HDAC inhibitors: applications in oncology and beyond

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Abstract
Inhibitors of the histone deacetylases (HDACIs), a class of small molecules primarily involved in the modification of epigenetic regulation, have been exploited widely for their anti-cancer effects and/or chemo/ radio-sensitization properties. The mechanisms of action of several HDACIs have been unraveled that include modifications of histone acetylation status influencing gene expression and/or changes in the post-translational modifications of proteins that collectively result in alterations in vital cell functions like proliferation, differentiation, death (apoptosis) and senescence manifesting in differential responses between pathological (cancer and others) and normal tissues (and cells). Reason for HDACI becoming the choice for anticancer therapeutics is their excellent differential action on normal and cancer cells at the therapeutic dosages. Some HDACIs like Zolinza (vorinostat or SAHA) and depsipeptide (Romidepsin or FK228) have been granted FDA approval for the treatment of various cancer types and many are at pre-clinical and clinical trial stages. Some of the non-oncologic effects have however unfolded interesting properties of HDACIs like cyto and neuroprotection, anti-inflammatory and radioprotective actions and indicated the possible applications of HDACIs in the treatment of nononcologic diseases like asthma, arthritis and other autoimmune disorders, neurodegeneration, diabetes, muscular dystrophy, bone marrow transplantation etc. We briefly review the current status of the applications of HDAC inhibitors in oncology and non-oncological diseases and the possibilities of developing them as radiation countermeasure agents, especially as mitigators of radiation injury.

Keywords: Epigenetic, HDAC inhibitor, radiosensitizer, radiation countermeasure

Introduction
The focus on expression and regulation of the genetic information by the epigenetic modulators has been the central theme of biomedical sciences today. The phenotype of different cell types remains different and different cell types express different genes despite the genetic information coded as DNA base sequences in all cells of a multi-cellular organism is identical. Cell type specific gene expression patterns are mostly determined at an early stage of embryonic development by several mechanisms like modification of nucleotide bases, local chromatin organization, modifications in transcription factors and other regulatory proteins, etc. collectively known as epigenetic landscape. Histones, a family of key chromosomal proteins, not only organize DNA in the chromosomes but also play an important role in the regulation of gene expression. Post-translational modifications of key amino acid residues of the histones facilitate processes including transcription, replication and repair [1]. Those modifications include methylation, acetylation, phosphorylation, ubiquitination and sumoylation in the N-terminal tail of the histone. Histone acetylation which is often transient and reversible in nature has been relatively well studied [2]. The acetylation status of histones, and some non-histone proteins, is a tightly regulated process maintained by the opposing actions of two enzymes, histone acetyl-transferases (HATs) and histone deacetylases (HDACs) [3]. The former can mediate the transfer of acetyl group from acetyl Co A to ε-amino tail of lysine residue in histone, thereby neutralizing the positive charge of histone and hence weakening the interaction between histone and DNA. The loosening of tightly wrapped DNA around the histone leads to a more open conformation and provide accessibility to many factors that help in the downstream processes like transcription, translation, replication, repair etc. [3-7].

The main focus of this review is to strengthen our hypothesis towards possible radiation countermeasure, especially mitigation of radiation injury, application of HDAC inhibitors based on available literature for other known applications of HDAC inhibitors. Therefore the review is organized in several sections starting with a brief description of HDAC inhibitors, their already widely exploited anticancer and radiosensitization properties and then the less explored applications of HDACIs in non-oncological conditions. The possible radiation
countermeasure applications will be more based on hypothesis and we try to derive support for the same in the light of observed differences in the actions of HDACIs in cancerous and non-cancerous cells.

