

# Effects of histone deacetylase inhibitors on ATP-binding cassette transporters in lung cancer A549 and colorectal cancer HCT116 cells

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**Abstract.** Histone deacetylase (HDAC) inhibitors and DNA alkylators are effective components used in combination chemotherapy. In the present study, the effects of HDAC inhibitors on the expression of ATP-binding cassette (ABC) transporters were investigated. It was observed that HDAC inhibitors induced the expression of multidrug-resistant ABC transporters differently in lung cancer A549 cells than in colorectal cancer HCT116 cells. In these two cell lines, the HDAC inhibitors suberoylanilide hydroxamic acid (SAHA) and trichostatin A (TSA) significantly increased ABCB1 expression at the mRNA and protein levels, whereas they had no evident effect on ABCG2 protein expression. SAHA and TSA decreased ABCG2 mRNA expression in A549 cells and had no evident effect on ABCG2 mRNA expression in HCT116 cells. Notably, SAHA and TSA increased the mRNA expression levels of ABCC5, ABCC6, ABCC10, ABCC11 and ABCC12, as well as the protein expression levels of ABCC2, ABCC10 and ABCC12. By contrast, these inhibitors decreased the mRNA expression levels of ABCC1, ABCC2, ABCC3 and ABCC4, as well as the expression of ABCC1 and ABCC3 proteins. Furthermore, SAHA and TSA were found to down-regulate HDAC3 and HDAC4, but not HDAC1 and HDAC2. Taken together, the results suggested that HDAC inhibitors work synergistically with DNA alkylators, in part, due to the inhibitory effect of these inhibitors on ABCC1 expression, which translocates these alkylators from inside to outside of

cancer cells. These results further suggested the possibility of antagonism when HDAC inhibitors are combined with anthracyclines and other ABCB1 drug ligands in chemotherapy.

## Introduction

Multidrug resistance (MDR) occurs when cancer cells are resistant to a number of functionally and structurally different chemotherapeutic agents, and represents a major obstacle for successful treatment of cancer (1,2). Efflux of anticancer drugs by ATP-binding cassette (ABC) transporters serves a crucial role in the development of the MDR phenotype (3-5). ABC transporters associated with chemoresistance include MDR-1, MDR-associated proteins and breast cancer resistance protein, which are coded for by ABCB1, ABCCs and ABCG2, respectively (3). ABCB1 is the most well-known ABC transporter, and is able to extrude natural toxins, anticancer drugs and drug metabolites across the plasma membrane to confer an MDR phenotype in cancer cells (6-8). Similarly, ABCC members have been reported to confer chemoresistance in cancer cells by translocating a variety of structurally diverse glutathione conjugates or therapeutic drugs (4,9). For instance, ABCC1, ABCC2 and ABCC3 cause resistance to hydrophobic anions, including several natural compounds, whereas ABCC4, ABCC5 and ABCC11 efflux cyclic nucleotides (10,11). ABCG2, another MDR-associated protein, is a ubiquitous ABC transporter with an important role in the distribution, absorption and elimination of its substrate (12,13). Collectively, these ABC transporters confer the MDR phenotype to cancer cells by reducing the intracellular concentrations of anticancer drugs to a nontoxic level. Furthermore, these ABC transporters are highly expressed in a number of human tumors and are often associated with poor prognosis (14,15).

Histone deacetylase (HDAC) inhibitors have emerged as a novel class of anticancer agents due to their significant anticancer activities, including angiogenesis inhibition, and the promotion of cell cycle arrest, differentiation and apoptosis (16,17). HDAC inhibitors may serve as potent anticancer drugs due to their broad antitumor activity and low toxicity in

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normal cells (18). Several HDAC inhibitors, including trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA; also known as vorinostat) and sodium butyrate, have exhibited potent anticancer activities in various cancer cells (19). SAHA has been approved by the US Food and Drug Administration as a treatment for cutaneous T-cell lymphoma (20). Furthermore, HDAC inhibitors have been demonstrated to work synergistically with a variety of antitumor agents, including gemcitabine, doxorubicin, etoposide, paclitaxel and cisplatin (21).

Our previous study and other previous research have demonstrated that treatment of cancer cells with HDAC inhibitors increases the expression of ABCB1, which results in the development of an MDR phenotype (22,23). However, the effects of HDAC inhibitors on other MDR-associated ABC transporters have not previously been reported. Thus, the aim of the present study was to investigate the effects of two HDAC inhibitors, namely SAHA and TSA, on the expression levels of ABCB1, ABCCs and ABCG2 in lung cancer A549 and colorectal cancer HCT116 cells. The results indicated that HDAC inhibitors are able to induce differential expression of these ABC transporters. The present study suggests that more attention should be paid to drug combinations with HDAC inhibitors, as ABC transporters have different substrates. DNA alkylators are substrates of ABCC1. A decrease in ABCC1 protein level may contribute to the synergism of HDAC inhibitors and DNA alkylating agents. Conversely, HDAC inhibitors may antagonize the efficacy of anticancer drugs that are substrates of ABCB1 (14). These differential effects of HDAC inhibitors on the expression levels of drug transporters support the necessity for caution in combining these drugs with other chemotherapeutic agents.

