Histone deacetylase inhibitors: Apoptotic effects and clinical implications (Review)

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Abstract. It has been shown that epigenetic modifications play an important role in tumorigenesis. Thus, affecting epigenetic tumorigenic alterations can represent a promising strategy for anticancer targeted therapy. Among the key chromatin modifying enzymes which influence gene expression, histone acetyltransferases (HATs) and histone deacetylases (HDACs) have recently attracted interest because of their impact on tumor development and progression. Increased expression of HDACs and disrupted activities of HATs have been found in several tumor types, with a consequent hypoacetylated state of chromatin that can be strictly correlated with low expression of either tumor suppressor or pro-apoptotic genes. Histone deacetylase inhibitors (HDACIs) represent a new and promising class of antitumor drugs that influence gene expression by enhancing acetylation of histones in specific chromatin domains. HDACIs have been shown to exert potent anticancer activities inducing cell cycle arrest and apoptosis. Notably, a high efficacy of these drugs has been selectively revealed in malignant cells rather than in normal cells. Moreover, the therapeutic potential of these agents is also supported by the evidence that HDACIs downregulate genes involved in tumor progression, invasion and angiogenesis. Several HDACIs are currently under clinical investigation, including vorinostat (SAHA), romidepsin (depsipeptide, FK-228), LAQ824/LBH589 and belinostat (PXD101), compounds that have shown therapeutic potential in many types of malignancies including solid tumors. Based on the ability of HDACIs to regulate many signaling pathways, co-treatment of these compounds with molecular targeted drugs is a promising strategy against many types of tumors.

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1. Introduction

Epigenetic modifications consist of the reversible covalent alteration of specific chromatin domains, such as acetylation, methylation, phosphorylation, ADP-ribosylation and ubiquitination, which influence the accessibility of transcription complexes to distinct DNA elements, thus regulating gene expression. These modifications exert a fundamental role in the control of gene expression during embryogenesis and are involved in silencing specific genes in adult differentiated cells and tissues (1). Tumor cells have been shown to reactivate expression of these genes during their malignant conversion leading to uncontrolled proliferation (2). On the other hand, alteration of gene expression in cancer due to epigenetic modifications has also been correlated with downregulation of genes involved in cell cycle control and apoptosis (3-5).

Various covalent modifications which influence chromatin state are involved in regulating the expression of cancer-related genes. In particular, enzymes that modulate chromatin structure through histone modifications are currently considered key regulators of tumorigenic events and can thus be considered as possible targets in cancer therapy (6). Histone modifications and DNA methylation maintain the equilibrium that regulates chromatin organization and function. Post-transcriptional modifications of histones play a fundamental role in regulating chromatin state and accessibility of transcription factors to the DNA and are, thus, considered to influence gene expression (7). The term 'histone code' is now widely used to describe complex patterns of N-terminal histone modifications by phosphorylation, acetylation, methylation and possibly ubiquitination and their effects on the expression of individual genes (8,9).

Balanced activities of histone acetyltransferases (HATs) and histone deacetylases (HDACs) determine the acetylation status of histones. HATs catalyse the addition of acetyl

groups to lysine residues present in the histone tails, thereby neutralizing this portion of the histones and consequently loosening the nucleosome. Histone acetylation is, therefore, correlated with a relaxed chromatin state that favors accessibility of transcription factors to DNA elements. HDACs, on the other hand, remove acetyl groups from histone tails leading to packaging of chromatin into a condensed, repressed form, thus preventing the contact of transcription factors, regulatory complexes and RNA polymerases with the DNA with consequent silencing transcription (10). These effects are normally localized to specific portions of DNA since both HATs and HDACs can be associated with particular transcription factors or modulators that orientate the position of these enzymes in specific encoding regions and allow them to cooperate in transcriptional control (Fig. 1). As a consequence, only a small subset of encoding genes, estimated to be approximately 2-5%, can be transcriptionally regulated by the histone acetylation state. Notably, such a limited percentage includes genes that exert a key role in cell cycle control and apoptosis (11). For this reason, modulating histone acetylation/deacetylation using specific inhibitors represents a novel and potential approach to cancer therapy. Intervening in modulating HAT/HDAC activity with a therapeutic purpose is also supported by the evidence that HATs are often downregulated or mutated in certain tumors whereas HDACs are typically overexpressed in tumor cells (12,13).