As many as 18 HDACs has been found till date in humans and these are classified in 4 classes of two different protein families- Classical Zinc dependent family and (nicotinamide adenine dinucleotide) NAD+ dependent family. Class I, II and IV HDACs form the Zinc dependent metalloproteins while class III (sirtuins) is NAD+ dependent. Class I HDAC which is mostly nuclear includes HDAC 1, 2, 3 and 8. These are smaller in size (350-500 amino acids) closely related to yeast transcription factor (Reduced Potassium Dependency) RPD3. Class I HDACs are thought to have critical role in cell survival and proliferation and are expressed in most cells. HDAC 3 also localize in cytoplasm [8]. Class II has two subgroups; Class IIA is represented by HDAC 4, 5, 7 and 9 while class IIB consists of HDAC 6 and 10. They are related to yeast (histone deacetylase A) HDA1, shuttle in and out of the nucleus and have more tissue specific function [9-15]. HDAC 6 is a major cytoplasmic HDAC with key substrate α-tubulin and is important target for protection and regeneration following central nervous system injury [16-17]. The NAD dependent group Class III HDACs consists of seven sirtuins, SIRT [1-7]. Finally HDAC11 is in its own class IV.

A number of small molecule modifiers (activators and inhibitors) of HDAC function have been identified so far. The chemical structure of inhibitors of HDACs (HDACI) consists of three parts- a zinc binding group (ZBG), a hydrophobic group (CAP) for protein recognition and interaction and a linker connecting both of them [18]. HDACI fall into the following five distinct classes based on their structure, origin and chemical properties [8,19]:

1. Short Chain Fatty acid (viz. Valproic acid, Sodium butyrate, Phenylbutyrate etc.)-inhibit HDAC at mM dose range in vitro.
2. Hydroxamic acid (viz. Trichostatin A or TSA, Vorinostat or SAHA, Panobinostat, Droxinostat)-inhibit HDAC activity at nano molar concentration in vitro. It is the largest group of HDACI.
5. Cyclic tetrapeptides (viz. Apicidin, Depsipeptide)-with effective concentration in the nanomolar range in vitro.

Applications of HDACI in cancer biology and oncology

A disruption or alteration of balance between histone acetylation and deacetylation leads to deleterious effect like tumorigenesis in cells, with the over expression of HDAC(s) as the main cause. Many studies have shown a major role of HDAC function in proliferation, apoptosis, migration and differentiation [20-23] with inhibition of individual HDAC resulting in the inhibition of tumor cell growth [24-25]. Such studies led to the development of HDACI as anticancer therapeutics. About 2-20% of genes in malignant cell lines have been shown to be altered by HDACI [4,6,26-27]. Of the several HDACIs developed, many have entered phase I, II and III clinical trials recently. Vorinostat (SAHA) and Romidepsin (depsipeptide or FK228) have got FDA approval for treatment of cutaneous T-cell lymphoma and peripheral T-cell lymphomas [19] in 2006 and 2009 respectively. Besides their potential use in cancer chemotherapy, several HDAC inhibitors have also been shown to synergize with chemo and radiotherapy in enhancing the local tumor control.

HDACIs increase the acetylation status of lysine residues of histones thereby neutralizing their positive charge and hence releasing the negatively charged DNA wrapped around it. This action of HDACI increases the accessibility of DNA damaging agents and (reactive oxygen species) ROS to the DNA and enhances potential of causing (and the levels) DNA damage. Several in vitro and in vivo preclinical studies have shown increased cancer cell killing by radiation when the cells or animals were administered HDACI before irradiation. In other words HDACI can cause radio-sensitization of tumor or cancer cells, coupled with minimal toxicity to the normal tissues and cells, an observation that makes HDACI as an attractive radio-sensitizer in the treatment of cancer. Accumulation of ROS has been believed to be an important event in HDACI induced DNA damage in cancerous cells leading to cell death [28-32]. Treatment of leukemia cells with Vorinostat (SAHA) has been shown to enhance ROS generation and to down regulate the expression of Mn-SOD (superoxide dismutase) [33], an antioxidant enzyme and thus enhancing oxidative damage to cells.

Cellular responses to DNA damage comprising DNA repair, checkpoint activation, apoptosis and senescence are regulated by several proteins are partly mediated by posttranslational modifications (PTMs) like phosphorylation, acetylation, ubiquitination, ribosylation etc. of proteins. Although UV irradiation of human cells results in a well regulated temporal pattern of acetylation of nuclear histones, ionizing radiation and other environmental DNA damaging agents do not acetylate all possible residues of H3 or H4 molecules but result in atypical acetylation pattern, which is not completely well understood [34]. There is a great deal of interdependency of different PTMs in the regulation of damage response pathways, particularly the DNA repair [35]. Since acetylation of proteins influences both transcriptionally and post-translationally controlled damage response pathways involving modifications of levels and compartmentalization as well as activities of histones and other proteins, modifications of protein acetylation status by HDACI are expected to significantly influence the cellular responses to damage caused by a variety of genotoxic and non-genotoxic agents.

