

Review

Competitive or noncompetitive, that's the question: research toward histone deacetylase inhibitors

Hong Su,¹ Lucia Altucci,² and Qidong You¹

¹Department of Medicinal Chemistry, China Pharmaceutical University, Jiangsu, People's Republic of China; and

²Dipartimento di Patologia generale, Seconda Università degli Studi di Napoli, Naples, Italy

Abstract

Histone deacetylase (HDAC) catalyze deacetylation of acetylated lysine residues on histones and a growing number of nonhistone proteins including many transcription factors, playing an important role in the upstream control of gene transcription, cell cycle progression, and apoptosis. It has been widely recognized that HDACs are promising targets for cancer therapy. At least 10 HDAC inhibitors are currently in clinical evaluation. However, none of them is practically isoform selective. More and more evidence suggests that acetylation modification occurring in ~85% of eukaryotic proteins should be a general mechanism for altering protein structures or protein-protein interactions. Unselectively inhibiting the deacetylation activity of HDACs and the consequent modulation of the acetylation status of so many substrates might have multiple mechanisms of action *in vivo*, resulting in both therapeutic responses and unanticipated side effects. Lack of selectivity for the existing HDAC inhibitors is somewhat logical for the highly conserved residues in the catalytic site and the malleable structure in the rim of the active site of HDAC enzymes. For further advancements in the development of HDAC inhibitors, clues for selectivity will have to be considered. [Mol Cancer Ther 2008;7(5):1007–12]

Introduction

Since the discovery of the correlation between gene transcription and acetylation level of histones in 1964 (1),

there has been a rapid development in the field of histone deacetylase (HDAC) research thus far. The first HDAC enzyme was isolated in 1996 (2), the first crystal structure of a HDAC homologue bound to inhibitor was elucidated in 1999 (3), and the first HDAC inhibitor suberoylanilide hydroxamic acid was approved by Food and Drug Administration in 2006. The last four decades have witnessed a considerable progress in the field of drug discovery.

The well-known substrates of HDACs are histones in nucleosome, fundamental repeating unit of chromatin, which explains why these enzymes have been named HDACs once identified. In general, hyperacetylation of histones is associated with increased transcriptional activity, whereas hypoacetylation is associated with repression of gene expression (4). The fact that acetylation is a key component in the regulation of gene expression has stimulated the study of HDACs as well as their inhibitors. The hypothesis of a "histone code" as major system of epigenetic modification further accelerated HDACs research (5, 6). Deacetylation is only part of the "histone code," which also includes methylation, phosphorylation, ubiquitination, SUMOylation, etc. These "languages" encoded in histone termini act in a combinatorial and hierarchical manner and modulate specific regulatory events. It should be pointed out that the biological properties of HDACs should not be restricted to the "histone code." As more and more nonhistone proteins have been identified as substrates of HDACs, the acetylation modification may represent a general mechanism for altering protein structures or protein-protein interactions and may play a crucial role in many biological processes *in vivo* (7).

HDACs appear to have diverged early in evolution and have been conserved in eukaryote, suggesting that these enzymes may play critical nonredundant functions (8). However, despite the fact that many HDAC inhibitors in clinical investigation have shown tremendous therapeutic benefits in diseases such as melanoma, leukemias, lymphomas, and myelodysplasia, the precise biological role of individual HDAC enzymes is largely unknown. The limited knowledge of HDAC biology and the role of individual HDACs in cancer have led to an empirical approach to the use of HDAC inhibitors, which threaten to slow the field's progress (9). Biological study of individual HDACs is a prerequisite task in the field of HDACs research.

HDACs and nonhistone substrates, including transcription factors, transcription regulators, signal transduction

Received 11/30/07; revised 2/4/08; accepted 2/20/08.

Grant support: Natural Science Foundation of Jiangsu Province BK2007171; PRIN 2006, European Union (CancerDip project 200620; Epitron LSHC-CT2005-518417), and Regione Campania, L.5 annualità 2005 (L. Altucci).