Histone deacetylase inhibitors (HDACIs) constitute a relatively new class of drugs with a high potential as anticancer agents. *In vitro* and *in vivo* evaluations have shown that these compounds have low toxicity for normal cells while selectively inducing apoptosis in several types of tumor cell lines (14,15). A number of HDACIs are currently undergoing phase I and phase II clinical trials; some are in phase III or are already used in the therapy for hematological malignancies (16). In most cases, HDACI treatment induces a rapid accumulation of hyperacetylated histone proteins, thereby causing modifications in chromatin state and consequently affecting gene expression.

Recent expression profiling studies have confirmed that genes that exert a key role in cell fate can be modulated by HDACIs. One of the most characterized effects of different HDACIs in tumor cells is the induction of p21$^{WAF1/CIP1}$, a cyclin-dependent kinase (cdk) inhibitor (17), together with downregulation of a number of other cell cycle regulatory genes (18,19). Oligonucleotide microarray analyses to characterize the transcriptional profile after treatment with HDACIs in multiple myeloma cells have demonstrated early changes in gene expression of certain regulatory factors involved in apoptosis and cell survival as well as drug resistance, DNA repair, proteasome function and cell cycle regulation (11,20).

However, HDACIs have been shown to induce growth arrest and apoptosis in a variety of human tumor cells by mechanisms that cannot only be attributed to histone hyper-acetylation and consequent gene transcription modulation. Several non-histone proteins, which exert a role in controling apoptosis, including p53, Hsp90, NF-κB and tubulin, can also interact with HDACs and behave as their substrates, thus being stabilized in an acetylated form after treatment with HDACIs (21).
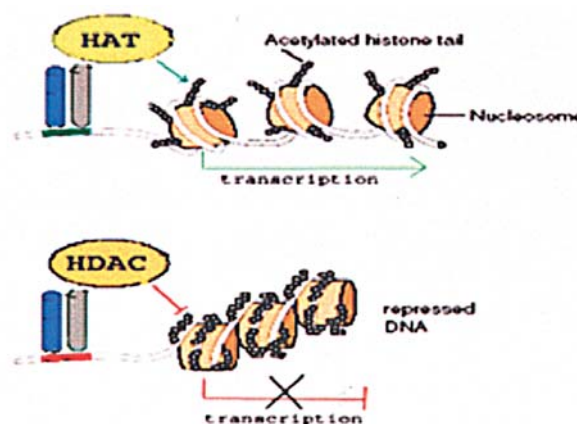

Figure 1. A simplified model of the influence of histone acetylation/deacetylation on transcriptional activity.

Recent findings have highlighted a role of HDACIs in counteracting angiogenesis (22-24), tumor progression and invasiveness (25-27), thus, supporting the therapeutic potential of these drugs.

The synergistic activity of HDACIs in combination with chemotherapy/radiotherapy as well as biological therapeutic agents has been widely described (28-30).

This review summarizes the results of recently published data on the effects of HDACIs in different tumor models, with a special focus on the molecular mechanisms of anti-tumor activity and their clinical applications.

2. The regulation of gene expression by histone modifications: HATs and HDACs

Eukaryotic DNA is organized into nucleosomes, which consist of 146 base pairs of DNA wrapped around a core of eight histone proteins, 2(H2A, H2B, H3, H4). Nucleosome packages create higher-order structures to form condensed chromatin. The N-terminal tails of the histones play a fundamental role in the regulation of chromatin structures. Acetylation of the lysine residues in the histone tails abrogates their positive charge, resulting in the destabilization of the association of histone and the negatively charged phosphate backbone of DNA. Thus, histone acetylation can result in the unwinding of the nucleosomal array, thereby favoring gene expression, since acetylation increases accessibility of transcription factors to DNA. Both HAT and HDAC activities can be recruited to target genes in complexes with sequence-specific transcription factors and their cofactors (Fig. 1).