In mammalian cells two major DNA repair pathways,
Many studies have shown an association between HDACs and proteins involved in signaling cascade in response to DNA damage [39-44]. Following the phosphorylation of H2AX in response to DNA damage, it is acetylated at K5 and ubiquitinated at K119, which is essential for the release of this histone from the chromatin and thus leading to chromatin remodeling essential for the sustenance of DNA repair [45]. Transcription of many genes involved in the DNA double strand break repair pathways is regulated by HDACI. For example, SAHA decreases the concentration of DNA repair proteins Rad51 and DNA-PKCs [46], sodium butyrate decreases the expression of the DNA repair protein Ku70, Ku86 and DNA-PKCs in melanoma cell lines [46]. Treatment with HDACI arrests cell cycle at G1/S and G2/M checkpoints [47-51]. It has been found that HDACI down regulate Cyclin D and A to arrest cell cycle in G1 [52], and Cyclin B1 to arrest cell cycle in G2/M phase [53]. One of the major targets of HDACIs is p21 that imparts cancer cell cycle arrest at G1 phase [54]. While most of the HDACIs enhance p21 expression in a p53 independence pathway, depsipeptide (FK228) increases p21 expression by activating the p53 pathways. HDACI negatively control angiogenesis, metastasis, and invasion by down regulating the expression of (hypoxia inducible factor) HIF-1α [55-57], matrix metallo-proteinases MMP-2 and MMP-9 [58-59], chemokine (C-X-C motif) receptor 4 (CXCR4) [57], endothelial nitric oxide synthase (e-NOS) [60], up regulate the expression of angiostatic protein ADAMTS1 [61].

There are two major pathways of apoptosis in mammalian cells; extrinsic or death-receptor pathway and intrinsic or mitochondrial pathway. HDACI has been reported to activate both intrinsic and extrinsic pathways of apoptosis in cancer cells [62-66]. SAHA and depsipeptide have been reported to up regulate pro-apoptotic genes like bax, apaf, caspase-3 and caspase-6. Both the HDACI also altered the expression of (tumor necrosis factor) TNF superfamly related genes as well as genes of death receptor signaling pathway [67]. HDACI have been shown to increase the expression of (TNF related apoptosis-inducing ligand) TRAIL, TRAIL-R2 (DR5), Fas, (Fas ligand) Fas-L, and TNF-α [68-70].

Of the several mechanisms proposed for chemotherapeutic and radio-sensitizing properties of HDACI, increased DNA damage, reduced DNA repair, increased cell death, cell cycle perturbations, alterations in signal transduction pathways are the prominent ones. Since cancer cells have higher levels and dependency on HDAC linked to their evolutionary process, HDACI are expected to have greater influence on the tumor responses to radiation and chemotherapeutic agents as opposed to normal cells. Furthermore, since the time course of molecular responses following the induction of DNA damage critically influence the outcome, presence of HDACI during damage causation has the maximum potential to influence all damage response pathways and hence differentially sensitize tumors to primary therapeutic agents like radiation and anti-cancer drugs. Potential mechanisms of radiosensitizing properties of HDACI are been shown in the schematic diagram (Figure 1a and b).