## Materials and methods

**Chemicals and reagents.** Oxaliplatin, 5-fluorouracil, SAHA and TSA were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). Primary antibodies against the following were purchased: ABCB1 (cat. no. 13978), purchased from Cell Signaling Technology, Inc. (MA, Danvers, USA);  $\alpha$ -tubulin (cat. no. sc-134237), obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA); ABCC2 (cat. no. 24893-1-AP), ABCC5 (cat. no. 19503-1-AP) and ABCC6 (cat. no. 27848-1-AP) obtained from ProteinTech Group, Inc. (Chicago, IL, USA); ABCC1 (cat. no. BS7474) and ABCG2 (cat. no. BS3482) from Bioworld Technology Co., Ltd. (Nanjing, China); ABCC3 (cat. no. ab3375), ABCC4 (cat. no. ab77184), ABCC10 (cat. no. ab91451), ABCC11 (cat. no. ab98979) and ABCC12 (cat. no. ab91453) from Abcam (Cambridge, MA, USA); HDAC1 (cat. no. ET1605-35), HDAC2 (cat. no. ET1607-78), HDAC3 (cat. no. ET1610-5) and HDAC4 (cat. no. ET1612-51), purchased from HuaAn Biotechnology Co., Ltd. (Hangzhou, China). Horseradish peroxidase (HRP)-conjugated secondary antibody (cat. nos. SH001X, SH002X and SH003X) were also purchased from DingGuo Biotechnology Co., Ltd. (Guangzhou, China). The PrimeScript<sup>®</sup> RT reagent and SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup> kits were purchased from Takara Bio, Inc. (Otsu, Japan).

**Cell culture.** A549 and HCT116 cells were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). HCT116 cells were maintained

in Dulbecco's modified Eagle's medium/F12 culture medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS). A549 cells were cultured in RPMI-1640 culture medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS. The two cell lines were cultured in an incubator at 37°C with an atmosphere of 5% CO<sub>2</sub>.

**Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) detection.** A549 and HCT116 cells were treated with SAHA (0.5  $\mu$ M) or TSA (100 nM) at 37°C for 24 h. Treatment with DMSO (equal volume added) was used as the control. Total mRNA was extracted from A549 and HCT116 cells using TRIzol reagent. RNA sample concentration was measured using a UV spectrophotometer, and optical density (OD)260/OD280 was limited to 1.8-2.0. A total of 500 ng RNA was used for cDNA synthesis with the PrimeScript<sup>®</sup> RT reagent kit. Subsequently, qPCR was performed on an ABI 7500 Real-Time PCR system (Thermo Fisher Scientific, Inc.) using the Takara SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup> kit to quantify the expression of target genes. Primers used in qPCR experiments are presented in Table I, with GAPDH serving as an internal reference. The thermal cycling conditions for qPCR were as follows: Holding stage conducted at 95°C for 30 sec; cycling stage conducted at 95°C for 5 sec and 60°C for 34 sec for 40 cycles; melt curve stage conducted at 95°C for 15 sec, 60°C for 60 sec, 95°C for 30 sec and 60°C for 15 sec. Following normalization to the GAPDH gene, the expression of each target gene was calculated using the comparative cycle quantification (Cq) method (24). In correlation analysis, the  $\Delta$ Cq values were calculated according to the following formula:  $\Delta$ Cq=Cq (gene of interest)-Cq (GAPDH). For determination of the relative expression, the  $2^{-\Delta\Delta Cq}$  value was calculated according to the following formula:  $\Delta\Delta Cq=\Delta Cq$  (control group)- $\Delta Cq$  (experimental group).

**Western blotting.** A549 and HCT116 cells were treated with SAHA (0.5  $\mu$ M) or TSA (100 nM) at 37°C for 24 h. DMSO treatment was used as the control. A549 and HCT116 cells were washed three times with ice-cold PBS and subsequently lysed using western blotting lysis buffer, containing 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.5% Na-deoxycholate, 5  $\mu$ g/ml aprotinin, 5  $\mu$ g/ml leupeptin and 1 mM phenylmethylsulphonyl fluoride. Cell lysates were cleared by centrifugation at 12,000  $\times$  g at 4°C for 30 min and denatured by boiling in Laemmli buffer. Bovine serum albumin was used as the standard for determining the protein concentration using a Bradford protein assay (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Equal amounts of protein samples were loaded onto 8% gels and separated by SDS-PAGE, following which they were electrophoretically transferred onto polyvinylidene difluoride (PVDF) membranes. Following blocking with 5% non-fat milk for 2 h at room temperature, the PVDF membranes were incubated with primary antibodies (ABCB1, ABCC1, ABCC2, ABCC4, ABCC5, ABCC6, ABCG2,  $\alpha$ -tubulin, HDAC1, HDAC2, HDAC3 and HDAC4 at a dilution of 1:1,000; ABCC3, ABCC10, ABCC11 and ABCC12 at a dilution of 1:50) at 4°C overnight. Membranes were then incubated with

