Review

Histone Deacetylase (HDAC) Inhibitors: Current Evidence for Therapeutic Activities in Pancreatic Cancer

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Abstract. *Pancreatic carcinoma is one of the leading causes of cancer death. Current standard treatments include surgical resection, chemotherapy and radiotherapy but patient's prognosis remains poor and present severe side-effects. Contemporary oncology found a wide variety of novel anticancer drugs that regulate the epigenetic mechanisms of tumor genesis. Histone deacetylases (HDACs) are enzymes with pleiotropic activities that control critical functions of the cell through regulation of the acetylation states of histone proteins and other non-histone protein targets. They are divided into four groups, each with different localization in the cell, role and structure. Histone deacetylase inhibitors (HDACIs) are substances, which inhibit the function of HDACs. We recognize four leading groups (hydroxamic acid, cyclic tetrapeptide, benzamide, aliphatic acid). There are many HDACIs currently in pre-clinical and two (vorinostat, romidepsin) in clinical stages of investigation for pancreatic cancer. Numerous studies argue for the use HDACIs as monotherapy, others suggest that combination of HDACIs with other antitumor drugs has better therapeutic results. This review focuses on the use of HDACIs as novel anticancer drugs and will explain the mechanisms of therapeutic effect on pancreatic cancer.*

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Pancreatic ductal adenocarcinoma (PDAC) is ranked fourth as leading cause of cancer death in USA and fifth in Europe. With an overall 5-year survival rate under 5% and a median survival of 6 months, pancreatic cancer is one of the most devastating human malignancies (1-3). Standard treatments for advanced disease include radiotherapy and/or chemotherapy. However, radiotherapy is often detrimental and chemotherapy with drug agents, such as 5-fluorouracil (5-FU) and gemcitabine (GEM), is either ineffective or effective for only a short period (4).

There is an ongoing interest in identifying signaling pathways and genes that play a key role in carcinogenesis and development of resistance to antitumoral drugs. Given that histone deacetylases (HDACs) interact with various partners through complex molecular mechanisms leading to the control of gene transcription, they are the subject of many researches.

It is widely accepted that the organization of chromatin is important for the regulation of gene expression. In eukaryotic cell nuclei, DNA is tightly wrapped around a central histone octamer comprising of two molecules of each of the core histones; thus, the nucleosome is formed. Histones allow DNA to tightly wrap into a coil of *ca*. 30 nm in diameter, which further builds upon itself, eventually forming chromosomes. The four core histones (H2A, H2B, H3 and H4) often undergo various modifications in their N-termini after protein synthesis. Acetylation, methylation, phosphorylation, ubiquitination and poly(ADP-ribosylation) of ε-amino groups, guanidino groups, carboxyl groups and hydroxyl groups are some of the modifications of the histones. Different patterns of modification that are observed in individual histone nucleosome core particles and

Table I. *Classification of HDACs.*

Class I	HDAC 1, HDAC 2, HDAC 3, HDAC 8
Class IIa	HDAC 4, HDAC 5, HDAC 7, HDAC 9
Class IIb	HDAC 6, HDAC 10
Class III	SIRT1, SIRT2, SIRT4, SIRT5, SIRT6, SIRT7
Class IV	HDAC 11

Table II. *Biological functions of HDACs.*

1.	Transcriptional regulation (and cell-cycle control)
2.	Modulation of apoptosis
3.	Modulation of DNA damage repair
4.	Modulation of autophagy
5.	Modulation of metabolism and senescence
6.	Chaperone function
7.	Modulation of angiogenesis

chromosomal domains play unique roles in chromatin structure, cell signaling and genomic stability (5-8).

Histone acetylation is the first modification identified and has since played a pivotal role in the transcriptional regulation of eukaryotic cells (8). The pattern of histone acetylation is a dynamic and reversible process that involves two groups of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs) (9). Histone acetylation effectively neutralizes the positive charge of lysine, which decreases the interaction between histone and DNA. This, in effect, loosens the nucleosome structure. This conformational change activates transcription by promoting the access of RNA polymerase, transcription factors, regulatory complexes and other transcriptional machinery to the DNA template. On the other hand, the presence of HDACs controls the amount of acetylation by opposing the action of HATs, thereby regulating the extent to which transcription can occur (6).

