Suberoylanilide Hydroxamic Acid Enhances Gap Junctional Intercellular Communication via Acetylation of Histone Containing Connexin 43 Gene Locus

Takahiko Ogawa,1 Tomonori Hayashi,1 Masahide Tokunou,1 Kei Nakachi,1 James E. Trosko,2 Chia-Cheng Chang,3 and Noriaki Yorioka4

1Department of Radiobiology and Molecular Epidemiology, Radiation Effects Research Foundation; 2Department of Molecular and Internal Medicine, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan; and 3National Food Safety Toxicology Center, Department of Pediatrics/Human Development, Michigan State University, East Lansing, Michigan

Abstract
A histone deacetylase (HDAC) inhibitor, suberoylanilide hydroxamic acid (SAHA), induces apoptosis in neoplastic cells, but its effect on gap junctional intercellular communication in relation to apoptosis was unclear. Therefore, we carried out a comparative study of the effects of two HDAC inhibitors, SAHA and trichostatin-A, on gap junctional intercellular communication in nonmalignant human peritoneal mesothelial cells (HPMC) and tumorigenic ras oncogene–transformed rat liver epithelial cells (WB-ras) that showed a significantly lower level of gap junctional intercellular communication than did HPMC. Gap junctional intercellular communication was assessed by recovery rate of fluorescence recovery after photobleaching. Treatment of HPMC with SAHA at nanomolar concentrations caused a dose-dependent increase of recovery rate without inducing apoptosis. This effect was accompanied by enhanced connexin 43 (Cx43) mRNA and protein expression and increased presence of Cx43 protein on cell membrane. Trichostatin-A induced apoptosis in HPMC but was less potent than SAHA in enhancing the recovery rate. In contrast, treatment of WB-ras cells with SAHA or trichostatin-A induced apoptosis at low concentrations, in spite of smaller increases in recovery rate, Cx43 mRNA, and protein than in HPMC. Chromatin immunoprecipitation analysis revealed that SAHA enhanced acetylated histones H3 and H4 in the chromatin fragments associated with Cx43 gene in HPMC. These results indicate that SAHA at low concentrations selectively up-regulates Cx43 expression in normal human cells without induction of apoptosis, as a result of histone acetylation in selective chromatin fragments, in contrast to the apoptotic effect observed in tumorigenic WB-ras cells. These results support a cancer therapeutic and preventive role for specific HDAC inhibitors. (Cancer Res 2005; 65(21): 9771-8)

Introduction
Histone deacetylase (HDAC) inhibitors have been suggested as potential cancer therapeutic agents because of their different effect on apoptosis in normal and cancer cells (1, 2). The prototype of hydroxamic acid–based hybrid polar molecules, suberoylanilide hydroxamic acid (SAHA), belongs to the second generation of this class of potential therapeutic cancer drugs. It displays a greater potency on a molar basis, as an inducer of differentiation and, therefore, is expected to be a safer analogue of trichostatin-A (3, 4). SAHA functions as a HDAC inhibitor, with ID50 values close to its optimal differentiation-inducing concentration (5). Acetylation of core nucleosomal histones is, in part, regulated by opposing activities of histone acetyltransferases and HDACs (6, 7); the increased acetylation of histones is associated with genes that are transcriptionally activated (8, 9). Hyperacetylation induced by HDAC inhibitors, such as SAHA, seems to be highly selective and changed the expression of only 2% to 5% of all genes (10). SAHA induces differentiation and/or apoptosis in certain transformed cells through the increased expression of selected genes involved in the cell cycle regulation, tumor suppression, differentiation, and apoptosis (5, 6). It has been reported that increased gene expression of the cell cycle kinase inhibitor p21WAF1 might account for the antitumor property of SAHA (6, 11), but the precise mechanism remains to be elucidated.