Table I. Primers used in reverse transcription-quantitative polymerase chain reaction assay.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
ABCB1	TGCTCAGACAGGATGTGAGTTG	AATTACAGCAAGCCTGGAACC
ABCC1	GCCAAGAAGGAGGAGACC	AGGAAGATGCTGAGGAAGG
ABCC2	TGGTGGCAACCTGAGCATAGG	ACTCGTTTTGGATGGTCGTCTG
ABCC3	CTTAAGACTTCCCTCAACATGC	GGTCAAGTTCCTCTTGCTC
ABCC4	GGTCCCCCTTGGAATCATTT	AATCCTGGTGTGCATCAAACAG
ABCC5	ACCCGTTGTTGCCATCTTAG	GCTTTGACCCAGGCATACAT
ABCC6	GTGGTGTGTTGCTGTCCACAC	ACGACACCAGGGTCAACTTC
ABCC10	ATTGCCCATAGGCTCAACAC	AGCAGCCAGCACCTCTGTAT
ABCC11	GGCTGAGCTACTGGTTGGAG	TGGTGAAAATCCCTGAGGAG
ABCC12	GGTGTTTCATGCTGGTGTGTTG	GCTCGTCCATATCCTTGGA
ABCG2	TATAGCTCAGATCATTGTCACAGTC	GTTGGTCGTCAGGAAGAAGAG
GAPDH	GCACCGTCAAGGCTGAGAAC	TGGTGAAGACGCCAGTGGA

ABC, ATP-binding cassette.

HRP-conjugated secondary antibodies (1:5,000 dilution) for 1.5 h at room temperature. Specific immune complexes were detected using Western Blotting Plus Chemiluminescence Reagent (Thermo Fisher Scientific, Inc.). Band intensity was quantified by densitometry analysis using Image-Pro Plus version 4.5 software (Media Cybernetics, Inc., Rockville, MD, USA).

**Cell Counting Kit (CCK)-8 assay.** Cell viability was measured using a CCK-8 assay. Briefly, A549 and HCT116 cells ( $1 \times 10^4$ /well) were seeded into 96-well plates in medium supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.). The cells were treated with different concentrations of SAHA (0, 0.25, 0.5, 1, 2 and 4  $\mu$ M) or TSA (0, 50, 100, 200, 400 and 800 nM) at 37°C for 48 h. Then, 10  $\mu$ l CCK-8 solution (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) was added to 100  $\mu$ l medium. The cells were incubated with CCK-8 in medium at 37°C for 2 h, and the absorbance was then measured at 450 nm using a microplate reader (Bio-Rad Laboratories, Inc.).

To investigate the effect of SAHA and TSA on chemoresistance, HCT116 cells were treated with SAHA (0.5  $\mu$ M) or TSA (100 nM) at 37°C for 24 h, then the media were removed and the cells were treated with different concentrations of oxaliplatin (0, 5, 10, 20, 40 and 80  $\mu$ g/ml) or 5-fluorouracil (0, 2.5, 5, 10, 20 and 40  $\mu$ g/ml) at 37°C for 48 h. DMSO treatment was used as a control. The cells were diluted with 10  $\mu$ l CCK-8 in medium at 37°C for 2 h, and the absorbance was then measured at 450 nm using a microplate reader.

**Statistical analysis.** Data are presented as the mean  $\pm$  standard deviation of three independent experiments. One-way analysis of variance was used to assess the differences among multiple groups. Data were analyzed using two-tailed unpaired Student's t-tests for differences between any two groups. These analyses were performed using GraphPad Prism software, version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**HDAC inhibitors induce drug resistance in HCT116 cells.** HDAC inhibitors are known to have a potent anticancer activity. The present study attempted to investigate the effect of two HDAC inhibitors, SAHA and TSA, on the expression of ABC transporters and drug resistance in cancer cells. The concentration of SAHA and TSA used to treat cancer cells should have no significant effect on cell survival. In order to determine the appropriate concentration, SAHA and TSA at concentrations of 0-4  $\mu$ M and 0-800 nM, respectively, were used in the cell viability assay, which are below their  $IC_{50}$  values. HCT116 and A549 cells were treated with different concentrations of SAHA or TSA for 48 h, and the cell viability was assessed using a CCK-8 assay. As shown in Fig. 1A, slight inhibition was observed for concentrations  $>0.5 \mu$ M for SAHA and  $>100$  nM for TSA. Therefore, 0.5  $\mu$ M SAHA and 100 nM TSA were selected for use in subsequent experiments.

Next, the study investigated whether HDAC inhibitors affect drug resistance by performing a CCK-8 assay. HCT116 cells were pretreated with 0.5  $\mu$ M SAHA or 100 nM TSA for 24 h, following which cells were exposed to various concentrations of oxaliplatin or 5-fluorouracil for 48 h, and cell viability was assessed using a CCK-8 assay. The results revealed that, compared with the DMSO-treated group, SAHA and TSA pretreatment significantly decreased the sensitivity of HCT116 cells to anticancer drugs (Fig. 1B).

**Effect of HDAC inhibitors on ABCB1, ABCC5, ABCC6, ABCC10, ABCC11 and ABCC12 expression.** ABC transporter-induced drug efflux is closely associated with acquisition of chemo-resistance in cancer cells (3). To investigate the effect of HDAC inhibitors on the expression of ABC transporters, HCT116 and A549 cells were treated with 0.5  $\mu$ M SAHA and 100 nM TSA for 24 h. The mRNA and protein expression levels of various ABC transporters were detected using RT-qPCR and western blotting, respectively. The RT-qPCR results indicated that SAHA markedly upregulated

