Gene Ther Mol Biol Vol 8, 61-74, 2004

Histone deacetylase inhibitors: promising candidates for chemotherapeutic drugs

Review Article

Ralph K. Lindemann and Ricky W. Johnstone*

Gene Regulation Laboratory, Cancer Immunology Division, Peter MacCallum Cancer Institute, Trescowthick Research Laboratories, East Melbourne 3002, Victoria, Australia

***Correspondence**: Ricky W. Johnstone; phone: +61-3-9656-3727; fax: +61-3-9656-1411;e-mail: ricky.johnstone@petermac.org,

Key words: Histone deacetylase inhibitors, Chromatin and histone, Cyclic peptides, Short-chain fatty acids, Benzamides, Ketones, apoptosis,

Abbreviations: acute promyelocytic leukemia, (APL); apoptosis protease activating factor-1, (Apaf-1); chorioallantoic membrane, (CAM); cinnamic acid hydroxamic acid, (CBHA); chronic myelocytc leukemia (CML); diallyl disulfide, (DADS); Fas ligand, (FasL); hexamethylene bisacetamide, (HMBA); histone acetyltransferases, (HAT); histone deacetylase inhibitors, (HDAC inhibitors); histone deacetylases, (HDAC); hybrid polar compounds, (HPCs); promyelocytic leukemia, (PML); promyelozytic zinc finger (PLZF); suberoylanilide hydroxamic acid, (SAHA); tumor necrosis factor-related apoptosis-inducing ligand, (TRAIL); Valproic acid, (VA)

Received: 25 February 2004; Accepted: 3 March 2004; electronically published: March 2004

Summary

Despite advances in the molecular understanding of tumorigenesis and tumor cell apoptosis, the efficacy of chemotherapeutic treatment has not significantly improved over the last decades. Conventional treatment regimens suffer from a small therapeutic window and are often associated with severe side effects. Recent data suggest that a novel class of compounds, the histone deacetylase inhibitors (HDAC inhibitors), displays potent cytotoxicity towards tumor cells with low or negligible effects on untransformed cells. In addition to causing cell cycle arrest and/or differentiation and programmed cell death in tumor cells, they can also inhibit tumor angiogenesis and display immunosuppressive properties. Excitingly, various HDAC inhibitors compounds show synergy with other anti-cancer drugs and are involved in early clinical trials or pre-clinical development.The mechanism of action of HDAC inhibitors has not been completely elucidated. They induce histone hyperacetylation associated with transcriptional modulation of a set of genes. Treatment of malignant cells with HDAC inhibitors has been reported to elicit upregulation of the cell cycle inhibitor p21(WAF1), and induction of apoptosis most likely is coincident with the cleavage and activation of the proapoptotic Bcl-2 family member Bid. In addition, reactive oxygen species seem to play an important role in HDAC inhibitor-mediated cell death.This article summarizes what is currently known about the molecular and systemic sequelae of HDAC inhibitor treatment and focuses on recent progress regarding potential applications for cancer treatment as stand-alone or combination therapy.

I. Introduction

A. Chromatin and histone modifications

Chromatin is a highly complex structure responsible for the spatiotemporal and functional organisation of the genome and consists of DNA, histones and non-histone proteins. One of the substructures of chromatin is the nucleosome which comprises 146 bp of DNA wrapped around an octamer of histones (Wu and Grunstein, 2000; Ito, 2003). The discovery that these histones are subject to extensive post-translational modifications with direct implications for transcriptional regulation and cell cycle progression has contributed to the view of chromatin being a dynamic regulatory element rather that a passive structural scaffold.

Acetylation and deacetylation are important modifications of lysine residues in N-terminal histone tails and in addition, certain amino acids may be phosphorylated, methylated, ubiquitinylated or sumoylated. These different alterations can be interdependent and are the basis of the so-called “histone code” model which suggests that multiple histone modifications can differentially specify downstream actions in transcriptional regulation. The molecular details of this model will not be discussed here, but have been addressed in a number of excellent reviews (Cheung et al, 2000; Fischle et al, 2003).

The acetylation state of histones is regulated by the opposing action of two classes of enzymes, the histone acetyltransferases (HAT) and histone deacetylases (HDAC). HATs transfer acetyl groups to N-terminal

lysine residues in histones, which results in local expansion of chromatin and increased accessibility of regulatory proteins to DNA (Roth et al, 2001). This explains the role of HATs as transcriptional coactivators, and DNA binding proteins including PCAF (p300/cyclic-AMP-response-element binding protein – associated factor) and members of the p300/CBP family of transcriptional coactivators can recruit them to their site of action (Roth et al, 2001). However, the acetyltransferase activity of HATs extends beyonds histones; various nuclear proteins including p53 and the transcriptional repressor Bcl-6 are also subject to HAT-mediated acetylation (Kouzarides, 2000).

HDACs counteract the activity of HATs and catalyse the removal of acetyl groups from lysine residues in histone N-termini leading to chromatin condensation and transcriptional repression (Gray and Ekstrom, 2001). For this purpose, they are part of either multiprotein transcriptional repressor complex or interact with DNA binding proteins. For example, HDAC1 and HDAC2 can be components of the SIN3-HDAC and the nucleosome-remodelling deacetylase NuRD-Mi2-NRD complex, whereas class II HDACs can interact with hormone receptors and other transcription factors (Gray and Ekstrom, 2001). To date, 18 HDACs have been identified in human, and they can be subdivided into three classes based on their homology to yeast HDACs (**Table 1**) (Khochbin et al, 2001). The class I HDACs HDAC1, HDAC2, HDAC3 and HDAC8 can generally be detected in the nucleus and show ubiqituous expression in various human cell lines and tissues. These enzymes function in multimeric protein complexes regulating e. g. transcription, cell cycle and DNA repair and interaction has been reported with other transcriptional regulators including Smads, glucocorticoid receptor and Sp1.

Table 1.

Class	HDAC	Subcellular localisation
Class I		
	HDAC1	nuclear
	HDAC2	nuclear
	HDAC3	nuclear/cytoplasmic
	HDAC8	nuclear
Class II		
	HDAC4	nuclear/cytoplasmic
	HDAC5	nuclear/cytoplasmic
	HDAC6	nuclear/cytoplasmic
	HDAC7	nuclear/cytoplasmic
	HDAC9	nuclear/cytoplasmic
	HDAC10	nuclear/cytoplasmic
	HDAC11*	nuclear/cytoplasmic
Class III (sirtuins)		
	SIRT1	nuclear
	SIRT2	
	SIRT3	
	SIRT4	
	SIRT5	
	SIRT6	
	SIRT7	

*HDAC11 shares features of class I and II HDACs.

Class II HDACs comprise HDAC4, 5, 6, 7, 9 and 10, which can shuttle between the nucleus and the cytoplasm (Fischle et al, 2001). This process involves site-specific phosphorylations and the sequestration of certain class II HDACs in the cytoplasm by 14-3-3 proteins, upon loss of which the respective HDACs can enter the nucleus and regulate gene expression. Recently, Gao et al reported the cloning of HDAC11, which shares features of class I and class II HDACs (Gao et al, 2002).

The founding member of class III HDACs ("sirtuins") is the yeast protein Sir2 (Buck et al, 2004). All the enzymes of this class require NAD+ for their activity, which could enable class III HDACs to regulate gene expression in response to changes in the cellular redox status. Although a complete understanding of the role of class III HDACs is still lacking, SIRT1 has been shown to interact with and deacetylate p53, resulting in repression of p53-mediated transcriptional activation (Luo et al, 2001; Vaziri et al, 2001).

B. Chromatin and cancer

Most living organisms rely on a complex mechanism of co-ordinated gene activation and repression as controlled by chromatin status to regulate cellular differentiation, cell cycle and programmed cell death. As chromatin remodelling through e. g. histone modification plays a key role regulating gene activation, erroneous chromatin-mediated trancriptional control can lead to aberrant proliferation of cells and, eventually, neoplasia.

Histone hypoacetylation has been implicated in a number of malignancies. For example, missense mutations in the gene encoding for the histone acetyltransferase p300 and loss of heterozygosity at the p300 locus are associated with colorectal and mammary tumors and glioblastomas (Giles et al, 1998). Furthermore, chromosomal translocations that involve HATs can be detected in leukemia, with the t(8;16)(p11:13) rearrangement in acute myelocytic leukemia fusing the *monocytic-leukemia zinc finger* gene to the amino-terminus of the HAT CBP. Not only can such translocations interfere with the physiologic function of HATs, but may also lead to irregular transcriptional activation of genes that otherwise might not be expressed.

The role of histone deacetylases as mediators of transcriptional repression during myeloid differentiation has been described in some detail (Fenrick and Hiebert, 1998). Physiologic differentiation and growth arrest in normal myeloid cells requires the interaction of retinoic acid (RA) with retinoic acid receptor-alpha (RARα (Gaines and Berliner, 2003). In the absence of RA, RARα recruits nuclear receptor corepressors and HDACs, which leads to nucleosome packaging and transcriptional repression. Addition of RA results in dissociation of the RARα-complex and subsequent transcriptional activation of genes necessary for differentiation (Fenrick and Hiebert, 1998). A hematological malignancy termed acute promyelocytic leukemia (APL) provides a well-examined example for deregulation of HDAC activity in human cancers. In that case, RARα is fused to the PML (promyelocytic leukemia) or PLZF (promyelocytic zinc

finger) proteins, respectively. These fusion proteins bind to retinoic acid response elements in regulatory regions of certain genes, recruit HDACs with high affinity and are unsresponsive to retinoids, leading to a constitutive repression of RAR-target genes (Lin et al, 2001; Zelent et al, 2001). A second example for HDAC involvement in tumorigenesis is the overexpression of the Bcl6 transcriptional repressor in non-Hodgkin's lymphoma, which occurs due to chromosomal rearrangements within the Bcl6 promoter. As Bcl6 recruits HDACs to negatively regulate transcription (Bereshchenko et al, 2002), tumor-associated increases in Bcl6 levels may lead to hyper-repression of certain genes, which have yet to be identified.

