Histone Methyltransferases as a New Target for Epigenetic Action of Vorinostat

Varvara Maksimova[1#], Julia Makus[1,2#], Valeriia Popova[1,3], Anzhelika Prus[1,4],
Olga Usalka[1,5], Ekaterina Trapeznikova[5], Ekaterina Zhidkova[1], Gennady Belitsky[1],
Marianna Yakubovskaya[1], and Kirill Kirsanov[1,2,a*]

[1]*Blokhin National Medical Research Center of Oncology, 115478 Moscow, Russia*
[2]*Peoples' Friendship University of Russia, 117198 Moscow, Russia*
[3]*Dmitry Mendeleev University of Chemical Technology of Russia, 125047 Moscow, Russia*
[4]*MIREA, Russian Technological University, 119571 Moscow, Russia*
[5]*Sechenov First Moscow State Medical University*
of the Ministry of Health of the Russian Federation (Sechenov University), 119991 Moscow, Russia
[a]*e-mail: kkirsanov85@yandex.ru*

Abstract—Epigenetic genome regulation during malignant cell transformation is characterized by the aberrant methylation and acetylation of histones. Vorinostat (SAHA) is an epigenetic modulator actively used in clinical oncology. The antitumor activity of vorinostat is commonly believed to be associated with the inhibition of histone deacetylases, while the impact of this drug on histone methylation has been poorly studied. Using HeLa TI cells as a test system allowing evaluation of the effect of epigenetically active compounds from the expression of the *GFP* reporter gene and gene knockdown by small interfering RNAs, we showed that vorinostat not only suppressed HDAC1, but also reduced the activity of EZH2, SUV39H1, SUV39H2, and SUV420H1. The ability of vorinostat to suppress expression of EZH2, SUV39H1/2, SUV420H1 was confirmed by Western blotting. Vorinostat also downregulated expression of SUV420H2 and DOT1L enzymes. The data obtained expand our understanding of the epigenetic effects of vorinostat and demonstrate the need for a large-scale analysis of its activity toward other enzymes involved in the epigenetic genome regulation. Elucidation of the mechanism underlying the epigenetic action of vorinostat will contribute to its more proper use in the treatment of tumors with an aberrant epigenetic profile.

INTRODUCTION

DNA damage, such as point mutations, gene amplification, and chromosomal translocations, can lead to the irreversible tumor transformation of cells. At the same time, tumor transformation is accompanied by extensive transcriptome reprogramming induced by alterations in the epigenetic modification patterns, in particular, histone methylation [1]. Histone methylation represents one of the key epigenetic mechanisms of transcription regulation and plays a critical role in DNA replication, DNA repair and recombination, gene transcription,

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cell cycle control, and spatial chromosomal organization [2-4]. Methylation mainly targets lysine and arginine residues in the N-terminal fragments of histones H3 and H4 and can lead to either transcriptional activation (H3K4me2/3, H3K36me2/3, H3K79me1/2/3) [3, 5, 6] or suppression of gene expression (H3K9me2/3, H4K20me3, H3K27me3) [7-9]. Various histone modifications play a key role in the formation of euchromatin and heterochromatin [10]. Histone methylation and demethylation are catalyzed by antagonistic enzymes — histone methyltransferases (HMTs) and demethylases, respectively, that function as components of multiprotein complexes containing chromatin-modifying enzymes, transcription factors, and transcription repressors [11, 12]. Aberrant histone methylation profiles emerge due to structural disturbances in methyltransferase and demethylase genes, altered expression of these enzymes caused by external factors, and/or indirect impact of genetic disorders causing irreversible cell transformation [13]. HMT overexpression and mutations are commonly observed in various types of tumors, as altered chromatin structure often affects signaling pathways and gene expression patterns that promote tumorigenesis [14].

Overexpression of methyltransferases EZH1/2, SETDB1, SUV39H1, SUV420H1/2, G9a, GLP, DOT1L, PRMT5, and PRMT6 is often involved in the emergence of tumors with a poor prognosis, such as breast, prostate, ovarian, pulmonary, cervical, and skin cancers [9, 15-28]. Hence, these enzymes might be promising targets for anticancer therapy. Currently, low-molecular-weight agents capable of modulating the activity of HMTs are actively searched for and investigated. Some of them, e.g., chaetocin (SUV39H1/H2 inhibitor), UNC0642, and BIX01294 (G9a/GLP inhibitors), undergo preclinical testing [29-32]. Pinometostat (DOT1L inhibitor), SHR2554 (EZH2 inhibitor), PRT543 (PRMT5 inhibitor), and EZM8266 (G9a inhibitor) have been tested in clinical trials for the treatment of lymphoid, hematopoietic, and solid tumors [33-35]. In 2020, tasemetostat (EZH2-suppressing agent) became the first HMT inhibitor approved for the cancer therapy [36].

