Histone Deacetylase Inhibitor as a Novel Anticancer Agent: A Review

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Abstract: Histone Deacetylase (HDAC) inhibitors are an exciting new class of drugs that are targeted as anti-cancer agents. These compounds can induce growth arrest, apoptosis and/ or terminal differentiation in a variety of solid and hematological neoplasms in patients with advanced disease. Accumulation of acetylated histones in both normal and tumour cells can be used as a marker of biological activity. Hydroxamic acid based compounds are among the most promising HDAC inhibitors as potential anti-cancer drugs. There is still much to be understood about the family of HDACs, including the varying functions of different HDACs and the range of HDAC substrates. The development of selective HDAC inhibitors might be important in defining their biological role and potential as therapeutic agents. Clinically, the optimal dose, timing and duration of therapy, as well as the most appropriate agents to combine with HDAC inhibitors, are still to be defined.

Key words: Nucleosome · Histone · Acetylase · Deacetylase · HDAC inhibitors

INTRODUCTION

Nucleosomes the fundamental unit of chromatin structure, provides the first order and, at least in part, the higher-order packaging and compaction of the DNA about 10,000 fold. The nucleosome core particle consists of a highly conserved basic proteins, histone around which 146 bp of DNA are wrapped. Over the past decade, extensive genetic, biochemical and cytological studies have revealed that in addition to their structural role, the histones proteins are also involved in regulation of gene expression. As the maintenance of health depends on the coordinated and tightly regulated expression of genetic information, this becomes a very important function of histones [1]. Post-translational modifications of histone tails, such as acetylation, phosphorylation and methylation has emerged as common denominators in regulating several biological functions.

Acetylation is probably the best understood of these modification reactions. The enzymes involved in this process are Histone acetyl transferases (HATs) and Histone deacetylases (HDACs). Thus HAT and HDAC activity control the level of acetylation and in turn, can regulate the gene expression and its biological functions. Any alterations in the enzyme activity leads to aberrant

acetylation. Aberrant acetylation has been linked to cellular transformation and in development of cancer suggesting that both HATs and HDACs play an important role in carcinogenesis.

Histone deacetylase inhibitors are small molecules and restore the acetylation to normal level, induce cell cycle arrest, differentiation and apoptosis, suggesting their promising anticancer activity. Results of clinical trials with several of these agents have indicated that they are well tolerated at dose that have anti-tumor activity [2. Apart from this, HDAC inhibitors are also investigated in other diseases such as polyglutamine disease [3], Huntington disease [4] and have shown promising result.

Histone deacetylase (HDAC) inhibitors have been shown to be potent inducers of growth arrest, differentiation and/or apoptotic cell death of transformed cells *in vitro* and *in vivo*.

Histones are part of the core proteins of nucleosomes. Acetylation and deacetylation of these proteins play a role in the regulation of gene expression [5]. There are two classes of enzymes involved in determining the state of acetylation of histones, histone acetyl transferases (HATs) and histone deacetylases (HDACs). There are several reports [6-9] that altered HAT or HDAC activity is associated with cancers.

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A histone is a basic protein that can be found in the nucleus of a eukaryotic cell. This protein is found as a complex with DNA and it is specifically found in chromatin and chromosomes and may function as a repressor of gene transcription. Because histones are involved in transcription, one of the first steps in cell division and cancer is caused, generally by uncontrollable cell replication, they are prime targets for cancer research. A histone can be in one of two forms: acetylated, or deacetylated.

Histone acetyltransferase causes the acetylation of histones, while histone deacetylase reverses this process. Deacetylation, in this case, involves the removal, through hydrolysis, of an acetyl group from the ε-amino group of the histone's lysine side chains. This process restores a positive charge to the lysine side chains [12], keeping the structure of the histone intact. When HDAC is inhibited, the counter-enzyme HAT becomes in excess and hyperacetylation occurs. The charge on the lysine tails then becomes neutralized, disrupting the histone structure and allowing its DNA to unfold. The unfolded state of the histone then permits transcription factors to reach previously hidden genetic information and the gene expression of the histone is changed [10].

