Published in final edited form as:
Mol Cell Pharmacol. 2011 ; 3(2): 39–43.

Effects of Histone Deacetylase Inhibitors on Modulating H3K4 Methylation Marks – A Novel Cross-Talk Mechanism between Histone-Modifying Enzymes

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Abstract

A recent study reports that histone deacetylase (HDAC) inhibitors, AR42 and MS- 275, upregulated H3K4 methylation marks in prostate cancer cells, leading to transcriptional activation of genes including those associated with roles in tumor suppression and cell differentiation (1). Evidence suggests that the crosstalk between histone deacetylation and histone H3K4 methylation is attributable to the ability of these HDAC inhibitors to repress the JARID1 family of histone H3 lysine 4 demethylases (H3K4DMs), including RBP2, PLU-1, SMCX, and LSD1, through the downregulation of Sp1 expression. This demonstrates the complexity of the functional roles of HDACs in the regulation of histone modifications as well as the activation of epigenetically silenced gene expression. Equally important is the ability of HDAC inhibitors to transcriptionally suppress H3K4DM gene expression which has therapeutic implications, in that several H3K4DMs such as LSD1 and PLU-1 have been implicated in the pathogenesis of many types of malignancies.

Keywords

Histone deacetylase; H3K4 methylation; H3K4 demethylases; Histone codes; Histone deacetylase inhibitors; AR42, MS-275

Introduction

Chromatin remodeling and other modifications of the cancer epigenome play an important role in gene regulation during cancer development (2). Gene silencing through DNA methylation, histone deacetylation, gain of repressive histones or replacement of active histones, has been shown to regulate gene expression and genome stability, by controlling the access of key regulatory factors and complexes to the chromatin (3–6). The function of histone methylation itself does not cause changes in the overall charge of the protein. Consequently, a number of lysine residues on histone H3 and H4, such as H3K4, H3K9, H3K27, H3K36, H3K79, and the H4K20, could be site-selectively methylated and reversibly demethylated, led to a dynamically balanced chromatin status with mono-, di-,

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Conflicts of Interest
No potential conflicts of interest to disclose.

and tri-methylation (7, 8). Among these residues, methylation marks of H3K4, H3K36, and H3K79 have been found in open chromatin and are related to transcriptional activation, while those of H3K9, H4K20, and H3K27 are associated with repressive histones (9). Histone acetylation, on the other hand, abolishes the positive charge of the lysine residues to unravel histones from DNA, to maintain the euchromatin, and to activate gene expression. It has been shown that several mechanisms of crosstalk exist between histone acetylation and histone methylation networks (4), which constitute to a complex network for epigenetic control of gene transcription during normal or pathogenic development (10). Specifically, for the H3K9 lysine residue that is subject to both types of post-translational modifications, acetylation can block subsequent methylation, and vice versa, due to mutual exclusivity. For the lysine residue at H3K4 where acetylation has not been found, it has been suggested that a functional link between H3 hyperacetylation and increased H3K4 methylation through different mechanisms. For example, the activity of the H3K4 methyltransferase MLL4 was stimulated by acetylated H3 peptides or HDAC inhibitors (11, 12), whereas that of the H3K4 demethylase LSD1 was diminished by HDAC inhibitors (13). More recently, the authors reported the effect of three distinct HDAC inhibitors, namely AR42 (1), MS-275, and vorinostat, on the upregulation of H3K4 methylation through the transcriptional repression of the JARID1 family of H3K4 demethylases (H3K4DMs) (1). This finding not only provides a novel crosstalk mechanism between class I HDACs and H3K4DMs, but also sheds light onto the mode of action of HDAC inhibitors in regulating global epigenetic homeostasis (14, 15), which might foster new strategies of using this important class of antitumor agents in cancer therapy.

