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# Histone deacetylase inhibitors: gathering pace

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Reversible histone acetylation is one of the key mechanisms involved in the epigenetic control of gene expression. A variety of recent studies has revealed a role for acetylation in a much broader repertoire of physiological processes, including proliferation control and protein folding, and has highlighted how a variety of non-histone regulatory proteins are influenced by acetylation. Inhibition of histone deacetylase (HDAC) prompts tumour cells to enter apoptosis and, as a consequence, several HDAC inhibitors have entered clinical trials. It is likely that HDAC inhibitor drugs will provide an important class of new mechanism-based therapeutics for cancer.

## Addresses

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## Introduction

Epigenetic modifications are increasingly recognised as having a substantial role to play in both normal cellular physiology and disease processes, particularly in cancer where inappropriate gene expression has long been known to play a fundamental role in the aetiology of the disease. Histone deacetylase (HDAC) enzymes, which regulate the level of histone acetylation, are one of the major groups mediating epigenetic control. In mammalian cells, they are responsible for the deacetylation of N-terminal lysine residues in histones, particularly the core histones H2A, H2B, H3 and H4. Deacetylation of histones is associated with a relatively compact and inaccessible chromatin state, which generally correlates with lower gene transcription [1].

There has been substantial progress in the development of drugs that target epigenetic control processes as a new class of mechanism-based cancer therapeutics [2,3], and

one of the fastest moving areas is the development of HDAC inhibitors (HDACIs). Cell-based studies have shown that HDACIs are anti-proliferative agents, causing cell-cycle arrest, apoptosis and, in some cases, differentiation [2]; the anti-proliferative effects, particularly apoptosis, are far more pronounced in tumour cells than in normal cells. As a consequence, various HDACIs have entered, and in some cases completed, early clinical trials where, importantly, they have been found to exhibit a generally favourable toxicity profile [3]. Currently, the most advanced trials are completing the Phase II stages. However, and somewhat surprisingly given their rapid clinical progress, there remains significant gaps in our knowledge of how HDACIs exert their effects on cells. Questions relating to the critical intracellular targets of HDACs, their precise cellular roles and the downstream effects of inhibiting their activity are important issues that remain to be addressed [3]. A clearer understanding of the mechanism of action of this interesting class of drugs is likely to be informative in clarifying the clinical utility of HDACIs.

In this review, we discuss recent developments in dissecting the mechanism of action of HDACIs as a new group of mechanism-based anti-cancer drugs, together with current progress in understanding their clinical application.

## The histone deacetylase family

The HDAC family is divided into Zn-dependent (Class I and II) and Zn-independent, NAD-dependent (Class III) enzymes. Class I and II enzymes have been subject to intense research, whereas Class III enzymes — the sirtuins — have only recently been implicated in proliferation control [3]. From the known human HDAC enzymes (Table 1), HDAC6 and HDAC10 are unusual in having two catalytic domains, with both domains of HDAC6 being required for deacetylase activity [4]. HDAC6 preferentially targets non-histone substrates, including  $\alpha$  tubulin and Hsp90, and its activity is important for the function of the aggresome [5].

In the cell, HDACs mediate their effects on transcription as components of large multi-protein complexes, often involving more than one HDAC subunit and frequently in association with the co-repressors Sin3, nuclear co-repressor (NCoR) and silencing mediator for retinoid and thyroid receptors (SMRT) [3]. Current studies suggest the Class I HDACs, rather than the Class II enzymes, are more significant in regulating cell proliferation [6], and HDAC2 might be involved in suppressing apoptosis in cancer cells [7]. For some Class II HDACs, a more defined intracellular role has been established; for example, HDAC5 and HDAC9 function in cardiomyocyte

