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Review Article

Histone deacetylase inhibitors: molecular mechanisms of action and clinical trials as anti-cancer drugs

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Abstract: **Abstract** Histone deacetylase (HDAC) inhibitors are a relatively new class of anti-cancer agents that play important roles in epigenetic or non-epigenetic regulation, inducing death, apoptosis, and cell cycle arrest in cancer cells. Recently, their use has been clinically validated in cancer patients resulting in the approval of two HDAC inhibitors, vorinostat and depsipetide, by the FDA. Also, clinical trials of several HDAC inhibitors for use as anti-cancer drugs (alone or in combination with other anti-cancer therapeutics) are ongoing. However, the molecular mechanisms underlying the response to HDAC inhibitors in cancer patients are not fully understood. In this review, we summarize our understanding of the molecular and biological events that underpin the anticancer effects of HDAC inhibitors and the outcomes of recent clinical trials involving these drugs.

Keywords: HDAC inhibitor, acetylation, cancer, vorinostat (SAHA), depsipeptide (FK228), MS-275

Introduction

Alterations in tumor suppressor genes or oncogenes are not always due to mutations. They may also be due to transcriptional regulation by epigenetic mechanisms, including DNA methylation or demethylation and/or histone acetylation or deacetylation. In this review, we focus on acetylation and deacetylation by histone acetyltransferases (HATs) and histone deacetylases (HDACs). The balance between histone acetylation and deacetylation, mediated by HATs and HDACs, respectively, is usually well regulated, but the balance is often upset in diseases such as cancer. Conventional HDACs are composed of 11 members which require Zn^{2+} as a cofactor for their deacetylase activity and are divided into four classes depending on their homology [1] . Class I comprises HDACs 1, 2, 3, and 8, which are located within the nucleus; class II comprises HDACs 4, 5, 6, 7, 9, and 10, which are located in both the nucleus and the cytoplasm; and class IV comprises HDAC 11. Unlike conventional HDACs, class III HDACs are composed of seven mammalian sirtuins (SIRT1–7) [2]. These are nicotinamide adenine dinucleotide (NAD^+)-dependent protein deacetylases, localized in the nucleus (SIRT1, SIRT6, and SIRT7), mitochondria (SIRT3, SIRT4, and SIRT5), and cytoplasm (SIRT2). The acetylation of histones is thought to neutralize their positive charges and loosen their interaction with negatively-charged DNA. This opens the chromatin structure to facilitate the binding of transcription factors and, subsequently, gene transcription. Deacetylation of histones by HDACs tightens their interaction with DNA, resulting in a closed chromatin structure and the inhibition of gene transcription [3] . Apart from regulating histone modification, HDACs also regulate the post-translational acetylation status of many non-histone proteins, including transcription factors, chaperones, and signaling molecules, resulting in changes in protein stability, protein-protein interactions, and protein-DNA interactions [4]. Deacetylation of non-histone proteins by HDACs results in degradation via the ubiquitin-proteasome pathway [5, 6]. The acetylation status of RUNX3, a tumor suppressor and transcription factor, is important for its stability and

transcriptional activity. Increased acetylation of RUNX3 by p300 or HDAC inhibitors improves protein stability and transcriptional activity [6]. HDAC6 physically interacts with the molecular chaperone heat shock protein (HSP) 90 (another non-histone target protein). Deacetylation of HSP90 by HDAC6 is essential for the stability and function of many client proteins such as Bcr-Abl, c-Raf, and AKT [7]. Accumulated acetylation of HSP90 due to HDAC inhibition leads to the release and degradation of these client proteins [7, 8]. Many studies have shown that HDAC inhibitors target non-histone proteins, as well as histones.

A range of much more potent, structurally diverse HDAC inhibitors has been identified. These are natural products or have been synthetically produced, and include pan-HDAC inhibitors and class-selective or isoform-selective inhibitors. Although the mechanisms of action of HDAC inhibitors are still unclear, they are emerging therapeutic agents that have been clinically validated in cancer patients with hematologic malignancies, including cutaneous T-cell lymphoma (CTCL). Two HDAC inhibitors, vorinostat (suberoylanilide hydroxamic acid, SAHA, Merck & Co., Inc.) and depsipeptide (Romidepsin, FK-228, Gloucester Pharmaceutical Inc.), have recently been approved by the FDA (in 2006 and 2009, respectively). Clinical trials involving several HDAC inhibitors as single agents in combination with conventional chemotherapies or as targeted drugs are currently underway. HDAC inhibitors are well tolerated and clinically effective against hematologic cancers, even though they have poor anti-cancer activity against solid tumors when used as a monotherapy [9-11]. In this review, we focus on understanding the molecular and biological effects of conventional HDACs and Zn^{2+}-binding HDAC inhibitors and summarize the clinical data from trials of HDAC inhibitors as anti-cancer drugs.

