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Histone deacetylase inhibitors open new doors in cancer therapy

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Abstract

Cancer drug development has moved from conventional cytotoxic chemotherapeutics to a more mechanism-based targeted approach towards the common goal of tumour growth arrest. The rapid progress in chromatin research has supplied a plethora of potential targets for intervention in cancer. Here, we focus on the histone deacetylase (HDAC) inhibitors, together with their current status of clinical development and potential utility in cancer therapy. HDACs have been widely implicated in growth and transcriptional control, and inhibition of HDAC activity using small molecules causes apoptosis in tumour cells. We discuss the rationale for the development of HDAC inhibitors as novel anti-cancer agents, the potential clinical application and explore ideas on how we may move towards patient stratification with the possibility of increasing efficacy in the clinic.

Keywords: Histone deacetylase; Chromatin; Cell cycle; Acetylation; Transcription

1. Introduction

The accessibility of DNA to proteins such as transcription factors and their co-factors is determined in part by a series of chromatin modifying enzymes. The text book ''beads-on-a-string'' structure of DNA wrapped around nucleosomes can be further condensed into higher order chromatin, known as heterochromatin and euchromatin, the latter being more transcriptionally active and containing most protein encoding genes [1]. The nucleosome itself is made up of an octamer of histone proteins, two each of H2A, H2B, H3 and H4, with 145 bp of DNA wrapped around it (Fig. 1). Modification of the highly charged lysine residues in the N-terminal histone tail is one mechanism whereby chromatin condensation is controlled. These lysine residues are subject to modification by acetylation and methylation, which are post-translational modifications that have a considerable impact on transcriptional activity [2].

Despite the fact that histone acetylation was shown some years ago [3], the enzymes controlling acetylation have only recently come to light [4–6]. Histone deacetylases (HDAC) are evolutionarily conserved and expressed in organisms from archaebacteria to man and, together with the conversely acting histone acetyl transferases (HATs), control the acetylation level of chromatin and subsequent transcriptional activity (Fig. 2). HDACs remove the acetyl group from histones using a charge-relay mechanism consisting of two adjacent histidine residues, two aspartate residues and one tyrosine residue, and crucial for this charge-relay system is a Zn^{2+} ion, which binds deep in the pocket of the enzyme [7]. Inhibitors such as trichostatin A (TSA), SAHA and PXD101, function by displacing the zinc atom [7].

The HDAC family is divided into the Zn-dependent (Class I and Class II) and Zn independent, NAD-dependent (Class III) enzymes (Table 1). The Zn-dependent enzymes have been the focus of intense research, whilst the Sir2 (silent information regulator) family recently have been implicated in acetylation and regulation of key cell cycle proteins such as p53 [8]. The HDAC inhibitors described here are inhibitors of Class I and Class II enzymes. To date, eleven HDAC family members in Classes I and II have been characterised; HDACs 1, 2, 3, 8 are Class I and HDACs 4, to 7, 9 and 10 are Class II, a grouping based on sequence similarity [9]. The most recently identified member, HDAC 11, is most likely Class I, although the similarity is weak.

Ultimately, the expression and activity profile of each family member in normal relative to diseased tissue, or

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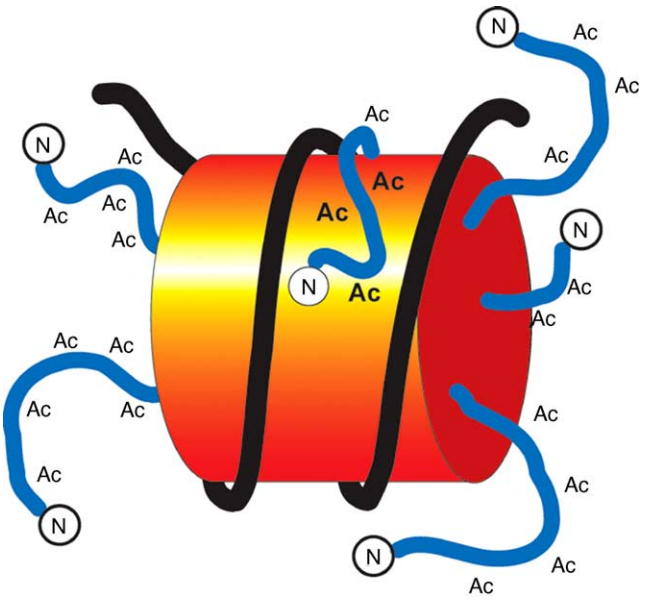


