Regulation of apoptosis-associated genes by histone deacetylase inhibitors: implications in cancer therapy

Ali R. Jazirehi

Anomalous gene regulation, dictated by epigenetic modifications, is a universal characteristic of cancer cells. Histone deacetylases (HDACs) are an important class of enzymes that influence gene expression by the removal of acetyl groups from histones leading to chromatin remodeling and transcriptional suppression of key apoptosis and cell cycle regulatory genes. Histone deacetylase inhibitors (HDACis) are a novel category of anticancer pharmacological agents developed to counter the actions of HDACs, thus, inducing an array of cellular consequences, such as apoptosis, cell cycle arrest, generation of reactive oxygen species, inhibition of angiogenesis, and autophagy. Suberoylanilide hydroxamic acid (SAHA, Zolinza, Vorinostat), is currently the only Food and Drug Administration-approved HDACi for the treatment of cutaneous T-cell lymphoma. SAHA and other HDACis have shown selective toxicity toward malignant cells while sparing the surrounding normal cells. In addition to this specificity, their regulation of apoptosis-associated genes and the synergistic augmentation of apoptotic events when used simultaneously with other anticancer agents such as conventional chemotherapies, radiation, inhibitors of DNA methylation, and proteasome inhibitors make HDACis potential novel arsenals in the battle against cancer. Herein I review epigenetic modifications, discuss the various mechanisms of HDACi-induced effects, in particular modulation of expression of apoptosis-associated gene products, and highlight SAHA and its antitumor functions. *Anti-Cancer Drugs* 21:805–813 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Department of Surgery, Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at UCLA, University of California, Los Angeles, California, USA

Correspondence to Dr Ali Jazirehi, CLS, PhD, 10833 Le Conte Avenue, Division of Surgical Oncology, CHS 54-140, Mail code: 178218, Los Angeles, CA 90095, USA
Tel: +310 206 8509; fax: +310 267 2679;
e-mail: ajazirehi@mednet.ucla.edu

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Introduction

The disturbance of the intricate balance of epigenetic modifications, heritable changes affecting gene expression carried out by means other than changes in the primary DNA sequence, such as DNA methylation and covalent histone modifications, leads to chromatin remodeling with significant impact on gene transcription [1]. For example, alterations in the pattern of DNA methylation resulting in DNA hypermethylation and/or hypomethylation alter the gene expression profile frequently seen in cancer [1]. Inside the nuclei, DNA is highly folded and compacted into a highly sophisticated molecular structure called chromatin. The basic repeating unit of chromatin is the nucleosome (measuring approximately 10 nm in diameter); 146 base pairs of DNA wrapped around an octamer of core histones [2]. Histones guide the interactions between DNA and other proteins such as transcription factors. The six classes of histones are divided into core histones (H2A, H2B, H3, and H4) and linker histones (H1 and H5). The linker histone, H1, binds the nucleosome at the entry and exit sites of the DNA and as a result can lock the DNA into place. The histone octamer can be dissociated into an $(H3\text{-}H4)_2$ tetramer and two H2A-H2B dimers [2,3]. Certain epigenetic alterations occur through covalent modification of histone amino (N)-terminal tails [3], which directly affect gene expression. Compared to normal cells, the expression levels of the genes encoding chromatin remodeling as well as histone tail-modifying enzymes are significantly modified (genes are mutated and/or overexpressed) in tumor cells leading to altered chromatin configuration as well as aberrant histone tail modifications with a direct impact on the regulation of the expression of genes involved in growth, apoptosis, and metastasis. This pattern of disturbed gene expression will ultimately confer an apoptosis resistance and growth advantage phenotype to the tumor cells, highlighting the need for the development of agents that can selectively reverse these epigenetic events to render tumor cells susceptible to various treatment modalities. The discovery of histone deacetylase inhibitors (HDACi) represents a novel approach toward achieving this goal. In this review, I will briefly highlight various enzymatic modifications of histone tails, effects of various HDACis with an emphasis on the Food and Drug Administration (FDA)-approved HDACi suberoylanilide hydroxamic acid (SAHA) in enhancing the efficacy of conventional therapies (chemo and radiation therapy), and their apoptosis gene regulatory effects.

Posttranslational histone modifications: impact on gene transcription

Often, almost immediately after or even during protein synthesis, the residues in a protein are chemically modified, which lead to changes in physical and chemical

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properties and stability, folding, activity, and function of the protein. Posttranslational modifications include addition of functional groups such as acetyl, methyl, phosphate groups, and SUMOylation [4].