HDAC Classification and Mechanism of Action

The primary role of HDACs is to oppose the activity of histone acetyltransferases (HATs) balancing the level of acetylation of the histones. HDACs are enzymes that catalyze the removal of the acetyl modification on lysine residues of proteins, including the core nucleosome histones H2A, H2B, H3 and H4. We recognize four classes of these enzymes: class I includes HDAC 1, 2, 3 and 8 (nuclear localization), class II includes HDAC 4, 5, 6, 7, 9 and 10 (exonuclear and nuclear localization), class III includes sirtuins (SIRT1-7) and class IV includes only HDAC 11, which exhibits features of both classes I and II. Classes I, II and IV HDACs share homology in both structure and sequence and require a Zinc (Zn^+) ion for their catalytic activity. Class III sirtuins share no similarities in their three-dimensional model or sequence with class I, II or IV HDACs and require a nicotinamide adenine dinucleotide (NAD^+) ion for their catalytic activity (Table I) (10). HDACs are localized either in cytoplasm or nucleus (11).

HDACs modulate the most important cellular processes (Table II) (8). It is known that the balance of acetylation of nucleosomal histones regulates the transcription of many genes. The repression of gene transcription is induced by a condensed chromatin structure associated with hypoacetylation of histones, whereas acetylated histones are associated with a less condensed chromatin structure and activation of transcription. Apart from histones, it has been shown that other acetylated proteins are substrates for the HDACs. These include NF-YA, p53 and GATA-1. There exists increasing evidence that alterations in HAT and HDAC activity occur in cancer. HAT activity can be disrupted by translocation, over-/ under-expression or mutation in a wide spectrum of cancers, including those of an epithelial and a hematological origin.

An imbalance in histone acetylation may lead to changes in chromatin structure and transcriptional deregulation of genes involved in the control of cell cycle progression, differentiation and apoptosis. Restraining HDAC activity and preventing histone deacetylation may favor their hyperacetylation, which leads to the unfolding of previously condensely ordered chromosome regions and promotes transcription factors combined with DNA. Thus, genes, which are inhibited, can now be expressed. If these genes belong to the family of oncogenes, then, cell proliferation is promoted and a tumor may arise. Therefore, a defect in the acetylation machinery appears to result in alterations of acetylation and perhaps the development of cancer. HDAC enzymes remove the acetyl group from the histone (hypoacetylation), thereby decreasing the space between the nucleosome and the DNA wrapped around it, thus lessening transcription factor access and leading to transcriptional repression (12). HDACs have been found to relate to aberrant transcription factors. As expected, it has been recently shown that HDACs/SIRTs are regulators of growth, differentiation and cell death (apoptosis). Inhibitors of these enzymes can induce arrest of growth, differentiation and apoptosis of many cultural transformed cells (13).

Indeed, modulation of expression levels of genes encoding HDACs (over- and/or under-expression) has been reported for different types of cancers. For example, over-expression of HDAC1 has been reported in gastric and HDAC2 and 3 in colorectal cancer. Additionally, the *HDAC5* gene reveals decreased transcription in colorectal cancer. Regarding class III HDACs, SIRT8 was found over-expressed in thyroid cancer, while *SIRT2* gene expression is down-regulated in human gliomas (14). Furthermore, it has been observed that HDAC7 is

over-expressed in pancreatic adenocarcinoma and HDAC6 is over-expressed in cutaneous T cell lymphoma (11, 15).

The catalytic domain of the HDAC is formed by a stretch of 390 aminoacids containing a set of conserved residues. The active site of the enzyme consists of a curved tubular pocket with a wider bottom. The removal of an acetyl group occurs *via* a charge-relay system, an important component of which is the zinc-binding site at the bottom of the pocket. The presence of a zinc ion at this site is an important factor in the mechanism of action of HDAC inhibitors (13, 16).

