Identification of Novel Isoform-Selective Inhibitors within Class I Histone Deacetylases

ERDING HU, EDWARD DUL, CHIU-MEI SUNG, ZUNXUAN CHEN, ROBERT KIRKPATRICK, GUI-FENG ZHANG, KYUNG JOHANSON, RONGGANG LIU, AMPARO LAGO, GLENN HOFMANN, RICARDO MACARRON, MAITE de los FRAILES, PALOMA PEREZ, JOHN KRAWIEC, JOHN K. WINKLER,1 and MICHAEL JAYE


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ABSTRACT

Histone deacetylases (HDACs) represent an expanding family of protein modifying-enzymes that play important roles in cell proliferation, chromosome remodeling, and gene transcription. We have previously shown that recombinant human HDAC8 can be expressed in bacteria and retain its catalytic activity. To further explore the catalytic activity of HDACs, we expressed two additional human class I HDACs, HDAC1 and HDAC3, in baculovirus. Recombinant HDAC1 and HDAC3 fusion proteins remained soluble and catalytically active and were purified to near homogeneity. Interestingly, trichostatin (TSA) was found to be a potent inhibitor for all three HDACs (IC50 value of 0.1–0.3 μM), whereas another HDAC inhibitor MS-27-275 (N-(2-aminophenyl)-4-[N-(pyridin-3-methyloxycarbonyl)-aminomethyl]benzamide) preferentially inhibited HDAC1 (IC50 value of 0.3 μM) versus HDAC3 (IC50 value of 8 μM) and had no inhibitory activity toward HDAC8 (IC50 value >100 μM). MS-27-275 as well as TSA increased histone H4 acetylation, induced apoptosis in the human colon cancer cell line SW620, and activated the simian virus 40 early promoter. HDAC1 protein was more abundantly expressed in SW620 cells compared with that of HDAC3 and HDAC8. Using purified recombinant HDAC proteins, we identified several novel HDAC inhibitors that preferentially inhibit HDAC1 or HDAC8. These inhibitors displayed distinct properties in inducing histone acetylation and reporter gene expression. These results suggest selective HDAC inhibitors could be identified using recombinantly expressed HDACs and that HDAC1 may be a promising therapeutic target for designing HDAC inhibitors for proliferative diseases such as cancer.

Histone acetylation modifies specific lysine residues on histones and other nuclear and cytoplasmic proteins and plays important roles in pivotal cellular functions such as chromosome remodeling, gene transcription, and cell proliferation. Histone acetyltransferase (HAT) and histone deacetylase (HDAC) control the addition and removal of acetyl groups on proteins and maintain a dynamic balance of steady-state protein acetylation. In addition to histone transcription activators (Src-1, p300, ACTR, and PCAF) and repressors (Sin3, pRB, YY1, and NcoR) have been shown to be associated with HAT and HDAC, respectively (Cress and Seto, 2000; Kouzarides, 2000). Several transcriptional activators even contain intrinsic HAT activities (CBP, p300, and PCAF) (Ogryzko et al., 1996; Krumm et al., 1998). Others (p53 and myoD) are acetylated and acetylation regulates their activities (Sartorelli et al., 1999; Luo et al., 2000). Oncogenes such as BRCA-1 and -2 have been shown to be associated with HDACs and HATs (Siddique et al., 1998; Yarden and Brody, 1999). In particular, the viral oncopene E1a has been shown to modulate histone acetyltransferase activity of p300 and PCAF (Chakravarti et al., 1999; Hamamori et al., 1999).