Asklund et al. (12) recently reported that 4-phenylbutyrate, an HDAC inhibitor, enhances gap junctional intercellular communication through increased levels of connexin 43 (Cx43) in malignant glioma cells, although precisely how this HDAC inhibitor up-regulates Cx43 has not been delineated. We previously reported that hexamethylen bisacetamide (H MBA), a hybrid polar molecule, enhanced gap junctional intercellular communication in human peritoneal mesothelial cells (HPMC), which are nontumorigenic primary cultured cells. This effect, induced by millimolar concentrations, was accompanied by an increased expression of both mRNA and phosphorylated isoforms of Cx43 (13, 14). Side effects, such as myelotoxicity, have been reported for HMBA (15).

Gap junction channels transport small molecules (<2,000 Da) important in growth regulation signaling between neighboring cells (16, 17). Gap junctional intercellular communication is involved in cell growth, differentiation, and apoptosis; aberrant control of gap junctional intercellular communication might also play an important role in cancer development (18–21). Several oncogene products have been shown to reduce gap junction channel permeability and connexin expression in vitro and in vivo (22, 23). It is anticipated that SAHA will work as an enhancer of gap junctional intercellular communication in both normal and cancer cells. It is then important to elucidate (a) whether SAHA enhances Cx43 expression and gap junctional intercellular communication in normal human cells and neoplastically transformed cells, with specific target molecules at lower concentrations than with trichostatin-A; (b) whether apoptosis is induced also in...
normal cells or only in neoplastic cells; and (c) if so, what the specific mechanisms are. Therefore, this study assesses the effects of SAHA, an HDAC inhibitor, on Cx43 expression and apoptosis in normal and neoplastically transformed cells, from the view of cancer prevention and cancer chemotherapy, along with the underlying molecular mechanisms.

**Materials and Methods**

Cells. HPMC was harvested from the omental tissues of three consenting patients who had undergone elective abdominal surgery. As described previously, the cells were isolated and cultured in M199 medium, supplemented with 1-glutamine, 10% FCS (Intergen, Co., Purchase, NY), penicillin, and streptomycin. All experiments were done using the initial primary culture or the third-passage cells. A cell line previously derived from WB-F344 rat liver epithelial cells was also used in this study (24). WB-ras cells are a neoplastically transformed line originating from infection of WB-F344 rat liver epithelial cells with retrovirus (raszip6) containing viral Ha-ras and the neomycin-resistant gene (25). WB-ras cells were cultured in MEM medium, supplemented with 1-glutamine, sodium pyruvate, essential amino acid, nonessential amino acid, MEM-vitamin solution, 7% FCS (Intergen), penicillin, and streptomycin.

**Drugs and chemicals.** SAHA was kindly provided by Aton Pharma, Inc. (Tarrytown, NY). Trichostatin A, DMSO, and bovine serum albumin (BSA) were purchased from Sigma Chemical Co. (St. Louis, MO). Trichostatin A and SAHA were prepared in a 100 mmol/L stock solution in DMSO and stored at −20°C.

**Experimental design.** Cells were seeded at a density of 1.0 × 10^4/cm^2 in growth medium. After confluence was reached, SAHA or trichostatin A was added and the culture was continued, whereas DMSO was used as solvent control. The incubation times and the concentrations of SAHA or trichostatin A used were based on the results of earlier studies (5–7, 26–28). Cells in the present study were incubated for 48 hours at 37°C with 50, 200, 800, and 2,000 mmol/L SAHA or trichostatin-A. These cells were then used for cell proliferation and apoptosis assays. Measurements of gap junctional intercellular communication and assessment of Cx43 protein and acetylated histones H3 and H4 were done by Western blotting and immunocytochemistry, along with mRNA quantitative analyses and chromatin immunoprecipitation assays. The p21WAF1 protein levels were also assessed.

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represent the average expression of target genes expression relative to GAPDH ± SD from three independent cultures.