Vorinostat (also known as SAHA or Zolinza®) is an inhibitor of class I, II, and IV histone deacetylases (HDACs) [37]. It is used to treat patients with advanced, recurrent, or chemotherapy-resistant cutaneous T-cell lymphoma [38]. Vorinostat binds zinc ion in the HDAC active site, thus inhibiting the catalytic activity of the enzyme. HDAC inhibition results in the stimulation of histone acetylation and transcription activation [37]. Moreover, vorinostat promotes acetylation of histone proteins and some non-histone transcription factors, as well as proteins involved in the regulation of cell proliferation, migration, and cell death [39]. Extensive *in vitro* and *in vivo* studies in various tumor models, including chemoresistance development models, have revealed strong antitumor effect of vorinostat, including cell cycle arrest

and initiation of apoptosis and autophagy [40]. Phase I and II clinical trials have demonstrated the positive effects of vorinostat in a combination therapy against multiple myeloma, colorectal carcinoma, sarcoma, myelodysplastic syndrome, and neuroblastoma [41-45].

Earlier studies have shown that vorinostat can also epigenetically regulate transcription by mechanisms other than HDAC inhibition. For instance, HDAC inhibitors, including vorinostat, were found to increase the level of histone 3 methylation at Lys4 (H3K4me1/2/3) due to the miRNA-mediated downregulation of expression of JARID1 lysine demethylases (RBP2, PLU-1, SMCX), as well as LSD1 of the KDM1 family [46]. Vorinostat was also shown to suppress expression of DNA methyltransferases DNMT1 and DNMT3b at both mRNA and protein levels [47]. Several studies have revealed that vorinostat triggers a small but significant decline in the level of H3K9me3 and H3K9me2 modifications [46, 47]. Moreover, vorinostat elicited a locus-specific effect on histone methyltransferases SUV39H1 and EZH2 [48, 49]. Despite these facts, the cumulative effect of vorinostat on the histone methylation by HMTs has not been comprehensively investigated.

Our study was aimed to evaluate the effect of vorinostat on histone methylation at the integral level. We used HeLa TI cells (HeLa trichostatin A-inducible cell population) bearing epigenetically repressed *GFP* (green fluorescent protein) reporter gene. GFP expression is initiated upon activation of 15 different epigenetic silencing factors that abolish gene repression, including genes encoding proteins regulating histone acetylation and methylation [50]. The knockdown of genes coding for certain epigenetic enzymes in HeLa TI cells allowed us to assess the impact of relevant proteins on the epigenetically regulated transcription. Alternatively, we analyzed the effects of vorinostat by assessing the content of studied enzymes by less laborious and less expensive Western blotting, which made it possible to extend our investigation of the effects elicited by the epigenetic agents. In our study, we (i) modelled the effects triggered by inhibitors with different modes of action on HDACs after *HDAC1* gene knockdown in HeLa TI cells to better understand the modality of vorinostat epigenetic action, (ii) assessed the effect of *HDAC1*, *EZH2*, *SUV39H1*, *SUV39H2*, and *SUV420H1* knockdown on the *GFP* reporter gene expression in vorinostat-treated HeLa TI cells, and (iii) evaluated the effect of vorinostat on the expression of histone methyltransferases *EZH2*, *SUV39H1*, *SUV39H2*, *SUV420H1*, *SUV420H2*, *G9a*, *GLP*, and *DOT1L*.

MATERIALS AND METHODS

Cell culture. HeLa TI cells derived from the parental HeLa cell line carried epigenetically repressed *GFP* reporter gene in an avian sarcoma virus vector. In 2008,

HeLa TI cells were obtained in the Anna Marie Skalka and Richard Katz laboratory at the Fox Chase Cancer Center (Philadelphia, USA) [50]. In 2011, HeLa TI cells were made available to the Department of Chemical Carcinogenesis, Blokhin National Medical Research Center of Oncology (Moscow, Russia), within a framework of a collaborative project. In 2021, our laboratory verified the possibility of HeLa TI cell application as a test system for the assessment of epigenetic activity of xenobiotics [51].

HeLa TI cells were cultured in 75-cm^2 flasks (Eppendorf, Germany) in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 4.5 g/liter glucose (PanEco, Russia), 10% (v/v) heat-inactivated fetal bovine serum (FBS; Biosera, France), antibiotic cocktail containing penicillin (50 units/ml) and streptomycin (50 µg/ml) (PanEco), and 2 mM L-glutamine (PanEco). The cells were cultured under standard conditions (37°C, 5% CO$_2$).