Requests for reprints: Qidong You, Department of Medicinal Chemistry, China Pharmaceutical University, 24 Tongjiang, Nanjing, Jiangsu, 210009 People's Republic of China. Phone: 86-25-83141351; Fax: 86-25-83141351. E-mail: youqidong@gmail.com

Copyright © 2008 American Association for Cancer Research.

doi:10.1158/1535-7163.MCT-07-2289

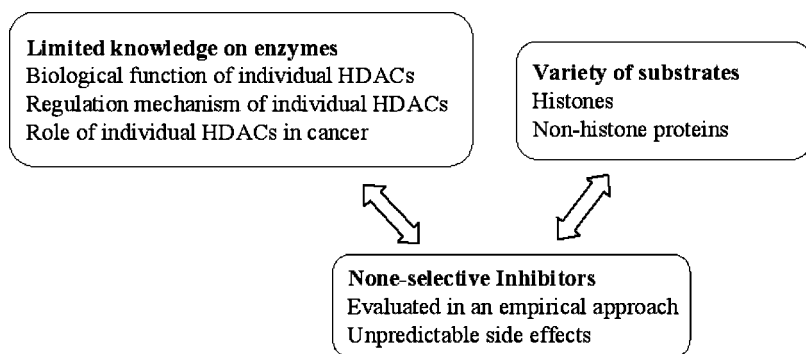


Figure 1. Interdependent relationship of knowledge on HDAC enzymes, substrates, and inhibitors. Nonselective HDAC inhibitors are evaluated in an empirical approach for limited knowledge on HDAC enzymes, and nonselective HDAC inhibitors are useless for identifying the biological roles of individual HDACs. There is a variety of HDAC substrates, but deacetylation identified thus far always occur on their specific lysine residues other than any other amino residues. Therefore, mimics of lysine tails that competitively inhibit HDACs are usually nonselective inhibitors. On the other hand, nonselective HDAC inhibitors should have lots of unpredictable side effects due to the wide distribution of HDAC substrates.

mediators, DNA repair enzymes, nuclear import regulators, chaperone proteins, structural proteins, inflammation mediators, and viral proteins (10), play key roles in many biological processes. Thus, unselectively inhibiting the deacetylation activity of HDACs and consequently modulating acetylation status of these substrates should have multiple mechanisms of action *in vivo*, resulting in both therapeutic responses and unanticipated side effects (Fig. 1). In fact, some clinically investigated HDAC inhibitors showed cardiac toxicity (11–14). Moreover, an electrocardiogram change (in particular, the prolongation of the QTc interval, a measure of the time required for polarization and depolarization of heart muscle cells) has been reported at least occasionally in patients taking almost every HDAC inhibitor (9, 14). Considering the diversified substrates of HDACs, selective HDAC inhibitors, which inhibit individual HDACs and affect the acetylation status of relative small number of HDACs substrates, may display more foreseeable therapeutic effects and less side effects.

HDAC Enzymes

HDACs belong to a deacetylase superfamily. There are at least 18 HDAC enzymes identified in human until now, which are categorized into four classes, based on their homology to yeast deacetylases (15).

Class I HDACs are well-known transcriptional corepressors and always associate with transcriptional repressors and cofactors *in vivo*. There are three well-known multicomponent complexes: Sin3, NuRD, and CoREST. These complexes all have HDAC1/2 as an indispensable component for deacetylation of acetylated histone tails. HDAC3 forms complexes with class II HDACs; however, reconstitution experiments *in vitro* indicate that HDAC4 and other class II HDACs are inactive in the context of the NCoR/SMRT-HDAC3 complex and do not contribute to its enzymatic activity (16, 17). These observations indicate that class II HDACs regulate transcription in the context of enzymatic active NCoR/SMRT-HDAC3 complex independent of any intrinsic HDAC activity.

Accumulating biological data suggest that class I HDACs are associated with cell cycle progression,

metastasis, and apoptosis and are promising targets for cancer therapy (18–22). Overexpression of class I HDACs has been reported in various cancer tissues (23–26). For example, studies showed that class I HDACs are expressed at significantly higher levels in ovarian cancers in comparison with normal ovarian tissues, whereas there is no significant difference in class II HDAC expression between the cancers tissues and normal tissues of ovary. Studies further showed that knocking down of genes encoding HDAC3 and other members of the class I HDAC family suppresses ovarian cancer cell growth (27). The role of individual class I HDACs in tumor cell proliferation was investigated using RNA interference-mediated protein knockdown. Cells can arrest either at the G₁ phase of the cell cycle or at the G₂-M transition in the absence of HDAC1, resulting in loss of mitotic cells, cell growth inhibition, and an increase in the percentage of apoptotic cells (28). Using different HDAC inhibitors together with small interfering RNA for HDAC1, HDAC2, HDAC3, and HDAC6, studies found that inhibition of class I HDAC but not class II HDAC is required for sensitization to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis of chronic lymphocytic leukemia cells and various cell lines (29).

