HDAC2 regulates chromatin plasticity and enhances DNA vulnerability

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Abstract

Histone deacetylases (HDAC) may have a prominent role in the development of cancer and the response to anticancer therapy. However, the therapeutic relevance and tissue specificity of individual HDAC enzymes remain largely unknown. HDAC inhibitors may function as sensitizing agents to chemotherapies that target DNA through their effects on chromatin structure and plasticity. Here, we report a new role for HDAC2 as a regulator of chromatin compaction status and the mediator of HDAC inhibitor-induced sensitization to chemotherapy. The selective depletion of HDAC2 by small interfering RNA led to reduced expression of heterochromatin maintenance proteins and morphologic changes indicative of chromatin decondensation. Furthermore, depletion of HDAC2 but not HDAC1 or HDAC6 was sufficient to sensitize breast cancer cells to topoisomerase inhibitor-induced apoptosis. The levels of HDAC2 expression appear to correlate with the degree of HDAC inhibitor-induced histone acetylation in a surrogate tissue in patients. These data suggest that HDAC2 may be a relevant pharmacologic and biological target for combination therapy involving drugs that target DNA. [Mol Cancer Ther 2009;8(4):794–801]

Introduction

One of the most studied regulatory processes of transcription is the acetylation status of histone. Histone acetyltransferases and histone deacetylases (HDAC) compete to add or remove acetyl groups on lysine residues of histone tails, respectively (1, 2). The acetylation of histones by histone acetyltransferases has been associated with chromatin decondensation and transcriptional activation, whereas the deacetylation of histones by HDACs has been associated with chromatin condensation and transcriptional silencing. It is now widely accepted that aberrant expression or activity of HDAC enzymes may lead to carcinogenesis and that specific HDAC enzymes are associated with particular malignancies.

The overexpression of HDAC1 has been associated with gastric cancer (3) and, together with HDAC3, hormone-sensitive breast tumors (4). In contrast, low levels of HDAC1 have been associated with invasive esophageal cancer (5). Although it has been suggested that targeting HDAC1 may play a role in hormonal therapy of breast cancer (6), a retrospective analysis suggested that HDAC6 expression may correlate with overall survival after hormonal therapy (7). In lung cancer, the expression of HDAC5 and HDAC10 may indicate poor prognosis (8), whereas, in colon cancer, HDAC2 may promote tumor formation and HDAC2 expression has been associated with mutations in the adenomatosis polyposis coli tumor suppressor gene (9, 10). In addition, increased levels of HDAC2 and HDAC1 have been associated with endometrial stromal sarcoma (11) and cervical dysplasia (10), respectively.

These studies suggested that individual or select groups of HDAC enzymes may be associated with specific cancer types and that the inhibition of HDAC enzymes can result in therapeutic benefits in certain cancer types. However, in some cancer types, HDAC inhibitors may better serve as sensitization agents to chemotherapy or hormonal intervention (12–20). HDAC inhibitors have been shown to induce cell cycle arrest, differentiation, and chromatin decondensation, to inhibit angiogenesis, and to induce programmed cell death (15, 17, 18, 21–28). These biological effects appear to vary with type and dose of HDAC inhibitor as well as with cell type and cell line, which may indicate distinct roles for individual HDAC enzymes in the various biological effects of HDAC inhibitors. Furthermore, this group and others have shown that that a sequence-specific administration of HDAC inhibitors followed by topoisomerase inhibitors enhanced the DNA binding of topoisomerase inhibitors and enhanced their cell death without exacerbating toxicity to noncancer cells (29).

Most of the available HDAC inhibitors are nonspecific, targeting multiple members within the class I and II HDAC enzymes (30, 31). However, clinical benefits of selective versus broad HDAC inhibitors are unknown and the appropriateness of inhibitor may depend on tumor HDAC enzyme expression, enzyme selectivity of the inhibitor, and the desired effects.

In this study, we evaluated the importance of individual HDAC enzymes in the regulation of chromatin compaction. It was shown previously that HDAC inhibitors may potentiate

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the cytotoxic effects of DNA-targeting agents in breast cancer cell lines by modulating the compaction state of chromatin to allow greater access of drug to targets on the DNA (17, 25, 26). Using small interfering RNA (siRNA) depletion methods, we show that selective inhibition of HDAC2 may lead to chromatin decondensation and may increase the sensitivity of cancer cells to cell death induced by topoisomerase inhibition. The expression of HDAC2 in peripheral blood mononuclear cells (PBMC) correlates with the increase in histone acetylation induced by a HDAC inhibitor.

Materials and Methods

Chemicals and Antibodies

Valproic acid (VPA) was purchased from Sigma-Aldrich and prepared as a 1 mol/L stock solution in PBS. Epirubicin was purchased from Pharmacia. All other reagents were of analytic grade and purchased from standard suppliers. Antibodies were purchased as follows: HDAC1 and HDAC2 from Upstate Biotechnology (now Millipore); structural maintenance of chromatin (SMC) 1, SMC3, heterochromatin protein 1 (HP1), and GAPDH from Chemicon (now Millipore); and HDAC6 and DNMT1 from Santa Cruz Biotechnology.

