Pathol. Oncol. Res. (2012) 18:713–720
DOI 10.1007/s12253-012-9499-7

RESEARCH

SAHA Inhibits the Growth of Colon Tumors by Decreasing Histone Deacetylase and the Expression of Cyclin D1 and Survivin

Jong-Shiaw Jin · Tang-Yi Tsao · Pei-Chang Sun · Cheng-Ping Yu · Ching Tzao

Received: 6 September 2011 / Accepted: 4 January 2012 / Published online: 20 January 2012

Abstract We studied the effects of suberoylanilide hydroxamic acid (SAHA), a histone deacetylase (HDAC) inhibitor, on colon cancer. The expression of HDACs in colorectal cancer specimens and the effects of SAHA on colon cancer cells and tumors of nude mice were assessed. Treatment with SAHA (3 μm) for 72 h induced downregulation of different subtypes of HDAC proteins and also induced acetylation of histone 3 and histone 4. SAHA significantly inhibited the expression of the oncogenic protein c-myc and also increased the expression of the p53 and Rb proteins. The immunohistochemical staining of HDACs, including HDAC1, HDAC2, HDAC3, and HDAC4, was significantly increased in colorectal adenocarcinoma specimens compared to healthy control tissues. In addition, murine studies showed that 100 mg/kg SAHA administered by intraperitoneal injection significantly induced tumor necrosis and inhibited the growth of colon tumors. Immunohistochemistry of the tumor tissues from nude mice revealed that SAHA inhibited the expression of different subtypes of histone deacetylase, the anti-apoptotic proteins cyclin D1, survivin, and also inhibited cell proliferative as determined by Ki67 expression. SAHA inhibited the growth of colon tumors by decreasing histone deacetylases and the expression of cyclin D1 and survivin in nude mice.

Keywords Histone deacetylase · Histone deacetylase inhibitor · Nude mice · Colon cancer

J.-S. Jin · T.-Y. Tsao
Department of Pathology, Tungs' Taichung MetroHarbor Hospital, Wuqi Township,
Taichung County, Taiwan, Republic of China

P.-C. Sun · C.-P. Yu
Department of Pathology, Tri-Service General Hospital, National Defense Medical Center,
Taipei County, Taiwan, Republic of China

C. Tzao (✉)
Division of Thoracic Surgery, Department of Surgery,
Tri-Service General Hospital, National Defense Medical Center,
No. 325, Sec. 2, Cheng-Kung Road, Neihu,
114, Taipei County, Taiwan, Republic of China
e-mail: doctor.jsjin@gmail.com

Introduction

The principle treatment for patients with colon adenocarcinoma is the surgical removal of the involved segment of colon tissue with free surgical margins [1]. In patients with metastatic lesions or high stage disease, surgery is followed by chemotherapy to ablate any remaining cancer cells [2]. Although the prognosis of patients with colon cancer has recently improved with advanced therapies, some cases remain refractory to these advanced approaches. Thus, development of new targeted therapies is necessary for the successful treatment of these patients.

Histone deacetylases (HDACs) and histone acetyltransferase are responsible for the covalent modification of histone proteins and consequential changes in chromatin architecture and gene expression in different cancers [3, 4]. Recent development of HDAC inhibitors (HDACi) represent a new approach for the treatment of cancers [5, 6]. One HDACi, suberoylanilide hydroxamic acid (SAHA), has promising anticancer activity. It functions via covalent modification of histone proteins, specifically inhibiting HDACs. We and other groups have recently demonstrated that SAHA can induce apoptosis and sub-G1 arrest in colon and other cancers [7, 8]. However, whether or not treatment with SAHA in nude mice is effective at inhibiting the growth of colon cancer is currently unknown.

In this current study, we analyzed the *in vitro* and *in vivo* effects of SAHA on colon cancer. SAHA efficiently inhibited

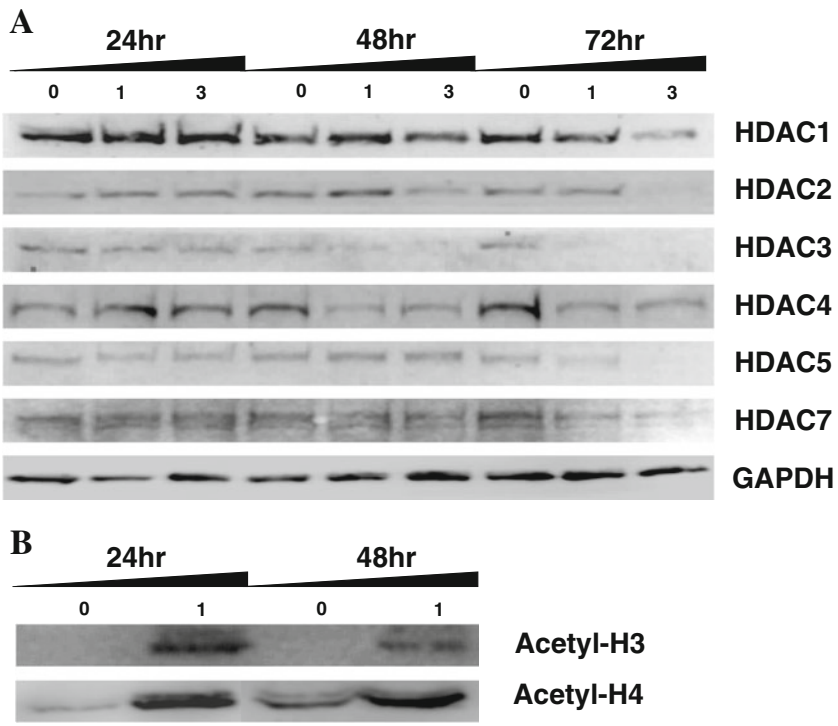

Fig. 1 SAHA alters the protein levels of HDACs, acetyl-histone 3, and acetyl-histone 4 in colon cancer cells. Western blot analysis of cells treated with or without SAHA (1 or 3 μM) for 24, 48, and 72 h

