Amino Acids (2004) 26: 435–441
DOI 10.1007/s00726-004-0087-3

Amino Acids
Printed in Austria

Acetylation of proteins as novel target for antitumor therapy: Review article

E. Di Gennaro[1,2], **F. Bruzzese**[1], **M. Caraglia**[2], **A. Abruzzese**[2], and **A. Budillon**[1]

[1] Dipartimento di Oncologia Sperimentale, Istituto Nazionale Tumori Fondazione G. Pascale, Napoli, Italy
[2] Dipartimento di Biochimica e Biofisica "F. Cedrangolo", Seconda Università di Napoli, Napoli, Italy

Received January 18, 2004
Accepted February 15, 2004
Published online June 11, 2004;

Summary. Imbalance in histone acetylation can lead to changes in chromatin structure and transcriptional dysregulation of genes that are involved in the control of proliferation, cell-cycle progression, differentiation and/or apoptosis. Histone acetyltransferases (HATs) and histone deacetylases (HDACs), are two classes of enzymes regulating histone acetylation and whose altered activity has been identified in several cancers. HATs and HDACs enzymes also target non histone protein substrates, including transcription factors, nuclear import factors, cytoskeleton and chaperon proteins. HDAC inhibitors are a novel class of anticancer agents which have been recently shown to induce growth arrest and apoptosis in a variety of human cancer cells by mechanism that cannot be solely attributed to the level of histone acetylation. Several clinical studies with HDAC inhibitors are ongoing, however the molecular basis for their tumour selectivity remains unknown and represent a challenge for the cancer research community.

Keywords: Cancer – Chromatin – Histone – Histone deacetylase – Histone deacetylase inhibitors – Transglutaminase

Introduction

In spite of the improvement of conventional medical therapy for cancer treatment, the impact on cancer related mortality in the last ten years has been modest especially for advanced disease in adults. On the other hand, understanding of oncogenomics and cancer signaling pathways have selected novel markers of molecular pathology and novel molecular targets for new drugs.

Recently, an increasing series of data are emerging about the involvement of chromatin structure in the control of cell proliferation and survival pathways in malignant cells, indicating that it can be an additional target for anti-cancer strategies (Marks et al., 2001). In particular, among multiple genetic alterations which characterize tumors, modification of epigenetic events such as methylation of DNA or histone acetylation are emerging as key events in cancer development.

It was Vincent G. Allfrey, several decades ago, who described how histones control gene activation in higher organism and who suggest for the first time the importance of their post-translational modification in the control of RNA synthesis (Allfrey et al., 1963). These pioneering studies from 1960s already indicated that active chromatin was hyperacetylated while inactive, silenced genes were deacetylated and often cytosine methylated. However, it is only in recent years that an emerging body of literature supports the role of chromatin structure in regulating cell-cycle progression, differentiation, and apoptosis, in normal and malignant cells, by modulation of gene transcription.

Four decades of research on the composition of eukaryotic chromatin has identified not only its basic structure, the nucleosome, composed of 146 base pairs of DNA coiled around an octamer of histones, but also the numerous post-translational modification on histone proteins (Richmond et al., 1984; Felsenfeld and Groudine, 2003). In particular, the NH_2-terminal tails of the four core histones (H2A, H2B, H3 and H4) emanate in all directions from the nucleosome, with the H3 NH_2-terminal tail being the longest, and undergo several modifications characterized by high versatility and density (Richmond et al., 1984; Felsenfeld and Groudine, 2003). Whereas additional sites of modification are still being uncovered and the full comprehension of all these modifications is still lacking, many direct and indirect connections between specific patterns of certain histone modifications and distinct biological phenomena such as appropriate regulation of gene transcription, chromosome

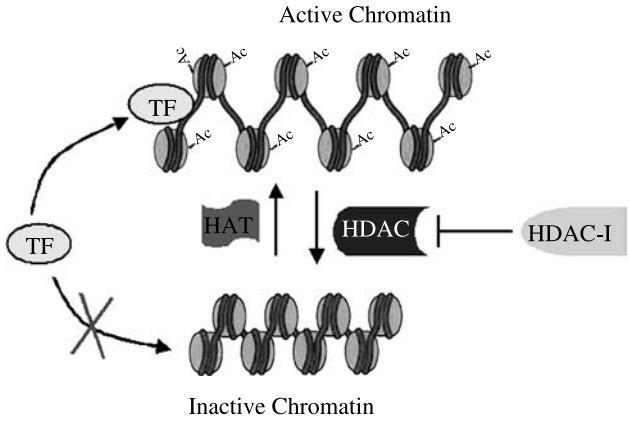

Fig. 1. Regulation of chromatin condensation and gene transcription by histone acetylation. The aminoterminal tails of core histones are potential acetylation (*AC*)/deacetylation sites for histone acetyltransferase (*HAT*) and histone deacetylase (*HDAC*). With inhibition of HDAC by HDAC inhibitors (*HDAC-I*) histones are acetylated and the DNA, that is tightly wrapped around the deacetylated histone core, relaxes, allowing the binding of transcription factor (*TF*)