Taken together, these data indicate that impaired chromatin function through dysregulated histone modifications could give rise to changes in cellular homeostasis, eventually leading to cancer development.

II. Histone deacetylase inhibitors: structurally diverse compounds

HDAC inhibitors bind to HDACs and inhibit their enzymatic activity, but do not alter their protein levels, the exception being valproic acid, which induces degradation of HDAC2. Crystallographic analysis of a complex formed between a homologue of mammalian HDAC and Trichostatin A or SAHA, respectively, indicated that the inhibitors bound to the catalytic pocket of the enzyme: the long aliphatic chain inserts into the pocket, whereas the polar hydroxamate group coordinates the catalytically indispensible zinc ion (Finnin et al, 1999).

The compound set of HDAC inhibitors that interact with histone deacetylases (Johnstone, 2002) can be subdivided into five groups (**Figure 1**), the characteristics of which will be discussed below:

1. Hydroxamic acid-derived compounds
2. Cyclic peptides
3. Short-chain fatty acids
4. Benzamides
5. Ketones

1. Hydroxamic acid-derived compounds

This group includes Trichostatin A, the first natural product hydroxamate to be discovered that directly inhibited HDACs. Other members are suberoylanilide hydroxamic acid (SAHA), oxamflatin, cinnamic acid hydroxamic acid (CBHA) and CBHA derivatives. Hydroxamic acid-derived HDAC inhibitors, especially SAHA, are part of pre-clinicial and clinical trials as anti-cancer agents.

2. Cyclic peptides

They constitute the most structurally complex class of HDAC inhibitors with depsipeptide, apicidin and the hydroxamic acid-containing peptides. These compounds display activity at the nanomolar range, but to date only depsipeptide has advanced to clinical trials (Piekarz et al, 2001; Sandor et al, 2002).

Figure 1.

3. Short-chain fatty acids

Among these are the least active HDAC inhibitors such as phenylbutyrate, phenylacetate and valproic acid, the last of which has been used in anti-epileptic treatment regimen. With the exception being valproic acid (Phiel et al, 2001), they may not be suitable for anti-tumor therapy because of their short plasma half-life and and the relatively high (milimolar) concentrations required for their action (Marks et al, 2001).

4. Benzamides

The benzamide class, including MS-275 and CI-994 generally operates at micromolar concentrations. Recently, the synthesis and characterization of sulfonamide anilides, their activities being in the low micromolar range, was reported (Bouchain and Delorme, 2003). They display tumorcidal activity *in vivo* and may qualify for use in anti-cancer therapy.

5. Ketones

The electrophilic ketones form a new class of HDAC inhibitors and include trifluoromethyl ketones and ketoamides, which are active in a comparable

concentration range as the benzamides (Frey et al, 2002). These recently developed compounds await examination of their suitability for chemotherapy.

III. Anti-tumor activities of HDAC inhibitors

HDAC inhibitors show a remarkable spectrum of anti-tumor effects. They are able to induce differentiation programmes in malignant cells, inhibit cell cycle progression and induce apoptosis. In doing so, they target processes that have often been observed to be deregulated in cancer cells. Furthermore, treatment of tumor-bearing animals showed that HDAC inhibitors could inhibit tumor growth, mediate activation of the host immune system and interfere with tumor-associated angiogenesis *in vivo*.

A. HDAC inhibitors can activate differentiation programs in tumor cells

More than two decades ago, several compounds were identified that could induce transformed cells to express their differentiated phenotype. Among these "polar organic compounds" was sodium butyrate, which could reproduce the changes in growth rate and morphology and upregulation of differentiation markers seen after DMSO or cAMP treatment of transformed cells (Kruh, 1982; Xiao et al, 1997). Another class of agents displaying this ability comprised the hybrid polar compounds (HPCs), of which hexamethylene bisacetamide (HMBA) was the prototype (Marks et al, 1996). Treatment of murine erythroleukemia cells with hybrid polar compounds led to rapid activation of the protein kinase C signalling pathway and subsequent upregulation of genes, including p21(WAF1) and certain differentiation-associated globin genes. This was accompanied by an elongation of the G1 phase of the cell cycle (Marks et al, 1996). Later, more potent derivatives of HMBA were developed, including SAHA and CBHA. It is noteworthy that although all of these three compounds were differentiation-inducing agents, only SAHA and CBHA conferred hyperacetylation of histones and inhibited HDAC1 and 3 *in vitro* (Richon et al, 1998). As cells resistant to SAHA could still be differentiated with HMBA, it is possible that HMBA stimulates the expression of differentiation-specific genes by a yet unrecognized mechanism or functions in the absence of altered gene expression.

All HDAC inhibitors induce upregulation of p21(WAF1), an important mediator of growth arrest and senescence in mammalian cells. Increased p21(WAF1) expression leads to growth arrest in both G1 and G2 phases of the cell cycle. This stems from its ability to inhibit cyclin-dependent kinase complexes that regulate transitions between different phases of the cell cycle (Sherr and Roberts, 1999). The mechanism of HDAC inhibitor-induced p21(WAF1) activation has been studied to some extent. (Johnstone, 2002). Elevated p21(WAF1) expression was independent of p53 and mediated through the Sp1/Sp3 transcription factor binding sites in the *p21(waf1)* promoter (Huang et al, 2000). This was accompanied by an increase in the acetylation of *p21(waf1)* promoter-associated histones and elevated DNaseI sensitivity and restriction enzyme accessibility of the gene (Gui et al, 2004). This clearly indicates that HDAC inhibitors directly induce transcription of the *p21(waf1)* gene. Furthermore, it has been demonstrated that cells lacking p21(WAF1) accumulate with a 4n DNA content upon HDAC inhibitor treatment, and that loss of p21(WAF1) induction renders cells hypersensitive to HDAC inhibitor-mediated cytotoxicity (Burgess et al, 2001). It is noteworthy that p21(WAF1) has been reported to interact with procaspase-3, which prevents its proteolytic activation (Suzuki et al, 2000; Suzuki et al, 1998). Altogether, this implies that p21(WAF1) could link cell cycle progression to sensitivity of cells towards apoptotic stresses. It cannot be ruled out that an increase in p21(WAF1) induced by HDAC inhibitors alters the sensitivity of cells towards these drugs by altering caspase-3 activity.

B. HDAC inhibitor-induced programmed cell death in transformed cells

At present, it is widely accepted that the anti-tumor effect of chemotherapeutic drugs depends greatly on their ability to induce programmed cell death in malignant cells (Johnstone et al, 2002). The apoptotic programme is a highly complex cascade consisting of basically two pathways that will eventually lead to the activation of caspases and execution of the final death program: an "extrinsic" pathway, which involves the cross-linking of certain death-inducing receptors (i. e. Fas) by their ligands (i. e. Fas ligand) and subsequent activation of caspases and an "intrinsic" pathway, engaging mitochondrial membrane perturbation and cytochrome c release as the central death signal (Johnstone et al, 2002).

HDAC inhibitor-induced apoptosis in tumor cells can involve either the death receptor-mediated or the intrinsic pathway. Several reports indicated that HDAC inhibitors sensitize cells to Fas ligand (FasL)/Fas-mediated apoptosis, possibly through upregulation of Fas ligand and Fas expression (Bonnotte et al, 1998; Glick et al, 1999; Maecker et al, 2002). In contrast, Wang et al showed that treatment of A1.1 T cell hybridoma with depsipeptide downregulated Fas ligand levels (Wang et al, 1998). Additionally, our work has demonstrated that blocking the death receptor pathway-activated caspases 8 and 10 by overexpression of the cowpox virus protein CrmA does not interfere with HDACi-mediated cytotoxicity (Bernhard et al, 1999; Ruefli et al, 2001; Peart et al, 2003). Taken together, these data indicate that different HDAC inhibitors might engage the FasL/Fas pathway to different extents.

A crucial step of the intrinsic pathway is the disruption of outer mitochondrial membrane integrity and subseqent release of cytochrome c and other pro-apoptotic molecules (Smac/DIABLO, AIF) into the cytoplasm. Cytochrome c is then believed to trigger the oligomerization of apoptosis protease activating factor-1 (Apaf-1) into a high-molecular complex ("apoptosome"), which recruits and activates procaspase-9 thus activating downstream caspases (Johnstone et al, 2002). Consistent

with other published data, our laboratory has reported that HDAC inhibitor-induced cell death involves release of cytochrome c into the cytoplasm. (Ruefli et al, 2000, 2002; Henderson et al, 2003; Peart et al, 2003). Interfering with the mitochondrial membrane disruption by overexpressing anti-apoptotic Bcl-2 protein, abrogates HDAC inhibitor-mediated apoptosis (Bernhard et al, 1999; Ruefli et al, 2001; Henderson et al, 2003; Peart et al, 2003). Moreover, in preliminary experiments using B cell lymphomas from Eμ-myc transgenic mice, we found that Apaf-1 is required for full activation of the cytotoxic response to HDAC inhibitors. Cell death was detectable only after prolonged treatment of Apaf1$^{-/-}$-lymphomas with HDAC inhibitors. (A. Cirnigliaro, R. K. Lindemann and R. W. Johnstone, unpublished). Similar results have just been published for the treatment of Apaf-1 – depleted lymphomas with etoposide and γ-irradiation (Scott et al, 2004). Evidence has been presented that the apoptosome might be dispensable for apoptosis in some cell types as thymocytes of apaf-1 knockout mice are sensitive to various death stimuli (Marsden et al, 2002). The precise role of Apaf-1 in apoptosis and whether it has an initiating role or rather acts as an amplifier for the apoptotic programme, is presently under debate (Adams, 2003; Lassus et al, 2002).

