Clin Transl Oncol (2008) 10:395-398
DOI 10.1007/s12094-008-0221-x

Histone deacetylase inhibitors: mechanism of action and therapeutic use in cancer

Olaia Martínez-Iglesias · Lidia Ruiz-Llorente · Ruth Sánchez-Martínez · Laura García · Alberto Zambrano · Ana Aranda

Received: 11 April 2008 / Accepted: 15 May 2008

Abstract Histone deacetylases (HDACs) remove the acetyl groups of lysine residues of histone tails leading to chromatin compaction and transcriptional repression. In addition, HDACs can also influence transcription-independent events such as mitosis or deoxyribonucleic acid (DNA) repair and deacetylate nonhistone proteins involved in cell proliferation and death, altering their function. Histone deacetylase inhibitors (HDACi) constitute a promising treatment for cancer therapy due to their low toxicity. HDACi have been shown to induce differentiation, cell-cycle arrest, and apoptosis and to inhibit migration, invasion, and angiogenesis in many cancer cell lines. In addition, these compounds inhibit tumor growth in animal models and show antitumor activity in patients. HDACi alone and in combination with a variety of anticancer drugs are being tested in clinical trials, showing significant anticancer activity both in hematological and solid tumors. SAHA (vorinostat, Zolinza) was the first HDACi approved by the US Food and Drug Administration to enter the clinical oncology market for treating cutaneous T-cell lymphoma (CTCL) and is being tested for other malignancies.

*Supported by an unrestricted educational grant from Pfizer.

O. Martínez-Iglesias · L. Ruiz-Llorente · R. Sánchez-Martínez · A. Zambrano · A. Aranda (✉)
Instituto de Investigaciones Biomédicas
CSIC-UAM
C/ Arturo Duperier, 4
ES-28029 Madrid, Spain
e-mail: aaranda@iib.uam.es

Keywords Histone deacetylases · Histone deacetylase inhibitors · Combination therapy

Histone deacetylases (HDACs)

Nucleosomes are the basic repetitive units of eukaryotic chromatin. Nucleosomes, which fold chromosomal deoxyribonucleic acid (DNA), contain two molecules each of the core histones H2A, H2B, H3, and H4. Almost two turns of DNA are wrapped around this octameric core, which provides a major impediment to transcription. Histone variants, distinct patterns of posttranslational modifications of histones (the histone code) [1], and histone-tail-binding proteins contribute to establishing various open or closed chromatin domains. Among histone modifications, histone acetylation has generated the most interest, as it provided an early link between gene transcription and chromatin modification [2]. Increased lysine acetylation in histone tails has been correlated with transcriptional activation. Acetylation neutralizes the positively charged lysine residues of the histone N-termini, decreasing their affinity for DNA, which causes nucleosome unfolding and can increase access of transcription factor to the promoter [3]. In contrast, histone hypoacetylation has been associated with chromatin compaction and transcriptional repression.

Relative levels of histone acetylation are known to be determined by the enzymatic activities of both histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDACs can be divided into two subfamilies [4] (Table 1): the Zn^{+2}-dependent HDACs (classes I, II, and IV) and the NAD^{+}-dependent sirtuins (SIRT) (class III). Class I, related to yeast RPD3 deacetylase, includes

Table 1 Histone deacetylases

Group	Class	Name
Zn^{2+}-dependent	I	HDAC1
		HDAC2
		HDAC3
		HDAC8
	II	
	IIa	HDAC4
		HDAC5
		HDAC7
		HDAC9
	IIb	HDAC6
		HDAC10
	IV	HDAC11
NAD^{+}-dependent	III	SIRT 1-7

HDACs 1, 2, 3, and 8 that are primarily nuclear and ubiquitously expressed, whereas class II HDACs are primarily localized to the cytoplasm but are known to shuttle in and out of the nucleus through association with 14-3-3 proteins. Class II HDACs are related to yeast Hda1 (histone deacetylase-1) [5]. This class is divided into class IIa, comprising HDACs 4, 5, 7, and 9; and class IIb, comprising HDACs 6 and 10, which contain two catalytic sites. Class III SIRTs are NAD+-dependent deacetylases, orthologous to yeast silent information regulator 2, that has been linked to regulation of caloric utilization in mammals and have nonhistone proteins as substrates [6]. Class IV is represented by HDAC 11, with limited identity to classes I and III, although is most related to HDACs 3 and 8.