Assessment of modality of vorinostat action in HeLa TI cells. *Reactivation of epigenetically repressed GFP gene in HeLa TI cells with HDAC1 knockdown after exposure to epigenetic modulators trichostatin A, 5-azacytidine, and curaxin CBL0137.* Trichostatin A (TSA; Selleckchem, USA), 5-azacytidine (5-aza; Merck, Germany), and curaxin CBL0137 (CBL0137; Inkuron, Russia) were dissolved in dimethyl sulfoxide (DMSO, PanEco) to obtain 10 mM stock solutions. HeLa TI cells were seeded into 24-well plates (Eppendorf) at 20,000 cells per well. After incubation for 10 h, the cells were transfected with 5 nM siRNA (small interfering RNA) against *HDAC1* gene (GS3065) mRNA using a set of 4 siRNAs (Qiagen, Germany) and HiPerFect Transfection Reagent (Qiagen) according to the manufacturer's protocol. The concentration of siRNAs was chosen to ensure a near complete (at least 80%) repression of the target gene. As a control, we used AllStars Negative Control siRNA kit (Qiagen) that included several siRNAs against mRNAs with no homology to any known mammalian gene. Selective knockdown of the *HDAC1* gene was verified by Western blotting and quantitative PCR (data not shown). The content of viable cells after *HDAC1* knockdown was at least 80%. On next day (24 h after transfection), the cells were treated with the non-toxic concentration of TSA (0.12 µM), 5-aza (10 µM), or CBL0137 (0.6 µM) (final DMSO concentration was below 0.1%). After incubation for 24 h, the transfection medium was replaced with fresh culture medium. Finally, 48 h later, the cells were detached using 0.25% (v/v) trypsin-EDTA solution (PanEco), washed with phosphate buffered saline (PBS), pelleted by centrifugation (250g, 5 min), and analyzed by flow cytometry using a BD FACSCanto II device (BD Biosciences, Belgium) with a blue laser (488 nm) and a FITC channel (530/30 nm).To maintain high cell viability in the cell suspension, PBS was supplemented with 2% FBS.

Reactivation of epigenetically repressed GFP gene by exposure to vorinostat and HDAC1/HMT gene knockdown in HeLa TI cells. Vorinostat (SAHA) (Selleckchem) was dissolved in DMSO to obtain 10 mM stock solution. HeLa TI cells were transfected with siRNAs specific to *HDAC1* (GS3065), *EZH2* (GS2146), *SUV39H1* (GS6839), *SUV39H2* (GS79723), *SUV420H1* (GS51111) genes as described above. A set of 4 siRNAs was used for knockdown of each gene. The concentration of siRNA was chosen to provide a near complete (at least 80%) repression of the target gene. The effects of selective gene knockdown were distinguished from non-specific effects by using AllStars Negative Control siRNA kit (Qiagen) as described above. The content of viable cells after transfection was at least 80%. Twenty-four hours after transfection, the cells were treated with a non-toxic concentration of vorinostat (5 µM) (cell viability was at least 95%); the final DMSO concentration was 0.1% (v/v). After 24 h of incubation, the culture medium was replaced with the fresh one, and the cells were analyzed by flow cytometry.

Analysis of HMT expression in vorinostat-treated HeLa TI cells. Vorinostat (SAHA) dissolved in DMSO to the concentration of 10 mM. HeLa TI cells were seeded in 6-well plates at 250,000 cells per well and incubated with vorinostat (5 µM) and DMSO [final concentration, 0.1% (v/v); negative control] for 24 h. To obtain the total protein fraction, the cells were lysed for 1 h at 4°C in RIPA buffer containing 50 mM Tris-HCl (PanEco), 150 mM NaCl (PanEco), 1% (v/v) Triton X-100 (Ferak, Germany), 0.5% (w/v) sodium deoxycholate (Diam, Russia), 0.1% (w/v) sodium dodecyl sulfate (SDS; Serva, Germany), and protease inhibitor cocktail (Roche, Switzerland). The lysate was centrifuged at 1790g for 5 min at 4°C; the supernatant was collected and proteins were denatured for 5 min in electrophoresis loading buffer (Merck). Protein concentration was determined by the Bradford method [52]. The proteins were separated in 10% polyacrylamide gel (PAG) in Tris-Gly buffer containing SDS (25 mM Tris, 190 mM Gly, 10% SDS) and transferred onto nitrocellulose membrane (Bio-Rad, USA) with 0.45-µm pore size for 1 h at 250 mA (25 to 100-kDa proteins) or 100 V (100 to 150-kDa proteins). The following rabbit antibodies (Abcam, UK) against HMTs were used: EZH2 (ab228697; dilution, 1 : 7000), SUV39H1 (ab245380; dilution, 1 : 3000), SUV39H2 (ab229493; dilution, 1 : 3000), DOT1L (ab64077; dilution, 1 : 500), G9a (ab183889; dilution, 1 : 3000), GLP (ab241306; dilution, 1 : 5000); as well as rabbit antibodies (Thermo-Fisher Scientific, Germany) specific to SUV420H1 (PA5-40926; dilution, 1 : 3000) and SUV420H2 (PA-109891; dilution, 1 : 3000). Rabbit antibodies against β-actin (Abcam; dilution, 1 : 10,000) were used for protein loading control. Horseradish peroxidase-conjugated goat antibodies (Abcam; dilution, 1 : 5000) were used as secondary antibodies.