Nuclear receptors of the steroid or retinoid superfamily, such as retinoic acid receptors (RAR) or thyroid hormone receptors are associated, in the absence of ligands, with corepressors and HDACs forming a large complex, thereby inhibiting transcription (31-33). Upon ligand binding, the complex dissociates and the corepressors are replaced by coactivator proteins, such as SRC-1 or CBP, which exert HAT activity (34). The ligand-induced switch of nuclear receptors from repression to activation thus reflects the change in the complex of corepressor with coactivator proteins and HDAC with HAT activity.
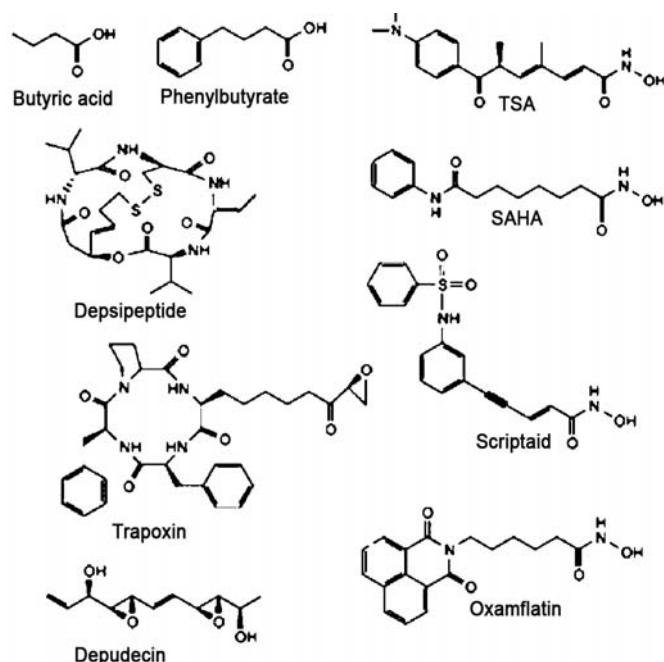
Figure 2. Chemical structure of selected histone deacetylase inhibitors.

Histone acetyltransferases (HAT) are enzymes that acetylate lysine residues present in the histone tails by transferring an acetyl group from acetyl CoA to lysine thereby forming ε-N-acetyl lysine (35). Mammalian HATs include two types, A and B, which are characterized on the basis of their differing roles. Type A HATs play a key role in the regulation of gene expression since they behave as transcriptional coactivators. Type B HATs are mainly involved in the assembly of nascent histones into chromosomes.

The most studied type A HATs
CBP/p300. CBP (CREB binding protein) and p300 are two closely related mammalian proteins that possess HAT activity (35) and are capable of acetylating all four core histones. Both proteins have been identified as coactivators of transcription and are known to interact with the histone acetyltransferase P/CAF (36).

The gene encoding for CBP is known to be mutated in patients with Rubinstein-Taybi syndrome, a well-characterized developmental disorder, which is associated with an increased risk of cancer (37).

Mutations in p300 have been described in epithelial malignancies (38). In addition, epigenetic mechanisms, such as p300 promoter methylation, have been found as a silencing modality of this gene in tumor models contributing to tumor invasion and metastasis (39).

Gcn5-P/CAF. Gcn5 was originally identified as a gene required for amino acid biosynthesis in yeast. Subsequent studies revealed that Gcn5 is a subunit of both the ADA (40) and SAGA protein complexes (41,42) and that it behaves like a transcriptional activator. P/CAF (p300/CBP associated factor) the human homolog of Gcn5 (43) was first identified as a protein that interacts with CBP protein. P/CAF exhibits histone acetyltransferase activity since it can acetylate free H3 and H4 and nucleosomal H3 (44). P/CAF associates with many other nuclear proteins and is also reported to acetylate several transcription factors (45).

MYST/SAS/Tip60. This group includes Esa1 and Tip60 proteins (46). Esa1, which is an essential protein in yeast, and its homolog MOF protein in *Drosophila* have been shown to acetylate histones H4, H3 and H2A (47). Tip60 (Tat interacting protein 60) was originally identified as an acetyl transferase which interacts with HIV-1 transactivator protein Tat (48).

TAFII250. TAFII250, a component of the general transcription factor TFIID, can act as a transcriptional co-activator and can contribute to the regulation of promoter selectivity (49). It has been recently demonstrated that this protein possesses histone acetyltransferase activity (50).