The classification of HDACs

Eighteen human HDAC enzymes have been identified and classified into four groups based on their homology with yeast HDACs [1, 2]. Classes I, II, and IV all require a zinc molecule as an essential cofactor in their active site and are inhibited by Zn^{2+}-binding HDAC inhibitors such as vorinostat and trichostatin A (TSA). However, class III HDACs are structurally homologous with the yeast Sir2 protein and require NAD^+ as a cofactor instead of Zn^{2+} [2]. Therefore, they are not inhibited by Zn^{2+}-binding HDAC inhibitors. Sir2 extends the life-span of budding yeast by repressing genomic instability, suggesting a key role in promoting the organism's health and survival [12, 13]. However, the role of sirtuins in tumorigenesis is still debatable because some SIRTs have dual roles as oncoproteins and tumor suppressors [14, 15]. Therefore, we will focus on the use of Zn^{2+}-dependent HDAC inhibitors as anti-cancer drugs. Class I HDACs comprise HDACs 1, 2, 3 and 8 and are homologous to yeast Rpd3. They are localized in the nucleus and are the most abundant and ubiquitously-expressed HDACs [16]. Class II HDACs are homologous with yeast Hda1 and are larger in size than the other two classes. Based on their sequence homology and domain organization, they can be further subdivided. Class IIa (HDACs 4, 5, 7, and 9) contains a highly conserved C-terminal deacetylase catalytic domain homologous to yHda1, but have an N-terminal domain with no similarity to that in HDACs in the other classes. Class IIb (HDACs 6 and 8) is characterized by having two deacetylase domains [17]. Class II HDACs can shuttle between the nucleus and the cytoplasm and their expression is tissue-specific [17]. HDAC11 is the sole member of class IV. The classification and structures of the eleven Zn^{2+}-dependent HDACs are shown in **Table 1**.

Biology of HDACs

Several diseases, especially cancer, are caused by aberrant epigenetic alterations in addition to genetic mutations. Chromatin remodeling by histone acetylation and/or deacetylation is an example of epigenetic regulation [3]. The acetylation of histones by HAT changes their charge from positive to negative, which reduces their interaction with negatively-charged DNA. This increases accessibility for the transcriptional machinery, resulting in transcriptional activation. This series of the events can be reversed by deacetylation by HDACs. Epigenetic changes caused by imbalances between HATs and HDACs can affect global transcriptional profiles. In fact, tumor suppressors, such as p53 and RUNX3, are suppressed in many cancers by aberrant epigenetic changes [18, 19]. Unlike classical tumor suppressors, such as Rb and p53, mutation of the RUNX3 gene is very rare and its inactivation is mainly caused by epigenetic changes rather than mutation [19]. This

Table 1. Classification, structures, and cellular localization of $Zn2^{+}$ dependent HDAC isoforms

	Structure of Zn2+ dependent HDACs	Cellular localization	References
Class I			
HDAC1	482 a.a	Nucleus	[16]
HDAC2	488 a.a		
HDAC3	428 a.a		
HDAC8	377 a.a		
Class IIa			
HDAC4	1084 a.a	Nucleus & cytoplasm	[17]
HDAC5	1122 a.a		
HDAC7	855 a.a		
HDAC9	1011a.a		
Class IIb			
HDAC6	1215 a.a	Nucleus & cytoplasm	[17]
HDAC10	669a.a		
Class IV			
HDAC11	347 a.a	Nucleus & cytoplasm	[17]

Zn2+ containing catalytic domain Nuclear localization sequence

suggests that RUNX3 may be an excellent molecular target for anti-cancer drugs that regulate epigenetic changes because its function as a tumor suppressor can be recovered by RUNX3-targeted drugs.

