



HDAC inhibitors: Targets for tumor therapy, immune modulation and lung diseases

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ABSTRACT

Histone deacetylases (HDACs) are enzymes that play a key role in the epigenetic regulation of gene expression by remodeling chromatin. Inhibition of HDACs is a prospective therapeutic approach for reversing epigenetic alteration in several diseases. In preclinical research, numerous types of HDAC inhibitors were discovered to exhibit powerful and selective anticancer properties. However, such research has revealed that the effects of HDAC inhibitors may be far broader and more intricate than previously thought. This review will provide insight into the HDAC inhibitors and their mechanism of action with special emphasis on the significance of HDAC inhibitors in the treatment of Chronic Obstructive Pulmonary Disease and lung cancer. Nanocarrier-mediated HDAC inhibitor delivery and new approaches for targeting HDACs are also discussed.

Introduction

Epigenetic mechanisms have a crucial role in determining the relative state of chromatin by several processes which include DNA methylation and post-translational modification of histones [1]. Long N-terminal extensions present in core histones undergo extensive post-translational modifications such as acetylation, methylation, and phosphorylation [2]. The histone acetylation process is being extensively investigated due to its ability to regulate gene transcription. Histone Deacetylases (HDACs) are enzymes that remove the acetyl group from histones. Thus they are considered critical regulators of gene expression [3]. HDACs are key regulators of gene expression and there are 18 HDACs identified to date. Based on their homology to yeast HDACs, they are categorized into four different classes. Class I HDACs (1, 2, 3, and 8) are generally present in the nucleus and have a ubiquitous expression in various cell lines and tissues. It shares homology to yeast RPD3 (reduced potassium dependency 3) protein. Class II HDACs include HDAC4, 5, 6, 7, 9 and 10 are homologous to yeast Hda 1 (histone deacetylase 1) protein and its shuttle between the nucleus and the cytoplasm. HDAC6 and 10, which are part of class IIb HDAC, are found in the cytoplasm and contain two deacetylase domains. The class III HDACs (SIRT1, 2, 3, 4, 5, 6, and 7) require NAD⁺ for their activity and homologs of the yeast protein Sir2. Depending on the change in cellular redox status, these classes of HDACs regulate the gene expression. Class IV has HDAC11 as their sole member and shares analogy with the

catalytic site of both class I and II enzymes, but not strong enough to classify in any of these two categories [4]. Different classes of HDACs, their function, and expression pattern are listed in the Table 1.

HDACs isoenzymes are dysregulated in a variety of malignancies and some inflammatory pulmonary diseases such as chronic obstructive pulmonary disease (COPD) [5]. Lung cancer and chronic obstructive pulmonary disease (COPD) are the leading causes of lung disease-related death worldwide. Studies have shown that COPD increases the susceptibility of lung tumorigenesis up to 4 to 5 fold [6]. COPD, which is characterised by chronic inflammation and is a possible cause of lung carcinogenesis, affects 50–70% of lung cancer patients. Inflammation is regulated by multiple mechanisms. It is regulated at the molecular level by post-translational modification most notably by acetylation and deacetylation of histone. HDAC-1 gene expression is linked with lung cancer progression and hypoacetylation of histone results in a more aggressive phenotype in adenocarcinoma of the lung [7]. Total HDAC activity is greatly reduced in alveolar macrophages, peripheral lung, and bronchial biopsies from COPD patients. This decreased expression of HDAC is directly associated with the disease severity and with increased acetylation of histones associated with NF- κ B binding site on CXCL8 promoter [8,9]. The therapeutic landscape for these two diseases has evolved dramatically with multiple molecular targeted therapies and immune checkpoint inhibitors. However, there are a small group of patients who do not respond to these therapies. This implies the necessity of developing novel therapeutic strategies. Inhibitors of HDAC

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enzymes are emerging as a potential therapeutic target in the pathophysiology of several diseases. This review mainly focuses on various research studies in recent years highlighting the significance of HDAC inhibitors in the treatment of lung cancer and COPD, with a special emphasis on nanoparticle-mediated administration of these inhibitors for optimum efficiency.

HDAC inhibitors (HDACi) as a potential therapeutic target

HDAC inhibitors are a group of prominent epigenetic drugs that are being tested in many clinical implications against several diseases. These may act specifically to one of the types of HDACs or it might target all the types of HDACs (pan-inhibitors). They are classified based on their chemical nature, Hydroxymates group of HDACi that can block all HDACs includes suberoylanilide hydroxamic acid (SAHA), Trichostatin A, LBH589 (panobinostat), and PXD101 (belinostat). Short-chain fatty acids, such as Valproic acid (VPA) and butyrate are specific to class I and IIa HDACs. MS 275 (entinostat), FK228 (romidepsin) belongs to benzamide, and the depsipeptides group is used to inhibit class I HDACs. Cyclic tetrapeptides such as trapoxin (TPX) inhibit a few classes I, IIa, and IV HDACs [10–13]. Classification of different HDAC inhibitors along with their specificity to HDAC is listed in Table 2:

Studies are showing that Histone deacetylase inhibitors inhibit the proliferation of a variety of transformed cells *in vitro*, including lymphoma, myeloma, leukemia, and Non-small cell Lung Carcinoma (NSCLC), and inhibit tumor progression in several solid tumors and hematological malignancies including lung cancer [14]. HDACi induces cell cycle arrest, differentiation, and cell death. They can also modulate the immune response and decrease angiogenesis (Fig. 1) SAHA/Vorinostat and romidepsin have been approved by FDA for the treatment of cutaneous T-cell lymphoma and peripheral T-cell lymphoma [15–17] Panobinostat shows clinical success in the treatment of multiple myeloma [18]. Common HDAC inhibitors and their target genes is listed in Table 3.

Modulation of cell cycle regulators by HDAC inhibitors

There are several mechanisms for HDACi mediated cell cycle arrest. The main mechanism reported in a majority of cancer cells is through increased expression of genes like CDKN1A (Cyclin-dependent kinase inhibitor p21). Treatment of leukemia cells with SAHA results in apoptosis with G0/G1 and S phase population. FR901228, a novel depsipeptide causes G1 arrest in addition to G2/M arrest in a different panel of cancer cell line [20–22]. HDACi can induce cellular differentiation and this is closely associated with cell cycle arrest at the G1/S stage mediated by retinoblastoma and other related protein. HDACi induces cell cycle arrest in a dose-dependent manner. Low concentration results in G1 arrest whereas high concentration induces G1 and G2/M arrests [23]. Expression of p21 is modulated by p53 protein which competes with HDAC1 for the interaction with p21 promoter thus decreasing the transcription of p21. Treatment of HDACi results in the release of HDAC1 protein from Sp1, which increases p21 expression and mediates cell

Table 1.

Classification of HDACs based on their homology to yeast HDACs.

Class activity	Enzyme	Tissue expression	Subcellular localization	Mechanism of deacetylase activity
I(RPD3 like)	HDAC 1& 2	Ubiquitous	Nucleus	Zn ²⁺ dependent
	HDAC3 & 8	Ubiquitous	Nucleus & cytoplasm	Zn ²⁺ dependent
	HDAC4	Tissue specific(Brain, heart, skeletal muscle, retina, neurons)	Nucleus & cytoplasm	Zn ²⁺ dependent
	HDAC5	Tissue specific(Brain, heart, skeletal muscle, retina, neurons)	Nucleus & cytoplasm	Zn ²⁺ dependent
II (HDA1 like)	HDAC7	Tissue specific(Thymus, heart, muscle, lung)	Nucleus & cytoplasm	Zn ²⁺ dependent
	HDAC9	Tissue specific (Heart, skeletal muscle, brain)	Nucleus & cytoplasm	Zn ²⁺ dependent
	HDAC6	Tissue specific (Muscle, brain, heart, liver, kidney)	Mainly cytoplasm	Zn ²⁺ dependent
	HDAC10	Tissue specific(Liver, spleen, kidney, skin)	Nucleus & cytoplasm	Zn ²⁺ dependent
III (SIR2 like)	SIRT1-7	Ubiquitous	Nucleus, cytoplasm & mitochondria	NAD ⁺ dependent
IV	HDAC11	Tissue specific(Brain, heart, skeletal muscle, kidney, T cells)	Nucleus & cytoplasm	Zn ²⁺ dependent

Table 2.

Classification of HDAC inhibitors and their specificity towards HDACs.

Class of HDAC inhibitors	HDAC inhibitors	HDAC specificity
Short-chain fatty acid	Butyrate	Class I, IIa
	Valproic acid (VPA)	Class I, IIa
Hydroxamate	Trichostatin A (TSA)	Class I, II
	Suberoylanilide hydroxamic acid (SAHA)	Class I, II
	PXD101	Class I, II
	Oxamflatin	Class I, II
	LAQ824	Class I, II
	LBH589	Class I, II
	Pyroxamide	Class I
	SK-7041	Class I, II
	SK-7068	Class I, II
	Tubacin	Class IIb
Benzamide	MS-275	HDACs 1, 2,3, 8
	Cyclic tetrapeptide	Class I
Cyclic tetrapeptide	Depsipeptide	Class I, IIa
	Trapoxin A	Class I, IIa
	Apicidin	HDACs 1 and 3
	CHAPs	Class I

cycle arrest and apoptosis [24–26]. In addition, HDACi inhibits the expression of cyclin D and cyclin A encoding genes and promotes the transcription of anti-apoptotic genes [27–30]. Trichostatin A (TSA) is a good example of an HDAC inhibitor that induces G1 arrest. It has been reported in human colon HCT116 cells via induction of p15 (INK4b) an inhibitor of cyclin D-dependent kinases [31]. The importance of CDKN1A expression in HDACi mediated cell cycle arrest is well understood by Mensah et al. They have observed based on the expression level of CDKN1A is important in determining the concentration of two novel HDACi ITF-A and ITF-B for cell cycle arrest [32].