**Immunoblotting.** Cells were grown to confluence in 6 cm dishes and were cultured with SAHA or DMSO. At the end of the given treatment period, the monolayers were rinsed thrice with ice-cold PBS and disposed of according to the extraction method. Nuclear extracts: Trypsinized cells were washed in PBS and resuspended in cell lysis buffer of Nuclear/Cytosol Fractionation Kit (BioVision, Inc., Mountain View, CA) and cells were then treated according to the protocol of the manufacturer. Whole cell samples: Lysates were prepared with ice-cold lysis buffer containing 20 mmol/L TBS (pH 7.5); 1% Triton X-100; 150 mmol/L NaCl; and 1 mmol/L each of EDTA, EGTA, β-glycerophosphate, Na3VO4, and phenylmethylsulfonyl fluoride, 2.5 mmol/L sodium PPi, and 1 μg/mL leupeptin. The lysates were then sonicated. The samples were diluted 1:4 in water, and their protein concentrations were determined using detergent-compatible protein assay (Bio-Rad Corp., Richmond, CA). Samples (30 μg for Cx43, 15 μg for histones and p21WAF1) of protein were then dissolved in Laemmli sample buffer, separated on 12.5% (for Cx43) and 15% polyacrylamide gels (for acetylated histones H3/H4 and p21WAF1), and transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad). As an internal control to determine whether equal amounts of protein had been loaded onto the gel, the PVDF membranes were stripped and reprobed with anti-α-tubulin (T5168, Sigma) mouse monoclonal antibody (p21WAF1, and Cx43). After being washed with distilled water, the membranes were scanned with a flatbed scanner, and total band density as amount of loaded protein was analyzed by NIH Image. The Cx43, acetylated histones H3/H4, or p21WAF1 contents of the various samples were determined by incubating them with anti-Cx43 monoclonal antibody (Chemicon International, Inc., Temecula, CA), antiacetylated histone H3 or H4 antibody (diluted 1:1,000; Upstate Biotechnology, Lake Placid, NY), and anti-p21WAF1 protein monoclonal antibody (F-5; Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Next, a horseradish peroxidase–conjugated secondary antibody (diluted 1:2,000; Amersham Co., Arlington Heights, IL) and an enhanced chemiluminescence detection reagent (Renaissance Western blot chemiluminescence reagent; NEN Life Science Products, Inc., Boston, MA) were added. The average control value was assigned an arbitrary value of 1 unit, and relative band intensities were standardized to this arbitrary unit. Exposed films were scanned using a flatbed scanner, and band density was quantified by NIH Image.

**Indirect immunofluorescence and confocal microscopy.** HPMC and WB-ras cells were cultured as described previously. The cells were plated on a Lab-Tek Chamber Slide (Nalge Nunc Int., Naperville, IL) before culture with SAHA or DMSO. The cells were then washed twice in PBS and fixed in 95% methanol/5% acetic acid for 1 minute at room temperature before being washed and permeabilized thrice with 0.1% Triton X-100–PBS (PBST), and then incubated in 5% BSA for 60 minutes. After this, slides were incubated overnight at 4 °C in anti-Cx43 monoclonal antibody (Chemicon) at a 1:400 dilution, and antiacetylated histone H3 antibody (diluted 1:1,000; Upstate Biotechnology, Lake Placid, NY), and with indicated concentrations of SAHA (2,000 nmol/L) for 0, 2, or 24 hours. Chromatin immunoprecipitation assay was done according to the protocol of the manufacturer (32). DNA extracted from both Zeiss LSM 510 laser-scanning confocal microscope (Carl Zeiss International, Jena, Germany).