Lysine (Lys; K) residues in the highly conserved histones H3 and H4 N-termini have a tendency to undergo chemical modifications, changing the chromatin structure, and affecting the dynamics of regulatory factors [5]. In acetylation, a process in which acetyl groups are introduced into a compound and later replace an active hydrogen atom, histones are acetylated on the N-terminal tail Lys residues, which neutralize a positively charged Lys residue, thereby decreasing its interaction with the negatively charged phosphate groups of the DNA backbone. Subsequently, chromatin switches from a condensed form to a more relaxed configuration, which is no longer bound to a histone octamer. Different components of the transcriptional machinery are then able to bind to the promoter sequence, with replication and transcription ensuing if the promoter sequence of the associated gene is not methylated. Acetylation affects transcription either by acting as physical barriers, preventing transcriptional repressors from binding to the nucleosome, or by functioning as docking sites for other proteins such as activators, both of which facilitate gene transcription. Specific examples of residues that are subject to acetylation include H3K9, H3K18, and H4K12 [6].

Genes are usually silenced through DNA methylation; the addition of methyl groups to the carbon residue located in the 5 position of cytosine nucleotides in the CpG dinucleotides by DNA methyl transferases (DNMT). Gene silencing is mediated by a class of methyl DNA binding proteins, which specifically recognize methylated DNA sequences and recruit repressive protein complexes. In some cases, methylation status of a gene has clinico-pathological significance, with no obvious gene regulatory effect. For instance, methylation-mediated silencing of the O^6-methyl guanine-DNA methyl transferase gene promoter (DNA repair protein) can serve as a predictor of a patient's responsiveness to alkylating agents in glioma [7]. O^6-methyl guanine is the carcinogenic lesion in the DNA induced by alkylating mutagens. This adduct is removed by the action of O^6-methyl guanine-DNA methyl transefrase and no gene regulatory effect has been reported by guanine methylation. In other cases, it cripples the function of tumor suppressors such as PTEN (see below); a prerequisite for tumor initiation. Genes can also be silenced upon methylation of specific histone residues (e.g. H3K9, H3K27) by creating a repressed chromatin structure making DNA inaccessible to the transcriptional machinery. Although methylation is usually associated with transcriptional repression, the effect of this modification actually depends on the particular Lys or arginine (R) residue involved. For instance, transcriptional repression is observed in H3K9 methylation in heterochromatin and repressed genes, whereas transcriptional activation is observed in H3K4 methylation of euchromatin and active genes [5]. H3K27 methylation is another epigenetic mark for transcriptional repression. In addition to the various H3 Lys-residue methylations, H4 can also undergo methylation at Lys 20 (H4K20) resulting in a silent chromatin structure [8]. Although R residues, such as demethylated H4R3, can only be methylated once or twice by peptidylarginine methyltransferases, K residues can be methylated once, twice, or three times by lysine methyltransferases, resulting in three distinct H3K9 methylation states: mono-methylated, di-methylated, and tri-methylated states – denoted as H3K9me1, H3K9me2, and H3K9me3, respectively [9].

Further discussion on methylation and its role in chromatin repression leads to the discussion of CpG island methylation, which also corresponds to heterochromatic regions [9]. A CpG island is a region of a genome characterized by a high frequency of C–G dinucleotides united through a phosphodiester bond. These 200 base-pair islands are usually located near or in the promoter regions of approximately 60% of mammalian genes. Methylation of CpG sites in promoter regions may result in gene repression. Furthermore, regions of silenced chromatin are produced when factors such as CpG methylation, the proteins that attach to them, and repressive histone modifications coalesce together, resulting in transcriptional repression. In cancer, gene silencing disables critical proteins, such as tumor suppressor PTEN [10], which directly correlates with tumor progression. Another posttranslational modification is phosphorylation, which is the addition of a phosphate group to a protein by a kinase. Phosphorylation plays a critical role in an extensive array of cellular processes such as the activation of cell signaling. Alternatively, dephosphorylation is mediated by the enzyme phosphatase. Phosphorylation and dephosphorylation result in turning various enzymes and receptors 'on' and 'off'. Phosphorylation usually occurs on serine (Ser), tyrosine (Tyr), and threonine (Thr) residues. The addition of a phosphate molecule to the polar R groups of these amino acids causes a conformational change by changing a protein's hydrophobic portion into a hydrophilic one. Lastly, SUMOylation involves the covalent attachment of one or more copies of the 101-amino acid polypeptide SUMO (small ubiquitin-like modifier) to Lys residues. SUMOylation is analogous to ubiquitination, but the results are different. SUMOylation functions in protein stability, apoptosis, nuclear cytosolic transport, and transcriptional regulation and has been linked to a variety of cancers, signifying that its manipulation could be one potential method of regulating cancer development. Ubc9 (ubiquitin-conjugating enzyme 9) plays a key role in SUMOylation, interacting with almost all of the effectors required for SUMOylation [11]. SUMOylation can potentially regulate the NF-κB signaling pathway. The NF-κB