HDAC inhibitors regulating tumor suppression

p53 is considered as the guardian of the genome and its activation results in the cell cycle arrest or programmed cell death which blocks uncontrolled growth of tumors. Most of the tumor cells express defective p53. Zhao et al. showed that depsipeptide FR901228 induces the expression of p21 via the p53 pathway and the acetylation of p53 at lysine 373/382 residue occurs after depsipeptide treatment. The acetylated p53 has a longer shelf-life and reduced ubiquitination thereby inducing cell cycle arrest *in vitro* lung cancer cells [33].

TSA is also shown to target tumor cells via activation of the p53 tumor suppressor pathway *in vitro* lung cancer A549, H1299, and human embryonal kidney HEK293 [34,35] TSA is found to inhibit the cell cycle progression upon targeting RUNX3 *in vitro* pancreatic endocrine tumors-insulinoma CM, carcinoid BON, somatostatinoma QCP-1, *in vitro* prostate cancer DU-145, PC3 [36,37].

HDAC inhibitor as inducers of apoptosis

HDACi promotes tumor cell death like other anti-cancer drugs with

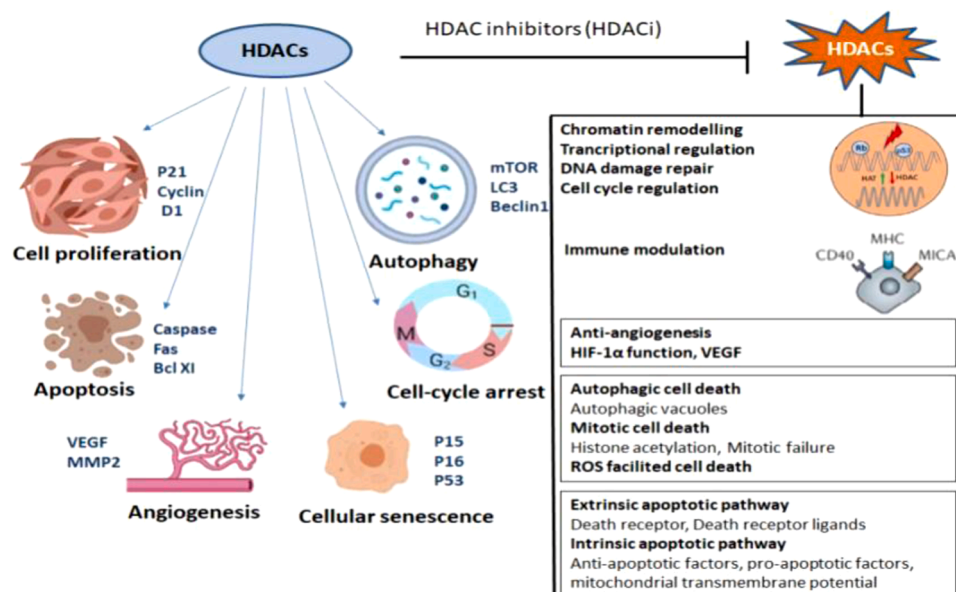


Fig. 1. Effect of HDAC inhibitors and their antitumor pathways: Dysregulated expression of HDAC aids in cell proliferation, angiogenesis and escape from autophagy, apoptosis of cancer cells. After treatment with HDAC inhibitor multiple anti-tumor pathways get activated and results in the destruction of cancer cells.

Table 3.
Mechanism of anti-cancer activity of HDAC inhibitors.

Mechanism	HDAC inhibitors	Targeted genes/pathway	References
Cell cycle arrest	SAHA	CDKN1A/p21	[19,20]
	TSA	p53,p21, RUNX3	[34,35]
	Sodium butyrate	p21	[156]
Apoptosis	MS275	intrinsic pathway, death receptor	[55]
	TSA	intrinsic pathway	[59,60]
	Apicidin	Death receptor	[54]
Modulation of immune response	VPA	Intrinsic pathway	[63]
	TSA	TAP1 & 2, LMP-2, tapasin, MHC class I	[83,88, 101]
	VPA	MHC genes	[157]
	Entinostat	Tumor associated antigens	[103]
	SAHA	Tumor associated antigens	[103,104]
	MGCD0103	MHC class I	[158]
Autophagy induction	LBH589	MHC class I	[158]
	SAHA	Akt/mTOR, ULK1, mTOR, p53, ULK1, NF- κB	[159,160]
	MS275	Akt/mTOR, ULK1, NF- κB	[159,160]
Effect on signal pathways	TSA	FOXO3, Atg5 & Beclin1, FOXO1, Akt/mTOR, ULK1	[159]
	Valproic acid	c-Jun, ERK, APC/β-catenin/ c-Myc	[161–163]

all morphological and cellular characteristics of apoptosis. Clinical trials and preclinical animal experiments revealed that HDACi shows selective apoptotic ability towards tumor cells. Upon treatment with HDACi, several effects have been demonstrated for apoptosis signaling in cancer cells. These compounds increase the expression of proapoptotic proteins (Bax or Bak) of the intrinsic pathway [38,39] or decrease the expression of anti-apoptotic proteins (Bcl-2) [40,41]. Other prominent mechanisms employed by HDACi are the generation of reactive oxygen species (ROS) [42,43] and increasing the expression of p53 or restoring the activity of p53 [44].