**Chromatin immunoprecipitation assay.** HPMC was plated at a density of 2 × 105 cells/cm2 dish and incubated overnight at 37°C with 5% CO2. The next day, cells were cultured with SAHA (2,000 nmol/L) for 0, 2, or 24 hours. Chromatin immunoprecipitation assay was done according to the protocol of the manufacturer (32). DNA extracted from both

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**Figure 1.** SAHA-induced growth suppression occurred only at high concentrations, and SAHA induced neither morphologic changes nor apoptosis even with accumulation of acetylated histones in HPMC. A1, SAHA time course and dose response in HPMC. Viable cell number was analyzed by WST-1 assay. Measurement at each time point was done in quadruplet. Points, mean; bars, SD. A2, phase-contrast micrographs. HPMC and WB-ras cells were cultured on Lab-Tek chamber slides with (b and d) or without (a and c) SAHA (2,000 nmol/L) for 48 hours. Bar, 100 μm. B, Western blot analysis of acetylated histones H3 and H4 in HPMC. Histones were isolated by nuclear extraction as described in Materials and Methods from the cells cultured for indicated hours and with indicated concentrations of SAHA (B1). Acetylation was detected by using antiacetylated H3 and H4 antibodies. Lane C, untreated HPMC or WB ras cells (control).
immunoprecipitation steps was purified by phenol/chloroform extraction and ethanol precipitation, and analyzed by real-time RT-PCR. The same Cx43 primers and probe for the real-time RT-PCR analysis were used to carry out real-time PCR of Cx43 DNA with samples obtained from chromatin immunoprecipitation experiments. The amplification and detection procedures were identical to the real-time RT-PCR analysis.

**Statistical analysis.** Data were analyzed using StatView II software (Apple Computer, Inc., Cupertino, CA). The two-tailed unpaired t test was used in comparing SAHA-treated cultures with control cultures; differences were considered significant at $P < 0.05$. Results are expressed as the mean ± SD.

**Results**

Suberoylanilide hydroxamic acid inhibits human peritoneal mesothelial cell growth without inducing morphologic changes. Figure 1A1 shows the results of the WST-1 assay of viable cell numbers. In preliminary experiments, WST-1 staining of HPMC (i.e., absorbance at 450 nm) was found to increase linearly with the number of viable cells from $1 \times 10^3$ to $1 \times 10^5$ per well in a 96-well plate (data not shown). SAHA, at 800 and 2,000 nmol/L, seemed to suppress cell proliferation of HPMC when compared with the cells incubated in the untreated control, but this was not associated with any loss of cell viability as determined by trypan blue exclusion (data not shown). Phase-contrast micrographs of control confluent HPMC revealed uniform monolayers of polygonal cells that clearly exhibited contact inhibition (Fig. 1A2, a); SAHA (2,000 nmol/L) did not change morphology of the HPMC even after 48 hours (Fig. 1A2, b). On the other hand, WB-ras cells revealed spindle-shaped morphologies and loss of contact inhibition (Fig. 1A2, c), and some of these cells became rounded with detached from the dishes (Fig. 1A2, d).

Suberoylanilide hydroxamic acid time and dose dependently accelerates acetylation of histones H3 and H4. We next determined the level of histone acetylation at each time point after culture with SAHA. Samples were collected from nuclear fractions of the cells cultured with SAHA (2,000 nmol/L) for 2, 4, 9, 24, and 48 hours, or with various concentrations (50, 200, 800, and 2,000 nmol/L) of SAHA for 48 hours. Western blot analysis showed that levels of acetylated histones H3 and H4 in untreated HPMC were low, and that accumulation of both acetylated histones...
occurred at 2 hours after SAHA addition. This accumulation continued for 48 hours (Fig. 1B1). Incubation for 48 hours with SAHA resulted in accumulation of acetylated histones, which reached a peak at 800 nmol/L and was sustained at 2,000 nmol/L (Fig. 1B1). In contrast, histone H3 was weakly acetylated in untreated WB-ras cells. Treatment of WB-ras cells with SAHA resulted in accumulated acetylated histones H3 and H4, which reached a peak at 2,000 nmol/L (Fig. 1B2).