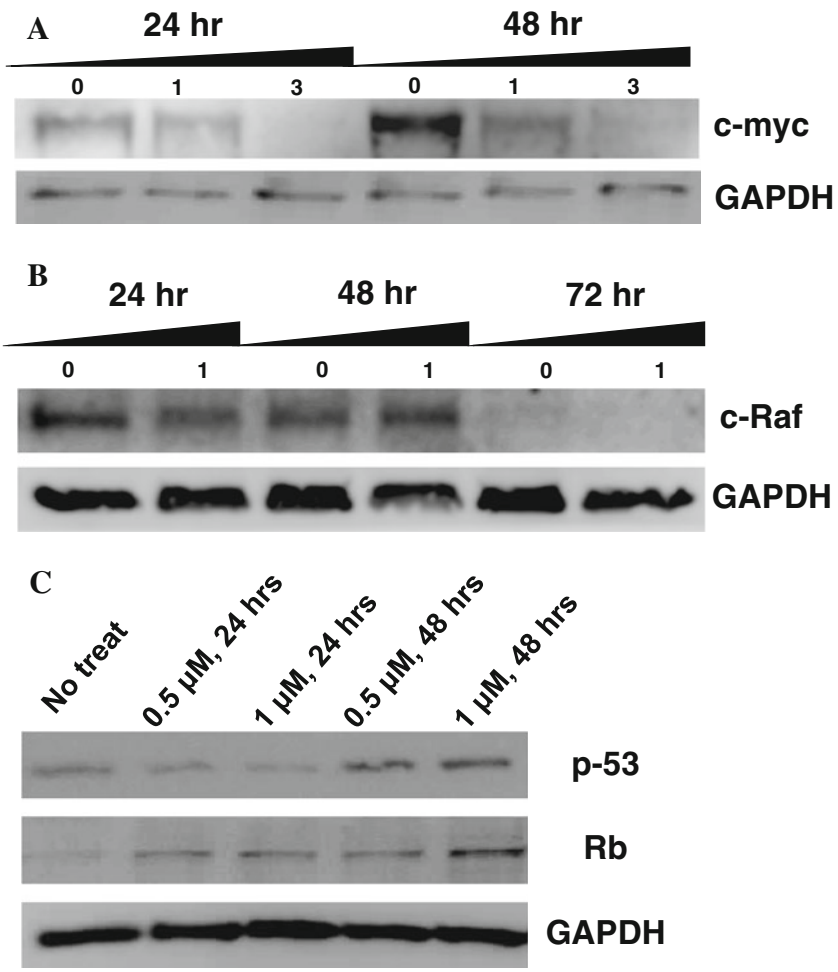

Fig. 2 SAHA alters the protein levels of c-myc **a**, c-Raf **b**, p53, and Rb **c** in colon cancer cells. Western blot analysis of cells treated with or without SAHA (0.5, 1, or 3 μM for 24, 48, and 72 h)

tumor growth in nude mice and induced tumor necrosis, indicating that SAHA might represent a promising adjuvant therapeutic agent for the treatment of colon cancer.

Materials and Methods

Cell Line

The human colon adenocarcinoma cell line Colo 320HSR (BCRC) was cultured in 90% RPMI 1640 medium supplemented with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate, and 10% heat-inactivated fetal bovine serum. Cell monolayers were routinely grown to maximum density of 70–80% of confluence at 37°C in 5% CO_2 prior to analysis. This study was approved by the Internal Review Board of Tri-Service General Hospital (No. 097-05-147).

Western Blot Analysis

The following antibodies and dilutions were used: HDAC antibody sample kit (1:100; Cell Signaling Technology, Beverly, MA, USA), rabbit anti-acetyl histone H3 (Lys 18; 1:1000; Cell Signaling Technology, Beverly, MA, USA), rabbit anti-acetyl histone H4 (Lys 12; 1:1000; Cell Signaling Technology, Beverly, MA, USA), rabbit anti-c–myc (Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-c–raf (Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-p53 (Upstate Biotechnology, Charlottesville, VA, USA), and mouse anti-Rb (Upstate Biotechnology, Charlottesville, VA, USA). Goat anti-rabbit (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) HRP-coupled secondary antibodies at a final concentration of 1 μg/mL was also used. Specific protein bands were visualized using enhanced chemiluminescence assays (Millipore, Billerica, MA, US). All Western blots were also immunoblotted with antibodies against GAPDH to demonstrate equal loading of protein samples.

