The development and potential clinical utility of biomarkers for HDAC inhibitors

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ABSTRACT: Drug discovery has always been a complex process including many phases from target validation to clinical development. Data from the Food and Drug Administration (FDA) has estimated that the elimination rate for investigational new drugs entering clinical trials is up to 80%. In recent years, many kinds of biomarkers have been used to predict response in cancer treatment and for evaluation of new drugs. By increasing the understanding of histone deacetylase (HDAC) inhibitors cellular mechanism of action, we have elucidated how HDAC inhibitors exert their effect by the use of proper biomarkers. In this paper, we mainly focus on the development and potential clinical utility of HDAC inhibitor biomarkers.

Keywords: Histone deacetylase, inhibitors, biomarker, cancer

1. Introduction

With the goal of finding mechanism-based agents, drug researchers have re-invented their agents during drug discovery and development in cancer research in recent years. They have tried their best to increase potency, improve tumor selectivity and reduce toxicity of these novel agents compared to classic cytotoxic drugs. D. Hanahan has pointed out that cancer has six hallmarks: insensitivity to growth inhibitory and differentiation signals, self-sufficiency in proliferative growth signals, evasion of apoptosis, acquisition of limitless replicative potential, induction of angiogenesis and induction of invasion and metastasis (1).

There are many clinically successful anti-cancer agents that target pathways which have something to do with the six hallmarks of cancer cells mentioned above, but we have to admit that these drugs are suitable for just a small number of patients despite their success. Development of anti-cancer agents to treat a wider population of cancer patients is particularly important. Data from the Food and Drug Administration (FDA) has estimated that the elimination rate for investigational new drugs entering clinical trials is up to 80% (2). The key question is therefore, how could cancer drug discovery be improved so that we can raise the success percentage of drug discovery in clinical trials? One feasible approach is to develop predictive biomarkers to help us identify responsive tumors.

Histone deacetylase (HDAC) is a good anti-cancer target. Until now, three drugs have been approved and more than 20 are in clinical studies. In many of the clinical trials underway, biomarkers are being assessed to elucidate how HDAC inhibitors exert their effect. In this paper we will focus on HDAC inhibitors and the development, and potential clinical utility of biomarkers.

2. HDAC biology

HDACs remove the acetyl moieties, which are transferred by the cofunction of histone acetyltransferases (HATs) and the cofactor acetyl-CoA, from the ε-amino groups of lysine residues present within the N-terminal extension of the nucleosomal histones. They belong to a family of metalloproteases found in bacteria, fungi, plants and animals.

While the function of HATs leads to a more open form of chromatin, the so-called euchromatin, the HDACs counteract the HATs and in turn lead to an increased positive charge of histones, so that the histones react with the negatively charged DNA and block the access of transcriptional machinery to the DNA template (3–6).

In this way, HDACs lead to a more condensed form of chromatin, the so-called heterochromatin, and gene silencing. HDACs work in concert with co-activators, corepressors, transcription factors and HATs to change the structure of histones and modulate transcription of genes (7,8).
3. HDAC family

So far, eighteen HDACs have been identified in humans, which are subdivided into four structurally and functionally different phylogenetic classes according to their homology to yeast HDACs, their subcellular location, their tissue specificity and their enzymatic activity (Figure 1) (9,10). The Class I HDACs (HDAC 1, 2, 3, and 8), which are generally nuclear, ubiquitously expressed in various human tissues, are closely related to yeast RPP3 protein. Class II HDACs (HDAC 4, 5, 6, 7, 9, and 10), which are selectively distributed among tissues, share domains with yeast HDAC-1 (11,12). Class IV HDACs (only comprising HDAC-11), which localize in the nucleus, exhibit properties of both Class I and Class II HDACs (13), but the overall sequence similarity is too low to be placed in either class (14).

All the above HDACs are zinc dependent proteases. The Class III HDACs (Sir 1-7), which are homologues of the yeast protein Sir 2, require the cofactor NAD+ for their deacetylase function, and are not targeted by the currently available HDAC inhibitors.