HDACs are so-named because they were first identified as enzymes that function to remove acetyl groups from lysine residues on the N-terminal tails of histones [16]. However, recent phylogenetic studies suggest that all four classes of HDACs preceded the evolution of histone proteins, indicating that the primary substrates of HDAC enzymes are not histone proteins but non-histone proteins [20]. At least 50 non-histone proteins have been identified as HDAC substrates, including transcription factors (RUNX3, p53, E2F, c-Myc, nuclear factor kB (NF-kB), hypoxia-inducible factor 1 alpha (HIF-1α), estrogen receptor alpha (ERα) and the androgen receptor (AR), MyoD, chaperones (HSP90), signaling mediators (Stat3 and Smad7), DNA repair proteins (Ku70) [6, 7, 21-31] (**Table 2**). HDAC-mediated deacetylation alters the transcriptional activity of nuclear transcription factors such as p53, E2F, c-Myc, NF-kB, HIF-1α, smad7, ER and AR [21-27, 30]. Recently, our group added RUNX3 and RUNX2, a tumor suppressor and a master transcription factor for bone development, respectively, to the list of HDAC substrates [6, 32]. We showed that the stability and transcriptional activity of both RUNX3 and RUNX2 are controlled by acetylation and deacetylation by p300 and HDACs, respectively. HDAC5 strongly interacts with RUNX3 and induces its degradation. However, pan-HDAC inhibitors, such as TSA, increase the stability and transcriptional activity of RUNX3 cooperatively with p300 [6], indicating that the reactivation of RUNX3 in RUNX3-inactivated cancers can be mediated through HDAC inhibitors. These results suggest that non-histone proteins such as RUNX3 become potent drug targets that are controllable with HDAC

Table 2. Non-histone protein substrates of HDACs

	Protein	Intracellular Function	HDAC implicated	References
Acetylation increases protein stability	RUNX3	Tumor suppressor	HDAC1, 5	[6]
	P53	Tumor suppressor	HDAC1	[5]
	c-Myc	Oncoprotein	Not determined	[23]
	AR	Nuclear receptor	HDAC1	[27]
	Smad7	Signal transducer	HDAC1	[30]
Acetylation decreases protein stability	HIF-1α	Transcription factor	Not determined	[25]
Acetylation promotes protein-protein interaction	STAT3	Signal transducer	HDAC1, 2, 3	[29]
	AR	Nuclear receptor	HDAC1	[27]
Acetylation disrupts protein-protein interaction	NFkB	Transcription factor	Not determined	[24]
	Ku70	DNA repair protein	Not determined	[31]
	HSP70	Chaperone	HDAC6	[7]
Acetylation increases DNA binding affinity	P53	Tumor suppressor	Not determined	[21]
	STAT3	Signal transducer	HDAC1, 2, 3	[29]
	E2F1	Transcription factor	HDAC1	[22]
	MyoD	Transcription factor	Not determined	[28]
Acetylation increases transcriptional activation	RUNX3	Tumor suppressor	HDAC1, 5	[6]
	P53	Tumor suppressor	Not determined	[5]
	AR	Nuclear receptor	HDAC1	[27]
	E2F1	Transcription factor	HDAC1	[22]
	MyoD	Transcription factor	Not determined	[28]

inhibitors.

HDACs are both directly and indirectly involved in many biological processes, including development, proliferation, differentiation, and cell death. HDAC knockout mice enable study of their biological functions and provide valuable insights into the development and side effects of selective inhibitors. Despite the 80% sequence homology between HDAC1 and HDAC2, HDAC1 knockout mice have an embryonic lethal phenotype and display severe proliferation defects and general growth retardation, which cannot be compensated for by the upregulation of HDAC2 [33, 34] . HDAC1-null embryonic stem cells show proliferation defects that are associated with increased expression of the cyclin-dependent kinase inhibitors p21 and p27 [35] . HDAC2 knockout mice are born alive but have severe cardiac defects and die within 24 hours [34]. HDAC3, HDAC5, and HDAC9 knockouts also have severe cardiac effects, including hypertrophy and fibrosis [36, 37]. Conditional knockout of HDAC3 in cardiomyocytes leads to a dramatic upregulation of ligand-induced lipid storage within the heart. The mice survive for 3–4 months, at which point they show massive cardiac hypertrophy and depression of the genes that control fatty-acid uptake and metabolism [36]. Mice lacking either HDAC5 or HDAC9 are viable, whereas mice lacking both genes show lethal ventricular septal defects and a thin-walled myocardium, which typically arise from abnormalities in growth and maturation of cardiomyocytes [37]. The transcription factor, MEF2, is a target for these HDACs [38]. HDAC4 knockouts show chondrocyte hypertrophy and die of excessive ossification [39], suggesting that HDAC4 has a central role in the formation of the skeleton. Vega et al. showed that HDAC4 interacts with and suppresses RUNX2 and MEF2C, both of which have pivotal roles in the control of chondrocyte hypertrophy and bone formation [39]. In the absence of HDAC4, transcriptional activation of these factors is uncontrolled, leading to excessive ossification [39]. HDAC7 knockouts are embryonic lethal due to the loss of vasculature [40]. HDAC8 knockout mice are viable but have craniofacial defects [41]. HDAC6 knockout mice