Class IIa HDACs are abundant in heart and repress cardiac gene expression by direct association with MEF2 or indirect interactions with SRF, NFAT, and natural killer family transcription factors (30). These interactions contribute to repression of fetal gene program and prevent hypertrophy. On phosphorylation by kinases of the CaMK superfamily, class IIa HDACs are bound to 14-3-3 chaperone protein and transported to cytoplasm, resulting derepression of target genes. Consistent with their repressive role, mutant mice lacking the class II HDACs are hypersensitive to hypertrophic signals (31).

One consideration for selective HDACs inhibitors is to avoid cardiac side effects, which may be result from unselective interaction with class II HDACs. Paradoxically, studies showed that nonselective HDAC inhibitors, such as trichostatin A and suberoylanilide hydroxamic acid, imposed a dose-dependent blockade to hypertrophy and fetal gene activation (32). There are also other evidences showing that nonselective HDACs inhibitors

block hypertrophy in cell-based model of pressure overload-induced cardiac hypertrophy and animal models of cardiac hypertrophy (33, 34). Explanation for these phenomena by retrospective analysis is that the catalytic activity of class II HDACs may not be required to repress the hypertrophic program, as MITR, a splice variant of HDAC9 that lacks a deacetylase catalytic domain, remains highly effective in suppressing MEF2 activity (35, 36).

In the past 5 years, a tremendous effort has been dedicated to the research of class IIa HDACs. The structure, regulatory mechanisms, biological functions, and therapeutic implications of class IIa HDACs have been elaborated recently (37). Despite the significant advances in this field, several essential questions remain unanswered. Class II HDACs are enzymatically inactive in transcriptional repression complex in the nucleus, and their catalytic domains have nothing to do with their already identified biological functions in nucleus; given their ability to shuttle between the nucleus and the cytoplasm, it may be speculated safely that they should have unidentified substrates in cytoplasm. What are the mysterious biological roles class II HDACs would play in cytoplasm? These are difficult but unavoidable issues in the process of HDACs research.

Selectivity of HDAC Inhibitors

HDAC inhibitors reported thus far are structurally diversified. They are divided into chemical classes including hydroxamic acid derivatives, benzamides, cyclic peptides, short-chain fatty acids, electrophilic ketones, and miscellaneous. Most of these inhibitors are equally active against HDAC of class I, II, and IV. Only a few inhibitors are class selective among class I and II enzymes; even fewer are member-selective within a specific class. All the reported selective HDAC inhibitors have been occasionally discovered or identified via high-throughput screening rather than following a rational design based on the structural differences of individual HDACs.

Most of HDAC inhibitors identified thus far are thought to be competitive inhibitors. Competitive inhibitors inhibit the enzymes by inserting into the same catalytic pockets as the normal enzyme substrates. Competitive HDAC inhibitors generally share the common features of a general pharmacophore model based on the crystal structures of inhibitor-enzyme (HDAC-like protein from *Aquifex aeolicus*) complexes (3). The pharmacophore model comprises a cap group, which is able to interact with the rim of the catalytic tunnel; a hydrophobic spacer, which allows the molecule to lie into the tunnel and interacts with the residues along the tunnel; and a zinc-binding group (ZBG), which is able to complex the zinc ion at the bottom of the catalytic cavity. Usually there is a polar connection unit linking the cap group to the hydrophobic spacer (38). However, inhibitors in inhibitor-enzyme crystal structures always have a hydroxamate group or a

trifluoromethyl ketone group (3, 39–41). As both hydroxamate and trifluoromethyl ketone groups are strong ZBGs, the binding in crystal structure should not be taken as the general binding mode for all HDAC inhibitors, especially for structurally different inhibitors or inhibitors which have weak and atypical ZBGs.

The catalytic domains consisted of ~400 amino acids are well conserved among class I, II, and IV HDAC enzymes (41). These enzymes all have an apparently identical Zn²⁺-dependent catalytic machinery (41, 42). Thus, it is not surprising that most of inhibitors lack class selectivity.