Multiple forms of HDAC have been identified in mammalian cells. In human, at least 11 HDACs have been uncovered. They are classified into two general classes: class I (HDAC1, 2, 3, 8, and 11) and class II (HDAC4, 5, 6, 7, 9, and 10) (Gray and Ekstrom, 2001; Gao et al., 2002; Kao et al., 2002). Class I enzymes are generally smaller polypeptides of around 500 amino acids, whereas class II HDACs are much larger proteins with approximately 1000 amino acids. Members of class II HDACs (HDAC4 and 5) have been shown to shuttle between the cytoplasm and nucleus and regulate (Siddique et al., 1998; Luo et al., 2000). Others (p53 and myoD) are acetylated and acetylation regulates their activities (Sartorelli et al., 1999; Luo et al., 2000). Oncogenes such as BRCA-1 and -2 have been shown to be associated with HDACs and HATs (Siddique et al., 1998; Yarden and Brody, 1999). In particular, the viral oncopene E1a has been shown to modulate histone acetyltransferase activity of p300 and PCAF (Chakravarti et al., 1999; Hamamori et al., 1999).

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ABBREVIATIONS: HAT, histone acetyltransferase; HDAC, histone deacetylase; TSA, trichostatin; GST, glutathione S-transferase; PCR, polymerase chain reaction; MTP, multiple titer plate; SV40, simian virus 40; PAGE, polyacrylamide gel electrophoresis; MS-27-275, N-(2-aminophenyl)-4-[N-(pyridin-3-methyloxycarbonyl)-aminomethyl]benzamide; FR901228, desipeptide; SB-499201, N-(4-phenoxypyridyl)-4-(3-phenylpropanoylamino)-benzamide; SB-379872-A, 4-pyridin-4-yl-1,3-imidazole-2-thione.
myogenesis (Lu et al., 2000; McKinsey et al., 2000). In contrast, most of the class I HDACs have been shown to be localized in the nucleus. In addition to these two main classes of HDACs, a third class of HDACs has been described that requires NAD as cofactor (Imai et al., 2000).

Several HDAC inhibitors, including sodium butyrate and trichostatin (TSA), have been shown to have a wide range of effects in cultured cells and in animals. These effects include specific gene activation, inhibition of cell proliferation and cell cycle arrest, as well as induction of cell differentiation (Kuo and Allis, 1998; Kouzarides, 1999). Importantly, two HDAC inhibitors (MS-27-275) (Saito et al., 1999) and FR901228 (Nakajuma et al., 1998) have demonstrated tumor growth suppression in vivo. These results suggest complex regulatory roles for HDACs in many vital cellular functions and potential therapeutic utilities of HDAC inhibitors.

With the identification of 11 human HDACs, it was suggested that these HDACs may play distinct roles in cellular functions. However, little is known about the catalytic properties of individual HDACs and their sensitivity to known inhibitors. We undertook an effort to express three class I human HDACs (HDAC1, 3, and 8), and studied their sensitivity to HDAC inhibitors. Our results demonstrated that soluble and active HDAC1 and 3 could be generated, and the recombinant proteins may be used to profile HDAC inhibitors.

Our data also demonstrated that individual HDACs displayed distinct inhibition profile toward known or novel HDAC inhibitors, and inhibition of HDAC1 correlated with the inhibition of cell proliferation and apoptosis. In addition, we examined the expression of HDAC1 in several normal and malignant human tissues using immunohistochemistry techniques.

Materials and Methods

Materials

Nylon membrane (Biotran) was purchased from ICN Biomedicals Inc. (Costa Mesa, CA). [α-32P]dATP (3000 Ci/mmol) was purchased from PerkinElmer Life Sciences (Boston, MA). Oligo-dT agarose was obtained from Amersham Biosciences Inc. (Piscataway, NJ). Routine molecular cloning and sequence analyses were performed as described previously (Sambrook et al., 1989). Reagents for subcloning and sequencing were purchased from Promega (Madison, WI). PCR reagents were obtained from PerkinElmer Instruments (Norwalk, CT). Random priming labeling kits were obtained from Promega. TSA and butyrate were obtained from Sigma-Aldrich (St. Louis, MO). [3H]histone was generated by labeling human embryonic kidney 293 cells with [3H]acetate in the presence of 10 mM butyrate as described previously (Yoshida et al., 1990). Computer programs used to analyze cDNA, and protein sequences were BLASTN, BLASTP, and Lasergene-DNA star programs. Polyclonal antibodies for HDAC3 and 8 were generated in rabbits by using the purified glutathione S-transferase (GST)-hHDAC3 and 8 as described below. Anti-HDAC1 antibody was obtained from Sigma-Aldrich.

