

Review

HDAC Inhibitors: A Potential New Category of Anti-Tumor Agents

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Over the past years, it has been found that the epigenetic silence of tumor suppressor genes induced by overexpression of histone deacetylases (HDACs) plays an important role in carcinogenesis. Thus, HDAC inhibitors have emerged as the accessory therapeutic agents for multiple human cancers, since they can block the activity of specific HDACs, restore the expression of some tumor suppressor genes and induce cell differentiation, growth arrest and apoptosis. To date, the precise mechanisms by which HDAC inhibitors induce cell death have not yet been fully elucidated and the roles of individual HDAC inhibitors have not been identified. Moreover, the practical uses of HDAC inhibitors in cancer therapy, as well as their synergistic effects with other therapeutic strategies are yet to be evaluated. In this review article, we discuss briefly the recent advances in studies of the developments of anti-cancer HDAC inhibitors and their potential clinical value. *Cellular & Molecular Immunology*. 2007;4(5): 337-343.

Key Words: HDAC inhibitor, cancer, clinical

Carcinogenesis is a complex process that is influenced by multi-factors and progresses in multi-steps. It has been well established that the occurrence and development of cancers involve a substantial change in functions of both oncogenes and tumor suppressor genes. Recent studies have revealed that apart from the genetic abnormality of these cancer-related genes, epigenetic regulation of genes is a major mechanism in carcinogenesis (1). The mechanisms of epigenetic control of genes involve changes of gene expression patterns fulfilled by modifications of DNA and/or histones, without the alteration of nucleotide sequence of the genes. These modifications include DNA methylation and the covalent modifications, i.e., acetylation, methylation, phosphorylation, ubiquitination, etc., of specific amino acid residues of the N-termini of core histones (1). Among these modifications, histone acetylation/deacetylation plays a central role in epigenetic regulation of genes. Typically, high acetylation level of the chromatin hallmarks the active transcription of the genes, whereas inactive chromatin is usually characterized by low acetylation level of histones. It has been discovered that the occurrence of many cancers are accompanied by a genome-wide histone hypoacetylation (2).

Recently, a great deal of research interest has been focused on the efforts that are aimed at the restoration of acetylation/deacetylation balance by using HDAC inhibitors, and this has evoked a hope that a new strategy of cancer treatment can be developed based on this mechanism.

Histone modification and the regulation of eukaryotic gene expression

Nucleosomes are the basic repeating units that constitute the eukaryotic chromatin. A typical nucleosome is composed of an octamer of the four pairs of core histones H2A, H2B, H3 and H4, and ~146 base pairs of DNA wrapped around them (3). The core histone N-terminal domains are rich in positively charged basic amino acids, which can actively interact with DNA (4). The chromatin barrier formed by histone-DNA interaction blocks the binding of the basic transcription complex to gene's promoter, and suppresses gene expression (5). It was reported that the dynamic process of histone acetylation was linked with gene transcription, acetylated histone were usually associated with transcriptionally active chromatin and deacetylated histones with inactive chromatin (6, 7).

Under normal physiological conditions, chromatin acetylation status is regulated by the balanced action between the histone acetyltransferases (HATs) and histone deacetylases (HDACs). The HATs transfer acetyl groups from acetyl coenzyme A (acetyl-CoA) onto the ϵ -amino groups of conserved lysine residues within the core histones (8). Acetylation can neutralize the positive charge of histones, loosening their interactions with the negatively charged DNA backbone, and leading to a more "open" active chromatin

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Table 1. The classification of HDACs in mammals

Classification		Location	Function	
Zn²⁺-dependent	Class I	HDAC1	Nucleus	
		HDAC2	Nucleus	
		HDAC3	Nucleus, rarely in cytoplasm	
		HDAC8	Nucleus	-
	Class IIa	HDAC4	Nucleus, cytoplasm	Interaction with SMRT/N-CoR and the co-repressors BcoR (Bcl-6-interacting co-repressor) and CtBP
		HDAC5	Nucleus, cytoplasm	
		HDAC7	Nucleus, cytoplasm	
		HDAC9	Nucleus, cytoplasm	Muscle differentiation
	Class IIb	HDAC6	Cytoplasm	Tubulin deacetylase
		HDAC10	Nucleus, cytoplasm	Recruitment other HDACs
	Class IV	HDAC11	Nucleus, cytoplasm	-
Zn²⁺-independent	Class III	SIRT1-7		

structure that favors the binding of transcription factors for active gene transcription (5). Contrarily, the re-establishment of the positive charge in the amino-terminal tails of core histones catalyzed by HDACs is thought to tighten the interaction between histones and DNA, blocking the binding sites on promoter thus inhibiting gene transcription (9). Obviously, a subtly orchestrated balance between the actions of HATs and HDACs is essential to the maintenance of normal cellular functions, and shifts of this balance might have dramatic consequences on the cell phenotypes such as carcinogenesis (10).