Tumor Xenograft Studies

Female congenital athymic BALB/c nude (nu/nu) mice were purchased from Charles River Laboratories (Boston, MA, USA). Cultured colon cancer cells (1 x 10^7) were injected subcutaneous into the flank region of the mice. Animals were divided into two groups: One group received intraperitoneal administration of SAHA (100 mg/kg) at seven doses per week for 5 weeks. The other group served as the vehicle control. The SAHA was diluted in DMSO. Tumor volume was determined every day by measurement of the length (L) and width (W) of the tumor mass. The tumor volume at day n (TVn) was calculated as TV (mm^3)=(LxW^2)/2 as previously published [7]. Xenograft tumors of the treated and vehicle control mice were harvested at the end of the experiment, fixed in 4% formalin, and then embedded in paraffin.

Immunohistochemistry Analysis of Tumors in Nude Mice

Xenograft tumors of the treated and vehicle control mice were harvested at the end of the experimental time period. Tumors were embedded in paraffin, and tissue microarray analysis was conducted according to our previous reports [9, 10]. The tissue microarray slides were cut in 4-μm sections

Table 1 HDAC immunostaining scores in colorectal adenocarcinomas

Differentiation status	Tumor		
	Intensity	% staining	Total score
HDAC1			
Well differentiated CA	2.2±0.4	93±6	204±22*
Moderatelyl differentiated CA	2.8±0.5	96±3	196±16*
Poorly differentiated CA	2.9±0.3	95±5	275±18*
Healthy tissue	1.2±0.4	63±8	76±6
HDAC2			
Well differentiated CA	1.6±0.4	45±8	72±18*
Moderatelyl differentiated CA	2.1±0.5	80±6	168±13*
Poorly differentiated CA	2.8±0.2	95±6	266±18*
Healthy tissues	1.4±0.5	74±11	103±15
HDAC3			
Well differentiated CA	2.6±0.5	92±11	239±21*
Moderatelyl differentiated CA	2.7±0.4	91±9	245±16*
Poorly differentiated CA	2.8±0.4	97±8	271±18*
Healthy tissue	2.5±0.4	60±8	150±19
HDAC4			
Well differentiated CA	2.7±0.6	96±7	259±22*
Moderatelyl differentiated CA	2.4±0.5	91±9	218±21*
Poorly differentiated CA	2.7±0.5	89±7	240±19*
Healthy tissue	0.6±0.2	11±6	70±8
HDAC5			
Well differentiated CA	0.4±0.4	5±1	2±2
Moderatelyl differentiated CA	0.5±0.2	7±3	4±3
Poorly differentiated CA	0.4±0.2	19±4	8±4
Healthy tissue	0.3±0.3	3±2	7±1
HDAC7			
Well differentiated CA	0.5±0.3	19±4	15±6
Moderatelyl differentiated CA	1.2±0.4	21±3	25±8
Poorly differentiated CA	0.6±0.2	60±5	36±12
Healthy tissue	0.7±0.5	57±5	36±11

Data are presented as the mean±standard error of the mean (SEM) of immunostaining scores for the different subtypes of HDACs in colorectal adenocarcinomas (CA). * Indicates a significant difference in HDAC scores between tumor and healthy colorectal epithelia ($P<0.05$)

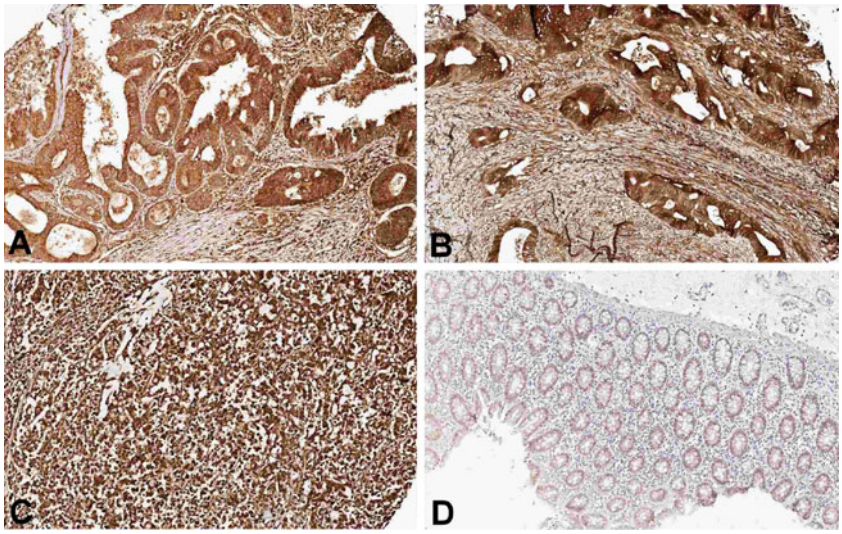

Fig. 3 Representative immunohistochemical analysis of HDAC1 in well differentiated **a**, moderately differentiated **b**, and poorly differentiated **c** colorectal adenocarcinomas and non-neoplastic colon tissue **d**. Original magnification, × 400

