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Targeting histones for degradation in cancer cells as a novel strategy in cancer treatment

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The anticancer therapies with the joint treatment of a histone deacetylase (HDAC) inhibitor and a DNA-damaging approach are actively under clinical investigations, but the underlying mechanism is unclear. Histone homeostasis is critical to genome stability, transcriptional accuracy, DNA repair process, senescence, and survival. We have previously demonstrated that the HDAC inhibitor, trichostatin A (TSA), could promote the degradation of the core histones induced by γ-radiation or the DNA-alkylating agent methyl methanesulfonate (MMS) in non-cancer cells, including mouse spermatocyte and embryonic fibroblast cell lines. In this study, we found that the joint treatment by TSA and MMS induced the death of the cultured cancer cells with an additive effect, but induced degradation of the core histones synergistically in these cells. We then analyzed various combinations of other HDAC inhibitors, including suberoylanilide hydroxamic acid and valproate sodium, with MMS or other DNA-damaging agents, including etoposide and camptothecin. Most of these combined treatments induced cell death additively, but all the tested combinations induced degradation of the core histones synergistically. Meanwhile, we showed that cell cycle arrest might not be a primary consequence for the joint treatment of TSA and MMS. Given that clinic treatments of cancers jointly with an HDAC inhibitor and a DNA-damaging approach often show synergistic effects, histone degradation might more accurately underlie the synergistic effects of these joint treatments in clinic applications than other parameters, such as cell death and cell cycle arrest. Thus, our studies might suggest that the degradation of the core histones can serve as a new target for the development of cancer therapies.

HDAC, histone deacetylase inhibitor, DNA damage, anticancer agent, histone degradation

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INTRODUCTION

Together with the surrounding DNA, the core histones form the basic unit of the chromatin, the nucleosome, which contains an octamer of H2A, H2B, H3 and H4. The homeostasis of histones, histone modifications and histone-modifying enzymes is critical to genome stability, transcriptional accuracy, DNA repair process, senescence, and survival (Altaf et al., 2007; Arnold et al., 2013; Dahlin et al., 2015; Dovey et al., 2013). Among various histone modifications, histone acetylation associates with actively transcribed chromatin domains and the relaxed chromatin following DNA double-strand breaks (Campos and Reinberg, 2009; Downs et al., 2004; Murr et al., 2006; Reinke and Hörz, 2003). The formation of histone acetylation is catalyzed by histone acetyltransferases (HAT), and its removal is catalyzed by histone deacetylases (HDAC) (Ge et al., 2013).

HDACs can be divided into class I (HDAC1, 2, 3, and 8), class IIa (HDAC4, 5, 7, and 9), class IIb (HDAC6 and 10),

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class III (SIRT1, 2, 3, 4, 5, 6, and 7) and class IV (HDAC11). Upregulation or the increased activity of HDACs has been associated with tumor development and progression (Dokmanovic and Marks, 2005). HDAC inhibitors (HDACi) can be categorized into four types according to their structures, including fatty acids, hydroxamates, cyclic peptides and benzamides. Some HDAC inhibitors can inhibit HDACs in several classes. For, example, trichostatin A (TSA), a hydroxamate, can inhibit HDACs in classes I, II, and IV (Carew et al., 2008). HDACi may cause cell cycle arrest, apoptosis, anti-angiogenesis and immune modulation. Notably, tumor cells are usually more sensitive to HDACi than normal cells (Chrun et al., 2017; Haberland et al., 2009). But, the underlying mechanism has not yet been fully exposed.

Many HDAC inhibitors are under clinical trials for treating different types of cancer as monotherapy or in combination with other anticancer modalities. Among them, three HDAC inhibitors, including SAHA (now an anticancer drug known as vorinostat), Belinostat, and Romidepsin, have been approved by FDA for treatment of T-cell lymphoma, and Panabiostat has been approved for treating multiple myeloma. In addition, Chidamide and HBI-8000 have been approved by China to treat Peripheral T-cell lymphoma (Suraweera et al., 2018). But, resistance has been developed against the clinical applications of HDACi due to various reasons. For example, elevated expressions of the cell cycle inhibitor p21 and thioredoxin (which reduces reactive-oxygen species-mediated DNA damage) as well as constitutive activations of NFκB and other signaling proteins, such as PI3K and STAT3, have all been suggested to contribute to the resistance of HDACi (Robey et al., 2011). Resistance to DNA-targeting chemotherapies has long been known. Treatments of cancers jointly with HDACi and DNA-damaging approach, such as chemotherapy or radiation therapy, often show synergistic effects (Suraweera et al., 2018). For example, the combination of valproic acid followed by the topoisomerase II inhibitor, epirubicin, shows responses in patients with anthracycline-resistant tumors and in heavily pretreated patients (ClinicalTrial.gov identifier: NCT00246103). However, numerous *in vitro* studies using cultured cancer cells show that the combined treatments of an HDACi and a DNA-damaging chemotherapeutic agents often induce cell death additively (Eckschlager et al., 2017; Shabason et al., 2011; Stiborova et al., 2012). We previously demonstrated that TSA potentiated the degradation of the core histones induced by radiation or the DNA-damaging agent methyl methanesulfonate (MMS) in mouse spermatocyte and embryonic fibroblast cell lines (Qian et al., 2013). It remains unclear whether the combined treatments with an HDAC inhibitor and a DNA-damaging agent facilitate degradation of the core histones in cancer cells, and whether histone degradation contributes to clinic consequences of the combined treatments. In this study, we analyzed histone degradation, and examined the influences on the expression of cell cycle inhibitors and the cell death rates in cancer cell lines following the combined treatments with various HDAC inhibitors and DNA-damaging agents.

RESULTS

Joint treatments with HDAC inhibitors and MMS induce cancer cell death additively

MMS may stall replication forks and cause DNA double-strand breaks by methylating DNA (Kumar et al., 2011; Lundin et al., 2005). Suberoylanilide hydroxamic acid (SAHA), an inhibitor of histone deacetylase, has been approved by FDA as an anti-cancer agent vorinostat. To examine the effects of the joint treatments with the HDAC inhibitor (TSA or SAHA) and MMS on cancer cells, we used the TUNEL (TdT-mediated dUTP Nick-End Labeling) kit to detect cell apoptosis in HeLa human epitheloid carcinoma cells, which were treated with either a single agent or the combination of an HDAC inhibitor and MMS for 4 h. The results showed that either TSA or SAHA could induce apoptosis together with MMS additively (Figure 1A and 1B).