Fig. 1. Organisation of the nucleosome. Diagram of the nucleosomes, indicating the DNA (black) wrapped around the core histone octamer (orange). The position of the histone tails are indicated (blue) together with sites of post-translational modification (Ac) which give rise to the "histone code".

tissue-specific profiles, will be crucial information in the design of new therapeutic strategies. Important questions such as those relating to which family member to target for

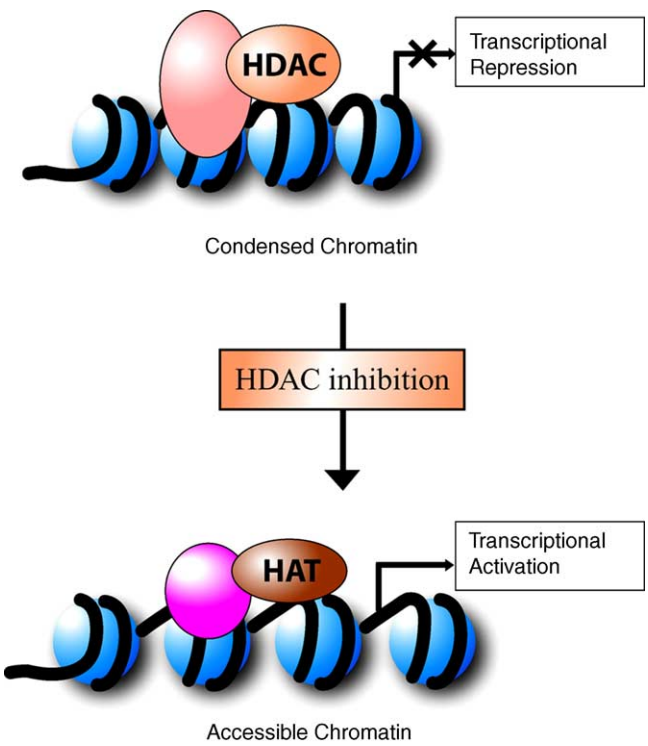


Fig. 2. Mechanism of action of HDAC inhibitors. HDACs remove the acetyl group from histones in nucleosomes which leads to condensed chromatin, whereas the conversely acting HATs add acetyl groups. HDAC inhibitors block HDAC activity, leading to increased levels of acetylation and accessible chromatin. Both HDACs and HATs are components of multicomponent protein complexes that interact with transcription factors.

Table 1
Classification of HDAC subtypes

Class I	Class II	Class III
Homologous of Rpd3	Homologues of Hda1	Homologues of Sir2, require NAD, not inhibited by TSA
HDAC 1	HDACs 4–7	SIRT1–7
HDAC 2	HDAC 9	
HDAC 3	HDAC 10	
HDAC 8		
HDAC 11		

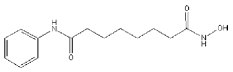
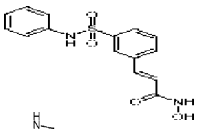
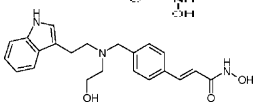
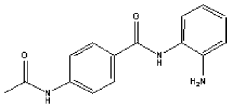
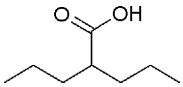
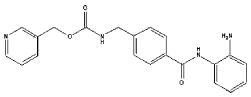
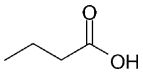
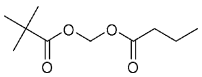
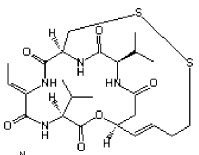
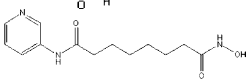
improved efficacy or reduced toxicity have yet to be addressed. Notably, the current HDAC inhibitors in clinical trials are generally regarded as pan HDAC inhibitors, on the basis of structural modeling, although with the caveat that robust enzyme assays that measure the activity of each family member have not yet been established. Against this background, a number of recent studies have allowed us to gain information about some aspects of the tissue-specific expression of HDACs; for example, HDAC 9 in heart, HDAC 7 in T cells, and HDAC 6 in breast [9–11]. In general, however, the expression of HDAC enzymes is fairly ubiquitous [9].