pathway is involved in a myriad of cellular responses, especially those of the immune system. Since the adaptive response is largely responsible for antitumor effects and tumor cell cytotoxicity, specific modulation of the NF-κB pathway has received much attention [12].

In addition to DNA methylation, histone deacetylation also corresponds to transcriptional silencing. In contrast to nucleosomal relaxation caused by histone acetyl-transferases, enzymes that catalyze the addition of acetyl groups to histone N-terminal Lys residues, histone deacetylases (HDACs) increase the ability of histones to bind to DNA, thus promoting DNA condensation and inhibition of chromatin expansion, which prevents gene transcription [4]. Histone acetylation plays a vital role in gene regulation, and the epigenetic modifications (reversibly altering the N-terminal tails of core histones, which remodels the higher-order chromatin structure and controls gene expression) displayed by HDAC activity may be brought to a standstill with the discovery of HDAC inhibitors (HDACis) [13]. On the basis of their homology to yeast HDACs, HDACs are classified into four classes. Class I includes HDAC-1, HDAC-2, HDAC-3, and HDAC-8 (related to the yeast RPD3 gene) and class II includes HDAC-4, HDAC-5, HDAC-6, HDAC-7, HDAC-9, and HDAC-10 (related to the yeast Hda1 gene). The two remaining classes include the sirtuins 1–7 that belong to class III, and HDAC-11, which has features of both of the first two classes, belongs to class IV [14]. Classes I, II, and IV HDACs, which are zinc-dependent deacetylases, could be impeded by several HDACis including SAHA, trichostatin A (TSA), and LBH589 [13]. However, class III HDACs, which are NAD^+-dependent could not be impeded by hydroxamic acid-based HDACis [15].

HDAC over-expression along with a disruption in histone acetyl-transferases activity leading to a hypoacetylated chromatin structure has been observed in cutaneous T-cell lymphoma (CTCL), acute myelogenous leukemia (AML) and chronic myelogenous leukemia, breast, colon, and prostate cancer. Hypoacetylated chromatin could decrease the expression of proapoptotic or tumor suppressor genes (e.g. retinoblastoma protein, p53, and PTEN) [4,10,13,14].

Acetylation of nonhistone proteins: role in regulation of gene expression

Reversible acetylation mediated by HDACis modifies the structure and function of histones and nonhistone proteins including those involved in transcription factor complexes that are involved in the regulation of gene expression. Specific examples of nonhistone proteins whose function is regulated by acetylation are heat shock protein (HSP) 90, p65 (Rel A) NF-κB subunit, and the tumor suppressor p53.

HSP90 is an ATP-dependent molecular chaperone that mediates active conformation of its client oncoproteins in cancer cells. Recent studies have shown a role of HSP90 acetylation in various tumor models. For instance, SAHA, LBH589, VPA, TSA, and sodium butyrate induce apoptosis in Kit-positive gastrointestinal stromal tumors and increase the efficacy of imitanib in imitanib-resistant gastrointestinal stromal tumors through Hsp90 acetylation and Kit mRNA downregulation [16]. Knock-down studies have shown in addition that HDAC6, through the acetylation of Hsp90, regulates androgen receptor hypersensitivity, impairs ligand-dependent nuclear localization of endogenous androgen receptor, and inhibits PSA expression and cell growth in the presence and absence of dihyrotestosterone [17]. Another study showed that knock-down of HDAC6 induces reversible acetylation and attenuates the ATP binding and chaperone function of Hsp90. In this study, LBH589 hyperacetylated Hsp90 on seven Lys residues in the middle domain and increased its binding to MMP-2, which was associated with in-vitro invasion and metastasis on tumors [18].

