Review

Histone tail modifications and noncanonical functions of histones: perspectives in cancer epigenetics

Annamaria Hadnagy,[1,3] Raymond Beaulieu,[1,2] and Danuta Balicki[1,2]

[1]Research Centre and Department of Medicine, Hôtel-Dieu du Centre hospitalier de l'Université de Montréal; and Departments of [2]Medicine and [3]Biology and Cellular Pathology, Université de Montréal, Montréal, Québec, Canada

Abstract

Over the past few years, the histone deacetylase (HDAC) inhibitors have occupied an important place in the effort to develop novel, but less toxic, anticancer therapy. HDAC inhibitors block HDACs, which are the enzymes responsible for histone deacetylation, and therefore they modulate gene expression. The cellular effects of HDAC inhibitors include growth arrest and the induction of differentiation. Early successes in cancer therapeutics obtained using these drugs alone or in combination with other anticancer drugs emphasize the important place of posttranslational modifications of histones in cancer therapy. Histone tail modifications along with DNA methylation are the most studied epigenetic events related to cancer progression. Moreover, extranuclear functions of histones have also been described. Because HDAC inhibitors block HDACs and thereby increase histone acetylation, we propose a model wherein exogenous acetylated histones or other related acetylated proteins that are introduced into the nucleus become HDAC substrates and thereby compete with endogenous histones for HDACs. This competition may lead to the increased acetylation of the endogenous histones, as in the case of HDAC inhibitor therapy. Moreover, other mechanisms of action, such as binding to chromatin and modulating gene expression, are also possible for exogenously introduced histones. [Mol Cancer Ther 2008;7(4):740–8]

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Requests for reprints: Danuta Balicki, Hôtel-Dieu du Centre hospitalier de l'Université de Montréal, Pavillon Masson, 3850, rue Saint-Urbain, Montréal, Québec, Canada H2W 1T7. Phone: 514-890-8000 ext. 15518; Fax: 514-412-7204. E-mail: danuta.balicki@umontreal.ca

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Histones

Histones are the most abundant proteins bound to DNA in eukaryotic cells and among the most evolutionary conserved proteins known (1). They are small basic proteins with a molecular weight between 11 and 20 kDa and they contain a high percentage of positively charged amino acids (~20%) such as lysine and arginine. Eukaryotic cells contain mainly five types of histones: histone linker H1 and core histones H2A, H2B, H3, and H4. All four core histones (i.e., histones H2A, H2B, H3, and H4) share a similar structure, with a central ''fold domain'' and terminal ''tails,'' NH_2-terminal and COOH-terminal (2). The fold domain contributes to octamer histone assembly and terminal tails are crucial for the normal functioning of cellular processes, including replication and transcription (3), because they are targets for posttranslational modifications (acetylation, methylation, phosphorylation, and ubiquitination; ref. 4). Linker histone H1 binds nucleosomes together and thus participates in a higher-order compaction of chromatin (5).

Besides these canonical histones, several histone variants have been described such as histone H2A.X, which is implicated in DNA repair and genomic stability (6), and histone H1.2, a proapoptotic protein that is translocated from the nucleus to mitochondria (7).

Epigenetics in Cancer

The term ''epigenetics'' was introduced by Conrad Waddington (Waddington, 1942 cited in ref. 8). Epigenetics is the study of heritable changes in gene expression that are not related to changes in DNA sequence (4, 8).

In eukaryotic cells, DNA is wrapped around histones and thus is limited in its accessibility in biological processes such as replication, transcription, and DNA repair. Therefore, cellular mechanisms of chromatin modulation were identified in cells, including recruitment of nucleosome remodeling factors such as SWI/SNF (9) and modulation of the contact between DNA and histones through posttranslational modifications of histones (3).

Epigenetics includes DNA methylation, histone modifications, chromatin remodeling, and microRNAs (10). These processes may be deregulated in several diseases including cancer, neurologic, and cardiovascular diseases.