Materials and Methods

Immunocytochemical detection of H3K4 trimethylation and H3K9 trimethylation

LNCaP cells were maintained in 10% FBS-supplemented RPMI 1640 medium, and 2×10^4/cm^2 cells were seeded on coverslips and incubated for 24 h. The cells were then treated with 1 μM AR42, 5 μM SAHA, and 5 μM MS275 for 24 h, washed with 1 X phosphate buffered saline (PBS), and fixed in 4% formaldehyde at 25°C for 20 min. After washing, the cells were incubated firstly with 0.1% Triton X-100/1% FBS in PBS for 1 h at 25 °C, and next with rabbit anti-H3K4Me3 (Cell Signaling Technology, Inc. Danvers, MA) and anti-H3K9Me3 (AbcamPlc, Cambridge, MA) antibodies at 1:100 dilutions for 1 h in an incubation buffer containing 0.1% Triton X-100 and 0.2% BSA in PBS. The cells were washed and then incubated with Alexa Fluor 555-conjugated goat anti-rabbit IgG (H+L) antibody (Invitrogen Corp. Carlsbad, CA) for 1 h at 25°C, and then washed with PBS before mounting the coverslips on slides with mounting solution containing 1 μg/mL 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories Inc., Burlingame, CA). The slides were then observed under a Zeiss LSM 510 inverted confocal laser scanning microscope operated with the Zeiss LSM 510 software.

Results

Differential effects of HDAC inhibitors on modulating histone H3K4 methylation in vitro

It is noteworthy that the pan-HDAC inhibitors AR42, vorinostat, and the class I inhibitor MS-275 altered the methylation state of H3K4 and H3K9 in LNCaP cells albeit with different potencies. AR42, MS-275, and, to a much lesser extent, vorinostat, upregulated the levels of H3K4 tri-methylation (H3K4Me3) (Fig. 1), di-methylation (H3K4Me2), and mono-methylation (H3K4Me), while attenuating H3K9 methylation levels as a result of increased acetylation (1).

From a mechanistic perspective, the differential effect of these three inhibitors on H3K4 methylation might underlie differences in their respective HDAC isozyme specificities. Moreover, the ability of MS-275 to increase H3K4 methylation suggests a functional link with class I HDACs.

HDAC inhibitors modulate intraprostatic H3K4 methylation in transgenic adenocarcinoma of the mouse prostate (TRAMP) mice

It has been shown that AR42 (16) and, to a lesser extent, MS-275 (17) could suppress prostate tumorigenesis and/or shift tumorigenesis to a more differentiated phenotype in the TRAMP chemoprevention model. In the present study, the authors hypothesized that this tumor-suppressive effect was, in part, due to the ability of HDAC inhibitors to change the prostate epigenome in TRAMP mice through histone modifications. The effects of daily oral administration of AR42 (25 mg/kg), vorinostat (50 mg/kg), and MS-275 (20 mg/kg) for two weeks on intraprostatic histone acetylation and methylation was evaluated in 6- week-old TRAMP mice that display early histological changes associated with androgen- driven tumorigenesis. The results showed that these HDAC inhibitors gave rise to robust H3 hyperacetylation and changes in the methylation profiles of H3K9 and H3K4 in the prostates paralleling those observed in LNCaP cells. Relative to vehicle control, AR42 and MS-275 caused significant increases in the expression of H3K4Me3, H3K4Me2, and H3K4Me, while in vorinostat-treated animals, of the three H3K4 methylation marks, only H3K4Me2 exhibited a significant increase. These data show that the premalignant lesions in the TRAMP prostate were as susceptible to modifications of histone methylation by HDAC inhibitors as malignant prostate cells.

H3K4Me3 is involved in the transcriptional activation of genes encoding the tumor suppressor Kruppel-like factor 4 (KLF-4) and the differentiation marker E- cadherin

The authors rationalized that changes in the H3K4 methylation status might underlie the tumor-suppressive activities of these HDAC inhibitors through the re-expression of key cancer genes that were silenced in the malignant tissue. Pursuant to this premise, the authors identified two tumor suppressor genes, *KLF4* and the *E-cadherin*, targeted by H3K4Me3. RT-PCR analysis revealed that both genes were differentially upregulated by AR42, vorinostat, and MS-275 in LNCaP cells, and chromatin immunoprecipitation (ChIP) demonstrated the accumulation of H3K4Me3 marks in the promoter DNA of *KLF4* and *E-cadherin* genes. These findings suggest that HDAC inhibitors can activate the expression of genes associated with tumor suppression and differentiation through changes in histone methylation status.