Strategies Toward HDAC Inhibitors

Sequence comparison indicates that main difference among the individual enzymes exists in the loops forming shallow grooves around the rim of enzyme pocket. The comparison of these crystal structures also suggests these enzymes differ considerably in the rim around the surface of the active site. These differences may be responsible for selectivity. A comparison of the structures of HDAC8 bound to four different inhibitors reveals considerable differences in the protein surface in the rim of enzyme pocket, which suggests that this region is highly malleable and is able to change to accommodate binding to different inhibitors (41). Recent researches focus mainly on modulating the cap group of inhibitors, which are supposed to be important for discrimination of residues in the rim of different HDAC enzymes.

Derivatives bearing hydroxamic acid group usually lack selectivity due to strong chelating activity with the zinc ion. The zinc-binding interaction outweighs other factors that may contribute to selectivity. However, a hydroxamic acid-based compound bearing a cyclic peptide mimic as cap group was recently discovered as potent HDAC inhibitor, showing 4-fold selectivity for HDAC1 over HDAC8 (43).

Some studies suggested that the 14 Å internal cavity immediately below the catalytic pocket should be explored as an additional binding element for HDAC inhibitor (44). A series of compounds bearing 2-amino-5-arylanilide-based benzamide pharmacophore were synthesized and these compounds lost activity against HDAC3 and are exclusively HDAC1 and HDAC2 inhibitors (45).

Our recent findings indicated open-chain bispyridinium dienes as selective HDAC inhibitors (46). In addition, some selective HDAC inhibitors with novel structures have also been identified (47). However, these compounds do not have any ZBG and their structures could not be dissected into the traditional ZBG-linker-cap pharmacophore. Considering the perfect selective profiles of these inhibitors, it may be supposed that they may possibly bind to HDAC enzymes in a noncompetitive manner, without interactions with the zinc ion. Although further biochemical experiments, such as photoaffinity labeling, crystallization of enzyme inhibitor complexes should be carried out to elucidate the mechanism of inhibition, as

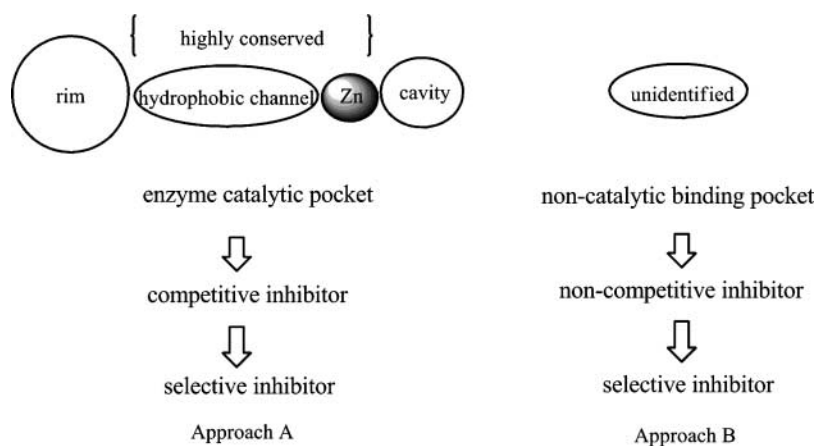


Figure 2. Two strategies toward selective HDAC inhibitor. Strategy A is common used nowadays for obtaining selectivity. For inhibitors that would interact with the catalytic pocket and inhibit HDAC competitively, the strategy starts from choosing an appropriate ZBG, which should not be too potent in zinc binding. Further optimization of the interactions with the rim of the enzyme counteracts the weak zinc-binding activity, gaining potency as well as selectivity. As more and more evidences suggest that substrate specificity mainly depend on cofactors that form various complexes with HDACs, inhibitors targeting noncatalytic binding pocket could affect the conformation of the HDACs and inhibit HDACs in a noncompetitive manner. Strategy B may be more feasible for obtaining selective HDAC inhibitor.

there was noncompetitive inhibitor of another family of zinc hydrolases matrix metalloproteinases already reported (48), noncompetitive HDAC inhibitors seems conceivable.

Until now, there are more than 400 zinc enzymes identified to be involved in all aspects of metabolism (49), such as RNA polymerase II, matrix metalloproteinases, carbonic anhydrases, etc. A bioinformatics survey on the zinc proteins encoded in the human genome has proposed that there are ~2,800 zinc-binding proteins in human (50). Thus, it is difficult for competitive HDAC inhibitors to avoid "off-target" effects *in vivo*, so noncompetitive inhibition of HDACs maybe a plausible approach for further development of HDAC inhibitors.