segregation, cell cycle progression, and the maintenance of genome integrity, have been established (Wolffe and Hayes, 1999; Cheung et al., 2000). Histone modifications can be highly reversible, such as histone acetylation (upon lysine residues) and histone phosphorylation (upon serine and threonine residues), or more stable such as histone methylation (upon lysine and arginine residues).

It has been demonstrated that in general DNA-histone interaction condense chromatin and repress transcription, whereas decreased DNA-histone interaction relax chromatin and enhance gene transcription. These interactions are regulated by the acetylation status of histones, which in eukaryotic cells play a pivotal role in chromatin remodeling and in the regulation of gene expression: hyperacetylation determines transcriptional activation while hypoacetylation transcriptional repression (Deckert and Struhl, 2001; Gregory et al., 2001; Felsenfeld and Groudine, 2003) (Fig. 1). Furthermore, it has been recently proposed that a cross-talk between different post-translational modifications, clustering on specific regions on histone tails, regulate the gene expression (Fischle et al., 2003). For example, it has been shown that modification of the H3 tail by phosphorylation and acetylation compromises its chromatin compaction activity, thereby enabling the transcriptional activation of local genes (Zhong et al., 2003).

HATs and HADCs

The acetylation state of histones is reversibly regulated by two classes of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs) (Gregory et al., 2001) (Fig. 1). In general, transcriptional activators can bind and recruit HAT and are associated with acetylated chromatin, while transcriptional repressors and co-repressors interact with HDAC, and their binding to promoters correlate with loss of histone acetylation (Gregory et al., 2001).

In particular, the acetylation by multiprotein complexes containing HAT enzymes neutralizes the positive charge associated with the ε-amino group of conserved lysine residues within the NH_2-terminal domains of the core histones. In general, this is thought to enhance the accessibility of nucleosomal DNA for transcription, by attenuating direct masking of the NH_2-terminal domains and also by destabilizing higher order folding of nucleosomal filaments mediated by internucleosomal contacts made by these domains (Gregory et al., 2001) (Fig. 1). HATs enzymes can be divided into several families on the basis of a number of highly conserved structural motifs. These include GNAT family, MYST group and p300/CBP family (Marks et al., 2001).

HDACs enzymes restore the positive charge to lysine residues upon hydrolysis of ε-amino acetyl moieties and are divided into three classes (Gray and Ekstrom, 2001; Marks et al., 2001) (Table 1). Class I human HDACs, that have homology to the yeast HDAC called Rpd3, are generally nuclear and associate with transcriptional repressors and co-factors. This class includes the ubiquitously expressed HDAC1, HDAC2, HDAC3 and HDAC8 (Gray and Ekstrom, 2001) and the recently discovered HDAC11 (Gao et al., 2002). Class II HDACs are larger proteins which can shuttle between the cytoplasm and nucleus, include HDAC4, HDAC5, HDAC6, HDAC7, and the recently discovered HDAC9 (Zhou et al., 2001) and HDAC10 (Fischer et al., 2002), are homologous to the yeast HDAC HdaI (Gray and Ekstrom, 2001) and their expression is tissue restricted. HDAC6 and HDAC10 contain two catalytic domains, one of which is specific for tubulin and not for histones (Hubbert et al., 2002), and can be considered a subclass of class II HDACs (class II b) as reported in Table 1. Class I and Class II HDACs have a catalytic pocket at the base, containing a critical zinc ion and a hydrophobic pocket allowing for the insertion of a lysine side chain. Class III of human HDACs consists of homologues of yeast and mouse Sir2 and requires nicotinamine adenine dinucleotide (NAD^+) as a 1:1 co-factor with substrate (Marks et al., 2001).

