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
Zn^{2+}-dependent	**Class I**	HDAC1	Nucleus	Participate in Sin3, NuRD (nucleosome remodeling and deacetylation) and Co-REST complex
		HDAC2	Nucleus	
		HDAC3	Nucleus, rarely in cytoplasm	Participate in SMRT (silencing mediator for retinoic acid and thyroid hormone receptors), N-CoR (nuclear receptor co-repressor) complex
		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	-
Zn^{2+}-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). Zn^{2+}-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 Zn^{2+}-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	Depsipeptide (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 actino-theraphy or chemotherapy for cancers and inhibit angio-genesis (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

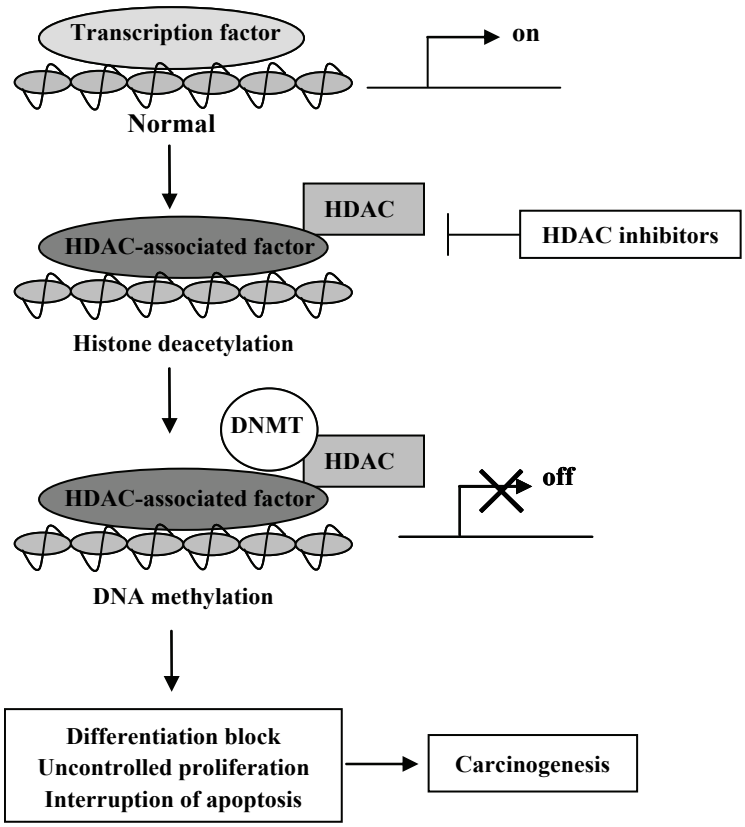

Figure 1. Roles of HDACs and HDAC inhibitors in carcinogenesis. In normal cells, transcription factors can bind to the promoter of tumor suppressor gene or other genes that participate in cell differentiation and proliferation, thus facilitating gene expression. The HDAC-associated factors may recruit 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 hydroximate 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 actinotheraphy 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 1α, 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.

References

1. Smith LT, Otterson GA, Plass C. Unraveling the epigenetic code of cancer for therapy. Trends Genet. 2007;23:449-456.
2. Mahlknecht U, Hoelzer D. Histone acetylation modifiers in the pathogenesis of malignant disease. Mol Med. 2000;6:623-644.
3. Luger K, Mader AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature. 1997;389:251-260.
4. Lenfant F, Mann RK, Thomsen B, Ling X, Grunstein M. All four core histone N-termini contain sequences required for the repression of basal transcription in yeast. EMBO J. 1996;15: 3974-3985.
5. Gregory PD, Wagner K, Horz W. Histone acetylation and chromatin remodeling. Exp Cell Res. 2001;265:195-202.
6. Grunstein M. Histone acetylation in chromatin structure and transcription. Nature. 1997;389:349-352.
7. Fletcher TM, Hansen JC. The nucleosomal array: structure/ function relationships. Crit Rev Eukaryot Gene Expr. 1996;6: 149-188.
8. Tanner KG, Trievel RC, Kuo MH, et al. Catalytic mechanism and function of invariant glutamic acid 173 from the histone acetyltransferase GCN5 transcriptional coactivator. J Biol Chem. 1999;274:18157-18160.
9. Roth SY, Denu JM, Allis CD. Histone acetyltransferases. Annu Rev Biochem. 2001;70:81-120.
10. Minucci S, Pelicci PG. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. Nat Rev Cancer. 2006;6:38-51.
11. Glaser KB. HDAC inhibitors: Clinical update and mechanism-based potential. Biochem Pharmacol. 2007;74:659-671.