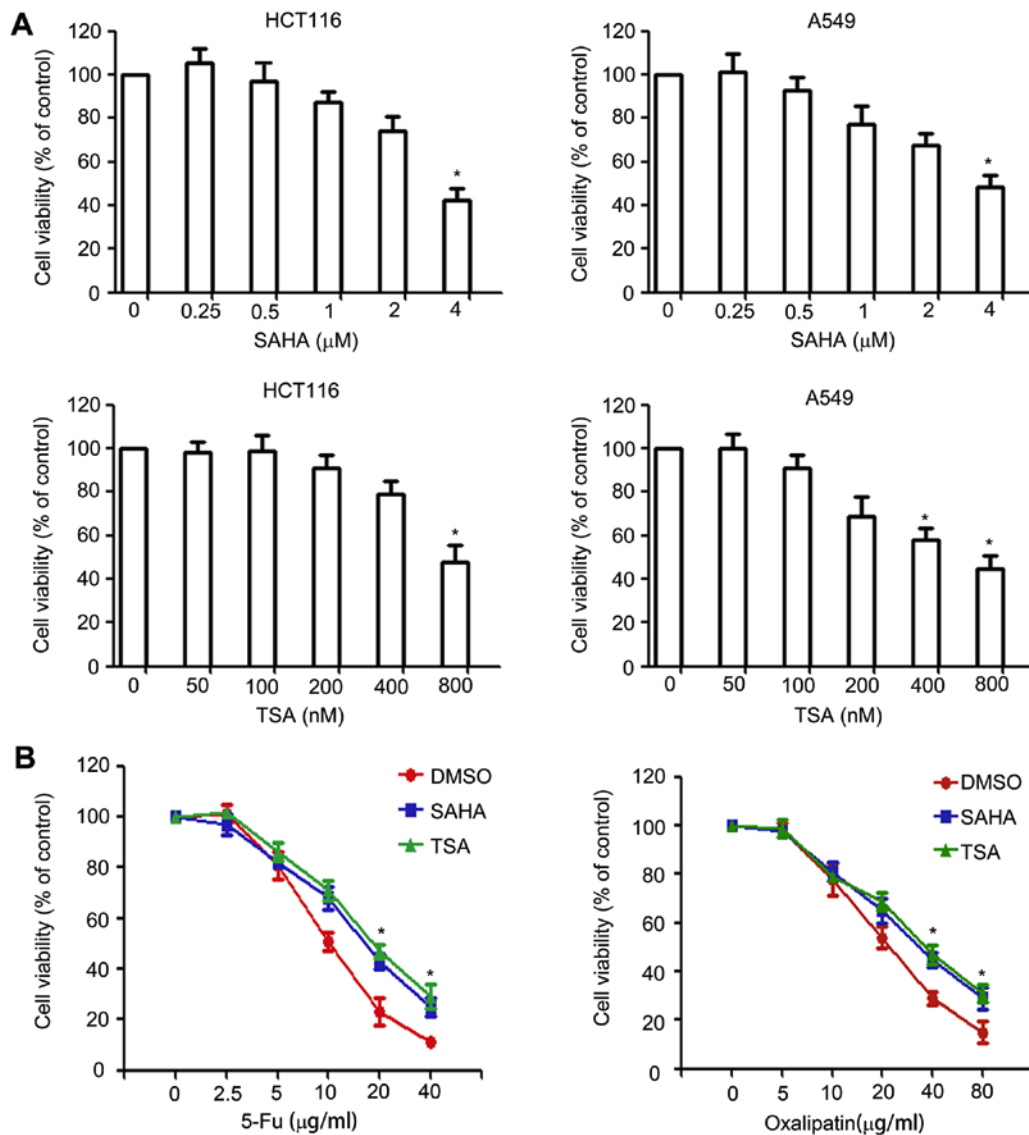


Figure 1. Histone deacetylase inhibitors induce drug resistance in HCT116 cells. (A) A549 and HCT116 cells were treated with various concentrations of SAHA or TSA for 48 h, and cell viability was detected using a CCK-8 assay. (B) HCT116 cells were pretreated with SAHA (0.5  $\mu$ M) or TSA (100 nM) for 24 h. Subsequently, the media were removed and the cells were treated with various concentrations of 5-Fu or oxaliplatin for 48 h. Cell viability was assessed using a CCK-8 assay. \* $P$ <0.05 vs. corresponding control group. ABC, ATP-binding cassette; SAHA, suberoylanilide hydroxamic acid; TSA, trichostatin A; CCK-8, Cell Counting Kit-8; 5-Fu, 5-fluorouracil.

mRNA expression of ABCB1, ABCC5, ABCC10, ABCC11 and ABCC12 in A549 cells and ABCB1, ABCC10 and ABCC12 in HCT116 cells. TSA markedly upregulated mRNA levels of ABCB1, ABCC5, ABCC6, ABCC10, ABCC11 and ABCC12 in A549 cells and HCT116 cells (Fig. 2A). Similarly, the results of western blotting revealed that SAHA and TSA upregulated ABCB1, ABCC10, ABCC11 and ABCC12 protein expression. However, SAHA and TSA had no clear effect on the expression levels of ABCC5 and ABCC6 (Fig. 2B).