Hat1. Hat1 represents a type B HAT (51) which participates in the acetylation of newly synthesized histones during the process of chromatin assembly.

The activities of HDACs determine a hypoacetylated state of chromatin by removing acetyl groups from the histone tails. Mammalian HDACs can be divided into three subclasses.

Class I enzymes are homologous to the yeast Rpd3 protein and include HDAC1, 2, 3 and 8 with molecular masses ranging from 40 to 55 kDa. Their activities are usually localized in the nucleus and seem to be ubiquitously expressed in human tissues (10). Class II enzymes include HDAC4, 5, 6 and 7 which are larger proteins (~120-130 kDa) related to the yeast Hda1 protein (22). Class II HDACs have been shown to localize either in the nucleus or cytoplasm thereby suggesting their involvement in deacetylation of non-histone proteins.

According to sequence homology and modular arrangement, class II can be subdivided into IIa (HDAC4, 5, 7 and 9) and IIb (HDAC6 and 10). Both class I and II HDACs possess a highly conserved catalytic domain of approximately 390 amino acids and appear to deacetylate their substrates by activating a water molecule with a divalent zinc cation in cooperation with histidine-aspartate residues (21).

Recently, a third non-classic group of HDACs with homology to the yeast SIR2 (silent information regulator 2) protein has been discovered. This group of deacetylases, named 'sirtuins', operates by a very different mechanism that requires NAD^+ as a substrate and is insensitive to inhibition by compounds such as hydroxamic acid derivatives (52). The sirtuin family is conserved from archaeobacteria to eukaryotes (52). SIR2 has been shown to regulate lifespan in yeasts and *Drosophila* and seems to regulate longevity by participating in the caloric restriction pathway (53). In mammals, there are seven homologs of the yeast Sir2 gene, SIRT1-7, each with significant diversity in function. Mammalian sirtuins can be localized to the nucleus, cytoplasm or mitochondria, with distinct substrates and a broad range of functions (54).

Since HDACs are typically overexpressed in tumor cells (55-57), inhibition of HDACs can be a selective manner for inducing differentiation of tumor cells or specifically stimulating apoptosis. Therefore, inhibition of HDACs represents a promising approach for the treatment of various cancers.

Moreover, some of the genes encoding HDACs have been shown to be rearranged in the context of chromosomal translocations in human acute leukemias and solid tumors

Table I. Several classes of HDAC inhibitors.

Class	Compound	Activity		
		Cell culture	Animal models	Clinical trials
Short chain fatty acids	Sodium butyrate	*	*	*
	Phenylbutyrate	*	*	*
	Phenylacetate	*	*	*
	Valproic acid	*	*	*
	Pivanex	*	*	*
Hydroxamic acids	Trichostatin A (TSA)	*	*	*
	Suberoylanilide hydroxamic acid (SAHA)	*	*	*
	M-carboxycinnamic acid (CBHA)	*	*	*
	Azelaic bis-hydroxamic acid (ABHA)	*	*	*
	LBH589	*	*	*
	Scriptait	*	*	*
	Pyroxamide	*	*	*
	Oxamflatin	*		
	PDX-101	*		
	LAQ-824	*		
	ITF 2357	*	*	*
Cyclic tetrapeptides	Depsipeptide (FK228)	*	*	*
	Apicidine	*	*	
	Trapoxin	*	*	
	Depudesin	*		
	CHAP	*		
Synthetic benzamides	MS-275	*	*	*
	CI-994	*	*	*
Ketones	Trifluoromethyl ketone	*		
	Ketomides	*		

(12). In addition, some HDACs are aberrantly recruited by proteins generated by rearranged chromosomal regions (58). Typical examples are PML-RARa, the protein of acute promyelocytic leukemia (APL), and AML1-ETO, the protein of acute myeloid leukemia (AML). They are two fusion proteins generated by translocations that aberrantly recruit HDACs to target genes leading to specific gene repression (16,58).

AML1-ETO, for instance, blocks retinoic acid signaling in hematopoietic cells thereby inhibiting cell differentiation. Importantly, treatment of AML or APL leukemia with retinoic acid plus HDAC inhibitors has been shown to result in growth arrest and myeloid differentiation (59,60).