Recombinant Expression and Purification of hHDAC1 and hHDAC3

All plasmid constructions were generated by standard cloning methods (Sambrook et al., 1989). The DNA sequence of all cloning insertions was confirmed on both strands by automated di-deoxy sequencing (Applied Biosystems, Foster City, CA).

Full-length histone deacetylase I (GenBank NM_004964) was PCR-amplified from a human cDNA library (BD Biosciences Clontech, Palo Alto, CA) from using the following primer set (forward: 5'-ATTAATATGGATCTTGGAAACAGCATATG-GCGCACAGCGCA-GGGCCACCACC-3' and reverse: 5' AATTAATATCGAGACGCG CGAACGGGGCG-AGCGC-CC-3') as underlined. GST encoding sequence was PCR-amplified from GSTHD3pFB using a primer set tailed 5' with EcoRI and 3' with BamHI (forward: 5'-GGCGGCGGAATTCTAGGCTAGCACCC-3' and reverse: 5' AATAATATAGGATCTTGGGATGAGTGTGGGC-CACCACCC-3'). The HDCA1 PCR fragment was digested with BamHI and Xhol, and GST PCR fragment was digested with EcoRI and BamHI, and these were ligated together with pFastbac-1 vector (Invitrogen, Carlsbad, CA) digested with EcoRI and Xhol. The three-way ligation generated GSTHD1pFB, containing an N-terminal GST fusion with HDAC1.

Histone deacetylase-3 (GenBank accession no. U75697) was cloned from Jurkat cell (ATTG CTTL-8130) polya RNA by reverse transcription-PCR using Superscript II reverse transcriptase for oligo-dt primed 1st cDNA synthesis according to the manufacturer's instructions (catalog no. 18089-011; Invitrogen) followed by PCR using the oligonucleotide primer set (forward: 5'-CATATGGCGCA-GACC-GTGGCCATATTTG-3' and reverse: 5'-ATCAATGATGGTGATGTTGAGTGTGGTCGGGACC-GGCGCGCC-3'). The HDAC3 coding sequence using the QuikChange mutagenesis kit (Stratagene, La Jolla, CA) to eliminate the internal restriction site underlined. The resultant PCR product was TA subcloned into pCR-2.1 vector (Invitrogen). Site-directed mutagenesis was used to introduce a silent change in HDAC3 coding sequence using the QuikChange mutagenesis kit (Stratagene, La Jolla, CA) to eliminate the internal Ndel for subcloning purposes. The following mutagenesis primer set was used containing the overlapping base change as underlined: (forward: 5'-ATGGTCGCCGCTGCTGACCTATAGA CACTGGCCTTCTTGTG-3' and reverse: 5'-CTTCTACCGACCGCTAGT-TCTCATAGGTCCAGACCGGACATT-3').

Full-length HDAC3 was transferred on an XbaI-Xhol fragment and ligated between the same sites in pFastbac-1 (Invitrogen). The resultant plasmid, HD3pFB, was further modified by adding an N-terminal GST tag (GenBank accession no. M14654). The GST encoding sequence was PCR-amplified from pGEX-2.1 using the following primer set tailed 5' with XbaI and 3' with Ndel as underlined: (forward: 5'-CCTGAAAGCCACCTACTAGTGGTAGAA-3' and reverse: 5'-CATATGGTGTTCCATTTGAGGATGTTGCCACCA-CC-3'). The resultant PCR product was subcloned into pCR-2.1 from where it was excised on an XbaI-Ndel fragment and ligated between the same sites of HD3pFB to generate GSTHD3pFB.