Like HATs, also HDACs are components of multiprotein complexes containing other proteins including transcriptional regulators. Both these key enzymes also target non-histone protein substrates involved in transcription,

Table 1. Characteristics of histone deacetylases

	Class I	Class IIa	Class IIb	Class III
Yeast HDAC	RPD3	HDA1	HDA1	SIR2
Human HDAC	HDAC 1, 2, 3, 8, 11	HDAC 4, 5, 7, 9	HDAC 6, 10	SIRT 1, 2, 3, 4, 5, 6, 7
Distribution	Ubiquitous	Tissue-restricted	Tissue-restricted	?
Localization	Nuclear	Nucl./cytopl.	Nucl./cytopl.	Nucl./cytopl./Mitochondrial
Targets	Histones p53 (HDAC1), NF-kB (HDAC3)	Histones	Histones, Tubulin	Histones, Tubulin (SIRT2), p53 (SIRT1), TAF(I)68 (SIRT1)
Co-factors	Zn^+	Zn^+	Zn^+	NAD^+
Inhibitor Sensitivity	S	S	S	NS/ND

Table 2. Acetylated protein substrates of HDACs

Histones	H2a, H2b, H3, H4
Transcription factors	TAT, p53, p73, TCF, GATA-1, RelA, C-Jun, E2F, EKLF, NF-Y, HMGI (Y), NF-kB
Nuclear import factors	Importin α, Reh1
Cytoskeleton proteins	αTubulin
Chaperon proteins	HSP90

nuclear transport, cytoskeleton and signal transduction (Grant and Berger, 1999) (Table 2).

Abberrant HDACs and/or HAT activity associated with cancer

Aberrant regulation of gene transcription is hallmark of many forms of cancer and there is increasing evidence that alterations in HAT and HDAC activity occur in several tumors (Cress and Seto, 2000). HDACs have been found to be associated with aberrant transcription factors and can mediate the function of oncogenic translocation products in specific forms of leukemia (Pandolfi, 2001) and lymphoma (Dhordain et al., 1998). For example, acute promyelocytic leukemia (APL) is associated with chromosomal translocation leading to PML-RARα and PLZF-RARα chimeric fusion oncoprotein functioning as aberrant transcriptional repressor in part by recruiting HDACs and altering normal gene regulation through modification of chromatin (Lin et al., 1998; He et al., 2001; Pandolfi, 2001). The repression of otherwise active genes lead to differentiation block and contribute to tumor development.

Moreover, specific regions of the genome of cancer cells are frequently aberrantly hypermethylated leading to silencing of tumor suppressor genes, such as $p16^{INK4a}$ in melanoma or other solid tumors, by the recruitment, on methylated cytosine residues, of multifactor repressor complex containing DNA methyltransferase and HDACs (Jones and Baylin, 2002).

HAT activity has been found to be disrupted by translocation, amplification, overexpression or mutation in a variety of cancers, including those of hematological and epithelial origin. In particular, missense mutations or deletion of p300 have been identified in colorectal and gastric primary tumors, and in other epithelial cancers, confirming a role of this regulatory enzymes in cell transformation (Gayther et al., 2000).

Re-expression of genes epigenetically inactivated can result in the suppression of tumor growth or sensitization to other anticancer therapies. This, together with epigenomic analysis of chromatin alterations such as DNA methylation and histone acetylation, opens up the potential to define epigenetic patterns of gene inactivation in tumors and to use drugs that target epigenetic silencing. If any component of the repression complex might be a therapeutic target, enzymes like HDACs are considerably among the more tractable, accordingly a number of HDAC inhibitors are now in preclinical development or in early clinical trials (Table 3).

Table 3. Examples of histone deacetylase inhibitors in clinical trials

Sodium butyrate (*Short-chain fatty acid*)
Depsipeptide (FR901228, FK228) (*Cyclic tetrapeptide*)
CI-994 (N-acetyl dinaline) (*Benzamide*)
MS-275 (*Benzamide*)
Suberoynalide hydroxamic acid (SAHA) (*Hydroxamic acid*)
LQ824 (*Hydroxamic acid*)
Pyroxamide (*Hydroxamic acid*)
Valproic acid

HDAC inhibitors as anticancer agents

Besides the cross talk between oncogenes and chromatin structures, the role of chromatin remodeling in carcinogenesis is based primarily on experiments with HDAC inhibitors. These compounds are novel agents that modulate chromatin structure and considerable efforts have recently focused on identifying and elucidating the mechanisms by which they mediate growth arrest and apoptosis in cancer cells.