Cancer is characterized by dysregulation of normal cell proliferation caused by genetic or epigenetic alterations. Genetic alterations of various genes implicated in normal cell proliferation such as tumor suppression genes lead to

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abnormal protein expression encoded by these genes and thus to loss of normal functioning of these proteins. Histones tail modifications such as acetylation, methylation, phosphorylation, and ubiquitination, along with DNA methylation, are the most studied epigenetic events related to cancer progression (4). Epigenetic events modulate gene expression without modification of primary gene sequence. For example, hypermethylation of DNA promoter region of genes that control normal cell proliferation, such as tumor suppression genes, is associated with gene silencing and, thus, with tumor progression (11). It was suggested that the DNA hypermethylome can be associated with tumor aggressiveness and it may be used as a clinical marker in cancer cell characterization. Fiegl et al. (12) suggested that HER-2/neu–positive aggressive breast cancer cells are characterized by a specific DNA methylation profile. They identified three genes, *PGR* (coding for the progesterone receptor), *HSD17B4* (coding for type 4 17-β-hydroxysteroid dehydrogenase, an enzyme involved in estrogen metabolism), and *CDH13* (coding for H-cadherin), whose DNA methylation correlates with Her2/neu status, and subsequently, they suggested that this methylation profile may explain the aggressiveness and reduced responsiveness to antiestrogen treatment.

Thus, epigenetic events play an important role in cancer development and progression and in recent advancements made in epigenetic therapy, as the epigenetic alterations are the targets of epigenetic drugs. Moreover, epigenetic modifications may also be implicated in prognosis and drug response of the patients. Thus, Esteller et al. (13) have shown that methylation of the promoter of DNA repair enzyme O^6-methyl-guanine-DNA methyltransferase, an enzyme involved in resistance to alkylating agents, can be used as a predictor of responsiveness to treatment with these drugs. Moreover, they suggested that O^6-methyl-guanine-DNA methyltransferase methylation may be a better prognostic factor than those used classically, such as tumor grade and patient age.

Histone Acetylation

Histone acetylation and deacetylation play an important role in chromatin remodeling and, thus, in gene expression. There is a fine balance between acetylation and deacetylation of histones in normal cells, and the enzymes catalyzing these modifications are histone acetyltransferases and histone deacetylases (HDAC), respectively (14). Whereas histone acetylation is associated with an open chromatin and enhanced transcription, histone deacetylation is associated with closed chromatin and transcriptional repression. For example, acetylation of NH_2-terminal core histones facilitates the recruitment of transcription factors such as transcription factor IIIA (15), and histone H3 and H4 acetylation is associated with an open $(H3\text{-}H4)_2$ tetrameric particle allowing the access of transcriptional machinery to DNA (3).

Along with transcriptional modulation, histone acetylation plays an important role in other biological processes such as replication and DNA repair, as histone acetylation facilitates the movement of the replication machinery along the DNA strand (16) and creates a favorable environment for DNA repair (17).

DNA Methylation

Methylation of CpG islands (DNA region characterized by a high percentage of cytosine and guanine) is an epigenetic event characterized by the transfer of a methyl group to the C-5 position of cytosine. This process is catalyzed by DNA methyltransferases (DNMT; ref. 18).

Alteration in DNA methylation can result in either DNA hypermethylation or DNA hypomethylation. Both modifications were identified in cancer cells. Gene promoter hypermethylation and global gene hypomethylation play an important role in tumorigenesis (4). Gene promoter hypermethylation was associated with the inhibition of cancer-related genes such as tumor suppressor genes and DNA mismatch repair genes (4, 11). DNA methylation is associated with chromatin compaction because the loss of DNA methylation alters the binding of the linker histone H1 (19). DNA hypometylation, the first epigenetic event identified in cancer cell (11), can lead to activation of oncogenes (11) and to genomic instability (4).

Whereas the human genome project, which is now completed, was designed to identify all human genes, a human epigenome project was designed to identify DNA methylation sites in human genes in major tissues (10). This project acknowledges the role of epigenetic modifications in human diseases such as cancer. Epigenetic modifications play an integral role in cancer. For example, Espada et al. (18) have shown that the loss of DNA methylation in cells lacking DNA methyltransferase 1 (DNMT1) is associated with an increase in the acetylation and a decrease in the methylation of histone H3. These results are not surprising because a specific interaction between DNMT1 and HDAC has also been reported (20). Moreover, DNMT1 itself is associated with deacetylase activity (20).