Table 3. The knockout phenotypes of HDAC isoforms

Isoforms	Phenotype	Related disease	References
HDAC1	Letheal (E10.5) Severe proliferation defects and generalgrowth retardation		[33]
HDAC2	Die within 24h after birth Severe cardiac defect	Cardiac diseases	[34]
HDAC3	Lethal (before E9.5) owing to defects in gastrulation Deletion in the liver-disruption of lipid and cholesterol homeostasis Deletion in cardiomyocytes – massive cardiac hypertrophy	Cardiac diseases	[36]
HDAC4	Lethal within 7 days owing to ectopic ossification of endochondral cartilage, which prevents expansion of the rib cage and leads to an inability to breathe	Skeletal diseases	[39]
HDAC5	Viable Cardiac defects	Cardiac diseases	[37]
HDAC6	Viable but no obvious phenotype		[42]
HDAC7	Embryonic lethality owing to a loss of integrity of endothelial-cell interactions and consequent rupture of blood vessels and haemorrhaging	Vascular disorders	[40]
HDAC8	Not determined		[41]
HDAC9	Viable Cardiac defects	Cardiac diseases	[37]
HDAC10	Not determined		
HDAC11	Not determined		

are viable with no obvious phenotype, except for increased tubulin acetylation [42] . HDAC10 and HDAC11 knockouts have not yet been reported. These different knockout phenotypes help to predict the side effects of HDAC isoform-specific inhibitors in the clinic, guiding better strategies for drug development. For example, severe cardiac side effects were reported in a few patients given vorinostat and depsipeptide, which correlate with the cardiac defects seen in HDAC2, 3, 5, or 9 knockout mice. Also, HDAC7-selective inhibitors might be useful for inhibiting tumor angiogenesis. The various HDAC knockout phenotypes are summarized in **Table 3**.

Classification of HDAC inhibitors and their mechanisms of action

A large number of HDAC inhibitors have been purified from natural sources, or have been synthesized. Recent FDA approval for two HDAC inhibitors for use as anti-cancer agents has encouraged the development of new HDAC inhibitors. HDAC inhibitors can be structurally grouped into at least four classes: hydroxamates, cyclic peptides, aliphatic acids and benzamides (**Figure 1**). TSA was the first natural hydroxamate found to inhibit HDACs [43]. Vorinostat is structurally similar to TSA and was the first FDA-approved HDAC inhibitor for the treatment of relapsed and refractory CTCL [9]. TSA and vorinostat are pan-HDAC inhibitors. The cyclic peptides are the most structurally complex group of HDAC inhibitors and include depsipeptide, apicidin, and the cyclic hydroxamic acid-containing peptide group of molecules. Depsipeptide is most important member of this class and was approved by the FDA for the treatment of CTCL in November 2009 (FDA, Office of Oncology Drug Products. "What's New

