Subject Review

Histone Deacetylase Inhibitors: Overview and Perspectives

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Abstract

Histone deacetylase inhibitors (HDACi) comprise structurally diverse compounds that are a group of targeted anticancer agents. The first of these new HDACi, vorinostat (suberoylanilide hydroxamic acid), has received Food and Drug Administration approval for treating patients with cutaneous T-cell lymphoma. This review focuses on the activities of the 11 zinc-containing HDACs, their histone and nonhistone protein substrates, and the different pathways by which HDACi induce transformed cell death. A hypothesis is presented to explain the relative resistance of normal cells to HDACi-induced cell death.
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Introduction

There is increasing evidence that the 18 histone deacetylases (HDAC) in humans are not redundant in function. The 18 HDACs are classified into three main groups based on their homology to yeast proteins. Class I includes HDAC1, HDAC2, HDAC3, and HDAC8 and have homology to yeast RPD3. HDAC4, HDAC5, HDAC7, and HDAC9 belong to class II and have homology to yeast HDA1. HDAC6 and HDAC10 contain two catalytic sites and are classified as class IIa, whereas HDAC11 has conserved residues in its catalytic center that are shared by both class I and class II deacetylases and is sometimes placed in class IV (refs. 1, 2; Table 1). These HDACs contain zinc in their catalytic site and are inhibited by compounds like trichostatin A (TSA) and vorinostat [suberoylanilide hydroxamic acid (SAHA)]. This review focuses on the activities of these zinc-containing HDACs. The class III HDACs include sirtuins, have homology to yeast Sir2, and have an absolute requirement for NAD^+; they do not contain zinc in the catalytic site and are not inhibited by compounds like TSA or vorinostat.

HDACs are known as HDACs because histones *were* considered the most important target of HDACs (3, 4). Phylogenetic analysis indicates that the evolution of HDACs preceded the evolution of histones, suggesting that primary HDAC targets may not be histones (3). To date, more than 50 nonhistone proteins have been identified that are substrates for one or another of the HDACs (refs. 4-8; Table 2). These substrates include proteins that have regulatory roles in cell proliferation, cell migration, and cell death. The enzymes may more properly be referred to as ''lysine deacetylases'' (8).

The sensitivity of tumor cells and relative resistance of normal cells to HDACi such as vorinostat may reflect the multiple defects that make cancer cells less likely than normal cells to compensate for inhibition of one or more prosurvival factors or activation of a pro-death pathway. Vorinostat inhibition of HDACs is relatively rapidly reversible, and we suggest that this provides normal cells with compensatory capabilities that translate into relative resistance to induced cell death, compared with the sensitivity of cancer cells (4).

HDAC Biological Activity

Class I HDACs are mostly localized within the nucleus whereas class II HDACs shuttle between nucleus and cytoplasm (Table 1; refs. 1, 5-17). Knockout analysis of different class I and class II HDAC proteins indicates that class I HDACs play a role in cell survival and proliferation, whereas class II HDACs may have tissue-specific roles. For example, HDAC1 knockout has a general proliferation and survival defect despite increased levels of HDAC2 and HDAC3 activity (10). HDAC1 and HDAC3 enhance hypoxia-inducible factor-1α stability via direct interaction with the transcription factor (11). HDAC2 modulates transcriptional activity through regulation of p53 binding activity (12). HDAC2 knockouts had cardiac defects (13). HDAC4 knockout mice have defects in chondrocyte differentiation (14); HDAC5 and HDAC9 knockouts have cardiac defects (15). HDAC7 knockouts have defects in the maintenance of vascular integrity (9). HDAC7 localization is controlled by its state of phosphorylation; myosin phosphate dephosphorylates HDAC7 and promotes its nuclear localization, causing repression of the HDAC7 target Nur77 (16).