Suberoylanilide hydroxamic acid induces apoptosis in WB-ras cells, but not in human peritoneal mesothelial cells. Analyses of the cell cycle and apoptosis, using propidium iodide and Annexin V/propidium iodide, respectively, were done at 24 and 48 hours after culturing confluent cells with SAHA or trichostatin-A. SAHA exerted minimal effects on cell cycle progression in HPMC. Treatment for 24 hours with SAHA reduced the S-phase fraction (8.4% control versus 2.6% SAHA 2,000 nmol/L) but did not significantly alter the G0-G1 and G2-M populations, whereas no subdiploid (apoptotic) population was detected. Treatment of HPMC with 50 to 2,000 nmol/L SAHA did not cause apoptotic cell death or reduce the living cell population in contrast to trichostatin-A, which dose-dependently induced apoptosis at >200 nmol/L. Both SAHA and trichostatin-A induced apoptotic cell death in WB-ras cells, although trichostatin-A showed greater potency (Fig. 2A).

Suberoylanilide hydroxamic acid induces transient expression of p21WAF1. The effect of SAHA on p21WAF1 protein levels was examined by Western blot analysis. HPMC was cultured with and without SAHA for 2, 4, 9, 24, and 48 hours. After culturing with SAHA, p21WAF1 protein levels slightly increased at 2 hours, reached a peak at 9 hours, and decreased to control level after 48 hours treatment (Fig. 2B).

Suberoylanilide hydroxamic acid enhances gap junctional intercellular communication in human peritoneal mesothelial cell and WB-ras cells. Figure 3A shows typical digitized images obtained by the fluorescence recovery after photobleaching assay. After photobleaching, sequential scans detected the recovery of fluorescence in the bleached cells: The dye was transferred to photobleached cells through gap junctional intercellular communication from surrounding nonbleached cells. Recovery of fluorescence after photobleaching was much more rapid in HPMC cultured with SAHA (Fig. 3A, SAHA) than in untreated cells (Fig. 3A, control). SAHA was more efficient than trichostatin-A in enhancing the recovery rate in HPMC in a concentration-dependent manner. In WB-ras cells, both SAHA and trichostatin-A treatments also increased the recovery rate. Although their recovery levels were much lower, their enhancing rates relative to the control were no less than those in HPMC (Fig. 3B).
Fig. 4A). Densitometric analysis of the results for HPMC showed that SAHA, at all concentrations, induced a significant dose-dependent increase in \( P_1 + P_2 \) (active form of Cx43) and \( P_0 + P_1 + P_2 \) (total Cx43), compared with control cells; the increase of \( P_0 \) with increased SAHA was less significant, compared with that of \( P_1 + P_2 \). As a result, \( \frac{P_1 + P_2}{P_0} \) was increased by the treatment. In untreated WB-ras cells, \( P_0 \) was predominant, and the active forms \( (P_1 + P_2) \) were minor. SAHA also induced an increase in active and total Cx43 protein in WB-ras cells, showing a peak at 800 nmol/L SAHA, although the ratio of \( P_1 + P_2 \) compared with that in HPMC (Fig. 4B).

The localization of Cx43 protein and acetylated histone H3 was then examined by indirect immunofluorescence cytochemistry. Figure 4C shows immunostaining of the cells for Cx43 (red) and acetylated histone H3 (green) after 48-hour incubation with or without SAHA in HPMC (a-e) or WB-ras cells (f-j). The negative control, in which mouse or rabbit IgG was substituted for the primary antibodies, showed no staining (data not shown). Control cells showed that few bright red spots (indicating Cx43 labeling) were dominant in cytoplasm rather than at the areas of intercellular contact. Incubation of HPMC with SAHA caused an increase in the number and size of the labeled regions, resulting in the cells displaying linear or dotted labeling along the membrane between cells, in contrast to control cells where a few positive spots were observed in cytoplasm. Although WB-ras cells showed altered immunostaining patterns in the same fashion as HPMC after treatment with SAHA, immunostaining was weaker for Cx43, displaying fewer spots in a nonlinear pattern along the membrane. The fluorescent levels of acetylated histone H3 seemed more prominent and concentrated in the nuclei in SAHA-treated HPMC and WB-ras cells compared with those in untreated cells.