Most of the biological effects of NF-κB transactivation are mediated through p65/Rel A containing dimers (mainly p65–p50). Signal-dependent phosphorylation and subsequent proteasomal degradation of IκB facilitates nuclear translocation of p65, which on binding to κB sites, regulates gene expression. Acetylation of p65 has been postulated as a postive regulatory mechanism. It was recently shown that p65 Lys acetylation and activation of NF-κB-HIF-1 regulatory loop contributes to the metastasis phenotype of human breast cancer cells [19]. IKK-α can phosphorylate both silencing mediator for retinoic acid and thyroid hormone receptor and p65 within the transactivation domain (S536), thus, removing the repression of silencing mediator for retinoic acid and thyroid hormone receptor-HADC3 leading to the acetylation of p65 at K310 by p300 and subsequent expression of NF-κB-dependent genes [20].

It was further shown that p65-HDAC association represses NF-κB activation. TSA treatment disrupts this association and leads to p65 acetylation and expression of IL-8 [21]. Taken together, these results suggest a positive gene regulatory role of acetylated p65.

Another example of a non-histone protein whose activity is, in part, regulated by acetylation is the transcription factor p53, which acts as a tumor suppressor. Protein stability, phosphorylation, and tetramerization also contribute to p53 activity. Recent studies on the effects of TSA, ITF2357, and SAHA on human hepatocellular carcinoma HepG2 cells have shown significant induction of apoptosis concordant with the acetylation of histones and p53. The direct role of acetylated p53 in the acetylation of histones and apoptosis was shown by small interfering RNA studies and pifithrina, a reversible p53 inhibitor [22].

Roy *et al.* [23] showed that p53 is constitutively acetylated at K320; however, HDACi CG1521 stablizes p53 Ac-K373 leading to increased p21 expression, cell cycle arrest, mitochondrial tranlocation of Bax and apoptosis induction in the LNCap prostate carcinoma line. Alternatively, TSA stablizes p53 Ac-K382 and increased p21 expression; however, no Bax mitochondrial translocation was observed. Therefore, only cell cycle arrest was observed. These results suggest different cellular consequences on specific p53 Lys acetylation. Metastasis-associated protein 2, a p53 target protein and a component of the NURD complex, whose expression diminishes acetylated p53; thus regulating p53-mediated cell cycle arrest and apoptosis. Although various HDACis contribute to p53 acetylation, p53 deacetylation is mediated by an HDAC1-containing complex [24].

Histone deacetylase inhibitors and their role in cancer therapy

HDACis have multiple effects on gene transcription and protein function, as their actions are not only limited to histone deacetylation, but also to altering the acetylation status of cytoplasmic proteins [16–24]. HDACis cause chromatin remodeling, differential gene expression, modulate the levels of regulatory molecules, promote protein-DNA interactions, and consequently induce apoptosis, cell cycle arrest, differentiation, and senescence [25,26]. Particularly, HDACi-induced hyperacetylation activates genes responsible for generating an intracellular proapoptotic milieu. Thus, HDACis are advantageous over traditional drugs as they regulate several genes and pathways involved in cell survival and apoptosis [27]. Another advantage of using HDACis in cancer therapy is their high selectivity for cancer cells. *In vitro* and *in vivo*, HDACis selectively induce apoptosis in several tumor types but have low toxicity toward normal cells [4,28,29], specifying the substantial potential of HDACis to regulate anomalous gene expression and restore normal growth [30,31]. The precise mechanism(s) by which HDACis cause cell death is still elusive and the particular functions of individual HDAC enzymes as therapeutic targets has yet to be determined [13,31,32]. Nonetheless, the use of HDACis could serve as a novel and promising approach in cancer therapy. Triggering apoptosis is associated with an increased expression of proapoptotic genes (e.g. Bcl-x_s, Bax, Bak, Bid, Bim) and a decreased expression of antiapoptotic genes (e.g. Bcl-x_L, Bcl-2, Mcl-1, Bfl1/A1, IAPs) shifting the cellular processes toward an irreversible genetic program designed to destroy malignant cells [14,33]. In addition, a prominent event that occurs during HDACi-mediated cell death is the generation of reactive oxygen species (ROS), which activates the intrinsic apoptotic pathway [13,14]. The mechanism of generation of these species, however, still remains unclear. Moreover, studies have indicated that HDACis can synergistically enhance the anticancer activity of a number of traditional drugs (e.g. bortezomib, etoposide, cisplatin, and gemcitabine) [14,34]. These studies have led to the discovery of various HDACis, such as MS-275, TSA, trapoxin (TPX), valproic acid, depsipeptide, and SAHA (Vorinostat, Zolinza) [35]. TSA and TPX act by inhibiting the cell cycle and provoking morphological deterioration of transformed cells (such as those seen in cancer), and inhibiting HDACis at nanomolar concentrations. FK228 (also known as FR901228 and depsipeptide) and MS-275 act by enhancing the expression of $p21^{Waf1}$ in a p53-independent manner. $P21^{Waf1}$ belongs to cyclin-dependent kinase inhibitors (CKIs), which are divided into two main classes that inhibit cyclin/CDK complexes. $P21^{Waf1}$ belongs to the category of CKIs, which functions by stoichiometric binding and the other category of CKIs, which functions by dissociating the cyclin/CDK complexes [36]. The orderly activation of certain CDKs is crucial for the progression of cells through the cell cycle. Thus, increased expression of $p21^{Waf1}$ caused by certain HDACis could lead to cell cycle arrest. TSA inhibits the catalytic reaction by binding a zinc ion in the active site through its hydroxamic acid group. Adversely, an epoxyketone group is the functional group of TPX, which ultimately alkylates the enzyme [37].