2. Chemistry strategies for the design of novel HDAC

The naturally occurring anti-fungal antibiotic TSA was one of the first HDAC inhibitors identified as an anti-proliferative agent, and although it has never progressed as a clinical candidate, has been an invaluable tool in validating HDAC enzymes as potential anti-cancer targets. TSA is a hydroxamic acid-based compound which fits into the HDAC active site, chelating the Zn^{2+} ion and inhibiting the enzyme at a low nM IC_{50}. Subsequent hydroxamic acid-based HDAC inhibitors, including SAHA, PXD101 and LAQ-824, are in clinical trials (Table 2) as anti-cancer agents and offer improved properties over TSA. The most advanced of these, SAHA, is currently in Phase II clinical trials and has demonstrated a well-tolerated safety profile in both intravenous and oral clinical studies [12]. Despite the relatively short half-life of these HDAC inhibitors, their pharmacodynamic effects are relatively long lived, perhaps suggesting a hit-and-run mechanism whereby a short exposure to relatively high concentrations of drug leads to a longer lasting anti-proliferative effect [13].

Less potent than the hydroxamates are the aliphatic acid derivatives such as valproic acid and butyrate (Table 2). Whilst in clinical trials for cancer and described as an HDAC inhibitor, valproic acid has been widely used as an anti-epileptic treatment and is a key regulator of many pathways in the cell [14]. A second natural product HDAC inhibitor is depsipeptide (FK-228), which is currently in Phase II trials for cutaneous T cell lymphoma [15,16]. This cyclic tetrapeptide was isolated from *Chromobacterium violceum* and needs to undergo intracellular reduction to

Table 2
HDAC inhibitors in clinical development

Molecule	Structure	Clinical trial status
SAHA		Phase II
PXD101		Phase I
LAQ-824		Phase I
CI-994		Phase II
Valproic acid		Phase I
MS-275		Phase II
Butyrate		Phase I/II
AN-9		Phase I/II
Depsipeptide (FK228)		Phase II
Pyroxamide		Phase I

generate the active HDAC inhibitor. Other cyclic tetrapeptides, such as apidicin and CHAPs, also represent potentially potent HDAC inhibitors [17,18]. Two further HDAC inhibitors in clinical development, MS-275 and CCI-994 (Table 2) are benzamide derivatives with only μM potency, although both are being studied in clinical trials [19,20].

3. Mechanism of action of HDAC inhibitors

There is a large body of literature which indicates that HDAC inhibitors block the cell cycle and induce apoptosis or differentiation depending on the cell-type and environmental factors [21–24]. It is consistent with the cell cycle arrest that SAHA regulates cell cycle control proteins, such as p21, p27, and gelsolin [25]. Whilst this may result from a transcriptional effect on the relevant target genes (Fig. 2), the activity of other proteins such as E2F, pRb, and p53 is directly influenced by acetylation and, moreover, proteins such as these are implicated in direct control of the cell cycle [26–28]. In this respect, it is important to note that although histones were the first proteins identified as targets of this enzyme family, and hence the name, a large and growing number of non-histone proteins in addition to the above are now known to be influenced by acetylation (Table 3).

Table 3
Non-histone substrates of HDAC enzymes

Substrate	Function
α-Tubulin	Cytoskeletal
Importin-α	Nuclear/cytoplasmic shuttling
β catenin	Cell adhesion/transcription
TCF	DNA binding
p53	Tumour suppressor
E2F	Transcriptional cell cycle
pRb	Tumour suppressor
Hmg1(y)	Transcription
Hsp90	Molecular chaperone
YY1	Transcription
Bcl6	Transcription
UBF	Transcription
Cart-1	Structural organisation
P50; relA	Transcription/inflammation
HIV-1 Tat	Replication
Rb	Cell cycle

HDAC inhibitors have been studied in transcriptional profiling by microarrays and, surprisingly, only a small percentage (∼2%) of mRNA transcripts are modulated by HDAC inhibitors. Therefore, in contrast to the idea of HDACs as master and global regulators of transcription, it appears that inhibition of HDAC leads to a fairly restricted alteration of gene expression profile which, in turn, may explain the apparent low toxicity seen in clinical trials to date [29,30]. To date, genes which are regulated by HDAC inhibitors, such as SAHA, suggest a pleiotropic effect on key pathways involved with proliferation, apoptosis, tumour suppressors, DNA synthesis and repair, and protein turnover [31].

