ANTICANCER RESEARCH *38*: 37-43 (2018)
doi:10.21873/anticanres.12189

Review

Histone Deacetylase Inhibitors as a Novel Targeted Therapy Against Non-small Cell Lung Cancer: Where Are We Now and What Should We Expect?

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Abstract. *Non-small cell lung cancer constitutes the most common type of lung cancer, accounting for 85-90% of lung cancer, and is a leading cause of cancer-related death. Despite the progress during the past years, poor prognosis remains a challenge and requires further research and development of novel antitumor treatment. Recently, the role of histone deacetylases in gene expression has emerged showing their regulation of the acetylation of histone proteins and other non-histone protein targets and their role in chromatin organization, while their inhibitors, the histone deacetylase inhibitors, have been proposed to have a potential therapeutic role in diverse malignancies, including non-small cell lung cancer. This review article focuses on the role of histone deacetylase inhibitors in the treatment of non-small cell lung cancer and the major molecular mechanisms underlying their antitumor activity recognized so far.*

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Key Words: Histone, deacetylase, HDAC, inhibitor, non-small cell, lung, cancer, targeted, epigenetics, review.

Primary lung cancer remains the most common malignancy after non-melanocytic skin cancer and is a leading cause of cancer-related death globally (1-3). Non-small cell lung cancer (NSCLC) accounts for 85-90% of lung cancer (1). The order of frequency of different histological subtypes has changed; incidence of adenocarcinoma has increased and nowadays represents the most frequent pulmonary malignancy, followed by squamous cell carcinoma (1, 3). Usually NSCLC is diagnosed in advanced stages, with poor

prognosis and limited therapeutic decisions. Despite the recent progress noted in the treatment of NSCLC with the introduction of immunotherapy (3-7) and the approval of tyrosine kinase inhibitors (TKIs) as first-line therapy (3, 8), there is an urgent need for further development of novel and more efficient antitumor treatment.

The role of histones and modifications in their *N*-termini, including acetylation, methylation, phosphorylation and ubiquitination, have been well recognized in chromatin organization and consequently in regulation of gene expression (9-12). More specifically, in eukaryotic cell nuclei, DNA is wrapped around proteins called histones. In this way, the structure of the nucleosome is formed, containing the wrapped DNA around a central histone octamer. Histones often present various modifications that permit the loosening of the nucleosome structure thereby allowing access of RNA polymerase and other transcription factors to their target genes in order to promote the transcriptional process (9-12).

Histone acetylation is the first among histone modifications identified and plays a principal role in the aforementioned regulation of transcription (12). It concerns a dynamic process, involving two groups of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs) (13-15). It is well known that histone acetylation modulates transcription in multiple ways. The result of histone acetylation is reduced interaction between histone and DNA. Increased histone acetylation has been associated with transcriptionally active genes, whilst low levels of acetylation correlate with decreased transcriptional action (13-16).

HDACs and Their Mechanisms of Action

HDACs are enzymes that remove acetyl groups from histone lysine residues of proteins, such as the core nucleosome histones, in this way not permitting DNA to loosen from the histone octamer and consequently preventing its transcription (Figure 1) (13, 16). Thus, histone deacetylation contributes to transcriptional repression favoring chromatin compaction (17). According to their homology, four classes of HDACs have been recognized: class I, which includes the nuclear localized HDAC-1, -2, -3 and -8; class II, which includes the exonuclear as well as the nuclear localized HDAC-4, -5, -6, -7, -9 and -10; class III, the sirtuins (SIRT-1-7); and class IV, HDAC-11, with features of both class I and II; in total there are 18 HDACs (18). However, they can further divided into Zn^{2+}-dependent classes (class I, II and IV) and NAD-dependent classes (class III) (Table I). The role of HDACs as transcriptional repressors has been examined in studies including biochemical analyses *in vitro*, cultured cells and HDAC knockdown models (19). Furthermore, recent studies have shown the role of HDACs in the progression of a variety of malignancies (20-23). Moreover, HDACs have been shown to contribute to cellular homeostasis and regulation of fundamental functions, such as cell-cycle progression, differentiation and apoptosis through deacetylation of non-histone proteins (24).