It is well established that HDACs catalyze the deacetylation of α-acetyl lysine that resides within the NH_2-terminal tail of core histones, but we know little about the sequence specificity of histones that determine the activity of different HDACs. Using a library of fluorigenic tetrapeptide substrates, HDACs

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Table 1. HDAC Characteristics

	Class I			
	HDAC1	HDAC2	HDAC3	HDAC8
Localization	Nucleus	Nucleus	Nucleus	Nucleus
Size (amino acids)	483	488	428	377
Chromosomal localization	1p34.1	6q21	5q31	Xq13
Catalytic sites	1	1	1	1
Tissue distribution	Ubiquitous	Ubiquitous	Ubiquitous	Ubiquitous? Smooth muscle differentiation
Substrates (partial list)	Androgen receptor, SHP, p53, MyoD, E2F-1, Stat3	Glucocorticoid receptor, YY-1, Bcl-6, Stat3	SHP, YY-1 GATA-1, RelA, Stat3, MEF2D	
Binding partners (partial list)			CDK9, SP1, PP4c	EST1B
Knockout phenotype	EL increased histone acetylation, increase in p21 and p27	Cardiac defect		

Abbreviations: H, heart; SM, skeletal muscle; B, brain; PL, placenta; PA, pancreas; L, liver; K, kidney; S, spleen. EL, embryonic lethal. Stat3, signal transducers and activators of transcription 3; CDK9, cyclin-dependent kinase 9; MMP10, matrix metalloproteinase 10; Hsp90, heat shock protein 90; HIF-1α, hypoxia-inducible factor-1α.

were ranked according to their substrate selectivity: HDAC8 > HDAC1> HDAC3> HDAC6 (17).

The acetylation status of the HDAC substrates is a determinant of their structure and, as a consequence, their activity (Table 2; refs. 1, 5-19). For example, HDAC6 was shown to interact with and deacetylate tubulin, causing modulation of cell migration (18, 19). HDAC1, HDAC2, and HDAC3 were shown to coimmunoprecipitate with the ATP-dependent chaperone protein heat shock protein 70 (20). Inhibition or knockdown of HDAC6 induces heat shock protein-90 acetylation and inhibits its chaperone activity (21, 22). The oncoproteins PLAG1 and PLAG2 are targets for deacetylation by HDAC7 (23).

As discussed below (''HDACi: Mechanisms of Action''), one or more pathways may be involved in HDACi-induced transformed cell death, which is a likely consequence of acetylation of multiple substrates of HDAC (Tables 1 and 2). There are still considerable gaps in our knowledge of the biological functions of the different HDACs.

Histone Acetyl Transferases and HDACs in Cancer

Several groups of transcription factors have intrinsic histone acetyltransferase activity. These include GCN5-related *N*-acetyltransferase, MYST, and cAMP response element binding protein (CREB/p300) families (24). Members of GCN5-related *N*-acetyltransferase family include GCN5, p300/CREB binding protein–associated factors, Elp3, and activating transcription factor-2. MYST family includes monocyte leukemia zinc-finger protein (MOZ), Ybf2/Sas3, Sas2, Tip60, Esa1, and MOF. Histone acetyltransferases and HDACs function within complexes that include multiple histone acetyltransferases, HDACs, transcription coactivators, and corepressors (25).

Alterations in both histone acetyltransferases and HDACs are found in many human cancers (1, 2, 5, 26-34). Individuals with Rubinstein-Taybi syndrome carry a mutation in CREB binding protein that inactivates its histone acetyltransferase activity. Loss of heterozygosity in *p300* gene has been described in 80% of glioblastomas, and loss of heterozygosity in CREB binding protein locus has been observed in a subset of lung cancers. CREB binding protein is fused to different proteins: MLL, MOZ, MYST4, and MORF in acute myeloid leukemia (31-34).

Structural mutations in HDACs associated with cancers are rare. However, changes in expression of different HDACs have been reported in various cancers. HDAC2 and HDAC3 proteins are increased in colon cancer samples (1, 2, 5, 27). HDAC1 is increased in gastric cancer, and reduced expression of HDAC5 and HDAC10 is associated with poor prognosis in lung cancer. HDACs are recruited by oncogenic translocation protein complexes in different types of lymphomas and leukemias (28, 29). A truncating mutation of HDAC2 has been discovered in two colon cancer cell lines and two endometrial cancer cell lines (30).