Suberoylanilide hydroxamic acid induces a higher level of Cx43 messenger RNA expression in human peritoneal mesothelial cell than in WB-ras cells. Cx43 mRNA levels, measured by real-time RT-PCR, also increased in both HPMC and WB-ras cells cultured with SAHA in a time-dependent manner. Cx43 mRNA in HPMC increased 3-fold over that of control cells after 48 hours culture with SAHA (Fig. 5A). Subsequently, we analyzed the Cx43 mRNA levels, at various SAHA concentrations in HPMC as well as in WB-ras cells. Cx43 mRNA levels in HPMC and WB-ras cells after 48 hours treatment with various concentrations of SAHA revealed a dose-dependent increase of Cx43 mRNA, which is much more remarkable in HPMC (3-fold increase at 2,000 nmol/L) than in WB-ras cells (1.3-fold increase at 2,000 nmol/L; Fig. 5B).

Suberoylanilide hydroxamic acid increases acetylated histones in chromatin fragments associated with Cx43 gene. Chromatin immunoprecipitation analysis was used to study the mechanism of SAHA-induced expression of Cx43. Chromatin fragments from HPMC cultured with SAHA for 2 and 24 hours were immunoprecipitated with antibodies against acetylated histones H3 and H4. DNA from the immunoprecipitates was isolated, and real-time PCR, using Cx43 primers, was done (Fig. 6A). The amounts of Cx43 gene in acetylated histones H3 and H4 increased remarkably with increasing hours of culture with SAHA (Fig. 6B). This observation confirms that histone acetylation is involved in the transcriptional regulation of Cx43 expression, and that Cx43 gene is a selective target for SAHA.

![Figure 5](image5.png)  
**Figure 5.** Real-time RT-PCR analysis of Cx43 mRNA expression in HPMC and WB-ras cells. A, HPMC was cultured with 2,000 nmol/L SAHA for the indicated hours. B, HPMC and WB-ras cells were cultured with the indicated concentrations of SAHA for 48 hours. The value from untreated control of HPMC was taken as a unit to determine fold increase after culturing with SAHA. Cx43 mRNA levels were normalized by GAPDH mRNA, whose levels did not change during culture with SAHA (data not shown). Results are means of at least three experiments; *P* values show significance levels compared with control (C). Columns, fold increase of Cx43 mRNA in HPMC (white) or WB-ras cells (black).

![Figure 6](image6.png)  
**Figure 6.** SAHA-induced accumulation of acetylated histones H3 and H4 in the chromatin fragments associated with Cx43 gene. Soluble chromatin from HPMC cultured with 2,000 nmol/L SAHA for 2 or 24 hours was immunoprecipitated with the antibodies against acetylated histone H3/H4. PCR primers used for Cx43 mRNA were also used to amplify the DNA isolated from immunoprecipitated chromatin as described in Materials and Methods. A, the diagrams of real-time PCR analysis of the Cx43 gene indicate that HPMC cultured with SAHA for 2 or 24 hours showed higher amplification levels when compared with untreated control cells. B, the relative amounts of DNA contained in acetylated histones were quantified by real-time PCR analysis. The value from an untreated control (C) was taken as a unit to determine fold increase.
Discussion

In this study, we showed that low concentrations (50-2,000 nmol/L) of SAHA enhanced gap junctional intercellular communication in normal HPMC via Cx43 gene–associated histone acetylation without incurring apoptosis, whereas the same SAHA treatment of tumorigenic WB-ras cells induced apoptosis along with an increase of gap junctional intercellular communication. When we used WB-vector control cells (WB-neo), 50-2,000 nmol/L SAHA induced no apoptosis in these cells as it was the case in HPMC (data not shown), implying little difference in response to SAHA between rat and human nonmalignant cells. We also showed that SAHA-induced Cx43 gene expression could be ascribed to histone H3/H4 acetylation. Our findings are the first demonstration of the efficacy of SAHA as an inhibitor of HDAC in nonmalignant cells to induce the transcriptional activation of the Cx43 gene through histone acetylation. Furthermore, we showed that SAHA induced apoptosis in ras-transformed cells at a low concentration despite a smaller increase in Cx43 mRNA and protein than in the case of HPMC.