To validate the above notion systematically, we tested the optimal concentrations of MMS in inducing death of more cancer cell lines, including HCT116 human colon carcinoma and MCF-7 human breast adenocarcinoma cells, in addition to HeLa cells. Trypan blue exclusion assay was instead used to detect cell death rates. All three cell lines responded similarly to MMS treatment for 24 h with median lethal concentrations (LC_{50}) of MMS at approximately 6 mmol L^{-1} (~0.05%) (Figure 1C). When HeLa cells were jointly treatment with 0.3 μmol L^{-1} of TSA and 1 mmol L^{-1} of MMS, cell death rates increased additively with the increasing time of treatments (Figure 1D). Valproate sodium (VPA) inhibits class I and class II of HDACs. Similarly, joint treatments with SAHA (Figure 1E–G) or VPA (Figure 1H–J) and MMS also induced cell death additively in HeLa cells.

Joint treatments with an HDAC inhibitor and MMS induce the degradation of the core histones synergistically

We have previously shown that the combined treatment of TSA and MMS induces the degradation of the core histones in GC-2spd spermatocyte cells (Qian et al., 2013). This degradation requires the proteasome activator PA200 (Qian et al., 2013). PA200 is highly expressed in testes and in spermatocytes and is present at relatively low levels in other tissue or cell types (Khor et al., 2006; Ustrell et al., 2002). To test whether the joint treatment of TSA and MMS can induce degradation of the core histones in cancer cells, HeLa cells were treated with TSA alone and with TSA and MMS jointly,

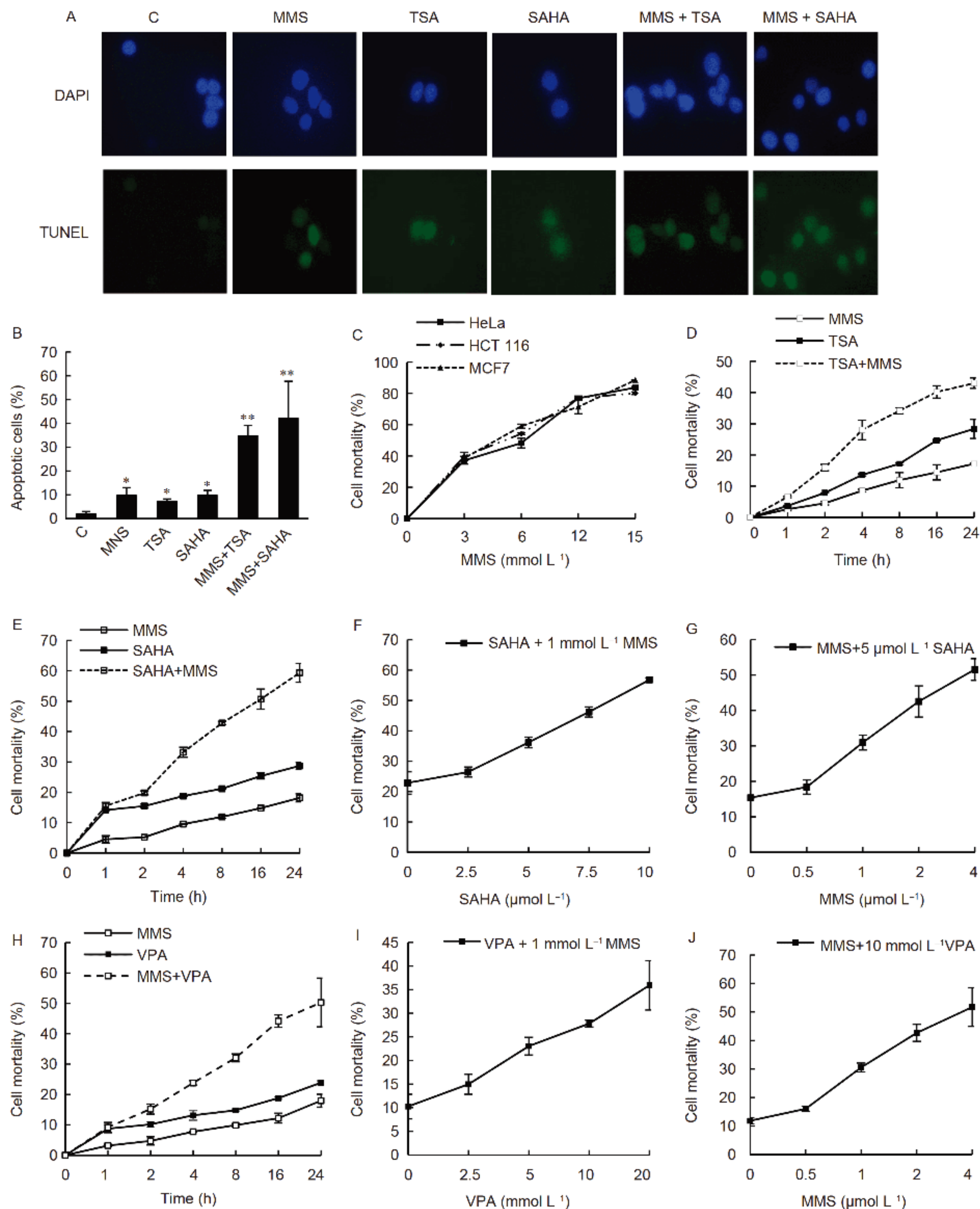

Figure 1 Joint treatments with an HDAC inhibitor and MMS induce cancer cell death additively. A, HeLa cells were treated with DMSO, 1 mmol L^{-1} MMS, 0.3 μmol L^{-1} TSA, 5 μmol L^{-1} SAHA, 1 mmol L^{-1} MMS and 0.3 μmol L^{-1} TSA, 1 mmol L^{-1} MMS and 5 μmol L^{-1} SAHA (from left to right), respectively, for 4 h, observed under a fluorescence microscope after TUNEL staining. B, Count the number of apoptotic cells stained by TUNEL relative to the number of nuclei stained by DAPI. C, HeLa, HCT116 and MCF-7 cells were treated with different concentrations of MMS for 24 h. D, Joint treatments of HeLa with 0.3 μmol L^{-1} TSA and 1 mmol L^{-1} MMS induce cell death additively. Cell death rates were analyzed by trypan blue staining. E, HeLa cells were treated with 1 mmol L^{-1} MMS and 5 μmol L^{-1} SAHA for different periods of time. F, HeLa cells were treated with 1 mmol L^{-1} MMS and different concentrations of SAHA for 4 h. G, HeLa cells were treated with 5 μmol L^{-1} SAHA and different concentrations of MMS for 4 h. H, HeLa cells were treated with 1 mmol L^{-1} MMS and 10 mmol L^{-1} VPA for different periods of time. I, HeLa cells were treated with 1 mmol L^{-1} MMS and different concentrations of VPA for 4 h. J, HeLa cells were treated with 10 mmol L^{-1} VPA and different concentrations of MMS for 4 h. Data are representative of one experiment with at least two independent biological replicates, mean±SEM, $^*P<0.05$, $^{**}P<0.01$ (two-tailed paired *t*-test).