**Applications of HDACI in nononcologic diseases**

Besides their use as anticancer therapeutics and/or as chemo and radiosentizers of cancer cells or tumors, HDACIs have also been exploited for their beneficial effects under several other pathophysiological conditions like neurodegenerative disorders and immune disorders and inflammation related pathologies where the mechanisms of action of the HDACIs appear to be unrelated to their cytotoxic or proapoptotic properties observed in tumors. Here their effects may be mediated through the modulation of various signal transduction pathways linked to modifications in the acetylation status of non-histone proteins, besides epigenetic alterations (histone acetylation) in relevant cells/ tissue in a
context dependent manner [71]. HDACi therapy to modulate T-cell associated inflammatory and immunologic disorders appears highly promising. HDACIs have been shown to enhance Treg (T regulatory cell) suppressive function and promote their development both in vivo and in vitro models [71]. HDACi have also been shown to attenuate airway hyper-responsiveness (AHR) and inflammation in animal models [72-75]. Sodium butyrate suppresses the expression of upstream signaling events for Caspase-11 regulating both inflammation and apoptosis at both m-RNA and protein level in mouse embryonic fibroblasts [76]. TSA has been shown to reverse the chronic renal injury by augmenting expression of (bone morphogenic protein) BMP-7 in Kidney side population cells of C57Bl/6 mouse. TSA prevents the progression of proteinuria, glomerulosclerosis, and interstitial fibrosis [77] and also down regulate MyoR expression [78]. Many HDACi including Trichostatin and Valproic acid prevent cardiac hypertrophy [78-81] in animal models.

There is considerable amount of evidence to suggest that disturbances in the epigenetic regulation is associated with neurodegenerative diseases like Rubinstein-Taybi Syndrome mental retardation, Huntington’s disease, Parkinsons disease, Alzheimer’s disease and ischemic brain injuries etc [82] in various preclinical systems. The role of epigenetic dysregulation pertaining to the CBP/CREB mediated gene regulation and its amelioration by HDAC inhibitor treatment has been extensively dissected out in the case of Huntington’s disease [82]. HDACi have shown beneficial effect in many neurodegenerative and neuropsychiatric diseases including Stroke, Huntington’s disease, Parkinson’s disease, Spinal cord and traumatic brain injury, Cerebellar degeneration, Muscular atrophy, Mood disorder and Alzheimer’s disease [83-87]. Their protective effect in middle cerebral artery occlusion (MCAO) has been recently demonstrated in animal models of stroke [88]. In ischemic brain, HDACi treatment has been found to over-express (heat-shock protein) HSP 70 and p53 that contributes to its anti-inflammatory action [88]. TSA can be used for the treatment of MCAO to restore long-term memory loss [89] where it is proposed that CBP/CREB mediated transcriptional activation plays a major role.

**Application of HDACi in radiation counter measures**

The term radiation countermeasures encompasses approaches that leads to effective medical management of radiation damage and includes assessment of absorbed dose and damage, diagnosis, and interventional strategies that reduce both acute and late harmful effects of radiation. Radiation countermeasure agents can be classified as: 1) protectors- agents that require their administration before the actual exposure for their action; 2) therapeutics- agents administered to counter the path-physiological consequences of radiation exposure based on the detectable or visible signs and symptoms after radiation exposure and 3) mitigators- agents that can counter the consequences of radiation exposure when administered during or within a short period after exposure and before the appearance of detectable symptoms of radiation exposure. Figure 2 depicts different sub classes of countermeasure agents with currently available agents, their time course of action and areas of application [90]. The aminothiol, WR-2721 or Ethyol, though to be used under stringent conditions of medical supervision, is the only clinically approved protector for external exposure other than potassium iodide available for internal decontamination purpose. There are guidelines for supportive care and therapeutic measures available for the management of subjects exposed to radiation which are mainly in the form of blood cell transfusion, cytokine and growth factor therapy etc. However, the field of mitigation of radiation injury is relatively unexplored and no definitive agent or modality is available as of now. Therefore the search of efficient and safe mitigators of radiation damage is an area of intense scientific interest.