Cell Lines

All cell lines used in this study were purchased from the American Type Culture Collection. Cell lines were maintained in DMEM supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol/L glutamine, and 50 units/mL penicillin and 50 μg/mL streptomycin (Life Technologies). Cells were incubated in a humidified atmosphere with 5% CO_2 at 37°C.

Evaluation of Apoptosis

Apoptosis was scored by the presence of nuclear chromatin condensation and DNA fragmentation and evaluated with fluorescence microscopy using bisbenzimide staining. Briefly, harvested cells were fixed in 4% paraformaldehyde for 10 min at room temperature and washed with PBS. Cell nuclei were stained with 0.5 μg/mL bisbenzimide trihydrochloride (Hoechst 33258; Molecular Probes). Two hundred cells were counted in five different fields each per experiment and scored for apoptosis (nuclei/all nuclei × 100). Each experiment was repeated at least three times, and the SE was calculated.

Western Blot Analysis

Samples were prepared with SDS lysis buffer [2% SDS, 10% glycerol, 0.06 mol/L Tris (pH 6.8)] and evaluated for protein concentration using the BCA method (Pierce). Proteins (50 μg) were separated on 8% SDS-PAGE gels and transferred to nitrocellulose membranes. Membranes were blocked in TBS-0.05% Tween 20-5% nonfat milk and incubated with primary antibody in TBS-0.05% Tween 20-5% nonfat milk overnight at 4°C. Membranes were washed three times for 10 min with TBS-0.05% Tween 20 and incubated with the appropriate secondary antibody in TBS-0.05% Tween 20-5% nonfat milk for 90 min at room temperature. Antibody binding was visualized by chemiluminescence on autoradiography film.

Transfection of siRNA

RNA duplexes for HDAC1 (ID 120418), HDAC2 (ID 120210), and HDAC6 (ID 120452) were purchased from Ambion. Cells were transfected by electroporation using the Nucleofector transfection kit according to the manufacturer's recommendations (Amaxa). Cells (4×10^6) were suspended in 0.1 mL electroporation buffer containing 1 μmol/L siRNA and pulsed with a cell line-specific protocol as described by the manufacturer. Pulsed cells were resuspended in 0.5 mL complete medium without antibiotics and incubated at 37°C for 15 min before experimentation. The *Silencer* negative control no. 2 siRNA (Ambion), a nonsense siRNA duplex, was used as a control.

Microarray

Alterations in gene expression were evaluated by microarray using Affymetrix U74Av2 Genechips (900343; Affymetrix) by standard protocols (Moffitt Molecular Biology Core Facility). Hybridization to Affymetrix chips was analyzed with Affymetrix Microarray Suite 5.0 software. Signal intensity was scaled to an average intensity of 500 before comparison analysis. The Microarray Suite 5.0 software uses a statistical algorithm to assess increases or decreases in mRNA abundance in a direct comparison between two samples (Statistical Algorithms description document). Two samples of each condition were evaluated, and each experiment was repeated at least twice. This analysis is based on the behavior of 16 different oligonucleotide probes designed to detect the same gene. Probe sets that yielded a change in $P < 0.04$ were identified as changed (increased or decreased) and those that yielded a P value between 0.04 and 0.06 were identified as marginally changed.

DNase Hypersensitivity

The vulnerability of DNA to nuclease digestion was evaluated with the *in situ* nick translation (ISNT) assay. Briefly, cells (2×10^6) were fixed with 1.5% paraformaldehyde for 15 min on ice, washed with cold PBS, and incubated for 16 h in 70% ethanol at −20°C. Cells were washed once with PBS and once with ISNT buffer [25 mmol/L $MgCl_2$, 50 mmol/L Tris-HCl (pH 8.0), 10 mmol/L β-mercaptoethanol, 10 μg/mL bovine serum albumin; ref. 32]. DNA nicks were generated by incubation with 5 units DNase I in 100 μL ISNT buffer for 25 min at room temperature. Cells were washed with PBS and incubated in 200 μL polymerase buffer (16 μmol/L dATP, 16 μmol/L dCTP, 16 μmol/L dGTP, 16 μmol/L dUTP- FITC, 7 units/mL DNase I) for 90 min at 37°C. Cells were washed with PBS and analyzed by fluorescence-activated cell sorting for the incorporation of dUTP-FITC.

Electron Microscopy

Cells were fixed with 2.5% glutaraldehyde for 16 h at 4°C, rinsed with PBS, and centrifuged. The resulting cell pellet was osmicated in 1% osmium tetroxide for 1 h at room temperature. The pellets were then dehydrated through a graded series of ethanol concentrations (30%, 50%, 70%, 95%, and 100%) and then embedded using Spurr's plastic in 100% acetone. The embedded cell pellets were sectioned with a diamond knife at 90 nm thickness

and stained for 2 min in 1% uranyl acetate and for 5 min with Reynold's lead citrate. Sections were examined with a Morgagni 268D FEI transmission electron microscope (FEI). Digital images of nuclei taken at ×4,400 magnification were used to estimate the average heterochromatin content of 50 nuclei per treatment group by point-count intercept morphometry as described by Weibel (33) and Hyde et al. (34).