respectively. As in GC-2spd cells, the joint treatment with TSA and MMS reduced the levels of histone H2B synergistically, whereas TSA alone at the same concentration could not reduce the levels of H2B (Figure 2A). No matter whether the MMS was added, the treatment with TSA increased the levels of H4K16ac, consistent with the note that the acetylation mediates the proteasomal degradation of the core histones (Qian et al., 2013). γ-H2AX, a marker for DNA damage (Turinetto and Giachino, 2015), was induced by the treatment of MMS (Figure 2A). More strikingly, the joint treatment with another HDAC inhibitor SAHA and MMS dramatically induced degradation of histone H2B, whereas SAHA alone at various concentrations could not affect the levels of H2B (Figure 2B). Thus, joint treatments with an HDAC inhibitor and MMS induce the degradation of the core histones synergistically.

Cell cycle arrest might not be a primary consequence for the joint treatment of TSA and MMS

Since TSA can also induce the expression of the cell cycle inhibitor p21 (Blagosklonny et al., 2002), we examined the levels of p21 together with H2B. As shown above, TSA itself could not cause the reduction in the levels of H2B, but its joint treatments with MMS reduced the levels of H2B synergistically (Figure 3A). In contrast, these joint treatments still induced cell death additively (Figure 3B). As reported, treatment with TSA induced expression of p21 sharply, but addition of MMS reduced the levels of p21, even though TSA was present (Figure 3A). To test whether the above phenomenon was cell-type specific, we further examined their effects in MEF cells. Interestingly, TSA alone slightly reduced the levels of H2B, and the addition of MMS potentiated this reduction in MEF cells (Figure 3C). In contrast to reducing the levels of p21, the combined treatment with TSA and MMS had almost no effect on the levels of p19, another cell cycle inhibitor (Capparelli et al., 2012), in MEF cells. Again, these joint treatments still induced cell death additively (Figure 3D). To test whether the reduced levels of p21 was due to the PA200-mediated proteasomal degradation, we employed the PA200-deficient MEF cells treated with TSA and MMS. It turned out that deletion of PA200 could not slow the degradation of p21 as examined in the presence of cycloheximide, an inhibitor of protein translation (Figure 3E). These results suggest that the cell cycle arrest might not be a primary consequence for the combined treatment with an HDAC inhibitor and a DNA-damaging agent.

Joint treatment of etoposide and TSA induces the degradation of the core histones synergistically

Topoisomerases, classified into types I and II, play a vital role in replication, transcription, recombination, DNA repair, and chromatin remodeling by modulating the DNA helix. Etoposide directly inhibits topoisomerase II to prevent religation of the DNA strands, and thus promotes DNA breaks. Etoposide is clinically used for the treatments of various

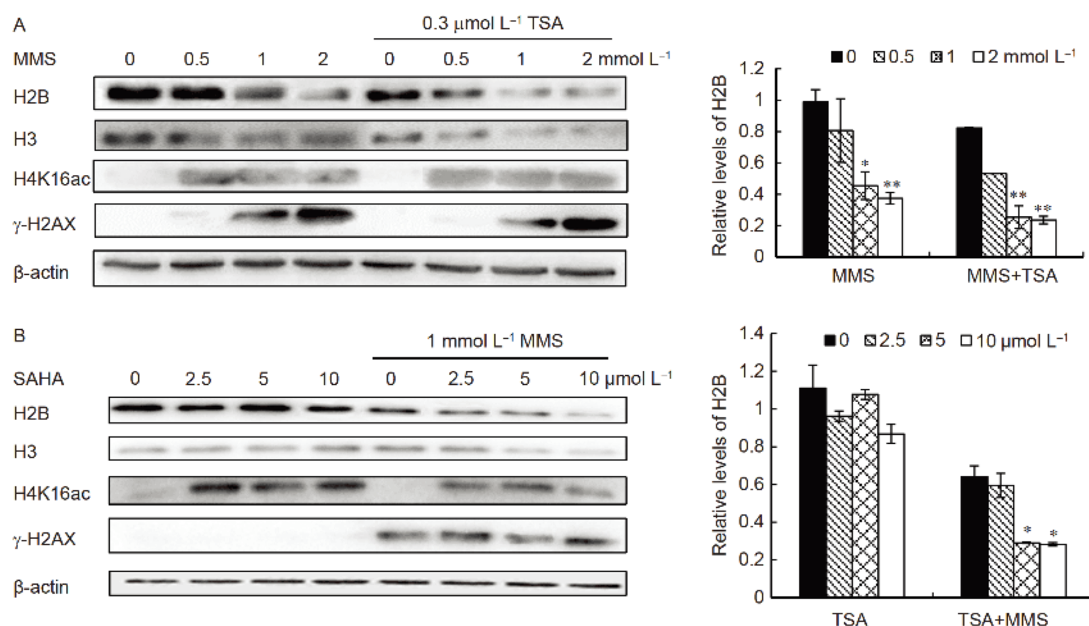

Figure 2 Joint treatments of HDAC inhibitors and MMS induce the degradation of the core histones synergistically. A, HeLa cells were treated with different concentrations of MMS and 0.3 μmol L^{-1} of TSA for 4 h. B, HeLa cells were treated with different concentrations of SAHA and 1 mmol L^{-1} MMS for 4 h. Protein levels in all panels were analyzed by immunoblotting. Data are representative of one experiment with at least two independent biological replicates, mean±SEM, *$P<0.05$, **$P<0.01$ (two-tailed paired *t*-test).