Table 1

## Characteristics of the human Class I and Class II HDACs

HDAC isoform <sup>a</sup>	Class	Size (kDa)	Cellular localisation	Evidence for cancer involvement?
1	I	22–55	Nuclear	Possible prognostic indicator (positive) in breast cancer [36] and lung cancer [37]
2	I	22–55	Nuclear	Upregulated in hormone-refractory prostate cancer [38], gastric cancer [39] and colorectal cancer [40]
3	I	22–55	Nuclear	Upregulated in colorectal cancer [40], gastric cancer [41], cervical dysplasia and invasive carcinoma [42**]
4	II	120–135	Nuclear/cytoplasmic shuttle	Loss of antigen-presenting cell expression in colorectal cancer leads to HDAC2 upregulation [4]
5	II	120–135	Nuclear/cytoplasmic shuttle	Upregulated in lung cancer [43] and a variety of solid tumours [44]
6	II	120–135	Nuclear/cytoplasmic shuttle	Not known
7	II	120–135	Nuclear/cytoplasmic shuttle	Downregulated in colon cancer and acute myeloid leukaemia [45]
8	I	22–55	Nuclear	Conflicting prognostic interpretations in breast cancer [46–48]
9	II	12–135	Nuclear/cytoplasmic shuttle	Not known
10	II	120–135	Nuclear/cytoplasmic shuttle	Not known
				Poor prognostic indicator in lung cancer [49]

<sup>a</sup> HDAC11 and the sirtuin proteins (SIRT1–7) differ markedly from the Class I and II HDACs. Unlike HDACs 1–10, the catalytic site of HDAC 11 is Zn-independent. HDAC11 and the SIRT proteins lie outside the scope of this review.

differentiation [8–10], whereas HDAC4 influences chondrocyte hypertrophy during skeletal development [11\*\*]. However, the degree of redundancy amongst the different HDAC proteins has been difficult to gauge, and this has been exacerbated by the lack of isoform-specific probes. For example, there is relatively little information available on the isoform specificity of the different inhibitors currently undergoing pharmaceutical development (Figure 1). Currently, our view is that the majority of HDACs are unlikely to be isoform specific [3,12]. It has been suggested, however, that, whereas the hydroxamic acid-based compounds, such as PXD101 and suberoylanilide hydroxamic acid (SAHA), inhibit the deacetylase activity of HDAC6, this is not the case for the majority of non-hydroxamates [12].

It is generally agreed that the rational design of isoform-specific inhibitors is a difficult task, as sequence comparison predicts a relatively high degree of similarity between the active site of different HDAC enzymes, especially within a given class. Rational design has been further hampered by the lack of crystal structures, as HDAC8 is to date the only mammalian isoform for which a crystal structure is available [13\*\*]. Several other inhibitors have been shown to exhibit subunit specificity, such as tubacin, which inhibits  $\alpha$ -tubulin deacetylase activity of HDAC6, without affecting the level of histone acetylation [14].

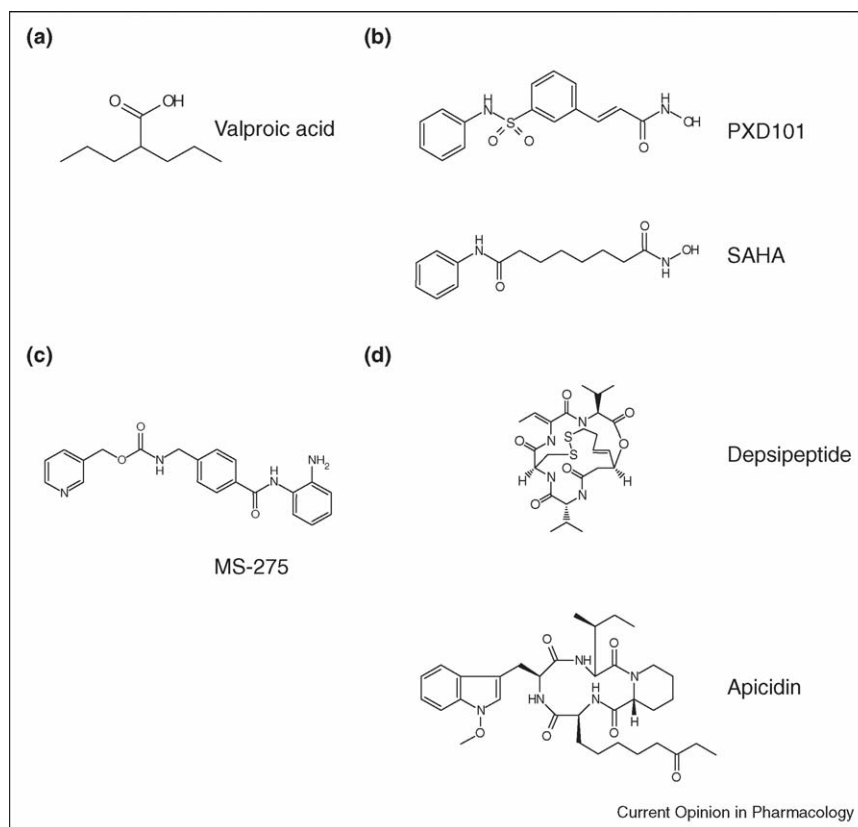
As already indicated, HDAC6 has dual substrate specificity, as it can deacetylate histones and tubulin, and has also been shown to deacetylate Hsp90 [14,15]. Indeed, it