Acetylation-deacetylation: Acetylation of specific lysine residues in the amino termini of the core histones plays a fundamental role in transcriptional regulation. All core histone proteins are reversibly and dynamically acetylated at multiple sites in their N-terminal tails (Lysine 14, 19, 22 and 27 in H3; and 4, 13, 17 and 21 in H4)). Hyperacetylated histones are generally found in transcriptionally active genes and hypoacetylated histones in transcriptionally silent regions, such as heterochromatin. The level of histone acetylation at a particular locus in chromatin reflects the competing activities of histone acetyltransferases and histone deacetylases [11].

Histone Acetyl Transferases (HATs): Histone acetyl transferases catalyze the transfer of acetyl groups from acetyl co-enzyme-A (Acetyl Co A) onto histone acceptors (i.e. the e-amino groups of conserved lysine residues within the core histones).There are two types of the HATs: cytoplasmic B-type HATs likely catalyze acetylation events linked to the transport of newly synthesized histones from the cytoplasm to the nucleus for deposition onto newly replicated DNA. Conversely nuclear A-type HATs likely catalyze transcription-related acetylation events [14].

Histone Deacetylase Transferases (HDACs): Histone deacetylases are involved in the reversible acetylation of histone and nonhistone proteins (p53, tubulin and various transcription factor). Mammalian HDACs have been ordered in to three classes based upon their similarity to known yeast factors.

Class I HDACs: It includes HDACs 1, 2, & 13 and are more similar to the transcriptional repressor yRpd3p and share homology in their catalytic sites.

Class II HDACs: It includes HDACs 3, 4, 11, 12, 14 & 16 and are more similar to yHDA1p. HDACs 3, 4, 12 and 14 share homology in two regions: the C-terminal regulatory domain and N-terminal regulatory domain. HDACs 13 and 17 have two regions homology with the class II catalytic site.

Class III HDACs: The third class of HDACs is the conserved nicotinamide adenine dinucleotide dependent Sir2 family of deacetylases and include SIRT-1 to SIRT-7 deacetylases. This class shows the homology with the yeast transcriptional repressor by Sir2 and no homology to class I & II HDACs.

Class I and II HDACs are inhibited by trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA) and related compounds; class III HDACs are not inhibited by these compounds. Sel

- Class II HDAC 7 is a regulator of T-cell differentiation in the thymus
- Class II HDACs regulate skeletal muscle differentiation and neural development.
- Class I is critically involved in regulation of p53 [2].

Compounds that are shown to inhibit HDACs activity fall into six structurally diverse classes. Butyrate was the first HDAC inhibitor to be identified and the related compound phenylbutyrate has been successfully employed in experimental cancer therapy. However, butyrates are far less potent than other HDAC inhibitors and only at mili molar concentrations do they inhibit HDACs *in vivo* by a nonspecific noncompetitive mechanism that is not fully understood. Other HDAC inhibitors are more specific and are active at much lower concentrations. Trapoxin and depudecin irreversibly bind to and inactivate HDAC enzymes and Hydroxamic acids such as TSA and SAHA and other HDAC inhibitors reversibly inhibit HDAC enzymes [1].

Identification of HDAC Inhibitors: The HDAC inhibitors that are currently in clinical trials were not discovered based on their ability to inhibit HDAC activity, rather they were identified based on their ability to change the behavior of transformed cells in culture. For example, depsipeptide is a fermentation product isolated from *Chromobacterium violaceum* that was identified during a screening project for agents that reverse the malignant phenotype of H-ras-transformed NIH 3T3 cells [22-29].