Histone Acetylation and HDAC Expression

PBMCs were isolated by Ficoll centrifugation (Ficoll-Paque; GE Healthcare). PBMCs were collected in conjunction with two clinical trials done at the Moffitt Cancer Center (MCC13991 and MCC13693) after obtaining approval from the institutional review board and under standard federal and institutional guidelines. Histone H3 and H4 acetylation and HDAC2 expression were evaluated by Western blot analysis as described above using 25 μg whole-cell lysate. Intrapatient protein expression was normalized to GAPDH. In addition, protein expression from 25 μg MCF-7 whole-cell lysates derived from the same source was used as an internal control for all Western blots. HDAC2 expression in PBMC and MCF-7 samples were normalized to lamin expression and depicted relative to HDAC2 expression levels in control MCF-7 cells. Histone acetylation was plotted against HDAC2 and HDAC6 expression.

Results

HDAC Inhibitors Sensitize Breast Cancer Cell Lines to DNA-Targeting Agents

As reported previously, HDAC inhibitors increase the sensitivity of cancer cells to DNA damage induced by topoisomerase II inhibitors *in vitro, in vivo* as shown in xenograft models, and likely in patients (17, 25, 26, 29). Sensitization of cells to topoisomerase II inhibitors in the presence of HDAC inhibitors occurred predominantly in transformed cells and involved enhanced DNA vulnerability and DNA access. These sensitization effects were seen in several tumor types and with both class I-specific and non-class-specific HDAC inhibitors (12, 14–18, 25, 26).

The synergistic induction of apoptosis after treatment with the fatty acid HDAC inhibitor VPA and the topoisomerase II inhibitor epirubicin is shown in the breast cancer cell lines MCF-7, T47D, and MDA-MB-468 (Fig. 1). Cells were incubated in the presence of 2 mmol/L VPA for 48 h followed by a 4 h treatment with 0.5 μmol/L epirubicin and evaluated for DNA condensation and fragmentation 24 h later. Although there was no significant increase in the apoptosis observed with either VPA or epirubicin alone compared with control, epirubicin induced a significant degree of apoptosis in cells after preexposure to VPA (Fig. 1). Similar results were seen with the HDAC inhibitors MS-275, trichostatin A, sodium butyrate, and vorinostat (data not shown).

HDAC2 Inhibition Mediates Sensitization to DNA-Targeting Agents

To determine whether the inhibition of a specific HDAC enzyme may mediate the sensitization of cancer cells to topoisomerase II inhibitors, breast cancer cell lines were depleted of select HDAC enzymes and evaluated for sensitivity to epirubicin (Fig. 2). The majority of the currently used HDAC inhibitors inhibit HDAC1, HDAC2, HDAC3, and HDAC6 (24, 30). However, the IC_{50} of VPA for HDAC3 is significantly higher than for HDAC1 and HDAC2 (30), rendering it an unlikely therapeutic target. Furthermore, although HDAC6 is a clinically relevant target of the hydroxamic acid HDAC inhibitors, it may not be sufficiently inhibited by VPA or the class I-specific HDAC inhibitors (e.g., MS-275 or MGCD0103). However, several investigators have reported effects on nonhistone targets of HDAC6 (35, 36); therefore, we also evaluated the effects of HDAC6 depletion on epirubicin sensitivity.

MCF-7, T47D, and MDA-MB-468 cells were independently depleted of HDAC1, HDAC2, and HDAC6 using siRNA and evaluated for apoptotic nuclei in the presence and absence of epirubicin. Transfection of siRNA resulted in the depletion of the target HDAC enzyme without affecting the expression of other enzymes (Fig. 2A). As shown in Fig. 2B, the combination of VPA and epirubicin induced significantly more apoptosis than treatment of any of the three cell lines with VPA alone. The combination effects were synergistic and exceeded both VPA alone (Fig. 2B, *left*) and epirubicin alone (data not shown). An enhancement of the apoptosis in the presence of epirubicin could be mimicked by the depletion of HDAC2 but not of HDAC1 or HDAC6 (Fig. 2B). Combined depletion of HDAC1 and HDAC2 did not enhance the observed effects (data not shown). The degree of epirubicin-induced apoptosis after HDAC2 depletion was comparable with the apoptosis seen with pharmacologic inhibition of HDACs by 2 mmol/L VPA in cells transfected with nonsilencing siRNA (Fig. 2B). Furthermore, after HDAC2 depletion, VPA (2 mmol/L) did not further enhance the epirubicin-induced apoptosis seen in these cells (data not shown).