HDACs are not redundant in function, as shown by the different phenotypes obtained in HDAC knockout mice. Thus, disruption of HDAC 1 causes early embryonic lethality, showing that this protein plays a key role in cell proliferation and survival, HDACs 2, 5, and 9 knockouts have cardiac defects, HDAC 4 knockouts have abnormal chondrocyte differentiation, and HDAC 7 knockouts have vascular defects [7].

A controlled equilibrium between histone acetylation and deacetylation appears to be essential for normal cell growth. Thus, tumorigenesis has been associated with HAT inactivation, whereas aberrant HDAC activity has been linked to development and maintenance of the transformed state of human tumors, which could be related to abnormal transcriptional silencing of tumor-suppressor-gene expression. HDACs do not bind to DNA directly but belong to multiprotein corepressor complexes. Different complexes contain different HDACs. For instance, HDACs 1 and 2 associate with Sin3-SAP, NuRD, and CoRest transcriptional corepressor complexes, whereas HDAC 3 is a component of nuclear receptor corepressor (NCoR) and silencing-mediator of retinoic and thyroid receptors (SMRT) complexes [8, 9]. These complexes are recruited by transcription factors to specific regions of the genome, leading to the gene expression profile characteristic of normal or transformed cells. Other epigenetic changes, such as promoter CpG island methylation, can differentiate cancer cells from their normal counterparts. Proteins that bind specifically to methylated DNA cause transcriptional repression. These proteins recruit HDACs, resulting in long-term silencing of target genes.

Alterations in both HATs and HDACs are found in many human cancers. Recently, specific changes in histone modifications that characterize cancer cells have been identified. For example, the loss of acetylation of histone H4 at lysine 16 and trimethylation of histone H4 at lysine 20 can be considered a common feature of cancer cells compared with normal cells [10].

In addition to histones, HDACs have many nonhistone proteins as substrates, including –among others– transcriptional regulators, nuclear receptors and other DNA-binding proteins, chaperone proteins, or cytoskeleton components [7, 11]. These HDAC substrates are known to be involved in gene expression, cell-cycle progression, cell differentiation, and cell-death regulation, indicating a complex multifunctional role for HDACs in normal proliferation and cancer that involves deacetylation of both histone and nonhistone proteins [5, 12–14].

Histone deacetylase inhibitors (HDACi)

HDACi are considered to be among the most promising agents in drug development for cancer therapy [12, 13]. These inhibitors present relatively low toxicity, most likely due to the fact that normal cells are relatively resistant to HDACi-induced cell death, whereas a broad variety of transformed cells are sensitive to HDAC-induced cell death.

Natural or synthetic HDACi that are able to inhibit the activity of classes I, II, and IV HDACs can be divided into several chemical groups: hydroxamic acid derivatives, cyclic peptides, short-chain aliphatic acids, and benzamides (Table 2) [7, 13, 15].

The first hydroxamate discovered to inhibit HDACs was the natural compound trichostatin A (TSA) [16]. TSA causes histone hyperacetylation and inhibits cell proliferation at nanomolar concentrations, but its high toxicity precludes its therapeutic use. However, other synthetic compounds derived from hydroxamic acids, such as SAHA (Zolinza, vorinostat), LAQ-824, LBH-589, PXD-101 (belinostat), LBH-589 (panobinostat), or IF-2357 with improved therapeutic profile have been generated. SAHA, a pan-inhibitor of classes I and II HDACs [17], is the first HDACi approved for clinical use in cancer patients for treating cutaneous T-cell lymphoma (CTCL) by the US Food and Drug Administration [18]. The cyclic peptide group of HDACi includes the natural products depsipeptide (FK-228) and apicidin, which are also being tested in clinical trials and inhibit HDAC activity at nanomolar concentrations.