Micromolar concentrations of SAHA have been shown to induce growth arrest and/or apoptosis in various transformed cells; the precise mechanism involved has been discussed with regard to variable transformed cells but not well defined (27, 35, 36). There seem to be two types of tumor cells based on their inability to have functional gap junctional intercellular communication: those whose connexin genes are not transcribed (37, 38) and those whose transcribed connexins have been rendered dysfunctional by a number of mechanisms, including posttranslational modification of connexin proteins by various activated oncogenes (18). Thus, one might expect to find differences in response to SAHA and trichostatin-A between normal cells that express connexins and tumor cells that express either very low levels of connexins or dysfunctional connexins. In confluent HPMC, nanomolar concentrations of SAHA generated a dose-dependent increase of Cx43 mRNA and protein, whereas SAHA induced neither cell cycling arrest nor apoptosis. On the other hand, trichostatin-A induced apoptosis at a concentration (200 nmol/L) that did not increase recovery rate. Because we observed that histone H3 and H4 acetylation was also pronounced in cells treated with nanomolar SAHA, it seems unlikely that this difference mirrors the potency of the two HDAC inhibitors.

The cell cycle checkpoint gene p21WAF1 was most commonly induced in various transformed cells cultured with SAHA (39) through histone acetylation (6, 40). In HPMC, p21WAF1 level was elevated soon after the addition of SAHA and reverted to control level in 48 hours: This time course profile did not parallel the change in Cx43 mRNA levels. Sodium butyrate, an HDAC inhibitor, has been shown to induce G1 arrest and pRb dephosphorylation in 3T3 cells lacking p21WAF1 (41). Richon et al. (6) found that the level of p21WAF1 mRNA decreased within 24 hours after the addition of SAHA similar to our observation in HPMC. In contrast, chromatin immunoprecipitation analysis showed that Cx43 gene-associated histone acetylation increased with increasing hours of culture with SAHA, similar to SAHA-induced expression of Cx43 mRNA, indicating a more probable cause-effect between the two.

Differential response between HPMC and WB-ras cells was noted for SAHA-induced apoptosis, although histones H3/H4 acetylation was observed in both cells treated with nanomolar SAHA. Previous reports have shown that mitochondria played a central role during HDAC inhibitor–mediated apoptotic response (42–45). The cellular pathways via mitochondria and other apoptotic genes, targeted by SAHA, might differ between normal and malignant cells. Our results indicate that SAHA might suppress cancer cell growth through up-regulation of gap junctional intercellular communication, but does not cause damage in surrounding normal cells.

The role of SAHA in enhancing gap junctional intercellular communication in nonmalignant cells without serious adverse effects could be a beneficial for cancer prevention. Zhang et al. (46) recently reported that Cx43 displayed gap junction–independent growth inhibition of various tumor cells. Another connexin gene (i.e., Cx26) has been previously shown to be a tumor suppressor gene (47). Therefore, up-regulation of Cx43 or other connexin genes could suppress tumor growth or progression by gap junction–dependent mechanism. Gap junctional intercellular communication is essential for maintaining homeostatic balance and normal differentiation through the modulation of cell growth and arrest. It will also be important to elucidate the role of histone acetylation and related proteins in the transcriptional regulation of Cx43 and other connexin genes in selective tissues or cells. Future study will likely provide some answers to these questions.

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