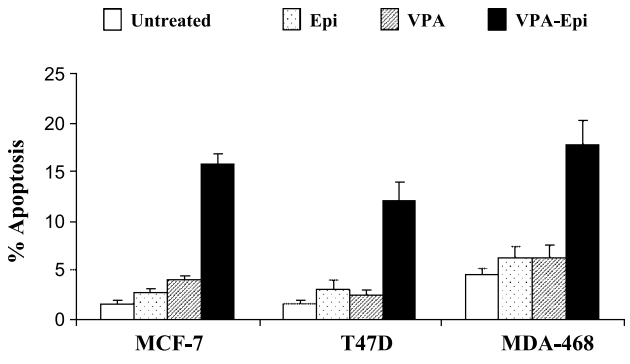

Figure 1. VPA sensitizes breast cancer cells to epirubicin-induced apoptosis. MCF-7, T45D, and MDA-MB-468 tumor cells were treated with 2 mmol/L VPA for 48 h followed by exposure to 0.5 μmol/L epirubicin (*Epi*) for 4 h. Cells were harvested 24 h later and evaluated for nuclear condensation and fragmentation. *Bars,* SE from at least three replicate experiments.

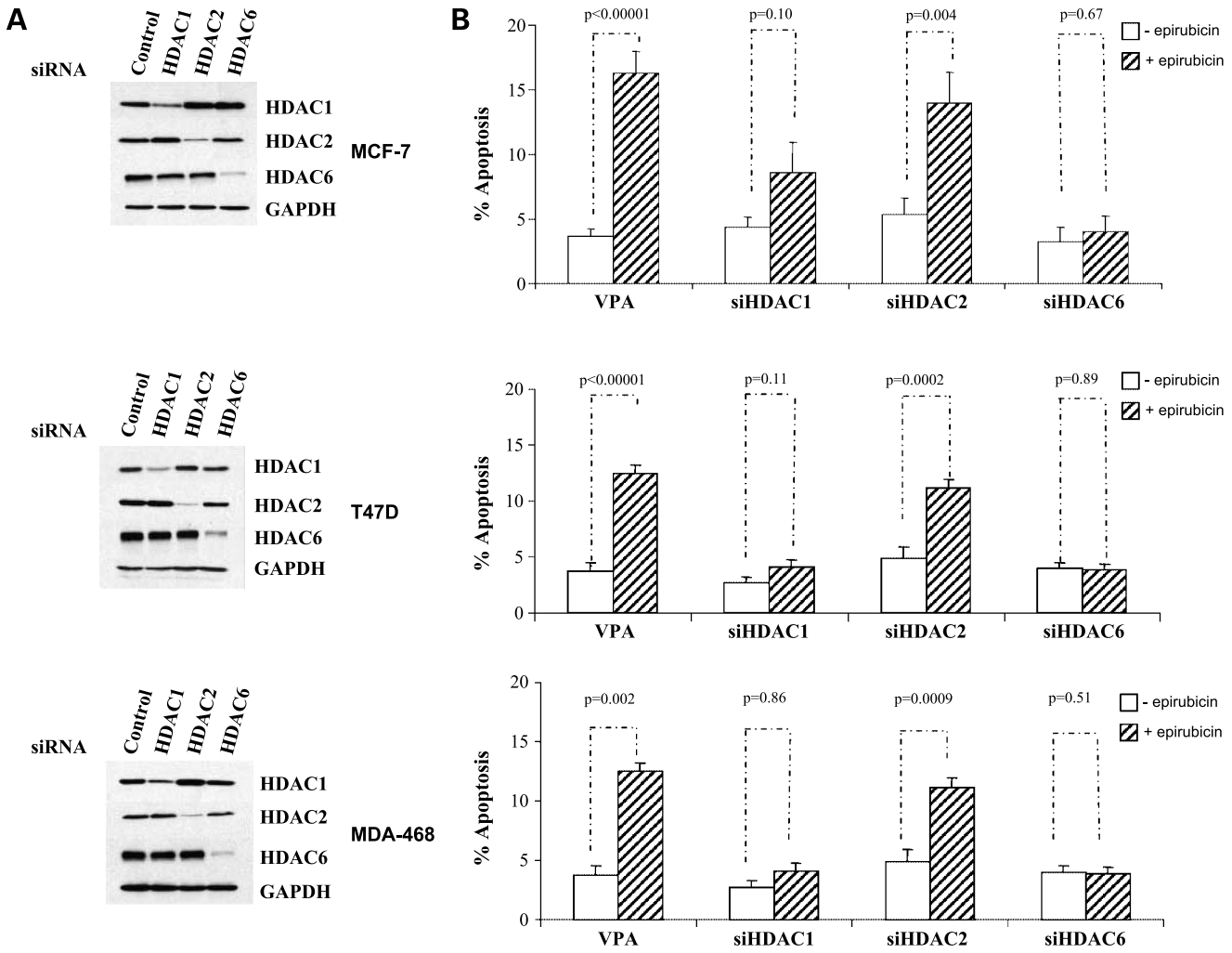

Figure 2. Depletion of HDAC2 sensitizes cells to epirubicin-induced apoptosis. **A,** Western blot showing select depletion of HDAC1, HDAC2, and HDAC6 by siRNA in MCF-7 (*top*), T47D (*middle*), and MDA-MB-468 (*bottom*) cells. **B,** comparison of epirubicin-induced apoptosis in cells transfected with nonsilencing siRNA versus cells depleted of HDAC1, HDAC2, or HDAC6 in MCF-7 (*top*), T47D (*middle*), and MDA-MB-468 (*bottom*) cells. As a positive control, cells transfected with nonsilencing siRNA were treated with 2 mmol/L VPA for 48 h before 0.5 μmol/L epirubicin for 4 h. The presence of apoptotic nuclei was evaluated 24 h after epirubicin treatment. Significance was by Student's *t* test. Bars, SE from a minimum of three replicate experiments.