For purification of recombinant HDACs, 2 liters of baculovirus-infected Sf9 cell pastes of HDAC1 or HDAC3 with GST fusion at the N terminus were lysed in 100 ml of lysis buffer (25 mM Tris, pH 7.8, 3 mM MgCl2, 150 mM NaCl, 10% glycerol, 0.5% Triton X-100, and protease inhibitor cocktail) and centrifuged at 20,000g for 1 h. The cell supernatant was allowed to bind to 8 ml of glutathione-Sepharose 4B resin at 4°C for 1 h before loading onto a column. The column was washed with buffer A (25 mM Tris, pH 7.8, 3 mM MgCl2, 150 mM NaCl, 10% glycerol) with 0.5% Triton X-100 extensively. The GST fusion protein was eluted with buffer A containing 10 mM reduced glutathione. The purified protein was dialyzed into final buffer A and kept at −70°C before activity assay. The HDAC1 was further purified using MonoQ column and eluted with linear gradient of 150 mM NaCl to 1 M NaCl. Purified HDAC1 was kept at −70°C before use.

HDAC Activity Assay

HDAC assays were performed essentially as described on previous work (Hu et al., 2000). The 200-μl HDAC assay reaction contained 10 mM Tris, pH 8, 150 mM NaCl, 1 mM MgCl2 (HDAC assay buffer), 5000 to 10,000 cpm of [3H]-histone or H4 peptide [SGRKGKGGKGLGK]-H.
acetylated(GGAKRHR), and varying amounts of purified HDACs. Assays were performed at 25°C. After 1 h of incubation, 50 µl of stop mixture (1 M HCl and 0.16 M acetic acid) was added to the sample, and 1 µl of ethyl acetate was added to extract released tritiated acetate. Eight hundred microliters of the ethyl acetate was counted and 1 ml of ethyl acetate was added to extract released tritiated mixture (1 M HCl and 0.16 M acetic acid) was added to the sample, after incubation at 4°C for 1 h, beads were recentrifuged, washed, and resuspended in 200 µl of HDAC assay buffer. After incubation, the glutathione-Sepharose beads were spun down, washed, and resuspended in 200 µl of HDAC assay buffer containing dimethyl sulfoxide vehicle or 1 µM HDAC inhibitors (TSA or MS-27-275). After incubation at 4°C for 2 h, beads were centrifuged, washed, and resuspended in 200 µl of HDAC assay buffer. HDAC activity was assayed as described above.

RNA isolation and Northern blot analysis were performed as described previously (Hu et al., 2000).

Densitometry Analysis. Analysis was performed using ImageJ (version 1.30) software package from National Institutes of Health (http://rsb.info.nih.gov/ij). Autoradiogram was scanned as JPEG image, and area under the curve was measured for each band and quantified.

Apoptosis Assay

Apoptosis Analysis. SW620 human colon carcinoma cell line was cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Cells (2 × 10⁶) were seeded in each well of a 24-well culture plates. Cells were starved overnight in Dulbecco’s modified Eagle’s medium without serum before addition of TSA and other agents. After overnight treatment, cell apoptosis was determined using the Cell Death Detection ELISA Plus kit (Roche Diagnostics) following the manufacturer’s protocol. Briefly, 1 ml of lysis buffer for each well was added after removing the medium, after 30-min incubation at room temperature, 20 µl of supernatant was transferred into each well of the streptavidin-coated MTP (96-well multiple titer plate). Then, 80 µl of immunoreagent mix was added to each well and incubated for 2 h at room temperature. Solution was removed and each well was rinsed three times with 250 µl of incubation buffer and then 100 µl of substrate solution was added into each well of the MTP modules and incubated on a plate shaker at 250 rpm for approximately 15 min or until the color development was sufficient for a photometric analysis. The optical density at 405 nm was measured. Samples were in triplicate, and the average value of each sample was interpreted against the positive and negative control samples.