The apoptotic process involves proteolytic breakdown of cellular constituents, and the key proteases in apoptosis, the caspases, have been well characterized (Philchenkov, 2003). Initiator caspases (caspase-2, -8, -9 and possibly –10) transactivate the executioner caspases (caspase-3, -7), which in turn hydrolyze intracellular substrates. Caspase-8 activation requires stimulation of the "extrinsic" pathway and transmission of the apoptotic signal through adaptor proteins, whereas caspase-9 is activated via the apoptosome (see above). The role of caspases in HDAC inhibitor-induced cell death could be model-dependent. Amin et al have shown that the poly-caspase inhibitor zVAD-fmk (N-tert-butoxy-carbonyl-Val-Ala-Asp-fluoromethylketone) inhibits SAHA-induced apoptosis in an acute promyelocytic leukemia cell line as judged by propidium iodide FACS analysis (Amin et al, 2001), arguing that activation of zVAD-sensitive caspases (i. e. caspase-3, -8, -9) is a necesessary event in HDAC inhibitor-induced cell death. However, whereas caspases were activated after treatment of CCRF-CEM cells with three different HDAC inhibitors, preincubation with zVAD-fmk did not antagonize mitochondrial membrane damage and induction of cell death by the inhibitors (Ruefli et al, 2001; Mitsiades et al, 2003; Peart et al, 2003). Caspase-2 is an initiator caspase residing in the nucleus and is only poorly inhibited by zVAD-fmk (Paroni et al, 2002). To date, caspase-2 is believed to be unable to directly initate the processing of initiator caspases, however, via cleavage of the Bid protein it elicits the release of cytochrome c (Guo et al, 2002). RNA interference-mediated depletion of caspase-2 prevented induction of cytochrome c release and programmed cell death by chemotherapeutic drugs (Lassus et al, 2002). Surprisingly, the suppression of apoptosis was comparable to Apaf-1 – depleted cells (Lassus et al, 2002), which may indicate that the mitochondria-apoptosome pathway and activation of caspase-2 are equally important for induction of cell death by anti-cancer therapeutics. Processing of Caspase-2 could be observed after HDAC inhibitor treatment of CCRF-CEM cells, this being partly inhibited by overexpression of Bcl-2. (Peart et al, 2003). Thus, HDAC inhibitors might initiate the apoptotic process by inducing cleavage and activation of caspase-2 in a yet uncharacterized manner. Whether this implies a feedback mechanism from the mitochondrium to caspase-2, as observed with other anti-cancer drugs (Lassus et al., 2002), needs to be determined. Further experiments with a specific inhibitor of caspase-2, zVDVADfmk, will facilitate the analysis of caspase-2 involvement in cell death mediated by HDAC inhibitors.

There is accumulating evidence that the pro-apoptotic BH3-only proteins, a subgroup of the Bcl-2 protein family, are important regulators of apoptosis. They most likely function by binding to and neutralizing their anti-apoptotic relatives, with the exception being Bid that, upon caspase-mediated cleagave, can additionally bind Bax and Bak and trigger their oligomerization in the mitochondrial membrane and subsequent release of cytochrome c into the cytoplasm (Cory and Adams, 2002; Esposti, 2002). Bid seems to be a mediator of HDAC inhibitor-induced apoptosis, as Bid cleavage has been shown to occur after treatment of cells with HDAC inhibitors (Ruefli et al, 2001; Henderson et al, 2003; Mitsiades et al, 2003; Peart et al, 2003). As this cleavage is also detected in cells overexpressing Bcl2 (Peart et al, 2003), the activation of Bid occurs upstream of mitochondrial membrane perturbation. It is tempting to speculate that HDAC inhibitor-activated caspase-2 is the protease responsible for Bid cleavage, this, however, has not yet been directly proven. Interestingly, Mitsiaides and colleagues recently reported that inhibition of the calcium-dependent protease calpain markedly reduced cell death induced by SAHA (Mitsiades et al, 2003). Whether this effect is a consequence of direct Bid cleavage by calpains is unclear, as the effect of calpain inhibition on SAHA-induced Bid cleavage was not investigated in this report. Of note, Bid has previously been described to be a calpain substrate (Gil-Parrado et al, 2002).

Another phenomenon observed in HDAC-inhibitor treated cells is the generation of radical oxygen species (ROS). We and others have reported an increase of ROS after exposure of cells to HDAC inhibitors, whereas preincubation of cells with antioxidants suppressed apoptosis induced by SAHA but not vincristine (Peart et al, 2003; Rosato et al, 2003; Ruefli et al, 2001). This indicates that production of ROS is an important effector of HDAC-mediated cell death.

Another differentiation-inducing agent possessing HDAC inhibitor activity, diallyl disulfide (DADS), inhibited proliferation of and induced apoptosis in cancer cells, the characteristics of which were generation of ROS, caspase activation and, intriguingly, an increase in intracellular Ca^{++} (Park et al, 2002; Filomeni et al, 2003). Preincubation with the calcium chelator BAPTA-AM abolished the DADS-induced elevation of intracellular Ca^{++}, blocked ROS generation and inhibited apoptosis (Park et al, 2002). Thus, there is a possibility that HDAC inhibitor-induced cell death is mediated, in part, by a Ca^{++}-

dependent signal, which could subsequently lead to calpain activation.

In summary, we postulate that HDAC inhibitors induce a novel mechanism of cell death that involves activation of the intrinsic pathway, generation of ROS and cleavage of the BH3-only protein Bid. To further understand HDAC inhibitor-induced cell death, it will be critical to identify the Bid-cleaving enzyme and how it is activated. The role of caspases in HDAC inhibitor-induced apoptosis is not well understood and may be cell-type dependent. Clearly, delineating the role of proteases in HDAC inhibitor-mediated apoptosis will help unveil the molecular mechanism of HDAC inhibitor-induced cell death.

IV. Which upstream events cause initiation of apoptosis in HDAC inhibitor-treated cells?

Efforts are currently being undertaken to elucidate which stimulus transmits the "death-signal" to the apoptotic machinery after HDAC inhibitor exposure. Due to the selective cytotoxicity of HDAC inhibitors towards transformed cells, the molecular mechanism underlying this effect may have enormous implications for the design of novel anti-cancer therapeutics.

First of all, HDAC inhibitors cause rapid histone hyperacetylation, which is accompanied by transcriptional modulation of 2-10% of all genes, depending on the cell type and HDAC inhibitor used ((Della Ragione et al, 2001; Glaser et al, 2003) and M. Peart and R. W. J., unpublished). It is thought that acetylation of histones and subsequent relaxation of chromatin lead to increased transcriptional activation. Therefore, if HDAC inhibitors were to regulate gene expression directly, one would assume that most if not all alterations in gene expression should be activating, rather than repressing, gene expression. This, however, is not the case as HDAC inhibitors clearly induce repression of a variety of genes. For example, in colon carcinoma cells, sodium butyrate treatment led to upregulation of 256 genes, whereas 333 genes were downregulated (Mariadason et al, 2000). This suggests that direct and indirect mechanisms are involved in HDAC inhibitor-regulated gene expression.

Do the genes whose expression is altered through HDAC inhibitors include regulators of cellular survival and death? Indeed, death-promoting Bak was upregulated by butyrates and TsA and both agents downregulated the survival protein Bcl-xL. SAHA induced expression of Caspase-9 and Apaf-1 and downregulated anti-apoptotic FLIP, Bcl-2 and survivin in a multiple myeloma cell line. Interestingly, expression of pro-apoptotic Caspase-8, Fas and Bid was also suppressed (Johnstone, 2002; Mitsiades et al, 2004). It is not clear whether these changes are necessary and/or sufficient for initiation of cell death in HDAC inhibitor-treated tumor cells. Supporting a role for altered gene expression in HDAC inhibitor-elicited cell death are data showing that preventing de novo transcription and translation through pretreatment of cells with actinomycin D and cycloheximide, respectively, interfered with SAHA-induced Bid cleavage and apoptosis (Ruefli et al, 2001; Peart et al, 2003). However, these findings need to be interpreted with caution as these two substances can be cytotoxic drugs themselves. To date, a comprehensive investigation comparing HDAC inhibitor-modulated gene expression among various structurally different compounds in healthy vs. malignant cells is still lacking. Glaser et al compared the effects of SAHA, TsA and MS-275, three structurally different HDAC inhibitors, on gene expression of two breast and one bladder carcinoma cell line and identfied a "core" set of genes that were up- or downregulated by all of the inhibitors in all of the cell lines: this set comprised 13 genes, eight up- and five downregulated (Glaser et al, 2003). Among those, only one was directly involved in regulation of apoptosis. This was clusterin, a certain form of which can be detected in the nucleus where it responds to DNA damage and promotes cell death (Yang et al, 2000). Downregulated genes of the "core" set included two genes involved in DNA synthesis (thymidylate synthetase and CTP synthetase), whose repression could potentially be mediating a HDAC inhibitor-induced cell cycle arrest.

Experiments with sychronized cells suggest that in tumor cells, disruption of a HDAC inhibitor-sensitive G2 checkpoint, which in untransformed cells acts as a protective mechanism responding to cellular stresses, allows cells to progress into mitosis (Qiu et al, 2000; Warrener et al, 2003). These mitotic cells were highly defective in that condensed chromosomes failed to migrate to the centerline of the spindle (Warrener et al, 2003). It has been hypothesized that HDAC inhibitors allow bypassing of a spindle checkpoint in these cells, subsequently leading to premature exit from mitosis and rapid apoptosis (Warrener et al, 2003). To date, the molecular target of HDAC inhibitors permitting the checkpoint bypass has not been identified. Again, more than one mechanism could playing a role. SAHA has been demonstrated to downregulate the gene encoding for Bub-3, a crucial regulator of the spindle checkpoint (Mitsiades et al, 2004). In addition, it could be speculated that aberrant hyperacetylation of centromeric chromatin interferes with kinetochore function thus compromising spindle checkpoint integrity. Both mechanisms, alone or in combination, could contribute to HDAC inhibitor-mediated bypass of the spindle checkpoint.