Tumor cells on exposure to HDACi result in acetylation of histone

and activation of gene expression thus enhance the chance of tumor cells to undergo apoptosis. Apoptosis or programmed cell death appears to be the predominant way of HDACi-induced cell death in cancer therapy [45,46]. HDAC inhibitors induce apoptosis by activation of either intrinsic or extrinsic pathways.

During extrinsic pathway, assembly of death-inducing signal complex (DISC) occurs after binding of tumor necrosis factors (TNF) superfamily death receptors like Fas, tumor necrosis factor receptor (TNFR), TNF-related apoptosis-inducing ligand receptor 1 (TRAIL-R1), and TRAIL-R2 to their respective TNF ligand. This complex is required for death signal transmission and includes adaptor proteins FADD (FAS-associated death domain) with procaspase 8 and 10. Cleavage of the downstream effector caspase -3 results in the induction of apoptosis signal [47–49].

HDACi have shown to facilitate the extrinsic pathway in different mechanisms which include increased expression of cell surface death receptors and reduction in the expression of cytoplasmic FLICE-like inhibitory protein (c-FLIP) which is known to prevent CD95L-induced apoptosis [50]. Several studies in malignant tumors demonstrate that HDACi treatment results in the upregulation of death receptor TRAIL-R2 and rapid DISC formation and activation of caspase-8 [51,52]. HDAC inhibitor FK228 can efficiently induce TRAIL-mediated apoptosis by active DISC formation leading to activation of caspase -8 in chronic lymphocytic leukemia (CLL) cells [53]. Other HDACi like apicidin and MS275 can activate the extrinsic pathway of apoptosis via Fas-L and TRAIL in human acute promyelocytic leukemia and acute myeloid leukemia cells [54,55].

Activation of intrinsic pathway requires the release of cytochrome C, from the intermembrane space of mitochondria to cytoplasm. The binding of cytochrome C to Apaf-1 facilitates the formation of the apoptosome, which then activates procaspase 9 and other downstream caspases include caspase 3 and 7. These events result in changes in the morphological changes associated with apoptosis [56]. The interplay between pro-and anti-apoptotic Bcl-2 superfamily proteins marks intrinsic pathway activation in the cell. HDACi activates the intrinsic pathway through decreased expression of anti-apoptotic proteins and enhanced expression of proapoptotic proteins. This increased expression of pro-apoptotic proteins by HDACi is due to hyperacetylation of H3 and H4 in their promoter regions [57,58]. Treatment with TSA and sodium butyrate leads to decreased transcription and expression of

anti-apoptotic Bcl-xL proteins in myeloma and mesothelioma cells [59, 60]. HDAC inhibitors like SAHA, TSA, VPA, MS 275 are known to induce the expression of pro-apoptotic genes involved in intrinsic pathways such as BAX, BAK, and APAF1 *in vitro* Leukemic cells Jurkat, ML-1, prostate cancer LNCaP, bladder cancer T24, breast cancer MCF7, neuroblastoma UKF-NB-4 and T cell leukemia cells [61–64].

Regulatory role of HDAC inhibitors in angiogenesis

Numerous studies show that HDACi can affect the steps of carcinogenesis that involve angiogenesis, invasion, and metastasis [65]. Treatment with HDACi decreases the expression of proangiogenic genes like basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), angiopoietin, tunica intima endothelial kinase 2 (TIE2), and endothelial nitric oxide synthase (eNOS) [66–70]. Treatment of VPA results in the increased production of inhibitors of angiogenesis such as thrombospondin-1 and activin A in neuroblastoma cell lines [71]. HDAC inhibitors can alter the angiogenic signaling pathway. Deroanne and his group demonstrated that exposure of TSA and SAHA results in the upregulation of semaphorin III, a competitor of VEGF at both mRNA and protein levels in HUVEC. These inhibitors work in a dose-dependent manner and are specific to endothelial cells [72]. VEGFR-2 signaling pathway is crucial for angiogenesis during tumor progression; HDACi can interfere and exert its anti-tumor activity by reducing the expression level of VEGFR-2. TSA, Sodium butyrate, and VPA treatment leads to the downregulation of VEGFR-2 along with VE-Cadherin suppression [73]. Crazzolaro et al. showed that expression of Chemokine receptor 4 (CXCR4) which is crucial for the homing of bone-marrow progenitor and endothelial cell circulation at the site of angiogenesis is significantly downregulated upon treatment with HDACi [74]. The anti-metastatic effect of HDACi is mainly through transcriptional repressions of metalloproteinases (MMPs) such as MMP2 and MMP9. It also induces the expression of RECK, a negative regulator of MMP2 activity [75–77].