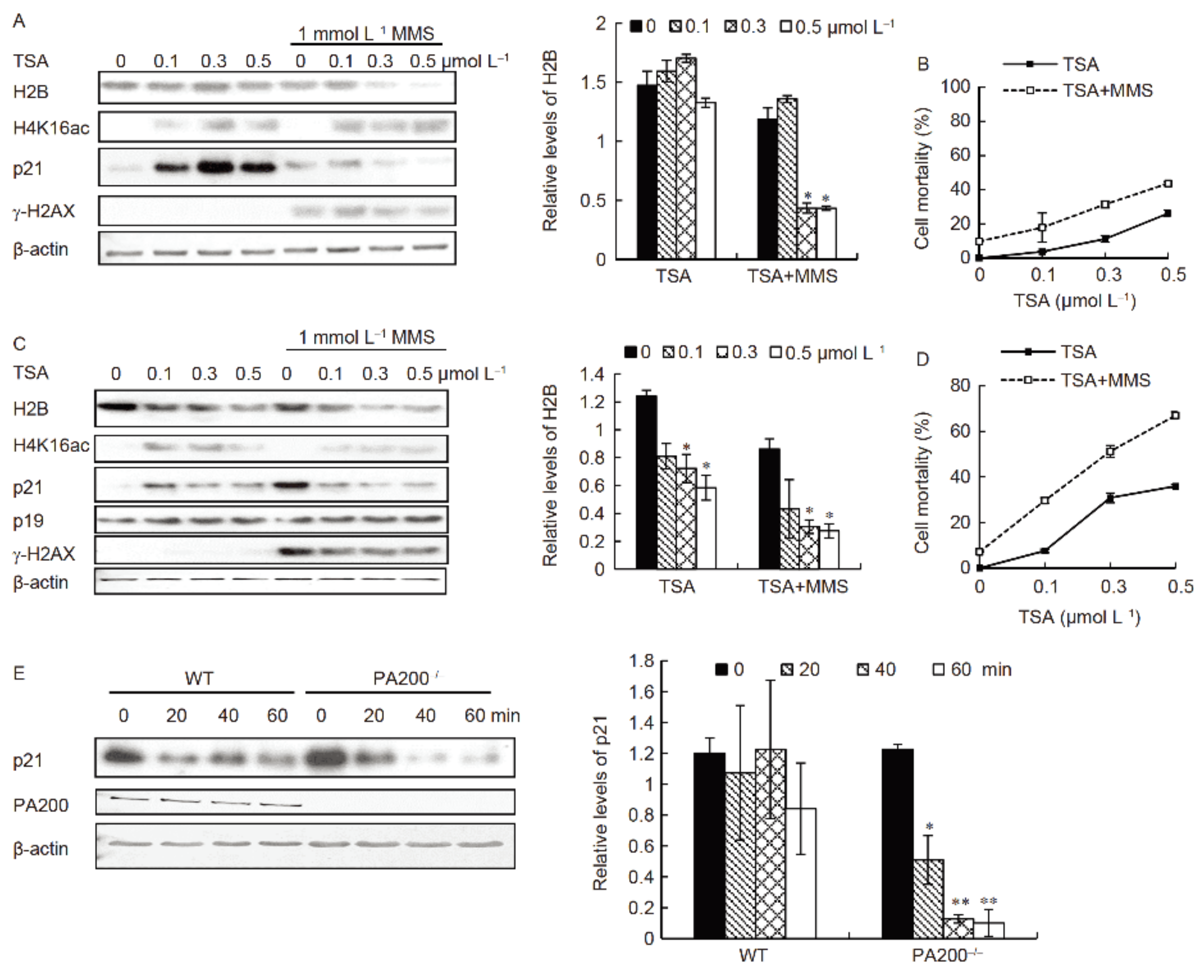

Figure 3 Cell cycle arrest might not be a primary consequence for the joint treatment of TSA and MMS. A, HeLa cells were treated with different concentrations of TSA and 1 mmol L^{-1} of MMS for 4 h. B, HeLa cells were treated as in (A), and cell death rates were analyzed by trypan blue staining. C, MEF cells were treated with different concentrations of TSA and with or without 1 mmol L^{-1} of MMS for 4 h. D, MEF cells were treated as in (C), and cell death rates were analyzed by trypan staining. E, WT and PA200$^{-/-}$ MEF cells were treated with 0.008% of MMS, 0.3 μmol L^{-1} of TSA and/or 25 μg mL^{-1} of CHX. Protein levels in all panels were analyzed by immunoblotting. Data are representative of one experiment with at least two independent biological replicates, mean±SEM, * $P<0.05$, ** $P<0.01$ (two-tailed paired *t*-test).

cancers, including testicular cancer, lung cancer, lymphoma, leukemia, neuroblastoma, and ovarian cancer (Montecucco et al., 2015; Pommier et al., 2010). To test whether other DNA-damaging anticancer agents can also act similarly to MMS, we tested the role of etoposide. Treatment with etoposide by itself reduced the levels of H2B in a dose-dependent manner in MEF cells (Figure 4A). Addition of TSA further strengthened the reduction dramatically, suggesting a synergistic effect (Figure 4A). But, these joint treatments induced cell death additively in MEF cells (Figure 4B). The treatment with etoposide alone or the joint treatment with etoposide and TSA reduced the levels of H2B in HeLa cells in a way similar to MEF cells (Figure 4C and D). These joint treatments also induced cell death additively in HeLa cells (Figure 4D). Thus, the joint treatment of etoposide and TSA promotes the degradation of the core histones synergistically, whereas it induces cell death in cancer cells additively.