In summary, the nature of the death-eliciting signals being transmitted to the apoptotic machinery after exposure of malignant cells to HDAC inhibitors remains elusive. Two scenarios are conceivable, which are not necessarily exclusive: One, HDAC inhibitors modulate the expression of a certain set of genes, which perturbs the homeostatic balance of pro-survival and pro-death factors ultimatively leading to programmed cell death. Two, hyperacetylation events trigger alterations in chromatin, which in untransformed cells activates mitotic checkpoints and cell cycle arrest, but results in apoptosis in tumor cells where these checkpoints are frequently disrupted. What is the molecular stimulus inducing rapid apoptosis seen after premature exit from mitosis? This question has not been answered yet, but changes in expression of pro- or anti-apoptotic proteins could modulate susceptibility towards

apoptotic insults. Clearly, depending on the experimental model HDAC inhibitors cause alterations in gene expression and perturb the cell cycle, but futher study need to show to which extent these factors contribute to induction of apoptosis.

V. Therapeutic use of HDAC inhibitors

Following the positive results obtained *in vitro*, the therapeutic efficacy of HDAC inhibitors was tested in various murine cancer models. These *in vivo* experiments demonstrated that HDAC inhibitors possessed potent anti-tumor activity against a variety of experimental tumors. **Table 2** lists the types of HDAC inhibitors used in both experimental models and clinical trials. As these preliminary results appeared very promising, the HDAC inhibitors have attracted much attention and interest and several pharmaceutical companies have now developed compounds with HDAC inhibitor activity.

HDAC inhibitors of the short-chain fatty acid group, i. e. butyrates and valproic acid, exerted a marked anti-tumor effect when used in carcinogen-induced cancers or xenograft models, respectively, of colon, endometrial, cervical and prostate malignancies (Johnstone, 2002). However, their applicability for chemotherapy is not without caveats. Butyrates were rapidly degraded after i.v. administration and required relatively high doses (Warrell et al, 1998). In addition, these compounds are not specific for HDACs as they also inhibit phosphorylation and methylation of proteins and DNA methylation (Newmark and Young, 1995). Valproic acid (VA) (Phiel et al, 2001) is a drug commonly used in the long-term therapy of epilepsy. Although it is very well tolerated, it can induce birth defects when administered during early pregnancy through a mechanism that is not well understood (Nau et al, 1991). VA promoted differentiation of leukemia cell lines and primary leukemic blasts from patients. Furthermore, when administered to tumor-bearing mice in a breast cancer xenograft model in high doses, tumor size of the primary tumor and lung metastasis burden were reduced by 50% (Gottlicher et al, 2001).

Table 2

Class of HDAC inhibitor	HDAC inhibitor	Application in experimental tumor models	Clinical trials	References
Short-chain fatty acids				
	Butyrates	colon, prostate, endometrial and cervical carcinomas	Phase I	Johnstone 2002
	Valproic acid	mammary carcinoma	Phase I/II in preperation	Gottlicher 2001
		neuroblastoma		Cinatl 1997
Hydroxamic acids				
	TsA	mammary tumor		Vigushin, 2001
	ABHA	melanoma		Qiu 1999
	HMBA	melanoma		Qiu 1999
	CBHA	neuroblastoma		Coffey 2001
	SAHA	prostate	Phase I	Butler 2000
		mammary tumor		Cohen 2002
	CI-994	carcinogen-induced colon carcinomas	Phase I and II	Seelig 1996
	oxamflatin	melanoma		Kim 1999
	pyroxamide	prostate	Phase I completed	Butler 2001
	NVP-LAQ824	myeloma	in preparation	Catley, 2003
	PXD101	ovarian and colon tumors		Plumb 2003
Cyclic peptides				
	Depsipeptide	acute promyelocytic leukemia	Phase I and II	
		lung, colon, stomach, prostate carcinoma, fibrosarcoma		Saito 1999, Ueda 1994
		prostate carcinoma renal carcinoma		Sasakawa 2003
	CHAP	melanoma		Komatsu, 2001
	apicidin	?		
Benzamides				
	MS-275	Ewing's sarcoma Neuroblastoma	Phase I	Jaboin 2002
	Sulfonamid anilides	Colon carcinoma		Fournel 2002

Despite the high levels of VA required for therapeutic activity and the reported side effects, these data suggest that it may be used for cancer treatment. A phase I/II clinical trial involving VA together with the DNA demethylating agent 5-aza-2'- Deoxycytidine for the treatment of relapsed/refractory leukemia or myelodysplastic syndromes will soon be underway (http://clinicaltrials.gov/show/NCT00075010).

Hydroxamic acid-type HDAC inhibitors reduced tumor burden of experimental mammary, prostate, ovarian, colon and skin cancers. When comparing three hydroxamic acid-type HDAC inhibitors TsA was found to be inferior to AAHA (azelaic-hydroxamate-anilide) and ABHA (azelaic bishydroxamate) on melanoma xenografts when administered i.p. at a dose of approximately 1 mg/kg over 30 days (Qiu et al, 1999). In contrast, TsA treatment (500 μg/kg s. c. over 4 weeks) reduced the frequency of adenocarcinomas and shifted tumor phenotype towards benign, more differentiated tumors in the N-methyl-N-nitrosurea – induced rat mammary carcinoma model (Vigushin et al, 2001). It remains to be seen whether the low stability of TsA in the presence of cultured cells (Komatsu et al, 2001; Qiu et al, 1999) will limit its suitability for therapy. SAHA proved effective in a murine xenograft model of prostate cancer (Butler et al, 2000). Application of an intermediate dose (50 mg/kg i.p.) caused almost complete suppression of xenograft growth over 21 days without side effects, whereas high-dose treatment (100 mg/kg) also showed anti-tumor efficacy but, in addition, side-effects including peritonitis and suppression of erythropoiesis in the spleen and bone marrow (Butler et al, 2000). Mice bearing human prostate tumor xenografts were treated with pyroxamide over 21 days showed decreased tumor volume to barely detectable levels (Butler et al, 2001). Interestingly, whereas SAHA and pyroxamide showed comparable anti-tumor activity, only SAHA caused a significant reduction of prostate serum antigen levels (Butler et al, 2000), a key diagnostic factor in prostate cancer. Further studies are necessary to clarify whether this reflects improved anti-tumor efficacy of SAHA compared to pyroxamide for prostate cancer treatment.

Of the cyclic peptides, depsipeptide appears to have very potent tumoricidal activity at relatively low concentrations against a variety of tumors. It suppressed the growth of murine (colon carcinoma) and human (lung and stomach adenocarcinoma) solid tumors implanted in normal and nude mice, respectively, some of which were refractory to mitomycin C or cisplatin (Ueda et al, 1994; Sasakawa et al, 2003). A cyclic hydroxamic acid-containing peptide with HDAC inhibitor activity was shown to be highly stable in the presence of cultured cells and exhibited anti-tumor activity in mice bearing melanoma cells and breast, stomach, melanoma and lung xenograft models (Komatsu et al, 2001).

HDAC inhibitors from the benzamide class have also been successfully used in animal tumor models. MS-275 inhibited growth of seven out of eight tumor xenografts (albeit to various extents), whereas 5-fluorouracil elicited an anti-tumor response in only one out of the eight cell lines (Saito et al, 1999). Moreover, a range of childhood tumors including Ewing's sarcomas and neuroblastoma responded to MS-275 in xenograft models with a marked reduction of tumor mass after oral administration of MS-275 over 4 weeks (Jaboin et al, 2002).

Taken together, these data show that HDAC inhibitors show therapeutic activity in various xenograft models, and intraperitoneal, subcutaneous or oral administration is feasible. The extent by which HDAC inhibitors repressed tumor growth did vary in the different tumor models, indicating that for some cancers complete suppression of tumor growth would require combination therapy. Severe side effects have rarely been reported. As a next step, the limitations of xenograft models need to be overcome and the therapeutic efficacy of HDAC inhibitors in immunocompetent tumor-bearing mice should be assessed. As HDAC inhibitors have been shown to induce expression of MHC class I and II molecules (see below), immune cells may possibly be part of the anti-tumor response elicited by these compounds.

Another important point also requires further investigation: What is the duration of the therapeutic response of HDAC inhibitor treatment? Once treatment ceases, how long would it take for tumors to relapse? Answers to these questions will provide valuable information for treatment strategies and combination therapy.

The first results of clinical trials employing HDAC inhibitors are encouraging. Analysis of tumor biopsies from patients demonstrated that HDAC inhibitors induced histone hyperacetylation *in vivo* and anti-tumor effects can be observed in several patients with minimal side effects (Johnstone, 2002). Two studies reported that phenylbutyrate treatment of refractory neoplasms was well tolerated by the patients with the side effects being fatigue, somnolence and hyperuricaemia and hypocalcemia, respectively. In one trial, disease stabilization for one out of four patients for more than six months was achieved. Piekarz and colleagues describe marked effects of depsipeptide on cutaneous T cell lymphomas where one out of four patients had a complete response and three had a partial response to treatment with minimal side effects (Piekarz et al, 2001). Subsequently, a phase I clinical trial with patients suffering from refractory neoplasms was conducted to define maximal tolerated dose and side effects. Depsipeptide was generally well tolerated, but patients showed signs of fatigue and nausea. Recently, the first results of a phase I clinical trial using SAHA were published. Patients with advanced solid tumors and hematological malignancies received i. v. administrations of SAHA (Kelly et al, 2003). While side effects included fatigue, leukopenia and thrombocytopenia, two patients with refractory metastatic bladder cancer showed minor responses. Antitumor activity was also observed in 2 patients with refractory Hodgkin's disease that previously had failed autologous transplants. Additionally, one patient with refractory Hodgkin's disease presented with complete remission after eight months of treatment, which has been converted to oral administration of SAHA (Kelly et al, 2003).

Overall, the remarkable data that were derived from the initial clinical trials demonstrate that HDAC inhibitors

have great potential for treatment of various malignancies and are generally well tolerated by patients.