3. HDAC inhibitors: Potent and selective anticancer drugs

There is ample evidence, both *in vitro* and *in vivo*, that HDACIs block the enzymatic activity of HDACs and induce hyperacetylation of histones.

Several classes of HDACIs have been widely described including i) short chain fatty acids, such as butyric and valproic acids; ii) hydroxamic acids, such as trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA) and oxamflatin; iii) cyclic peptides, such as depsipeptide and trapoxin; and iv) benzamides such as CI-994 (Table I).

Originally, dimethyl sulphoxide was the first chemical of natural origin that was shown to induce histone hyperacetylation and cell differentiation (61). However, successively, owing to its toxicity, the attention was given to other agents.

Among the HDACIs of natural origin, sodium butyrate, a short chain fatty acid derived from the bacterial fermentation of fibers in the colon (62), and tricostatin A (TSA), a hydroxamic acid derivative isolated from *Streptomyces* (63), represent the most studied compounds. Sodium butyrate has been shown to induce cell cycle arrest and apoptosis in a wide variety of tumor cell types (64-66). However, butyrate displays a low clinical potential because of its instability when administered *in vivo* (67). Therefore, more stable butyrate analogs with a clinical potential, such as phenylbutyrate and tributyrine, have been developed and used as apoptotic inducers in many tumor models (68-70). TSA, although very effective as an HDACI and apoptotic inducer in tumor cells, has been shown to be unstable under *in vivo* conditions and to exert excessive toxicity (20). However, the apoptotic efficacy

displayed by TSA *in vitro* has lead to the production of synthetic hydroxamic acid compounds with high clinical potential.

The hydroxamic acid class of HDACIs has attracted the attention of oncologists because of their remarkable and selective efficacy in cancer cells (71-74). Specifically, suberoylanilide hydroxamic acid (SAHA), which is already approved for patients with hematological malignancies (vorinostat, Zolinza®), is currently of interest due to its capability of inducing apoptosis in a number of tumor cell types, whereas normal cells appear to be relatively resistant (75,76). Clinical investigations have provided evidence that SAHA exhibits a high therapeutic potential for different forms of tumors at doses that are well tolerated by patients (77). In addition, SAHA seems to exert antitumor effects in a synergistic manner with various compounds such as anti-cancer drugs (29,30), topoisomerase inhibitors (29,78), proteasome inhibitors (79-82), TRAIL (83) and Imatinib (84). Other hydroxamic acids, including scriptaid (85), ITF 2357 (15,86,87) and LBH589 (88,89), currently have a high impact on clinical applications because of their relative low toxicity profile and selective antitumor activitiy.

Cyclic peptides include trapoxin A and B, depudecin and cyclic tetrapeptides such as apicidin and FK228, also known as depsipeptide. All of these compounds have been shown to inhibit cell proliferation in several human cancer cell lines at nanomolar ranges (75).

The benzamide class of HDACIs include two compounds, MS-275 (90) and CI-994 (91,92), which seem to have a high potential in the treatment of different types of tumors.

Considering that tumor cells are often characterized by a hypoacetylated state of chromatin associated with transcriptional repression of pro-apoptotic genes, the use of HDACIs can be considered as a general approach to specifically stimulate apoptosis in tumor cells.

4. Molecular aspects of HDACI pleiotropic antitumor action

The molecular mechanisms responsible for the selective susceptibility of cancer cells to the effects of HDACIs have not been completely elucidated. However, a number of relevant studies have been performed.

Many lines of evidence suggest that tumor cells are characterized by histone hypoacetylation and that over-expression of HDACs is involved in tumorigenesis of various human malignancies (12,22). In accordance, it has been shown that histone hypoacetylation induces repression of tumor suppressor gene expression (93).

Treatment of tumor cells with HDACIs can be responsible for acetylation of histones, thus inducing derepression of gene expression leading to increased susceptibility of tumor cells to apoptosis (20,55).

Other authors have proposed that the different behavior of HDACIs in tumor vs non-tumor cells is most likely correlated with differential expression of thioredoxin, a factor present in normal cells but poorly expressed in tumor cells, a condition that in normal cells could represent a defensive mechanism against oxidative stress and apoptosis induced by HDACIs (94,95).