**Effect of HDAC inhibitors on ABCC1, ABCC2, ABCC3, ABCC4 and ABCG2 expression.** Treatment with HDAC inhibitors affected the expression of ABCC1, ABCC2, ABCC2 and ABCC4 differently, as compared with ABCB1, ABCC5, ABCC6, ABCC10, ABCC11 and ABCC12. It was observed that SAHA and TSA treatment downregulated the expression levels of ABCC1, ABCC3 and ABCC4 mRNA (Fig. 3A). Similarly, the

western blotting results indicated that the ABCC1 and ABCC3 protein expression levels were also decreased (Fig. 3B). Notably, while SAHA and TSA decreased ABCC2 mRNA expression, its protein expression in A549 and HCT116 cells was found to be increased (Fig. 3A and B). SAHA and TSA exerted no obvious effects on the protein expression levels of ABCC4 (Fig. 3B).

The effect of HDAC inhibitors on ABCG2 expression was further investigated in A549 and HCT116 cells. As shown in Fig. 3A, SAHA and TSA downregulated ABCG2 mRNA expression in A549 cells, while no marked effect was observed in HCT116 cells. Furthermore, western blotting revealed that SAHA and TSA had no evident effect on the expression of ABCG2 protein in A549 and HCT116 cells (Fig. 3B).

**Effect of HDAC inhibitors on the expression of HDAC1, HDAC2, HDAC3 and HDAC4.** Since HDACs serve an important role in the regulation of gene transcription, the effect of HDAC

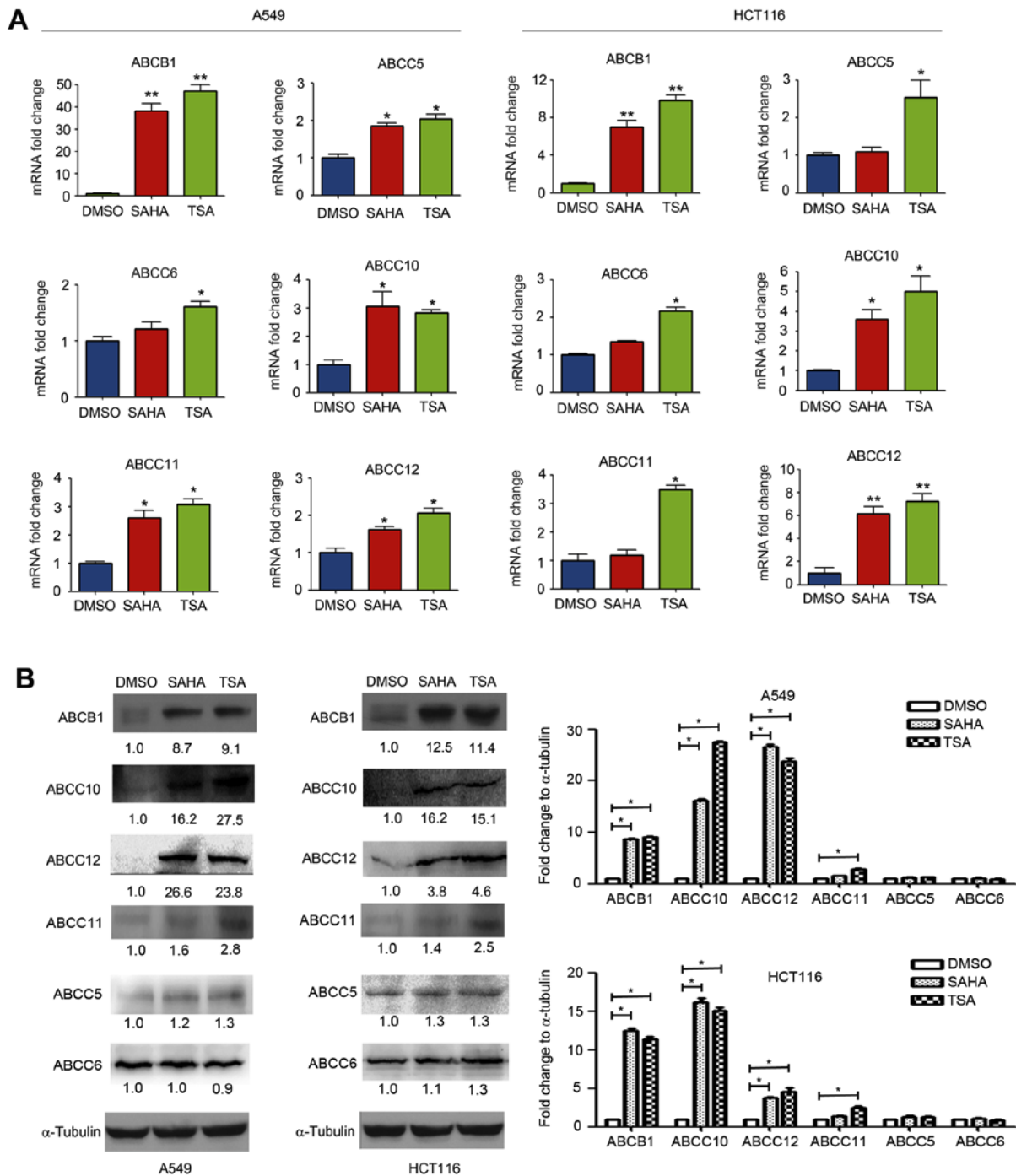


Figure 2. Histone deacetylase inhibitors increased the ABCB1, ABCC5, ABCC6, ABCC10, ABCC11 and ABCC12 expression levels. A549 and HCT116 cells were treated with DMSO, SAHA (0.5  $\mu$ M) or TSA (100 nM) for 24 h. (A) mRNA expression levels of ABCB1, ABCC5, ABCC6, ABCC10, ABCC11 and ABCC12 were measured using reverse transcription-quantitative polymerase chain reaction. (B) Protein expression levels of ABCB1, ABCC5, ABCC6, ABCC10, ABCC11 and ABCC12 were detected using western blotting. \* $P$ <0.05 and \*\* $P$ <0.01, vs. DMSO group. ABC, ATP-binding cassette; SAHA, suberoyl-anilide hydroxamic acid; TSA, trichostatin A.