The Role of HDACs in Cancer

The levels of many HDAC isoenzymes have been found increased in diverse malignancies (25). In particular, overexpression of HDAC-1 has been described in gastric cancer, while HDAC-2 and -3 in colorectal cancer. Similarly, activity of HDAC-6 and HDAC-7 have also been found increased in cutaneous T-cell lymphoma and pancreatic adenocarcinoma, respectively (26, 27). The complete mechanisms of action of HDACs have not been fully elucidated yet, as the involved pathways are considerably complex. However, an example of the exact meaning of the increased expression of HDACs and the subsequent transcriptional suppression of genes of hematopoietic differentiation has been well described in certain types of leukemia. For example in acute promyelocytic leukemia, chromosomal rearrangements of the retinoic acid receptor (RAR) transcription factor and the modified interactions with other transcriptional co-regulators, including nuclear receptor co-repressor (NCoR) and silencing mediator for retinoid or thyroid-hormone receptors (SMRT), result in recruitment of HDACs and contribute to transcriptional repression of specific target genes (28).

HDAC Inhibitors and Their Role as Anticancer Agents in NSCLC

Recent data has shown that HDAC inhibitors contribute to tumor cell growth arrest, enhancing cell apoptosis and promoting cell-cycle arrest (29-33). Their capacity to enhance the acetylation of cellular proteins by blocking HDAC activity reflects the importance of their potential anticancer action. Diverse studies have highlighted their role in diverse malignancies, including breast cancer (34, 35), and medullary thyroid (36) and pancreatic cancer (37, 38). The potential actions of HDAC inhibitors as novel anticancer agents have been supported based on their capacity to induce apoptosis and autophagy in cancer cells, by activating intrinsic mitochondrial pathways (39). In addition, they have been shown to up-regulate tumor suppressor genes, such as Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and Death Receptor 5 (*DR5*), involved in the apoptotic pathways and, on the contrary, to inhibit the expression of pro-survival genes, including B-cell lymphoma 2 (BCL2) (39, 40). Moreover, HDAC inhibitors also exhibit indirect actions, by enhancing immune responses and up-regulating major histocompatibility complex class I and II

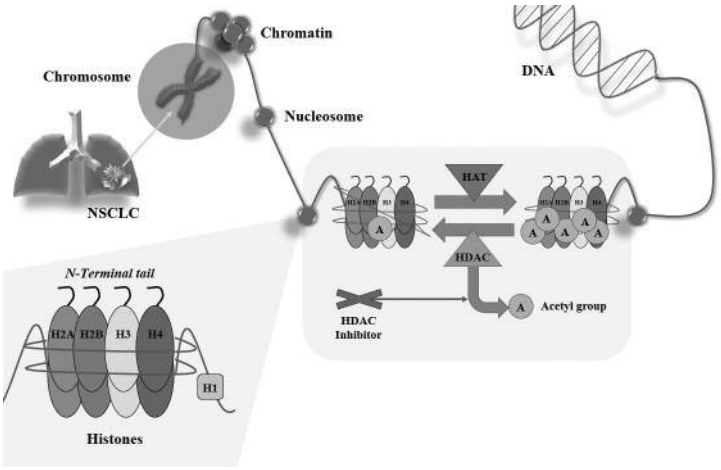

Figure 1. *Mechanism of histone deacetylase (HDAC) activity. NSCLC: Non-small cell lung cancar; HAT: histone acetyltransferase.*

Table I. *Classification of histone deacetylases (HDAC).*

Class	HDAC	Dependence
I	HDAC-1, -2, -3 and -8	Zn^{2+}
II	HDAC-4, -5, -6, -7, -9 and -10	
III	SIRT-1-7	NAD
IV	HDAC-11	Zn^{2+}

SIRT: Sirtuins.

proteins and cytokine secretion, as well as other co-stimulatory molecules, including CD80 and CD86. There exist some evidence that they also have anti-angiogenetic effects (39, 40). Furthermore, treatment of cells with HDAC inhibitors such as butyrate, trichostatin A (TSA), and trapoxin A, has been shown to contribute to the transcription of target genes (41). To date three HDAC inhibitors, namely vorinostat, romidepsin and panobinostat (LBH-589, PS), have received US Food and Drug Administration approval and belong to the therapeutic armamentarium against cutaneous and peripheral T-cell lymphoma and multiple myeloma (42).