To date, several structural classes of HDAC inhibitors have been identified, including short chain fatty acids such as phenylbutyrate and valproic acid (VPA); cyclic tetrapeptide such as trapoxin A, cyclic peptide such as depsipeptide (FK228) and apicidin; benzamides such as MS27-275 and CI-994; hydroxamic acids such as suberoylanilide hydroxamic acid (SAHA), oxamflatin, trichostatin A and the recently developed LAQ824 and PXD101 (Gottlicher et al., 2001; Marks et al., 2001; Fuino et al., 2003; Plumb et al., 2003). Crystallographic studies revealed that the histone deacetylase inhibitors SAHA and trichostatin A, fit very well into the catalytic pocket of histone deacetylase. The functional group of the hydroxamic acid binds to the zinc atom while the carbon aliphatic chain mimics a lysine side chain of common HDAC substrate, and the hydrophobic cap moiety interacts with the edge of the catalytic pocket and could play a role in HDAC selectivity (Finnin et al., 1999; Marks et al., 2001). In contrast, HDAC inhibitors with lower potency such as phenylbutyrate and VPA possess an acyl group that may interact with the catalytic zinc ion, but cannot make significant contact with the catalytic pocket due to their very short side chains (Johnstone and Licht, 2003). Most HDAC inhibitors are able to inhibit the activity of all class I/II HDACs (Table 1) with few exception, such as the recently identified tubacin which specifically targets the tubulin-specific catalytic domain of class II b HDAC6 (Haggarty et al., 2003). As today, inhibitors of class III HDAC have not been selected yet.

It has been proposed that HDAC inhibitors, by inducing the hyperacetylation of nucleosomal histones, activate the expression of repressed genes that produce growth arrest, terminal differentiation, and/or apoptosis in a variety of cancer cell types (Fig. 1) (Marks et al., 2001; Johnstone and Licht, 2003). In some cases HDAC inhibitors induce gene repression by a mechanism that involve the recruitment of a repressor protein rather than transcriptional activator, as a consequence of histones acetylation.

It is of particular interest the finding that these compounds act very selectively to alter the transcription of fewer than 2% of expressed genes (Van Lint et al., 1996) and by mechanism that cannot be solely attributed to the level of histone acetylation (Johnstone and Licht, 2003). Acetylation of other proteins such as transcription factors, rather than histones, has been described (Table 2) including p53 (Gu and Roeder, 1997), GATA-1 (Boyes et al., 1998) and NF-Y (Jin and Scotto, 1998). The acetylation could either repress or activate the transcription factor leading to either the repression or activation of transcription, respectively. For example, acetylation of p53 and E2F1 increases DNA binding and transcription (Gu and Roeder, 1997; Martinez-Balbas et al., 2000) while acetylation of high mobility group I (HMGI) transcription factors inhibit their binding to DNA (Munshi et al., 1998).

A recent paper by Glaser et al. analyzed the gene expression patterns of three different HDAC inhibitors in three different cell lines using microarray studies, and identified a ''core'' set of genes that are regulated by all HDAC inhibitors in all cell lines examined (Glaser et al., 2003). The ''core'' set, 8 upregulated and 5 down-regulated genes, were predominantly involved in cell cycle, apoptosis and DNA synthesis.

Hierarchical nature of epigenetic transcriptional control, with DNA and histone methylation providing a dominant effect over histone acetylation, can, at least in part, explain the relative low number of genes affected by HDAC inhibitors. In general, methylated genes cannot be reactivated by histone hyperacetylation alone. Re-expression of methylated genes can be obtained, in some cases, by treatment with 5-Azacytidine (5Aza) which sequesters DNA methyltransferase. Interestingly, while HDAC inhibitors or 5Aza alone induced a different set of genes, combination therapy with both drugs synergistically activated a set of methylated repressed genes in different cell models (Jones and Baylin, 2002; Yamashita et al., 2002). Similar approaches has been successfully used in therapy-resistant acute promyelocitic leukemia by using HDAC inhibitors in combination with a all-trans retinoic acid (ATRA) (He et al., 2001).

One of the most commonly induced genes by HDAC inhibitors is the cell cycle kinase inhibitor $p21^{waf1}$ which is transcriptionally activated by a p53 independent mechanism (Sambucetti et al., 1999). Other commonly induced genes include gelsolin, $p16^{ink4a}$ and $p27^{kip1}$ (Hoshikawa et al., 1994; Kim et al., 1999). Among several genes repressed by HDAC inhibitors are cyclin D1 (Kim et al., 1999) and thymidilate synthase (Glaser et al., 2003). The induction of inhibitors of the cyclin dependent kinase 4 (CDK4) and CDK2, such as $p21^{waf1}$, $p16^{ink4a}$ and $p27^{kip1}$, as well as the repression of cyclin D1, resulted in

G1 cell cycle arrest and differentiation, common mechanism of the antiproliferative effect induced by HDAC inhibitors.