The short-chain fatty acid butyrate has been known for several decades to cause histone hyperacetylation and to

Table 2 Class I and II histone deacetylase inhibitors (HDACi)

Chemical class	Name
Short-chain fatty acids	Butyrate Phenylbutyrate Valproate Phenylacetate
Hydroxamic acid derivatives	Trichostatin A (TSA) SAHA LAQ-824 LBH-589 PXD-101 LBH-589 IF2357
Cyclic peptides	Depsipeptide (FK-228) Apicidin
Benzamides	MS-275 MGCD0103 CI-994

inhibit malignant cell growth [7]. Similar effects have now been demonstrated for other structurally similar compounds such as phenylbutyrate, phenylacetate, valproic acid, and AN-9 (a butyric-acid precursor), which act at millimolar concentrations to inhibit HDAC activity. Both valproic acid and phenylbutyrate are drugs with low toxicity that were in the market for various therapeutic uses before being identified as HDACi [12].

MS-275 and MGCD-0103 are synthetic benzamide derivatives. Interestingly, MS-275 preferentially inhibits HDAC 1 compared with HDAC 3 and has little or no effect against HDACs 6 and 8 [19]. In contrast, tubacin, a selective inhibitor of HDAC 6, does not affect acetylation of histones and does not inhibit cell-cycle progression, but causes tubulin acetylation [20]. Isoform-specific HDACi are under development. However, whether or not selective inhibition will be advantageous in cancer treatment over paninhibition of HDACs remains to be determined.

HDACi target genes

The mechanisms of HDACi-induced transformed-cell-growth arrest and cell death are complex and not completely elucidated. HDACi can cause accumulation of acetylated histones and many nonhistone proteins that are involved in regulation of gene expression, cell proliferation, cell migration, and cell death. Gene-profiling studies with DNA microarrays show that HDACi treatment results in altered expression of as many as 2–10% of genes in various cancer cell lines [21, 22]. The extent of changes depends on the HDACi used and on the concentration and time of exposure to the inhibitors. Intriguingly, although histone acetylation is associated with transcriptional activation, many genes are repressed in response to HDACi treatment. In some cases, repression may be secondary to HDACi-mediated activation of transcriptional repressors, but it should be remembered that HDACi can also induce acetylation of transcription factors and other transcription-regulatory proteins that can result in altered transcriptional activity.

As expected, many of the HDACi-regulated genes are involved in growth arrest, differentiation, and apoptosis [12, 22]. Treatment of cancer cells with HDACi normally causes growth arrest that consistently involves induction of the cyclin-kinase inhibitor (CKI) $p21^{Waf1/Cip1}$. The increase in $p21^{Waf1/Cip1}$ levels is due to transcriptional stimulation. Sp1 binding motifs in the $p21^{Waf1/Cip1}$ promoter mediate induction by HDACi and an increase in the amount of acetylated histone H3 associated with the promoter, and reduction in HDAC 1 binding has been obtained by chromatin immunoprecipitation assays after treatment with HDACi [23]. In addition to p21, HDACi also induce expression of other growth inhibitory CKIs. In contrast, HDACi coordinately downregulate expression of several proproliferative genes, including multiple cyclins, cyclin-dependent kinases, and transcription factors (myc, myb, and E2F family members) involved in cell-cycle progression. HDACi have also been shown to cause terminal cell differentiation and induce polyploidy in cancer cells that loose their ability to proliferate and commit to senescence, indicating a further mechanism by which HDACi can exert growth arrest [12, 22].

HDACi potently induce apoptosis in cancer cells via both the intrinsic/mitochondrial- and the extrinsic/death-receptor pathways [24, 25], although death could also occur by nonapoptotic mechanisms, including mitotic failure and autophagic cell death. HDACi-induced apoptosis may involve transcriptional alteration of the balance between expression of pro- and antiapoptotic Bcl-2 family members, which regulate mitochondrial membrane integrity. HDACi also modulate expression of genes involved in the extrinsic apoptotic pathway, including upregulation of the proapoptotic genes and downregulation of antiapoptotic caspase inhibitors. Consistent with these effects, HDACi sensitize cancer cells to tumor necrosis factor alpha related apoptosis inducing ligand (TRAIL) and fatty acid synthase (FAS)-induced apoptosis in cells resistant to these agents. HDACi-induced apoptosis has also been linked to increased reactive oxygen species (ROS) production. HDACi treatment promotes accumulation of ROS in tumor cells, and treatment with free-radical scavengers can suppress apoptotic activity of HDACi [25].