HDACs and HDAC inhibitors

Based on their homologies to yeast HDACs, mammalian HDACs can be divided into four classes (Table 1) (11). Class I comprises HDAC1, 2, 3 and 8, which are related to the yeast HDAC rpd3, and these HDACs are located in the nuclei of the cells. Class IIa/b HDACs, homologous to yeast hda1, are primarily localized to the cytoplasm but they can shuttle to nucleus. Specifically, HDAC4, 5, 7 and 9 fall into Class IIa, whereas Class IIb contains the HDAC6 and 10, which have two catalytic sites (12). There have been reported that HDAC11 has a conserved domain in the catalytic region of both Class I and Class II enzymes (13), and it has been grouped to Class IV HDAC (11). Zn²⁺-independent and NAD-dependent Class III HDACs are yeast sir2 homologies, and they are virtually unaffected by the HDAC inhibitors that are now in clinical trials. At present, the applications of HDAC inhibitors in therapy of cancer or other diseases are

mainly pointed to Zn²⁺-independent Class I and II HDACs.

HDAC inhibitors reported to date can be divided into four groups based on their structures (15, 16), including hydroximates, cyclic peptides, aliphatic acids and benzamides (Table 2). Trichostatin A (TSA), which belongs to hydroximates group is the first discovered natural product (17). Low concentration (nM) of TSA and its structural analog, suberoyl anilide hydroxamic acid (SAHA), can induce cell differentiation and inhibit growth in tumors, with little effects on normal cells (18). Recent studies have shown that TSA and SAHA are able to induce cell differentiation of lymphoma, and they inhibit the activity of Class I and II HDACs (19, 20). Another HDAC inhibitor, depsipeptide (romidepsin, FK-228 or FR901228), belonging to the cyclic peptides group, is a natural product extracted from *Chromobacterium violaceum* (21). It has been under the multiple Phases I and II trials for cancer therapy. Aliphatic acid group contains butyrate, phenylbutyrate and valproic acid and their derivatives. This group has been limited in clinical application due to its high effective millimolar concentrations when assessed in cells (22). Benzamide HDAC inhibitors such as MS-275 and CI-994 are now in Phase I and II clinical trials. MS-275 preferentially inhibits HDAC1 over HDAC3, while it has little effect on HDAC8 (23).

HDAC inhibitors as anti-tumor agents

There have been indications that histone hypoacetylation frequently occurs in tumor cells, while the disorder of histone

Table 2. Histone deacetylase inhibitors in clinical development

Group	Compound	Effective concentration	Phase I trial	Phase II trial	Phase III trial
Hydroxamate	Trichostatin A (TSA)	nM	-	-	-
	Suberoyl anilide hydroxamic acid (SAHA)	nM	√	√	√
	CBHA	μM	-	-	-
	LAQ-824 / LBH 589	nM	√	√	√
	PXD-101	nM	√	√	-
Cyclic peptide	Depsiptide (FK-228)	nM	√	√	-
Aliphatic acid	Valproic acid	μM	√	√	-
	Phenylbutyrate	μM	√	√	-
Benzamide	MS-275	μM	√	√	-
	CI-994	μM	√	√	-
	MGCD0103	nM	√	√	-

acetylation level is associated with carcinogenesis (2). Abnormal transcriptional silencing of certain cancer-related genes mediated by overexpression of HDACs that are recruited by transcription factors may be a cause of carcinogenesis (Figure 1) (24). Sequentially, deregulation of gene expression induces cancer or other diseases. HDAC inhibitors are thought to be able to interact with the catalytic domain of histone deacetylases to block the substrate recognition ability of these HDACs, resulting in restoration of the expression of relevant genes (25). The main biological effects of HDAC inhibitors are the induction of differentiation of tumor cells, cell cycle arrest and promotion of apoptosis (26, 27). Moreover, there has been evidence that HDAC inhibitors can enhance the sensitivity of actinotherapy or chemotherapy for cancers and inhibit angiogenesis (28, 29). The sources, natures and structures of known HDAC inhibitors so far vary greatly, and this has raised the question whether these different HDAC inhibitors affect tumor occurrence and development through different mechanisms.