HDAC Inhibitors as Anticancer Agents

With the finding that HDAC inhibition is the cause of the selective differentiation of malignant cells, the field of HDACIs was deeply studied (17-19). Extensive research showed that HDACIs are chemical compounds that may block tumor cell proliferation by restoring the balance of histone acetylation, thus resulting in proper gene expression. These compounds are potent inducers of growth arrest, apoptosis and/or differentiation of transformed cells *in vitro* and *in vivo*. They can also urge cancer cells to apoptosis, whereas normal cells are relatively resistant to HDACI-induced cell death (20).

The field of HDACIs is moving into a new phase of evolution. The exponential growth in the level of research activity surrounding the HDACIs witnessed over the past decade has now started to achieve successful results in clinical practice, particularly in the field of oncology. Although the study of HDACIs is a new field, an impressive body of data describes the ability of these molecules to modulate a wide variety of cellular functions, including cell differentiation and proliferation, apoptosis, cytoskeletal modifications and angiogenesis (8, 21).

HDACIs are grouped into four main classes based on their structure (hydroxamic acid, cyclic tetrapeptide, benzamide, aliphatic acid) (22). Trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA) and sodium butyrate (NaB) are commonly studied HDACIs (23). These inhibitors induce cell growth arrest and apoptosis in a broad spectrum of transformed cells. Because of these characteristics, HDACIs are being tested in clinical trials for cancer therapy (24).

Several HDACIs are already used in clinical trials and have shown significant activity against a spectrum of both hematological and solid tumors at doses that are well-tolerated by patients. Two HDACIs, SAHA and romidepsin, have been approved by FDA for the treatment of cutaneous T-cell lymphoma (8, 23). Other HDACIs in clinical trials include LAQ-824, a hydroxamate, and a sulfonamide derivative called PXD-101. Most compounds inhibit class I and class II HDACs at nanomolar concentrations. The cyclic peptides are the most structurally complex group of HDACIs. The natural product depsipeptide (also known as FK-228) is currently being tested in phase I and II clinical trials. Other HDACIs include the aliphatic acids, such as phenylbutyrate and its derivatives valproic acid and MS-275, which are in phase I and II clinical trials (25, 26).

However, HDACIs as monotherapeutics are only effective in a defined subset of hematological tumors. There is considerable evidence showing that rational- and molecular-defined HDACI-based combination therapies are more useful for the treatment of solid cancers. Overall, the field of HDACIs is relatively new and unexplored. Early successful results, such as those with SAHA and romidepsin, have set the bar high for possible future drugs and revealed the wide potential of the activity of the HDACs' (7).

Table III. *Molecular actions of HDACIs in pancreatic cancer.*

TRICHOSTATIN A (TSA) (pan-HADCI)
• Increased p21 and p27 levels, decreased cyclin B1 levels and induction of G2/M-phase cell cycle arrest (42-44)
• Increased levels of BIM, decreased levels of MCL1, BCL(W), BCL(XL) and induction of caspase-dependent apoptosis (30, 42, 43, 45)
• E-cadherin expression in mesenchymal cells (46)
• Synergistic action with oxaliplatin, gemcitabine, bortezomib, gefitinib and CPT11 (30, 31, 47, 48)
PANOBINOSTAT (LBH-589) (pan-HADCI)
• Increased p21 and p27 levels, decreased cyclin B1 levels and induction of G2/M-phase cell cycle arrest (49, 50)
• Induction of apoptosis (49, 51)
• Down-regulation of CHK1 (52)
• Inhibition of PI3K/mTOR pathway (50)
• Synergistic action with gemcitabine (49)
SUBEROYLANILIDE HYDROXAMIC ACID (SAHA) (pan-HADCI)
• Increased p21 levels, decreased cyclin B1 levels and induction of G2/M-phase cell cycle arrest (53)
• Induction of apoptosis (53, 54)
• Synergistic action with gemcitabine and SANT-1 (55, 56)
BELINOSTAT (PDX-101) (pan-HADCI)
• Increased p21 levels (57)
• Induction of apoptosis (58)
• Inhibition of PI3K/mTOR pathway (32)
• Synergistic action with gemcitabine (57)
SK-7041 (class 1 HADCI)
• Increased p21 levels, decreased cyclin B1 levels and induction of G2/M-phase cell cycle arrest (43)
• Induction of apoptosis (43)
• Decreased levels of BCL(XL) and MCL1 (43)
SODIUM BUTYRATE (class 1 HADCI)
• Induction of FAS-mediated apoptosis (59)
• Decreased levels of BCL(XL) (59)
• Synergistic action with gemcitabine (60)
FR901228 (class 1 HADCI)
• Increased p21 levels and induction of G2/M-phase cell cycle arrest (61)
• Induction of caspase-dependent apoptosis (61)
• Decreased levels of surviving (61)
VALPROIC ACID (VPA) (class 1 HADCI)
• E-cadherin expression in mesenchymal cells (46)
• Synergistic action with etoposide (62)