Classification of HDAC Inhibitors: On the basis of mechanism of inhibition [1];
- Reversible inhibitors e.g. TSA, SAHA
- Irreversible inhibitors e.g. Trapoxin, Depudecin

On the basis of chemical structure [22];
- Hydroxamates e.g. TSA, SAHA, Oxamflatin, LAQ-824
- Cyclic tetrapeptide e.g. Depsipeptide, Apicidine
- Aliphatic acid e.g. Valproic acid
- Benzamide e.g. MS- 27-275
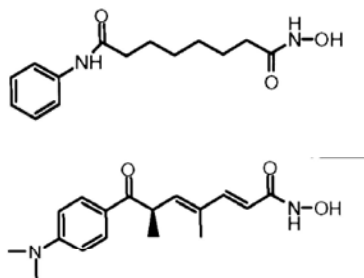- Electrophilic ketones e.g. Trifluromethyl ketone
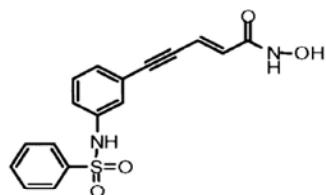- Miscellaneous e.g.Depudecin.

Butyric Acid
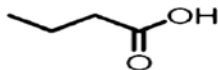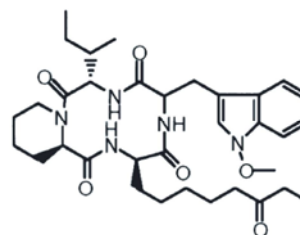
MS-27-275
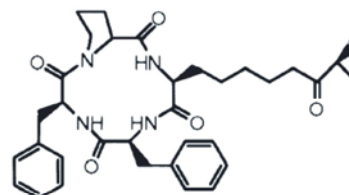**SUBEROYLANILIDE HYDROXAMIC ACID (SAHA)**
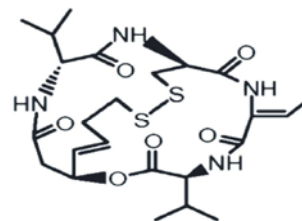**Trichostatin A**

**Oxamflamtin**

Apicidin

Depsipeptide

Trapoxin

The essential characteristics of hydroxamic acid-based HPCs are a polar site, the hydroxamic group, a six-carbon hydrophobic methylene spacer, a second polar site and a terminal hydrophobic group. Substitution of the hydroxamic acid with a carboxylic acid or amide oxime group results in inactive compounds. Modification of the hydroxamic acid, such as introduction of a methyl group on an adjacent carbon or *N*-methylation, results in inactive compounds. The benzene ring in the hydrophobic moiety can be modified in the meta and para positions without loss of activity; however, in general, larger substituents are associated with loss of activity. The optimal methylene spacer is six methylenes, five- and seven-carbons spacers being less active.
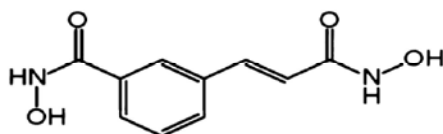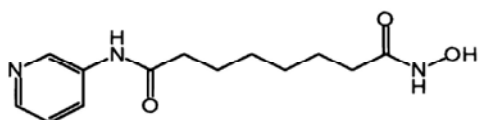
SBHA
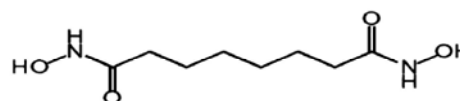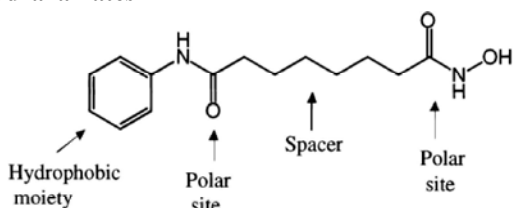
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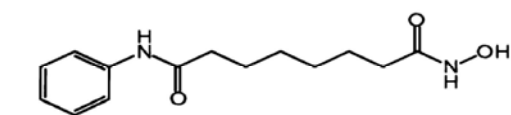
CBHA

Pyroxamide

Hydraxamates

HDAC inhibitors can induce growth arrest, differentiation and/or apoptotic cell death in a wide variety of cultured transformed cells, including neuroblastoma, melanoma and leukemia cells, as well as cells from breast, prostate, lung, ovary and colon cancers [30-38].