inhibitors on HDAC1, HDAC2, HDAC3 and HDAC4 expression was measured in A549 and HCT116 cells. HDAC1-4 exert their function depending on the protein; therefore, protein expression was detected by western blotting. As shown in Fig. 4, SAHA and TSA decreased the protein expression levels of HDAC3 and HDAC4, compared with the DMSO-treated group. By contrast, SAHA and TSA had no marked effects on HDAC1 and HDAC2 expression levels in A549 and HCT116 cells (Fig. 4).

## Discussion

Chemotherapy is a common strategy used for the treatment of malignant tumors. However, the efficacy of chemotherapy is limited by the development of an MDR phenotype due to overexpression of ABC transporters in cancer cells. Chemoresistance-associated ABC transporters mainly include ABCB1, ABCC1-6, ABCC10-12 and ABCG2 (3).



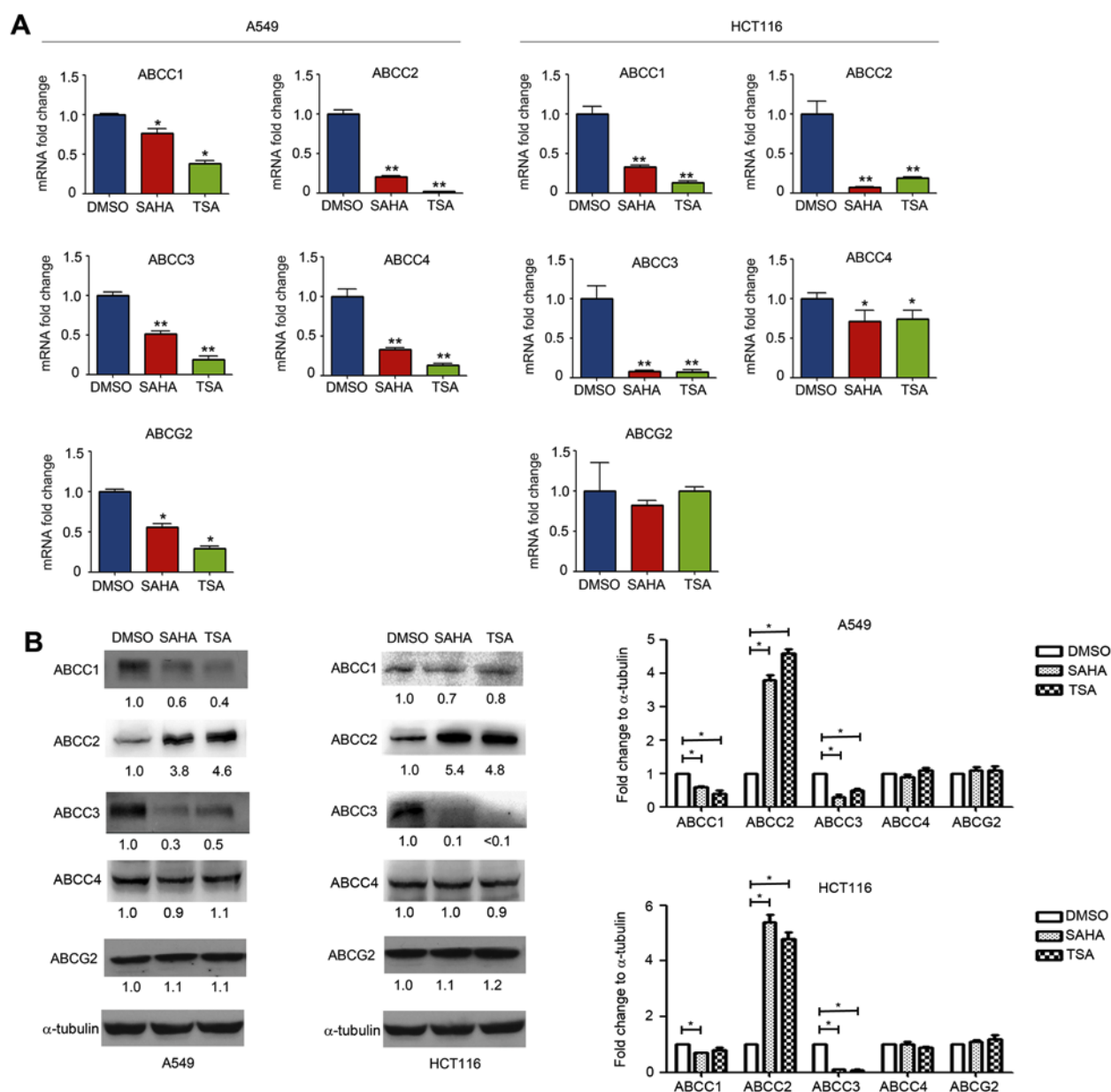


Figure 3. Effect of histone deacetylase inhibitors on ABCC1, ABCC2, ABCC3, ABCC4 and ABCG2 expression levels. A549 and HCT116 cells were treated with DMSO, SAHA (0.5  $\mu$ M) or TSA (100 nM) for 24 h. (A) mRNA and (B) protein expression levels of ABCC1, ABCC2, ABCC3, ABCC4 and ABCG2 were measured using reverse transcription-quantitative polymerase chain reaction and western blotting, respectively. \* $P$ <0.05 and \*\* $P$ <0.01, vs. DMSO group. ABC, ATP-binding cassette; SAHA, suberoylanilide hydroxamic acid; TSA, trichostatin A.