Apoptosis occurs through the 'extrinsic' or the death receptor (DR) and the 'intrinsic' or the mitochondrial pathway. Most HDACis can activate one or both of these pathways in various models (38 and references therein). For example, comparing the effects of SAHA and depsipeptide revealed that several genes involved in both cell survival and apoptosis, such as Apaf-1, myc, TGF-β, cyclin/cyclin-dependent kinases, TNF-α, Bcl-2, and caspases, are modulated by these HDACis [27]. Suberic bishydroxamate, in a capase-3-dependent manner, selectively induces apoptosis in melanoma cells and not in normal melanocytes and fibroblasts by the upregulation of Bim, Bax, and Bak, while downregulating the expression of anti-apoptotic X-linked inhibitor of apoptosis, Bcl-$_{xL}$, and Mcl-1 [38].

Another great advantage of using HDACis in cancer therapy is their high selectivity for cancerous tissue. On treatment with HDACi, leukemic cells show increased surface expression of DRs (TRAIL, DR5, FasL, and Fas) independent of p53. As a result, apoptosis was selectively induced in leukemic cells and not in normal hematopoietic progenitor cells [39], with TRAIL being the main mechanism [40]. In addition, HDACis can induce mitochondrial- and caspase-independent apoptosis, suggestive of their massive potential benefits for treating cancers with inherent defects in apoptosis [41].

TSA increases the transcription and surface expression of major histocompatibility complex (MHC)-I and MHC-II, and CD40 on tumor cells [42]. Decreased surface HLA-I expression, because of hypermethylation of specific DNA

regions, greatly inhibits tumor destruction by cytotoxic T-lymphocytes. Treatment of human melanoma cells with the demethylating agent, 5′-aza-2′-deoxycytidine, restores HLA-A and HLA-B gene transcription and reinstates surface HLA-I expression, making them susceptible to CTLs [43]. By increasing the surface expression of HLA-I in cancer cells, HDACi makes these cells attractive targets for natural killer (NK) cell-mediated killing through a NK group 2, member D (NKG2D)-dependent mechanism [44]. This finding was supported by the treatment of hepatocellular carcinoma cells sodium valproate (VPA), which increased surface MHC-I expression leading to increased NK cell cytotoxicity through an NKG2D-restricted mechanism. This effect was not seen in normal primary human hepatocytes, indicating tumor specificity and lower toxicity of VPA compared with conventional drugs [45].

Accumulation of ROS within the cell on treatment with HDACis has been reported [46–49]. Although the mechanism of ROS generation is not yet fully understood, it is believed that ROS activate the intrinsic apoptotic pathway [50].