HDACs, HDACi, and Gene Expression

The chromatin structure is complex and composed of DNA, histones, and nonhistone proteins. The basic repeating unit of chromatin is the nucleosome, ~146 bp of DNA wrapped around the histone octamer composed of two copies of each of four histones: H2A, H2B, H3, and H4. Posttranslational modifications of histones, including acetylation, phosphorylation, methylation, ubiquitination, and sumoylation, play an important role in regulating gene expression (25, 35). The two groups of enzymes, histone acetyltransferases and HDACs, determine the pattern of histone acetylation. It has been proposed that histone modifications, acting alone, sequentially, or in combination, represent a ''code'' that can be recognized by nonhistone proteins, which form complexes that are important for regulation of gene transcription (35).

HDACs and histone acetyltransferases do not bind to DNA directly, but rather interact with DNA through multiprotein complexes that include corepressors and coactivators (24, 25). Class I and class II HDACs form multiprotein complexes containing transcription factors with diverse functions, including

Table 1. HDAC Characteristics (Cont'd)

Class IIA				Class IIB		Class IV
HDAC4	HDAC5	HDAC7	HDAC9	HDAC6	HDAC10	HDAC11
Nucleus/cytoplasm	Nucleus/cytoplasm	Nucleus/cytoplasm	Nucleus/cytoplasm	Mostly cytoplasm	Mostly cytoplasm	Nucleus/cytoplasm
1,084	1,122	855	1,011	1,215	669	347
2q37.2	17q21	12q13	7p21-p15	Xp11.22-23	22q13.31-q13.33	3p25.2
1	1	1	1	2	1	2
H, SM, B	H, SM, B	H, PL, PA, SM	B, SM	H, L, K, PA	L, S, K	B, H, SM, K
GCMa, GATA-1, HP-1	GCMa, Smad7, HP-1	PLAG1, PLAG2		α-Tubulin, Hsp90, SHP, Smad7		
ANKRA, RFXANK	CAMPTA, REA, estrogen receptor	FOX3P, HIF-1α, Bcl-6, endothelin receptor, α-actinin 1, α-actinin 4, androgen receptor, Tip60	FOX3P	Runx2		
Defects in chondrocyte differentiation	Cardiac defect	Maintenance of vascular integrity, increase in MMP10	Cardiac defect			

corepressors like Sin3, nuclear receptor corepressor (N-CoR), silencing mediator for retinoic acid and thyroid hormone receptor (SMRT), as well as activators and chromatin-remodeling proteins (25).

Despite the ubiquitous distribution of HDACs in chromatin, HDACi selectively alter a relatively small proportion of expressed genes (2-10%) in transformed cells (36-40). The effect on gene transcription may be a consequence of acetylation of a particular complex of histones and other proteins regulating gene expression. Studies with lymphoid cell lines found that TSA alters only 2% of 340 expressed genes (36). Recent studies using DNA arrays have shown that as many as 7% to 10% genes were altered in their expression in cell lines of diverse origins (37-40). In these studies, roughly similar number of genes are down-regulated and up-regulated by the HDACi. For example, in a study with CEM cells, a total of 2,205 (22.1%) of expressed genes were altered by vorinostat (as defined by at least 1.5-fold change) by 16 h, with roughly similar numbers being up-regulated and repressed (40).

In a multiple myeloma ARP-1 cell line, vorinostat down-regulated genes of the insulin like growth factor/insulin-like growth factor-I receptor and interleukin-6 receptor signaling cascades and antiapoptotic genes such as caspase inhibitors, oncogenic kinases, DNA synthesis and repair enzymes, transcription factors such as E2F-1, subunits of proteasome, and ubiquitin conjugating enzymes (37). Thymidylate synthetase is commonly repressed by HDACi treatment, including TSA (38).

p21 cyclin-dependent kinase inhibitor is one of the most commonly induced genes by HDACi (41). HDACi induction of p21 is independent of p53. It does correlate with a specific increase in histone acetylation of H3K4 in the p21 promoter region. This change did not occur in the histones associated with the promoter regions of the p27 or of the ε-globin genes whose expression is not altered by the HDACi. The protein complex associated with the proximal region of p21 promoter includes HDAC1, HDAC2, myc, BAF155, Brg-1, GCN5, p300, and SP1. Vorinostat caused a marked decrease in HDAC1 and myc and recruitment of RNA polymerase II to this complex. These findings suggest that HDACi-induced selective alteration of transcription of a gene may be determined by the composition and configuration of proteins in the transcription factor complex, including HDACs.