Epigenetic Drugs

The implications of epigenetic events in gene expression and DNA repair, and therefore in tumorigenesis process, make them a valuable target for cancer therapy. Tumorigenesis is associated with genetic and epigenetic alterations. Whereas genetic alterations such as gene deletions cannot be reversed, certain epigenetic alterations can. Thus, the rationale for epigenetic therapy is reactivation of the expression of several genes silenced by epigenetic events during tumorigenesis. As we previously mentioned, abnormal histone acetylation and DNA methylation were identified in cancer cells. Thus, drugs that target these epigenetic alterations are studied and are termed epigenetic drugs. There are two classes of epigenetic drugs that are currently being investigated: HDAC inhibitors and DNMT inhibitors.

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HDAC Inhibitors

Alterations of histone acetylation are reported in cancers (21). As previously mentioned, two types of enzymes, HDAC and histone acetyltransferase, modulate histone acetylation. The dysregulation of HDAC functions has been associated with hematologic cancers [e.g., HDACs are recruited by the acute promyelocytic leukemia fusion protein (PML-RARα); ref. 22]. They have also been associated with solid tumors; for example, the breast and ovarian cancer susceptibility gene *BRCA1* is associated with HDACs because BRCA1 interacts with components of the HDAC complex; ref. 23.

The mechanism of action of HDAC inhibitors is not entirely understood. The rationale behind the development of HDAC inhibitors was that HDAC inhibitors lead to an increased acetylation of histones and, thus, might reactivate genes, such as the cell cycle inhibitor p21, which are silenced during carcinogenesis (24). However, recently, other mechanisms of action of HDAC inhibitors have been identified, such as the generation of oxidative stress (25) and induction of premature sister chromatid separation that renders the mitotic spindle assembly checkpoint ineffective (26).

Several HDAC inhibitors are currently being investigated, including suberoylanilide hydroxamic acid (27), suberoyl-3-aminopyridineamide hydroxamic acid (pyroxamide; ref. 28), and the benzamide derivate MS-275 (29). Suberoylanilide hydroxamic acid obtained U.S. Food and Drug Administration approval for clinical use for the treatment of cutaneous T-cell lymphoma,[4] and currently, it is in a phase III clinical trial for the treatment of advanced mesothelioma.[5]

DNMT Inhibitors

Although the global low level of gene methylation is associated with cancer, hypermethylation was observed in the promoter region of several genes implicated in carcinogenesis, which correlates with gene silencing (4). DNMT inhibitors, nucleoside and nonnucleoside analogues, are epigenetic drugs that target DNA hypermethylation. Whereas nucleoside analogues require DNA incorporation for DNMT inhibition, nonnucleoside analogues can block DNMT directly without DNA incorporation (4). Inhibition of enzymes responsible for DNA methylation during the process of tumorigenesis results in reactivation of previously silenced cancer-related genes such as tumor suppression genes, DNA mismatch repair genes, and cell cycle–related genes (30).

Recently, it has been suggested that epigenetic modifications such as methylation can regulate the expression of microRNAs (31). They are small RNA molecules encoded in the genome and they control expression of several genes by translational repression. For example, Saito et al. (31) have shown that DNMT inhibitor treatment increases the expression of microRNA-127, a member of the microRNA family. The target of this microRNA is a proto-oncogene, and therefore, the authors suggested that up-regulation of microRNA by epigenetic therapy may be a novel strategy in cancer treatment.

There are several DNMT inhibitors under preclinical and clinical investigation. Two of them, members of nucleoside analogue family, 5-azacytidine and 5-aza-2′-deoxycytidine (5-aza-CdR), obtained Food and Drug Administration approval for clinical use in the treatment of myelodysplastic syndrome. Zebularine (1-[β-D-ribofuranosil]-1,2-dihydropyrimidin-2-1) is a 5-azacytidine derivative that has recently been described as a novel DNMT inhibitor that is more stable and less toxic compared with 5-azacytidine and 5-aza-CdR (32). Because these drugs are incorporated into DNA, they are associated with cytotoxicity (33).