4. Clinical utility of HDAC inhibitors

An important finding in predicting the potential utility of HDAC inhibitors in the clinic is their activity in cell-lines that are resistant to existing chemotherapeutics. For example, Gleevec-resistant Bcr/Abl human chromic myelogenous leukaemia (CML) cells are sensitised to Gleevec upon co-treatment with SAHA [32]. In addition, CD34-positive progenitor cells from patients with Gleevec refractory CML respond to SAHA treatment and exhibit increased apoptosis and histone acetylation levels [33]. In other examples, TSA enhances the expression of the oestrogen receptor in breast cancer cells, with subsequent re-sensitization to tamoxifen [34]. Similar combination studies where HDAC inhibitors have been combined with DNA methyltransferase inhibitors [35,36] suggest that HDAC

inhibitors may synergise with other inhibitors that target DNA regulating processes, altering the pattern of transcriptionally activate genes in favour of a drug sensitive profile [35]. These studies have potentially expanded the utility of HDAC inhibitors in clinical disease, and suggest potential widespread applicability in cancer in regulating drug resistance. Overall, it is generally regarded that HDAC inhibitors will be used as combination agents in the clinical environment.

5. Future development of HDAC inhibitors

The first generation of HDAC inhibitors in clinical trials (Table 2) have shown encouraging anti-tumour effects, with well-tolerated safety profiles. None of the agents in clinical trials have been developed to selectively target individual HDAC family members. Consequently, these agents are generally viewed as pan-HDAC inhibitors, although future experiments are required to substantiate this view. Nevertheless, there is considerable interest in developing molecules with selectivity towards individual family members, or more generally specificity for Class I or Class II enzymes. This has proven to be a difficult task since recombinant HDAC enzymes are frequently poorly active and in cells HDAC enzymes are usually found in multicomponent complexes [37].

Class I enzymes are primarily nuclear [38] and Class II enzymes generally shuttle between the nucleus and the cytoplasm [39]. An added level of complexity occurs in the interaction between different HDAC family members; for example, HDAC 6 and HDAC 11 associate in the cytoplasm [40]. Furthermore, a screen for HDAC 8 inhibitors [41] identified three compounds, namely Scriptaid (SB-556629), SB-429201 and SB-379872-A, the latter having a degree of specificity for HDAC 8, albeit with a relatively high IC50 (0.5 μM). Studies on existing HDAC inhibitors in Class I enzyme assays demonstrated that MS-275, currently in Phase II trials, inhibits HDACs 1 and 3 to varying degrees, but was inactive against HDAC 8 [42]. Further, the depsipeptide FK-228 has activity against Class I (HDACs 1 and 2) but not against Class II (HDACs 4 and 6) enzymes [43]. Thus, it appears that HDAC inhibitors which are already in clinical trials have some level of selectivity for HDAC enzymes. As further HDAC subunit assays are developed, the selectivity profile of other molecules may allow us to correlate anti-tumour specificity with HDAC enzyme selectivity and their expression profile.

There are also a number of molecules recently identified which have given insights into the roles played by individual HDAC subunits. The most appropriate examples of this are inhibitors that exhibit specificity for HDAC 6. The HDAC 6 subunit has some unusual properties in the context of the whole HDAC family; it is exclusively cytoplasmic, contains two HDAC catalytic homology domains, a ubiquitin-binding zinc finger and co-localises with components of the microtubule-associated dynein motor complex [44,45]. Interestingly, HDAC 6 is resistant to Trapoxin B (TPX) and sodium butyrate, whereas tubacin [46] inhibits HDAC 6 selectively. The finding that HDAC 6 is a tubulin deacetylase has allowed information to be gathered on HDAC 6 activity by measuring tubulin acetylation in comparison with histone acetylation [46]. Similar information on substrate specificity is not yet available for other HDAC subunits, although as HDAC inhibitors appear that exhibit selectively it may be possible to deduce similar levels of information.

A detailed study used siRNA to understand which HDAC isotypes are potential therapeutic targets. The levels of HDACs 1, 2, 3, 4 and 7 were knocked-down in HeLa cells, where HDACs 1 and 3 were identified as important in proliferation, arguing perhaps that Class I HDACs may be more relevant targets for intervention in oncology [37]. This hypothesis remains to be borne out in clinical conditions and we await the development of Class I and Class II selective drugs to address these fundamentally important questions.

6. Conclusions

Chromatin modifying enzymes have provided a number of important and increasingly validated therapeutic targets for oncology. There is now compelling evidence from the number of HDAC inhibitors in clinical studies that these molecules exhibit efficacy in human disease. It is the hope that their application, most probably in combination with chemotherapeutics, will lead to broad clinical utility in many tumour types. We can confidently anticipate that this field will continue to expand as researchers glean more information about new levels of HDAC biology, and their relevance to human disease.

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