HDACi treatment inhibited the induction of IFN-stimulated gene expression with little or no effect on their basal expression (42). Selective inhibition of HDAC6 protein by small interfering RNA attenuated the IFN-induced gene expression response (43).

In addition to inhibiting catalytic sites of HDACs, HDACi caused selective changes in expression of class II HDAC proteins. For example, HDAC7 is selectively down-regulated by at least two structurally different HDACi: vorinostat and depsipeptide (44). Repression of HDAC7 is associated with down-regulation of HDAC7 mRNA and transformed cell growth arrest.

Regulation of HDACs

Activities of HDACs are regulated on multiple levels, including protein-protein interactions, posttranslational modifications (sumoylation, phosphorylation, and proteolysis), subcellular localization, and availability of metabolic cofactors (25). Regulation of promoter activity of HDAC genes has been studied for certain HDACs. For example, murine HDAC1 promoter is autoregulated by TSA, which involves several sites including SP1 binding sites and CCAAT box (45). Murine HDAC1 promoter is also inducible by interleukin-2 in T cells. Human HDAC4 promoter activity was repressed after treatment with mithramycin SP1/SP3 transcription factors, indicating their role in HDAC4 regulation (46).

Phosphorylation and subsequent association with 14-3-3 regulate subcellular localization of HDAC4 and HDAC5 (46). Phosphorylation and association with 14-3-3 also regulate HDAC7 localization. Protein kinase D1 is important for phosphorylation of HDAC7 and its nuclear export (47). Myosin phosphatase dephosphorylates HDAC7 and thus promotes its nuclear localization (16). CaMKIV is important for HDAC4 phosphorylation and its nucleocytoplasmic shuttling (48). Tetradecapeptide repeat domain of HDAC6 plays a role in cytoplasmic retention of HDAC6 (49).

Table 2. Protein Substrates of HDACs (Partial List)

Functional Group	Protein	HDAC Implicated	References
Structural protein	α-Tubulin	HDAC6	(19)
Chaperone protein	Hsp90	HDAC6	(84)
DNA binding nuclear receptors	Androgen receptor	HDAC1	(85)
	Glucocorticoid receptor	HDAC2	(86)
	Estrogen receptor α	ND	(87)
	SHP	HDAC1, HDAC3, HDAC6	(88)
DNA binding transcription factors	p53	HDAC1	(89)
	p73	ND	(90)
	MEF2D	HDAC3	(91)
	GCMa	HDAC1, HDAC3, HDAC4, HDAC5	(92)
	YY1	HDAC1, HDAC2, HDAC3	(93)
	GATA-1	HDAC3, HDAC4, HDAC5	(94)
	GATA-2	HDAC3, HDAC5	(95)
	GATA-3	ND	(96)
	MyoD	HDAC1	(97)
	E2F-1	HDAC1	(98)
	E2F-2	ND	(89)
	E2F-3	ND	(89)
	RelA (in NF-κB)	HDAC3	(99)
	PLAG1, PLAG2	HDAC7	(23)
	Bcl-6	HDAC2	(100)
	c-Myc	ND	(89)
	EKLF	ND	(89)
	HIF-1α	ND	(101)
Transcription coregulators	Rb	ND	(102)
	PGC-1α	Class III	(103)
	DEK	ND	(104)
Chromatin remodeling	HMG-A1	ND	(105)
	HMG-B1	ND	(105)
	HMG-B2	ND	(105)
	HMG-N2	ND	(106)
	HMGI(Y)	ND	(106)
	SRY	HDAC3	(107)
Signaling mediators	Stat3	HDAC1, HDAC2, HDAC3	(108)
	Smad7	HDAC1, HDAC3, HDAC2, HDAC5, HDAC6	(109)
	IRS-1	ND	(110)
	β-Catenin	ND	(111)
DNA repair enzymes	Ku70	ND	(112)
	WRN	ND	(113)
Nuclear import	Importin-α7	ND	(114)

Abbreviations: ND, not determined. HMG, high mobility group.