Immunomodulatory effect of HDAC inhibitors

There is substantial evidence that HDACi can increase the antitumor immunity by making the tumor cell immune targets or by altering the immune cell activity or by cytokine production [78]. Some immunoregulatory effects of HDAC inhibitors are discussed below.

Role of HDACi in tumor cell recognition by T and natural killer (NK) cells

Tumor cell recognition is the most important step in cancer immunosurveillance. T and NK cells are the key players in the recognition and elimination of tumor cells based on the expression of tumor-associated antigen (TAA) and MHC Class I (MHCI) molecules by them [79]. Reports have demonstrated that HDACi results in the upregulation of genes involved in the antigen presentation and/or costimulatory molecule expression by cancer cells [80,81] TSA treatment facilitates the surface expression of MHC I in murine cervical cell lines which have an impaired antigen processing machinery [82]. In murine plasmacytoma cells, exposure to TSA can also induce MHC class II expression through activation of promoter III of Class II Trans activators (PIII-CIITA) which results in enhanced proliferation of CD4 T cells [83]. *In vitro* studies of several human and murine melanoma cell lines show increased expression of several tumor-associated antigens, MHCI & II and co-stimulatory molecule upon treatment with panobinostat [84]. Increase histone H3 acetylation upon HDACi treatment is playing a critical role in the modulation of antigen processing, expression of tumor-associated antigens, and other components which facilitate the tumor cell recognition and destruction by tumor-specific T cells [85]. Tumor cell recognition by NK cells depends on the expression of ligands by the tumor cells. MHC class I-related chain A (MICA) and B molecules (MICB) and UL16-binding proteins (ULBPs) are some of the examples of stress-induced activating ligand expressed by the tumor cell which is recognized by receptors of NK cells [86]. NK cell-mediated tumor cell

recognition and killing is increased in hepatocellular carcinoma by upregulation of MICA, MICB, and ULBP upon treatment with Valproic acid [87]. TSA treatment leads to the release of HDAC3 mediated repression on the promoter of ULBP thereby facilitate its expression by tumor cells [88]. Treatment panHDAC inhibitors result in increased expression of ULBPs in osteosarcoma, leukemia cells and activate NKG2D receptors on NK cells [89–92]. Skov et al. showed that HDACi treated hepatocellular carcinoma cells are efficiently eliminated by natural killer cells with increased expression of MICA and MICB [93,94]. It can also increase the expression of MHC class I and II proteins and co-stimulatory / adhesion molecules [95,96].

HDAC inhibitors as enhancers of immune responses

HDACi also enhances immune responses by altering the activity of immune cells via variations in cytokine and chemokine secretion [97]. Tumor Necrosis Factor (TNF) is an inflammatory cytokine involved in various biological processes. TNF signaling pathway results in the activation of NF- κ B and MAPK pathway which aid in the survival of several malignant cells [98]. TRAIL [TNF- α -related apoptosis-inducing ligand] has been shown to elicit the antitumor activity in several cancer cells. HDAC inhibitors along with TRAIL block the cell cycle and lead to apoptosis in several malignant tumors [99,100]. Type 1 interferons include IFN- α and IFN- β possess anti-tumor and immunoregulatory functions. Treatment with TSA and romidepsin results in the reduction of transcriptional responses of IFN- α by targeting the C-terminal STAT2 transcriptional activation domain [101].

Treating melanoma cells with tubastatin A, a potent inhibitor of HDAC6 results in the upregulation of tumor-associated antigen and MHC class I expression and thus improving the immunogenicity of the cells [102].

Exposure to SAHA and entinostat induces the immune system to recognize tumor cells more efficiently by cytotoxic T cells. *In vitro* studies show that pancreas (AsPC-1) breast (MDA-MB-231) and prostate (LNCaP) cell lines become more sensitive to T-cell mediated lysis after treating with SAHA and entinostat [103]. Exposure to SAHA has been shown to increase the expression of HLA-related genes class I/ epitope complexes and increased antigen-specific cytotoxic T-lymphocyte lysis [104]. Extensive clinical studies should be done to determine the immunomodulatory effect of HDACi.

Role of HDAC inhibitors in COPD treatment

The increasing prevalence of people with COPD insists on developing some novel therapeutic approaches. As Class I HDAC is playing a crucial role in the pathogenesis of COPD by inflammatory responses, utilizing HDAC inhibitors to target those HDAC will be an efficient method for treatment [105].