4. Alteration of HDACs

Alteration of HDACs has been found in both hematological malignancies and solid tumors for a long time (15). Genes coding for HDACs have been always found normal in such cancer cells (16), but altered expression and aberrant recruitment of HDACs in tumors have been found. In colon, breast, prostate, thyroid, cervical, and gastric cancers, some HDACs such as HDAC1, HDAC2, HDAC3, HDAC6, and SIR 7 have been found overexpressed (17,18). Aberrant recruitment of HDACs results from chromosomal translocations has been found to have a causal role in tumorigenesis. For example, the retinoic acid receptor (RAR) is an important component in the differentiation pathway in myeloid cells, in acute promyelocytic leukemia (APL). The aberrant promyelocytic leukemia (PML)-RARα fusion protein, which is generated by chromosomal translocation, recruits HDACs to RARα target genes, and this leads to constitutive repression of these target genes (17-19). The translocation, commonly found in acute myelogenous leukemia (AML), generates a fusion protein containing N-terminal AML1 and C-terminal ETO amino acids. Normal AML1 is required as a transcription factor for differentiation of hematopoietic cells. The fusion protein AML1-ETO, which is formed by translocation, recruits HDACs to AML1 target genes and constitutively represses their expression (17-19). A transcription repressor LAZ3/BCL6 (lymphoma-associated zinc finger 3/B cell lymphomas 6) is overexpressed in non-Hodgkin’s lymphoma resulting in recruitment of HDACs (such as HDAC2) to target genes, leading to the repression of specific genes such as growth regulatory genes and so on (18,20). These fusion proteins, which eventually lead to tumorigenesis, are generally transcription regulators that repress their target genes (genes encoding proteins for cell differentiation or tumor suppression) through the aberrant recruitment of HDAC.

5. Biomarkers for HDAC-targeted drug development

HDAC inhibitors were found to have an anti-tumor function as a novel therapeutic class of drugs in many types of cancers (21-25). According to their chemical structure, these inhibitors can be subdivided into four different classes, including hydroxamates, cyclic peptides, aliphatic acids and benzamides (19). Suberoyl anilide hydroxamic acid (SAHA) was the first approved HDAC inhibitor for clinical treatment of T cell lymphoma by FDA. Among these four different classes of HDAC inhibitors, there are 13 hydroxamates including SAHA, LBH-589, resminostat, PXD-101, ITF-2357, SB-939, AR-42, R306465, CRA024781, CUDC-101, JNJ-26481585, CHR-3996, and CHR-2845; 1 cyclic peptide romidepsin; 4 benzamides including entinostat, mocetinostat, tacedinaline, and chidamide; 3 aliphatic acids including valproic acid, sodium phenylbutyrate, and AN-9 (26) undergoing clinical trials (including those that have been approved by FDA). Their structures and clinical phases are shown in Figures 2 and 3.

Among all the HDAC inhibitors there are three HDAC inhibitors in total approved by FDA so far, including SAHA approved October 2006 for the treatment of advanced forms of cutaneous T-cell lymphoma (CTCL), romidepsin (FK228) approved 6
Figure 2. Hydroxamate HDAC inhibitors approved and undergoing clinical trials.

Figure 3. Cyclic peptide, aliphatic acid and benzamide HDAC inhibitors approved and undergoing clinical trials.
November 2009 for CTCL and resminostat approved 6 September 2011 for Hodgkin’s Lymphoma (26).