Increased H3K4 methylation is attributable to the transcriptional repression of H3K4 demethylases in response to HDAC inhibitors

Recent evidence indicates that histone methylation is a reversible process that is regulated by a dynamic balance between histone methyltransferase and histone demethylase activities (18). Therefore, increases in H3K4 methylation levels might arise from the upregulation of histone H3K4 methyltransferases (H3K4MTs) and/or the downregulation of H3K4DMs. In this study, the authors obtained evidence that the functional link between HDAC inhibition and H3K4 methylation was attribute the suppressive effect of HDAC inhibitors on the expression of the JARID1 family of H3K4DMs, including RBP2, PLU-1, SMCX, and LSD1, at both mRNA and protein levels.

HDAC inhibitors mediate transcriptional repression of H3K4 demethylases via the downregulation of Sp1 expression

Sp1 has been reported to play a critical role in regulating the promoter activity of the *PLU-1* (19). In addition, sequence analysis revealed that the promoters of *RBP2* and *LSD1* also contained putative Sp1 binding elements (GGCGGG or GGGCGG). Thus, based on the finding that HDAC inhibitors suppressed the expression of Sp1, the authors hypothesized that Sp1 downregulation was involved in the transcription repression of *PLU-1* and other H3K4DMs in response to HDAC inhibitors. The functional role of Sp1 in regulating the transcription of H3K4DM genes was supported by several lines of evidence. First, ChIP analysis indicates that treatment with AR42 led to a dose-dependent decrease in the amount of Sp1 associated with the promoters of *RBP2*, *PLU-1*, and *LSD1*. Second, ectopic expression of Sp1 led to dose-dependent upregulation of the protein levels of these H3K4DMs with concomitant decreases in the level of H3K4Me3/Me2/Me. Third, luciferase reporter assays indicate that replacement of the GGC sequence of the Sp1 binding site abrogated the effect of ectopic Sp1 on the transcriptional activation of RBP2 and PLU-1 genes.

The class I HDAC isozymes are responsible for the Sp1-mediated downregulation of H3K4 demethylases

Considering the suppressive effect of MS-275 on the transcription of H3K4 demethylases, class I HDACs might represent the key targets by which HDAC inhibitors modulated H3K4 methylation through Sp1 downregulation. This premise was corroborated by the ability of shRNA-mediated knockdown of HDAC1, 2, 3, and 8, but not HDAC6, to mimic the effect of HDAC inhibitors on H3K4 methylation (i.e., increased expression of H3K4/Me3/Me2/Me in conjunction with suppression of H3K9Me3/Me2 levels) as well as the expression of Sp1 and the H3K4DMs RBP2, PLU-1, SMCX, and LSD1. Moreover, using luciferase reporter assays, the authors demonstrated that shRNA-mediated knockdown of any of the four class I HDAC isozymes led to a significant reduction in the luciferase activities of the RBP2, PLU-1, and LSD1 reporter constructs, and that this HDAC silencing effect could be partially restored by the ectopic expression of Sp1. Together, these findings underscore the important role of class I HDAC isozymes in mediating the effects of HDAC inhibitors on H3K4 methylation through Sp1-dependent transcriptional repression of H3K4 demethylases.

Discussion

Substantial evidence demonstrated that both HDACs and histone demethylases play central roles in cell differentiation and pathogenesis of many diseases including cancer (20, 21). The functional crosstalk between these two histone-modifying systems in regulating the complex pattern of histone codes, however, remains elusive (4). In the present study, the authors demonstrate that through the inhibition of class I HDACs, HDAC inhibitors promote H3K4 methylation by suppressing the expression of the JARID1 family of H3K4DMs, and that reduced Sp1 expression represents the mechanistic link between HDAC inhibition and the transcriptional repression of H3K4DMs (Fig. 2)

An important issue that remains undefined is the mechanism by which HDAC inhibition down-regulates Sp1 expression. It is plausible that HDAC inhibitor-induced increases in chromatin acetylation leads to the expression of a factor that represses Sp1. Alternatively, the acetylation of a nonhistone HDAC substrate could stimulate pathways leading to suppression of Sp1 expression. Moreover, a recent study showed that in the context of KIT-driven acute myeloid leukemia, HDAC inhibitors can disrupt the repressive transcriptional complex that binds to *miR-29b* regulatory elements leading to *miR-29b* upregulation and consequent inhibition of Sp1 expression (22).