Treatments with an HDAC inhibitor in conjunction with either etoposide or camptothecin induce cancer cell death additively

Next, we further analyze the effects of joint treatments with HDAC inhibitors and etoposide on cell death rates. In comparison to MMS, additional treatment with etoposide much less efficiently increased the rates of cell death in the presence of either SAHA (Figure 5A–C) or VPA (Figure 5D–F). But, joint treatment with etoposide and SAHA still induced the degradation of the core histones synergistically in HeLa cells (Figure 5G). Camptothecin (CPT) is an inhibitor of DNA topoisomerase I. However, the additive effects of CPT on cell death rates were as remarkable as those for MMS in the presence of TSA (Figure 6A–C), SAHA (Figure 6D–F), or VPA (Figure 6G–I). Thus, all joint treatments with any tested DNA-damaging agent and any tested

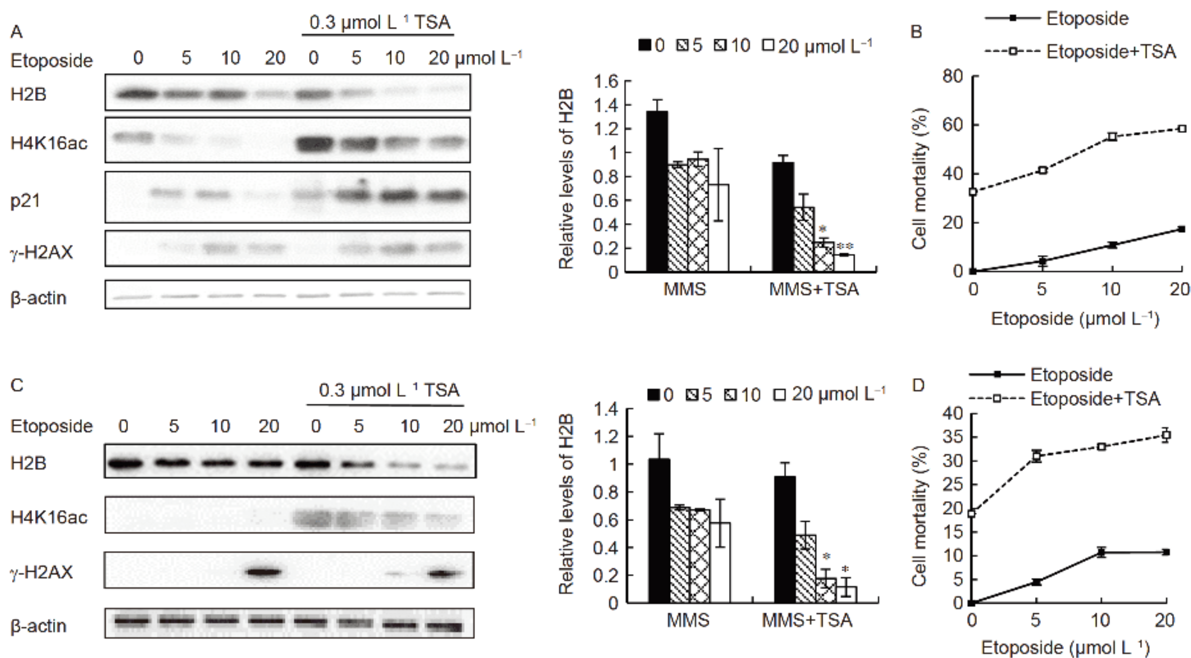

Figure 4 Joint treatment with etoposide and TSA induces degradation of the core histones in HeLa and MEF cells synergistically. A, MEF cells were treated with etoposide and 0.3 μmol L^{-1} of TSA for 24 h. Detection the degradation of the core histones by immunoblotting. B, MEF cells were treated with different concentrations of etoposide and with or without 0.3 μmol L^{-1} TSA for 24 h. Cell death rates were analyzed by trypan staining. C, HeLa cells were treated with etoposide and 0.3 μmol L^{-1} of TSA for 24 h. Detection the degradation of the core histones by immunoblotting. D, HeLa cells were treated with different concentrations of etoposide and with or without 0.3 μmol L^{-1} TSA for 24 h. Cell death rates were analyzed by trypan blue staining. Data are representative of one experiment with at least two independent biological replicates, mean ± SEM, *P<0.05, **P<0.01 (two-tailed paired t-test).

HDAC inhibitor induce cell death additively, but induce degradation of the core histones synergistically.

DISCUSSION

The joint treatments with an HDAC inhibitor and a DNA-damaging agent can finally cause cell cycle arrest and apoptosis (Feng et al., 2015; Meng et al., 2012). Although the combined treatments of an HDACi and a DNA-damaging chemotherapeutic agents often induce cell death additively (Eckschlager et al., 2017; Shabason et al., 2011; Stiborova et al., 2012), these joint treatments mostly show synergistic effects in clinic investigations (Suraweera et al., 2018). The results from this study further support that all joint treatments with any tested DNA-damaging agent (including MMS, etoposide and CPT) and any tested HDAC inhibitor (including TSA, SAHA, and VPA) induce cell death additively. But, we show here that all these joint treatments surprisingly induce degradation of the core histones synergistically in tumor cells, in a way similar to their clinical consequences. The joint treatment of a DNA-damaging agent and an HDAC inhibitor could also induce histone degradation synergistically in non-tumor MEF cells (Qian et al., 2013). Given that upregulation or the increased activity of HDACs has been associated with tumor development and progression (Dokmanovic and Marks, 2005), inhibition of HDAC might elucidate its enhanced activity in the tumor cells with the increased activity of HDACs. TSA alone induced the expression of the cell cycle inhibitor p21, but not another cell cycle inhibitor p19. Moreover, addition of MMS reduced the levels of p21, suggesting that cell cycle arrest is probably not a primary mechanism for the joint treatment of TSA and MMS. Given that loss of the core histones can be critical to genome stability, transcriptional accuracy, DNA repair process, senescence, and survival (Altaf et al., 2007; Arnold et al., 2013; Dahlin et al., 2015; Dovey et al., 2013), our results reveal the induction of histone degradation as a new mechanism underlying the effect of these joint treatments against cancer cells. Thus, we propose that targeting the core histones for degradation could be a novel approach for the development of cancer therapy.

MATERIALS AND METHODS

Cell culture

HeLa human epitheloid carcinoma cells, MCF-7 human breast adenocarcinoma cells, and HCT116 human colon carcinoma cells were maintained in the Dulbecco's modified Eagle's medium (DMEM, Gibco), supplemented with 10% fetal bovine serum (FBS), 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin. All the above cell lines were