Whether these compounds structurally different from pharmacophore model of HDAC inhibitors may inhibit HDAC by binding to the catalytic pocket of HDACs in a noncompetitive manner or by binding to an unidentified noncatalytic pocket of HDACs needs further study. However, based on the recruitment mechanism of HDACs, we suppose noncatalytic pockets of HDACs may be explored for mechanism-based development of inhibitors. It has been described that HDACs are recruited to their substrates in four mechanisms (51, 52). The first three mechanisms involve recruiting HDACs to genes via interactions with specific DNA-binding proteins, whereas the fourth mechanism suggests a higher-order regulatory system in which HDACs are inactivated by sequestration (8). Thereby, targeting noncatalytic pocket responsible for the interaction of HDACs and DNA-binding proteins may disrupt recruitment of HDACs to specific site or specific substrate. Inhibitors designed based on the regulation mechanism should be more selective than competitive inhibitors.

The fourth recruitment mechanism is basically for class IIa HDACs. Class IIa HDACs are bound to 14-3-3 chaperone protein and transported to cytoplasm, resulting in derepression of target genes (30). Recent findings indicate that interaction with 14-3-3 proteins protects class IIa HDACs from dephosphorylation, and small molecules that displace this interaction would promote access of the phosphatases and consequently tip the balance toward

hypophosphorylation and increased repression of MEF2-dependent transcriptional activity (37). Targeting the noncatalytic pocket responsible for interaction of class IIa HDACs and 14-3-3 proteins could selectively modulate the biological effects of class IIa HDACs.

Therefore, there should be two strategies for the development of HDAC inhibitors as well as selective HDAC inhibitors. "Competitive" inhibitors target the catalytic pocket of HDACs, whereas the noncompetitive inhibitors would target the noncatalytic pocket responsible for the interaction of HDACs and DNA-binding proteins or other regulatory proteins. Many HDAC inhibitors are designed based on the catalytic pocket of HDACs and are structurally similar with pharmacophore model. Noncompetitive inhibitors selectively interrupting the interaction of HDACs with specific DNA-binding proteins or other regulatory proteins (e.g., 14-3-3 protein) may have better selective profiles (Fig. 2).

Conclusion

Currently, there are at least 10 HDAC inhibitors in clinical investigation. Most of them are hydroxamates and inhibit HDACs in a competitive manner, with quite limited selectivity (9). As more and more pharmaceutical companies and research institutes are devoting to the development of selective HDAC inhibitors, there are some advances in biological methods for evaluating HDAC inhibitors. For example, direct cellular HDAC enzymatic activity assay for robotic screening and spectrophotometric method for screening HDAC8-selective inhibitors (53, 54). In addition, novel markers based on biological roles of individual HDACs should be employed for the assessment of HDAC inhibitors. Information on biological roles and regulatory mechanisms of individual HDACs and gene profiling studies from tumor samples obtained from responders and nonresponders might lead to the identification of these markers (55). These novel predictive markers could be employed for identification of selective HDAC inhibitors and further accelerate the progress toward selective HDAC inhibitors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