TSA, a compound belonging to hydroximates, is the first natural product that has been discovered to possess the HDAC inhibitor activity (17). Studies have demonstrated that TSA is able to inhibit the growth of the non-small-cell lung cancer (NSCLC) at concentrations ranging from 0.01 to 0.04 mM, while a concentration of TSA as high as 0.7 mM is effective for normal lung fibroblast cell inhibition (30). It has also been indicated that the effect of TSA in tumor cells is to induce apoptosis, whereas in normal cells it predominantly arrests cell cycle progression (30). In NSCLC cells or malignant melanoma cells, TSA induces the expression of p21 that is independent of p53 (30, 31). TSA increases histone H4 acetylation and expression of p21 without significant effect on p16, p27, CDK2 and cyclin D1 in NSCLC cells (30). TSA treatment results in a transient G2/M phase delay and in accumulation of Rb (31). Furthermore, TSA reduces Cyclin A expression but elevates Cyclin E level, while it has little effect on p27, CDK4 and CDK2 expression

(31). It has been demonstrated that TSA inhibits Cyclin D1 expression in an NF-κB dependent manner in JB6 mouse epidermal cells (32). TSA enhances p52 acetylation and increases p52 expression, which is a negative regulator of NF-κB, and consequently prevents the p65 hetero-dimer from binding to the NF-κB sites on DNA (32). The latest studies reveal that TSA inhibits the telomerase activity in the brain cancer cell lines and human normal hTERT-immortalised fibroblasts (hTERT-BJ1) cell line and elevates the expression of p53 and p21 with a decrease in Cyclin-D level (33). This evidence implicates that TSA may have the potential use as a telomerase inhibitor in cancer therapy (33). Recent data also demonstrate that TSA treatments result in a dose-dependent inhibition of growth in DMS53 small cell lung cancer (SCLC) cells, along with the elevated p21 and p27, cleaved poly(ADP-ribose) polymerase and decreased Bcl-2 (34). Also, TSA causes morphological differentiation and growth inhibition *via* cell cycle arrest and subsequent apoptosis (34). Data from a wide spectrum of studies suggest that TSA can induce cell cycle arrest or apoptosis in a variety of cell lines, along with the accumulation of p21 and a decrease of Cyclin D. Furthermore, we speculate that TSA induces cell cycle arrest or apoptosis by delaying the transition of G1/S phase (30). In spite of the accumulation of evidence that as a common HDAC inhibitor, TSA has a wide range of anti-cancer effects; it has not so far been used in the clinical trials, probably due to its unidentified possible side effects.

A hydroximate compound suberoyl anilide hydroxamic acid (SAHA), on the other hand, has shown the most promising prospect of application in cancer therapy. SAHA has been proven to inhibit the growth of pancreatic cancer cells in a dose-dependent manner, associated with induction of apoptosis, G2/M cell cycle arrest and cell differentiation (35). It upregulates the expression of p21, C/EBPα, RARα and E-cadherin, while it decreases the Cyclin B1, c-myc and Cyclin D1 levels (independent of an active β-catenin pathway) (35). The latest data also show that the proliferation of glioblastoma multiforme (GBM) cell lines and explants