Nonnucleoside DNMT inhibitors are also under investigation. For example, MG98, a phosphorothioate antisense oligodeoxynucleotide that is a specific inhibitor of mRNA for human DNMT1, is currently being tested in clinical trials (34). Fini et al. (35) have evaluated the anticancer effect of *Annurca* polyphenol extract in sporadic colorectal cancers. They showed that *Annurca* polyphenol extract acts as a DNMT inhibitor with comparable effects to those obtained with 5-aza-CdR, but with no side effects, such as myelossupression as reported for 5-aza-CdR.

Although epigenetic drugs can be used as monotherapy, their effects can be optimized by combination therapies, such as combinations of demethylating agents and HDAC inhibitors (30, 36). Mongan et al. (36) have shown that the combination of valproic acid, a short-chain fatty acid structurally related to the butyrate class of HDAC inhibitors, with 5-aza-CdR, a DNMT inhibitor, and retinoic acid leads to reactivation of the tumor suppression gene *RARβ2*, which is epigenetically silenced in breast cancer cells.

Epigenetic drugs can be associated not only with each other but also with chemotherapeutic agents. For example, it has been shown in a phase I trial that the association of valproic acid, a HDAC inhibitor, with epirubicin, an anthracycline antitumor antibiotic, can improve the patients' response to epirubicin. Moreover, an antitumor response was obtained in patients with anthracycline-resistant tumors (37).

A promising application of combination therapy is the reactivation of the estrogen receptor in breast cancer, followed by antiestrogen treatment (38). In general, estrogen receptor–positive tumors are characterized by a better prognosis and treatment outcome compared with estrogen receptor–negative tumors. Loss of estrogen receptor expression in breast cancer is caused not only by genetic events such as DNA sequence mutation (30) but also by epigenetic events such as hypermethylation of estrogen receptor promoter (38). Sharma et al. (38) have shown that treatment of the MDA-MB-231 estrogen receptor–negative breast cancer cell line with a combination of trichostatin, a HDAC inhibitor, and 5-aza-CdR, a

[4] U.S. Food and Drug Administration; http://www.fda.gov/.
[5] U.S. NIH http://clinicaltrials.gov/.

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DNMT inhibitor, can restore estrogen receptor expression, and thus become sensitive to hormonal therapy such as tamoxifen, an antiestrogen agent.

An interesting combination therapy includes epigenetic drugs and cancer immunotherapy. Development of tumor-associated antigen-directed vaccines is one strategy involved in cancer immunotherapy. However, tumor-associated antigens are characterized by a limited and heterogeneous expression in tumors. Sigalotti et al. (39) have shown that the expression of cancer antigens is related to methylation status of their promoter. Therefore, they have shown that DNMT inhibitor treatment induces expression of cancer/testis antigens, and thus, it may improve cancer/testis antigen–directed immunotherapy. Thus, it is hoped that combination therapy including epigenetic drugs will overcome some of the resistance to therapy that we currently face.

Most conventional cancer therapies, such as chemotherapy and radiation therapy, are highly toxic and nonspecific. Advances made in our understanding of the molecular basis of cancer facilitated the development of novel drugs directed more specifically to cancer cell. This is the case of imatinib mesylate, an ABL kinase inhibitor effective against chronic myeloid leukemia, and trastuzumab, a monoclonal antibody against HER-2 receptor, which is effective against breast cancer cells that overexpress HER-2 receptor. It has been suggested that cancer epigenetic therapy may also be specific to cancer cells. Thus, Ungerstedt et al. (40) have shown that HDAC inhibitors induce cell death in transformed cells, whereas normal cells are relatively resistant. One possible explanation for this resistance is the increased expression of thireodoxin, a protein implicated in cell protection against oxidative stress. Thus, increased expression of thireodoxin in normal cells, but not in cancer cells, may protect cells against the cytotoxic effects of HDAC inhibitors.

The induction of DNA damage is the mechanism underlying cancer cell death following chemotherapy or radiation therapy, which will activate DNA cell damage response and triggers apoptosis (7). Besides apoptosis, other strategies may be used to induce cancer cell growth arrest, including cellular differentiation and senescence. This review will focus on differentiation and senescence because these two mechanisms were often reported to be associated with epigenetic drugs.