HDAC inhibitors can induce also apoptosis and their ability to induce proapoptotic genes such as FAS or BAK suggests that transcription regulation may be involved in this process (Johnstone and Licht, 2003). However, in several systems it has been shown that cell death induced by HDAC inhibitors was characterized by alteration of cell cycle profile with a loss of cells in G1 and S phase and accumulation of cells with a 4n DNA content, consistent with a G2-M cell cycle arrest (Peart et al., 2003). Moreover, while a number of different mechanism were described for HDAC-induced cell death, including death receptor-activated caspase cascade, usually the intrinsic apoptotic pathway, mediated by mitochondrial membrane disruption, is commonly activated by all the HDAC inhibitors studied (Ruefli et al., 2001; Peart et al., 2003; Rosato et al., 2003).

It is common knowledge that subsequent phosphorylation and acetylation of p53 promote different interactions with other proteins and with target gene regulatory elements, to facilitate cell-cycle arrest, apoptosis, or adaptation in response to DNA damage (Ashcroft and Vousden, 1999). HDAC inhibitors, by blocking deacetylation and destabilization induced by class I and II HDACs, could inhibit MDM2-mediated ubiquitination of p53 and enhance sequence-specific DNA binding of this protein (Ashcroft and Vousden, 1999). However, the importance of p53 in HDAC inhibitors-mediated cell death is uncertain. It is of particular interest the finding that HDAC inhibitors, such as SAHA and Depsipeptide, can downregulate the expression of mutant, but not wild-type, p53 (Di Gennaro et al., unpublished observations; Yu et al., 2002).

The HDAC inhibitor SAHA is also able to up-regulate the expression of tissue transglutaminase (TGase) (Di Gennaro et al., unpublished observations), another enzyme involved in the regulation of several biological events including cellular differentiation and apoptosis (Fesus and Piacentini, 2002). TGase catalyzes covalent cross-linking of proteins by forming isopeptide bonds between peptide-bound glutamine and lysine residues (Fesus and Piacentini, 2002). Histones are substrates for TGase and a recent paper suggests that histone cross-linking by TGase may play a role in chromatin remodeling and gene transcription regulation (Sato et al., 2003).

Suppression of telomerase gene expression by HDAC inhibitors has been also demonstrated, indicating that these agents may induce antiproliferative effect also by interfering with cell senescence programs (Nakamura et al., 2001).

Furthermore, a novel mechanism of antitumor activity can be attributed to the recently described ability of HDAC inhibitors to deplete the level of several oncoproteins that are normally stabilized by binding to the Hsp90 multichaperone complexes, including Bcr-Abl, ErbB2, Raf, or AKT (Yu et al., 2002; Nimmanapalli et al., 2003). It has been shown that HDAC inhibitors could promote directly the acetylation of Hsp90, with destabilization of the ATP-dependent active Hsp90-client protein complex. As a consequence, client proteins are driven to poly-ubiquitination and proteosomal degradation. Interestingly, this findings suggest that HDAC inhibitors has the potential to improve the efficacy of drugs targeting Hsp-90 client oncoproteins. On this regard, Fuino et al. have shown how the HDAC inhibitor LAQ824, by downregulating ErbB2, sensitizes human breast cancer cells to the anti-ErbB2 antibody Trastuzumab and to convention chemotherapeutics (Fuino et al., 2003). Moreover, the same group have shown that LAQ824, by lowering expression and promoting proteosomal degradation of Bcr-Abl, induced apoptosis of imatinib mesylate (Gleevec)-sensitive or refractory chronic myelogenous leukemia blast crisis cells (Nimmanapalli et al., 2003).

Finally, other mechanisms of antitumor activity of HDAC inhibitors include antiangiogenic properties, through alteration of VEGF signaling (Deroanne et al., 2002) and suppression of tumor invasion, through negative regulation of matrix metalloproteinases expression (Liu et al., 2003).