These transporters function as efflux pumps that energetically translocate chemotherapeutic drugs from inside to outside of cancer cells, reducing the intracellular drug concentration and resulting in an MDR phenotype (3). In order to overcome the MDR phenotype induced by HDAC inhibitors, it is crucial to understand the effect of HDAC inhibitors on the expression of chemoresistance-associated ABC transporters. In the present study, it was demonstrated that treatment with HDAC inhibitors increased the expression levels of ABCB1, ABCC2, ABCC5, ABCC6, ABCC10, ABCC11 and ABCC12 in cancer cells, whereas it decreased the expression levels of ABCC1, ABCC3 and ABCC4. To the best of our knowledge, this is the first systematic investigation on the effect of HDAC inhibitors on the expression of chemoresistance-associated ABC transporters.

ABCB1, the most well-known ABC transporter, extrudes natural toxins, anticancer drugs and drug metabolites across the plasma membrane, conferring an MDR phenotype in various cancer cells (25,26). A number of studies have reported that HDAC inhibitors may promote ABCB1 expression in several types of cancer cells (27,28). For instance, the HDAC inhibitors SAHA, TSA and phenylbutyrate were reported to increase ABCB1 expression in acute myeloid leukemia (29). Our previous study revealed that SAHA, TSA and sodium butyrate induced ABCB1 expression by transcriptional activation of STAT3, and stabilized ABCB1 mRNA in lung and colorectal cancer (22,23). Consistently, the present study also demonstrated that SAHA and TSA significantly increase ABCB1 mRNA and protein expression

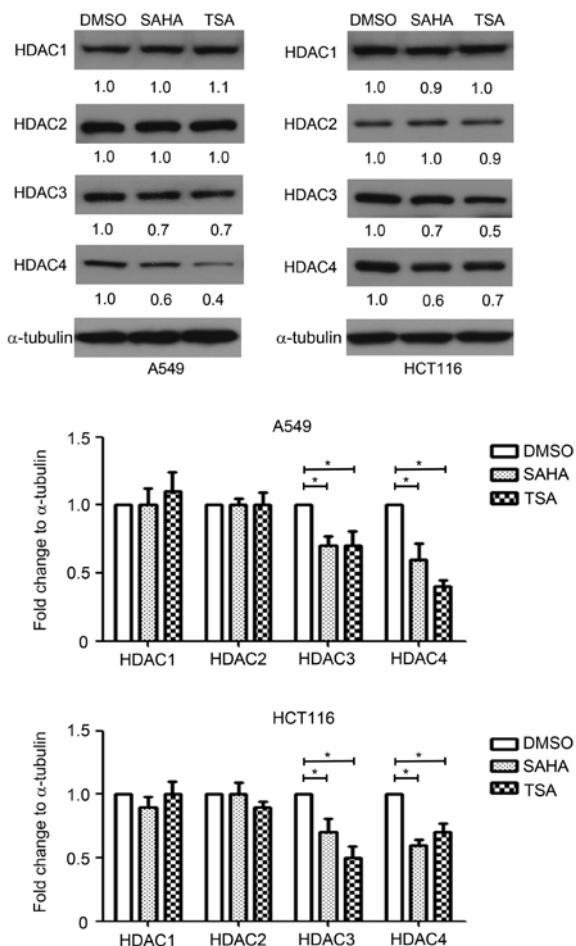


Figure 4. Effect of HDAC inhibitors on the HDAC1, HDAC2, HDAC3 and HDAC4 expression levels. A549 and HCT116 cells were treated with DMSO, SAHA (0.5  $\mu$ M) or TSA (100 nM) for 24 h, following which HDAC1, HDAC2, HDAC3 and HDAC4 expression was detected using western blotting. \* $P$ <0.05 vs. DMSO group. HDAC, histone deacetylase; SAHA, suberoylanilide hydroxamic acid; TSA, trichostatin A.

in A549 and HCT116 cells. Recent research has suggested that continuous stimulation with the HDAC inhibitor FK228 may active ABCG2, another ABC transporter, at the mRNA and protein levels in renal and colon cancer cells to give an MDR phenotype (30). Furthermore, SAHA, TSA and phenylbutyrate have been reported to increase ABCG2 expression in acute myeloid leukemia (19,29). However, in the present study it was observed that SAHA decreased ABCG2 mRNA expression in A549 cells, while neither SAHA nor TSA had any evident influence on ABCG2 protein expression in A549 and HCT116 cells.