Differentiation Therapy

The absence of the cellular differentiation, or anaplasia, is a hallmark of malignant tumors, and it is associated with morphologic and functional changes. The morphologic changes of anaplastic cells include alterations in the size and cellular morphology (pleomorphism) and a higher nuclear-cytoplasmic ratio as compared with normal cells. A highly transformed cell is undifferentiated and loses the functional characteristics of the normal cell of origin. In contrast, differentiated cells preserve the functional properties of normal cells.

As it is well accepted that undifferentiated tumors are associated with a poor prognosis, the induction of differentiation is a promising strategy in cancer therapy. The rationale behind differentiation therapy lies in the finding that undifferentiated cancer cells are characterized by an unlimited potential to proliferate and, thus, the induction of differentiation will halt their proliferative capacity. The underlying molecular mechanisms include the induction of specific gene expression (e.g., p21, a cell cycle inhibitor; ref. 29).

One of the most successful differentiation agents tested thus far is all-*trans* retinoic acid, which is used in the treatment of acute promyelocytic leukemia (22). Under normal physiologic conditions, retinoic acid binds to retinoic acid receptor α and releases the HDAC complex, leading to transcriptional activation and hematopoietic cell maturation. Whereas this pathway is disrupted in acute promyelocytic leukemia, high levels of retinoic acid can restore this pathway and release the maturation arrest (22). However, after promising initial remissions obtained in the patients treated with retinoic acid, many of these patients acquire a retinoid resistance. Lin et al. (41) made the assumption that this resistance is the result of the constitutive association of HDACs, and subsequently, they showed that the association of tichostatin A, a HDAC inhibitor, with retinoic acid enhances the differentiating effect of retinoic acid and may overcome retinoic acid resistance.

Induction of differentiation by HDAC inhibitors is not limited to hematopoietic tumors but can also be used in the treatment of solid tumors. For example, Munster et al. (27) have shown that suberoylanilide hydroxamic acid induces growth arrest and differentiation in human breast cancer cells.

Induction of Cellular Senescence

Cellular senescence is a state of irreversible growth arrest associated with morphologic and functional changes. Senescent cells are characterized by the presence of the senescence-associated β-galactosidase marker. This enzyme is a lysosomal hydrolase active at pH 4 in normal cells, but also active at pH 6 in senescent cells. This increase in senescence-associated β-galactosidase activity is a result of its increased lysosomal content in senescent cells (42). Hayflick and Moorhead (43) made the initial suggestion that cells can divide in culture a finite number of times, and beyond that they will stop dividing. This phenomenon is called replicative senescence. Two signals can trigger senescence: telomere shortening, which is associated with replicative senescence, and cellular stress exposure, which is associated to stress- or aberrant signaling–induced senescence (44).

One important step in the carcinogenesis process is overcoming normal cellular senescence and acquiring limitless replicative potential. Maintenance of telomere length is mandatory for a cell to acquire immortal phenotype. Two mechanisms are implicated in this process.

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The first one is the reactivation of telomerase, the enzyme responsible for the maintenance of telomere length in most cancer cells, and the second is the alternative lengthening of telomeres (ALT) by intratelomeric recombination mechanism in cancer cells that do not express the telomerase. Epigenetic mechanisms seem to be involved in both processes. Whereas telomerase reactivation is associated with histone H3 and H4 hyperacetylation, lack of telomerase expression in ALT cells is associated with histone H3 and H4 hypoacetylation (45).

It has been that suggested that because cellular senescence is associated with growth arrest, the induction of senescence can be applied to cancer therapeutics (46). It has been shown that chemotherapeutic drugs, such as etoposide (47), and differentiation agents, such as retinoic acid (48), can trigger cellular senescence. The induction of cellular senescence has also been reported for epigenetic drugs [i.e., HDAC inhibitors (49) and DNMT inhibitors (50)].

"Noncanonical" Functions of Histones: Perspectives in Cancer Therapeutics

Whereas most of the focus on histones is classically involved in chromatin organization in the nucleus, the extracellular localization of histones has also been identified. For example, extracellular histones associated with DNA (i.e., nucleosomes) are found in the circulation under various pathologic conditions such as autoimmune disease (51). Patients with malignant diseases undergoing chemotherapy also have higher levels of circulating nucleosomes, which correlates with a higher rate of cellular death caused by chemotherapy (52).