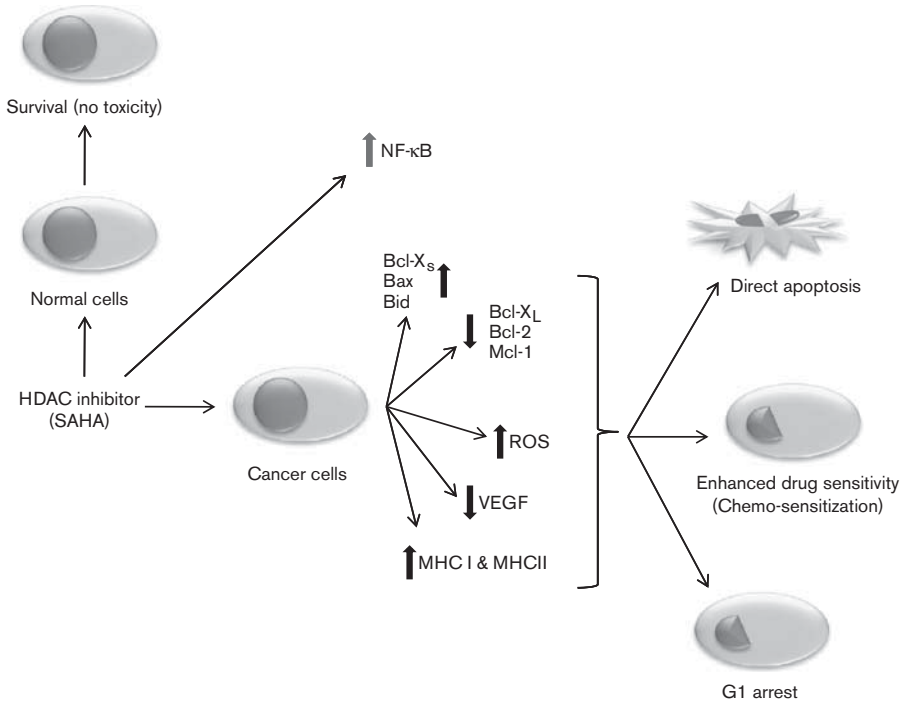
Autophagy is another cellular mechanism that has recently been emphasized in cancer treatment. It involves a series of signaling events, which ultimately degrade cellular components, usually large cytoplasmic materials and organelles that provide the cell with energy and molecular building blocks necessary for various biosynthetic pathways [51]. Autophagy is induced on starvation or when cells are situated in hypoxic conditions: two universal characteristics of the tumor microenvironment. Autophagy is a key player in resistance to certain apoptotic stimuli [52]. Moreover, HDACis not only induce apoptosis in malignant cells, but also augment autophagy. On the basis of these findings, it was hypothesized that simultaneous treatment of malignant cells with an HDACi and agents disrupting autophagy pathways could potentially enhance the apoptotic effects of HDACi. Evaluation of the effects of two well-established inhibitors of autophagy, namely 3-methyladenine and chloroquine, on the anticancer effects of an HDACi (SAHA) on imatinib (Gleevec)-resistant chronic myelogenous leukemia showed that both chloroquine and 3-methyladenine synergistically enhance the proapoptotic activities of SAHA on malignant cells while sparing the normal tissue [52].

HDACis also exert antiangiogenic activities. TSA and SAHA prevent vascular endothelial growth factor (VEGF)-stimulated human HUVEC cells from entering type I collagen gel and forming capillary-like structures [53]. Both SAHA and TSA inhibit the VEGF-induced formation of a CD31-positive capillary-like network in embryoid bodies and blocked VEGF-induced angiogenesis in the CAM assay. In addition, TSA prevents the development of capillaries from rat aortic rings. Another study showed that treatment with an HDACi lowers the expression of VEGF and hypoxia-inducible factor-1α [54] giving HDACis another cancer-apprehending property.

HADCis cause hyperacetylation of histones, thereby remodeling chromatin structure, which may override cell cycle checkpoint responses to DNA damage and enhance radiation-induced and/or chemotherapy-induced tumor cell death. Several recent reports have shown the superior efficacy of a combination of HDACis with various chemotherapeutic agents [14,34]. In addition, LBH589 (panobionostat) exhibited potent activity against AML cell lines and primary patient-derived AML cells and significantly enhanced the cytotoxic effects of doxorubicin through the mitochondrial apoptotic (upregulation of proapoptotic Bcl-2 members particularly Bad) pathway and induction of DNA double-strand breaks [55]. In addition, a combination of LBH589 plus dexamethasone and either bortezomib or lenalidomide significantly improved treatment efficacy in multiple myeloma cell lines, freshly isolated plasma cells and murine models of myeloma. In-vivo LHB589 provided a marked benefit in bone disease [56]. Zuco *et al.* [57] have recently reported that the novel HDACi RC307 enhances the sensitivity of ovarian carcinomas to synthetic atypical retinoid ST1926 as a result of enhanced DNA damage response measured by prolonged expression of DNA damage markers, such as H2AX, p53 and phosphorylation of RPA-2 and that marked phosphorylation of Chk1 (DNA damage checkpoint kinase). Another recent report showed that TSA upregulates the expression of Mcl-1 and Gadd45 genes (by activating the DNA damage checkpoint pathway) through ataxia telangiectasia mutated signaling pathway [58].