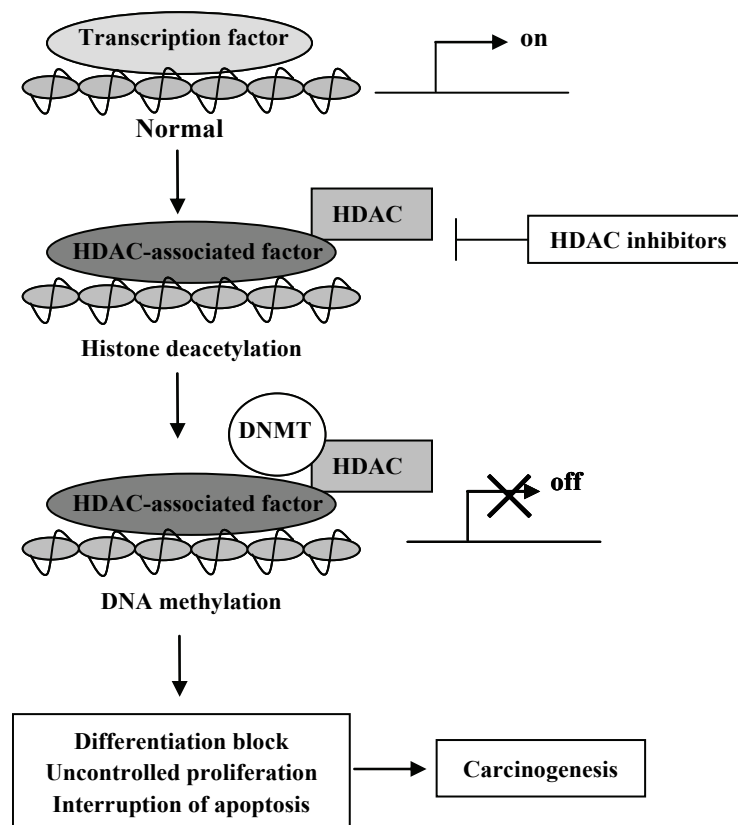


Figure 1. Roles of HDACs and HDAC inhibitors in carcinogenesis. In normal cells, transcription factors can bind to the promoter of tumor suppressor gene or other genes that participate in cell differentiation and proliferation, thus facilitating gene expression. The HDAC-associated factors may recruit HDACs, resulting in increase of histone deacetylation level. The HDACs can in turn recruit DNMTs, which induce DNA methylation, resulting in formation of an inhibitory chromatin structure. The silencing of these genes may contribute to differentiation block, uncontrolled proliferation and interruption of apoptosis, etc, which may cause carcinogenesis. HDAC inhibitors can restore the gene expression.

can be inhibited *in vitro* by SAHA, along with cell cycle G2/M arrest, and accumulation of p21 (36). Besides, SAHA treatments inhibit cell proliferation in a dose-dependent manner and arrest cell cycle at the G2/M phase transition in NSCLC (37).

Other HDAC inhibitors currently in clinical trials include FK-228, a cyclic peptide, as well as MS-275 and MGCD0103 that are benzamide compounds. The clinical application of aliphatic acid HDAC inhibitors has been hampered, to a large extent, due to their high functional concentrations at the millimolar scale. FK-228 has been reported to induce cell cycle arrests and apoptosis in NSCLC, colon cancer, and chronic myelogenous leukemia (38-40). In HT29 colon cancer cells, caspase-3, -7 and -8, and serine protease can be activated by FK-228, and the expression of p21, p27 can be induced, along with cell cycle arrest at G0/G1 or G2/M phase (38). Also, FK-228 treatment leads to the upregulation of p21 and a substantial decrease in the expression of Cdc2/Cdk-1, cyclin B1 and phosphorylated pRb in A549 NCSLC cells, and subsequently induces cell cycle arrest at G2/M (39). A recent study demonstrates that FK-228 inhibits the expression of EGFR in lung cancer cells,

resulting in the restrained EGFR-related pathways and the activation of p38 MAPK pathway (40). Besides, in A549 lung cancer cells, FK-228 treatment decreases the expressions of MMP-2 and MMP-9 in a dose-dependent manner (41). Another compound, MS-275, which belongs to the benzamide HDAC inhibitor group, has been shown to induce apoptosis of B-chronic lymphocytic leukemia cells, Jurkat lymphoblastic T cells and prostate cancer cells (42-44), and this HDAC inhibitor has been used in clinical trials in combination with other anti-tumor agents (42).