Extracellular histones have also been located on the surface of the human T-cell line HPB-ALL and phytohemaglutinin-activated human peripheral blood lymphocytes (53). Rose et al. (54) have shown that ileal epithelial cells release histone H1 while undergoing apoptosis and that histone H1 possesses antimicrobial activity.

Histone participation in chromatin organization, gene expression, and DNA repair is well documented. For example, histone H2B participates in postreplicational DNA repair (55) and in the cellular response to double-stranded DNA breaks (56). It was also shown that histone H2B is phosphorylated in apoptotic cells and, thus, it may be used as a hallmark of apoptotic cells (57). The increased acetylation of histones H3 and H4 has been systematically reported after treatment with HDAC inhibitors (24, 28), and it was associated with transcriptional activation of several genes implicated in tumor growth suppression (24).

Additional ''noncanonical'' histone functions include applications in nonviral gene therapy. It has been shown that linker histone H1 and core histones H2A, H2B, H3, and H4 can be used as transfection agents to deliver DNA into various cells (reviewed in ref. 58). In addition to their functions in DNA metabolism, histones H1 and H2A and histone variants H1.2 and H2A.X display additional activities that may have important repercussions in cancer therapy.

Histone H1

Besides its participation in chromatin organization, linker histone H1 possesses other functions. Widlak et al. (59) have shown that the COOH-terminal domain of histone H1 activates the apoptotic nuclease DNA fragmentation factor DFF40/CAD via protein-protein interactions. It has also been shown that histone H1 suppresses tumor cell growth *in vitro* in the Burkitt's lymphoma Daudi cell line and the lymphoblastic leukemia IM-9 cell line, as well as *in vivo* in a mouse xenograft model (60). Histone H1 inhibits cellular proliferation and induces apoptosis in the human breast adenocarcinoma cell lines MCF-7 and MDA-MB-231 (61). Class et al. (60) suggested that the mechanism underlying histone H1 cytotoxicity is the presence of histone H1-binding proteins on the cell surface that will trigger cellular responses such as apoptosis. Moreover, the entire histone H1 molecule may be required to obtain the observed cellular response because different peptides derived from histone H1 have no inhibitory effect (60).

Histone H1.2

Histone H1.2 is a member of the histone H1 family (5). Pohlmeyer et al. (62) have shown that histone H1 derived from calf thymus possesses cytotoxic effects on leukemia cells. The authors identified histone H1.2 as a major component of the histone H1 preparation, and thus they concluded that histone H1.2 is responsible for the cytotoxic effects observed. Moreover, exogenous recombinant histone H1.2 triggers apoptosis in human cervical carcinoma HeLa cells.[6]

Recently, it has been shown that histone H1.2 is involved in X-ray–induced apoptosis (7). Histone H1.2, along with histone H1.1, binds with the lowest affinity to chromatin, and thus it was suggested that histone H1.2 may be a very sensitive DNA double-strand break sensor (5). Indeed, Konishi et al. (7) have shown that after X-ray irradiation, histone H1.2 translocates from the nucleus to the cytoplasm. The mechanism underlying this histone H1.2 translocation and the activation of apoptosis is not completely understood. However, they suggest that histone H1.2 triggers apoptosis by the release of cytochrome *c* from mitochondria following activation of Bak, a member of the Bcl-2 family. Thus, histone H1.2 becomes a proapoptotic protein once it reaches the cytoplasmic compartment and translocates to mitochondria.

Based on these reports, Gine et al. (63) analyzed the cytosolic release of histone H1.2 in primary tumoral chronic lymphocytic leukemia cells after treatment with genotoxic

[6] A. Hadnagy, R. Beaulieu, D. Balicki, unpublished data.