Lung cancer still remains the leading cause of cancer-related deaths worldwide with approximately 221,200 estimated new cases (39). NSCLC accounts for 85%-90% of lung cancer. Usually the diagnosis is established in advanced stages, with poor prognosis and limited therapeutic decisions (39). Current research focuses on the potential utility of HDAC inhibitors as monotherapy or in combination with other chemotherapeutic regimens in NSCLC in an effort to increase their efficacy or reduce tumor resistance.

More specifically, TSA is an antifungal antibiotic and has reversible HDAC inhibitory activity, regulating apoptosis, angiogenesis and cell differentiation. Studies based on NSCLC cell lines have reported the antitumor action of vorinostat and TSA by inhibiting tumor cell growth. Mukhopadhyay *et al*., evaluated the role of TSA in NSCLC lines compared to normal lung fibroblasts (41). Their results confirmed the apoptotic action of TSA. Moreover, the authors concluded that TSA increased histone H4 acetylation and the expression of p21, whilst no significant effect on p16, p27, cyclin-dependent kinase 2 (CDK2), and cyclin D1 were observed (41). All the above findings of the TSA-treated NSCLC cells were consistent with the apoptotic effects that TSA has on tumor cells. The exact mechanism of the induced apoptosis is not clear. However, the authors proposed that TSA may induce expression of p21, which is associated with G_1 phase arrest (41). In addition, it has been demonstrated that lung cancer cells exhibit diverse histone H4 modifications, characterized by either hyperacetylation of H4K5/H4K8 or hypoacetylation of H4K12/H4K16. Seligson *et al*. demonstrated that decreased levels of histone modifications are associated with more aggressive cancer phenotype in lung adenocarcinoma (43). In parallel to the above results, diverse studies have found that HDAC-1 gene expression is associated with lung cancer progression (44, 45). Kim *et al*. found that treatment with TSA resulted in a dose-dependent reduction of H157 lung cancer cells (46). The proposed mechanism of TSA action was the induction of apoptosis by activating the intrinsic mitochondrial and extrinsic/Fas/FasL system death pathways. TSA seems to act synergistically with other HDAC inhibitors, including vorinostat (47) (Table II). Furthermore, Piao *et al*. demonstrated that combination of TSA with a mechanistic

Table II. *Proposed actions and involved molecular pathways of histone deacetylase (HDAC) inhibitors in non-small cell lung cancer (NSCLC).*

HDAC inhibitor	Action
Trichostatin A (TSA)	Inhibits tumor cell growth
	Promotes apoptosis in cancer cell lines by activating the intrinsic mitochondrial and extrinsic/Fas/FasL system death pathways
	Increases histone H4 acetylation and the expression of p21
	Sensitizes cancer cells to chemotherapy *in vitro*
Vorinostat	Exhibits antiproliferative activity
	Up-regulates the cyclin-dependent kinase inhibitor p21
	Synergistic action with carboplatin and paclitaxel and epidermal growth factor receptor tyrosine kinase inhibitors
	Sensitizes NSCLC cell lines to ionizing radiation through activation of caspase-8
Valproic acid	Synergistic action with cisplatin –vinorelbine
	Enhances the efficacy of ionizing radiation
Belinostat	Synergistic action with cyclin-dependent kinase inhibitor CYC202
	Antiproliferative action
	Increases apoptosis in NSCLC cell lines
Panobinostat	Synergistic action with erlotinib
	Induction of apoptosis

Table III. *Studies of histone deacetylase (HDAC) inhibitors in non-small cell lung cancer (NSCLC).*

Molecule	Phase	Author	Line of treatment	Sample size	Limitations
Vorinostat	Phase II study	Ramalingam *et al*. (48)	In patients with NSCLC in combination with carboplatin and paclitaxel	94	Increased toxicity
	Phase II study	Traynor *et al*. (51)	In patients with relapsed NSCLC	16	No objective antitumor activity was seen
Valproic acid	Pre-clinical	Shirsath *et al*. (57)	In NSCLC cell lines	-	*In vitro* study, further studies needed
	Pre-clinical	Chen *et al*. (55)	In NSCLC cell lines	-	*In vitro* study, further studies needed
	Pre-clinical	Gavrilov *et al*. (56)	In NSCLC cell lines	-	*In vitro* study, further studies needed
Belinostat	Pre-clinical	Ong *et al*. (58)	In NSCLC cell lines	-	*In vitro* study, further studies needed
Panobinostat	Pre-clinical	Greve *et al*. (42)	In EGFR-mutated and wild-type NSCLC cells.	-	*In vitro* study, further studies needed
	Phase I trial	Gray *et al*. (59)	In patients with advanced NSCLC and head-and-neck cancer	42	Small number of participants