for histologic study using hematoxylin and eosin (H&E) staining and immunostaining. The slides were incubated with the primary antibody for 1 h and then rinsed. Staining was carried out using a streptavidin-biotin labeling kit (DakoCytomation, Glostrup, Denmark). The primary antibodies used were as follows: mouse monoclonal anti-cyclin D1 (1:100; Oncogene Research Products, Cambridge, MA, USA), anti-survivin (1:100; DakoCytomation, Glostrup, Denmark), anti-Ki–67 (1:50; DakoCytomation, Glostrup, Denmark), and anti-p53 (1:100; DakoCytomation, Glostrup, Denmark). Positively stained cells were counted in 3–4 random high-power fields (×400). The HDAC antibody sample kit (including HDAC1, HDAC2, HDAC3, HDAC4, HDAC5, HDAC7 were also used in this study (1:100; Cell Signaling Technology, Beverly, MA, USA). For evaluation of the immunohistochemical scores of different subtypes of HDAC, the intensity of nuclear immunostaining was scored on a scale of 0 (no staining) to 3 (strongest intensity), and the percentage of cells with staining of the nucleus was estimated at each intensity. As in our previous study (4, 5), the percentage of cells (from 0 to 100) was multiplied by the corresponding immunostaining intensity (from 0 to 3) to obtain an immunostaining score ranging from 0 to 300.

Immunohistochemistry Analysis of Colorectal Adenocarcinoma Specimens

Paraffin-embedded tumor tissues were obtained from the Department of Pathology, Tri-Service General Hospital, and tissue microarray slides were constructed. We selected

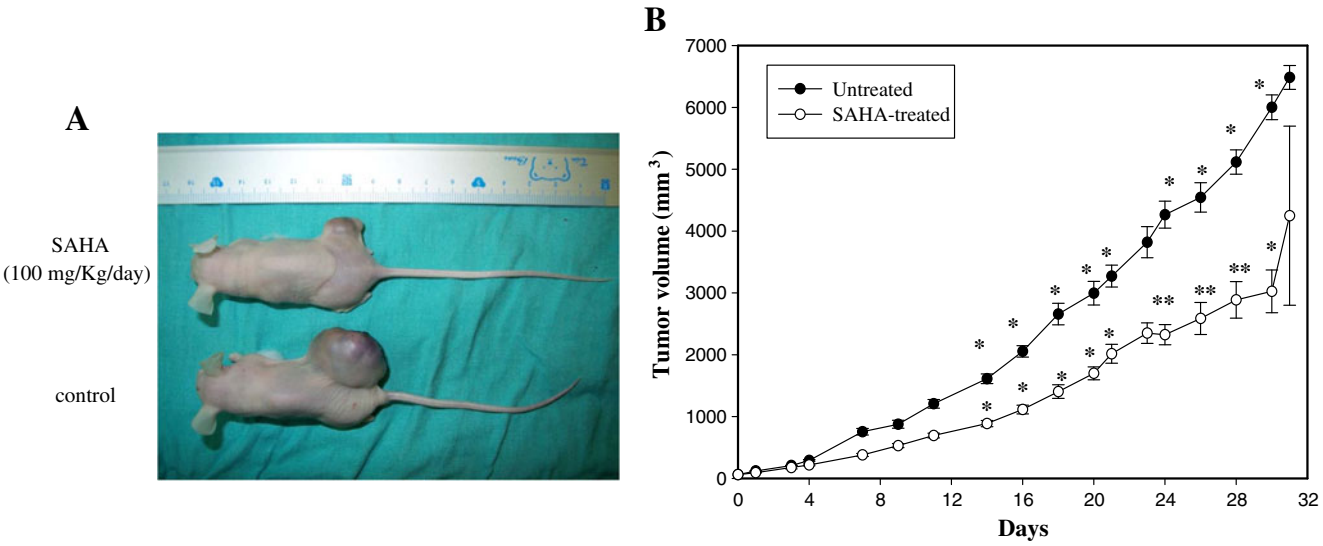

Fig. 4 Effect of SAHA on xenograft colon tumor growth in nude mice. Mice carrying colon tumors were intraperitoneally administered SAHA (100 mg/kg) for 5 weeks. **a** Representative mice from control (n=7) and SAHA-treated (n=7) groups. **b** Tumor volume curve of the colon tumors with or without SAHA treatment. * Indicates a significant difference, P<0.05 versus control

blocks of 91 primary colorectal adenocarcinomas, including 32 that were well differentiated (glandular structure >95%), 33 moderately differentiated (glandular structure between 50–95%), and 26 poorly differentiated tumors (glandular structure <50%).

One core was taken from a selected area of each paraffin-embedded tumor tissue, and a tissue microarray was constructed. Normal colon tissues were obtained from nine specimens, with the tissues taken 4 cm from the neoplasm. Tissue microarray sections were dewaxed in xylene, rehydrated in alcohol, and immersed in 3% hydrogen peroxide for 5 min to suppress endogenous peroxidase activity. Antigen retrieval was performed by heating each section at 100°C for 30 min in 0.01 mol/L sodium citrate buffer (pH 6.0). After three rinses (each for 5 min in phosphate buffered saline [PBS]), the sections were incubated for 1 h at room temperature according to the protocol included with the HDAC antibody sample kit (1:100; Cell Signaling Technology, Beverly, MA, USA). After three washes (each for 5 min in PBS), sections were incubated with biotin-labeled secondary immunoglobulin (1:100; DAKO, Glostrup, Denmark) for 1 h at room temperature. After three additional washes, peroxidase activity was developed with AEC+substrate chromogen (DAKO, Glostrup, Denmark) at room temperature. The immunohistochemical scores for different subtypes of HDAC were calculated as previously described.