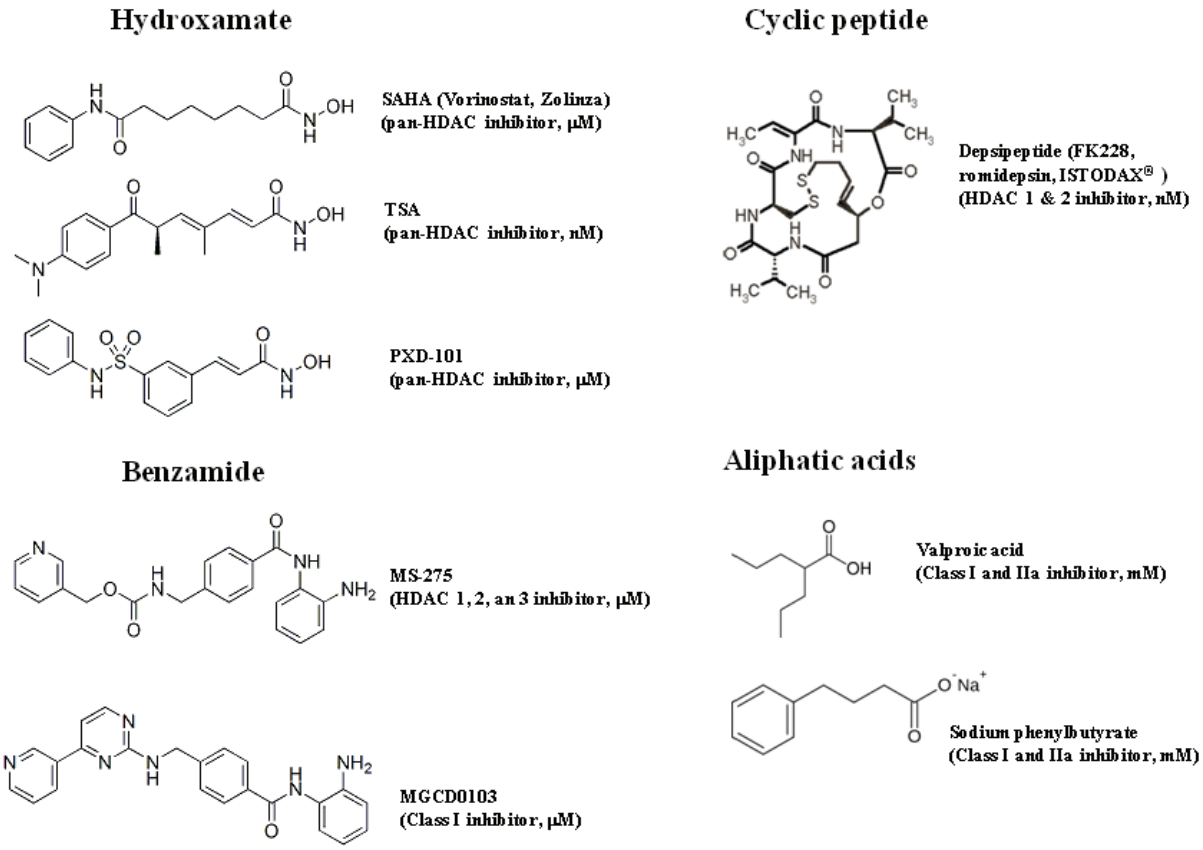

Figure 1. Structures of major classes of HDAC inhibitors. Suberoylanilide hydroxamic acid (SAHA/Vorinostat/Zolinza), Trichostatin A (TSA), and PXD-101 are hydroxamic acid-based pan-HDAC inhibitors. Depsipeptide (FK228/ romidepsin/ISTODAX®) is a natural cyclic peptide product of prodrug type, which inhibits HDAC1 and 2 selectively. Both MS-275 and MGCD0103 are synthetic benzamide derivatives. MS-275 is selective to HDAC 1, 2, and 3, and MGCD0103 is a class I selective HDAC inhibitor. Aliphatic acids include valproic acid and Sodium phenylbutyrate which have relatively low HDAC inhibitory potency.

From the Office of Oncology Drug Products"). It is a prodrug, converted intracellularly to a reduced form containing a functional sulfydryl group able to interact with the zinc in the active site pocket of class I HDACs, particularly HDAC1 and HDAC2 [44] . The aliphatic acids, such as butyrate, phenylbutyrate, and valproic acid, are relatively weak HDAC inhibitors, with activity at millimolar concentrations [45-47]. Because of their weak inhibitory effects, they are the least attractive agents. SNDX-275 (formally MS-275, Syndax Pharmaceutical Inc.) is a synthetic benzamide derivative with activity against HDAC1, 2, and 3 (class I) in the μM range. MGCD0103 (Methylgene Inc.) is an isoform-selective aminophenyl benzamide that inhibits HDAC classes I and IV, with almost no effect on class II [48].