HDAC Inhibitors in Pancreatic Adenocarcinoma

Pancreatic cancer cells are highly effective in escaping cell death by up-regulation of pro-survival and inhibition of pro-death pathways. Progress in the therapy of pancreatic cancer may come from many areas, including a better understanding of survival mechanisms that allow pancreatic cancer cells to escape cell death. Cell transformation to tumor cells involves the silencing of tumor suppressor epigenetic alteration, which includes DNA hyper-/hypo-methylation of promoter CpG islands and/or changes in associated post-translational histone modifications leading to transcriptional repression (27).

HDACI-based combinations are particularly important in the treatment of PDAC. A recent phase II clinical trial disproved the effectiveness of the weak HDACI CI-994 combined with the current standard chemotherapy, which includes GEM (20). Recently, it was shown that specific depletion of HDAC2, but not HDAC1, sensitizes PDAC cells towards tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-induced apoptosis suggesting a new therapeutic strategy (28). TRAIL is included in the tumor necrosis factor (TNF) superfamily and is responsible for the induction of apoptosis of many cancer cell lines (23).

HDAC inhibitors induce apoptosis in pancreatic adenocarcinoma cell lines irrespective of their intrinsic resistance to conventional anti-neoplastic agents. This is a result of a serine protease-dependent and caspase-independent mechanism. Initially, HDACIs increase BAX protein levels without affecting BCL-2 levels. Then, the apoptosis-induced factor (AIF) and Omi/HtrA2 are released from the mitochondria inducing programmed cell death.

Several HDACIs have been studied against pancreatic cancer cell lines. TSA can strongly inhibit the cellular growth of pancreatic cancer cell lines. The mechanism underlying this effect consists of cell-cycle arrest at the G_2 phase and apoptotic cell death. HDACI FK228 strongly inhibits the growth of five human pancreatic adenocarcinoma cell lines through cell-cycle arrest and apoptosis; this is followed by caspase-3 activation, surviving degradation and p21WAF1 cleavage. FK228 seems to be clinically useful for the treatment of refractory pancreatic cancers (9).

The ability of HDACIs to synergize with classic chemotherapeutic agents, as well as newer signal transduction pathway modulators and angiogenesis inhibitors, has become a highly appreciated quality. In contrast to the current wave of targeted-therapies, the utility of HDACIs could span multiple cancers and be used alongside a broad range of therapeutics. This is a similar function to that advanced for lead apoptosis modulators. However, the additional effects of HDACIs on cell-cycle progression and angiogenesis should make HDACIs the partner of choice in combinatorial approaches to cancer (21).

According to a recent survey, histone modification levels indicate that patients who suffered from pancreatic cancer and were treated with adjuvant chemotherapy were more or less likely to derive a significant survival benefit from adjuvant fluorouracil when compared with GEM. Although the differences were moderate requiring further validation, they increase the possibility that histone modification levels could serve as predictive biomarkers for adjuvant treatment.