Conclusions

Several HDAC inhibitors exhibit antitumor effects also in preclinical animal models at amounts that have little or no toxicity and some of them, are in clinical trials (Table 3). First clinical studies have shown that histone hyperacetylation can be achieved safely in humans and that treatment of cancer with such agents seems to become possible. Thus, HDAC inhibitors remains one of the most promising class of new anticancer agents. Further studies are needed in order to delineate the optimal dosage, the duration of therapy and, possibly, the efficacy of other agents able to synergize with HDAC inhibitors in the fight against cancer. Based on the studies we have presented here, some example of combination therapy are already moving to the design of novel clinical trials. A recent paper suggests that loosening-up the chromatin structure by histone acetylation can increase the efficiency of several conventional anticancer drugs targeting DNA (Kim et al., 2003). However, the precise mechanism by which

these agents mediate growth inhibition and apoptosis, preferentially in cancer cells, remains uncertain; histone acetylation alone appears to be insufficient to account for the potent cytotoxic effects of HDAC inhibitors. Moreover, the gene expression profile regulated by these novel agents has yet to be completed. In addition, what factors establish whether a cancer cell undergoes G1 or G2-M cell cycle arrest, differentiation or cell death in response to HDAC inhibitors remain to be defined. Drugs levels as well as tissue type seem to play a role (Johnstone and Licht, 2003). Moreover, the basis of the selected toxicity toward cancer cells is unclear. One possible explanation could be ascribed to the fact that cancer cells lose cell cycle checkpoints and are more sensitive to agents whose proapoptotic activity is related to abberant mitosis, as described above. The few clinical studies showed no major toxicity in patient treated with HDAC inhibitors with good response in selected tumor subtype such as renal cell carcinoma (Sandor et al., 2002), and T cell lymphoma (Kelly et al., 2002; Piekarz et al., 2002). HDAC inhibitors that specifically target selected HDACs might be useful if ongoing studies can demonstrate if a particular HDAC play a role in a specific type of cancer.

Future translation studies are critical for the correlation between clinical response, accumulation of acetylated chromatin and biological phenomena, such as specific gene expression regulation or apoptosis, and should determine clinical utility and mechanism of action of these agents.

References

Allfrey VG, Littau VC, Mirsky AE (1963) On the role of histones in regulation ribonucleic acid synthesis in the cell nucleus. Proc Natl Acad Sci USA 49: 414–421

Ashcroft M, Vousden KH (1999) Regulation of p53 stability. Oncogene 18: 7637–7643

Boyes J, Byfield P, Nakatani Y, Ogryzko V (1998) Regulation of activity of the transcription factor GATA-1 by acetylation. Nature 396: 594–598

Cheung P, Allis CD, Sassone-Corsi P (2000) Signaling to chromatin through histone modifications. Cell 103: 263–271

Cress WD, Seto E (2000) Histone deacetylases, transcriptional control, and cancer. J Cell Physiol 184: 1–16

Deckert J, Struhl K (2001) Histone acetylation at promoters is differentially affected by specific activators and repressors. Mol Cell Biol 21: 2726–2735

Deroanne CF, Bonjean K, Servotte S, Devy L, Colige A, Clausse N, Blacher S, Verdin E, Foidart JM, Nusgens BV, Castronovo V (2002) Histone deacetylases inhibitors as anti-angiogenic agents altering vascular endothelial growth factor signaling. Oncogene 21: 427–436

Dhordain P, Lin RJ, Quief S, Lantoine D, Kerckaert JP, Evans RM, Albagli O (1998) The LAZ3(BCL-6) oncoprotein recruits a SMRT/mSIN3A/histone deacetylase containing complex to mediate transcriptional repression. Nucleic Acids Res 26: 4645–4651

Felsenfeld G, Groudine M (2003) Controlling the double helix. Nature 421: 448–453

Fesus L, Piacentini M (2002) Transglutaminase 2: an enigmatic enzyme with diverse functions. Trends Biochem Sci 27: 534–539

Finnin MS, Donigian JR, Cohen A, Richon VM, Rifkind RA, Marks PA, Breslow R, Pavletich NP (1999) Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors. Nature 401: 188–193

Fischer DD, Cai R, Bhatia U, Asselbergs FA, Song C, Terry R, Trogani N, Widmer R, Atadja P, Cohen D (2002) Isolation and characterization of a novel class II histone deacetylase, HDAC10. J Biol Chem 277: 6656–6666

Fischle W, Wang Y, Allis CD (2003) Binary switches and modification cassettes in histone biology and beyond. Nature 425: 475–479