EGFR: Epidermal growth factor receptor.

target of rapamycin inhibitor had an increased synergistic therapeutic antitumor effect on NSCLC cell migration and invasion *in vitro*, suggesting their additional role in sensitizing cancer cells to chemotherapy (48).

Vorinostat represents another HDAC inhibitors with potential therapeutic action in NSCLC. Vorinostat exhibited antiproliferative activity by repressing telomerase activity *via* up-regulation of the cyclin-dependent kinase inhibitor *p21*. The induction of *p21* resulted in G_0-G_1 cell-cycle arrest when human lung cancer lines were treated with vorinostat (46, 49). The potential therapeutic benefit of the addition of vorinostat to the first-line carboplatin and paclitaxel was evaluated in pre-clinical and clinical studies, which showed improved response rates (50, 51). Lately, research interest has been focused on the benefit of the use of HDAC inhibitors in combination with epidermal growth factor receptor (EGFR) TKIs (52). The Wisconsin Oncology Network phase II study by Traynor *et al*. showed that monotherapy with vorinostat in patients with relapsed NSCLC provided significant benefit regarding time to progression, however, no objective antitumor activity was observed (53). Vorinostat has been also shown to sensitize NSCLC cell lines to ionizing radiation through activation of caspase-8. These results could imply a potential clinical

benefit of HDAC inhibitors in improving response to radiotherapy in NSCLC (54) (Table II).

Valproic acid (VPA) represents another HDAC inhibitor. Recently, Chen *et al*. demonstrated that despite the fact that VPA did not affect NSCLC cell proliferation, it did induce increased sensitivity to cisplatin (55). VPA has also been also proposed to enhance the efficacy of the anticancer activity of combination therapy with cisplatin-vinorelbine and ionizing radiation and also reduce their side-effects (56) (Table II). Recently, it was shown that VPA had a potential synergistic therapeutic effect on NSCLC cell lines when combined with a CDK inhibitor, supporting more evidence of a potential novel antitumor activity (57).

Belinostat is a composite class I and II HDAC inhibitor. Recently, the novel combination of belinostat and CDK inhibitor CYC202 was evaluated as a potential anticancer strategy in NSCLC in an *in vitro* study of *p53* wild-type A549 cells. Concurrent treatment led to significant reduction in cell proliferation and an increase in apoptosis (58) (Table II).

Panobinostat is a novel HDAC inhibitor that was recently demonstrated to sensitize *EGFR*-mutated and wild-type NSCLC cells to the antiproliferative activity of erlotinib. Furthermore, this combination enhanced the induced acetylation of histone H3 (42). A small phase I trial of combination therapy with panobinostat and TKI in patients with advanced NSCLC and head-and-neck cancer showed that is a well-tolerated therapeutic regimen. However, larger randomized controlled studies are needed to elucidate its clear benefits in erlotinib-resistant NSCLC (59) (Table II). Table III summarizes current studies of HDAC inhibitors in NSCLC.

Conclusion

Despite much progress, poor prognosis remains a crucial issue in the therapeutic management of NSCLC. Alterations in histone acetylation result in crucial changes in gene expression and HDACs play a vital role in this process, underlying the potential importance of HDAC inhibitors as alternative or additional targets of antitumor therapy. However, further pre-clinical and clinical studies of HDAC inhibitors in NSCLC are required for evaluating their antitumor activity. Clinical trials should also assess the potential benefit of combining HDAC inhibitors with other antitumor drugs. The approach of utilizing HDAC inhibitors in combination with standard chemotherapy deserves further assessment since it could potentially enhance efficacy and reduce the side-effects of current anticancer regimens.

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Received September 24, 2017
Revised October 23, 2017
Accepted October 25, 2017