During the 1990s, a clear link between the suppression of tumor cell growth and survival and the inhibition of HDAC activity was established [43, 49]. The expression of individual HDACs is altered in tumors. HDAC1 is overexpressed in prostate, gastric, colon and breast carcinomas [50-53], whereas HDAC2 is overexpressed in colorectal, cervical and gastric cancers [52, 54-56]. Normal cells are relatively resistant to the treatment with HDAC inhibitors [57], whereas tumor cells are more sensitive and undergo growth arrest, inhibited differentiation and cell death. The mechanisms of action of HDAC inhibitors are thought to be related to altered gene expression and to changes in non-histone proteins via regulation at the epigenetic and post-translational modification levels, respectively. In many tumor cell lines, HDAC inhibitors cause upregulation of the cell cycle gene p21, blocking the cyclin /CDK complexes, leading to

cell cycle arrest and inhibiting differentiation [58, 59]. HDAC inhibition modulates the balance between pro- and anti-apoptotic proteins, causing tumor cell death [60]. HDAC inhibition upregulates the intrinsic and extrinsic apoptosis pathways through the induction of the proapoptotic genes, Bmf and Bim [61, 62], and TRAIL and DR5, respectively [63]. Also, hyperacetylation stabilizes the p53 protein, promoting both cell cycle arrest and the expression of proapoptotic genes [64]. Similar to the post-translational modification of p53, HDAC inhibition by HDAC inhibitors increases the stability and transcriptional activity of RUNX3, which induces p21 and Bim, leading to cell cycle arrest and apoptosis of tumor cells [6, 65, 66].

HDAC inhibition may also affect tumor cell survival by blocking tumor angiogenesis and by inhibiting intracellular stress response pathways. HIF-1α, a pro-angiogenic transcription factor, is hyperacetylated by HDAC inhibitors, resulting in its degradation [25]. In addition, HDAC inhibitors decrease the expression of the vascular endothelial growth factor receptor (VEGFR) [67], increase the generation of intracellular reactive oxygen species, and impair the handling of misfolded proteins by influencing endoplasmic reticulum stress responses [68, 69]. When hypoacetylated, the chaperone protein HSP90 protects client proteins such as Bcr-Abl, epidermal growth factor receptor and ErbB2 from degradation [7]. Hyperacetylation of HSP90 by HDAC inhibitors leads dysfunctional chaperone activity, resulting in the degradation of cancer-related client proteins. Further understanding of the mechanisms of action of HDAC inhibitors may make it possible to use them in combination with other drugs such as HSP90 inhibitors, tyrosine kinase inhibitors, and proteasome inhibitors.

HDAC inhibitors in clinical development

After vorinostat, depsipeptide was the second HDAC inhibitor, but the first from the cyclic peptide HDAC inhibitor class, approved for the treatment of CTCL (in November 2009). More than 15 HDAC inhibitors have been tested in preclinical and clinical studies. In the following sections, we discuss the available data on agents from the three different HDAC classes, vorinostat, depsipeptide, and MS-275, and evaluate the evidence for anticancer activity in these trials.

Vorinostat

Vorinostat is the most advanced HDAC inhibitor and was approved by the FDA in October 2006 for the treatment of advanced forms of CTCL that could not be treated with multiple or systemic drugs [70]. Vorinostat has also been investigated in other phase I and II clinical trials for other hematological malignancies and solid tumors [10, 71-74]. Vorinostat can be given orally, with a maximum tolerated dose (MTD) of 400 mg once daily or 200 mg twice daily, for hematological malignancies. It can also be given at a dose of 300 mg twice a day for 3 consecutive days per week in a 4-week cycle to treat solid tumors [75]. In a phase IIb trial, 74 patients with progressive, persistent, or recurrent CTCL, who had received at least two prior therapies, were treated with oral vorinostat at 400 mg/day until disease progression or intolerable toxicity was observed [76]. The objective response rate (ORR) was 29.7%. The median time to progression was 4.9 months overall and ≥ 9.8 months for stage IIB or higher responders. Thirty two percent of patients experienced relief of pruritus. The most common drug-related adverse effects (AE) were diarrhea, fatigue, and nausea. Some patients had a pulmonary embolism and thrombocytopenia. Eleven patients required dose modification and nine patients stopped taking the drug due to AE. The post hoc study provides evidence for the long-term safety and clinical benefit of vorinostat in heavily pretreated patients with CTCL, regardless of previous treatment failures [77]. Six of the 74 patients remained on vorinostat for 2 years or longer with continued clinical effect (one with complete response (CR), four partial responses (PR), and one with stable disease SD)) and minimal toxicity.