Fuino L, Bali P, Wittmann S, Donapaty S, Guo F, Yamaguchi H, Wang HG, Atadja P, Bhalla K (2003) Histone deacetylase inhibitor LAQ824 down-regulates Her-2 and sensitizes human breast cancer cells to trastuzumab, taxotere, gemcitabine, and epothilone B. Mol Cancer Ther 2: 971–984

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

Gayther SA, Batley SJ, Linger L, Bannister A, Thorpe K, Chin SF, Daigo Y, Russell P, Wilson A, Sowter HM, Delhanty JD, Ponder BA, Kouzarides T, Caldas C (2000) Mutations truncating the EP300 acetylase in human cancers. Nat Genet 24: 300–303

Glaser KB, Staver MJ, Waring JF, Stender J, Ulrich RG, Davidsen SK (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

Gottlicher M, Minucci S, Zhu P, Kramer OH, Schimpf A, Giavara S, Sleeman JP, Lo Coco F, Nervi C, Pelicci PG, Heinzel T (2001) Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. EMBO J 20: 6969–6978

Grant PA, Berger SL (1999) Histone acetyltransferase complexes. Semin Cell Dev Biol 10: 169–177

Gray SG, Ekstrom TJ (2001) The human histone deacetylase family. Exp Cell Res 262: 75–83

Gregory PD, Wagner K, Horz W (2001) Histone acetylation and chromatin remodeling. Exp Cell Res 265: 195–202

Gu W, Roeder RG (1997) Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell 90: 595–606

Haggarty SJ, Koeller KM, Wong JC, Grozinger CM, Schreiber SL (2003) Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. Proc Natl Acad Sci USA 100: 4389–4394

He LZ, Tolentino T, Grayson P, Zhong S, Warrell RP Jr, Rifkind RA, Marks PA, Richon VM, Pandolfi PP (2001) Histone deacetylase inhibitors induce remission in transgenic models of therapy-resistant acute promyelocytic leukemia. J Clin Invest 108: 1321–1330

Hoshikawa Y, Kwon HJ, Yoshida M, Horinouchi S, Beppu T (1994) Trichostatin A induces morphological changes and gelsolin expression by inhibiting histone deacetylase in human carcinoma cell lines. Exp Cell Res 214: 189–197

Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, Yoshida M, Wang XF, Yao TP (2002) HDAC6 is a microtubule-associated deacetylase. Nature 417: 455–458

Jin S, Scotto KW (1998) Transcriptional regulation of the MDR1 gene by histone acetyltransferase and deacetylase is mediated by NF-Y. Mol Cell Biol 18: 4377–4384

Johnstone RW, Licht JD (2003) Histone deacetylase inhibitors in cancer therapy: is transcription the primary target? Cancer Cell 4: 13–18

Jones PA, Baylin SB (2002) The fundamental role of epigenetic events in cancer. Nat Rev Genet 3: 415–428

Kelly W, O'Connor O, Richon VM, Tong W, Rao TD, Chiao J, O'Brien JP, Rifkind RA, Marks PA, Scher HI (2002) A phase I clinical trial of an

oral formulation of the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA). Eur J Cancer 38[Suppl 7]: 88 (#286) (Proceedings of 14th EORTC-NCI-AACR Symposium, Frankfurt, Germany, 19–22 November 2002)

Kim MS, Blake M, Baek JH, Kohlhagen G, Pommier Y, Carrier F (2003) Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA. Cancer Res 63: 7291–7300

Kim YB, Lee KH, Sugita K, Yoshida M, Horinouchi S (1999) Oxamflatin is a novel antitumor compound that inhibits mammalian histone deacetylase. Oncogene 18: 2461–2470

Lin RJ, Nagy L, Inoue S, Shao W, Miller WH Jr, Evans RM (1998) Role of the histone deacetylase complex in acute promyelocytic leukaemia. Nature 391: 811–814

Liu LT, Chang HC, Chiang LC, Hung WC (2003) Histone deacetylase inhibitor up-regulates RECK to inhibit MMP-2 activation and cancer cell invasion. Cancer Res 63: 3069–3072

Marks P, Rifkind RA, Richon VM, Breslow R, Miller T, Kelly WK (2001) Histone deacetylases and cancer: causes and therapies. Nat Rev Cancer 1: 194–202

Martinez-Balbas MA, Bauer UM, Nielsen SJ, Brehm A, Kouzarides T (2000) Regulation of E2F1 activity by acetylation. EMBO J 19: 662–671