Results

Effect of SAHA on Protein Expression

The effects of SAHA treatment (0, 1, or 3 μM) on the expression of HDAC proteins are showed in Fig. 1a. Treatment of colon cancer cells with SAHA (3 μM) for 72 h significantly downregulated the protein levels of HDAC1, 2, 3, 4, 5 and 7. In addition, treatment with SAHA (1 μM) for 24 and 48 h significantly increased the protein levels of acetyl-histone 3 and acetyl-histone 4 (Fig. 1b).

The expression of proteins related to tumor oncogenes is displayed in Fig. 2. Treatment of colon cancer cells with SAHA (3 μM) for 24 and 48 h significantly reduced the expression of the c-myc protein; expression of the c-Raf protein was not significantly altered in SAHA treated cells. In addition, increased p53 and Rb protein expression was observed after a 48 h exposure to 1 μM SAHA.

HDAC Expression in Colorectal Adenocarcinomas

The immunostaining results for HDACs in colorectal adenocarcinoma specimens are presented in Table 1, and representative images for HDAC1 are showed in Fig. 3. The non-neoplastic colonic glands (Table 1) demonstrated low expression of HDAC1, HDAC2, HDAC3, and HDAC4. In all 91

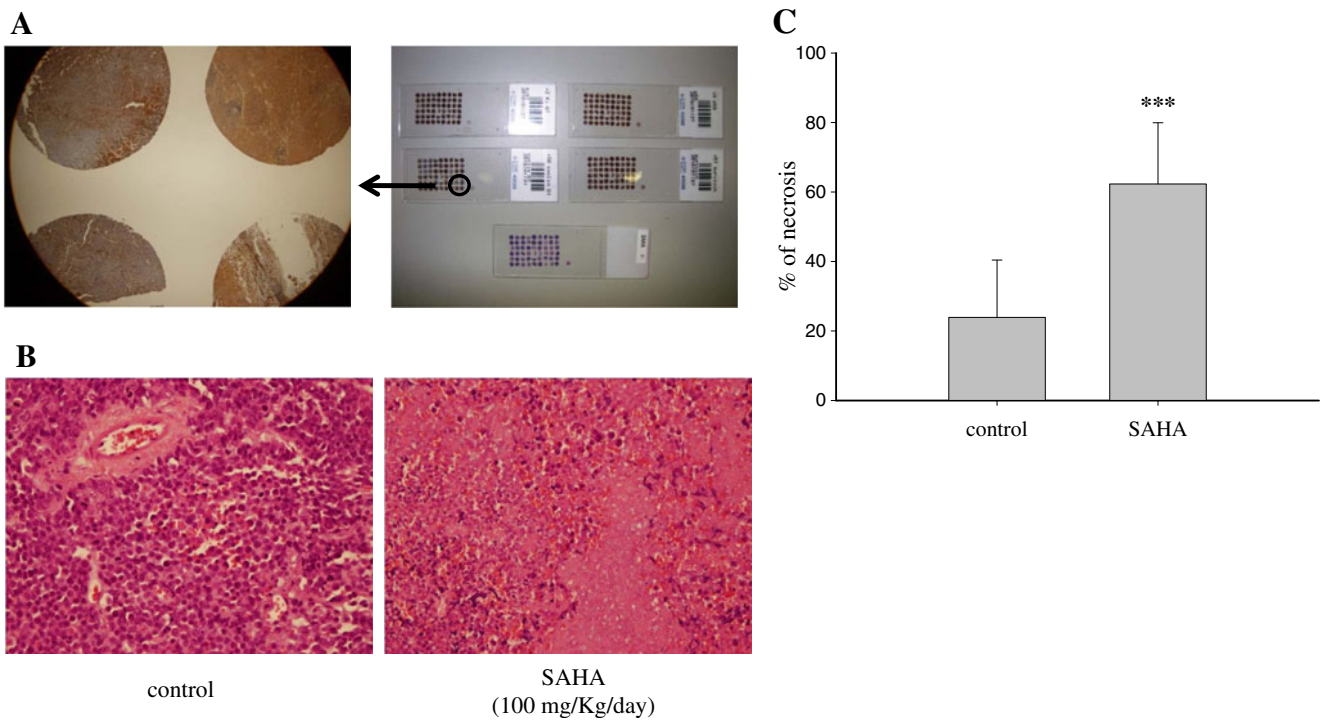

Fig. 5 Necrotic effect of SAHA on xenograft colon tumor growth in nude mice. **a** Representative H&E staining of tissue microarray slides, with each representative core measuring 2 mm in diameter. **b** Tumor necrotic effects in control and SAHA-treated mice (original magnification, ×400). **c** Percentage of cells undergoing tumor necrosis at the end of the experimental time period. *** Indicates a significant difference, $P<0.001$ versus control

colorectal adenocarcinoma specimens, immunoreactivity for HDACs was present in the nucleus and also occasionally observed in the cytoplasm; however, only the nuclear staining was analyzed. The scores for HDAC1 in well-differentiated (Table 1, 204±22), moderately differentiated (196±16), and poorly differentiated (275±18) colorectal adenocarcinomas were significantly higher than those in healthy colon tissues (76±6). In addition, the immunohistochemistry scores for HDAC2, HDAC3, and HDAC4 were also significantly increased compared to those of healthy colon tissues. The expression of HDAC5 and HDAC7 was low in both the colorectal adenocarcinoma and healthy colon tissues.