5.1. Histone acetylation

The most extensively used biomarker in HDAC inhibitor trials to date has been histone acetylation, in particular H3 and H4. Preclinical and clinical studies have shown that there are several advantages of measuring histone acetylation. First, histone acetylation is a direct downstream modification regulated by HDAC, which can be detected within the tumor tissue. Second, histone acetylation can be measured in peripheral blood mononuclear cells (PBMCs), which is often taken as a surrogate tissue for tumors where biopsies are unobtainable without invasive procedures (27). In research conducted by Melinda and coworkers, they found that, in human subjects, a dose of 68 g of Brussel Sprouts inhibited HDAC activity significantly in PBMCs 3 and 6 h following consumption. Their findings provide evidence that one mechanism through which sulforaphane (a novel dietary HDAC inhibitor) acts as a cancer chemoprevention agent in vivo is through the inhibition of HDAC activity. Such findings maybe have profound historical significance that HDAC activity in PBMCs may be a potential biomarker for assessing exposure to novel dietary HDAC inhibitors in human subjects (28).

The use of the biomarker for hyperacetylation of histones (both in blood lymphocytes and tumor cells) has been useful as a guide to target specificity in early studies of HDAC inhibitors, and this biomarker has been the most extensively developed so far. Changes of this biomarker can be determined via Western blot, flow cytometry analysis or immunohistochemical methods. V. Novotny and coworkers produced a sensitive Western blot assay to quantitate histone H3 acetylation on lysine 9 and 14 which was developed to measure target efficacy of SB939 (29).

This biomarker was used in many clinical trials. Hyperacetylation of target proteins was detected in basically all patients treated with an HDAC inhibitor, but a dose-dependent and time-dependent increase in acetylation levels could be observed at least. Because of this fact drug effects were found to be reversible at the lowest dose levels and histone acetylation returned to basal levels within 2 h of drug infusion, while at higher doses, histone H4 acetylation was found to plateau. Consequently, at the maximum tolerated dose (MTD), further increases in histone acetylation were not observed in a phase I trial using PXD101 (30). Though higher doses did not produce an increase in the level of histone acetylation, a longer duration of hyperacetylation was observed. Moreover, hyperacetylation was shown in posttreatment tumor biopsies, although no correlation between acetylation status and tumor response was reported. So far, this and numerous other studies have failed to show a correlation between the level of hyperacetylation and response, and although hyperacetylation of blood lymphocytes is a useful biomarker to show that HDAC inhibitors hit the point (the so called target), it is likely that there are numerous other targets and mechanisms of response and resistance that impact an antitumor effect.

5.2. Induction of p21, HSP90

p21 is also commonly used as a biomarker of HDAC inhibition in clinical trials. According to the study of Arts and coworkers, it was found that numerous HDAC inhibitors, including R306465, SAHA, PXD101, and MS-275, increased the levels of p21 in a concentration-dependent manner (31). It has also been possible to measure HSP72, a protein related to HSP90, which is induced upon inhibition of HSP90 (when HDAC6 is inhibited) and also c-Raf, an HSP90 client protein, the levels of which decrease when HSP90 is unable to function in response to cell stress (32,33). In a phase I pharmacokinetic and pharmacodynamic study of LAQ824, thirty-nine patients were treated at seven dose levels (mg/m²): 6 (3 patients), 12 (4 patients), 24 (4 patients), 36 (4 patients), 48 (4 patients), 72 (19 patients), and 100 (1 patient). Dose-escalation used a modified continual reassessment method. Peripheral blood mononuclear cell lysates showed consistent accumulation of acetylated histones posttherapy from 24 mg/m²; higher doses resulted in an increased and longer duration of pharmacodynamic effect. Changes in HSP90 client protein and HSP72 levels consistent with HSP90 inhibition were observed at higher doses (33).

