Induction of Polyploidy by Histone Deacetylase Inhibitor: A Pathway for Antitumor Effects

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Abstract

Histone deacetylase (HDAC) inhibitors can induce various transformed cells to undergo growth arrest and/or death. Suberoylanilide hydroxamic acid (SAHA) is an HDAC inhibitor which is in phase I/II clinical trials and has shown antitumor activity in hematologic and solid tumors at doses well tolerated by patients. HDAC is the target for SAHA, but the mechanisms of the consequent induced death of transformed cells are not completely understood. In this study, we report that SAHA induced polyploidy in human colon cancer cell line HCT116 and human breast cancer cell lines, MCF-7, MDA-MB-231, and MBA-MD-468, but not in normal human embryonic fibroblast SW-38 and normal mouse embryonic fibroblasts. The polyploid cells lost the capacity for proliferation and committed to senescence. The induction of polyploidy was more marked in HCT116 p21WAF1−/− or HCT116 p53−/− cells than in wild-type HCT116. The development of senescence of SAHA-induced polyploidy cells was similar in all colon cell lines. The present findings indicate that the HDAC inhibitor could exert antitumor effects by inducing polyploidy, and this effect is more marked in transformed cells with nonfunctioning p21WAF1 or p33 genes. (Cancer Res 2005; 65(17): 7832-9)

Introduction

Histone deacetylase (HDAC) inhibitors, a promising class of targeted anticancer agents, can block the proliferation and induce cell death in a wide variety of transformed cells (1). Suberoylanilide hydroxamic acid (SAHA) is an HDAC inhibitor which is in phase I/II clinical trials and has shown antitumor activity in hematologic and solid tumors at doses well tolerated by patients (2, 3). The antitumor mechanism of HDAC inhibitors has not been completely understood.

HDAC inhibitors selectively affect gene transcription by acetylation of histones and components of transcription factor complexes (4). HDAC inhibitors block the activity of class I and II HDACs (1), leading to accumulation of acetylated histones, relaxation of chromatin structure, which, in general, enhances the accessibility of transcription machinery to DNA (5). The acetylation of transcription factors alters their activities and can alter gene transcription (6, 7). Contrary to what might be anticipated by the widespread distribution of HDACs in chromatin, inhibition of HDAC activity by SAHA or trichostatin A results in an alteration in transcription of a limited number of expressed genes (~2-10%) in various transformed cell lines (8-11). Among these genes, p21WAF1 (to be referred to as p21) is frequently increased in expression (5, 12). p21 mediates growth arrest in the G1 phase of the cell cycle by inhibiting cyclin-dependent kinase complexes that regulate cell cycle progression (13). p21 can protect cells against apoptosis (14, 15). Induction of proapoptotic proteins such as BAX, PIG3, and NOXA via p53 acetylation contributed to HDAC inhibitor induced cell death in cells with competent p53 (16, 17), but these agents can induce transformed cell death in p53 null cells. The mechanisms by which HDAC inhibitors result in cell death remain incompletely understood.

It has been shown that HDAC inhibitors can induce mitotic defects by causing aberrant acetylation of histones in heterochromatin and centromere domains perse (18-22). The newly replicated chromatin contains acetylated histones (23, 24). In culture with trichostatin A, in both yeast and mammalian cells, histones in the newly synthesized chromatin remain acetylated, and this disrupts the structure and function of the centromere (18-22). The mitotic defect induced by HDAC inhibitors can result in cell death by either apoptosis or mitotic death/catastrophe (25, 26).

Generation of reactive oxygen species may play a role in HDAC inhibitor–induced cell death (27-29). SAHA and MS-275 can induce transformed cells (27-29), but not normal cells (29), to accumulate reactive oxygen species, increase caspase activity and undergo cell death. Complete inhibition of the caspase activity did not inhibit SAHA-induced transformed cell death (28-32), but inhibition of reactive oxygen species by N-acetyl cysteine attenuated cell death (27, 28). The resistance of normal cell to SAHA-induced death is associated with high levels of thioredoxin, a potent scavenger of reactive oxygen species (29).