Munshi N, Merika M, Yie J, Senger K, Chen G, Thanos D (1998) Acetylation of HMG I(Y) by CBP turns off IFN beta expression by disrupting the enhanceosome. Mol Cell 2: 457–467

Nakamura M, Saito H, Ebinuma H, Wakabayashi K, Saito Y, Takagi T, Nakamoto N, Ishii H (2001) Reduction of telomerase activity in human liver cancer cells by a histone deacetylase inhibitor. J Cell Physiol 187: 392–401

Nimmanapalli R, Bali P, O'Bryan E, Fuino L, Guo F, Wu J, Houghton P, Bhalla K (2003) Arsenic Trioxide Inhibits Translation of mRNA of bcr-abl, Resulting in Attenuation of Bcr-Abl Levels and Apoptosis of Human Leukemia Cells. Cancer Res 63: 7950–7958

Pandolfi PP (2001) *In vivo* analysis of the molecular genetics of acute promyelocytic leukemia. Oncogene 20: 5726–5735

Peart MJ, Tainton KM, Ruefli AA, Dear AE, Sedelies KA, O'Reilly LA, Waterhouse NJ, Trapani JA, Johnston RW (2003) Novel mechanisms of apoptosis induced by histone deacetylase inhibitors. Cancer Res 63: 4460–4471

Piekarz RL, Robey R, Sandor V, Bakke S, Wilson WH, Dahmoush L, Kingma DM, Turner ML, Altemus R, Bates SE (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 JA, Finn PW, Williams RJ, Bandara MJ, Romero MR, Watkins CJ, La Thangue NB, 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

Richmond TJ, Finch JT, Rushton B, Rhodes D, Klug A (1984) Structure of the nucleosome core particle at 7 A resolution. Nature 311: 532–537

Rosato RR, Almenara JA, 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

Ruefli AA, Ausserlechner MJ, Bernhard D, Sutton VR, Tainton KM, Kofler R, Smyth MJ, Johnstone RW (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 USA 98: 10833–10838

Sambucetti LC, Fischer DD, Zabludoff S, Kwon PO, Chamberlin H, Trogani N, Xu H, Cohen D (1999) Histone deacetylase inhibition selectively alters the activity and expression of cell cycle proteins leading to specific chromatin acetylation and antiproliferative effects. J Biol Chem 274: 34940–34947

Sandor V, Bakke S, Robey RW, Kang MH, Blagosklonny MV, Bender J, Brooks R, Piekarz RL, Tucker E, Figg WD, Chan KK, Goldspiel B, Fojo AT, Balcerzak SP, Bates SE (2002) Phase I trial of the histone deacetylase inhibitor, depsipeptide (FR901228, NSC 630176), in patients with refractory neoplasms. Clin Cancer Res 8: 718–728

Sato N, Ohtake Y, Kato H, Abe S, Kohno H, Ohkubo Y (2003) Effects of polyamines on histone polymerization. J Protein Chem 22: 303–307

Van Lint C, Emiliani S, Verdin E (1996) The expression of a small fraction of cellular genes is changed in response to histone hyperacetylation. Gene Expr 5: 245–253

Wolffe AP, Hayes JJ (1999) Chromatin disruption and modification. Nucleic Acids Res 27: 711–720

Yamashita K, Upadhyay S, Osada M, Hoque MO, Xiao Y, Mori M, Sato F, Meltzer SJ, Sidransky D (2002) Pharmacologic unmasking of epigenetically silenced tumor suppressor genes in esophageal squamous cell carcinoma. Cancer Cell 2: 485–495

Yu X, Guo ZS, Marcu, MG, Neckers L, Nguyen DM, Chen GA, Schrump DS (2002) Modulation of p53, ErbB1, ErbB2, and Raf-1 expression in lung cancer cells by depsipeptide FR901228. J Natl Cancer Inst 94: 504–513

Zhong S, Goto H, Inagaki M, Dong Z (2003) Phosphorylation at serine 28 and acetylation at lysine 9 of histone H3 induced by trichostatin A. Oncogene 22: 5291–5297

Zhou X, Marks PA, Rifkind RA, Richon VM (2001) Cloning and characterization of a histone deacetylase, HDAC9. Proc Natl Acad Sci USA 98: 10572–10577

Authors' address: Alfredo Budillon, Istituto Nazionale Tumori G. Pascale, Via M. Semmola, 80131 Napoli, Italy,
Fax: 39-081-5903814, E-mail: budillon@fondazionepascale.it