VI. HDAC inhibitors in combination therapy

In addition to stand alone-therapeutics for chemotherapy, HDAC inhibitors seem to be suitable for combination therapy as "sensitizer drugs", enhancing the anti-tumor effect of specific chemotherapeutics. In fact, a proportion of the clinical trials using HDAC inhibitors involve a combination of an established anti-tumor compound together with a HDAC inhibitor. For example, butyrates are part of clinical trials in combination with gancyclovir (treatment of lymphoproliferative disorders), retinoic acid (treatment of AML, CML) or fluorouracil/indomethacin (treatment of advanced colorectal carcinomas) (Johnstone, 2002). Lately, the more recently developed HDAC inhibitors have also been reported to be effective with other anti-cancer drugs. The action of Bcr/Abl kinase inhibitor STI571 (Gleevec, imatinib mesylate) can be markedly enhanced by SAHA in chronic myelocytc leukemia (CML), and this combination is effective against STI571-refractory cells (Yu et al, 2003a). Excitingly, a recently developed HDAC inhibitor, LAQ824, can induce proteasomal degradation of Bcr/Abl and apoptosis in both STI571-sensitive and –resistant cells as a single agent (Nimmanapalli et al, 2003), reflecting the progress and the potential of HDAC inhibitors as chemotherapeutic drugs.

HDAC inhibitors also synergize with another anti-cancer drug termed bortezomib (Velcade™) (Yu et al, 2003b), a proteasome inhibitor also known as PS-341. Exposure of CML cells to both compounds resulted in the generation of reactive oxygen species, degradation of Bcr/Abl and cell death (Yu et al, 2003b).

The tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is a type II transmembrane protein belonging to the TNF superfamily. At least five receptors for TRAIL have been identified in humans and two of them, DR4 and DR5, are capable of transducing an apoptotic signal. TRAIL has lately received attention because soluble recombinant versions of the TRAIL molecule display selective cytotoxicity towards malignant cells (Smyth et al, 2003). In a number of experimental tumor models, TRAIL administration significantly reduced tumor growth *in vivo* (Smyth et al, 2003). It has been suggested that chemotherapeutic drugs could act synergistically with TRAIL, possibly through the upregulation of certain TRAIL receptors. Milimolar levels of sodium butyrate downregulated FLIP levels in human colon cancer cell lines, upon which a modest sensitization towards TRAIL-mediated cytotoxicity was measured (Hernandez et al, 2001). Inoue and colleagues reported that TsA and sodium butyrate rendered TRAIL-resistant adenocarcinoma cell lines sensitive to TRAIL, but do not provide detailed insight into the molecular mechanism (Inoue et al, 2002). Recently, SAHA and TRAIL were demonstrated to induce cell death in human leukemia cells in a synergistic manner, and only the combination treatment downregulated Bcl-xL expression (Neuzil et al, 2004). These data could imply that in certain cancer cell types, a cooperative cytotoxic effect through TRAIL and HDAC inhibitors functions through depletion of anti-apoptotic proteins. Clearly, further work with a larger number of cell lines derived from different cancers and with primary tumor cells is required to further elucidate a potential TRAIL/HDAC inhibitor synergism.

Taken together, HDAC inhibitors can augment the cytotoxic effect of conventional chemotherapeutics, the therapeutic efficacy of which is often subverted by resistance mechanisms. Therefore, in addition to their inherent tumoricidal activity, HDAC inhibitors show great promise as "amplifier" drugs in cancer therapy.

VII. Other anti-tumor effects of HDAC inhibitors

Besides the direct effect of HDAC inhibitors on tumor cell growth and survival, there is evidence that they might exert indirect anti-tumor effects through antagonizing neo-angiogenesis, counteracting invasive behaviour of cancer cells and activation of the host immune system. These are often associated with transcriptional modulation of one or more specific target genes.

In chorioallantoic membrane (CAM) and Matrigel plug assays that serve as experimental models for angiogenesis, trichostatin A markedly decreased new vessel formation without any signs of thrombosis and hemorrhage (Kim et al, 2001). Of note, TsA reduced hypoxia-induced VEGF (vascular endothelial growth factor) mRNA and protein expression to background levels (Deroanne et al, 2002; Williams, 2001). Furthermore, TsA caused a reduction in the expression of eNOS (endothelial nitric oxide synthase), the activity of which is coupled to angiogenesis through the production of nitric oxide (Rossig et al, 2002). Recently, similar results have been obtained using depsipeptide. Notably, depsipeptide-mediated repression of VEGF expression was accompanied by hyperacetylation of histones associated with regulatory regions of the *vegf* gene (Hernandez et al, 2001).

Evidence for another anti-metastatic effect of HDAC inhibitors has been provided recently. TsA treatment of lung cancer cells caused an upregulation of the metastasis-suppressor RECK, which subsequently inhibited MMP-2 activity and invasive behavior of these cells in a Matrigel assay (Liu et al, 2003). If this would be reproducible using other HDAC inhibitors, it would imply that these compounds do not only cause cell death in tumor cells, but possibly interfere with tumor spreading by antagonizing invasive properties specifically acquired by metastasizing cells.

Recent evidence suggests that HDAC inhibitors may have immunomodulatory effects, some of which could indirectly impinge on tumorigenesis. Magner and colleagues noticed that several cancer cell lines showed upregulated levels of MHC (major histocompatibility complex) class I and II proteins upon HDAC inhibitor treatment (Magner et al, 2000). In addition, costimulatory molecules CD40, CD80 and CD86 and ICAM-1

(intracellular adhesion molecule-1), respectively, were also found to be transcriptionally induced by HDAC inhibitors (Magner et al, 2000). Importantly, in an allo-mixed lymphocyte reaction assay sodium butyrate-treated HL60 cells showed enhanced capacity to induce lymphocyte proliferation as compared to untreated controls (Maeda et al, 2000).

Finally, treatment of leukemia cells with butyric acid and SAHA resulted in reduced expression of the chemokine receptor CXCR4, which plays a role in leukemia cell trafficking and survival (Crazzolara et al., 2002).

In summary, these data show that in addition to inducing differentiation and programmed cell death in cancer cells, HDAC inhibitors possess supplementary anti-tumor effects. Tumorigenesis is a multi-stage process with various tumor-host interactions, both on the molecular (e. g. tumor-stroma) and the systemic (e. g. tumor-immune system) level. As discussed above, HDAC inhibitors may exert their anti-tumor effect on the tumor entity *per se*, but also indirectly by affecting host tissues. This tumoricidal "double-strike" is unique among chemotherapeutic drugs.

VIII. Perspective

HDAC inhibitors comprise an expanding set of compounds that induce histone hyperacetylation and display potent anti-tumor acitivity *in vitro* and *in vivo*. Initially, it was observed that HDAC inhibitors were able to elicit differentiation and growth arrest in transformed cells, and due to remarkable progress, they are currently part of numerous clinical trials. Part of their story of success is the selective cytotoxicity towards transformed cells, despite the fact that they rapidly cause accumulation of hyperacetylated histones in both untransformed and malignant cells. That directly leads to to an important question: What is the molecular basis for the selective anti-tumor activity of HDAC inhibitors? Several options could be discussed. Do cell cycle phase-specific safety mechanisms that are often defective in tumor cells determine sensitivity to HDAC inhibitors? Is it the up- or downregulation of certain genes that tip the intracellular balance of life and death in favor of death? Activated oncogenes can cause stress in tumor cells from which they need to be "rescued" by a second mutation, most likely disabling the apoptotic apparatus. For example, in the Eμ-myc model of B-cell lymphoma, a murine model of myc-driven tumorigenesis, early stages of disease show B cell proliferation being counterbalanced by p53-dependent apoptosis. However, mutations in genes encoding the Arf or p53 tumor suppressor shut off this safety switch enabling myc to drive cell proliferation and lymphomagenesis (Nilsson and Cleveland, 2003). Consequently, if HDAC inhibitors augment expression of pro-apoptotic proteins, it could be speculated that this re-sensitized tumor cells to internal (and external) stress stimuli.

Finally, HDAC inhibitors could assist us on another path to improved tumor therapy, which is to try and adjust chemotherapy treatment regimens to "individual" tumor genotypes. The potential of this approach has been demonstrated in the case of APL, where chemotherapeutic drugs (i. e. HDAC inhibitors) are specifically targetting a protein (i. e. HDACs recruited by RARα fusion proteins) that is causal in the leukemogenic process (Zelent et al, 2001). If we appreciate that tumorigenesis is often driven by the same mutations that interfere with effective anti-cancer therapy (i. e. mutations in genes encoding critical apoptotic regulators) (Johnstone et al, 2002), tailored strategies for cancer therapy would be desirable. We hypothesize that the growing entity of HDAC inhibitors with their structural diversity may qualify for such an approach. They could be tested for anti-tumor activity in an experimental tumor model with defined genetic alterations in apoptotic regulators and pathways. Possibly, eradicating cells with different tumorigenic mutations would require the use of different HDAC inhibitors or a combination of them.

In conclusion, although their precise mechanism of action is yet incompletely understood, HDAC inhibitors represent a novel and promising class of anti-tumor compounds, whose efficacy in chemotherapy as single agent and in combination therapy is currently emerging.

References

Adams, J.M. (**2003**) Ways of dying: multiple pathways to apoptosis. ***Genes Dev***, **17**, 2481-2495.

Amin, H.M., Saeed, S. and Alkan, S. (**2001**) Histone deacetylase inhibitors induce caspase-dependent apoptosis and downregulation of daxx in acute promyelocytic leukaemia with t(15;17). ***Br J Haematol***, **115**, 287-297.

Bereshchenko, O.R., Gu, W. and Dalla-Favera, R. (**2002**) Acetylation inactivates the transcriptional repressor BCL6. ***Nat Genet***, **32**, 606-613.

Bernhard, D., Ausserlechner, M.J., Tonko, M., Loffler, M., Hartmann, B.L., Csordas, A. and Kofler, R. (**1999**) Apoptosis induced by the histone deacetylase inhibitor sodium butyrate in human leukemic lymphoblasts. ***Faseb J***, **13**, 1991-2001.

Bonnotte, B., Favre, N., Reveneau, S., Micheau, O., Droin, N., Garrido, C., Fontana, A., Chauffert, B., Solary, E. and Martin, F. (**1998**) Cancer cell sensitization to fas-mediated apoptosis by sodium butyrate. ***Cell Death Differ***, **5**, 480-487.