SAHA Inhibits Colon Cancer Cell Growth in Nude Mice

We tested the ability of SAHA to kill human colon cancer cells growing in nude mice. The flank region of immunodeficient mice was injected with colon cancer cells and subjected to intraperitoneal injection of SAHA at 100 mg/kg, 7 days/week for 5 weeks. Figure 4a shows two representative mice from each group at the end of the experimental period. Control mice had obviously larger tumors, whereas the mice from the SAHA-treated group possessed much smaller tumors. The tumor volume was calculated using the formula described in Materials and Methods. Figure 4b shows the mean increase in tumor volume for each group. SAHA treatment significantly decreased the tumor volume in the nude mice.

Tumor Necrosis and Immunohistochemistry Analysis of Tumors from Nude Mice

The tumors from nude mice were fixed in formalin and embedded in paraffin. Figure 5a shows a representative tissue microarray slide. The representative H&E stained tumor slide (Fig. 5b) shows that SAHA treatment significantly increased tumor necrosis in nude mice compared to the tumor slide from control mice (63%±16 for the SAHA group and 23±15 for the control group; Fig. 5c).

To characterize the effects of SAHA on cell-cycle proteins and cell proliferation, tissue microarray slides were immunostained for cyclin D1, survivin (an anti-apoptosis marker), Ki-67 (a proliferation marker), and p53 (Fig. 6a). In untreated cells, the proportion of positively staining cells was 66% for cyclin D1, 63% for survivin, 82% for Ki-67, and 38% for p53. For the tumors from SAHA treated mice, the proportion of positively staining cells was 26% for cyclin D1, 30% for survivin, 26% for Ki-67, and 73% for p53. A significant decrease in cells positive for cyclin D1, survivin, and Ki-67 was noted in SAHA-treated tumors. In contrast, the number of cells positive for the p53 protein was significantly increased in SAHA treated tumors.

We further characterized the effects of SAHA on immunohistochemical scores of different subtypes of histone deacetylase in tumors from nude mice (Fig. 6b). In untreated tumor cells, the immunohistochemical scores were 210±22 for HDAC1, 146±21 for HDAC2, 96±24 for HDAC3, 154±32 for HDAC4, 78±18 for HDAC5, and 114±32 for HDAC7. For the tumors from SAHA treated mice, the immunohistochemical scores were 180±36 for HDAC1, 88±16 for HDAC2, 45±11 for HDAC3, 113±21 for HDAC4, 45±13 for HDAC5, and 108±27 for HDAC7. A significant decrease in immunohistochemical scores for HDAC2, HDAC3, and HDAC5 was noted in SAHA-treated tumors (Fig. 6b).

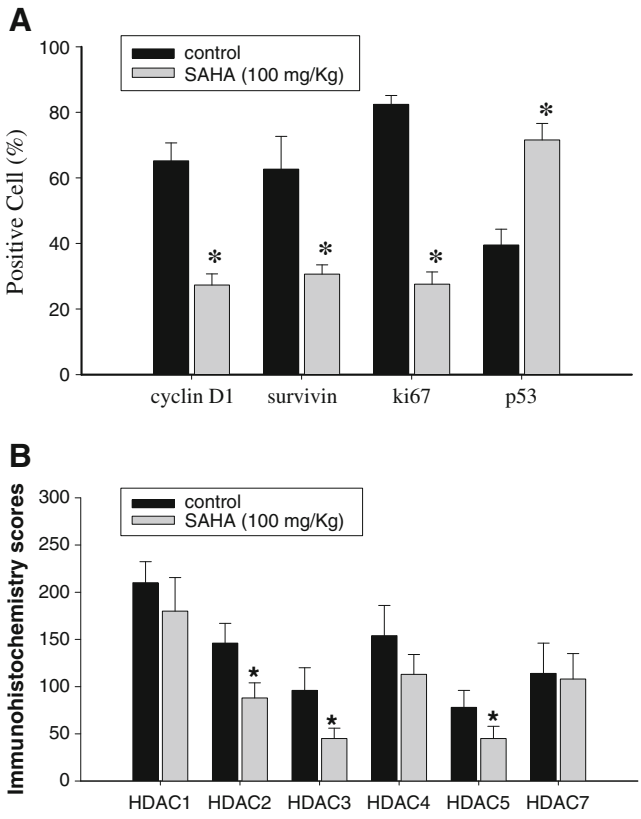

Fig. 6 Immunohistochemical analysis of cyclin D1, survivin, Ki-67, and p53 expression in xenograft colon tumors of nude mice **a**. **b**, immunohistochemical scores of HDAC1, 2, 3, 4, 5 and 7 in xenograft colon tumors of nude mice. * Indicates a significant difference, $P<0.05$ versus control

Taken together, these results indicated that SAHA inhibited the growth of colon tumor cells in nude mice via inhibiting the expression of HDAC and induction of tumor necrosis. Furthermore, SAHA inhibited tumor growth by decreased cell proliferation and expression of cyclin D1 and survivin in nude mice.