SV40 Promoter Reporter Assays. SV40 promoter construct (2 µg; pGL3-promoter luciferase reporter vector; Promega) was transiently transfected into Rat-2 cells. The SV40 promoter region in pGL3 (nucleotide 44–244) contains transcription initiation sites as well as multiple Sp1 binding sites. This promoter region corresponds to bases 5180 to 5240 of the SV40 viral genome (GenBank accession no. J02400). Empty vector DNA was used to normalize the amount of transfected DNA to 20 µg/100-mm cell culture dish. Our previous experiments demonstrated that this construct, when transiently transfected into Rat-2 cells, can be activated by TSA (Hu et al., 2000). Transfections were performed as described previously (Hu et al., 2000). Forty-eight hours after transfection, cells were trypsinized and resuspended into a 96-well dish at approximately 10,000 cells/well. After overnight incubation, the luciferase reporter activity was measured on a Top Counter (TopCounter NXT; PerkinElmer Life Sciences) using a LucLite luciferase assay kit (PerkinElmer Life Sciences). When cells were treated with TSA or MS-27-275 at various concentrations, an additional 24-h incubation was allowed before luciferase activities were measured.

Results

Expression of Recombinant Human HDAC1 and HDAC3. We previously have successfully expressed hHDAC8 in Escherichia coli using histag fusion (PET-30) vectors (Hu et al., 2000). However, when similar constructs were made to express HDAC1 and HDAC3, the proteins were insoluble and inactive. Thus, a baculovirus expression system was chosen for expression of hHDAC1 and hHDAC3. GST-fusion expression vectors were constructed as outlined in Fig. 1. Western blot was used to assess the expression level and solubility (data not shown). Approximately 50% of the GST-HDAC1 and GST-HDAC3 were soluble. HDAC1 was purified using GST affinity chromatography and monQ high-performance liquid chromatography. HDAC3 was purified using GST affinity chromatography alone. Both purified proteins remained soluble, and over 80% purity was achieved for both HDACs (Fig. 2). Purified GST-hHDAC1 and GST-hHDAC3 corresponded to ~100 and ~85 kDa, respectively. A small amount of cleaved GST (29 kDa) was visible on the stained gel (Fig. 2, star). This could be due to cleavage by a presumed endogenous protease in Sf9 cells. We attempted to cleave the GST moiety from both fusion proteins; however, cleavage resulted in rapid aggregation and precipitation of both HDACs. Thus, purified GST-HDAC1 and GST-HDAC3 was used for further enzyme assays and analysis of inhibitors without further manipulation. As described previously (Hu et al., 2000), hHDAC8 was expressed as histag fusion protein in E. coli and was purified to near homogeneity via nitrilotriacetic acid-Ni²⁺ affinity chromatography (Fig. 2).

Enzymatic activity of recombinant hHDAC1, hHDAC3, and hHDAC8 was examined using a conventional HDAC assay. Both recombinant hHDAC1 and hHDAC3 displayed concentration-dependent HDAC activity toward ³H-labeled acetylated-histone H4 peptide. This activity was completely suppressed by 1 µM TSA (Fig. 3A). Furthermore, when metabolically ³H-labeled, acetylated histones were incubated with all three hHDACs and resolved in an SDS-PAGE, significant reduction in ³H radioactivity was observed, indicating active deacetylase activity (Fig. 3B). Densitometry measurement indicated that reduction of ³H radioactivity after incubation were approximately 65% for 1 µg of HDAC1, 44% for 1 µg of HDAC3, and 40% for 1 µg of HDAC8 after 1-h incubation (Fig. 3B, bottom). These data indicated that purified HDACs were active toward acetylated histone peptide as well as isolated histones.