- Short chain fatty acids (eg.sodium butyrate, phenylbutyrate; valproic acids;)
- Hydrosyaminic acids (SAHA, pyrocamide, TSA, oxamflatin and CHAPAs);
- Synthesis benzamides derivatives (eg.MS-275 AND CL 994)
- Cyclic tetrapeptides (such as depsipeptide, trapoxin, apicidin.) [39-40].

Short Chain Fatty Acids: Compounds of this class, particularly sodium butyrate have been the subject of interest. Sodium butyrate is a non toxic short chain fatty acid found naturally in the gastrointestinal tract and appears to be responsible for the protective effects associated with high fiber diets [41]. It is well known that butyrate, at concentrations similar to those encountered within the colonic lumen causes growth inhibition differentiation and apoptosis in a variety of colon-cancer cell lines [42]. Moreover in a rat model of colon

carcinogenesis interracial administration of butyrate was effective in reducing the incidence of cancers.In human leukemia cells, butyrate is also a parent inducer of growth arrest and differentiation [43]. However, the short sodium butyrate half life and difficulties in achieving mili molar plasma concentrations mimicking those shown to be active *in vitro* studies have limited its use. To overcome these difficulties, other derivatives have been investigated. For example, arrest and differentiation in primary leukemia cell *in vitro* [44, 45].

Recently, a well - tolerated antiepileptic drug, valproic acid, has also been shown to posses HDAC inhibitor activity *in vitro* and to induce differentiation of carcinoma cells, transformed hematopoitic progenitor cells and leukemia blasts from acute myeloid leukemia patients. In addition, it was also effective in reducing tumor growth and metastasis formation in animal studies [46].

Hydroxyamic Acid Derivatives: SAHA (suberoylanidile hydroxamic acid) is a second -generation polar planar compounds that induces growth arrest, differentiation and / of apoptosis that is currently undergoing Phase I clinical evaluation in both hematologic and non hematologic malignancies [47-49]. SAHA is approximately 1000-fold more potent in a molar basis than HMBA (hexamethyl enebisacetamide), a first generation hybrid polar compound,inducing maturation in murine erythroleukemia cells (MEL) [50]. In malignant human hematopoietic cells, SAHA related maturation was relatively limited, although accompanied by marked cytoxicity. In studies involving human breast cancer cells SAHA inhibited clonogenic growth and ultimately induced apoptosis [51].

Recently, a trapoxin analog has been synthesized in which the epoxyketone group was replaced by a hydroxamic acid. The hybrid compound, designated cyclic hydroxamic acid -containing peptide 1(CHAP1), is a reversible inhibitor of HDACs at low nanomolar concentrations [52]. A series of CHAP derivatives have been obtained and assayed for structure activity. Among them, CHAP31 displayed several promising characteristic including increased stability relative to established HDAC inhibitors like TSA and impressive antitumor activity in nude mice bearing various tumor cell types [53]. Another novel hybrid polar compounds that has recently been described is pyroxamide.At micromolar concentrations, pyroxamide induced terminal differentiation in murine erythroleukemia (MEL) cells and caused growth inhibition by cell cycle arrest and/or apoptosis in MEL, prostate carcinoma bladder carcinoma and neruoblastoma cells [54].

Synthetic Benzamide Derivatives: This class of compound consists of a structurally diverse group of agents that contain a benzamide moiety. This group is postulated to enter the catalytic site and bind the active zinc [39]. Two compounds have been described as members of this group, MS-275and CI-994. MS-275 is a novel agent with HDAC-inhibitory activity that is structurally dissimilar from other HDAC-inhibitors [55, 56]. As in the case of other compounds of this class, MS-275-associated HDAC-inhibitory activity is accompanied by an increase in expression of the CDKI p21$^{CIPI/WAFI}$ and accumulation of cell in GI-phase [56]. MS-275 displays antiproliferative activity toward several human cancer cell lines including breast, colorectal, leukemia, lung, ovary and pancreas [56].The second compound with a benzamide structure is CI-994. CI-994 is an investigation anticancer drug with a board spectrum of activity in marine and human tumor xenografts, although it's specific mechanism of action remains unknown [57].