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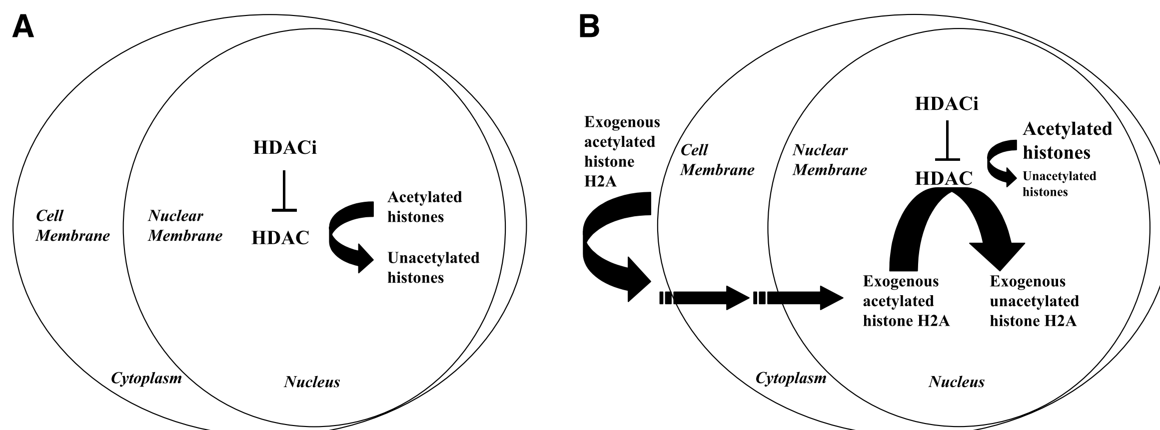

Figure 1. **A,** normal homeostasis where deacetylation of endogenous histones is accomplished by HDAC and inhibited by HDAC inhibitors (*HDACi*). **B,** exogenous histone H2A translocates into the nucleus and disrupts the normal activities of HDAC and HDAC inhibitors by serving as an additional substrate for HDAC. As a result of this competition for HDAC, less HDAC is available to deacetylate histones H3 and H4, leading to an accumulation of the acetylated forms of these histones, as is also described with HDAC inhibitors. The increased acetylation of histones H3 and H4 has been associated with the transcriptional activation of several genes involved in the suppression of tumor growth. Other mechanisms of action of exogenously introduced histones are also possible.

and nongenotoxic agents. They have shown that resistance to treatment with genotoxic agents is associated with the lack of histone H1.2 release. Therefore, the release of histone H1.2 may indicate the treatment outcome.

Another interesting function of histone H1.2 is its antimicrobial activity. Jacobsen et al. (64) have shown *in vitro* and *in vivo* the efficacy of histone H1.2 against burn wound infection pathogens. Moreover, because of its low hemolytic effect, it has been suggested that histone H1.2 may be used as a systemic antimicrobial agent.

Histone H2A

Histone H2A contains a histone fold domain and NH_2- and COOH-terminal tails, just like other core histones. In contrast to other core histones, histone H2A has the largest family of variants (65). It contains a cluster of DNA binding sites localized near the NH_2-terminal tail (66) and it possesses the largest consensus COOH-terminal tail (67).

Exogenous histone H2A inhibits cellular proliferation in several cancer cell lines including the human MCF-7 noninvasive adenocarcinoma cell line and the human MDA-MB-231 invasive adenocarcinoma cell line. Histone H2A blocks cell cycle progression and induces differentiation and cellular senescence in these cells. The mechanism of action underlying these effects is the increase of the cell cycle inhibitor p21.[7,8]

Histone H2A.X

Histone H2A.X represents 2% to 25% of the histone H2A family (68) and its COOH-terminal motif SQ(D/E)(I/L/Y) distinguishes it from other H2A variants (68). The serine (S) in this motif is Ser[139] and is the site of a γ-phosphorylation. Recently, Celeste et al. (6) have shown that the loss of one H2A.X allele compromises genomic integrity and increases cancer incidence in the absence of the tumor suppresor gene *p53*.

Phosphorylation of histone H2A.X is catalyzed by members of the phosphatidylinositol 3-kinase family, including ataxia telangiectasia mutated, ataxia telangiectasia mutated and Rad3-related, and DNA-dependent protein kinase (69). However, ataxia telangiectasia mutated may be the major kinase that contributes to H2A.X phosphorylation in response to DNA double-strand breaks (69). This phosphorylation rapidly occurs in response to DNA double-strand breaks induced by ionizing radiation (68). Ionizing radiation is used in radiotherapy of cancer and it has been shown that it induces histone H2A.X phosphorylation (70). Taneja et al. (70) have shown that the level of the phosphorylated form of histone H2A.X (γH2A.X) after ionizing radiation exposure may predict tumor response to radiotherapy. Moreover, they suggested that histone γH2A.X might represent a biological target in therapy of radioresistant tumors because blocking histone H2A.X phosphorylation increases ionizing radiation–induced apoptosis in cancer cells.