HDACi: Chemistry

HDACi can be divided into several structural classes including hydroxamates, cyclic peptides, aliphatic acids, and benzamides (Table 2; refs. 1, 5, 50, 51). TSA was the first natural hydroxamate discovered to inhibit HDACs (52). Vorinostat is structurally similar to TSA (53). A series of aminosuberoyl hydroxamic acids have recently been discovered to inhibit HDACs and transform cell proliferation at nanomolar concentrations (54). Vorinostat is the first HDACi to be approved for clinical use by the Food and Drug Administration (4, 55). Vorinostat is a pan-inhibitor of class I and class II HDAC proteins (4). M-Carboxycinnamic acid bishydroxamate is a potent HDACi (53) and is the structural basis for several derivatives including LAQ-824, LBH-589, and a sulfonamide derivative, belinostat (PXD-101), TopoTarget AS/Cure Gen Coop; ref. (51). These HDACi inhibit class I and class II HDACs. Panobinostat (LBH-589; Novartis AG) is a cinnamic hydroxamic acid analogue of M-carboxycinnamic acid bishydroxamate. IF2357 (Italfarmaco SpA) is an HDACi that contains a hydroxamic acid moiety linked to an aromatic ring (56). A series of aryloxyalkanoic acid hydroxamides have been synthesized that are HDACi at nanomolar concentrations (57).

The cyclic peptide class is a structurally complex group of HDACi, which includes the natural product depsipeptide (Romidepsin, FK-228, Gloucester Pharmaceutical Inc.), apicidin, and the cyclic hydroxamic acid–containing peptide group of molecules, all active at nanomolar concentrations (58). FK-228 is a prodrug of an active agent, red FK. Among newer HDACi is a cyclic peptide mimic linked by an aliphatic chain to a hydroxamic acid, which is active at millimolar concentrations (59).

The aliphatic acids, such as butyrate, phenylbutyrate, and valproic acid, are relatively weak inhibitors of the HDACs, with activity at millimolar concentrations (5, 8, 51). Both valproic acid and phenylbutyrate are drugs that have been in the market for non-oncological uses and were recently shown to have activity as HDACi. AN-9 (pivaloyloxymethyl butyrate; Titan Pharmaceutical, Inc.) is a novel prodrug of butyric acid (51). 5 NOX-275 (MS-275; Syndax Pharmaceutical Inc.) is a synthetic benzamide derivative. MGCD0103 (Methylgene Inc. Pharmion Corp.) is dihydrobromide salt of a substituted 2-aminophenyl benzamide (60). Some HDACi show relative selectivity in the HDACs inhibited (Tables 1 and 3). For example, MS-275 preferentially inhibits HDAC1 compared

with HDAC3 and has little or no effect against HDAC6 and HDAC8. Two novel synthetic compounds, SK7041 and SK7068, preferentially target HDAC1 and HDAC2. A small molecule, tubacin, selectively inhibits HDAC6 activity and causes accumulation of acetylated tubulin, but does not affect acetylation of histones and does not inhibit cell cycle progression (61). It remains to be determined whether selective inhibition of HDACs would be advantageous over pan-inhibition of HDACs in cancer treatment (4, 50, 62).

Combination of HDACi with Other Antitumor Agents

HDACi is synergistic or additive with different anticancer agents, including radiation therapy (1), chemotherapy, differentiation agents, epigenetic therapy, and new targeted agents (1, 5, 63-65). Chemotherapeutic agents with additive or synergistic effects with HDACi therapy include antitubulin agent docetaxel; topoisomerase II inhibitors doxorubicin, etoposide, and ellipticine; and DNA cross-linking reagent cisplatin (1, 5, 51). Some of the new targeted agents include Bcr-Abl inhibitor imatinib, heat shock protein-90 inhibitor 17-*N*-allylamino-17-demethoxygeldanamycin (64), proteasome inhibitor bortezomide (PS-341; ref. 65), and Her2 receptor inhibitor trastuzumab (Herceptin). Elucidation of downstream antitumor pathways engaged by HDACi may enable the development of more effective therapeutic strategies with these drugs (5, 8, 51).