is becoming increasingly apparent that HDAC enzymes are not exclusively directed towards histones, and that a variety of other proteins are now known to be regulated through acetylation (Table 2). Some of these proteins are themselves therapeutic targets in cancer, such as Hsp90, and their regulation by acetylation could highlight potentially important drug combinations to be pursued in the clinical context; in this respect, promising *in vitro* results have been obtained with the combination of SAHA and Hsp90 inhibitors [16,17]. Other proteins that take on important regulatory roles, such as pRb and p53, are also influenced by acetylation, and it is through these substrates that HDACs might mediate their anti-proliferative effects [3].

### Histone deacetylase inhibitors and cancer

Because of the profound anti-proliferative activity of HDACs, a variety of high potency HDACI compounds have reached clinical trials, where they are being assessed in a number of different cancer indications. A frequently argued rationale for their use has been that HDACs cause the de-repression of genes whose reactivation would promote an anti-proliferative outcome. One such example is the gene encoding the cyclin-dependent kinase inhibitor p21, which is upregulated by HDACI treatment and is responsible for cell-cycle arrest and differentiation [18]. Other genes upregulated by HDACI treatment include *TRAIL-R2* [19], *p19ARF* [20], *Bmf* [21] and *Rap1* [22]. HDACs also induce activation of the pro-apoptotic enzyme caspase-3 [23]; however, there are many examples of genes whose expression is

Figure 1



Structure of representative compounds from the major classes of HDACIs. **(a)** Small chain fatty acids: valproic acid is in Phase II oncology trials. **(b)** Hydroxamate small molecule inhibitors SAHA and PDX101 are both in Phase II oncology trials. **(c)** Non-hydroxamate small molecule inhibitors: MS-275 is in Phase II oncology trials. **(d)** Cyclic peptides: depsipeptide is in Phase III oncology trials.

downregulated in response to HDACI treatment, such as those encoding thymidylate synthetase [24], Bcr-Abl and c-Myc [25<sup>\*</sup>] and the interleukin (IL)-6 receptor [26]. Interestingly, HDACI treatment influences the expression of HDACs themselves: treatment of chronic myeloid

leukaemia cell lines with SAHA leads to a decrease in HDAC3 expression [25<sup>\*</sup>], whereas valproic acid treatment results in proteasomal degradation of HDAC2 [27]. This suggests a complex response to HDACIs. Although it is possible that these effects are all primarily mediated through general effects on chromatin, resulting in the activation of genes such as *p21*, it is equally possible that some of these effects occur through specific mechanisms involving the deacetylation of non-histone targets, such as p53 E2F and pRb [3].

Table 2

**Non-histone proteins shown to be direct substrates for HDAC enzymes.**

Substrate	Function	References
$\alpha$ -tubulin	Cytoskeletal	[1,50,51 <sup>**</sup> ,52]
$\beta$ catenin	Cell adhesion/transcription regulator	[53,54]
TCF	DNA binding	[55]
p53	Tumour suppressor	[56]
E2F	Transcription/cell cycle	[57,58]
Hsp90	Molecular chaperone	[59,60 <sup>*</sup> ]
YY1	Transcription factor	[61,62]
Bcl6	Transcription (oncogene)	[63,64]
UBF	Transcription factor	[65]
Rb	Cell cycle (tumour suppressor protein)	[66,67]

Bcl6, B cell lymphoma 6; Rb, retinoblastoma protein; TCF, T cell factor; UBF, upstream binding factor; YY1, Yin Yang 1.

Numerous groups have reported the results of microarray experiments, using unbiased approaches, to identify genes transcriptionally regulated by HDACIs in cancer cell lines [28–30]. In general, HDACI treatment leads to changes in RNA levels of a surprisingly small number of genes (typically 2–5% of the genome), and often as many genes are downregulated as are upregulated. One interpretation of these microarray studies is that transcription might not be the primary mechanism through which HDACIs mediate anti-proliferative effects, a view compatible with the increasing number of regulatory proteins that are being found to be subject to acetylation control.