Studies on gene expression profiles induced by different HDACIs demonstrated that these drugs modulated a set of genes involved in several pathways regulating cell cycle and apoptosis (20).

A typical effect of HDACIs in many tumor cells is the induction of the tumor suppressor p21[WAF1/CIP1], which plays a major role in growth arrest as well as in apoptosis (96). Increased p21 expression has been correlated with both p53-dependent and -independent pathways (97). Moreover, it has been reported that HDACIs upregulate, apart from p21, other genes with antiproliferative functions including p16, p57[Kip2] and p19, while they downregulate several genes with proliferative functions, which are frequently overexpressed in tumor cells, including CDK4, CDC25; various cyclins, proliferating cell nuclear antigen (PCNA) and Ki-67 (11,20,98). Finally, HDACIs have been also shown to induce upregulation of several pro-apoptotic genes, such as Apaf-1 and caspase-9, and downregulation of anti-apoptotic genes such as Bcl-2 and survivin (11).

A wide description of the apoptotic mechanism induced by SAHA was recently described in hepatocarcinoma cells in comparison with primary human hepatocytes (PHH) (79). In HCC cells SAHA has been shown to potently induce the extrinsic apoptotic pathway, increasing the expression of both FasR and FasL, caspase-8 and t-Bid production, and contemporaneously the apoptotic intrinsic pathway by up-regulating pro-apoptotic members of the Bcl-2 family, such as Bim isoforms and Bcl-Xs, which is an isoform derived by alternative splicing of the Bcl-X transcript. In HCC cells SAHA was also shown to synergistically interact with the proteasome inhibitor bortezomib with a complex pleiotropic mechanism. Notably, neither SAHA alone nor the combination SAHA/bortezomib has been shown to exert any effect in PHH (79).

Recently, it has been proposed that HDACI-induced chromatin remodeling cannot be the only mechanism responsible for the antiproliferative/pro-apoptotic action of these drugs. An increasing list of non-histone proteins, including p53, NF-κB, Hsp90, α-tubulin p300 and FOXO-1, have been identified as HDAC substrates (21) thereby suggesting that acetylation can directly modulate effectors of apoptotic/cell survival mechanisms. Moreover, evidence has been provided that interaction of HDACIs with HDACs can modulate the formation or dissociation of protein complexes thus influencing key regulatory events determining cell fate. One significant example is given by the relationship between HDACs, the PP1 phosphatase and the pro-survival kinase Akt. Disruption of HDAC-PP1 complexes, induced by HDACIs, has been demonstrated to lead to an increased association of PP1 with phospho-Akt, resulting in Akt dephosphorylation and inactivation with consequent cell death (99).

Acetylation of p53 by HDACIs has been reported in several tumor systems (100) and has been correlated with p53 stabilization and cell cycle arrest and apoptosis (101). It has been recently suggested that acetylated p53 favors the production of nuclear complexes by recruiting HATs that are involved in mediating histone acetylation with the consequent activation of a set of pro-apoptotic genes (102).

HDACIs have also been shown to promote anti-angiogenic effects in different tumor cells (103,104) and inhibit cell

invasiveness (105). Evidence has also been provided that HDACIs are capable of downregulating metalloproteinases (MMPs) (106,107).

Notably, several HDACIs seem to exert an antitumor effect in a synergistic manner with different anticancer compounds and to overcome the resistance induced by conventional chemotherapeutic drugs (79-83).

In this regard, evidence has indicated that HDACIs are capable of sensitizing tumor cells to TRAIL-mediated death signaling. It has been shown that many types of cancers fail to undergo apoptosis in response to TRAIL treatment due to overexpression of decoy receptors or anti-apoptotic components such as Flip or IAPs. Therefore, combination therapies of TRAIL and HDACIs can result in sensitization of tumor cells towards TRAIL-mediated death signaling. Several classes of HDACIs have been shown to synergize with exogenously added TRAIL to induce apoptosis in different tumor cell lines (86,108).

In summary, several lines of evidence indicate that HDACIs exert complex and multiple mechanisms leading to inhibition of cell proliferation and induction of apoptosis in different tumor models. HDACIs seem to exert a selective efficacy in tumor cells and a remarkable tendency to synergistically act when administered together with chemotherapeutic drugs or apoptotic compounds with low toxicity.