Though HDAC inhibitors possess intrinsic anticancer properties they act differentially on normal and cancerous cells. HDAC induce cancer cell death at concentrations which are either non toxic to normal cells or induce pro-survival responses in normal cells. For example the anticancer doses of SAHA (Vorinostat) and MS-275 increase the (thioredoxin) Trx protein level to protect normal cells and increase the concentration of ROS and activation of caspase in transformed cells but not in normal cells [30]. HDACi has ability to selectively induce apoptosis in malignant cells while sparing the normal tissue [91-92]. Moreover, HDACi induce DNA double strand breaks that get repaired in normal cells but not in transformed cells. This has been indicated by the decreased level of γ-H2AX in normal cells
A and valproic acid have been reported to protect mice 2-benzanthracene) induced oral carcinogenesis in hamsters. Sodium Butyrate (NaB) significantly reduce the expression of the repair related genes Ku 70 and Ku 86 and DNA dependent protein kinase catalytic sub unit in melanoma cells at protein and m-RNA levels where as normal human fibroblast showed no change in the levels of DNA repair proteins [39]. Therefore, it appears that HDACI produce more cytotoxic effect in malignant cells than the normal cells. Another HDAC inhibitor H6CAHA has been shown to induce radiosensitization of cancer cells by disrupting the mechanism of DNA double strand break repair, but have potential to facilitate the damage repair in normal cells [95]. Furthermore, some of the HDACI have been found to induce the transcription of DNA double strand break repair proteins like RAD 50, MRE 11 etc. in normal cells [93] and may therefore reduce the damage to the genome. HDACI may also lead to the up-regulation in the levels of cell cycle inhibitors such as p21 cip1, p27 kip1 and p16 INK4, and the repression of inflammatory cytokines like (interleukins) IL-1, IL-8, TNF-α, TGF-β [96-101]. These are known to be involved in the inflammatory response to radiation. Particularly, prolonged secretion of TNF-α and (transforming growth factor) TGF-β from epithelial, endothelial and connective tissue cells is implicated in cutaneous radiation syndrome [102]. Phenylbutyrate has been shown to reduce radiation and DMBA (dimethyl-1, 2-benzanthracene) induced oral carcinogenesis in hamsters [103]. Recent studies have shown that HDACI protects from cutaneous radiation syndrome in vivo [102-105] by reducing the levels of inflammatory cytokines, while Trichostatin A and valproic acid have been reported to protect mice from radiation induced skin injuries after TBI (total body irradiation) exposure [104].

HDACI treatment decreased the loss of BM cells and stimulated the proliferation of bone marrow stem cells [106] and also increased endogenous spleen colony formation, intestinal crypts, and increase villus height [95] in irradiated mice. We recently reported that diallyl sulfide (DAS) and TSA mitigate radiation damage in mouse by significantly reducing the acute radiation syndrome in the form of reduction in the injuries to the hematopoietic system and reducing levels of radiation induced proinflammatory cytokines. Our recent observations on the lymphocytes of accidentally radiation exposed individuals have also shown that sulforaphane, a dietary organosulfur compound and an established HDAC inhibitor, efficiently mitigates radiation-induced genotoxicity, when administered at G0 or G1 phase of lymphocytes after stimulation of cell cycle with (phytohemagglutinin) PHA.

Conclusion
HDACIs have not only emerged as a pharmacologically important class of molecules for anticancer therapy in the recent years but also have shown promise for the treatment of several otherwise incurable autoimmune and neurodegenerative disorders. In the field of oncology HDACIs have been particularly attractive as their effective anticancer dose render negligible normal tissue toxicity, one of the limiting factors in case of other anticancer therapeutics. HDACIs have also been found to be highly specific to the target cell population under other stress conditions like their profound ability to kill inflammatory cells while sparing cells with normal immune function. The radiosensitizing property of HDACI, at least in part, can be attributed to its ability to disturb chromatin remodeling by hyper-acetylating the histones thereby increasing the susceptibility of cells radiation induced DNA damage. A relaxed chromatin after irradiation possibly would facilitate assembly of repair proteins contributing to mitigation of radiation injury (depicted schematically in Figure 3a and b). The extreme low effective concentrations of HDACI required for radiation mitigation have no report to exert any normal tissue damage. Besides, at such low concentration may not lead to random hyperacetylation causing aberrant signal transduction and hence cell death. Since most of the HDAC inhibitors studied so far render little or no HDAC isoform specificity and have several non-histone protein targets their administration can initiate alterations in several biochemical or physiological functions or pathways leading to some or other degree of toxicity or side effects. The development of more class
specific HDACIs (for example the HDAC 6 specific inhibitor tabucin or tubulin acetylation inhibitor) may help overcome the problem of toxicity and enhance therapeutic gains and also lead to the better understanding of the underlying mechanism(s).

In summary, although HDACIs may appear to have radio-mitigating potentials more detailed studies are required to unravel the underlying mechanisms as well as the optimization of protocol for their administration to maximize their mitigative effects.

Competing interests

The authors declare that they have no competing interests.

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