Several studies have shown that HDAC expression and activity are altered in COPD. Patients with severe COPD express less than 5% of the HDAC2 that nonsmokers do [106]. This reduced expression of HDAC2, which deacetylates histone (H4) on IL-8 promoter, correlates with disease development and can be used as a biomarker [107,108]. Barnes et al. showed that on exposure to cigarette smoke, phosphorylation of serine residues on HDAC2 leads to ubiquitination and proteasomal degradation. Reactive oxygen species from cigarette smoke results in the activation of phosphoinositide-3-kinases, the class I PI3K- δ isoform in particular. Downstream kinase AKT phosphorylates the serine residue on HDAC2 [109]. Decreased expression of HDAC2 abolishes the effect of glucocorticoid in patients with COPD [110]. The expression level of HDAC 1, 3, 4, 6, and 7 in COPD are unaltered whereas SIRT1 activity is decreased and HDAC5 and 8 expressions are low [111,112].

There is accumulating evidence suggest that HDACi can effectively modulate inflammatory response at a concentration of 10–100 fold lower than their cell cytotoxicity observed in cancer [105]. Considering that HDAC3, positive regulator of NF- κ B mediated inflammation, inhibitors against HDAC3 become a promising therapeutical strategy to

target inflammation in COPD [113]. RGF966 a selective inhibitor of HDAC3 can reduce the transcriptional activity of NF- κ B in LPS/IFN- γ stimulate macrophages and shows an anti-inflammatory property. At high concentration (10 μ M) RGF966 also inhibit the activity of HDAC1 and HDAC2 [114]. Treatment with entinostat reduces cigarette smoke mediated airway inflammation in mice and can be used as a potential drug for the treatment of COPD. Selective inhibition of HDAC6 and HDAC8 with HDACi aids in the reduction of inflammation and airway remodeling in COPD [115]. PCI-34501, a selective inhibitor of HDAC8 is shown to reduce airway hyperresponsiveness and inflammation. Pan HDACi TSA treatment results in reduced inflammation by increasing Treg cell activation and inhibiting the expression of IL-17 and Th17 cells in human precision-cut lung slices and *in vivo* animal models [116,117]. Other non-selective HDACi also induce apoptosis in macrophages [118]. Current research focuses on exploring the importance of specific HDAC isoforms in the context of their catalytic and structural role. This offers a novel therapeutic strategy for treating inflammatory diseases like COPD.

HDAC inhibitors and their anti-cancer activity in lung cancer therapy

The anti-tumor activity of HDACi is greatly employed as a potential therapeutic strategy in treating lung cancer.

Diverse studies show increased expression of HDACs observed in several malignancies and it has been associated with poor outcomes in patients [119,120]. Histone modifications play a crucial role in lung carcinogenesis. Lung cancer cells possess an abnormal histone modification pattern when compared with normal lung cells, which includes hyperacetylation of H4K5/H4K8, hypoacetylation of H4K12/H4K16, and loss of trimethylation [121]. Exposure to cigarette smoke greatly influences histone modification. Induction of H3K4methylation by tobacco smoke affects the expression of tumor suppressor genes and results in malignant transformation [122]. HDAC3 expression is elevated in a majority of NSCLC (Non-Small Cell Lung Cancer) tumors [123]. Increased expression of HDAC 1 and HDAC3 is related to poor prognosis of lung adenocarcinoma, whereas reduced expression of HDAC 5, 6, 10 is associated with poor prognosis of NSCLC [124,125].

In H157 lung cancer cells, Trichostatin A treatment results in activation of the intrinsic mitochondrial apoptotic pathway in a dose-dependent manner [126]. CG200745 shown to increase the global level of histone acetylation and block the proliferation of NSCLC via epigenetic modification of critical genes involved in cancer cell survival [127]. Inhibition of HDAC6 leads to increased apoptosis, G2 arrest in NSCLC cell lines [128]. Few novel inhibitors such as SL142, SL325, HTPB, and CG0006 result in apoptosis via induction of caspase-3 activity [129]. HDACi may employ its therapeutic role by reducing TNF- α mediated activation of NF- κ B pathway. This property is of great significance in tumors that are mainly associated with inflammation, particularly in many smoking-associated NSCLC tumors [130]. In addition to that, combination therapy of HDACi with other chemotherapeutic agents, immune checkpoint inhibitors, Tyrosine kinase inhibitors is of growing interest. Several studies have shown that combination therapy increases the efficacy of HDACi [131]. Some of the clinical trials involves combination therapy employed in the treatment of NSCLC is listed in Table 4.

Nanoparticle mediated delivery of HDAC inhibitors

HDAC inhibitors are potential therapeutic targets as it plays a significant role in targeting the expression of HDACs. Major disadvantages of employing HDAC inhibitors are poor pharmacokinetics which includes short half-life, fast metabolism, and clearance. It has a limited specificity which leads to fashionable off-target and adverse secondary effects, and finally low solubility and permeability of HDAC inhibitors which limit intra tumor delivery [132,133]. These limitations imply the necessity of developing a better carrier for delivering HDAC inhibitors.

Table 4.
Ongoing clinical trials employing HDACi in NSCLC.