Cyclic Tetrapeptides: Depsipeptides (FK228, FK901228) is a novel HDAC inhibitor isolated from chromobacterium violaceum that possesses potent antitumor activity against human cancer cell lines and inhibits the growth of tumor generated in mice [58]. In human leukemia cells (U937), depsipeptide was a strong inhibitor of cell growth with IC50 at nanomolar concentrations [59] and proved very active in inducing apoptosis in cells from patients with chronic lymphocitic leukemia [60].

Apicidin is another novel cyclic tetrapepide compound whose structure is related to that of trapoxin. It has a potent broad spectrum of antiprotozoal activity against aplcomplexan parasites, which appear to involve HDAC inhibitor [61]. Apicidin displayed marked antiproliferative effects in a wide variety of human cancer cell lines including breast osteosarcoma, stomach and v-ras-transformed NIH3T3 cells [62].

These are the agent or drugs which prevents excessive growth of tumor cells. Next to heart disease, cancer is the major killed of mankind. The majority of antineoplastic drugs appear to act by affecting either enzyme of substrates acted upon by enzyme system [62].

They may be classed into six major groups [63]:
- Aklylating agents;
- Antimetabolites;
- Natural products;
- Hormones and antagonists;
- Radioactive isotopes;
- Misscellaneous agents.

Histone deacetylase (HDAC) inhiibitors are currently being tested in clinical trials as anti cancer agents [2] HDAC inhibitors represent a new approach for anticancer drugs and are an exciting prospect for the tretmeant of cancer [79].

The link between altered HDAC activity and tumorigenesis is probably best demonstrated in acute promyelotic leukemia (APL) the retinoic acid receptors (RAR) transcription factors RAR and its heterodimerization partner RXR bind to retinoic acid response element(RARES) and in the absence of retinoid, repress transcription through a complex involving sin3/HDAC, NCOR and SMRT. Addition of retinoic acid enables HATs (such as TIF2 and CBP) to replace the HDACs, thereby activating transcription. This step is important for myeloid cell development [79, 80].

Histone deacetylase inhibitors are emerging as an exciting new class of potential anticancer agents for the treatment of solid and malignancies. Several HDAC inhibitors have shown impressive antitumor activity *in vivo* with remarkably littly toxicity in preclinical studies and are currently in phase1 clinical trial [39].

It has been show that the level of histone acetylation directly correlates to a wide variety of biological activity. Specifically, inhibition of HADC can causes over expression of variety of genes [64]. HDACs are typically over expressed in tumor cell, thus inhibition of HDACs can be a selective mean for inducing differentiation of tumor cells, converting them form a malignant to abnormal phenotype. This makes inhibition of HDAC a promising approach for the treatmeant of various cancers [80].

Phase I and Phase II clinical trials with HDAC inhibitors either as monotherapy or in combination with cytotoxic and differentiation agents are ongoing.

HDAC Inhibitors Currently in Clinical Trials [64]:

Molecule Clinical trial status	SAHA Phase II
PXD101	Phase I
LAQ-824	Phase I
CI-994	Phase I
Valproic acid	Phase I
MS-275	Phase II
Butyrate	Phase I/II
Depsipeptide	Phase II
Pyroxamide	Phase I

Suberoylanilide Hydroxamic ACID: Suberoylanilide hydroxamic acid (SAHA) is a hydroxamate-containing small-molecule HDAC inhibitor that directly interacts with the hydrophobic catalytic site of HDACs [22]. SAHA has reached Phase II clinical trials for the treatment of both

solid tumors and hematological malignancy. *in vitro* and *in vivo* studies demonstrated the potential for synergy using combinations of HDAC inhibitors with several mechanistically different antitumor agents.