Bouchain, G. and Delorme, D. (**2003**) Novel hydroxamate and anilide derivatives as potent histone deacetylase inhibitors: synthesis and antiproliferative evaluation. ***Curr Med Chem***, **10**, 2359-2372.

Buck, S.W., Gallo, C.M. and Smith, J.S. (**2004**) Diversity in the Sir2 family of protein deacetylases. ***J Leukoc Biol***.

Burgess, A.J., Pavey, S., Warrener, R., Hunter, L.J., Piva, T.J., Musgrove, E.A., Saunders, N., Parsons, P.G. and Gabrielli, B.G. (**2001**) Up-regulation of p21(WAF1/CIP1) by histone deacetylase inhibitors reduces their cytotoxicity. ***Mol Pharmacol***, **60**, 828-837.

Butler, L.M., Agus, D.B., Scher, H.I., Higgins, B., Rose, A., Cordon-Cardo, C., Thaler, H.T., Rifkind, R.A., Marks, P.A. and Richon, V.M. (**2000**) Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, suppresses the growth of prostate cancer cells in vitro and in vivo. ***Cancer Res***, **60**, 5165-5170.

Butler, L.M., Webb, Y., Agus, D.B., Higgins, B., Tolentino, T.R., Kutko, M.C., LaQuaglia, M.P., Drobnjak, M., Cordon-Cardo, C., Scher, H.I., Breslow, R., Richon, V.M., Rifkind, R.A. and Marks, P.A. (**2001**) Inhibition of transformed cell growth and induction of cellular differentiation by

pyroxamide, an inhibitor of histone deacetylase. ***Clin Cancer Res***, **7**, 962-970.

Catley, L., Weisberg, E., Tai, Y.T., Atadja, P., Remiszewski, S., Hideshima, T., Mitsiades, N., Shringarpure, R., LeBlanc, R., Chauhan, D., Munshi, N.C., Schlossman, R., Richardson, P., Griffin, J. and Anderson, K.C. (**2003**) NVP-LAQ824 is a potent novel histone deacetylase inhibitor with significant activity against multiple myeloma. ***Blood***, **102**, 2615-2622.

Cheung, P., Allis, C.D. and Sassone-Corsi, P. (**2000**) Signaling to chromatin through histone modifications. ***Cell***, **103**, 263-271.

Cinatl, J., Jr., Cinatl, J., Driever, P.H., Kotchetkov, R., Pouckova, P., Kornhuber, B. and Schwabe, D. (**1997**) Sodium valproate inhibits in vivo growth of human neuroblastoma cells. ***Anticancer Drugs***, **8**, 958-963.

Coffey, D.C., Kutko, M.C., Glick, R.D., Butler, L.M., Heller, G., Rifkind, R.A., Marks, P.A., Richon, V.M. and La Quaglia, M.P. (**2001**) The histone deacetylase inhibitor, CBHA, inhibits growth of human neuroblastoma xenografts in vivo, alone and synergistically with all-trans retinoic acid. ***Cancer Res***, **61**, 3591-3594.

Cohen, L.A., Marks, P.A., Rifkind, R.A., Amin, S., Desai, D., Pittman, B. and Richon, V.M. (**2002**) Suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, suppresses the growth of carcinogen-induced mammary tumors. ***Anticancer Res***, **22**, 1497-1504.

Cory, S. and Adams, J.M. (**2002**) The Bcl2 family: regulators of the cellular life-or-death switch. ***Nat Rev Cancer***, **2**, 647-656.

Crazzolara, R., Johrer, K., Johnstone, R.W., Greil, R., Kofler, R., Meister, B. and Bernhard, D. (**2002**) Histone deacetylase inhibitors potently repress CXCR4 chemokine receptor expression and function in acute lymphoblastic leukaemia. ***Br J Haematol***, **119**, 965-969.

Della Ragione, F., Criniti, V., Della Pietra, V., Borriello, A., Oliva, A., Indaco, S., Yamamoto, T. and Zappia, V. (**2001**) Genes modulated by histone acetylation as new effectors of butyrate activity. ***FEBS Lett***, **499**, 199-204.

Deroanne, C.F., Bonjean, K., Servotte, S., Devy, L., Colige, A., Clausse, N., Blacher, S., Verdin, E., Foidart, J.M., Nusgens, B.V. and Castronovo, V. (**2002**) Histone deacetylases inhibitors as anti-angiogenic agents altering vascular endothelial growth factor signaling. ***Oncogene***, **21**, 427-436.

Esposti, M.D. (**2002**) The roles of Bid. ***Apoptosis***, **7**, 433-440.

Fenrick, R. and Hiebert, S.W. (**1998**) Role of histone deacetylases in acute leukemia. ***J Cell Biochem Suppl***, **30-31**, 194-202.

Filomeni, G., Aquilano, K., Rotilio, G. and Ciriolo, M.R. (**2003**) Reactive oxygen species-dependent c-Jun NH2-terminal kinase/c-Jun signaling cascade mediates neuroblastoma cell death induced by diallyl disulfide. ***Cancer Res***, **63**, 5940-5949.

Finnin, M.S., Donigian, J.R., Cohen, A., Richon, V.M., Rifkind, R.A., Marks, P.A., Breslow, R. and Pavletich, N.P. (**1999**) Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. ***Nature***, **401**, 188-193.

Fischle, W., Kiermer, V., Dequiedt, F. and Verdin, E. (**2001**) The emerging role of class II histone deacetylases. ***Biochem Cell Biol***, **79**, 337-348.

Fischle, W., Wang, Y. and Allis, C.D. (**2003**) Histone and chromatin cross-talk. ***Curr Opin Cell Biol***, **15**, 172-183.

Frey, R.R., Wada, C.K., Garland, R.B., Curtin, M.L., Michaelides, M.R., Li, J., Pease, L.J., Glaser, K.B., Marcotte, P.A., Bouska, J.J., Murphy, S.S. and Davidsen, S.K. (**2002**) Trifluoromethyl ketones as inhibitors of histone deacetylase. ***Bioorg Med Chem Lett***, **12**, 3443-3447.

Gaines, P. and Berliner, N. (**2003**) Retinoids in myelopoiesis. ***J Biol Regul Homeost Agents***, **17**, 46-65.

Gao, L., Cueto, M.A., Asselbergs, F. and Atadja, P. (**2002**) Cloning and functional characterization of HDAC11, a novel member of the human histone deacetylase family. ***J Biol Chem***, **277**, 25748-25755.

Giles, R.H., Peters, D.J. and Breuning, M.H. (**1998**) Conjunction dysfunction: CBP/p300 in human disease. ***Trends Genet***, **14**, 178-183.

Gil-Parrado, S., Fernandez-Montalvan, A., Assfalg-Machleidt, I., Popp, O., Bestvater, F., Holloschi, A., Knoch, T.A., Auerswald, E.A., Welsh, K., Reed, J.C., Fritz, H., Fuentes-Prior, P., Spiess, E., Salvesen, G.S. and Machleidt, W. (**2002**) Ionomycin-activated calpain triggers apoptosis. A probable role for Bcl-2 family members. ***J Biol Chem***, **277**, 27217-27226.

Glaser, K.B., Staver, M.J., Waring, J.F., Stender, J., Ulrich, R.G. and Davidsen, S.K. (**2003**) Gene expression profiling of multiple histone deacetylase (HDAC) inhibitors: defining a common gene set produced by HDAC inhibition in T24 and MDA carcinoma cell lines. ***Mol Cancer Ther***, **2**, 151-163.

Glick, R.D., Swendeman, S.L., Coffey, D.C., Rifkind, R.A., Marks, P.A., Richon, V.M. and La Quaglia, M.P. (**1999**) Hybrid polar histone deacetylase inhibitor induces apoptosis and CD95/CD95 ligand expression in human neuroblastoma. ***Cancer Res***, **59**, 4392-4399.

Gottlicher, M., Minucci, S., Zhu, P., Kramer, O.H., Schimpf, A., Giavara, S., Sleeman, J.P., Lo Coco, F., Nervi, C., Pelicci, P.G. and Heinzel, T. (**2001**) Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. ***Embo J***, **20**, 6969-6978.

Gray, S.G. and Ekstrom, T.J. (**2001**) The human histone deacetylase family. ***Exp Cell Res***, **262**, 75-83.

Gui, C.Y., Ngo, L., Xu, W.S., Richon, V.M. and Marks, P.A. (**2004**) Histone deacetylase (HDAC) inhibitor activation of p21WAF1 involves changes in promoter-associated proteins, including HDAC1. ***Proc Natl Acad Sci U S A***.

Guo, Y., Srinivasula, S.M., Druilhe, A., Fernandes-Alnemri, T. and Alnemri, E.S. (**2002**) Caspase-2 induces apoptosis by releasing proapoptotic proteins from mitochondria. ***J Biol Chem***, **277**, 13430-13437.

Henderson, C., Mizzau, M., Paroni, G., Maestro, R., Schneider, C. and Brancolini, C. (**2003**) Role of caspases, Bid, and p53 in the apoptotic response triggered by histone deacetylase inhibitors trichostatin-A (TSA) and suberoylanilide hydroxamic acid (SAHA). ***J Biol Chem***, **278**, 12579-12589.

Hernandez, A., Thomas, R., Smith, F., Sandberg, J., Kim, S., Chung, D.H. and Evers, B.M. (**2001**) Butyrate sensitizes human colon cancer cells to TRAIL-mediated apoptosis. ***Surgery***, **130**, 265-272.

Huang, L., Sowa, Y., Sakai, T. and Pardee, A.B. (**2000**) Activation of the p21WAF1/CIP1 promoter independent of p53 by the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA) through the Sp1 sites. ***Oncogene***, **19**, 5712-5719.