In the present study, we showed that SAHA can induce polyploidy and, in turn, cell senescence in transformed cells. We determined the effect of HDAC inhibitor on human colon cancer cell line HCT116, and p21 null (p21−/−) and p53 null (p53−/−) derivative cell lines. The HDAC inhibitor induced the accumulation of polyploid cells in which there was continued DNA synthesis with failure of cytokinesis. The large polyploid cells showed loss of clonogenicity and evidence of senescence. The effect was more marked in p21−/− cells than in the p53−/− cells and in both the degree of induced polyploidy was greater than in the wild-type cells. We also found that HDAC inhibitor induced an increased accumulation of polyploid cells in human breast cancer cell lines, MCF-7, MDA-MB-231, and MDA-MB-468, but not in normal human fibroblast SW-38 and mouse normal embryonic fibroblasts. The present findings indicate that SAHA, in addition to causing tumor cell growth arrest, apoptosis, mitotic cell death, or reactive oxygen species–induced cell death, may exert antitumor effect via pathway-inducing polyploidy, particularly in cells with nonfunctioning p21 or p53.

Materials and Methods

Cell lines. Human colon cancer cell lines HCT116 p21−/− and HCT116 p53−/− and the parental HCT116 cell line from which each was derived (33) were gifts from Dr. Bert Vogelstein (Johns Hopkins University, Baltimore, MD).
and were grown in McCoy's medium supplemented with 10% fetal bovine serum (HyClone Laboratory Inc., Logan, UT). Human breast cancer cell lines MCF-7, MDA-MB-231, MDA-MB-468, and human embryonic lung fibroblast cell line SW-38 were obtained from American Type Culture Collection (Rockville, MD), and cultured in DMEM with 10% fetal bovine serum. Three mouse embryonic fibroblast cell lines (339, 364, and a pooled cell line) were gifts from Dr. Andrew Koff (Memorial Sloan-Kettering Cancer Center, New York), and grown in DMEM with 10% fetal bovine serum. The effects of SAHA on cell growth and viability were determined as described previously (34). The expression of p21 and p53 proteins in HCT116 cell lines were determined with a Western blotting technique as described previously (12), using antibodies against p21 (Ab-11; NeoMars, Fremont, CA) and p53 (BP-53-12; Upstate, Lake Placid, NY). The antibody to α-tubulin (Ab-1) was used to evaluate loading of gels (EMB Biosciences Inc., San Diego, CA). The morphology of HCT116 cells cultured without or with SAHA was examined on slides prepared with ~5000 cells by cytospin and Giemsa staining.

Cell cycle analysis. Cells were seeded in 10 cm cell culture dishes at a density of 10^5 cells/dish. Cells were allowed to attach overnight, and then treated with 0.4 to 1 μmol/L (p21+/+ and p21−/− cells) or 0.8 to 4 μmol/L (p53+/+ and p53−/− cells) SAHA for 3 days. For human breast cancer cell lines, cells were cultured with 2.5 to 20 μmol/L for 5 days. For human and mouse embryonic fibroblast cell lines, cells were cultured with 2.5 to 20 μmol/L for 3 days. On each day, the medium, together with cells suspended in the medium, was removed and either combined with the attached cells or discarded. The attached cells were collected by trypsinization. The cells were centrifuged at 1,000 rpm for 5 minutes, washed with PBS, fixed with 2 volumes of ethanol, treated with 0.2 mg/ml RNase A and 20 μg/ml propidium iodide for 30 minutes at 37°C, and analyzed by fluorescence-activated cell sorter analysis. The data were analyzed with Flowjo software (Tree Star, Inc., Ashland, OR).