This has evoked a good deal of research interest in the issue whether other HDAC inhibitors besides MS-275, will work more effectively when used in combination with existing anti-tumor drugs. Studies reveal that hydroxamate HDAC inhibitors such as TSA, LAQ824 and its analog LBH589, when used together with the multiple receptor tyrosine kinase inhibitor AEE788, can induce apoptosis in non-small cell lung cancer (MV522, A549), ovarian cancer (SKOV-3) and leukemia (K562, Jurkat, and ML-1) cells (45). Similarly, the combined application of HDAC inhibitor LBH589 and the other tyrosine kinase inhibitor AMN107, exhibits a synergistic role in Bcr-Abl-expressing human

leukemia cells (46). LBH589 can enhance the effect of chemotherapeutic drug bortezomib, dexamethasone and melphal in myeloma. This may emerge as a new strategy to unravel the problem of drug resistance (47). Moreover, LBH589 has been reported to increase the irradiation-induced apoptosis in NSCLC cells (28). The cyclic peptide HDAC inhibitor FK-228 is capable of enhancing the effect of gemcitabine on hormone refractory prostate cancer cells (47). The combinatorial use of HDAC inhibitors and multiplicate chemotherapeutic drugs has now been under clinical trials (11). Apparently, the synergistic effects generated from the combinatorial use of HDAC inhibitors and chemotherapy or actinotherapy will be one of the hot issues in researches of cancer therapy. In this regard, workout of a suitable therapeutic strategy and determination of correct concentrations of both HDAC inhibitors and chemotherapeutic drugs are critical to the successful cure of a particular cancer.

Additionally, over the past years, vitamin D has been discovered to have the anti-proliferation effect besides its known functions of maintaining normal blood levels of calcium and phosphorus (48, 49). HDAC inhibitors such as TSA can upregulate the target genes of vitamin D receptors (VDR) to reverse the uncontrolled proliferation induced by overexpression of NcoR1 (50, 51). Combined use of vitamin D3 and TSA can remarkably inhibit cell growth in breast and prostate cancers (50, 52, 53). These studies implicate that the use of HDAC inhibitors and vitamin D in combination can be an effective therapeutics for cancers.

Problems and perspectives

In recent years, HDAC inhibitors have attracted a great deal of interests attributing to their potential as a new category of anti-cancer agents. Specific HDAC inhibitors can regulate the expression of certain cancer-related genes by increasing the histone acetylation level, thus in turn induce cell cycle arrest, differentiation and apoptosis in cancer cells, while they have little effects on normal cells. This property has aroused hopes to use HDAC inhibitors as anti-cancer therapeutic agents in clinics. In this regard, it is vital to distinguish the differences in response to HDAC inhibitors between the normal and carcinoma cells, as well as the pathways underlying this response. These data will lay the basis for the determination of new therapeutic targets for HDAC inhibitors. Moreover, it is now clear that the effects of different HDAC inhibitors on different kinds of cancers vary significantly. Both Class I and Class II HDACs exhibit a considerable extent of tissue-specific expression. Clearly, a good understanding of this background knowledge is extremely important to the development of efficient and specific HDAC inhibitors for the treatments of particular cancers.

The existing data support the view that the gene regulation effects of HDAC inhibitors are selective. It has been shown that in cells treated with TSA, only a small proportion of the genes (2%) have been changed in their expression pattern (54), though the underlying mechanism of this selectivity is unclear. Extensive researches are needed to

elucidate the mechanisms of HDAC inhibitors in cancer therapy. Efforts that are aimed at the discovery of common characteristics of the HDAC inhibitor-regulated genes, e.g., their chromosomal locations, function of proteins encoded by these genes, etc., may be helpful in unraveling the mechanisms of HDAC inhibitor action. Also, as mentioned earlier in this article, the combinatorial application of HDAC inhibitor with other agents have shed light on the hope of a more effective therapy strategy. It has been shown that the combined use of TSA and the methylase inhibitor 5-aza-dC can reduce the toxicity of 5-aza-dC in leukemia therapy (55). Treatment with TSA and 1 α , 25-dihydroxyvitamin D3 in combination induces cancer cell differentiation *in vitro* and inhibited the tumor growth *in vivo* (50, 52, 53). Studies carried out in our laboratory indicate that TSA enhances the apoptosis induced by doxorubicin (56). These data suggest that HDAC inhibitors may work synergistically with methylase inhibitors, vitamin D and traditional chemotherapy drugs to inhibit the growth in tumor cells. Collectively, information regarding the toxicity, effecting concentrations and the interactions and synergies with other agents, is critical for the workout of an optimal treatment practice involving the use of HDAC inhibitors.

Finally, a world-wide effort is constantly being made to screen and discover more new HDAC inhibitors, of both synthetic and natural sources, with high efficiency and low toxicity, for cancer therapy. We would hopefully expect new breakthroughs in this aspect in the future.

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