HDAC2 Regulates the Expression of Chromatin-Associated Genes

We reported previously that HDAC inhibitors potentiate the antitumor effects of topoisomerase II inhibitors by inducing chromatin decondensation, thereby facilitating access of drug to targets on the DNA substrate (17, 26). Furthermore, we showed that chromatin decondensation, observed after HDAC inhibitor treatment, correlated with the down-regulation of genes and proteins involved in the maintenance of heterochromatin. To determine whether the effects of HDAC inhibitors on the expression of heterochromatin maintenance proteins and chromatin plasticity are mediated by HDAC2, microarray analysis was done on T47D cells transfected with siRNA to selectively inhibit HDAC2. For comparison, we first established an expression profile from T47D cells transfected with nonsilencing siRNA and incubated cells with 2 mmol/L VPA for 48 h.

Pharmacologic inhibition of HDAC enzymes in control transfected T47D cells resulted in the down-regulation of 63 genes predicted through gene ontology to be associated with chromatin.[1] The down-regulation of these chromatin-associated genes is shown in Fig. 3A as a clustering to the right of the diagonal line. Accumulation on the diagonal is associated with no change from baseline, whereas clustering to the left is associated with an increase in mRNA copy numbers of selected genes. Ninety percent (57 of 63) of the chromatin-associated genes affected by VPA were also

[1] http://www.geneontology.org/index.shtml

down-regulated by siRNA depletion of HDAC2 (Fig. 3A). The effects of VPA treatment and HDAC2 depletion on the expression of select heterochromatin maintenance genes previously correlated with chromatin plasticity (26) are shown in Fig. 3B. VPA treatment and HDAC2 depletion similarly reduced mRNA expression of SMC1 to SMC5 and HP1 (Fig. 3B). The reduction in mRNA levels translated into reduced protein expression (Fig. 3C). Control transfected T47D cells incubated with VPA showed reduced expression of SMC1 to SMC3 and HP1 as well as the hyperacetylation of histone H4 while having no effect on the expression of HDAC1 or HDAC2 (Fig. 3C). Similarly, the select depletion of HDAC1 or HDAC2 resulted in the reduced expression of SMC2 and SMC3. However, only the depletion of HDAC2 resulted in the reduced expression of SMC1 and HP1 as well as increased acetylation of histone H4 (Fig. 3C).

HDAC2 Controls Chromatin Plasticity

The reduced expression of heterochromatin maintenance protein mRNA and protein after VPA treatment or HDAC2 depletion correlated with increased sensitivity of DNA to nuclease digestion. Figure 4A shows results from the ISNT DNA hypersensitivity assay. In this assay, DNase I is used to generate nicks in the DNA, which are then filled with FITC-labeled dUTP. A more open chromatin structure would allow for increased DNase I activity, resulting in a greater incorporation of FITC-labeled dUTP and increased fluorescence. As shown, the selective depletion (>90% of baseline) of HDAC2, but not HDAC1, resulted in a fluorescence shift when compared with cells transfected with nonsilencing siRNA.

The effects of HDAC2 depletion on decondensation of chromatin were qualitatively and quantitatively assessed by electron microscopy (Fig. 4B). Electron micrographs of cells transfected with nonsilencing siRNA showed dense areas of heterochromatin clusters (Fig. 4B, *arrows*). Similar results were observed in cells depleted of HDAC1. In contrast, T47D cells depleted of HDAC2 showed a more even distribution of chromatin fibers and a reduced number of dense heterochromatin clusters indicative of chromatin decondensation (Fig. 4B). To quantitatively assess the degree