Bromodeoxyuridine labeling assay. Cells were cultured without or with 0.4 to 1 μmol/L (p21+/+ and p21−/− cells) or 0.8 to 4 μmol/L SAHA (p53+/+ and p53−/− cells) SAHA for 24, 48, and 72 hours. At each time point, bromodeoxyuridine (BrdU; Amersham Biosciences, Buckinghamshire, England) was added into the medium to a final concentration of 10 μmol/L and cells were cultured for an additional hour. Cells were harvested, washed with PBS, fixed with 2 volumes of ethanol, treated with 2 N HCl to denature DNA, incubated with FITC-labeled anti-BrdUrd antibody (Becton Dickinson Immunocytometry System, San Jose, CA) followed by propidium iodide staining according to the manufacturer’s protocol, and subjected to fluorescence-activated cell sorter analysis.

MPM-2 labeling assay. A similar procedure for BrdUrd labeling assay, except for HCl denaturation step, was adapted to simultaneously measure the DNA content and MPM-2-positive cells with a fluorescein-succinimidyl ester-conjugated MPM-2 antibody (Upstate), which recognizes phosphohistones on proteins in mitotic cells (35).

Assay for cell division using cell membrane label, PKH2. Assay of cell division was done using PKH2, a cell membrane label. HCT116 p21−/− cells (10^7) cultured without or with 0.8 μmol/L SAHA for 3 days were trypsinized, recovered as indicated above, and labeled with PKH2 green fluorescent cell linker kit (Sigma-Aldrich Chemical Co., St. Louis, MO) according to the manufacturer’s protocol. The PKH2 labeled cells were plated at 2 × 10^5 per 6 cm plate and PKH2 fluorescence signal was measured for each of the 5 consecutive days using fluorescence-activated cell sorter analysis with propidium iodide staining to exclude dead cells.

Clonogenicity analysis. HCT116 p21−/− cells were cultured without or with 0.8 μmol/L SAHA for 3 days, and then sorted according to size into small and large cells using MoFlo cell sorter (DakoCytomation Inc., Carpinteria, CA). The cells cultured without SAHA and the sorted small and large cells were plated in soft agar (2,000 cells/10 cm plate), and after 14 days, the colonies were stained with Giemsa and counted.

Senescence analysis. HCT116 cells were cultured without or with 0.8 μmol/L SAHA (p21+/+ and p21−/− cells) or 2 μmol/L SAHA (p53+/+ and p53−/− cells) for 5 days and then grown in fresh medium without inhibitor for up to 5 days. Cells were stained with a senescence β-galactosidase staining kit (Cell Signaling Technology, Inc., Beverly, MA), which detects senescence-associated β-galactosidase (SA-β-gal).

SAHA sensitivity assay. HCT116 p21−/− cells were treated with 0.8 μmol/L for 3 days and sorted as described above. The sorted small cells were grown in SAHA-free medium for 5 days to allow for recovery from SAHA treatment. The sensitivity to SAHA of the sorted small cells and the untreated control cells was determined as follows: 5 × 10^3 cells were seeded in triplicate in 24-well plate in 1 mL medium. After 24 hours, the cells were incubated with 0.125 to 4 μmol/L SAHA. At 48 hours, 0.1 μl Alamar blue (BioSource International Inc., Camarillo, CA) was added to the cell culture. The cells were incubated at 37°C for an additional 4 hours. The fluorescence signal was then measured with GeminiXS microplate reader (Molecular Devices, Sunnyvale, CA).

Statistical analysis. The significance of differences between experimental conditions was determined using Student’s t test for unpaired observations.