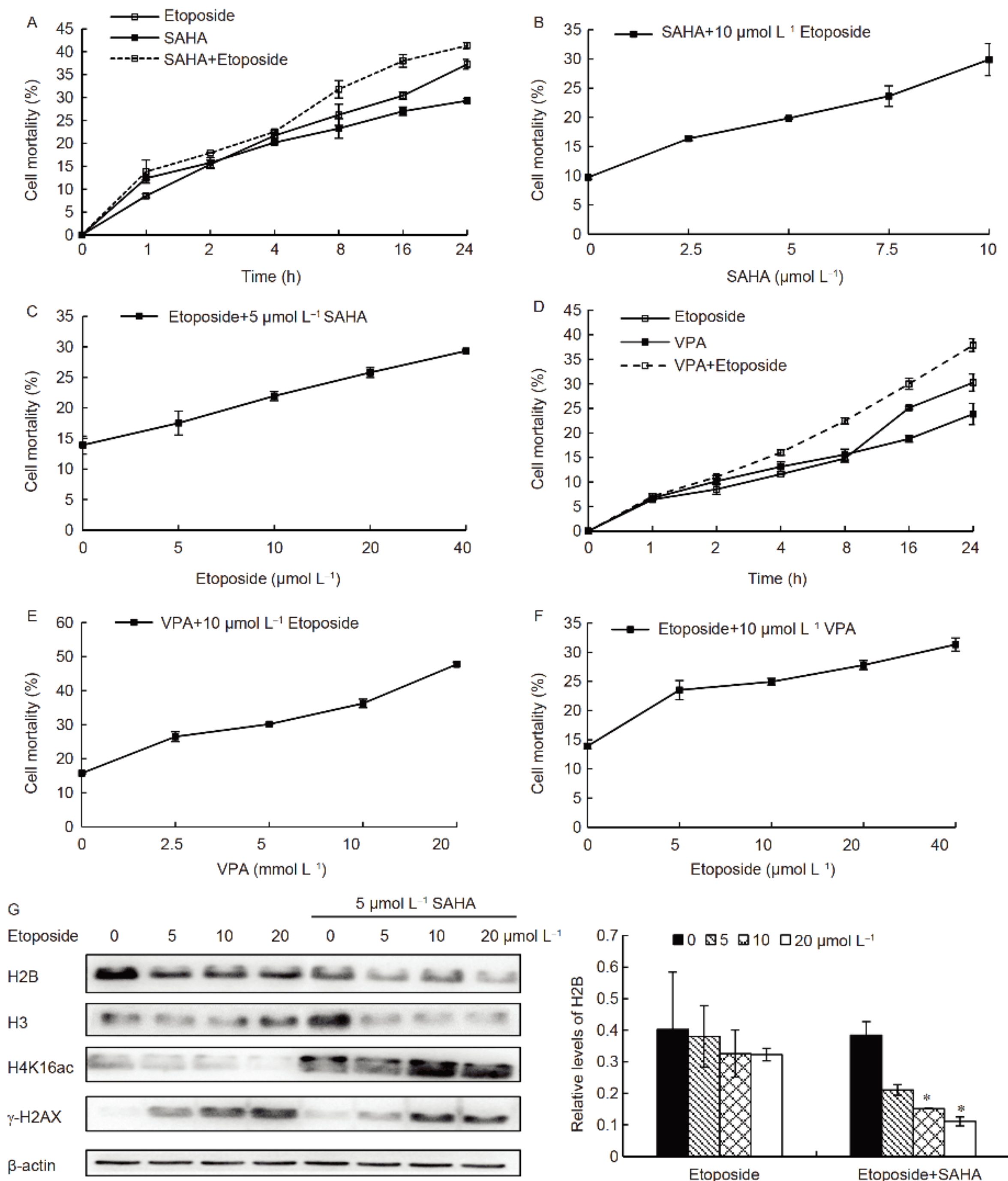

Figure 5 Joint treatments with an HDAC inhibitor and etoposide induce tumor cell death with a weak additive effect. A, HeLa cells were treated with 10 μmol L^{-1} etoposide and 5 μmol L^{-1} SAHA for different time. B, HeLa cells were treated with 10 μmol L^{-1} etoposide and different concentrations of SAHA for 4 h. C, HeLa cells were treated with 5 μmol L^{-1} SAHA and different concentrations of etoposide for 4 h. D, HeLa cells were treated with 10 μmol L^{-1} etoposide and 10 mmol L^{-1} VPA for different time. E, HeLa cells were treated with 10 μmol L^{-1} etoposide and different concentrations of VPA for 4 h. F, HeLa cells were treated with 10 mmol L^{-1} VPA and different concentrations of etoposide for 4 h. G. HeLa cells were treated with 5 μmol L^{-1} SAHA and with or without different concentrations of etoposide for 4 h. The levels of the core histones were analyzed by immunoblotting. Data are representative of one experiment with at least two independent biological replicates, mean ± SEM, *P<0.05, **P<0.01 (two-tailed paired t-test).

obtained from the American Type Culture Collection. MEF cells were obtained as described (Qian et al., 2013), and were cultured in the above DMEM additionally supplemented with 1% non-essential amino acids and 200 μmol L^{-1} of β-mercaptoethanol. They were cultured at 37°C with 5% CO_2. We have used PCR to detect the contamination of mycoplasma, and confirmed that there was no contamination for any cell lines used in this study.