Besides inhibiting tumor cell proliferation and causing cell death, HDACi also inhibit tumor development and progression by blocking angiogenesis, migration, and invasion [26–28]. HDACi prevent new vessel formation in tumors through downregulation of proangiogenic factors such as hypoxia-inducible factor-1 alpha (HIF-1), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiopoietin, or endothelial nitric oxide

synthase (eNOS), and activation of antiangiogenic factors such as p53 or the von Hippel Lindau protein. In addition, HDACi increase expression of the intercellular adhesion molecules ICAM 1 and E-cadherin, suppress transcription of matrix metalloproteinases (MMPs) while upregulating MMP inhibitors, and inhibit expression of the chemokine receptor 4 (CXCR 4), which is important for colonization of target tissues by metastatic cells [11, 12, 22].

Combination therapy

Based upon the preclinical findings of HDACi *in vitro* and in animal models *in vivo*, a number of HDACi have been used in clinical trials for treating hemopoietic and solid tumors [4, 7, 12, 15, 29]. Data from these clinical trials demonstrate that HDACi treatment leads to tumor regression and improvement in patients at an advanced stage, with unexpectedly few side effects, since, as stated above, HDACi show selective cytotoxicity against tumor cells with little toxicity for normal cells. However, although HDACi are rather well tolerated and show promise for use as monotherapy, it is likely that for improved therapeutic effects, they should be used in combination with other drugs. Preclinical studies have demonstrated that a combination of HDACi with different drugs can synergistically enhance cell killing *in vitro* and inhibit tumor growth in xenograft models, creating a rationale for this approach. Already, HDACi have been shown to cooperate with radiation therapy, antitubulin agents, topoisomerase I and II inhibitors, cisplatin, the kinase inhibitor imanitib, proteasome inhibitors such as bortezomide, the heat shock protein-90 inhibitor 17-N-allylamino-17-demethoxygeldanamycin, the Her2 receptor inhibitor trastuzumab, retinoids, inhibitors of DNA methylation, estrogen receptor antagonists, dexamethasone, etc. More than 100 clinical trials with HDACi either as monotherapy or in combination therapy are ongoing in patients with lung, breast, pancreatic, renal, and bladder cancers, melanoma, neuroblastoma, glioblastoma, leukemias, lymphomas, and multiple myeloma. Results of these clinical trials have recently been reviewed [4, 15, 29], and information about ongoing studies are available online [30]. From these studies, it can be deduced that several HDACi have activity in hematologic malignancies and solid tumors at doses that are well tolerated by patients.

Acknowledgements

This laboratory is supported by grants BFU2007-62402 from MEC, RD06/0020/0036 and PIO40682 from FIS and from the EU Project CRESCENDO (FP6-018652).