Liu et al. (71) have shown that imatinib mesylate treatment of gastrointestinal stromal tumor cells induces an increase in histone H2A.X level. Moreover, this up-regulation is critical for imatinib mesylate efficiency, and thus, novel therapeutic strategies designed to increase histone H2A.X levels, such as proteasome inhibition, might prevent imatinib resistance of gastrointestinal stromal tumor cells.

[7] M. Kaouass, A. Hadnagy, S. Mansour, R. Beaulieu, D. Balicki. Post-translational modifications of histone H2A are pivotal in its inhibition of human breast cancer cell proliferation via senescence. Poster presentation at San Antonio Breast Cancer Symposium, 2006.
[8] A. Hadnagy, M. Kaouass, S. Mansour, R. Beaulieu, D. Balicki, in preparation.

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Conclusion and Future Goals

Epigenetic drugs represent a promising strategy in cancer therapy as monotherapy as well as combination therapies. The intense focus on HDAC inhibitors was rewarded by the recent introduction of suberoylanilide hydroxamic acid in the clinical setting. Whereas the classic substrates of HDAC are acetylated histones, it has been suggested that not only histone proteins but acetylated nonhistone proteins may also be HDAC substrates (72).

Exogenous Proteins Enter the Nucleus

It has been shown that exogenous proteins may be taken up by *Physarum* cells, an eukaryotic organism implicated in cellular metabolism. This is the case of exogenous H2A/H2B dimers. Thus, Thiriet and Hayes (73) have shown that these exogenous dimers are assembled into nucleosomes. Moreover, it has been shown that the NH_2-terminal tails of H2A/H2B dimers are not required for their nuclear import but that they are pivotal for efficient chromatin assembly.

Balicki et al. (66) have shown that exogenous histone H2A enters the cytoplasm and nucleus within 24 hours. Subsequently, Hariton-Gazal et al. (74) showed that core histones translocate directly across mammalian cell membranes and that this translocation is temperature and energy independent and uninhibited by endocytosis inhibitors such as colchicine, nocodazole, cytochalasin D, brefeldin A, chlorochine, and nystatin. Rosenbluh et al. (75) have shown that core histones penetrate lipid bilayers and *Mycoplasma* membranes. As several proteins have the ability to penetrate cellular membranes, this property was related to the presence of a specific domain rich in arginine and lysine residues, termed the protein transduction domain. Proteins containing this domain were termed cell-penetrating proteins. It has been suggested that histones may be considered cell-penetrating proteins (75). Thus, all these studies have shown that exogenous histone H2A penetrates the nuclear compartment of the cell.

HDAC Competition Model

We propose a model (Fig. 1) in which exogenous acetylated histone H2A, once it enters the nuclear compartment, becomes a substrate for HDAC and thereby competes with endogenous nuclear histones for HDAC. The consequence of this competition will be an increase in the acetylation status of endogenous histones, analogous to treatment with HDAC inhibitors. Our unpublished data about the effects of exogenous histone H2A on cancer cells support this model, as we have observed similarities between HDAC inhibitor and histone H2A effects. Inhibition of cellular proliferation, induction of cell cycle arrest, increase of p21 expression, and initiation of cellular differentiation and senescence are the common effects of HDAC inhibitors (24, 28, 49) and exogenous histone H2A that we have observed and which support this model.[9] However, we do not exclude the possibility that exogenously introduced histones may bind directly to chromatin and modulate gene expression.

In conclusion, the acetylation status of histones plays an important role in cancer progression and treatment. Therefore, epigenetic drugs such as HDAC inhibitors have been studied and they are being introduced in cancer therapy (e.g., suberoylanilide hydroxamic acid). We propose a model whereby the histone acetylation status can be modulated not only by HDAC inhibition but also by competition for HDAC. As HDAC substrates may potentially be histone and nonhistone proteins, the development of acetylated substrates of HDACs capable of reaching the nuclear compartment holds promise as an alternative strategy in epigenetic therapy.

[9] A. Hadnagy, M. Kaouass, S. Mansour, R. Beaulieu, D. Balicki, in preparation.

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