Ample evidence suggests that HADCis can enhance the antitumor effects of radiation in both in-vitro and in-vivo models. The novel HDACi, scriptaid, in combination with TSA enhances killing of the radioresistant SQ-20B human head and neck squamous carcinoma line, which was accompanied by prolonged retention of γ-H2AX foci suggesting inhibition of double-strand break repair [59]. In a xenograft model of colon cancer and in cell lines, SAHA and radiation delayed tumor growth [60] and induced apoptsis in prostate and glioma cancer cell lines [61]. MS-275 and VA reduced colonogenic cell survival of prostate carcinoma xenografts and human brain tumor cell lines accompanied by prolonged expression of γ-H2AX, an indication of double-strand break [62,63]. Similarly, SAHA radiosensitized melanoma cell lines by substantially reducing the survival fraction by blocking the DNA repair mechanism, a non-homologous end-joining pathway, and reducing the expression of DNA repair-related genes Ku70, Ku80, Rad50, and increased expression of γ-H2AX [64]. Both *in vitro* and *in vivo* SAHA perturbs cell cycle distribution, causes apoptosis, and radiosensitizes (evaluated by colonogenic assay) breast

Fig. 1



Postulated molecular mechanisms of action of suberoylanilide hydroxamic acid (SAHA). SAHA is a potent inhibitor of histone deacetylases (HDACs) that selectively causes growth arrest, and/or apoptosis in many types of tumors, while sparing normal cells. Treatment of cancer cells with SAHA leads to increased expression of certain silenced pro-apoptotic gene products (such as Bcl-$_{XS}$, Bax, Bad, and Bid), and inhibition of anti-apoptotic proteins (such as Bcl-$_{XL}$, Bcl-2, and Mcl-1). In addition, posttreatment cancer cells tend to exhibit augmented immunogenicity due to increased surface expression of both classes of the major histocompatibility complex (MHC) molecules. Angiogenesis is also inhibited in SAHA treated tumors due to repression of vascular endothelial growth factor (VEGF). The generation of reactive oxygen species (ROS) is another major event occurring upon SAHA treatment, which leads to apoptosis. SAHA also enhances the activity of the NF-κB survival/anti-apoptotic signaling pathway, which acts to lower the overall pro-apoptotic effects of the drug when administered alone. Some histone deacetylase inhibitor (HDACi)-treated cancer cells are also arrested in G1 phase. Altering the ratio of pro/anti-apoptotic gene products favors a pro-apoptotic milieu and the cells exhibit sensitivity to chemotherapy.

cancer brain metastasis (intracranial) xenograft models [65]. These studies support the notion that DNA damage is a primary event leading to the activation of apoptotic pathways, which increases the efficacy of chemotherapeutics and radiation in various tumor models.

DNA hypomethylation induces the reactivation of tumor suppressor genes that are silenced by methylation-mediated mechanisms prompting the investigators to evaluate the potential antitumor effects of HDACis and DNMT inhibitors. A combination of HDAC inhibitors such as SAHA and TSA with the DNMT inhibitors, 5-aza-cytidine (5-aza-CR) 5-aza-2′-deoxycytidine (5-aza-CdR), has enhanced the antiproliferative and apoptotic effects on human pancreatic and prostate cancer cells compared with each agent used alone [66,67] providing a possible molecularly targeted therapeutic strategy. Taken together, the above data suggest that the overall level of chromatin compaction is an important mechanism of drug sensitivity and radiosensitivity, and modifications of histone deacetylation and DNA methylation may increase treatment efficacy by altering chromatin compaction.

Suberoylonilide hydroxamic acid in cancer therapy

The orally administered drug SAHA is the only FDA-approved HDACi used for the treatment of CTCL [26]. The pivotal study leading to its granted FDA approval included 74 patients with stage IV and higher CTCL. Patients received SAHA orally at a dose of 400 mg/day; it was well tolerated and an overall 30% response rate was observed [68].