Proteins were detected with Clarity™ Western ECL Substrate (Bio-Rad) and ImageQuant LAS 4000 digital image processing system (GE Healthcare, USA) and quantified with the ImageJ software. All experiments were performed in four independent replicates.

Statistical data analysis was performed with the GraphPad Prism 8.3.0 software. The normality of data distribution was assessed with the Kolmogorov−Smirnov test. The significance of difference in the percentage of GFP$^+$ cells after exposure to TSA, 5-aza, and CBL0137 and after *HDAC1* knockdown was analyzed using one-way ANOVA with the Tukey's *post hoc* test for multiple comparisons. The significance of differences in the percentage of vorinostat-exposed GFP$^+$ cells with knocked down *HDAC1*, *EZH2*, *SUV39H1*, *SUV39H2*, and *SUV420H1* genes was assessed using the two-way ANOVA with the Tukey's *post hoc* test. The significance of differences in the expression levels of HMTs in vorinostat-treated cells was assessed with the Student's *t*-test. In all cases, the differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The major epigenetic effect of vorinostat is non-selective HDAC suppression. Vorinostat is most efficient against HDAC1, HDAC2, HDAC3, and HDAC6. It inhibits HDAC1, HDAC3, and HDAC6 at similar concentrations, with the most prominent decline in expression observed for HDAC1 [53, 54].

HeLa TI cells are a population of HeLa cells carrying epigenetically repressed *GFP* reporter gene in an avian sarcoma virus-based vector. Epigenetic repression of *GFP* in HeLa TI cells is driven by more than 15 factors, including chromatin-modifying enzymes and histone chaperones [50]. HDAC enzyme that contributes most to the *GFP* epigenetic silencing in HeLa TI cells is HDAC1 [55]. Previously, HeLa TI cells were shown to be sensitive to a wide range of epigenetic modulators, including HDAC inhibitors, as well as inhibitors of DNMTs, HMTs, and BRD (bromodomain)-containing proteins. This test system can also be used to analyze synergistic epigenetic effect of chemical agents (epimodulators) with different mechanism of action, as reactivation of *GFP* expression occurs according to the individual contribution of each tested agent [51]. Based on these data, we proposed that HeLa TI cells with the knocked down *HDAC1* could be used for detection of the epigenetic effects of vorinostat unrelated to the HDAC inhibition.

To test this hypothesis, we assessed the effects of HDAC inhibitors with different mechanisms on HeLa TI cell deficient by the *HDAC1* gene expression to further use the information to determine the modality of vorinostat effect. Reactivation of the *GFP* expression was analyzed by determining the content of GFP$^+$ cells

after *HDAC1* knockdown and treatment with (i) HDAC inhibitor (trichostatin A), (ii) agent with a mixed mechanism of action (curaxin CBL0137), and (iii) agent not affecting the HDAC activity (5-azacytidine). Trichostatin A is an inhibitor of class I and II HDACs that belongs to a group of natural hydroxamic acid derivatives [56]; 5-azacytidine is a cytidine analog that causes DNA demethylation via DNMT inhibition [57]; CBL0137 (low-molecular-weight second-generation curaxin) activates p53 tumor suppressor and inhibits FACT histone chaperone, resulting in the chromatin remodeling and transcription activation [58]. Zhou et al. [59] demonstrated that CBL0137 also upregulates histone H3 acetylation and lowers the level of H3K9me3 and H3K27me3 modifications in the promoters of interferon signaling genes. We found that regardless of the cell treatment, incubation with a set of siRNAs bearing no homology to any known mammalian gene (siNEG) caused no significant alterations in the level of *GFP* reactivation compared to the non-transfected (NT) cells: the extent of *GFP* expression reactivation in the NT cells and siNEG-treated cells was 4.2 and 4.5%, respectively (Fig. 1a; Fig. S1, see Online Resource 1).