![Fig. 1. Construction of baculoviral expression vector for hHDAC1 and hHDAC3. pFB is viral polyhedrin promoter. HDAC1 and HDAC3 were inserted as an in-frame fusion to GST.](image-url)
Differential Inhibition of hHDAC1, hHDAC3, and hHDAC8 by TSA and MS-27-275. To assess the sensitivity of recombinant human HDAC1, 3, and 8 toward HDAC inhibitors, we used two known HDAC inhibitors TSA and MS-27-275. TSA inhibited all three HDACs with similar IC50 values (100–300 nM) (Fig. 4A); however, MS-27-275 displayed differential inhibition of the three HDACs (Fig. 4B). Recombinant hHDAC1 was highly sensitive to MS-27-275, with an IC50 value of ~300 nM, similar to that of TSA. hHDAC3 is moderately sensitive to MS-27-275, with IC50 value of ~8 μM, whereas hHDAC8 was insensitive to MS-27-275, with only ~30% inhibition observed at 100 μM concentration.

To test whether the inhibition of HDAC1 is reversible, HDAC1 was first bound to GST beads and incubated with 1 μM TSA or MS-27-275. Subsequently, the beads were washed and assayed for HDAC activity. As shown in Fig. 4C, washing eliminated the inhibitory effect of both TSA and MS-27-275, suggesting that both compounds are reversible inhibitors.

Effects of TSA and MS-27-275 on SW620 Colon Cancer Cells. Because TSA and MS-27-275 displayed different profiles with regards to inhibition of individual HDACs, we compared the effect of both inhibitors on SW620 human colon cancer cells. Northern and Western blot analysis (Fig. 5, A and B) demonstrated that HDAC1, 3, and 8 were all expressed in these cells. The level of expression of HDAC1 (mRNA and protein) was significantly higher compared with HDAC3 and 8, and addition of TSA did not affect the expres-
sion of HDAC1. In contrast, TSA treatment of SW620 cells modestly induced HDAC3 mRNA (1.3-fold) and protein (1.5-fold) (Fig. 5, A and B), consistent with a previous report (Dangond and Gullans, 1998). When SW620 cells were treated with TSA and MS-27-275, both compounds dose-dependently induced histone H4 acetylation (Fig. 6A) and apoptosis (Fig. 6B). TSA-induced accumulation of histone H4 acetylation seemed to plateau at 0.3 to 1 μM, whereas MS-27-275-induced acetylation reached maximum at approximately 3 μM. For TSA, the maximum apoptosis was reached at approximately 1 μM, whereas 10 μM MS-27-275 was required to reach maximal level.

HDAC inhibitors have been shown to activate artificial promoters containing Sp1 site (Sowa et al., 1997). We thus compared the effect of TSA and MS-27-275 on the SV40 early promoter. Both compounds significantly induced the promoter activity in a dose-dependent manner (Fig. 6C). TSA was more potent with an EC50 value of ~100 nM, whereas the EC50 value for MS-27-275 was approximately 300 nM.

**Novel HDAC Inhibitors.** In an attempt to identify novel and selective HDAC inhibitors, an HDAC8 enzyme-based high-throughput screen (Hofmann et al., 2000) was developed and used to screen a chemical compound collection. In addition, a luciferase reporter assay using thymidine kinase promoter was also used to screen a similar chemical collection. Resulting compounds were analyzed for HDAC inhibitory activities as well as for activation of a SV40 early promoter (Hu et al., 2000). Three representative compounds were identified (Fig. 7A). SB-556629, also known as Scriptaid (Su et al., 2000), demonstrated inhibitory activity toward all three class I HDACs tested (IC50 values of 0.6 μM for HDAC1 and 3 and ~1 μM for HDAC8). SB-429201 preferentially inhibited HDAC1 (IC50 value of ~1.5 μM), and SB-379872-A only inhibited HDAC8 (IC50 value of ~0.5 μM) (Fig. 7B). When these compounds were added to SW620 cells, dose-dependent increases in histone acetylation were observed for Scriptaid. A modest dose-dependent increase was also seen for SB-429201, whereas no effect in histone acetylation was observed for SB-379872-A (Fig. 7C). A similar activation in SV40 early promoter in response to these three
compounds was also observed (Fig. 7D). These results suggest that the inhibition of HDAC1 could underlie the observed increase in H4 acetylation as well as the increase of Sp-1-mediated SV40 promoter activity in these cell types. However, our data do not exclude the possibility that other members of class I or class II are involved in either histone acetylation or SV40 promoter activation. Further experiments are required to examine the effect of these inhibitors on the activities of other members of HDAC family.