Inoue, H., Shiraki, K., Ohmori, S., Sakai, T., Deguchi, M., Yamanaka, T., Okano, H. and Nakano, T. (**2002**) Histone deacetylase inhibitors sensitize human colonic adenocarcinoma cell lines to TNF-related apoptosis inducing ligand-mediated apoptosis. ***Int J Mol Med***, **9**, 521-525.

Ito, T. (**2003**) Nucleosome assembly and remodeling. ***Curr Top Microbiol Immunol***, **274**, 1-22.

Jaboin, J., Wild, J., Hamidi, H., Khanna, C., Kim, C.J., Robey, R., Bates, S.E. and Thiele, C.J. (**2002**) MS-27-275, an inhibitor of histone deacetylase, has marked in vitro and in vivo antitumor activity against pediatric solid tumors. ***Cancer Res***, **62**, 6108-6115.

Johnstone, R.W. (**2002**) Histone-deacetylase inhibitors: novel drugs for the treatment of cancer. ***Nat Rev Drug Discov***, **1**, 287-299.

Johnstone, R.W., Ruefli, A.A. and Lowe, S.W. (**2002**) Apoptosis: a link between cancer genetics and chemotherapy. ***Cell***, **108**, 153-164.

Kelly, W.K., Richon, V.M., O'Connor, O., Curley, T., MacGregor-Curtelli, B., Tong, W., Klang, M., Schwartz, L., Richardson, S., Rosa, E., Drobnjak, M., Cordon-Cordo, C., Chiao, J.H., Rifkind, R., Marks, P.A. and Scher, H. (**2003**) Phase I clinical trial of histone deacetylase inhibitor: suberoylanilide hydroxamic acid administered intravenously. ***Clin Cancer Res***, **9**, 3578-3588.

Khochbin, S., Verdel, A., Lemercier, C. and Seigneurin-Berny, D. (**2001**) Functional significance of histone deacetylase diversity. ***Curr Opin Genet Dev***, **11**, 162-166.

Kim, Y.B., Lee, K.H., Sugita, K., Yoshida, M. and Horinouchi, S. (**1999**) Oxamflatin is a novel antitumor compound that inhibits mammalian histone deacetylase. ***Oncogene***, **18**, 2461-2470.

Kim, M.S., Kwon, H.J., Lee, Y.M., Baek, J.H., Jang, J.E., Lee, S.W., Moon, E.J., Kim, H.S., Lee, S.K., Chung, H.Y., Kim, C.W. and Kim, K.W. (**2001**) Histone deacetylases induce angiogenesis by negative regulation of tumor suppressor genes. ***Nat Med***, **7**, 437-443.

Komatsu, Y., Tomizaki, K.Y., Tsukamoto, M., Kato, T., Nishino, N., Sato, S., Yamori, T., Tsuruo, T., Furumai, R., Yoshida, M., Horinouchi, S. and Hayashi, H. (**2001**) Cyclic hydroxamic-acid-containing peptide 31, a potent synthetic histone deacetylase inhibitor with antitumor activity. ***Cancer Res***, **61**, 4459-4466.

Kouzarides, T. (**2000**) Acetylation: a regulatory modification to rival phosphorylation? ***Embo J***, **19**, 1176-1179.

Kruh, J. (**1982**) Effects of sodium butyrate, a new pharmacological agent, on cells in culture. ***Mol Cell Biochem***, **42**, 65-82.

Lassus, P., Opitz-Araya, X. and Lazebnik, Y. (**2002**) Requirement for caspase-2 in stress-induced apoptosis before mitochondrial permeabilization. ***Science***, **297**, 1352-1354.

Lin, R.J., Sternsdorf, T., Tini, M. and Evans, R.M. (**2001**) Transcriptional regulation in acute promyelocytic leukemia. ***Oncogene***, **20**, 7204-7215.

Liu, L.T., Chang, H.C., Chiang, L.C. and Hung, W.C. (**2003**) Histone deacetylase inhibitor up-regulates RECK to inhibit MMP-2 activation and cancer cell invasion. ***Cancer Res***, **63**, 3069-3072.

Luo, J., Nikolaev, A.Y., Imai, S., Chen, D., Su, F., Shiloh, A., Guarente, L. and Gu, W. (**2001**) Negative control of p53 by Sir2alpha promotes cell survival under stress. ***Cell***, **107**, 137-148.

Maecker, H.L., Yun, Z., Maecker, H.T. and Giaccia, A.J. (**2002**) Epigenetic changes in tumor Fas levels determine immune escape and response to therapy. ***Cancer Cell***, **2**, 139-148.

Maeda, T., Towatari, M., Kosugi, H. and Saito, H. (**2000**) Up-regulation of costimulatory/adhesion molecules by histone deacetylase inhibitors in acute myeloid leukemia cells. ***Blood***, **96**, 3847-3856.

Magner, W.J., Kazim, A.L., Stewart, C., Romano, M.A., Catalano, G., Grande, C., Keiser, N., Santaniello, F. and Tomasi, T.B. (**2000**) Activation of MHC class I, II, and CD40 gene expression by histone deacetylase inhibitors. ***J Immunol***, **165**, 7017-7024.

Mariadason, J.M., Corner, G.A. and Augenlicht, L.H. **(2000**) Genetic reprogramming in pathways of colonic cell maturation induced by short chain fatty acids: comparison with trichostatin A, sulindac, and curcumin and implications for chemoprevention of colon cancer. ***Cancer Res***, **60**, 4561-4572.

Marks, P., Rifkind, R.A., Richon, V.M., Breslow, R., Miller, T. and Kelly, W.K. (**2001**) Histone deacetylases and cancer: causes and therapies. ***Nat Rev Cancer***, **1**, 194-202.

Marks, P.A., Richon, V.M. and Rifkind, R.A. (**1996**) Cell cycle regulatory proteins are targets for induced differentiation of transformed cells: Molecular and clinical studies employing hybrid polar compounds. ***Int J Hematol***, **63**, 1-17.

Marsden, V.S., O'Connor, L., O'Reilly, L.A., Silke, J., Metcalf, D., Ekert, P.G., Huang, D.C., Cecconi, F., Kuida, K., Tomaselli, K.J., Roy, S., Nicholson, D.W., Vaux, D.L., Bouillet, P., Adams, J.M. and Strasser, A. (**2002**) Apoptosis initiated by Bcl-2-regulated caspase activation independently of the cytochrome c/Apaf-1/caspase-9 apoptosome. ***Nature***, **419**, 634-637.

Mitsiades, C.S., Mitsiades, N.S., McMullan, C.J., Poulaki, V., Shringarpure, R., Hideshima, T., Akiyama, M., Chauhan, D., Munshi, N., Gu, X., Bailey, C., Joseph, M., Libermann, T.A., Richon, V.M., Marks, P.A. and Anderson, K.C. (**2004**) Transcriptional signature of histone deacetylase inhibition in multiple myeloma: biological and clinical implications. ***Proc Natl Acad Sci U S A***, **101**, 540-545.

Mitsiades, N., Mitsiades, C.S., Richardson, P.G., McMullan, C., Poulaki, V., Fanourakis, G., Schlossman, R., Chauhan, D., Munshi, N.C., Hideshima, T., Richon, V.M., Marks, P.A. and Anderson, K.C. (**2003**) Molecular sequelae of histone deacetylase inhibition in human malignant B cells. *Blood*, **101**, 4055-4062.

Nau, H., Hauck, R.S. and Ehlers, K. (**1991**) Valproic acid-induced neural tube defects in mouse and human: aspects of chirality, alternative drug development, pharmacokinetics and possible mechanisms. ***Pharmacol Toxicol***, **69**, 310-321.

Neuzil, J., Swettenham, E. and Gellert, N. (**2004**) Sensitization of mesothelioma to TRAIL apoptosis by inhibition of histone deacetylase: role of Bcl-x(L) down-regulation. ***Biochem Biophys Res Commun***, **314**, 186-191.

Newmark, H.L. and Young, C.W. (**1995**) Butyrate and phenylacetate as differentiating agents: practical problems and opportunities. ***J Cell Biochem Suppl***, **22**, 247-253.

Nilsson, J.A. and Cleveland, J.L. (**2003**) Myc pathways provoking cell suicide and cancer. ***Oncogene***, **22**, 9007-9021.

Nimmanapalli, R., Fuino, L., Bali, P., Gasparetto, M., Glozak, M., Tao, J., Moscinski, L., Smith, C., Wu, J., Jove, R., Atadja, P. and Bhalla, K. (**2003**) Histone deacetylase inhibitor LAQ824 both lowers expression and promotes proteasomal degradation of Bcr-Abl and induces apoptosis of imatinib mesylate-sensitive or -refractory chronic myelogenous leukemia-blast crisis cells. ***Cancer Res***, **63**, 5126-5135.

Park, E.K., Kwon, K.B., Park, K.I., Park, B.H. and Jhee, E.C. (**2002**) Role of Ca(2+) in diallyl disulfide-induced apoptotic cell death of HCT-15 cells. ***Exp Mol Med***, **34**, 250-257.

Paroni, G., Henderson, C., Schneider, C. and Brancolini, C. (**2002**) Caspase-2 can trigger cytochrome C release and apoptosis from the nucleus. ***J Biol Chem***, **277**, 15147-15161.

Peart, M.J., Tainton, K.M., Ruefli, A.A., Dear, A.E., Sedelies, K.A., O'Reilly, L.A., Waterhouse, N.J., Trapani, J.A. and Johnstone, R.W. (**2003**) Novel mechanisms of apoptosis induced by histone deacetylase inhibitors. ***Cancer Res***, **63**, 4460-4471.

Phiel, C.J., Zhang, F., Huang, E.Y., Guenther, M.G., Lazar, M.A. and Klein, P.S. (**2001**) Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. ***J Biol Chem***, **276**, 36734-36741.

Philchenkov, A.A. (**2003**) Caspases as regulators of apoptosis and other cell functions. ***Biochemistry*** *(Mosc)*, **68**, 365-376.