In human pancreatic adenocarcinoma cell lines, TSA and SAHA induce cell death by apoptosis and are caspase-independent (29). Other investigations have shown that TSA can synergize with GEM (30) or the proteasome inhibitor PS-341 (31) to induce apoptosis of human pancreatic cancer cells. Interestingly, it has been demonstrated that Class III HDACIs, such as sirtinol and nicotinamide, may also induce pancreatic cell death. Despite that, whether these HDACIs are efficient *in vivo* anticancer drugs has yet to be proved (20).

Belinostat and panobinostat are in the same group of HDACIs as TSA and SAHA (hydroxamic acid HDACIs). Chien *et al*. show that belinostat and panobinostat may be antitumor agents for some human pancreatic cancer cells potentially able to suppress proliferation of human pancreatic cells by multiple molecular pathways (32, 33).

The anticancer actions of HDACIs are also attributed to transcriptional inhibition of certain genes that are mainly related to histone acetylation. N-myc downstream regulated gene 1 (*NDRG1*) has been described as a potential tumor suppressor gene in various human cancers and may be associated with tumor's grade of aggressiveness and possibility for metastasis. NDRG1 expression in pancreatic cancer is linked to cellular differentiation and patient survival. *NDRG1* promoter contains a large CpG island augmenting the possibility that it could be silenced by DNA hypermethylation in cancer. Several HDACIs enhance NDRG1 expression in human colon cancer cell lines. In a mouse embryonic cell line, TSA was shown to repress the inhibition of *NDRG1* by N-myc/Max, thus potentially enhancing *NDRG1* transcription too. It became clear, moreover, that NDRG1 expression can be regulated by the pharmacologic inhibition of DNA methylation and histone deacetylation in pancreatic cancer cells (26).

As has also been demonstrated, under hypoxic conditions, various cell lines *in vitro* (malignant and primary) exhibit increased expression of *HDAC1*, *HDAC2* and *HDAC3 mRNA*, as well as HDAC1, HDAC2 and HDAC3 protein levels. Furthermore, experiments showed that over-expression of HDAC1 mediated the reduction of the p53 and pVHL expression. The suppression of these two antioncogenes resulted in the over-expression of HIF-1a and VEGF, which was inversed by the use of TSA. Mahon *et al*. also proved that the reduction in p53 and pVHL expression resulted in reduction of factor inhibiting HIF-1a (FIH) as well, thus allowing the stimulation of angiogenesis in endothelial cells. This means that

HDACs indirectly regulated HIF-1a activity under hypoxic conditions. With the use of several methods, it was shown that HDACIs could lead to increased interaction and degradation of HIF-1a by Hsp70, thus inhibiting tumor angiogenesis (34).

Numerous studies have been conducted for the identification of potential biomarkers, such as *HDAC* and *SIRT* genes, which are highly expressed in pancreatic cancer compared to normal pancreas. So far, an increased number of *SIRT5* mRNA transcripts have been observed in most of the pancreatic adenocarcinoma samples. Approximately 81% of the PDAC tissue samples displayed an increased expression of *HDAC7* mRNA transcripts and its corresponding protein. Although it is difficult at this stage to determine whether the up-regulation of HDAC7 in PDAC is a cause or a consequence of malignant progression, its over-expression in cancerous tissues and not in their normal counterparts is a promising field of future research for new approaches in the design of antipancreatic cancer therapy (14, 15).

Finally, of crucial importance is the fact that many clinical studies, in which HDACIs are used, have not shown any antitumor effect because of the HDAC pleiotropic activities. Peulen *et al*. suggest that simultaneous inhibition of cyclooxygenase-2 and HDAC may have a better result than the exclusive use of HDACIs (15). In addition, there is a high rate of combining HDACIs with other anticancer agents to improve the therapeutic result in various cancer types (35-40) or combining inhibitors of HDACs from different classes (41).

Conclusion

Well-designed trials are important to fully exploit HDACIs agents. The use of surrogate markers of activity would be an extremely effective method to identify the optimal clinical application of HDACIs. The fact that two HDCAIs (SAHA and romidepsin) have already been approved as anticancer agents and several others are in the early stages of clinical trials means that we can anticipate new studies in the near future as to their efficacy and potential clinical application.

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