In the limited number of clinical trials reported, vorinostat showed either modest activity or no response when used to treat solid tumors. None of 16 patients with either relapsed/refractory breast, colorectal, or non-small cell lung cancer achieved a CR according to the Response Evaluation Criteria in Solid tumors (RECIST) criteria [72]. In a single center, open-label, non-randomized phase II trial, oral vorinostat (400 mg once daily) was used to treat patients with squamous cell carcinoma of the head and neck. The drug was generally well-tolerated and had an acceptable safety profile; however, it was ineffective [73]. In another phase II study using

the same regimen, vorinostat was well tolerated, but had minimal activity as a single agent in unscreened patients with recurrent platinum-refractory ovarian or primary peritoneal carcinoma [10]. A phase II trial of oral vorinostat (200 mg b.i.d. for 14 days in a 3 week cycle) for the treatment of metastatic breast cancer showed no CR or PR; however, four patients had SD [11]. Although vorinostat shows either modest activity or no efficacy when used as a monotherapy for solid tumors, the preclinical data strongly suggests that further study of vorinostat as a combination therapy with either chemotherapeutic or targeted agents would be prudent and further combinational clinical studies are ongoing.

Depsipeptide

Depsipeptide is a unique HDAC inhibitor prodrug, which is converted intracellularly to a reduced form containing a functional sulfhydryl group able to bind the zinc in the active site pocket of class I HDACs [44]. In a phase I study of depsipeptide at a dose of 12.7 or 17.8 mg/m^2 given as a 4-hour infusion on Days 1 and 5 of a 21-day cycle, three patients with CTCL showed a PR, and one patient with peripheral T-Cell lymphoma showed a CR [78]. Although this trial was conducted on only four patients, the clinical outcome encouraged further trials. After a phase II multi-institutional trail, Piekarz and colleagues reported the final results of 71 CTCL patients treated in a multicenter NCI study with depsipeptide administered as a 4-hour infusion at a starting dose of 14 mg/m^2 on Days 1, 8, and 15 of a 28 day cycle [79]. The ORR was 34%, with a CR (6%) observed in four patients, a PR (28%) in 20, and SD (37%) in 26. Commonly observed toxicities were similar to those observed in the phase I trials and to those reported for other HDAC inhibitors [80]. They included nausea, vomiting, fatigue, and transient thrombocytopenia and granulocytopenia. Because asymptomatic T-wave flattening and ST segment depression were observed in the phase I trials, cardiac evaluation was incorporated into the phase II study. Testing revealed no evidence of acute or cumulative cardiac damage. However, 1/71 patients died unexpectedly due to severe valvular heart disease. The protocol was then amended to exclude patients with heart disease. Similarly to vorinostat, depsipeptide had minimal antitumor activity in patients with prostate, renal cell, lung and colorectal cancers [81-84].

MS-275

MS-275 (Entinostat, SNDX-275) is a synthetic benzamide derivative that inhibits HDACs and has been used to treat patients with leukemia, lymphomas, or solid tumors in phase I and II clinical trials [85-88]. Preclinical pharmacokinetics indicated that MS-275 had good oral bioavailability, with a half life (T1/2) of about 1 hr (similar in rats, mice and dogs; MS-275 (NSC-706995) preclinical toxicity summary, NCI Drug development group, 2000). However, a phase I trial in patients with solid tumors showed that MS-275 had a much longer half-life (30–50 hrs), leading to an early change in the proposed schedule from daily treatment to treatment every 14 days. The MTD was 10 mg/m^2 and the dose limiting toxicities were gastrointestinal side effects and fatigue [85]. In two other phase I studies in patients with solid tumors and leukemia, the MTD was 8 and 6 mg/m^2, respectively, and the drug was given once a week for 4 weeks, with 6 weeks between treatment cycles. The reported dose limiting toxicities at a dose of 8 mg/m^2 were infections and neurological toxicity manifesting as an unsteady gait and somnolence. At a dose of 6 mg/m^2, toxicity manifested as reversible grade 3 hyposphophatemia, hyponatremia and hypoalbuminemia [86, 87]. While the regimens were well tolerated, MS-275 appeared to have limited antitumor activity in these phase I trials. Phase II clinical trials are still ongoing.