DMSO-treated cells (vehicle control) had the same percentage of GFP$^+$ cells as untreated (NT-C, siNEG-C) cells. The *HDAC1* knockdown resulted in 43.5% reactivation of *GFP* expression, which was an order of magnitude higher that in the NT cells. Exposure to TSA and treatment with siNEG resulted in the increased percentage of GFP$^+$ cells that was 25 and 27% in the non-transfected (NT-TSA) and siNEG-treated cells, respectively. At the same time, *HDAC1* gene knockdown followed by TSA treatment increased GFP reactivation by 50%, which was similar to the effect of siHDAC alone. The percentage of GFP$^+$ cells after exposure to 5-aza was 14% vs. 59% in the *HDAC1*-knockdown HeLa TI cells, which suggests a cumulative effect of *HDAC1* and DNMT downregulation. CBL0137 alone increased reactivation of GFP expression to 28-30%. However, when added to *HDAC1*-deficient cells, CBL0137 added increased the content of GFP$^+$ cells to 65.5%, which was 2 and ~1.5 times higher than in the cells exposed either to CBL0137 or siHDAC1. These data demonstrate that the agents that do not affect HDAC, but act on other factors of epigenetic repression in HeLa TI cells (e.g., 5-aza) should potentiate the effects elicited by the *HDAC1* gene knockdown. The crossover effects of modulators and *HDAC1* knockdown can result in their partial or full overlapping at the level of GFP reactivation, as it was shown for TSA. Exposure as a broad-spectrum epigenetic modulator not limited to HDAC (CBL0137) would lead to a significant reactivation of GFP expression.

Next, we analyzed the modality of vorinostat epigenetic action in HeLa TI cells with the knocked down *HDAC1* and HMT genes (*EZH2*, *SUV39H1*, *SUV39H2*, *SUV420H1*). As a KMT6 (lysine methyltransferase 6),
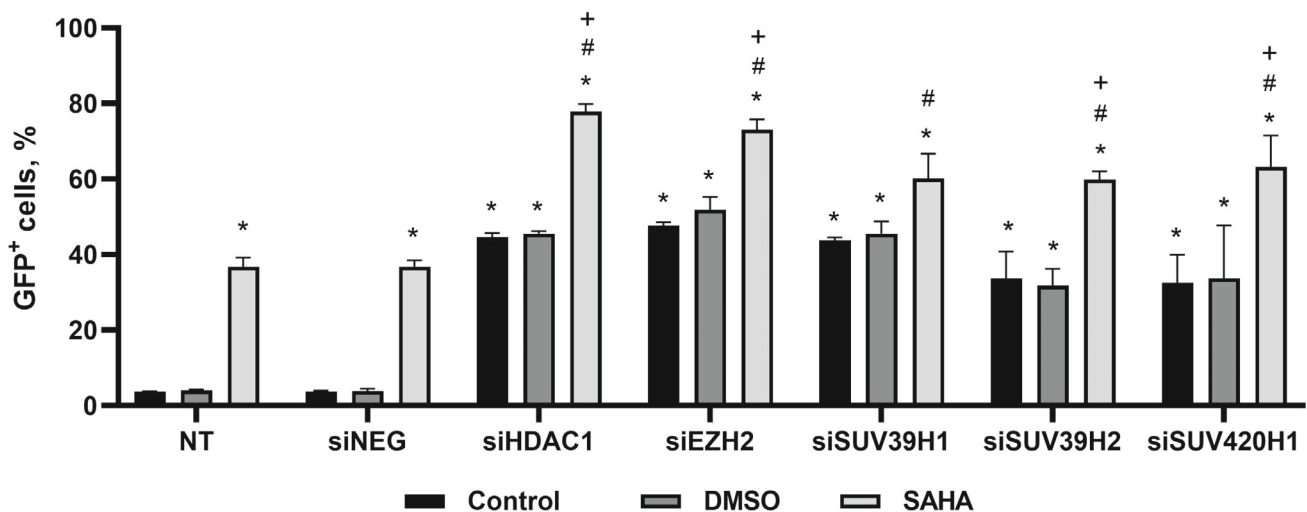
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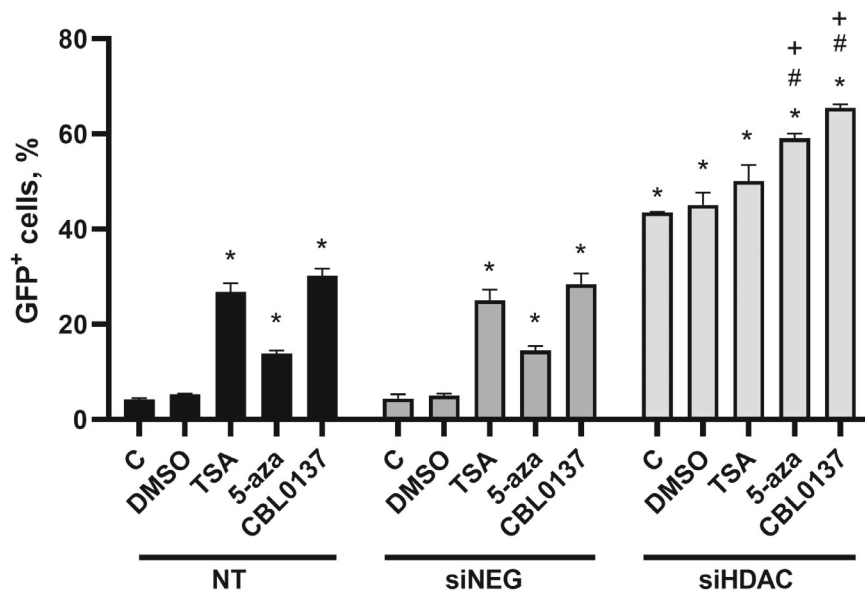
b