In breast cancer cells, SAHA induces growth inhibition, cell cycle arrest and apoptosis by regulating genes such as p21, p27 Rb and gelsolin, contrasting with the growth arrest seen in prostate cells *in vitro* and *in vivo* [64].

PXD101: PXD101 is a highly potent HDAC inhibitor that blocks proliferation of diverse tumor cell lines at low macromolecular potency (IC 50 0.08-2.43ìM) and HDAC enzyme activity (IC50 9-110 nM). In xenograft models, PXD101 slows tumor growth in a dose-dependent manner and is particular active in leukemic mouse models. As with other HDAC inhibitors PXD101 causes cell cycle arrest and apoptosis in rapidly proliferating cells and could have widespread applications in diseases other than cancer that are marked by aberrant proliferation [63].

LAQ-824: The clinical candidate LAQ-824 is a hydroxamate-based HDAC inhibitor. *in vitro*, LAQ-824 inhibits HDAC enzyme activity (IC 10 nM), inhibits tumor cell growth at submicromolar csssoncentrations and induces apoptosis. *In vivo*, the molecule possesses antitumor activity in several xenograft models, including breast (MDA-MA-435), colon (HCT116) and lung (A549). LAQ- 824 is in phase l trial for solid tumors [63].

Depsipeptide (FR-901228): This natural HDAC inhibitor is currently progressing through Phase II clinical trials for coetaneous T cell lymphoma. It is a natural product purified from *Chromobacterium violceum* that undergoes intracellular reduction to generate an active HDAC inhibitor. Initial toxicity (cardiac and inflammatory responses) has been overcome by using intermittent dosing schedules as opposed to daily dosing, allowing higher drug administration with reduced side effects. As with other HDAC inhibitors, this cycle peptide is a pro-apoptotic, anti-proliferative and ant-angiogenic. Despite bearing an anti-tumor activity and results from clinical trials against T cell lymphoma have demonstrated encouraging activity [63].

HDAC Inhibitors and Conventional Cytotoxic Drugs: The use of HDAC inhibitors in combination with established cytotoxic agent has shown potentially promising results in human colon cancer cells. The treatment of advanced colorectal cancer has resisted most therapeutic efforts and continues to rely heavily on the

use of fluoropyrimidines such as 5-fluorouracil-(5-FU) [64, 65]. Studies performed with several colon cancer cell lines showed that exposures to phenyl butyrate markedly reduced the recovery of 5-FU-pertreated cells [66, 67].

HDAC Inhibitor and Differentiation-inducing Agents [Retinoic Acid (RA); Phorbol Myristate Acetate (PMA)]: The concept of combining HDAC inhibitors with retinoids such as all transretinoic acid (ATRA) is based on dual consideration:both groups of compounds are potent differentiation inducing agents; and retinoids exert their effects via a nuclear receptor complex that interacts with the promoters of RA-responsive genes.

Furthermore, an HDAC subunit is an integral part of this corepressor complex, which is involved in transcriptional silencing in the absence of the ligand [68]. The use of RA as an inducer of growth arrest and/or differentiation in neuroblastoma has been extensively studied and has potentially important clinical implications [69]. Recent studies indicate that RA-induced decrease in neuroblastoma cell number could be significantly enhanced by coexposure to the HDAC inhibitor CBHA [48]. In general, relatively low doses of both drug achieved considerably more dramatic results than either agent alone. The effects of the drugs combination involved induction of apoptosis and G1 growth arrest. The combination CHBA-RA was also effective *in vivo*, in suppressing the growth of tumor xenotgrafts in a dose dependent manner [70].

Other HDAC inhibitors tested in combination with RAs include phenylbutyrate, sodium butyrate, TSA and depsipeptide.