12. Dokmanovic M, Marks PA. Prospects: histone deacetylase inhibitors. J Cell Biochem. 2005;96:293-304.
13. 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.
14. Frye RA. Phylogenetic classification of prokaryotic and eukaryotic Sir2-like proteins. Biochem Biophys Res Commun. 2000;273:793-798.
15. Miller TA, Witter DJ, Belvedere S. Histone deacetylase inhibitors. J Med Chem. 2003;46:5097-5116.
16. Marks PA, Richon VM, Miller T, Kelly WK. Histone deacetylase inhibitors. Adv Cancer Res. 2004;91:137-168.
17. Yoshida M, Kijima M, Akita M, Beppu T. Potent and specific inhibition of mammalian histone deacetylase both *in vivo* and *in vitro* by trichostatin A. J Biol Chem. 1990;265:17174-17179.
18. Marks PA, Dokmanovic M. Histone deacetylase inhibitors: discovery and development as anticancer agents. Expert Opin Investig Drugs. 2005;14:1497-1511.
19. Wegener D, Hildmann C, Schwienhorst A. Recent progress in the development of assays suited for histone deacetylase inhibitor screening. Mol Genet Metab. 2003;80:138-147.
20. Marks PA, Richon VM, Kelly WK, Chiao JH, Miller T. Histone deacetylase inhibitors: development as cancer therapy. Novartis Found Symp. 2004;259:269-281; discussion 281-288.
21. Nakajima H, Kim YB, Terano H, Yoshida M, Horinouchi S. FR901228, a potent antitumor antibiotic, is a novel histone deacetylase inhibitor. Exp Cell Res. 1998;241:126-133.
22. Kelly WK, Marks PA. Drug insight: Histone deacetylase inhibitors--development of the new targeted anticancer agent suberoylanilide hydroxamic acid. Nat Clin Pract Oncol. 2005;2: 150-157.
23. Hu E, Dul E, Sung CM, et al. Identification of novel isoform-selective inhibitors within class I histone deacetylases. J Pharmacol Exp Ther. 2003;307:720-728.
24. Scneider-Stock R, Ocker M. Epigenetic therapy in cancer: Molecular background and clinical development of histone deacetylase and DNA methyltransferase inhibitors. IDrugs. 2007; 10:557-561.
25. Finnin MS, Donigian JR, Cohen A, et al. Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. Nature. 1999;401:188-193.
26. Kim YB, Ki SW, Yoshida M, Horinouchi S. Mechanism of cell cycle arrest caused by histone deacetylase inhibitors in human carcinoma cells. J Antibiot (Tokyo). 2000;53:1191-1200.
27. Mai A, Massa S, Rotili D, et al. Histone deacetylation in epigenetics: an attractive target for anticancer therapy. Med Res Rev. 2005;25:261-309.
28. Geng L, Cuneo KC, Fu A, Tu T, Atadja PW, Hallahan DE. Histone deacetylase (HDAC) inhibitor LBH589 increases duration of γ-H2AX foci and confines HDAC4 to the cytoplasm in irradiated non-small cell lung cancer. Cancer Res. 2006;66: 11298-11304.
29. Qian DZ, Kato Y, Shabbeer S, et al. Targeting tumor angiogenesis with histone deacetylase inhibitors: the hydroxamic acid derivative LBH589. Clin Cancer Res. 2006;12:634-642.
30. Mukhopadhyay NK, Weisberg E, Gilchrist D, Bueno R, Sugarbaker DJ, Jaklitsch MT. Effectiveness of trichostatin A as a potential candidate for anticancer therapy in non-small-cell lung cancer. Ann Thorac Surg. 2006;81:1034-1042.
31. Florenes VA, Skrede M, Jorgensen K, Nesland JM. Deacetylase inhibition in malignant melanomas: impact on cell cycle regulation and survival. Melanoma Res. 2004;14:173-181.
32. Hu J, Colburn NH. Histone deacetylase inhibition down-regulates cyclin D1 transcription by inhibiting nuclear factor-κB/ p65 DNA binding. Mol Cancer Res. 2005;3:100-109.
33. Khaw AK, Silasudjana M, Banerjee B, Suzuki M, Baskar R, Hande MP. Inhibition of telomerase activity and human telomerase reverse transcriptase gene expression by histone deacetylase inhibitor in human brain cancer cells. Mutat Res. 2007; in press.
34. Platta CS, Greenblatt DY, Kunnimalaiyaan M, Chen H. The HDAC inhibitor trichostatin A inhibits growth of small cell lung cancer cells. J Surg Res. 2007;142:219-226.
35. Kumagai T, Wakimoto N, Yin D, et al. Histone deacetylase inhibitor, suberoylanilide hydroxamic acid (Vorinostat, SAHA) profoundly inhibits the growth of human pancreatic cancer cells. Int J Cancer. 2007;121:656-665.
36. Yin D, Ong JM, Hu J, et al. Suberoylanilide hydroxamic acid, a histone deacetylase inhibitor: effects on gene expression and growth of glioma cells *in vitro* and *in vivo*. Clin Cancer Res. 2007;13:1045-1052.
37. Komatsu N, Kawamata N, Takeuchi S, et al. SAHA, a HDAC inhibitor, has profound anti-growth activity against non-small cell lung cancer cells. Oncol Rep. 2006;15:187-191.
38. Choudhary S, Wang HC. Pro-apoptotic activity of oncogenic H-Ras for histone deacetylase inhibitor to induce apoptosis of human cancer HT29 cells. J Cancer Res Clin Oncol. 2007;133: 725-739.
39. Radhakrishnan V, Song YS, Thiruvengadam D. Romidepsin (depsipeptide) induced cell cycle arrest, apoptosis and histone hyperacetylation in lung carcinoma cells (A549) are associated with increase in p21 and hypophosphorylated retinoblastoma proteins expression. Biomed Pharmacother. 2007; in press.
40. Yu XD, Wang SY, Chen GA, et al. Apoptosis induced by depsipeptide FK228 coincides with inhibition of survival signaling in lung cancer cells. Cancer J. 2007;13:105-113.
41. Vinodhkumar R, Song YS, Ravikumar V, Ramakrishnan G, Devaki T. Depsipeptide a histone deacetlyase inhibitor down regulates levels of matrix metalloproteinases 2 and 9 mRNA and protein expressions in lung cancer cells (A549). Chem Biol Interact. 2007;165:220-229.
42. Lucas DM, Davis ME, Parthun MR, et al. The histone deacetylase inhibitor MS-275 induces caspase-dependent apoptosis in B-cell chronic lymphocytic leukemia cells. Leukemia. 2004;18:1207-1214.
43. Maggio SC, Rosato RR, Kramer LB, et al. The histone deacetylase inhibitor MS-275 interacts synergistically with fludarabine to induce apoptosis in human leukemia cells. Cancer Res. 2004;64:2590-2600.
44. Qian DZ, Wei YF, Wang X, Kato Y, Cheng L, Pili R. Antitumor activity of the histone deacetylase inhibitor MS-275 in prostate cancer models. Prostate. 2007;67:1182-1193.
45. Yu C, Friday BB, Lai JP, et al. Abrogation of MAPK and Akt signaling by AEE788 synergistically potentiates histone deacetylase inhibitor-induced apoptosis through reactive oxygen species generation. Clin Cancer Res. 2007;13:1140-1148.
46. Fiskus W, Pranpat M, Bali P, et al. Combined effects of novel tyrosine kinase inhibitor AMN107 and histone deacetylase inhibitor LBH589 against Bcr-Abl-expressing human leukemia cells. Blood. 2006;108:645-652.
47. Kanzaki M, Kakinuma H, Kumazawa T, et al. Low concentrations of the histone deacetylase inhibitor, depsipeptide, enhance the effects of gemcitabine and docetaxel in hormone refractory prostate cancer cells. Oncol Rep. 2007;17:761-767.
48. Holick MF. Resurrection of vitamin D deficiency and rickets. J Clin Invest. 2006;116:2062-2072.
49. Tian J, Liu Y, Williams LA, de Zeeuw D. Potential role of active