- Allfrey VG, Faulkner R, Mirsky AE. Acetylation and methylation of histones and their possible role in the regulation of RNA synthesis. *Proc Natl Acad Sci U S A* 1964;51:786–94.
- Taunton J, Hassig CA, Schreiber SL. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science* 1996;272:408–11.
- Finnin MS, Donigian JR, Cohen A, et al. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. *Nature* 1999;401:188–93.
- Forsberg EC, Bresnick EH. Histone acetylation beyond promoters: long-range acetylation patterns in the chromatin world. *BioEssays* 2001;23:820–30.
- Jenuwein T, Allis CD. Translating the histone code. *Science* 2001;293:1074–80.
- Egger G, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004;429:457–63.
- Kouzarides T. Acetylation: a regulatory modification to rival phosphorylation? *EMBO J* 2000;19:1176–9.
- Grozinger CM, Schreiber SL. Deacetylase enzymes: biological functions and the use of small-molecule inhibitors. *Chem Biol* 2002;9:3–16.
- Garber K. HDAC inhibitors overcome first hurdle. *Nat Biotechnol* 2007;25:17–9.
- Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene* 2007;26:5541–52.
- Piekarz RL, Frye AR, Wright JJ, et al. Cardiac studies in patients treated with depsipeptide, FK228, in a phase II trial for T-cell lymphoma. *Clin Cancer Res* 2006;12:3762–73.
- Shah MH, Binkley P, Chan K, et al. Cardiotoxicity of histone deacetylase inhibitor depsipeptide in patients with metastatic neuroendocrine tumors. *Clin Cancer Res* 2006;12:3997–4003.
- Molife R, Fong P, Scurr M, et al. HDAC inhibitors and cardiac safety. *Clin Cancer Res* 2007;13:1068; author reply 9.
- Kell J. Drug evaluation: MGC0-0103, a histone deacetylase inhibitor for the treatment of cancer. *Curr Opin Investig Drugs* 2007;8:485–92.
- Gregoretti IV, Lee YM, Goodson HV. Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. *J Mol Biol* 2004;338:17–31.
- Fischle W, Dequiedt F, Hendzel MJ, et al. Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. *Mol Cell* 2002;9:45–57.
- Fischle W, Dequiedt F, Fillion M, et al. Human HDAC7 histone deacetylase activity is associated with HDAC3 *in vivo*. *J Biol Chem* 2001;276:35826–35.
- Xia Y, Wang J, Liu TJ, et al. c-Jun downregulation by HDAC3-dependent transcriptional repression promotes osmotic stress-induced cell apoptosis. *Mol Cell* 2007;25:219–32.
- Wang N, Lin KK, Lu Z, et al. The LIM-only factor LMO4 regulates expression of the BMP7 gene through an HDAC2-dependent mechanism, and controls cell proliferation and apoptosis of mammary epithelial cells. *Oncogene* 2007;26:6431–41.
- Bu Y, Gelman IH. v-SRC-mediated downregulation of the SSeCKS metastasis suppressor gene promoter by the recruitment of HDAC1 into a USF1/Sp1/Sp3 complex. *J Biol Chem* 2007;282:26725–39.
- Scott SA, Dong WF, Ichinohasama R, et al. 5-Aza-2'-deoxycytidine (decitabine) can relieve p21WAF1 repression in human acute myeloid leukemia by a mechanism involving release of histone deacetylase 1 (HDAC1) without requiring p21WAF1 promoter demethylation. *Leuk Res* 2006;30:69–76.
- Gui CY, Ngo L, Xu WS, Richon VM, Marks PA. Histone deacetylase (HDAC) inhibitor activation of p21WAF1 involves changes in promoter-associated proteins, including HDAC1. *Proc Natl Acad Sci U S A* 2004;101:1241–6.
- Liby P, Kostrouchova M, Pohludka M, et al. Elevated and deregulated expression of HDAC3 in human astrocytic glial tumours. *Folia Biol (Praha)* 2006;52:21–33.
- Song J, Noh JH, Lee JH, et al. Increased expression of histone deacetylase 2 is found in human gastric cancer. *APMIS* 2005;113:264–8.
- Halkidou K, Gaughan L, Cook S, et al. Upregulation and nuclear recruitment of HDAC1 in hormone refractory prostate cancer. *Prostate* 2004;59:177–89.
- Wilson AJ, Byun DS, Popova N, et al. Histone deacetylase 3 (HDAC3) and other class I HDACs regulate colon cell maturation and p21 expression and are deregulated in human colon cancer. *J Biol Chem* 2006;281:13548–58.
- Khabele D, Son DS, Parl AK, et al. Drug-induced inactivation or gene silencing of class I histone deacetylases suppresses ovarian cancer cell growth: implications for therapy. *Cancer Biol Ther* 2007;6:795–801.
- Senese S, Zaragoza K, Minardi S, et al. A role for histone deacetylase 1 in human tumor cell proliferation. *Mol Cell Biol* 2007;27:4784–95.
- Inoue S, Mai A, Dyer MJ, Cohen GM. Inhibition of histone deacetylase class I but not class II is critical for the sensitization of leukemic cells to tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis. *Cancer Res* 2006;66:6785–92.
- McKinsey TA. Derepression of pathological cardiac genes by members of the CaM kinase superfamily. *Cardiovasc Res* 2007;73:667–77.
- Zhang CL, McKinsey TA, Chang S, et al. Class II histone deacetylases act as signal-responsive repressors of cardiac hypertrophy. *Cell* 2002;110:479–88.
- Antos CL, McKinsey TA, Dreitz M, et al. Dose-dependent blockade to cardiomyocyte hypertrophy by histone deacetylase inhibitors. *J Biol Chem* 2003;278:28930–7.
- Kong Y, Tannous P, Lu G, et al. Suppression of class I and II histone deacetylases blunts pressure-overload cardiac hypertrophy. *Circulation* 2006;113:2579–88.
- Kee HJ, Sohn IS, Nam KI, et al. Inhibition of histone deacetylation blocks cardiac hypertrophy induced by angiotensin II infusion and aortic banding. *Circulation* 2006;113:51–9.
- Hamamori Y, Schneider MD. HATs off to Hop: recruitment of a class I histone deacetylase incriminates a novel transcriptional pathway that opposes cardiac hypertrophy. *J Clin Invest* 2003;112:824–6.
- Sparrow DB, Miska EA, Langley E, et al. MEF-2 function is modified by a novel co-repressor, MITR. *EMBO J* 1999;18:5085–98.
- Martin M, Kettmann R, Dequiedt F. Class IIa histone deacetylases: regulating the regulators. *Oncogene* 2007;26:5450–67.
- Mai A, Massa S, Rotili D, et al. Synthesis and biological properties of novel, uracil-containing histone deacetylase inhibitors. *J Med Chem* 2006;49:6046–56.
- Nielsen TK, Hildmann C, Riestler D, et al. Complex structure of a bacterial class 2 histone deacetylase homologue with a trifluoromethylketone inhibitor. *Acta Crystallograph Sect F Struct Biol Cryst Commun* 2007;63:270–3.
- Vannini A, Volpari C, Filocamo G, et al. Crystal structure of a eukaryotic zinc-dependent histone deacetylase, human HDAC8, complexed with a hydroxamic acid inhibitor. *Proc Natl Acad Sci U S A* 2004;101:15064–9.
- Somoza JR, Skene RJ, Katz BA, et al. Structural snapshots of human HDAC8 provide insights into the class I histone deacetylases. *Structure* 2004;12:1325–34.
- Vanommeslaeghe K, De Proft F, Loverix S, Tourwe D, Geerlings P. Theoretical study revealing the functioning of a novel combination of catalytic motifs in histone deacetylase. *Bioorg Med Chem* 2005;13:3987–92.
- Liu T, Kapustin G, Etkorn FA. Design and synthesis of a potent histone deacetylase inhibitor. *J Med Chem* 2007;50:2003–6.
- Wang DF, Wiest O, Helquist P, Lan-Hargest HY, Wiech NL. On the function of the 14 Å long internal cavity of histone deacetylase-like protein: implications for the design of histone deacetylase inhibitors. *J Med Chem* 2004;47:3409–17.
- Moradei OM, Mallais TC, Frechette S, et al. Novel aminophenyl benzamide-type histone deacetylase inhibitors with enhanced potency and selectivity. *J Med Chem* 2007;50:5543–6.
- Perez-Balado C, Nebbioso A, Rodriguez-Grana P, et al. Bispyridinium diones: histone deacetylase inhibitors with selective activities. *J Med Chem* 2007;50:2497–505.
- Hu E, Dul E, Sung CM, et al. Identification of novel isoform-selective