Discussion

In this study, we demonstrated that the different subtypes of the HDAC proteins were expressed in colon cancer cell lines and in colorectal adenocarcinomas specimens. SAHA also

had a profound antigrowth activity on colon tumor cells in nude mice via inhibition of the expression of the anti-apoptosis proteins survivin and cyclin D1.

Previous studies have shown that HDAC inhibitors inhibit deacetylation of histones H3 and H4 and induce tumor apoptosis in various types of cancers [6, 11, 12]. Our results demonstrated that SAHA increased the levels of acetyl-histone H3 and acetyl-histone H4. The anticancer effects of SAHA are mediated through covalent modification of histone proteins, specifically inhibition of HDACs. Our results are consistent with a recent study reporting that SAHA induces the accumulation of acetylated histones by direct interaction with HDACs [13].

Currently, four classes of HDACs comprising more than 18 isoenzmes have been identified in humans [14]. Most of the recent studies were focused on class I isoforms HDAC1, HDAC2, and HDAC3 which are the target of SAHA [2]. A previous study has showed that the class I HDACs are highly expressed in colorectal adenocarcinoma with positivity for HDAC1 in 36.4%, HDAC2 in 57.9%, and HDAC3 in 72.9% of cases [2]. It is unclear whether SAHA has effects on the expression of class I HDACs and other HDACs. Our results showed that the different subtypes of HDAC proteins were expressed in colon adenocarcinoma cells and also in colorectal adenocarcinoma specimens. Furthermore, SAHA significantly inhibited the expression of HDAC proteins in colon adenocarcinoma cells (HDACs 1, 2, 3, 4, 5 and 7) and also in tumors (HDACs 2, 3, 5) of nude mice. These results suggested that SAHA may be effective for the treatment of patients with colorectal adenocarcinoma.

The effect of SAHA on the expression different HDACs could be explained by different subtypes of HDAC may possess minor difference in catalytic site [15]. As suggesting by our resuls, SAHA is a broad set and non-selective inhibitor of HDACs. Further understanding of structural information on the molecular architecture of human HDACs could have a significant impact on the design of subtype selective inhibitors.

Multiple mechanisms have been proposed to describe the effects of SAHA in different cancers [1]. Previous studies have indicated that SAHA downregulates certain anti-apoptosis proteins, such as Bcl-2 and Bcl-xL [1], and upregulates pro-apoptotic protein expression [16, 17]. In this current study, immunohistochemistry analysis of tumors from nude mice revealed a decreased number of cells positive for cyclin D1, survivin, and Ki67 in the SAHA-treated group, suggesting an apoptotic function for SAHA.

Our previously published results demonstrated a 65% inhibition of colon cancer cell growth following treatment with 5 μM SAHA [8]. Other clinical studies have shown that these levels can be achieved in individuals receiving this drug [18]. Studies in humans have also found that SAHA induces only minor side effects [19], suggesting that use of SAHA as an adjuvant targeted therapy may be beneficial.

The antiproliferative effects of SAHA have been studied in a thyroid cancer cell line [5], human lymphoma cells [19], breast cancer [20], and non–small cell lung carcinoma [21] as well as endometrial and ovarian cancer cells [22, 23]. These studies indicate that the inhibitory activity of SAHA on cancer cell growth spans many tissue types, suggesting it could be a useful agent for the treatment of a wide variety of malignancies.

Previous studies have demonstrated that the survivin protein regulates cell-cycle progression in mitosis as a passenger protein and blocks apoptotic pathways [13]. SAHA-induced mitotic defects can be mediated by modulation of survivin [24, 25]. In the present study, downregulation of survivin protein expression in the tumors of nude mice following SAHA treatment likely contributed to the tumor necrotic effect of SAHA.

Our results showed that SAHA treatment increased the expression of the p53 protein in colon cancer cell lines and tumors in nude mice. p53 is a tumor suppressor protein that is encoded by the *TP53* gene [26–28]. p53 is crucial for the regulation of the cell cycle and functions as a tumor suppressor in many cancers [26–28]. The tumor necrotic effect of SAHA in nude mice may be mediated in part by increased expression of the p53 protein.

We demonstrated that SAHA inhibited the growth of colon adenocarcinoma cells via induction of tumor necrosis, and inhibition of HDAC, cyclin D1 and survivin expression. Thus, SAHA should be considered as a potential adjuvant therapy for the treatment of colon adenocarcinomas.

Acknowledgments This study was supported by a grant from the Tri-Service General Hospital (TSGH-C100-007-009-10-S04), R. O. C.

Declaration of competing interests The authors declare that they have no competing interests.