HDAC Inhibitors and Demethylating Agents: Reexpression Strategies: A growing number of tumor-suppressor and other cancer-related genes have been shown to be silenced by aberrant methylation of CpG islands in their respective promoter region [71, 72]. Numerous studies have shown that several pyrimidine nucleoside analogs including 5-aza-2'-deoxycytidine and 5-azacytidine, by inhibiting DNA methayltransferases (DMTs), can induce DNA demethylation and thereby reverse the silencing of tumor suppressor genes [73, 74]. Both depsipeptide and TSA have also been shown to synergistically enhance 5-aza-dC apoptotic effects in human long cancer cells [75].

HDAC Inhibitors and Cell Cyclic Modulators (Flavopiridol): As noted previously, HDAC-inhibitors induce the expression of the CDK inhibitor p21 [WAFI/CIPI],

Which leads in turn to growth arrest in the G1 phase of the cell cycle and ultimately cellular differentiation [44, 76]. Works from various laboratories have focused on the factors that determine whether HDAC-inhibitors such as SB and SAHA induce apoptosis versus differentiation in leukemia cells with a particular emphasis on p21 [WAFI/CIPI] [43, 50, 75]. These studies demonstrated a functional role for p21 WAFI/CIPI in promoting differentiation and preventing apoptosis induced by sodium butyrate in human leukemic cells [43]. Similar result was observed in cells exposed to SAHA [50].

HADC Inhibitors and Other Signal Transduction Cell Survival Modulators: Two novel classes of compound have recently been examined in combination with HDAC inhibitors:

- Activation of the extrinsic, receptor mediated apoptotic pathway including TRAIL and TNFα; and
- Signal transduction modulators, such as STI571.

Recent studies have characterized interactions between HDAC inhibitors and TRAIL (Tumor necrosis factor -related apoptosis including ligand) in human colon cancer cell lines [76, 77]. TRAIL unlike FAS-L, appears to induce apoptosis in tumor cell preferentially versus normal cells [78]. Sodium butyrate sensitized colon cancer cells to TRAIL by decreasing FLIP (Flice-like inhibitory protein) protein expression while significantly increasing cell death in all cell lines analyzed [76, 77]. Similar effects were observed with other TNF family members, such as TNFα and anti-FAs antibody and the HDAC inhibitor TSA [77].

HDAC inhibitors are an exciting new class of drugs that are targeted as anti-cancer agents. These compounds can induce growth arrest, apoptosis and/ or terminal differentiation in a variety of solid and hematological neoplasms in patients with advanced disease. Accumulation of acetylated histones in both normal and tumor cells can be used as a marker of biological activity. Hydroxamic acid based compounds are among the most promising HDAC inhibitors as potential anti-cancer drugs. The members of different classes of HDACs are shown to involve in a particular function or in a specific tissue. Thus, the development of the inhibitors for a particular HDAC will help in treatment of the condition involving these functions or the tissue. Currently many efforts are being made to expand our knowledge of the HDACs and to develop potent and stable HDAC inhibitors [17].

CONCLUSIONS

HDAC inhibitors are an exciting new class of drugs that are targeted as anti-cancer agents. These compounds can induce growth arrest, apoptosis and/ or terminal differentiation in a variety of solid and hematological neoplasms in patients with advanced disease. Accumulation of acetylated histones in both normal and tumour cells can be used as a marker of biological activity. Hydroxamic acid based compounds are among the most promising HDAC inhibitors as potential anti-cancer drugs. There is still much to be understood about the family of HDACs, including the varying functions of different HDACs and the range of HDAC substrates. The development of selective HDAC inhibitors might be important in defining their biological role and potential as therapeutic agents. Clinically, the optimal dose, timing and duration of therapy, as well as the most appropriate agents to combine with HDAC inhibitors, are still to be defined. The search for more potent HDAC inhibitors will continue.

The members of different classes of HDACs are shown to involve in a particular function or in a specific tissue. Thus, the development of the inhibitors for a particular HDAC will help in treatment of the condition involving these functions or the tissue. Currently many efforts are being made to expand our knowledge of the HDACs and to develop potent and stable HDAC inhibitors. In the future, this might give rise to the tailored use of HDAC-specific inhibitors in order to dissect the complex functions of HDACs in a cell-type specific manner.

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