HDAC inhibitor Combination drugs	Summary of the study	Estimated completion date	Clinical trial identifier
Entinostat	Pembrolizumab November 2023	Compare the effects of the combination of two drugs pembrolizumab and vorinostat with the effects of pembrolizumab alone in advanced lung cancer patients.	NCT02437136
	Azacitidine & Nivolumab August 2022	Epigenetic therapy with Entinostat and Azacitidine with concurrent nivolumab in Metastatic Non-Small Cell Lung Cancer	NCT01928576
Panobinostat	Anti PD-1 antibody August 17, 2022	The purpose of this study is to combine the PDR001 checkpoint inhibitor with immunomodulatory activity to identify the doses and schedule for combination therapy in NSCLC patients	NCT02890069
Abexinostat	Pembrolizumab May 31, 2023	This phase I trial studies the best dose and side effects of abexinostat and how well it works with given together with pembrolizumab in treating participants with advanced stage of lung cancer	NCT03590054
Mocetinostat	Nivolumab December 2021	The study will evaluate the clinical activity of nivolumab in combination with receptor tyrosine kinase inhibitor and HDACinhibitor mocetinostat.	NCT02954991

Nanoparticle-based therapeutics like nanocarriers and nano drugs are emerging technologies to deliver HDAC inhibitors effectively and address the abovementioned shortcomings.

Nanoparticles of smaller size are more likely to accumulate into the tumors to achieve passive targeting via enhanced permeability and retention effect (EPR). During this effect, nanomaterials along with encapsulated macromolecules tend to distribute in the tumor due to the leaky neovasculature and poor lymphatic drainage [134].

Nanomaterials include liposomes, bio-nanocapsules, and polymeric nanoparticles which offer the greatest advantages such as a controlled release system, biocompatibility, and low toxicity profiles. Both organic and inorganic nanoparticles can be used for the successful delivery of HDAC inhibitors (Fig. 2).

There are a plethora of studies that insist on the need for nanoparticle-mediated delivery of HDAC inhibitors. PEGylated liposomes are used to load HDAC inhibitors like SAHA, TSA, LAQ824, CG1521, PXD101, and it has been observed that the efficacy of these inhibitors is greatly enhanced [135,136]. Some of the potential HDACi and their nanocarriers that show promising anti-cancer therapeutics are listed in Table 5.

Efficacy of nanocarrier based HDACi delivery in lung cancer therapy

There are diverse treatments strategies employed to treat several malignancies, among that chemotherapy are the most prominent and

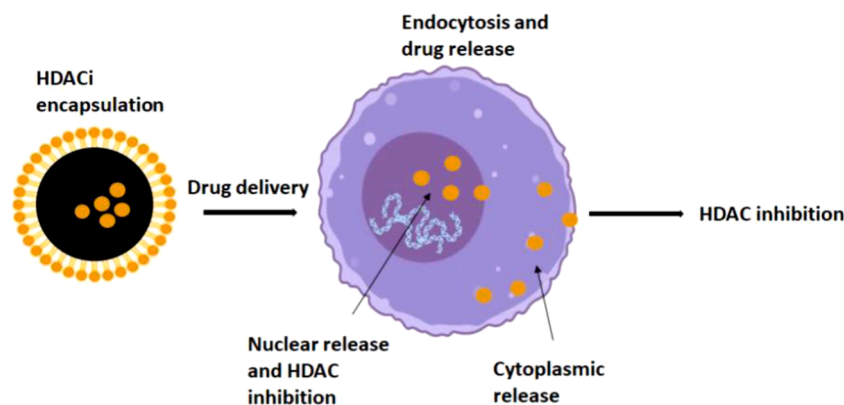


Fig. 2. Schematic diagram for the delivery and release of HDAC inhibitors: HDAC inhibitors are encapsulated in organic and inorganic nanoparticles and absorbed by the cells by endocytosis. They get delivered either in cytoplasm or nucleus.

Table 5.

Potential HDAC inhibitors and their nanocarrier that shows promising anti-cancer effects.

HDACinhibitors	Type of Nanoparticle delivery system	Reference
Trichostatin	A Polymeric Nanoparticle azido-polyethylene oxide-norbomene macromonomers.	[149]
SAHA	Poly(lactide-co-glycolide) PLGA nanoparticle	[150]
Vorinostat	Hyaluronan based nanoparticles	[142]
Valporic acid	Polysaccharide nanoparticle	[142]
MS-275	Silver Nanoparticle	[151]
Sodium butyrate	Polymer polyethylene glycol (PEG)- coated luminescent nanoparticles	[152]
CI-994	Norbomene polyethylene oxide macromonomer	[153]
Quisinostat	PLA-PEG Nanoparticle	[142]
Belinostat	PLGA nanoparticle	[154]
CG-1521	Polymeric Starch Nanoparticle	[155]