Fig. 1. Reactivation of epigenetically repressed *GFP* expression in HeLa TI cells. a) Exposure to epigenetic modulators TSA, 5-aza, CBL0137 and *HDAC1* knockdown. b) Exposure to vorinostat (SAHA) and knockdown of *HDAC1*, *EZH2*, *SUV39H1*, *SUV39H2*, *SUV420H1* genes. Flow cytometry data (mean ± SD): C, untreated cells, negative control; NT, non-transfected cells; siNEG, cells transfected with siNEG (negative transfection control); siHDAC, cells with knocked down *HDAC1*; * significant difference in the percentage of GFP+ cells after exposure to TSA/5-aza/CBL0137/SAHA or siRNA compared to the negative control (siNEG-C), $p < 0.01$; # significant difference in the percentage of GFP+ cells after exposure to TSA/5-aza/CBL0137/SAHA in the cells with the gene knockdown compared to siNEG-treated cells, $p < 0.05$; + significant difference in the percentage of GFP+ cells after exposure to TSA/5-aza/CBL0137/SAHA compared to the negative control in the siHDAC1/siEZH2/siSUV39H1/siSUV39H2/siSUV420H1 group, $p < 0.05$.

EZH2, a component of the PRC2 repressive protein complex, mediates H3K27 di- and trimethylation associated with transcriptional repression [13]. SUV39H1 and SUV39H2 belong to KMT1 methyltransferases that catalyze trimethylation at H3K9. SUV420H1 and its homologue SUV420H2 are key enzymes in H4K20me3 trimethylation, which plays an important role in DNA replication and repair, as well as cell cycle regulation [60]. H3K9me3 and H4K20me3 modifications contribute to the initiation of DNA methylation by recruiting heterochromatic protein HP1 and DNMT,

thus promoting transcriptional repression [61, 62]. H3K9me3 and H4K20me3 play a critical role in the formation of condensed chromatin in the pericentric and telomeric repeats and are involved in euchromatin silencing [63, 64].