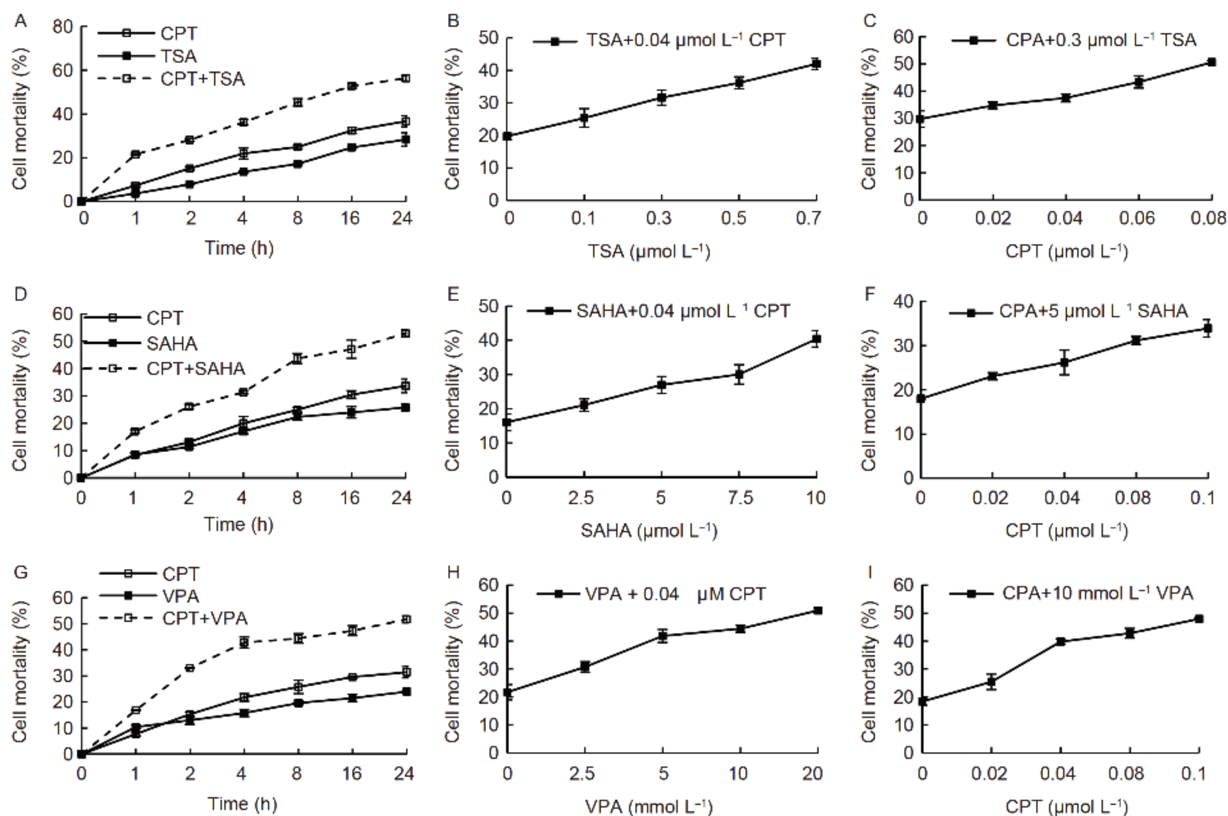

Figure 6 Joint treatments with an HDAC inhibitor and camptothecin induce tumor cell death additively. A, HeLa cells were treated with 0.04 μmol L^{-1} CPT and 0.3 μmol L^{-1} TSA for different time. B, HeLa cells were treated with 0.04 μmol L^{-1} CPT and different concentrations of TSA for 4 h. C, HeLa cells were treated with 0.3 μmol L^{-1} TSA and different concentrations of CPT for 4 h. D, HeLa cells were treated with 0.04 μmol L^{-1} CPT and 5 μmol L^{-1} SAHA for different time. E, HeLa cells were treated with 0.04 μmol L^{-1} CPT and different concentrations of SAHA for 4 h. F, HeLa cells were treated with 5 μmol L^{-1} SAHA and different concentrations of CPT for 4 h. G, HeLa cells were treated with 0.04 μmol L^{-1} CPT and 10 mmol L^{-1} VPA for different time. H, HeLa cells were treated with 0.04 μmol L^{-1} CPT and different concentrations of VPA for 4 h. I, HeLa cells were treated with 10 mmol L^{-1} VPA and different concentrations of CPT for 4 h. Data are representative of one experiment with at least two independent biological replicates, mean ± SEM, *P<0.05, **P<0.01 (two-tailed paired t-test).

Trypan blue exclusion

After drug treatments, cells were collected in centrifuge tubes, and centrifuged at 500×g for 5 min at 4°C. The cell pellet was resuspended in 1 mL of medium. Cell suspension was mixed with 0.04% trypan blue, and the mixture was added to a hemacytometer for counting.

Whole cell extract and immunoblotting

Wash cells with cold PBS once, scrape the cells in PBS, and collect the cells in a pre-cold 1.5-mL tube. Add 1.2×SDS sample buffer with urea into cells from a 6-cm plate, mix well by flipping, and spin briefly to make sure that all the samples are at the bottom. After adding 1.2×SDS sample buffer into the frozen cells, all the steps are performed at RT. Sonicate at 200 W for 4 s twice, and then use pipette tips to check whether the samples are sonicated completely. Heat sonicated samples at 97°C for 6 min and spin them at 16,000 at RT for 10 min. Transfer the supernatant into a new 1.5-mL tube, and place the samples at RT.

Separate the samples in SDS-PAGE at 150V for ~90 min. Transfer at 60V for 3 h using ice-cold transfer buffer. After blocking, incubate the membrane with primary anti-bodies for 1 h. Then perform the second antibody incubation and ECL detection as usual.

TUNEL assay

Dilute 0.4% PFA and 0.1% Triton X-100 by PBS, and collect the treated HeLa cells. Resuspend the cells in the prepared diluent and add them to the slides. Wash the slides twice with PBS for 5 min, add 100 μL equilibration buffer to cover the slides for 10 min. Prepare the rTdT incubation buffer, which contains 45 μL equilibration buffer, 5 μL Nucleotide Mix and 1 μL rTdT Enzyme. Cover the slides with 50 μL rTdT incubation buffer in a dark box in 37°C for 1 h. Dilute 20×SSC to 2×SSC with H_2O. Termination of the reaction by 2×SSC for 15 min. Wash the slides three times with PBS for 5 min. Dilute DAPI with Mountain solution (DAPI: Moun-

tain solution=1:20,000). Cells were stained by DAPI for 20 min. Observe cells under a fluorescence microscope.

Statistical analysis

Unless stated elsewhere, significance levels for comparisons between two groups were determined by one-way ANOVA tests, mean±SEM (*P<0.05 and **P<0.01), normal distribution. Sample size was based on empirical data from pilot experiments. No additional randomization or blinding was used to allocate experimental groups.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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Altaf, M., Saksouk, N., and Côté, J. (2007). Histone modifications in response to DNA damage. Mutat Res/Fundamental Mol Mech Mutagenesis 618, 81–90.

Arnold, C., Stadler, P.F., and Prohaska, S.J. (2013). Chromatin computation: epigenetic inheritance as a pattern reconstruction problem. J Theor Biol 336, 61–74.

Blagosklonny, M.V., Robey, R., Sackett, D.L., Du, L., Traganos, F., Darzynkiewicz, Z., Fojo, T., and Bates, S.E. (2002). Histone deacetylase inhibitors all induce p21 but differentially cause tubulin acetylation, mitotic arrest, and cytotoxicity. Mol Cancer Ther 11, 937–941.

Campos, E.I., and Reinberg, D. (2009). Histones: annotating chromatin. Annu Rev Genet 43, 559–599.

Capparelli, C., Chiavarina, B., Whitaker-Menezes, D., Pestell, T.G., Pestell, R.G., Hulit, J., Andò, S., Howell, A., Martinez-Outschoorn, U.E., Sotgia, F., et al. (2012). CDK inhibitors (p16/p19/p21) induce senescence and autophagy in cancer-associated fibroblasts, "fueling" tumor growth via paracrine interactions, without an increase in neo-angiogenesis. Cell Cycle 11, 3599–3610.