The effects of SAHA have been tested on different cell types including CTCL [68], pituitary adenomas [33], cervical cancer [69], oral squamous cell carcinoma [70], prostate cancer [71], mesenchymal stem cells (MSCs) [72], neuroblastoma [73], retinoblastoma [74], effector and regulatory T-cells [75], acute lymphatic leukemia [76], hepatoma [22], breast cancer [77], mantle cell lymphoma [78], and liver cancer [79]. Pituitary adenoma accounts for 15–20% of intracranial tumors. Although radiotherapy and surgery continue to serve as the main options, the treatment of this invasive tumor requires novel therapeutics. A recent study has indicated that

the treatment of the GH3 pituitary adenoma cells, with 500 nmol/l to 4 μmol/l SAHA, induces growth arrest and promotes cell cytotoxicity because of poly-ADP-ribose polymerase cleavage and caspase-3 activation. In addition, SAHA downregulates X-linked inhibitor of apoptosis, survivin, Bcl-2, and Bcl-$_{xL}$ with no changes in Bax [33]. Results of another study indicate a dose-dependent suppression of the in-vitro propagation of oral squamous cell carcinoma cell lines treated with SAHA because of cell cycle arrest at the G1 phase and reduction in the percentage of S-phase cells. In addition, hyperacetylation of p53 subsequent to treatment with SAHA (0.7–1.7 μmol/l) was observed [70,80]. A current study showed the potential of HDACis in treating retinoblastoma in transgenically rb/rb mice. The proapoptotic effects of SAHA, TSA, and MS-275 were analyzed by caspases-3/7 activity, Annexin V translocation, and Bim expression levels. All three HDACis promoted apoptosis in a dose-dependent manner and the mice showed reduced RB cell survival and decreased tumor burden [74] highlighting the potential of using HDACis in retinoblastoma. Despite having relatively low toxicity on normal cells, SAHA is suspected of having some adverse effects on the bone marrow and the pleuripotency of stem/progenitor cells. The BM microenvironment contains several types of stem cells, including MSCs. Experimentally, treating MSCs with SAHA induces cell cycle arrest and apoptosis [72]. Using human hepatoma HepG2 cells, SAHA (10 μmol/l)-induced apoptotic effects after a lag phase of 12–16 h and more than 80% of the SAHA-treated cells were in G0–G1 phase. While inducing apoptosis, SAHA also activates NF-κB [76]. The NF-κB pathway is often activated in cancer cells and contributes to tumor survival and proliferation. The results of one study suggest that simultaneous treatment of the cells with the proteasome inhibitor MG-132 and SAHA dramatically reduces enhanced NF-κB activity [77]. Another study further investigated the effects of a combination of SAHA and bortezomib, which showed synergistic apoptosis and higher levels of caspases-3, -8, and -9 along with cytoplasmic accumulation of IκBα resulting in lower NF-κB activity [78]. Moreover, SAHA induces the re-expression of DR5 in TRAIL-insensitive malignant cells and subsequently the cells become sensitive to TRAIL [78]. Postulated molecular mechanisms of action of SAHA are depicted in Fig. 1.

Aside from its antitumor benefits SAHA can downmodulate the immune system by upregulating the $CD4^+$ $CD25^+$ $Foxp3^+$ T-regulatory cells while having antiproliferative effects on CTLs resulting in dampening the immune response [75]. Thus, SAHA can be used as a means of immunosuppression to allow for increased chances of tolerance in graft transplantation. Altogether, numerous clinical and pre-clinical investigations have verified that SAHA is capable of inducing cell cycle arrest and triggering apoptosis.

Concluding remarks

HDACis are newly developed targeted drugs that have shown vast and selective anticancer activities. In addition, as significant antitumor activity can be established by these drugs at doses that are well tolerated by patients, they have an advantage over conventional chemotherapeutic agents. Although HDACi is one of the newest and most promising drugs in cancer therapy, it is not an ideal drug. For instance, HDACis cannot induce adequate apoptosis in non-small-cell lung cancer. Molecular analysis has shown that even though this drug successfully inhibits the deacetylase activity in non-small-cell lung cancer, it also activates the antiapoptotic NF-κB pathway in an Akt-dependent manner [81], suggesting the use of an Akt/PI3 K or NF-κB inhibitor in combination with HDACi for future anticancer therapeutic endeavors [82]. Future studies are warranted to optimize the clinical use of HDACi. It is believed that combining individual anticancer drugs with distinct intracellular targets, leads to better results in the treatment of different types of cancer as synergistic and targeted therapy are among the most powerful anticancer treatment options and HDACis represent a novel class of drugs that can be used in these settings.

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