HDACi in Clinical Trials

At least 12 different HDACi are undergoing clinical trials as monotherapy or in combination with retinoids, Taxol, gemcitabine, radiation, etc., in patients with hematologic and solid tumors, including lung, breast, pancreas, renal, and bladder cancers, melanoma, glioblastoma, leukemias, lymphomas, and multiple myeloma (5, 51, 64; Table 2). There are well over 100 clinical trials ongoing with HDACi as monotherapy or in combination therapy. The available results of these clinical trials have recently been reviewed (51). From these studies, one can conclude that HDACi, including vorinostat, depsipeptide, LBH-589, PDX-101, and several others, have activity in hematologic malignancies and solid tumors at doses that are well tolerated by patients.

Information about ongoing studies are available at several websites.[1]

Hydroxamic Acids

Vorinostat (SAHA) is the first of the new HDACi to be approved by the Food and Drug Administration for clinical use in cancer patients for the treatment of cutaneous T-cell lymphoma. In a phase II clinical trial, 33 previously treated patients with refractory cutaneous T-cell lymphoma received orally administered vorinostat up to 400 mg od. Partial responses were observed in 8 (24.2%) patients and 14 of 31 (45.2%) patients had relief from pruritis. Vorinostat-related toxicities included anemia, thrombocytopenia, fatigue, and diarrhea (55). Vorinostat was evaluated in phase I clinical trials as an i.v. (66) and orally administered drug (67). Patients included those with hematologic (Hodgkin's lymphoma, non-Hodgkin's lymphoma, and multiple myeloma) and solid malignancies (prostate, bladder, breast, colon, ovarian, and renal). In both trials, there was evidence of significant anticancer activity at doses that were well tolerated by patients. In the study with oral formulation, 30% of patients remained on study for 4 to >37 months. Of those patients, there was one complete response in a diffuse large B-cell lymphoma patient and three partial responses in *de novo* diffuse large B-cell lymphoma, laryngeal cancer, and papillary thyroid cancer patients. Stable disease was seen in all other responsive patients (30% of original patient cohort; ref. 67).

In the clinical trial with oral vorinostat in pretreated mesothelioma patients, 2 of 13 patients had a partial remission (68). More than 50 clinical trials with combination therapy with vorinostat and various agents (carboplatin, paclitaxel, 5-fluorouracil, etc.) in patients with advanced hematologic and solid tumors are in progress (see websites indicated earlier).

LBH-589 is a hydroxamic acid–based HDACi with a structure similar to vorinostat (Table 3). It is in phase I clinical trials for cutaneous T-cell lymphoma as an oral agent administered on a Monday, Wednesday, Friday schedule. It has a longer half-life than vorinostat. Responses were seen in 6 or 10 patients including two complete responses and one partial response. Toxicities were similar to those observed with vorinostat (51).

ITF2357, another hydroxamic acid–based HDACi, is in clinical trail for refractory multiple myeloma (56).

PXD-101 (Bellinostat) has completed a phase I open-label study with i.v. administration and is undergoing further studies with an oral preparation. Preliminary data indicate that the oral doses are well tolerated and anticancer activity in the form of stable disease in patients with advanced cancer was observed (57).

Cyclic Peptides

Depsipeptide (Romidepsin; Table 3) is currently in phase II clinical trials including a pivotal trial in cutaneous T-cell lymphoma and peripheral T-cell lymphomas (51, 63). In cutaneous T-cell lymphoma, an objective response was observed in 10 of 28 evaluable patients, including 3 complete responses and 7 partial responses, for an overall response rate of 36%. Adverse events included myelotoxicity, nausea, vomiting, and cardiac dysrhythmias.

Depsipeptide is also in clinical trials as monotherapy and in combination therapy with various anticancer agents in patients with hematologic and solid malignancies (63).