widely employed. Though it is considered a quality treatment, the efficacy to treat tumors is not satisfactory. Nanocarriers have been gained interest in recent days as it overcomes the limitation of conventional drug delivery strategies. The Nano carrier-based delivery system includes nanoparticles of submicron particle size which effectively deliver the anti-cancer drug by exploiting the pathophysiology of the tumor microenvironment [137]. Epigenetic modulation, including HDAC inhibition, is a prospective therapeutic approach for the treatment of NSCLC. One of the widely used HDACi suberoylanilide hydroxamic acid (SAHA) shows active uptake and favorable biodistribution patterns when loaded with PLGA nanoparticles. PLGA polymeric nanoparticles possess enhanced permeability and retention effect (EPR), which results in the delivery of drug specifically at the tumor site. Studies from Gurunathan et al. showed that SAHA loaded PLGA nanoparticle is biocompatible and readily taken up by lung cancer cell and tumor models [138]. Encapsulation of MS-275 with silver nanoparticles maintains the drug's mechanism of action in the lung cancer cell line model. This work explains that this combination of silver nanoparticles and HDACi induces apoptosis, accompanied by increased expression of reactive oxygen species and induction of DNA fragmentation [139].

Anticancer potential of curcumin is greatly enhanced when it is loaded with 4-phenyl butyric acid (PBA)-installed hyaluronic acid (HA)-based nanoparticle HAPBA10 for lung cancer therapy. The HDACi is released from the conjugate through an esterase-responsive cleavage of the ester bond in cancer cells resulting in sustained drug release and cytotoxicity in both *in vitro* and *in vivo* lung cancer models. Further, it has been reported that this HAPBA nanoparticle can specifically target CD44 receptors which can be implemented in the treatment of CD44 receptor-expressed cancers [140].

Along with HDAC inhibitors, some chemotherapeutic agents are also loaded with nanoparticles for efficient targeting of cancer cells.

Hyaluronan-based nanoparticles of 30 nm diameter encapsulate both gefitinib and vorinostat which effectively targets CD44 receptors in lung adenocarcinoma cell lines [141].

Thus HDACi nanomedicines showed an enhanced anti-tumor efficacy with limited systemic toxicity. In addition to that, nanotechnology has also been used to predict the therapeutic efficacy of HDACi and a gold nanoparticle nanosensor was developed for the detection of histone deacetylase [142]. However, inorganic nanoparticles are expected to be limited for therapeutic purposes until research claims that they are safe and biodegradable.

Conclusion and future perspectives

HDAC inhibitors are emerging as a successful epigenetic therapy and there are only five HDACi which have been approved by FDA for therapeutic use. In some subtypes of hematological malignancies, they appear to be clinically advantageous, whereas their efficacy against solid tumors is not well studied [143]. Complete structural analysis and molecular mechanism of HDAC should be done to improve the efficacy of the treatment. Combination therapy which includes oncoprotein inhibitors, DNA methylation inhibitors, or autophagy inhibitors should be seriously considered for individuals with malignancies that have not responded to conventional therapies. Further studies should mainly focus on improving the selectivity of HDACi to enhance their accumulation in tumor cells at a lower concentration which will reduce the adverse effect on normal cells. The most common HDAC inhibitors used to treat lung cancer and COPD are pan HDAC inhibitors that target signaling pathways involved in inflammation. Understanding the link between these two diseases will aid in the development of an effective treatment. COPD patients are more likely to acquire lung cancer; as a result, treatment measures should be enhanced to prevent COPD from progressing to lung cancer. Nanocarrier-based HDACi delivery is a huge step forward in improving the targeted delivery of these drugs. Further future development of these drugs, more selective HDACi must be developed, as well as a predictive biomarker to aid patient selection for HDACi-based therapy. One of the most exciting and emerging fields is histone deacetylase degraders - HDAC PROTACs. Proteolysis targeting chimeras (PROTACs) are small molecules that can effectively degrade the target protein via the ubiquitin-proteasome system. This strategy eliminates the limitation of conventional chemotherapeutics by selectively targeting a protein that is specific to a cancer cell (but not in normal cells). Schiedel et al. and Yang et al. in 2018 developed HDAC-PROTAC that degrades NAD⁺-dependent histone deacetylase sirtuin 2 and classical Zn²⁺-dependent HDACs in 2018 [144,145]. These HDAC degraders provide better anticancer effects compared to classical HDAC inhibition. dHDAC-6 showed efficient degradation of HDAC6 in MCF-7 breast cancer cells [146] and ARV-110 and ARV-471, two PROTAC probes, are in phase I clinical trials for prostate and breast

cancer, respectively (NCT03888612 and NCT04072952 clinicaltrials.gov) [147,148]. Thus these new PROTACs will be the most promising strategy in nearby future. In conclusion, HDAC inhibitors can be key players in future targeted solid tumor therapy.

Author's Contributions

Geetha Shanmugam has done data collection and wrote the manuscript; Ms. Sudeshna Rakshit was involved in writing the manuscript; Dr. Koustav Sarkar designed the review article and also wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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