inhibitors within class I histone deacetylases. *J Pharmacol Exp Ther* 2003; 307:720–8.

48. Fujita M, Nakao Y, Matsunaga S, et al. Ageladine A: an antiangiogenic matrixmetalloproteinase inhibitor from the marine sponge *Agelas nakamurai*. *J Am Chem Soc* 2003;125:15700–1.

49. Lipscomb WN, Strater N. Recent advances in zinc enzymology. *Chem Rev* 1996;96:2375–434.

50. Andreini C, Banci L, Bertini I, Rosato A. Counting the zinc-proteins encoded in the human genome. *J Proteome Res* 2006;5:196–201.

51. Burke LJ, Baniahmad A. Co-repressors 2000. *FASEB J* 2000;14: 1876–88.

52. Wade PA. Transcriptional control at regulatory checkpoints by histone deacetylases: molecular connections between cancer and chromatin. *Hum Mol Genet* 2001;10:693–8.

53. Ciossek T, Julius H, Wieland H, Maier T, Beckers T. A homogeneous cellular histone deacetylase assay suitable for compound profiling and robotic screening. *Anal Biochem* 2008;372:72–81.

54. Fatkins DG, Zheng W. A spectrophotometric assay for histone deacetylase 8. *Anal Biochem* 2008;372:82–8.

55. Minucci S, Pelicci PG. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nat Rev Cancer* 2006;6: 38–51.