Piekarz, R.L., Robey, R., Sandor, V., Bakke, S., Wilson, W.H., Dahmoush, L., Kingma, D.M., Turner, M.L., Altemus, R. and Bates, S.E. (**2001**) Inhibitor of histone deacetylation, depsipeptide (FR901228), in the treatment of peripheral and

cutaneous T-cell lymphoma: a case report. ***Blood***, **98**, 2865-2868.

Plumb, J.A., Finn, P.W., Williams, R.J., Bandara, M.J., Romero, M.R., Watkins, C.J., La Thangue, N.B. and Brown, R. (**2003**) Pharmacodynamic response and inhibition of growth of human tumor xenografts by the novel histone deacetylase inhibitor PXD101. ***Mol Cancer Ther***, **2**, 721-728.

Qiu, L., Burgess, A., Fairlie, D.P., Leonard, H., Parsons, P.G. and Gabrielli, B.G. (**2000**) Histone deacetylase inhibitors trigger a G2 checkpoint in normal cells that is defective in tumor cells. ***Mol Biol Cell***, **11**, 2069-2083.

Qiu, L., Kelso, M.J., Hansen, C., West, M.L., Fairlie, D.P. and Parsons, P.G. (**1999**) Anti-tumour activity in vitro and in vivo of selective differentiating agents containing hydroxamate. ***Br J Cancer***, **80**, 1252-1258.

Richon, V.M., Emiliani, S., Verdin, E., Webb, Y., Breslow, R., Rifkind, R.A. and Marks, P.A. (**1998**) A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases. ***Proc Natl Acad Sci U S A***, **95**, 3003-3007.

Rosato, R.R., Almenara, J.A. and Grant, S. (**2003**) The histone deacetylase inhibitor MS-275 promotes differentiation or apoptosis in human leukemia cells through a process regulated by generation of reactive oxygen species and induction of p21CIP1/WAF1 1. ***Cancer Res***, **63**, 3637-3645.

Rossig, L., Li, H., Fisslthaler, B., Urbich, C., Fleming, I., Forstermann, U., Zeiher, A.M. and Dimmeler, S. (**2002**) Inhibitors of histone deacetylation downregulate the expression of endothelial nitric oxide synthase and compromise endothelial cell function in vasorelaxation and angiogenesis. ***Circ Res***, **91**, 837-844.

Roth, S.Y., Denu, J.M. and Allis, C.D. (**2001**) Histone acetyltransferases. ***Annu Rev Biochem***, **70**, 81-120.

Ruefli, A.A., Ausserlechner, M.J., Bernhard, D., Sutton, V.R., Tainton, K.M., Kofler, R., Smyth, M.J. and Johnstone, R.W. (**2001**) The histone deacetylase inhibitor and chemotherapeutic agent suberoylanilide hydroxamic acid (SAHA) induces a cell-death pathway characterized by cleavage of Bid and production of reactive oxygen species. ***Proc Natl Acad Sci U S A***, **98**, 10833-10838.

Ruefli, A.A., Bernhard, D., Tainton, K.M., Kofler, R., Smyth, M.J. and Johnstone, R.W. (**2002**) Suberoylanilide hydroxamic acid (SAHA) overcomes multidrug resistance and induces cell death in P-glycoprotein-expressing cells. ***Int J Cancer***, **99**, 292-298.

Ruefli, A.A., Smyth, M.J. and Johnstone, R.W. (**2000**) HMBA induces activation of a caspase-independent cell death pathway to overcome P-glycoprotein-mediated multidrug resistance. ***Blood***, **95**, 2378-2385.

Saito, A., Yamashita, T., Mariko, Y., Nosaka, Y., Tsuchiya, K., Ando, T., Suzuki, T., Tsuruo, T. and Nakanishi, O. (**1999**) A synthetic inhibitor of histone deacetylase, MS-27-275, with marked in vivo antitumor activity against human tumors. ***Proc Natl Acad Sci U S A***, **96**, 4592-4597.

Sandor, V., Bakke, S., Robey, R.W., Kang, M.H., Blagosklonny, M.V., Bender, J., Brooks, R., Piekarz, R.L., Tucker, E., Figg, W.D., Chan, K.K., Goldspiel, B., Fojo, A.T., Balcerzak, S.P. and Bates, S.E. (**2002**) Phase I trial of the histone deacetylase inhibitor, depsipeptide (FR901228, NSC 630176), in patients with refractory neoplasms. ***Clin Cancer Res***, **8**, 718-728.

Sasakawa, Y., Naoe, Y., Noto, T., Inoue, T., Sasakawa, T., Matsuo, M., Manda, T. and Mutoh, S. (**2003**) Antitumor efficacy of FK228, a novel histone deacetylase inhibitor, depends on the effect on expression of angiogenesis factors. ***Biochem Pharmacol***, **66**, 897-906.

Scott, C.L., Schuler, M., Marsden, V.S., Egle, A., Pellegrini, M., Nesic, D., Gerondakis, S., Nutt, S.L., Green, D.R. and Strasser, A. (**2004**) Apaf-1 and caspase-9 do not act as tumor suppressors in myc-induced lymphomagenesis or mouse embryo fibroblast transformation. ***J Cell Biol***, **164**, 89-96.

Seelig, M.H. and Berger, M.R. (**1996**) Efficacy of dinaline and its methyl and acetyl derivatives against colorectal cancer in vivo and in vitro. ***Eur J Cancer***, **32A**, 1968-1976.

Sherr, C.J. and Roberts, J.M. (**1999**) CDK inhibitors: positive and negative regulators of G1-phase progression. ***Genes Dev***, **13**, 1501-1512.

Smyth, M.J., Takeda, K., Hayakawa, Y., Peschon, J.J., van den Brink, M.R. and Yagita, H. (**2003**) Nature's TRAIL--on a path to cancer immunotherapy. ***Immunity***, **18**, 1-6.

Suzuki, A., Ito, T., Kawano, H., Hayashida, M., Hayasaki, Y., Tsutomi, Y., Akahane, K., Nakano, T., Miura, M. and Shiraki, K. (**2000**) Survivin initiates procaspase 3/p21 complex formation as a result of interaction with Cdk4 to resist Fas-mediated cell death. ***Oncogene***, **19**, 1346-1353.

Suzuki, A., Tsutomi, Y., Akahane, K., Araki, T. and Miura, M. (**1998**) Resistance to Fas-mediated apoptosis: activation of caspase 3 is regulated by cell cycle regulator p21WAF1 and IAP gene family ILP. ***Oncogene***, **17**, 931-939.

Ueda, H., Manda, T., Matsumoto, S., Mukumoto, S., Nishigaki, F., Kawamura, I. and Shimomura, K. (**1994**) FR901228, a novel antitumor bicyclic depsipeptide produced by Chromobacterium violaceum No. 968. III. Antitumor activities on experimental tumors in mice. ***J Antibiot (Tokyo)***, **47**, 315-323.

Vaziri, H., Dessain, S.K., Ng Eaton, E., Imai, S.I., Frye, R.A., Pandita, T.K., Guarente, L. and Weinberg, R.A. (**2001**) hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase. ***Cell***, **107**, 149-159.

Vigushin, D.M., Ali, S., Pace, P.E., Mirsaidi, N., Ito, K., Adcock, I. and Coombes, R.C. (**2001**) Trichostatin A is a histone deacetylase inhibitor with potent antitumor activity against breast cancer in vivo. ***Clin Cancer Res***, **7**, 971-976.

Wang, R., Brunner, T., Zhang, L. and Shi, Y. (**1998**) Fungal metabolite FR901228 inhibits c-Myc and Fas ligand expression. ***Oncogene,*** **17**, 1503-1508.

Warrell, R.P., Jr., He, L.Z., Richon, V., Calleja, E. and Pandolfi, P.P. (1998) Therapeutic targeting of transcription in acute promyelocytic leukemia by use of an inhibitor of histone deacetylase**.** ***J Natl Cancer Inst***, **90**, 1621-1625.

Warrener, R., Beamish, H., Burgess, A., Waterhouse, N.J., Giles, N., Fairlie, D. and Gabrielli, B. (**2003**) Tumor cell-selective cytotoxicity by targeting cell cycle checkpoints. ***Faseb J***, **17**, 1550-1552.

Williams, R.J. (**2001**) Trichostatin A, an inhibitor of histone deacetylase, inhibits hypoxia-induced angiogenesis. ***Expert Opin Investig Drugs***, **10**, 1571-1573.

Wu, J. and Grunstein, M. (**2000**) 25 years after the nucleosome model: chromatin modifications. ***Trends Biochem Sci***, **25**, 619-623.

Xiao, H., Hasegawa, T., Miyaishi, O., Ohkusu, K. and Isobe, K. (**1997**) Sodium butyrate induces NIH3T3 cells to senescence-like state and enhances promoter activity of p21WAF/CIP1 in p53-independent manner. ***Biochem Biophys Res Commun***, **237**, 457-460.

Yang, C.R., Leskov, K., Hosley-Eberlein, K., Criswell, T., Pink, J.J., Kinsella, T.J. and Boothman, D.A. (**2000**) Nuclear clusterin/XIP8, an x-ray-induced Ku70-binding protein that signals cell death. ***Proc Natl Acad Sci U S A***, **97**, 5907-5912.

Yu, C., Rahmani, M., Almenara, J., Subler, M., Krystal, G., Conrad, D., Varticovski, L., Dent, P. and Grant, S. (**2003a**) Histone deacetylase inhibitors promote STI571-mediated apoptosis in STI571-sensitive and -resistant Bcr/Abl+ human myeloid leukemia cells. ***Cancer Res***, **63**, 2118-2126.

Yu, C., Rahmani, M., Conrad, D., Subler, M., Dent, P. and Grant, S. (**2003b**) The proteasome inhibitor bortezomib interacts synergistically with histone deacetylase inhibitors to induce

apoptosis in Bcr/Abl+ cells sensitive and resistant to STI571. ***Blood***, **102**, 3765-3774.

Zelent, A., Guidez, F., Melnick, A., Waxman, S. and Licht, J.D. (**2001**) Translocations of the RARalpha gene in acute promyelocytic leukemia. ***Oncogene***, **20**, 7186-7203.