5.3. HDAC enzyme activity

Bonfils and coworkers reported in a paper to determine the pharmacodynamic effects of an isotype-selective inhibitor of HDAC-MGCD0103 in preclinical models and patients with a novel whole-cell HDAC enzyme assay in 2008 (34). This biomarker counts on the measurement of HDAC enzyme activity in living cells. The substrate they used, which is converted by HDACs, is a small, cell-permeable molecule. The deacetylated substrate is then fixed to a fluorophor with a longer wavelength shifted emission and a lysine moiety by a protease like trypsin, in which way they can quantitate the fluorescence intensity to show the correlation. The first results obtained in this way indicated that the measurement of the HDAC enzyme activity seems to be a biomarker with a greater dynamic range than the former biomarker of histone acetylation levels (35). There is still a lot work to be done to determine whether a correlation between the HDAC enzyme activity and the therapeutic response exists or not.
5.4. The gene signatures

Recently, some groups put forward the imagine that the gene signatures could be determined to reflect the response to an HDAC inhibitor treatment. Stimson and coworkers showed that there are indeed distinct changes in gene expression of some genes (36) during treatment with HDAC inhibitors. Consequently, La Bonte and coworkers treated two different colon cancer cell lines with vorinostat and panobinostat and had the same conclusion (37).

A study was carried out on a group of six patients with CTCL, who were treated with orally administered LBH-589. The biopsies taken from them were then analyzed by DNA microarrays. The results showed that distinct gene expression profiles can be observed as time progresses. A total of 23 genes revealed statistical significance, and these genes included some involved in angiogenesis, apoptosis and immune regulation. Four of these genes, including two angiogenesis related genes: GUCY1A3 (guanylate cyclase 1A3) and angiopeptin-1 (endothelial Tie2/Tek ligands ANGPT1) and two cell progression genes: CCND1 (cyclin D1) and NR2F2 (transcription factor COUP-TF II). The DNA microarray studies above are just a small example of the vast literature on HDAC inhibitors. Although it is possible to identify a gene signature for HDAC inhibitors, the signature is likely to vary because of tumor type difference, duration of exposure and inhibitor concentration. Lots of work still needs to be done to identify gene changes that can be used as a prognostic signature rather than just a response signature of HDAC inhibitor effects for future clinical trials.

Due to the multiple roles of the HDAC enzymes in different pathways, it may be questionable whether a defined gene signature can be identified at least for a certain HDAC subtype selectivity profile. However, Monks and coworkers gave evidence to negate the above questions. They found that gene signature is selectively induced by HDAC inhibitors compared to classical anti-cancer agents in a study based on a microarray of Belinostat (38).

5.5. HDAC enzymes expression

HDAC inhibitors can inhibit the HDAC enzymes, so that the expression of HDAC enzymes themselves was suggested to serve as a predictive biomarker. It should be the most direct method as a biomarker.

In view of that HDAC enzymes themselves are linked to tumorigenesis, therefore it is plausible to identify responsive tumor types by measuring levels of HDAC enzymes. Immunohistochemistry (IHC) is the easiest way to compare and contrast HDAC levels so far, and is able to identify cell localization and tissue distribution. Weichert and coworkers found that Class I HDACs were highly expressed in the nuclei of many patients in a study containing a cohort of 140 colorectal carcinomas. The results are as follows: HDAC1 (36.4%), HDAC2 (57.9%), and HDAC3 (72.9%). It was also found that HDAC enzyme expression was highest in proliferating, dedifferentiated tumor cells, which correlated with patients that had reduced survival times (39). A subsequent study in 192 prostate carcinomas by IHC (the data: HDAC1 (69.8%), HDAC2 (74%), HDAC3 (94.8%)) confirmed the conclusion above (40). These observations imply that assessing HDAC enzyme levels in patients may help identify patient sub-groups who will benefit from HDAC inhibitor treatment.

CTCL has been shown to be the malignancy most responsive to HDAC inhibitors to date (41). HDAC1, HDAC2 and also the Class II enzymes, HDAC6 were analyzed along with histone H4 acetylation to prove if they were of prognostic value in a panel of 73 CTCL biopsies. It was concluded that in CTCL, high expression of HDAC2 and histone H4 acetylation were more common in aggressive CTCL compared to indolent forms of the disease. HDAC6 expression was the only HDAC enzyme whose high expression was correlated to a favorable outcome independent of CTCL subtype. These IHC investigations indicate that assessing HDAC enzyme levels in patients may help us to identify patient sub-groups. However, for that to be clinically possible, a more comprehensive analysis of measuring HDAC enzyme levels across a variety of tumor cells will be necessary because different tumor cells are likely to be dependent on specific HDAC enzymes (32).