5. Clinical investigations and therapeutic impact

A number of HDACIs which exhibit remarkable antitumor activity *in vivo* and low toxicity profiles are currently in clinical trials (Table I). Most of these drugs are in phase I and phase II evaluation for the treatment of hematological malignancies as well as solid tumors. HDACIs are administered either as monotherapy or in combination with other drugs. Some HDACIs are also used for other medical conditions including urea cycle disorder (109), sickle cell anemia (110-112) adrenoleukodystrophy (113), cystic fibrosis (114) and Fragile X syndrome (115). Valproic acid is well known in the field of neurology and widely used as an anti-epileptic (116).

The HDACIs in clinical trials against tumors include butyric acid derivatives (mostly sodium phenylbutyrate); valproic acid; hydroxamic acids such as SAHA, LAQ-824 and pyroxamide; benzamides including MS-275 and CI-994; and depsipeptide.

Short chain fatty acids, such as butyric acid derivatives and valproic acid, are very well tolerated in patients but show short plasma half-life due to rapid metabolism, thus requiring high doses for therapeutic effects (117). Among the butyric acid derivatives, sodium phenylbutyrate has been the most extensively investigated. As suggested by a phase I clinical study, intravenous or oral administration of phenylbutyrate, at concentrations that induce histone acetylation *in vitro*, is clinically well tolerated in patients with refractory solid tumors (118) or with AML (119).

Significantly, the use of phenylbutyrate or other HDACIs has been shown to sensitize leukemic blasts from AML patients to the differentiating effect of retinoic acid (21). Similarly, retinoic acid plus phenylbutyrate therapy has been reported to provide a good response in pro-myelocytic leukemia patients with PML-RARa fusion protein (120).

Valproic acid has been shown to induce differentiation of breast and colon carcinoma cells, as well as leukemic blasts from AML patients, and to cause reduction in tumor growth and metastasis formation in animal models (121). Clinical studies are ongoing and the preliminary results suggest a promising activity of valproic acid both in solid and hematopoietic malignancies (122,123).

Among the compounds belonging to the hydroxamate class, SAHA (vorinostat, Zolinza®) has been extensively considered, either alone or in combination with other drugs, for clinical studies in several neoplastic forms (71,72,78-82, 124,125) and is already approved for therapy in patients with follicular lymphomas (126,127). SAHA has been shown to be well tolerated at doses that caused biological activity. Phase I studies of orally administered SAHA to patients with advanced cancer demonstrated that SAHA can be administered safely for prolonged periods of time (77). Currently, there are multiple phase II studies in patients with hematologic and solid tumor malignancies that will aid in ascertaining the most optimal dosing regimen.

Depsipeptide (FK-228, romidepsin), a bicyclic peptide isolated from *Chromobacterium violaceum*, showed significant antitumor properties and is undergoing phase I-II clinical studies in hematopoietic malignancies and solid tumors (128,129).

CI-994 (N-acetyldinalin) is a novel oral compound with a wide spectrum of antitumor activity. *In vivo* studies of CI-994 exhibited activity in tumors normally refractory to conventional anticancer agents. Cytostatic *in vivo* activity of CI-994 has been demonstrated in several tumor models such as colon, osteosarcoma, prostate carcinoma, mammary carcinoma and pancreatic ductal adenocarcinoma (93,130).

Further studies need to define the optimal dosing schedule and elucidate the biological effects of HDAC inhibition in patients.

6. Future directions/conclusions

Considering that a hypoacetylated state of chromatin is a condition that can favor neoplastic transformation, regulating chromatin structure and function by histone deacetylase inhibitors can be a potential targeted therapy. It has recently been shown that HDACIs exert pleiotropic effects on cancer cells. This might be due to both transcriptional activation of specific gene targets, primarily including those involved in growth arrest and apoptosis, and post-transcriptional modifications of key regulatory proteins that promote apoptotic commitment. It remains to be elucidated how these mechanisms are less significant or even absent in normal cells.

In addition, further studies are needed to determine the exact therapeutic window of HDACIs in clinical applications and to identify proper combinations with other antitumor drugs already used in therapy or undergoing clinical evaluations.

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