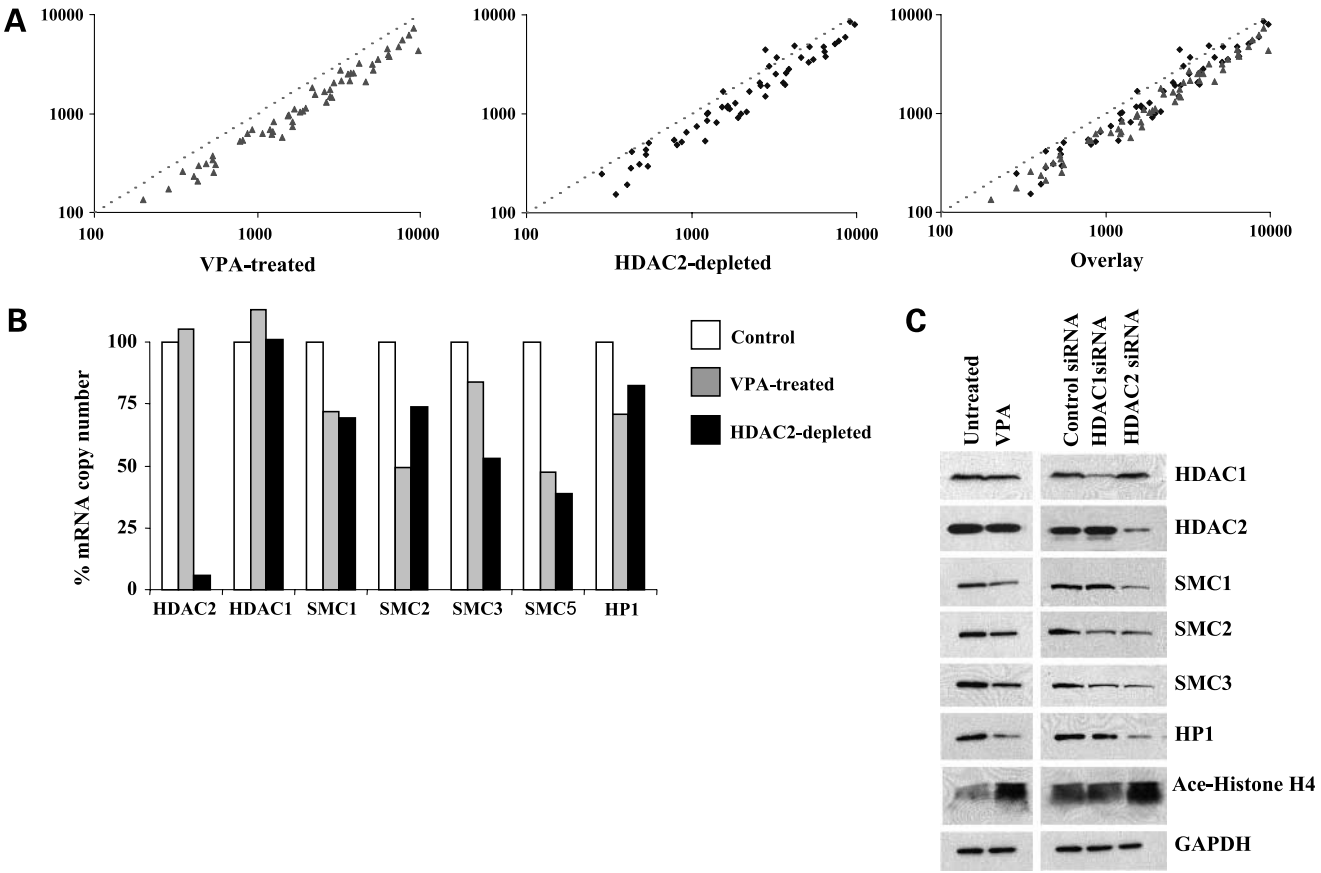

Figure 3. HDAC2 regulates the expression of chromatin-associated genes. **A,** scatter plots of microarray data depicting changes in mRNA expression from T47D cells transfected with nonsilencing siRNA and treated with 2 mmol/L VPA for 48 h compared with cells depleted of HDAC2. To compare between treatments, scatter plots were enriched for genes associated with chromatin structure and dynamics that were down-regulated in the presence of VPA. **B,** bar graph showing similarity in the down-regulation of genes encoding the heterochromatin maintenance proteins previously found associated with HDAC inhibitor-induced chromatin decondensation (26), including SMC1 to SMC3, SMC5, and HP1 after a 48 h treatment with 2 mmol/L VPA or siRNA depletion of HDAC2. **C,** changes in the expression of heterochromatin maintenance proteins in T47D cells after VPA treatment or siRNA depletion of HDAC1 and HDAC2 by Western blot.