The concomitant increases in histone H3 acetylation and H3K4 methylation underlie the ability of HDAC inhibitors to activate the transcription of a broad range of genes associated with tumor suppression and differentiation. This epigenetic activation of tumor-suppressing genes might, in part, account for the ability of AR42 and MS-275 to suppress tumor progression and, in the case of AR42, to shift tumorigenesis to a more differentiated phenotype in the TRAMP model (16).

Moreover, the ability of HDAC inhibitors to transcriptionally suppress H3K4 demethylase genes has potential therapeutic implications as LSD1 and PLU-1 have been suggested as targets for the treatment of various types of malignancies, including prostate cancer (23), breast cancer (24), and neuroblastoma (25). A recent study shows that patients with a Gleason score of less than 7 have a lower 10-year recurrence rate if the percentage of cells with H3K4Me2 staining is above the 60th percentile (26). This correlation is consistent with findings that over-expression of LSD1 in prostate carcinoma is sufficient to induce androgen receptor-dependent transcription in the absence of androgens (23, 27), and that LSD1 and PLU-1 could regulate the transcriptional activity of the androgen receptor (28). Thus, understanding the mode of action of AR42 and MS-275 in upregulating H3K4 methylation by suppressing the expression of H3K4DMs may foster new therapeutic strategies for cancer therapy.

Acknowledgments

This work was supported by the National Institutes of Health National Cancer Institute (CA112250), the Department of Defense Prostate Cancer Research Program (W81XWH-08-1-0663).

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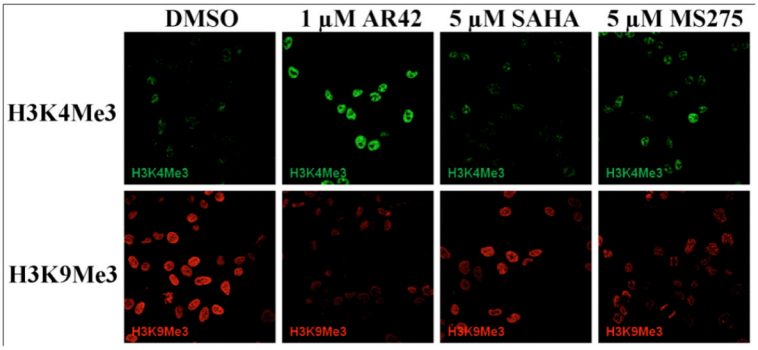

Figure 1.
Immunocytochemical analysis of the effects of AR42, SAHA, and MS-275 at the indicated concentrations on H3K4Me3 (upper panels) and H3K9Me3 (lower panels) after 24 h of treatment in LNCaP cells.

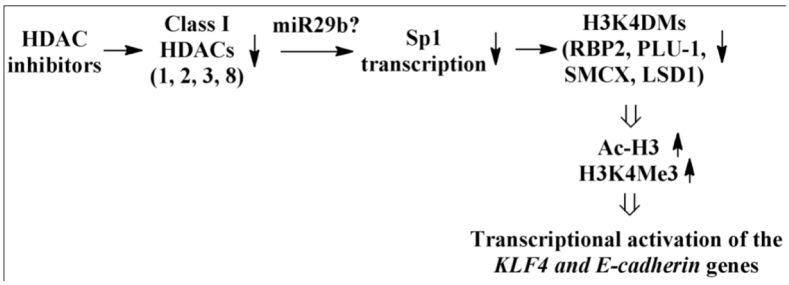

Figure 2.

Schematic diagram depicting the effect of HDAC inhibitors on H3K4 methylation and subsequent transcriptional activation of *KLF4* and *E-cadherin* gene expression through the transcriptional repression of H3K4DMs.