Discussion

HDACs belong to a large and expanding protein family that now encompasses at least 11 members. They are classified into class I and class II HDACs, based on 1) size and sequence characteristics, and 2) distinct association with different transcription cofactors. It has been suggested that distinct HDACs play specific role in diverse cellular processes, including regulation of gene expression, chromosome remodeling, and cell proliferation. Because almost all of the class I and class II mammalian HDACs identified thus far are sensitive to sodium butyrate and TSA inhibition, there is intense interest in identifying isomor-specific inhibitors for each HDACs, and such tool inhibitors would facilitate the functional analysis of HDACs.

In this article, we demonstrated that recombinant human HDAC1, 3, and 8 can be used to identify such isoform-specific inhibitors. All three HDACs were expressed as soluble enzymes and thus were amenable to biochemical and enzymatic analysis. In vitro, purified HDAC1, 3, and 8 demonstrated deacetylase activity toward purified histone, and this activity was completely inhibited by TSA. Our results demonstrated that MS-27-275, a compound originally isolated by its ability to suppress tumor growth (Saito et al., 1999), was in fact an HDAC1-selective inhibitor. MS-27-275 inhibited HDAC1 with IC50 value of approximately 300 nM, whereas its IC50 value for HDAC3 was 8 μM, and it only marginally inhibited HDAC8. These results confirm the notion that HDACs display differential sensitivity toward different pharmacological inhibitors and that a highly selective HDAC inhibitor could be obtained via high-throughput screening of diverse chemical banks and medicinal chemical approaches. This point is further strengthened by the identification of isoform-selective as well as nonselective HDAC inhibitors using high-throughput screening technology. A relatively nonselective inhibitor Scriptaid, a selective HDAC1 inhibitor SB-429201, and a potent HDAC8 inhibitor SB-379278-A were identified in this manner. Interestingly, we observed a dramatic increase of histone acetylation when SW620 cells were treated with Scriptaid and SB-429201, but not with SB-379278-A. Moreover, very little SV40 promoter activation was observed for HDAC8 inhibitor SB-379278-A, whereas all the other inhibitors elicited strong activation. It is noteworthy that Scriptaid and SB-429201 were identified from a reporter-based screen, whereas SB-379278-A was found via a HDAC8 enzyme activity based screen. It is thus possible that a functional screen such as reporter assay could skew toward HDAC1 and thus identify selective set of inhibitors. On the other hand, HDAC enzyme activity-based screening could alleviate such bias and may be useful to identify compounds with selectivity toward individual HDACs. Selectivity of HDAC inhibitors could help reduce the potential adverse effects of a pan-HDAC inhibitor on cardiovascular and other systems (Rossig et al., 2002; Zhang et al., 2002).