Conclusions and Future perspectives

Preclinical and clinical trials show that HDAC inhibitors have varying antitumor activity. Both of the FDA-approved HDAC inhibitors (vorinostat and depsipetide) have great clinical benefits and minimal AE when used to treat hematological malignancies such as CTCL. However, the clinical outcomes of HDAC inhibitors, including vorinostat and depsipeptide, when used as a single agent to treat solid tumors are disappointing. Based on clinical trials and the mechanisms of action of HDAC inhibitors, HDAC inhibitor therapy for hematologic and solid tumors is likely to take the form of combined therapy with other agents that have synergistic or additive effects. Since many studies show that HDAC inhibitors alter the balance in favor of proapoptotic pathways, they have been clinically tested

with conventional cytotoxic chemotherapeutic agents such as carboplatin, paclitaxel, fluorouracil, and gemcitabine to treat solid tumors [89]. In one phase I trial, vorinostat in combination with paclitaxel and carboplatin was used to treat 25 patients with advanced solid tumors. Eleven patients showed a PR and seven showed an SD [90], demonstrating that HDAC inhibitors have promising antitumor activity when used in combination with other drugs. Also, HDAC inhibitors have been used in patients with advanced solid tumors or hematologic cancers in combination with the DNA methylation inhibitor azacitidine, the differentiating agent all-trans-retinoic acid, and with bortezomib [91-93]. HDAC inhibition leads to the loss of HSP90 chaperone function and enhanced degradation of client proteins, such as Bcr-Abl, ErbB2/neu, and FLT3. This suggests that there may be potential synergistic effects between HDAC inhibitors and imatinib, traztuzumab, or FLT3 inhibitors [94].

At present, clinical trials of HDAC inhibitors have been focused on cancer treatment. This approach was based on extensive *in vitro* and *in vivo* data showing excellent anticancer activity of HDAC inhibitors. However, there is growing evidence that HDAC inhibitors have potential therapeutic effects against nonmalignant diseases. HDAC inhibitors have therapeutic benefit in neurodegenerative diseases such as stoke, Huntington's disease, spinal muscular atrophy, Parkinson's disease and Alzheimer's disease [95-99]. TSA and SAHA have anti-arthritic activity in rodent models [100, 101]. We have also suggested that HDAC inhibitors might be used to treat bone diseases such as osteoporosis and fractures by regulating the stability and transcriptional activity of RUNX2 [32]. Many studies have also implicated that HDAC inhibitors may be used to treat diabetes, sickle-cell anemia, inflammation, and HIV infection.

Since there are eleven HDAC isoforms, there are also multiple protein targets. It is, perhaps, to be expected that HDAC inhibition causes a variety of biological effects, resulting in them having a narrow therapeutic window and several adverse side effects. To solve this problem, many medicinal chemists have been making efforts to develop isoform-selective HDAC inhibitors. Although several class-selective HDAC inhibitors and one isoform-specific HDAC inhibitor (tubacin against HDAC6) have been developed, it is still questionable whether more selective or specific HDAC inhibitors will result in improved efficacy and minimized AE compared with pan-HDAC inhibitors. Comparing the anticancer activity and AE of pan-HDAC inhibitors, such as SAHA, and class I-selective HDAC inhibitors, such as depsipeptide and MS-275, shows that they have similar ORRs for anticancer activity and similar AE (though class specificity does lead to certain specific AE). Therefore, as there are no significant differences between them in terms of anti-tumor activity or AE, it seems that new strategies for developing HDAC inhibitors for medical purposes are needed in addition to developing HDAC isoform-selective inhibitors with better HDAC inhibitory potency. One example is the targeting of non-histone proteins regulated by HAT or HDAC. Non-histone proteins, such as the RUNX3 tumor suppressor, that are downregulated by HDAC can be targeted. The strategy is to find HDAC inhibitors strongly and selectively able to reactivate RUNX3 in cancer cells. Simultaneously, HDAC inhibitors should have mild HDAC inhibitory potency to avoid the broad biological effects caused by the strong inhibition seen when HDACs are targeted to histones.

In conclusion, based on the results of recent clinical trials, HDAC inhibitors are promising therapeutic agents, even though their exact targets and mechanisms of action are still unclear. Also, expansion of their therapeutic application beyond the treatment of cancers has encouraged further development of HDAC inhibitors. Combination therapy with other medicines will yield improved clinical outcomes over those seen with single agents. If new strategies are applied to develop HDAC inhibitors for therapeutic use, new classes of HDAC inhibitors with defined targets, improved therapeutic effects and minimal adverse effects will be anticipated.

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