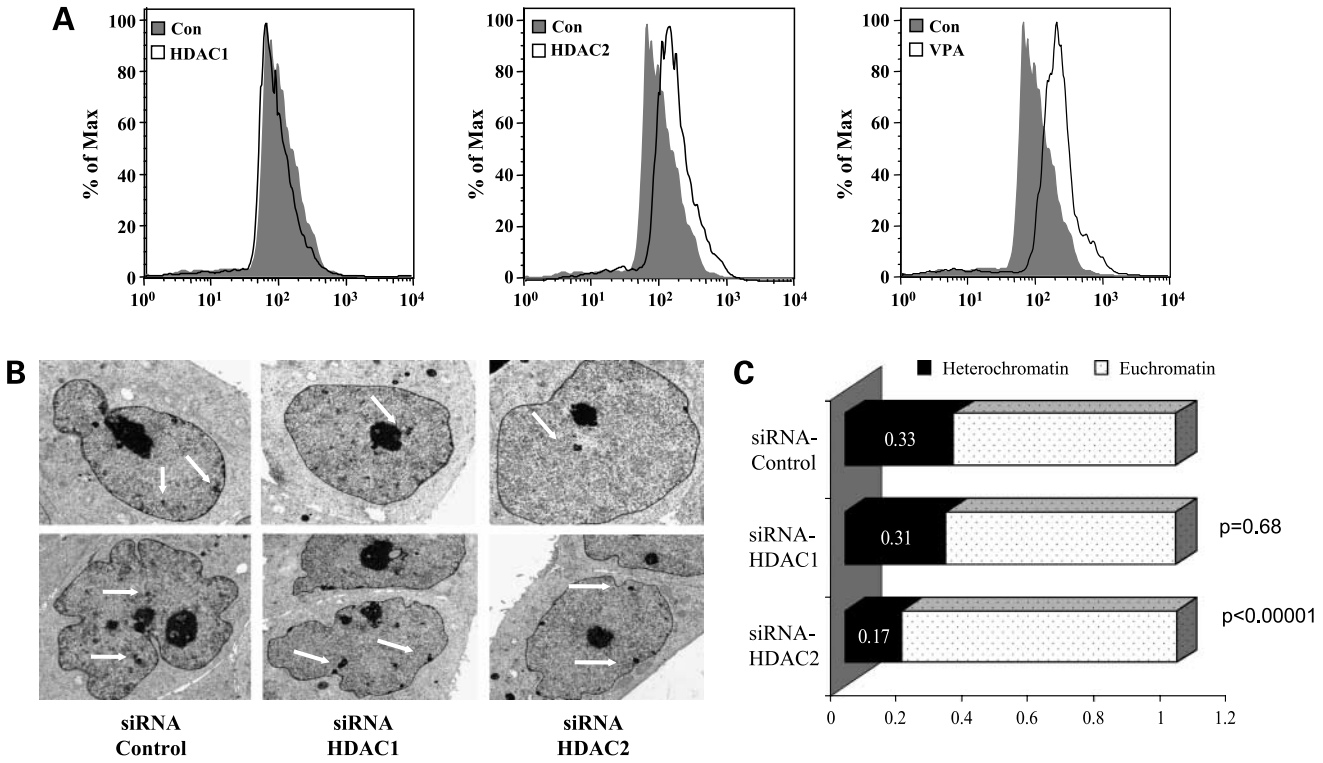

Figure 4. HDAC2 modulates chromatin plasticity. **A,** T47D cells depleted of HDAC2, but not of HDAC1, showed increased DNA sensitivity to nuclease digestion by the ISNT DNA hypersensitivity assay. Cells transfected with nonsilencing siRNA followed by incubation with 2 mmol/L VPA for 48 h served as a positive control. **B,** electron micrographs of cells transfected with nonsilencing siRNA or depleted of HDAC1 or HDAC2. *Arrows,* dense areas of heterochromatin clusters. **C,** stacked bar graph depicting the mean heterochromatin content (*shaded*) per cell per transfection condition. Heterochromatin content was determined by point-count intercept morphometry analysis of the electron micrographs for 50 cells per transfection condition. Statistical analysis by Student's *t* test indicated a significant ($P < 0.0001$) reduction in the heterochromatin content in cells depleted of HDAC2 but not of HDAC1 compared with cells transfected with nonsilencing siRNA.

of chromatin decondensation, electron micrographs of 50 cells for each siRNA transfection condition were analyzed by point-count intercept morphometry as described by Weibel (33) and Hyde et al. (34). This analysis is expressed as a stacked bar graph showing the mean percent heterochromatin content per cell (*shaded*; Fig. 4C). As shown, the select inhibition of HDAC2 but not HDAC1 decreased cellular heterochromatin content suggestive of chromatin decondensation (Fig. 4C). Student's *t* test indicated that the changes in chromatin structure due to HDAC2 depletion were significant ($P \leq 0.0001$).

To further delineate the possible role of HDAC2 as a therapeutic target and potential biomarker, we compiled data from two clinical trials correlating HDAC2 expression with histone H3 and H4 acetylation in PBMCs. We have shown previously that histone acetylation induced by HDAC inhibitors is required for chromatin decondensation (26). In the experimental data presented thus far, we show that HDAC2 appears to be a relevant target for VPA or other HDAC inhibitors, and its inhibition is essential for the HDAC inhibitor-induced chromatin decondensation. In a first step, we have now evaluated the relevance of HDAC2 expression in a surrogate system gathered from two clinical trials. If histone acetylation is an indirect measure of HDAC enzyme inhibition and HDAC2 is a relevant target, one would expect that higher expression levels of HDAC2 should result in a higher degree of H3 and H4 acetylation. As shown in Fig. 5A and B, there is a statistically significant correlation between histone acetylation and HDAC2 expression. Such a correlation was not found with HDAC6 (data not shown).

Discussion

Individual HDAC enzymes may have a distinct biological function during development and may be regulated independently (37, 38). Although HDAC1 was shown to be required for the development of the mouse embryo (39), HDAC4 was associated with bone development (40, 41). HDAC5 and HDAC7 may be involved in muscle cell differentiation (42, 43), whereas HDAC8 and HDAC9 have been linked to the differentiation of smooth and skeletal muscle cells, respectively (44). HDAC6 has been associated with cytoskeletal regulation (45) and may be required for the autophagic response (46). In addition to skeletal muscle cell differentiation, HDAC9 may play a role in cardiac development in conjunction with HDAC5 (47). In addition,

individual or select groups of HDAC enzymes may have other functions that have yet to be recognized.