With the expanding family of HDACs, a logical question is which one(s) of these 11 HDACs might be primarily responsible for the diverse effects of HDACs, such as cell proliferation and/or differentiation and therefore, most relevant to cancer cell growth? Using the HDAC1-selective inhibitor MS-27-275, we observed a good correlation between HDAC1 inhibition, cellular histone acetylation, SV40 promoter activation, and apoptosis. Similar findings were also observed for a novel HDAC1-selective inhibitor SB-429201. In contrast, a potent HDAC8 inhibitor, SB-379278-A, did not affect cellular histone acetylation or SV40 promoter activity, suggesting either that proteins other than histones may be the physiological substrate of HDAC8 or that HDAC8 is unimportant for these activities in SW620 cells. These data also suggest...
Fig. 7. A, structures of novel HDAC inhibitors. B, effects of selective HDAC inhibitors on deacetylase activity of purified HDAC1, 3, and 8. C, effect of novel HDAC inhibitors on histone H4 acetylation in SW620 cells. Control (0) and TSA (T, 300 nM) were used as positive controls. Increasing concentration of SB-556629 (Scriptaid), SB-429201 and SB-379278A (0.01, 0.03, 0.1, 0.3, 1, 3, 10, and 30 μM) were used. D, effect of novel HDAC inhibitors on SV40 early promoter activity. Individual experiments were repeated at least three times and a representative data set is shown.
that of the class I HDACs evaluated in this study, HDAC1 is more essential for cancer cell growth and may be the therapeutical target for intervention. HDAC1 is the first histone deacetylase identified (Taunton et al., 1996) and there is considerable evidence linking this enzyme to cell growth and tumorgenic transformation. HDAC1 is also known to be induced by growth stimuli (Bartl et al., 1997); it is involved in deacetylation of acetylated p53, thereby modulating its activity (Luo et al., 2000). HDAC1 expression is increased in human gastric and prostate cancers (Choi et al., 2001; Patra et al., 2001). More recently, HDAC1 has been shown to be induced by hypoxia (Kim et al., 2001). This evidence, combined with our results with selective HDAC1 inhibitors suggests the important role of HDAC1 in tumorgenesis and its potential as a therapeutical target.

It is worth noting that our study did not examine the effect of the novel HDAC inhibitors on class I HDACs, and it is unknown whether these compounds also inhibit any of the class II HDACs. Class II HDACs have more tissue-restricted distribution and may participate in biological functions related to a particular tissue or cell type rather than cell proliferation per se. For example, HDAC4 and 5 are highly expressed in cardiomyocytes and are involved in cardiac hypertrophy (Zhang et al., 2002). Interestingly, a selective HDAC6 inhibitor, tubacin, has been recently identified that seems to specifically increase α-tubulin acetylation, whereas it has no effect on histone acetylation, TSA-inducible gene expression and cell cycle progression (Haggarty et al., 2003). In contrast, FK901228, an HDAC inhibitor undergoing phase I clinical trial for cancer, showed strong inhibition for HDAC1 and HDAC2 but weak activity toward HDAC4 and 6 (Furumai et al., 2002). These data are consistent with the notion HDAC1 may be more important for tumor growth. Further studies are warranted to identify inhibitors that can distinguish class I and class II HDACs and examine their cellular effects.

HDACs are known to form complexes with other cofactors such as p300, MEF2, and even other HDACs. For instance, HDAC1 and 2 are present in Sin3, NuRD, and coREST nuclear complexes, whereas HDAC3 is found in Neor complex (Grozinger and Schreiber, 2002). Recent data also showed that class I HDACs associate with chaperone proteins such as Hsp70 and the formation of such complexes enhances deacetylase activity (Johnson et al., 2002). It is currently unknown whether HDAC activity is modulated in other complexes and whether their sensitivity to various inhibitors is different compared with purified HDACs. The availability of selective inhibitors and purified components of these complexes will allow us to begin to explore these questions.

Currently, several HDAC inhibitors are in clinical trials (Kelly et al., 2003) for antitumor therapy; however, most of these have not been tested for selectivity versus individual purified HDACs. Many, if not all, of these compounds inhibit multiple HDACs. For example, hybrid polar compounds have been shown to suppress both HDAC1 and 3 activities at similar potencies (Richon et al., 1998). All of these inhibitors increased histone acetylation, thus suggesting that at least HDAC1 inhibition is involved. The method of expressing recombinant HDACs that we described here could be used to systemically examine the effect of these inhibitors on HDAC enzymatic activity and correlate their effects on gene expression, cell proliferation, and apoptosis. This would allow identification of compounds with better therapeutic potency and reduced side effects.

References


Address correspondence to: Dr. Erding Hu, Rm. 25-1084, Department of Vascular Biology, GlaxoSmithKline, 709 Swedeland Rd., King of Prussia, PA 19406. E-mail: erding_hu-1@gsk.com