Benzamides

MS-275 (Table 2) is in clinical trials as an oral preparation with evidence of antitumor activity (51).

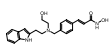
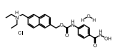
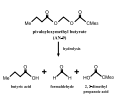
Another benzamide-based HDACi, MGCD0103 is in early combination trials (60).

Aliphatic Acids

Aliphatic acids such as valproic acid (VA) or AN-9 (Table 3) generally are weaker HDACi than hydroxamic acid– or cyclic peptide–based agents. Valproic acid had some therapeutic effect as monotherapy in myelodysplastic syndromes (69).

[1] http://clinicaltrials.gov; http://cancer.gov/clinicaltrials

Table 3. HDACi (Partial List)

Class	Compound	Structure	HDAC Target (Potency)	Effects on Transformed Cells	Stage of Development (Reference)
Hydroxamates	TSA		Class I and II (nmol/L)	TD; GA; A; AI; AE	N/C
	SAHA, Zolinza, vorinostat		Class I and II (μmol/L)	TD; GA; AI; AE; MF; AU; S; PP; ROS-CD	Merck Food and Drug Administration approved for CTCL (4)
	CBHA		N/A (μmol/L)	GA; A; AI; AE	Merck (4)
	LAQ-824		Class I and II (nmol/L)	GA; A; AI	Novartis phase I (discontinued)
	PDX-101		Class I and II (μmol/L)	GA; A	TopoTarget phase II (57)
	LBH-589		Class I and II (nmol/L)	GA; A; ROS-CD	Novartis phase I (51)
	ITF2357		Class I and II (nmol/L)	GA; A; AI	Italfarmaco phase I (56)
	PCI-24781	NA	Class I and II (NA)	N/A	Pharmacyclics phase I
Cyclic peptide	Depsipeptide (FK-228)		Class I (nmol/L)	TD; GA; A; AI; AE; MF; ROS-CD	Gloucester Pharmaceuticals phase IIb for CTCL and PTCL (63) phases I and II
Aliphatic Acids	Valproic Acid		Class I and IIa (mmol/L)	TD; GA; A; S	Abbot phase II
	Phenyl butyrate		Class I and IIa (mmol/L)	TD; GA; A; AI; AE	Phase II
	Butyrate		Class I and IIa (mmol/L)	TD; GA; A; AI; AE	Phase II
	AN-9		N/A (μmol/L)	TD; GA; A	Titan Pharmaceuticals phase II
Benzamides	MS-275		HDAC1, HDAC2, HDAC3 (μmol/L)	TD; GA; A; AI; AE; ROS-CD	Schering AG phase II (51)
	MGCD0103		Class I (μmol/L)	TD; GA; A	Methylgene phase II (60)

Abbreviations: GA, growth arrest; TD, terminal differentiation; A, apoptosis; AI, cell death by activating intrinsic apoptotic pathway; AE, cell death by activating extrinsic apoptotic pathway; MF, mitotic failure; AU, autophagic cell death; S, senescence; PP, polyploidy; ROS-CD, reactive oxygen species–facilitated cell death; N/A, not available; CBHA, M-carboxycinnamic acid bishydroxamate; CTCL, cutaneous T-cell lymphoma; CTCL, peripheral T-cell lymphoma.

Clinical trials with phenylacetate have generally shown little anticancer activity (5, 51, 70).

HDACi: Mechanisms of Action

The mechanisms of HDACi-induced transformed cell growth arrest and cell death are complex and not completely elucidated (1, 4-8). HDACi can cause the accumulation of acetylated histones and many nonhistone proteins that are involved in regulation of gene expression, cell proliferation, cell migration, and cell death.

Normal cells are relatively resistant to HDACi-induced cell death (71, 72), whereas a broad variety of transformed cells are sensitive to inhibitor-induced cell death.