In cancer cells, the biological consequences of HDAC inhibition are cell cycle arrest, chromatin decondensation, differentiation, growth arrest, and apoptosis (15, 17, 18, 21–28). We and others have reported that HDAC inhibitors may act as sensitizing agents to certain chemotherapies that target the DNA (16, 18) by inducing chromatin decondensation, thereby facilitating access of drug to targets on the DNA substrate (15, 17, 26). In two clinical trials, the feasibility and early clinical efficacy of a sequence-specific administration of HDAC inhibitors and topoisomerase inhibitors were shown (29, 48). The chromatin decondensation observed after HDAC inhibitor treatment was further linked to the hyperacetylation of histones and the reduced expression of genes and proteins involved in the maintenance of heterochromatin (26).

Here, we report a new role for HDAC2 as a regulator of chromatin compaction status and the primary mediator of HDAC inhibitor-induced chromatin decondensation and sensitization to chemotherapy. The depletion of HDAC2 by siRNA, while not affecting the expression of other HDAC enzymes, resulted in the reduced expression of several genes and proteins associated with chromatin, including those involved in the formation and maintenance of heterochromatin, such as SMC1 to SMC5 and HP1, and resulted in the increased acetylation status of histone H4. In addition, cells depleted of HDAC2 were hypersensitive to DNase I digestion by the ISNT assay and showed reduced heterochromatin content by point-count intercept morphometry of electron micrographs. The results of both assays are indicative of decondensed chromatin. Furthermore, HDAC2-depleted cells, but not HDAC1- or HDAC6-depleted cells, were more vulnerable to epirubicin-induced apoptosis. In this respect, the selective depletion of HDAC2 was sufficient to mimic the effects of pharmacologic HDAC inhibition.

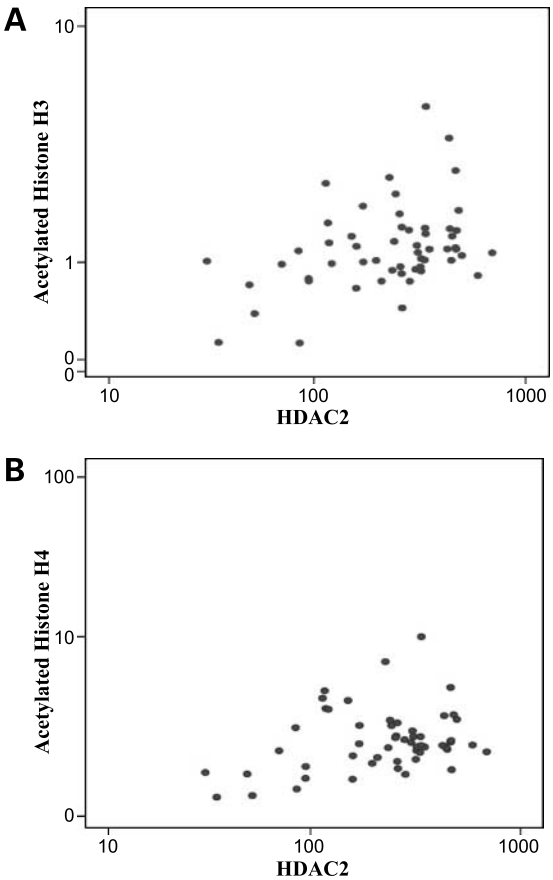

Figure 5. Histone acetylation induced by VPA is directly related to the expression of HDAC2 in PBMCs. Scatter plots showing the expression of HDAC2 in PBMCs plotted against fold change in acetylation of histone H3 (**A**) and H4 (**B**). HDAC expression and histone acetylation were evaluated by immunofluorescence analysis from >50 patients participating in two separate phase I clinical trials who were treated with VPA for 48 h.

The evaluation of HDAC2 in PBMCs suggested that the relevance of HDAC2 inhibition may be directly linked to its expression. In this case, if the chromatin plasticity and its remodeling induced by HDAC inhibitor is mediated through HDAC2, one would expect the expression levels of HDAC2 to correlate with the change in histone H3 and H4 acetylation induced by a HDAC inhibitor. These findings lend further support that HDAC2 should be considered as a therapeutic target and biomarker.

Although there are several published studies and numerous ongoing clinical trials involving HDAC inhibitors, very little is known about the roles of individual HDAC enzymes, and a formal assessment of HDAC enzyme expression is not routinely done. Emerging data suggest that the currently used HDAC inhibitors may differ significantly with regard to target selectivity. In addition, the expression of HDAC enzymes may vary considerably between normal and tumor tissues and likely between different tumor tissues. This report therefore may be relevant in the development of large-scale biomarker assays as well as predictive markers of response when using HDAC inhibitors to modulate chromatin plasticity or to enhance the DNA vulnerability to chemotherapeutic or radiation therapy. Furthermore, the data generated in this study support the development of more selective HDAC inhibitors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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