ABCC members have been demonstrated to confer chemoresistance in cancer cells by translocating a variety of structurally diverse glutathione conjugates or therapeutic drugs (4). A previous study suggested that SAHA, TSA and valproate were able to increase ABCC11 expression by promoting its transcription in acute myeloid leukemia (29). In the present study, the data revealed that SAHA and TSA increased ABCC5, ABCC6, ABCC10, ABCC11 and ABCC12 mRNA expression levels, as well as ABCC10, ABCC12 protein expression levels. By contrast, SAHA and TSA significantly

decreased ABCC1, ABCC3 and ABCC4 mRNA expression levels, as well as ABCC1 and ABCC3 protein levels. A recent study reported that the HDAC inhibitors SAHA and belinostat downregulated ABCC1 and upregulated ABCB1 expression in T-cell lymphoma and T-cell prolymphocytic leukemia (31).

Of the nine ABCC proteins investigated in the present study, ABCC4, ABCC5, ABCC11 and ABCC12 have a typical ABC structure, with four domains (MSD1, MSD2, NBD1 and NBD2). The other ABCCs, namely ABCC1, ABCC2, ABCC3, ABCC6 and ABCC10 have an additional fifth domain, MSD0 (4). It can thus be speculated that HDAC inhibitors may induce different effects on ABCC expression in cancer cells due to their different structures. Notably, the current study results revealed that SAHA and TSA significantly decreased ABCC2 mRNA expression, but evidently increased its protein expression in A549 and HCT116 cells. Post-transcriptional regulation, such as mRNA stability alternations and protein stability modifications, serves an important role in protein expression (20,32,33). Therefore, HDAC inhibitors may influence the post-transcriptional regulation processes that regulate ABCC2 mRNA and protein expression.

According to the findings of the current study, it can be speculated that the underlying mechanisms responsible for the differential effects of HDAC inhibitors on ABC transporters may be associated with differences in site-specific acetylation/methylation of histones. For instance, acetylation and mono-methylation of histone 3 at Lys-9 activates gene transcription, whereas di- and tri-methylation of the same residue results in gene suppression (34-36). Several HDAC family members are aberrantly expressed in various cancer types and may have potential as target molecules for anticancer treatments (37). Valdez *et al* (31) also reported that the HDAC inhibitors SAHA, TSA and phenylbutyrate downregulated the expression levels of HDAC3, HDAC4 and HDAC6 in T-cell lymphoma and T-cell prolymphocytic leukemia. In the present study, it was observed that SAHA and TSA treatment decreased HDAC3 and HDAC4 expression levels, but had no significant effect on HDAC1 or HDAC2 expression. Furthermore, an earlier study indicated that the HDAC3 and HDAC4 complex stimulated the transcriptional activity of mineralocorticoid receptor (MR), and HDAC4 served an important role as a scaffold between MR and HDAC3 (38). Acetylation occurs, in part, due to decreased HDAC3 and HDAC4 protein expression, while methylation is most likely to occur due to the functional interaction between histone methyltransferases and deacetylases (39). Nevertheless, it remains unclear which histone modifications contribute to the differential effects of HDAC inhibitors on the expression of ABC transporters.

ABC transporters have different substrates; for instance, ABCC1 is known to pump GSH-conjugated DNA alkylators out of cells (4,40), while decreased ABCC1 activity may cause cellular accumulation of DNA alkylating agents and thus enhanced cytotoxicity. Cancer cells with high levels of ABCC1 are more resistant to DNA alkylating agents, such as busulfan and chlorambucil. It has been reported that busulfan exerts synergistic cytotoxicity when used in combination with HDAC inhibitors (41,42). However, the present study demonstrated that SAHA and TSA increased ABCB1 expression, which is known to pump its substrates, such as doxorubicin, vincristine and prednisone, out of cells (25,30). The increased

ABCB1 expression induced by HDAC inhibitors may lead to reduced anticancer activity of ABCB1 substrates. Therefore, the results of the present study indicate that it is important to select appropriate drugs in combination with HDAC inhibitors.

In conclusion, the present study demonstrated that HDAC inhibitors have different effects on the expression of ABC transporters in A549 and HCT116 cells. SAHA and TSA increased the expression levels of ABCB1, ABCC2, ABCC5, ABCC6, ABCC10, ABCC11 and ABCC12, whereas they downregulated ABCC1, ABCC3 and ABCC4. Furthermore, SAHA and TSA induced drug resistance in HCT116 cells, and decreased HDAC3 and HDAC4 expression levels. In future studies, drug resistance mediated by SAHA and TSA in A549 cells will be investigated. ABC transporters have different substrates (14). Differential effects of HDAC inhibitors on the expression of drug transporters support the necessity for caution in combining these drugs with other chemotherapeutic agents. The present study seems timely in light of an ongoing clinical trial (no. NCT01280526) testing the value of combining romidepsin, a HDAC inhibitor, with cyclophosphamide, doxorubicin, vincristine and prednisone (31). Particularly, efflux of doxorubicin, vincristine and prednisone may increase with HDAC inhibitor-mediated upregulation of ABCB1 (43). The present study highlighted the importance of understanding the mechanism of drug combination to achieve more efficient cytotoxicity to cancer cells. However, the molecular mechanisms of the different effects of ABC transporter expression induced by HDAC inhibitors remain unclear. It is necessary to investigate the underlying mechanisms in future studies.

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## Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

## Authors' contributions

HW contributed to the study design, data acquisition and analysis, and drafted the manuscript. BJW participated in the study design, data acquisition and revision of the manuscript. CHC, YZ, BS and RJ assisted in the performance of the statistical analysis. All authors have read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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