Carew, J.S., Giles, F.J., and Nawrocki, S.T. (2008). Histone deacetylase inhibitors: mechanisms of cell death and promise in combination cancer therapy. Cancer Lett 269, 7–17.

Chrun, E.S., Modolo, F., and Daniel, F.I. (2017). Histone modifications: a review about the presence of this epigenetic phenomenon in carcinogenesis. Pathol - Res Practice 213, 1329–1339.

Dahlin, J.L., Chen, X., Walters, M.A., and Zhang, Z. (2015). Histone-modifying enzymes, histone modifications and histone chaperones in nucleosome assembly: lessons learned from Rtt109 histone acetyltransferases. Critical Rev Biochem Mol Biol 50, 31–53.

Dokmanovic, M., and Marks, P.A. (2005). Prospects: histone deacetylase inhibitors. J Cell Biochem 96, 293–304.

Dovey, O.M., Foster, C.T., Conte, N., Edwards, S.A., Edwards, J.M., Singh, R., Vassiliou, G., Bradley, A., and Cowley, S.M. (2013). Histone deacetylase 1 and 2 are essential for normal T-cell development and genomic stability in mice. Blood 121, 1335–1344.

Downs, J.A., Allard, S., Jobin-Robitaille, O., Javaheri, A., Auger, A., Bouchard, N., Kron, S.J., Jackson, S.P., and Côté, J. (2004). Binding of chromatin-modifying activities to phosphorylated histone H2A at DNA damage sites. Mol Cell 16, 979–990.

Eckschlager, T., Plch, J., Stiborova, M., and Hrabeta, J. (2017). Histone deacetylase inhibitors as anticancer drugs. IJMS 18, 1414.

Feng, W., Cai, D., Zhang, B., Lou, G., and Zou, X. (2015). Combination of HDAC inhibitor TSA and silibinin induces cell cycle arrest and apoptosis by targeting survivin and cyclinB1/Cdk1 in pancreatic cancer cells. Biomed PharmacoTher 74, 257–264.

Ge, Z., Nair, D., Guan, X., Rastogi, N., Freitas, M.A., and Parthun, M.R. (2013). Sites of acetylation on newly synthesized histone H4 are required for chromatin assembly and DNA damage response signaling. Mol Cell Biol 33, 3286–3298.

Haberland, M., Montgomery, R.L., and Olson, E.N. (2009). The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nat Rev Genet 10, 32–42.

Khor, B., Bredemeyer, A.L., Huang, C.Y., Turnbull, I.R., Evans, R., Maggi, L.B., White, J.M., Walker, L.M., Carnes, K., Hess, R.A., et al. (2006). Proteasome activator PA200 is required for normal spermatogenesis. Mol Cell Biol 26, 2999–3007.

Kumar, V., Ara, G., Afzal, M., and Siddique, Y.H. (2011). Effect of methyl methanesulfonate on hsp70 expression and tissue damage in the third instar larvae of transgenic Drosophila melanogaster (hsp70-lacZ) Bg9. Interdisciplinary Toxicol 4, 159–165.

Lundin, C., North, M., Erixon, K., Walters, K., Jenssen, D., Goldman, A.S.H., and Helleday, T. (2005). Methyl methanesulfonate (MMS) produces heat-labile DNA damage but no detectable *in vivo* DNA double-strand breaks. Nucleic Acids Res 33, 3799–3811.

Meng, J., Zhang, H.H., Zhou, C.X., Li, C., Zhang, F., and Mei, Q.B. (2012). The histone deacetylase inhibitor trichostatin A induces cell cycle arrest and apoptosis in colorectal cancer cells via p53-dependent and -independent pathways. Oncol Rep 28, 384–388.

Montecucco, A., Zanetta, F., and Biamonti, G. (2015). Molecular mechanisms of etoposide. EXCLI Journal 14, 95–108.

Murr, R., Loizou, J.I., Yang, Y.G., Cuenin, C., Li, H., Wang, Z.Q., and Herceg, Z. (2006). Histone acetylation by Trrap-Tip60 modulates loading of repair proteins and repair of DNA double-strand breaks. Nat Cell Biol 8, 91–99.

Pommier, Y., Leo, E., Zhang, H.L., and Marchand, C. (2010). DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. Chem Biol 17, 421–433.

Qian, M.X., Pang, Y., Liu, C.H., Haratake, K., Du, B.Y., Ji, D.Y., Wang, G.F., Zhu, Q.Q., Song, W., Yu, Y., et al. (2013). Acetylation-mediated proteasomal degradation of core histones during DNA repair and spermatogenesis. Cell 153, 1012–1024.

Reinke, H., and Hörz, W. (2003). Histones are first hyperacetylated and then lose contact with the activated PHO5 promoter. Mol Cell 11, 1599–1607.

Robey, R.W., Chakraborty, A.R., Basseville, A., Luchenko, V., Bahr, J., Zhan, Z., and Bates, S.E. (2011). Histone deacetylase inhibitors: emerging mechanisms of resistance. Mol Pharm 8, 2021–2031.

Shabason, J.E., Tofilon, P.J., and Camphausen, K. (2011). Grand rounds at the National Institutes of Health: HDAC inhibitors as radiation modifiers, from bench to clinic. J Cellular Mol Med 15, 2735–2744.

Stiborova, M., Eckschlager, T., Poljakova, J., Hrabeta, J., Adam, V., Kizek, R., and Frei, E. (2012). The synergistic effects of DNA-targeted chemotherapeutics and histone deacetylase inhibitors as therapeutic strategies for cancer treatment. CMC 19, 4218–4238.

Suraweera, A., O'Byrne, K.J., and Richard, D.J. (2018). Combination therapy with histone deacetylase inhibitors (HDACi) for the treatment of cancer: achieving the full therapeutic potential of HDACi. Front Oncol 8, 92.

Turinetto, V., and Giachino, C. (2015). Multiple facets of histone variant H2AX: a DNA double-strand-break marker with several biological functions. Nucleic Acids Res 43, 2489–2498.

Ustrell, V., Hoffman, L., Pratt, G., and Rechsteiner, M. (2002). PA200, a nuclear proteasome activator involved in DNA repair. EMBO J 21, 3516–3525.