### Clinical utility of HDAC inhibitors

The vast majority of anti-cancer drugs are used as combination therapies in the clinical setting. Thus, it is considered likely that HDACs will find their greatest utility not as monotherapies but as components of combination drug regimens. Identifying relevant drug combinations and responsive cancer types for which HDACs will be most efficacious is an important but complex task, and one that has to progress hand-in-hand with functional studies, ideally through the use of bio-markers, together with cellular and molecular approaches. For example, there is a strong mechanistic rationale for the use of HDACs in combination with retinoic acid, on the basis of the known interactions of the retinoic acid receptor/promyelocytic zinc finger (RAR $\alpha$ /PLZF) fusion protein and the Sin3/HDAC/NCoR/SMRT complex at the retinoic acid response element in acute promyelocytic leukaemia [31]. The downregulation of thymidylate synthetase induced by HDAC treatment suggests that the combination of HDACs and 5-fluorouracil might be of therapeutic benefit, as high thymidylate synthetase levels correlate with a relatively poor response to 5-fluorouracil [24]. HDACs have been shown to enhance the cytotoxic effects of camptothecin, a topoisomerase I inhibitor [32\*\*], and to potentiate DNA damage caused by topoisomerase II inhibitors [33]. Importantly, both of these studies highlight the potential impact of scheduling drug delivery in combination therapies, an area in which more work is required and which is likely to respond favourably to the application of bio-markers.

An important milestone in understanding the clinical utility of HDACs will come from the identification of bio-markers that predict tumour sensitivity patterns to HDACs. PRAME (originally identified as preferentially amplified in melanoma) is a dominant repressor of retinoic acid receptor signaling [34]. Recent research has suggested that its activity confers resistance to HDACs by acting as an 'HDAC-independent pathway' that leads to transcriptional repression and insensitivity to retinoic acid (René Bernards, personal communication). Melanoma cell lines express high levels of PRAME, and show reduced sensitivity to growth inhibition by HDACs [34].

The opposite type of marker — namely 'sensitivity' markers — has yet to be reported. One approach to tackling this issue was to pursue a genome-wide screen and, as a result, genes have been identified and implicated in regulating sensitivity to HDAC inhibition (La Thangue, unpublished). Although we must await the implementation of resistance and sensitivity genes in the clinical setting, it is a reasonable prediction that such bio-markers will have a significant impact on defining the clinical utility of HDACs. They will, for example, allow some of the most important outstanding questions to be addressed, such as the identification of responsive

tumours, and in the longer term could allow patients to be stratified into likely responder groups.

### Conclusions

HDAC inhibition as a therapeutic regimen in cancer is generating intense interest in both the scientific and medical arenas, with a number of potent compounds having demonstrated good safety profiles and hints of clinical activity. Efficacy has yet to be established although, based on the encouraging clinical results to date, it seems likely that HDACs will reach regulatory approval and become marketed drugs. However, the clinical application and response will be significantly improved both by a greater understanding of how HDACs kill cells and by the identification of bio-markers, which in turn could lead to optimised identification of target tumours, susceptible patients and appropriate drug combinations and scheduling. Indeed, a recent report suggests that HDACs may be more effective in tumours expressing mutant *p53*, rather than in tumours lacking this protein [35]. However, the clinical reality of predicting responses in humans is still far from being realised.

To some extent, the development of HDACs has swum against the tide of drug discovery, in which there has been a constant drive towards highly selective target-specific compounds. To date, the majority of HDACs appear to be fairly promiscuous in the HDAC proteins that they inhibit. Although more specific compounds would certainly provide interesting pharmacological tools for elucidating functions of individual HDACs, it is far from clear if they would have therapeutic advantages over the pan inhibitors already being pursued. Although it could be argued that a more specific compound would have an improved side effect profile compared with a pan inhibitor, this remains a theoretical assumption and has yet to be established in the clinical setting. Interestingly, although existing HDACs are predominantly pan-specific, the results from microarray analysis of the gene expression profile suggest that there are subtle differences in cellular responses, the mechanistic basis for which is currently unclear [28–30] but could reflect activity against different HDACs or cell line differences.

Finally, and in common with so many proteins, HDACs were so-named because histones were the first target substrates identified for these enzymes. It has become increasingly clear that this is a somewhat inappropriate designation, given the increasing number of non-histone targets. Even if it is too late to re-name these enzymes, perhaps it is time for a mental shift into thinking of them as PDACs — protein deacetylases.

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