vitamin D in retarding the progression of chronic kidney disease. Nephrol Dial Transplant. 2007;22:321-328.
50. Banwell CM, MacCartney DP, Guy M, et al. Altered nuclear receptor corepressor expression attenuates vitamin D receptor signaling in breast cancer cells. Clin Cancer Res. 2006;12: 2004-2013.
51. Gommersall LM, Khanim FL, Peehl DM, Doherty AH, Campbell MJ. Epigenetic repression of transcription by the Vitamin D3 receptor in prostate cancer cells. J Steroid Biochem Mol Biol. 2004;89-90:251-256.
52. Banwell CM, O'Neill LP, Uskokovic MR, Campbell MJ. Targeting 1α, 25-dihydroxyvitamin D3 antiproliferative insensitivity in breast cancer cells by co-treatment with histone deacetylation inhibitors. J Steroid Biochem Mol Biol. 2004;89-90:245-249.
53. Banwell CM, Singh R, Stewart PM, Uskokovic MR, Campbell MJ. Antiproliferative signalling by 1,25$(OH)_2D_3$ in prostate and breast cancer is suppressed by a mechanism involving histone deacetylation. Recent Results Cancer Res. 2003;164:83-98.
54. Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK. Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer. 2001;1:194-202.
55. Gore SD. Combination therapy with DNA methyltransferase inhibitors in hematologic malignancies. Nat Clin Pract Oncol. 2005;2 Suppl 1:S30-35.
56. Pan L, Lu J, Wang X, et al. Histone deacetylase inhibitor trichostatin A potentiates doxorubicin-induced apoptosis by up-regulating PTEN expression. Cancer. 2007;109:1676-1688.