Vorinostat and other HDACi can induce transformed cell cycle arrest and terminal cell differentiation (2), cell death by activating the intrinsic apoptotic pathway (73), activating the extrinsic apoptotic pathway, mitotic failure, autophagic cell death, polyploidy, and senescence, and reactive oxygen species–facilitated cell death (8). HDACi can block angiogenesis (5-8). The induction of a particular response in

transformed cells seems to depend on "cell content" (i.e., molecular changes in the transformed cell), the HDACi used, and the concentration of and time of exposure to the inhibitors.

HDACi have been discovered that are up to 2 or more logs more active than vorinostat in inhibiting partially purified class I and class II HDACs (Table 1; ref. 4). These more active HDACi have generally been more toxic in *in vivo* studies in tumor-bearing animals. Vorinostat has only medium potency in inhibiting HDACs (4). This reflects its binding constant and the relatively rapid reversibility of its binding to HDACs.

Compounds that are more strongly bound are released from the binding pocket more slowly in a first-order process unaffected by the concentration of free ligand. Stronger binding might lead to a more lasting inhibition of the activity of HDAC in both normal and cancer cells and could cause undesirable effects. Vorinostat binding to HDACs is sufficient to cause accumulation of acetylation of target proteins, and its relatively rapid release from the binding site allows for some level of deacetylation activity.

Essentially all cancer cells have multiple defects in expression and/or structure of proteins that regulate cell proliferation, migration, and death. Among transformed cells, even of the same clinical diagnosis, there is heterogeneity in the multiple defects (73). The plurality of target proteins of HDAC and, thus, of HDACi, such as vorinostat, may be important for the efficacy of these agents against a broad spectrum of hematologic and solid tumors. The relative resistance of normal cells to vorinostat, we suggest, could be owing to the ability of normal cells to recover from the inhibitor because of its rapidly reversible binding in intervals of nonexposure to the drug.

Consistent with this hypothesis is the observation that in patients receiving vorinostat, once a day (half-life, ~4-6 h), there is accumulation of acetylated histones in normal peripheral mononuclear cells, which is transient (demonstrable up to 8-10 h following the oral dose; ref. 67). This vorinostat-induced transient accumulation of acetylated histones in normal cells occurred in patients with significant anticancer effects, but had no detectable effect on leukocyte count.

Non-Oncologic Potential of HDACi

There is growing evidence that HDACi have potential therapeutic application in nonmalignant diseases. For example, several reports indicate that HDACi have selective anti-inflammatory and specific immune modulator activity (74, 75). HDACi, in preclinical studies, have been reported to have therapeutic benefit in neurodegenerative diseases associated with memory impairment (76). HDACi have been shown to slow the progression of Huntington-like syndrome in mice (77, 78). HDACi are potent inducers of γ globin gene expression, which has implications for treatment of sickle-cell anemia (79). TSA has been shown to cause functional and morphologic recovery of dystrophic muscles in mice (80). Vorinostat has antirheumatic activity in mouse and rat models (81). HDACi can modulate stem cell survival and mobilization in *in vitro* studies (82). Valproic acid can activate latent HIV infection, and thus HDACi may have a therapeutic use in treating HIV (83).

Conclusions and Perspectives

Vorinostat is the first HDACi to be approved for clinical use in treating patients with malignancy (e.g., cutaneous T-cell lymphoma; ref. 4, 55). The molecular basis for the antitumor effects of vorinostat and other HDACi is not completely understood. It is not clear if more selective targeting of a particular HDAC as compared with pan-HDACi (such as vorinostat) will result in improved HDACi efficacy (4, 62).

We present a hypothesis for the basis of the sensitivity of transformed cells and relative resistance of normal cells to vorinostat. We suggest that the rapid reversal of the binding of the HDACi to its target may provide normal cells with the ability to compensate for the inhibitory effects of these agents, whereas cancer cells with multiple defects altering proteins regulating cell proliferation, survival, death, and migration are less able to compensate for the effect of the HDACi. This hypothesis, if it has validity, suggests that clinical therapeutic strategies involving intermittent dosing may be a most effective regimen to achieve selective anticancer activity. Further, the multiple protein targets of HDACs and, therefore, of HDACi and the preclinical evidence of synergy and additive activity with many other anticancer agents suggest that a therapeutic strategy using HDACi with other anticancer agents may be most promising.

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