References

1. Jenuwein T, Allis CD (2001) Translating the histone code. Science 293(5532):1074–
2. Riggs MG, Whittaker RG, Neumann JR, Ingram VM (1977). n-Butyrate causes histone modification in HeLa and Friend erythroleukaemia cells. Nature 268(5619):462–464
3. Struhl K (1998) Histone acetylation and transcriptional regulatory mechanisms. Genes Dev 12 (5):599–606
4. Glaser KB (2007) HDAC inhibitors: clinical update and mechanism-based potential. Biochem Pharmacol 74(5):659–671
5. Marks PA, Dokmanovic M (2005) Histone deacetylase inhibitors: discovery and development as anticancer agents. Expert Opin Investig Drugs 14(12):1497–1511
6. Blander G, Guarente L (2004) The Sir2 family of protein deacetylases. Annu Rev Biochem 73:417–435
7. Dokmanovic M, Clarke C, Marks PA (2007) Histone deacetylase inhibitors: overview and perspectives. Mol Cancer Res 5(10):981–989
8. Fischle W, Dequiedt F, Hendzel MJ et al (2002) Enzymatic activity associated with class II HDACs is dependent on a multiprotein complex containing HDAC3 and SMRT/N-CoR. Mol Cell 9(1):45–57
9. Li J, Wang J, Wang J et al (2000) Both corepressor proteins SMRT and N-CoR exist in large protein complexes containing HDAC3. Embo J 19 (16):4342–4350
10. Fraga MF, Ballestar E, Villar-Garea A et al (2005) Loss of acetylation at Lys16 and trimethylation at Lys20 of histone H4 is a common hallmark of human cancer. Nat Genet 37(4):391–400
11. Glozak MA, Seto E (2007) Histone deacetylases and cancer. Oncogene 26(37):5420–5432
12. Bolden JE, Peart MJ, Johnstone RW (2006) Anticancer activities of histone deacetylase inhibitors. Nat Rev Drug Discov 5(9):769–784
13. Minucci S, Pelicci PG (2006) Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. Nat Rev Cancer 6(1):38–51
14. Rosato RR, Grant S (2005) Histone deacetylase inhibitors: insights into mechanisms of lethality. Expert Opin Ther Targets 9(4):809–824
15. Marks PA, Breslow R (2007) Dimethyl sulfoxide to vorinostat: development of this histone deacetylase inhibitor as an anticancer drug. Nat Biotechnol 25(1):84–90
16. Yoshida M, Kijima M, Akita M, Beppu T (1990) Potent and specific inhibition of mammalian histone deacetylase both in vivo and in vitro by trichostatin A. J Biol Chem 265(28):17174–17179
17. Richon VM, Emiliani S, Verdin E et al (1998) A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases. Proc Natl Acad Sci U S A 95(6):3003–3007
18. Duvic M, Talpur R, Ni X et al (2007) Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). Blood 109(1):31–39
19. Hu E, Dul E, Sung CM et al (2003) Identification of novel isoform-selective inhibitors within class I histone deacetylases. J Pharmacol Exp Ther 307 (2):720–728
20. Haggarty SJ, Koeller KM, Wong JC et al (2003) Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. Proc Natl Acad Sci U S A 100(8): 4389–4394
21. Peart MJ, Smyth GK, van Laar RK et al (2005) Identification and functional significance of genes regulated by structurally different histone deacetylase inhibitors. Proc Natl Acad Sci U S A 102(10):3697–3702
22. Xu WS, Parmigiani RB, Marks PA (2007) Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene 26(37):5541–5552
23. Gui CY, Ngo L, Xu WS et al (2004) Histone deacetylase (HDAC) inhibitor activation of p21 WAF1 involves changes in promoter-associated proteins, including HDAC1. Proc Natl Acad Sci U S A 101(5):1241–1246
24. Insinga A, Monestiroli S, Ronzoni S et al (2005) Inhibitors of histone deacetylases induce tumor-selective apoptosis through activation of the death receptor pathway. Nat Med 11(1):71–76
25. Ruefli AA, Ausserlechner MJ, Bernhard D et al (2001) The histone deacetylase inhibitor and chemotherapeutic agent suberoylanilide hydroxamic acid (SAHA) induces a cell-death pathway characterized by cleavage of Bid and production of reactive oxygen species. Proc Natl Acad Sci U S A 98(19):10833–10838
26. Kim MS, Kwon HJ, Lee YM et al (2001) Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. Nat Med 7(4): 437–443
27. Mori T, Kim J, Yamano T et al (2005) Epigenetic up-regulation of C-C chemokine receptor 7 and C-X-C chemokine receptor 4 expression in melanoma cells. Cancer Res 65(5):1800–1807
28. Whetstine JR, Ceron J, Ladd B et al (2005) Regulation of tissue-specific and extracellular matrix-related genes by a class I histone deacetylase. Mol Cell 18(4):483–490
29. Karagiannis TC, El-Osta A (2006) Clinical potential of histone deacetylase inhibitors as stand alone therapeutics and in combination with other chemotherapeutics or radiotherapy for cancer. Epigenetics 1(3):121–126
30. US National Institutes of Health, US National Library of Medicine (2008). www.clinicaltrials.gov. Accessed