Results

SAHA inhibits cell growth and induces cell death in HCT116 cells. We first determined the effect of SAHA on the growth and viability of HCT116 p21+/+, HCT116 p21−/−, HCT116 p53+/+, and HCT116 p53−/− cells. SAHA ≥0.8 μmol/L inhibited cell growth of both the HCT116 p21+/+ and the p21 knock-out cells (Fig. 1A and B). HCT116 p21−/− cells were more sensitive to SAHA-induced cell death than HCT116 p21+/+ cells (Fig. 1E and F). For example, 1.0 μmol/L SAHA after 72 hours of culture induced 30.5 ± 2.1% cell death in p21+/+ cells compared with 55.3 ± 6.1% cell death in p21−/− cells (P < 0.05). For HCT116 p53+/+ and the p53−/− cells, SAHA ≥2.0 μmol/L inhibited their growth (Fig. 1C and D), whereas HCT116 p53−/− cells were less sensitive to SAHA-induced cell death than HCT116 p53+/+ cell (Fig. 1G and H). For example, 4 μmol/L SAHA after 72 hours of culture induced 69.2 ± 5.7% cell death in HCT116 p53+/+ cells, but caused 49.5 ± 3.3% cell death in p53−/− cells (P < 0.05). This was consistent with previous reports that wild-type p53 increased the sensitivity to HDAC inhibitor-induced cell death (16, 17).

HCT116 p21+/+ cells (parent cell line of p21−/− cells) are more sensitive to SAHA-induced cell death than HCT116 p53+/+ cells (parent cell line of p53−/− cells; Fig. 1A, C, E, G). The difference between the two parental cells HCT116 p21+/+ and HCT116 p53+/+ may reflect the genomic instability of HCT116 cell line, which is defective in DNA mismatch repair machinery (36). To minimize the effect of possible genetic drift between the parental and p21 or p53 knock-out cells, only early passages (<20) of cells were used in this study, and knock-out cell lines were compared with their own parental cell lines. We determined the expression of p21 and p53 in each cell cultured without or with SAHA. As expected, p21 and p53 proteins were expressed in the parental cells, but not in p21−/− and p53−/− cells, respectively (Fig 1I). SAHA dramatically induced expression of p21 in both parental cells and in the p53−/− cell line but not in p21−/− cells. Knockout of p53 reduced the basal level of p21 expression, but did not affect p21 induction by SAHA. This is consistent with the finding that SAHA can induce p21 expression in a p53-independent manner (37).

SAHA-induced polyploidy in HCT116 cells. We next determined the effects of SAHA on cell cycle progression of the HCT116 p21+/+ and p21−/− cells (Table 1). With increasing concentrations of SAHA from 0.4 to 1.0 μmol/L, there was an increasing proportion of cells with 4 N DNA content (4 N cells). With increasing SAHA concentration and time in culture, there was an increase in the percentage of polyploid cells (>4 N), which was more marked in p21−/− cells, >80% by day 3 at ≥0.8 μmol/L SAHA. The accumulation of 4 N cells decreased with time with the increase in proportion of polyploid cells.
We then determined the effect of SAHA on cell cycle progression of HCT116 p53+/+ and p53−/− cells (Table 1). SAHA induced a marked G1 arrest at low concentrations (0.8 and 1 μmol/L) in HCT116 p53+/+ cells with a slight accumulation of 4 N cells and polyploid cells, whereas it induced an accumulation of 4 N cells at higher concentrations (2 and 4 μmol/L) with increased polyploidy accumulation. SAHA induced an accumulation of 4 N cells in HCT116 p53−/− cells with accumulation of polyploid cells, but no G1 arrest, suggesting that p53 may be required for SAHA-induced G1 arrest. SAHA induced more polyploid cells in HCT116 p53−/− cells than in p53+/+ cells, but this difference was not as great as that between HCT116 p21−/− cells and p21+/+ cells. The proportion of 4 N cells in p53−/− cells decreased with time.

We determined if the relative increase in polyploid cells in culture with SAHA could result from a loss of diploid cells. p21+/+ and p21−/− cells cultured with 0.4 to 0.6 μmol/L SAHA or p53+/+ and p53−/− cells cultured with 0.8 to 1.0 μmol/L SAHA caused little cell death of the four cell lines (<10%), but induced several fold more polyploidy cells in the knock-out cells than in the wild-type (Table 1).

