooth muscle origin (8). A recent study shows that human brown adipocytes possess molecular features similar to those of rodent beige cells (68). We used the brown fat cell BAT1 in this study. Thus, it is not clear whether the role of Haad1 in regulating brown adipocyte function can be extrapolated to beige cells. Additional studies, involving knockdown or overexpression of Haad1 in beige lineage cells, will be warranted to determine the role of Haad1 in the regulation of beige cell function.

In the present study, we investigated the role of HDAC1 in brown fat gene expression at the mature adipocyte stage. It has been reported that HDAC1 inhibits white adipocyte differentiation by deacetylating H4 at the promoter of Cebp, an important regulator of adipogenesis (39). In addition, we find that HDAC1 RNA and protein levels are down-regulated during brown adipocyte differentiation. Thus, it would be interesting to study whether HDAC1 also regulates brown adipogenesis and subsequently affects the brown fat thermogenic program.

In summary, we have identified Haad1 as a negative regulator of brown adipocyte thermogenic program. Our data show that HDAC1 is down-regulated during brown adipocyte differentiation and is also suppressed by sympathetic activation in BAT. Overexpressing Haad1 blocks, whereas knocking down Haad1 further enhances, β-adrenergic agonist-stimulated BAT-specific gene expression in brown adipocytes. Remarkably, HDAC1 physically interacts with PRC1/2 complexes, and activation of β-adrenergic signaling dissociates HDAC1 along with PRC1/2 complexes from Ucp1 and Pgc1α promoters and concomitantly recruits UTX to these promoters, leading to increased H3K27 acetylation and decreased H3K27me3 levels in these promoters. These coordinated changes switch the transcriptional repressive state to the transcriptional active state at the promoters of Ucp1 and Pgc1α, which in turn activates the brown thermogenic program. Thus, our data demonstrate that HDAC1 negatively regulates brown adipocyte gene expression by coordinated regulation of H3K27
deacetylation and methylation, and inhibiting HDAC1 promotes the brown adipocyte thermogenic program. Targeting HDAC1 may be a novel therapeutic target in the treatment of obesity by promoting brown adipocyte thermogenesis and energy dissipation.

Author Contributions— F. L., R. W., X. C., and L. Z. performed most experiments. L. Y. contributed to discussion and reviewed/editied the manuscript. B. X., H. S., and F. L. conceived the hypothesis, designed the study, analyzed the data, and wrote the manuscript.

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