The content of GFP+ cells in the negative control (NT-C) and transfection control (siNEG-C) groups was 3.7% and 3.4%, respectively (Fig. 1b; Fig. S2, see Online Resource 1). Similar to previous data, siNEG had no effect on the *GFP* gene reactivation profile as compared to the NT cells. Exposure to DMSO (vehicle control) also
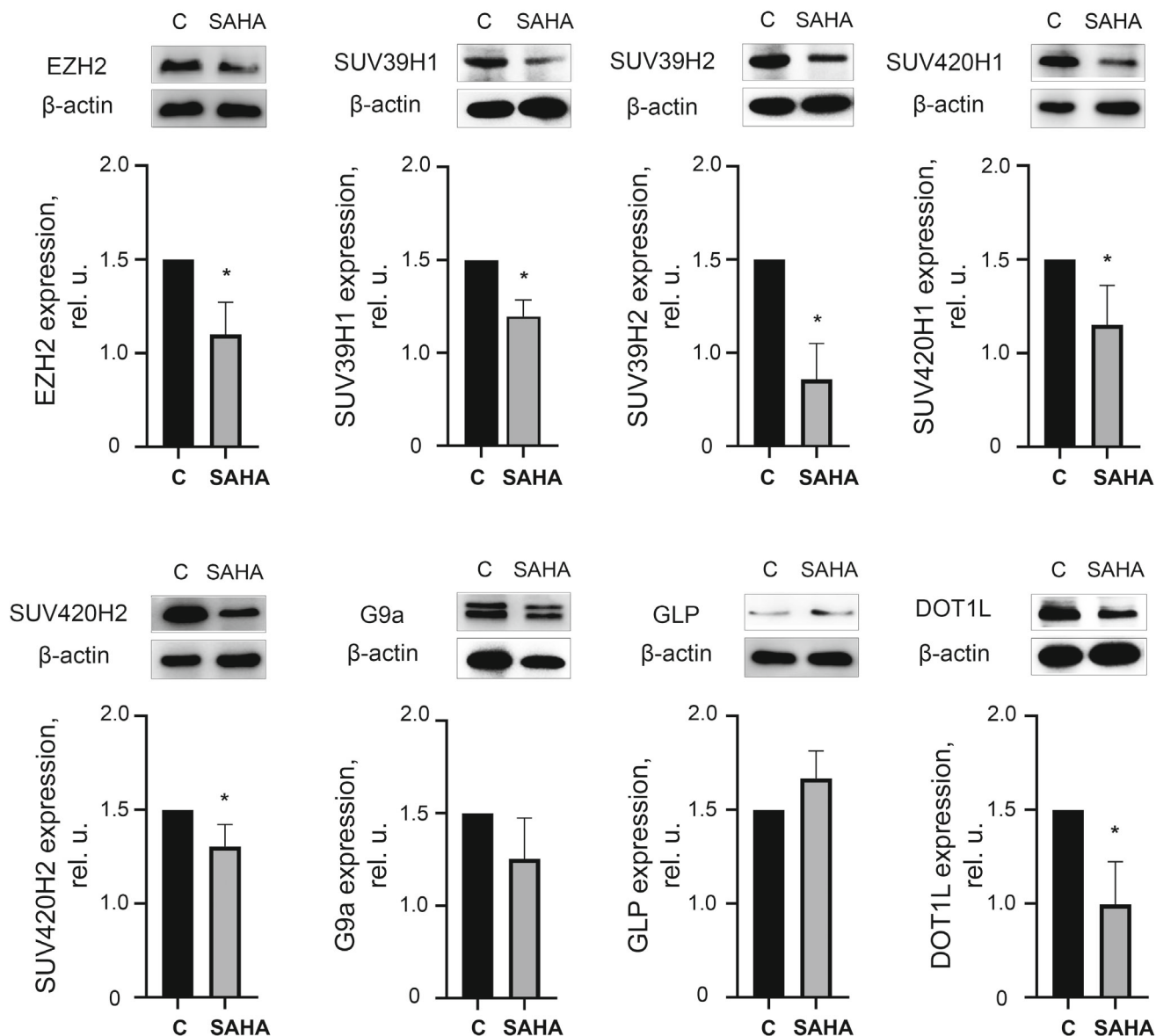
Fig. 2. Expression of HMTs after exposure to vorinostat (SAHA) analyzed by Western blotting and densitometric analysis (mean ± SD). C, negative control; * $p < 0.05$, significant difference with the negative control. Protein expression was normalized against β-actin.

caused no significant changes in the percentage of GFP⁺ cells. *HDAC1* knockdown triggered reactivation of *GFP* expression in 45% cells, whereas vorinostat induced it in 37% cells (Fig. 1b). Exposure of *HDAC1*-knockdown cells to vorinostat increased the content of GFP⁺ cells to 78%, which exceeded the levels of *GFP* reactivation after exposure to vorinostat alone (2.1-fold) and *HDAC1* knockdown (1.7-fold). Therefore, the use of vorinostat as an HDAC inhibitor in the *HDAC1*-knockdown HeLa TI cells resulted in the elevated percentage of GFP⁺ cells, thereby indicating an existence of an HDAC-independent epigenetic effect of vorinostat.

Compared to the negative control, the knockdown of *EZH2*, *SUV39H1*, *SUV39H2*, and *SUV420H1* genes resulted in a significantly elevated percentage of GFP⁺ cells: *EZH2* (48%; 12.9-fold), *SUV39H1* (44%; 11.8-fold),