References

1. Mayer RJ (2009) Targeted therapy for advanced colorectal cancer-more is not always better. N Eng J Med 360:623–625
2. Wilko W, Annika R, Silvia N, Aurelia N, Ann-Christin B, Manfred D, Volker G, Boehm M, Thomas B, Carsten D (2008) Class I histone deacetylase expression has independent prognostic impact in human colorectal cancer: specific role of class I histone deacetylases in vitro and in vivo. Clin Cancer Res 14:1669–1677
3. Ito K, Adcock IM (2002) Histone acetylation and histone deacetylation. Mol Biotechnol 20:99–106
4. Kuo MH, Allis CD (1998) Roles of histone acetyltransferases and deacetylases in gene regulation. Bioessays 20:615–626
5. Kim DH, Kim M, Kwon HJ (2003) Histone deacetylase in carcinogenesis and its inhibitors as anti-cancer agents. J Biochem Mol Biol 36:110–119

6. Marks PA, Richon VM, Breslow R, Rifkind RA (2001) Histone deacetylase inhibitors as new cancer drugs. Curr Opin Oncol 13:477–483
7. Luong QT, O'Kelly J, Braunstein GD, Hershman JM, Koeffler HP (2006) Antitumor activity of suberoylanilide hydroxamic acid against thyroid cancer cell lines in vitro and in vivo. Clin Cancer Res 12:5570–5577
8. Sun PC, Tzao C, Chen BH, Liu CW, Yu CP, Jin JS (2010) Suberoylanilide hydroxamic acid induces apoptosis and sub-G1 arrest of 320 HSR colon cancer cells. J Biochem Sci 17:76–85
9. Jin JS, Hsieh DS, Loh SH, Chen A, Yao CW, Yen CY (2006) Increasing expression of serine protease matriptase in ovary tumors: tissue microarray analysis of immunostaining score with clinicopathological parameters. Mod Pathol 19:447–452
10. Jin JS, Wu WY, Lin YF (2006) Higher expression of epidermal growth factor receptor is associated with extracellular matrix metalloprotease inducer in colorectal adenocarcinoma: tissue microarray analysis of immunostaining score with clinicopathological parameters. Dis Markers 22:309–316
11. Jung M (2001) Inhibitors of histone deacetylase as new anticancer agents. Curr Med Chem 8:1505–1511
12. Lindemann RK, Gabrielli B, Johnstone RW (2004) Histone-deacetylase inhibitors for the treatment of cancer. Cell Cycle 3:779–788
13. Truchet I, Jozan S, Baron S (2008) Estrogen and antiestrogen-dependent regulation of breast cancer cell proliferation in multicellular spheroids: Influence of cell microenvironment. Int J Oncol 32:1033–1039
14. Minucci S, Pelicci PG (2006) Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. Nat Rev Cancer 6:38–51
15. Alessandro V, Cinzia V, Gessica F, Elena CC, Mirko B, Debora R, Prasun C, Chantal P, Raffaele DF, Paola G, Christian S, Stefania DM (2004) Crystal structure of a eukaryotic zinc-dependent histone deacetylase, human HDAC8, complexed with a hydroxamic acid inhibitor. PNAS 101:15064–15069
16. Wang S, Yan-Neale Y, Cai R, Alimov I, Cohen D (2006) Activation of mitochondrial pathway is crucial for tumor selective induction of apoptosis by LAQ824. Cell Cycle 5:1662–1668
17. Zbar AP, Kennedy PJ, Singh V (2009) Functional outcome following restorative rectal cancer surgery. Acta Chir Iugosl 56:9–16
18. Kelly WK, O'Connor OA, Krug LM (2005) Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. J Clin Oncol 23:3923–3931
19. Sakajiri S, Kumagai T, Kawamata N, Saitoh T, Said JW, Koeffler HP (2005) Histone deacetylase inhibitors profoundly decrease proliferation of human lymphoid cancer cell lines. Exp Hematol 33:53–61
20. Bali P, Pranpat M, Swaby R (2005) Activity of suberoylanilide hydroxamic acid against human breast cancer cells with amplification of Her-2. Clin Cancer Res 11:6382–6389
21. Rundall BK, Denlinger CE, Jones DR (2005) Suberoylanilide hydroxamic acid combined with gemcitabine enhances apoptosis in non-small cell lung cancer. Surgery 138:360–367
22. Takai N, Desmond JC, Kumagai T (2004) Histone deacetylase inhibitors have a profound antigrowth activity in endometrial cancer cells. Clin Cancer Res 10:1141–1149
23. Takai N, Kawamata N, Gui D, Said JW, Miyakawa I, Koeffler HP (2004) Human ovarian carcinoma cells: histone deacetylase inhibitors exhibit antiproliferative activity and potently induce apoptosis. Cancer 101:2760–2770
24. Altieri DC (2006) The case for survivin as a regulator of microtubule dynamics and cell-death decisions. Curr Opin Cell Biol 18:609–615
25. Noh EJ, Lim DS, Jeong G, Lee JS (2009) An HDAC inhibitor, trichostatin A, induces a delay at G2/M transition, slippage of spindle checkpoint, and cell death in a transcription-dependent manner. Biochem Biophys Res Commun 378:326–331
26. Kim DH, Kundu JK, Surh YJ (2011) Redox modulation of p53: mechanisms and functional significance. Mol Carcinog 50:222–234
27. Shen F, Kirmani KZ, Xiao Z, Thirlby BH, Hickey RJ, Malkas LH (2011) Nuclear protein isoforms: implications for cancer diagnosis and therapy. J Cell Biochem 112:756–760
28. Smith RA, Tang J, Tudur-Smith C, Neoptolemos JP, Ghaneh P (2011) Meta-analysis of immunohistochemical prognostic markers in resected pancreatic cancer. Br J Cancer 104:1440–1451