5.6. Predictive biomarkers: HR23B

Fotheringham and coworkers found that the protein HR23B had the ability to sensitize tumor cells to HDAC inhibitors in a genome-wide loss-of-function screen (42). Almost at the same time Chen and coworkers showed us that HR23B plays a role in shutting ubiquitinated cargo proteins to the proteasome (43). In an attempt to identify predictive biomarkers, a genome-wide loss-of-function screen using shRNA of 8000 genes identified a group of genes that when silenced in the tumor cell prevented HDAC inhibitor-induced apoptosis (42). Then in 2010, Omar Khan and coworkers evaluated the role of HR23B in CTCL cells. The results showed that HR23B governs the sensitivity to HDAC inhibitors of CTCL cells. Furthermore, through a mechanism dependent upon HR23B, proteasome activity is deregulated in HDAC inhibitor-treated CTCL cells. Through an analysis of a unique collection of CTCL biopsies taken from a phase II clinical trial the predictive power of HR23B for clinical response to HDAC inhibitors was investigated. In such clinical trials there was a frequent coincidence between
HR23B expression and clinical response to HDAC inhibitors (44).

5.7. Tolerances to oxidative stress

During a phase I clinical trial, Garcia and coworkers found a relationship between increased tolerance to oxidative stress and SAHA resistance (45). Another investigation using cDNA microarray analysis performed during a phase I clinical trial of SAHA in patients with advanced leukemia, revealed an upregulation of expression of genes mainly coding for antioxidants in SAHA resistant patients. The same results were also found in an HDAC inhibitor-resistant leukemia cell line by the same group (46). Furthermore, they found that addition of β-phenylethyl isothiocyanate, a compound that causes a decrease in cellular glutathione levels, resulted in enhanced toxicity of SAHA in leukemia cell lines and primary leukemia cells. Thus, the combination of an HDAC inhibitor with an inhibitor of the antioxidant pathway may sensitize non-responder patients to an HDAC inhibitor therapy.

5.8. Level of phosphocholine (PC)

Studies from Christopher and coworkers reported that PC, which can be detected by magnetic resonance spectroscopy (MRS), is elevated following SAHA treatment (47). They investigated the response of SAHA on MCF-7 breast cancer cells to monitor choline uptake and phosphorylation to PC using 13C MRS. The cancer cells were treated with 10 μM SAHA. Such a dosage can lead to a 50% inhibition in cell proliferation. The results showed that the level of PC synthesis was significantly higher (54 ± 19% of control) in treated cells (48). Although such a finding is promising, the use of PC as a validated biomarker still needs a lot of work to understand the mechanism of metabolic modulation (47).

6. Conclusion and future perspectives

In many of the clinical trials underway, biomarkers are being assessed to elucidate how HDAC inhibitors exert their effect. By increasing understanding of HDAC inhibitors cellular mechanisms of action, several important biomarkers are summarized in the paper, including histone acetylation, induction of p21 and Hsp90, HDAC enzyme activity, gene signatures, HDAC enzymes expression, predictive biomarkers HR23B, tolerances to oxidative stress, and the level of PC.

By using proper biomarkers, identifying tumors and stratifying patients into groups that may undergo an improved clinical response to HDAC inhibitor-based therapy we can make an individual operative therapy for the benefit of the patients.

These biomarkers were used in many clinical trials but the correlation between the therapeutic response and the biomarkers or any other target proteins was not found precisely. So these biomarker in clinical trials have been questioned, and we have to admit that this is a very ill presage (49). While a nice bit of previous studies have focused on the use of biomarkers, the search for more clinically relevant biomarkers must be continued.

References

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