SUV39H2 (34%; 9.1-fold), *SUV420H1* (33%; 8.8-fold) (Fig. 1b). In a population of *EZH2*-knockdown cells, vorinostat increased the content of GFP⁺ cells up to 73%, which 1.5 and 2 times higher than in cells transfected with siEZH2 and exposed to vorinostat, respectively. The knockdown of *SUV39H1* followed by the treatment with vorinostat also increased the percentage of GFP⁺ cells compared to knockdown cells and cells exposed to vorinostat alone (1.4- and 1.6-fold, respectively). Reactivation of *GFP* expression was found in 60% of *SUV39H2*-knockdown cells treated with vorinostat, which was 1.8- and 1.6-times more than for the individual effects of either siSUV39H2 and vorinostat, respectively. Exposure to vorinostat of *SUV420H1*-knockdown cells increased the percentage of GFP⁺ cells to 63.2%, which exceeded 1.9 and 1.7 times the effects elicited by either

siSUV420H1 and vorinostat alone, respectively. These data show that the effect of vorinostat overlapped with the effects of *EZH2*, *SUV39H2*, and *SUV420H1* knockdowns, suggesting that vorinostat might have an impact on these enzymes. It should be noted that the knockdown of *SUV39H1*, *SUV39H2*, and *SUV420H1* genes in HeLa TI cells followed by vorinostat treatment decreased the percentage of viable cells to 65%. Such high toxicity may be explained by the fact that SUV39H1, SUV39H2, and SUV420H1 methyltransferases are important in the catalysis of H3K9me3 and H4K20me3 modifications essential for the chromosome structure organization, so that their loss may be critical even upon a short-term treatment with siRNA [65].

The results obtained in the HeLa TI cells suggested that vorinostat nonselectively inhibited expression of some HMTs. In view of this, we used Western blotting to assess the impact of vorinostat not only on enzymes that we found to be affected in the HeLa TI model system, but also on some other HMTs (SUV420H2, G9a, GLP, and DOT1L). Related methyltransferases G9a and GLP act in a content of heterodimeric complex and mediate H3K9 mono- and demethylation, as well as induce DNA methylation [66]. As KMT4 methyltransferase, DOT1L catalyzes mono-, di-, and trimethylation of H3K79. DOT1L is involved in multiple cellular events, including genomic imprinting, DNA damage response, and processes, such as erythropoiesis, cell differentiation, and embryonic development [67]. DOT1L overexpression results in the activation of gene transcription, including transcription of telomeric sequences [68]. Moreover, DOT1L-mediated H3K79 methylation limits recruitment of repressive proteins to heterochromatin regions [69].

Exposure to vorinostat elicited an overall decline (1.8-fold) in the EZH2 protein expression (Fig. 2). These results are in good agreement with the data reported by Nordstrom et al. [49] who demonstrated that treatment with 5 µM vorinostat decreased the content of EZH2 as well as the level of H3K27me3 modification in the promoter of the *SOX11* gene encoding SRY-box transcription factor 1l. Also, Shi et al. [70] reported an increased sensitivity to vorinostat of non-small cell lung cancer cells with upregulated EZH2 expression, as well as a positive correlation between the expression of EZH2 and HDAC1.

It was found that exposure to vorinostat resulted in a significant decrease in the content of SUV39H1 (1.6-fold) and SUV39H2 (2.8-fold) proteins responsible for the H3K9me3 modification (Fig. 2). The effect of vorinostat on SUV39H1 correlated with the data by Natarajan et al. [48] on the downregulated expression of methyltransferases DNMT3A, SUV39H1, and PRMT1 in cultured ovarian cancer cells exposed to vorinostat at a comparable dose (7.5 µM). Expression of GLP and G9a, components of the complex mediating H3K9 dimethylation, was changed insignificantly, although the content of

G9a was slightly (1.4-fold) decreased. Vorinostat caused a decline in the expression of SUV420H1 and SUV420H2 involved in the methylation of H4K20me3 repressive mark (1.3- and 1.5-fold, respectively). The observed impact of vorinostat on these proteins may provide a deeper insight into its potential to induce chromatin reorganization which might be related to the genotoxic effect of this compound [71, 72]. We also demonstrated that exposure to vorinostat inhibited 2 times the expression of DOT1L, a protein that methylates H3K79. No effects of vorinostat toward SUV39H2, SUV420H1, SUV420H2, and DOT1L have been previously described.

CONCLUSION

Here, we demonstrated that along with the HDAC inhibition, the epigenetic effect of vorinostat (SAHA) involves its action on HMTs. It was shown for the first time that vorinostat decreases the total level of SUV39H2, SUV420H1, SUV420H2, and DOT1L that are commonly found to be overexpressed in various tumors. The new data on the mechanism of epigenetic activity of vorinostat will ensure a proper use of this agent in the antitumor therapy, as well as provide deeper insight into molecular mechanisms behind its side effects. Taken together, the obtained data can serve as a mechanistic basis for expanding the use of vorinostat and increasing the efficacy of treatment of tumors with aberrant epigenetic profiles.

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