To address whether the accumulation of 4 N cells induced by SAHA represented a G2-M arrest, we analyzed the 4 N cells in cultures without or with SAHA (2 μmol/L for p53+/+ and p53−/− cells, 0.8 μmol/L for p21+/+ and p21−/− cells) using a mitosis-specific antibody, MPM-2, which recognizes a subset of mitotic phosphoproteins (35). SAHA induced a transient accumulation of MPM-2-positive mitotic cells in all four cell lines within 12 to 24 h, with 26% in p53+/+, 32% in p53−/−, 28% in p21+/+, and 21% in p21−/− cells being MPM-2-positive at 12 hours (Fig. 2). By 24 hours, the percentage of MPM-2-positive cells in SAHA-treated cells returned to the control level. Cells (4 N) accumulated at 24 hours in all four cell lines cultured with SAHA (Table 1). Taken together, the data suggests that after 24 hours in culture with SAHA, most 4 N cells are not in mitosis. Morphologic analysis showed that cells cultured with SAHA became polymorphic, and the polyploid cells contained giant nuclei and/or multiple varied size nuclei.
including micronuclei (Fig. 2C). These are features of abnormal mitosis which can be associated with failure of cytokinesis.

**Induction of polyploidy by SAHA is associated with DNA replication but without cell division.** To confirm that the polyploidy cells were induced by SAHA, we evaluated DNA replication with BrdUrd labeling in SAHA-treated HCT116 p21+/+, p21−/−, p53+/+, and p53−/− cells. A portion of cells in each of these cell lines cultured with SAHA continued to replicate DNA without cell division and became polyploid cells (Fig. 2H-K), whereas few cells cultured without SAHA became polyploid (Fig. 2D-G). This is indicated by the incorporation of BrdUrd into DNA of cells with >4 N DNA content.

SAHA induced polyploidy in other tumor cells, but not in normal cells. We next examined whether SAHA can induce polyploidy in other transformed cells. We analyzed the cell cycle profile of human breast cell lines MCF-7, MDA-MB-231, and MDA-MB-468 cultured without or with SAHA. SAHA induced polyploidy in each of these cell lines, although the effect in MCF-7 cells was small (0.5 μmol/L SAHA at 5 days, 6% versus 3% in control), greater in MDA-MB-231 (2.5 μmol/L SAHA at 5 days, 16% versus 1% in control) and MDA-MB-468 (2.5 μmol/L SAHA at 5 days, 10% versus 6% in control). Because MDA-MB-231 and MDA-MB-468 each contain a mutant p53 whereas MCF-7 has a wild-type p53, loss of p53 function may contribute to the difference in these cells. SAHA did not increase polyploidy in any of the four normal fibroblasts examined, including human embryonic lung fibroblast SW-38 and three mouse embryonic fibroblast cell lines (data not shown).

Polyploidy cells lose the capacity to proliferate. To evaluate whether polyploidy cells can divide, proliferation was assayed by labeling cells with PKH2, which is a fluorescent dye that binds to membranes (38). HCT116 p21−/− cells were cultured without or with 0.8 μmol/L SAHA for 3 days at which time medium was removed, cells labeled with PKH2 and cultures continued for 5 days in fresh medium without SAHA. As cells divide, the PKH2 distributes to daughter cells and the fluorescent signal decreases in the progeny cells. This was observed in cells in prior culture without SAHA (Fig. 3A). In cells in prior culture with SAHA, two peaks of fluorescent signal were apparent, one peak remained relatively unchanged in intensity indicating that cells were not dividing (Fig. 3B), and a second peak lost intensity of fluorescence with time, indicating that a portion of the cells in prior culture with SAHA resumed proliferation or cell division (Fig. 3B).

### Table 1. Percentage of the cells in different phases of cell cycle in HCT116 cells cultured without or with SAHA

| HCT116 p21+/+ | G1 | day 0 | day 1 | day 2 | day 3 | S | day 0 | day 1 | day 2 | day 3 | 4 N | day 0 | day 1 | day 2 | day 3 | >4 N | day 0 | day 1 | day 2 | day 3 |
|--------------|----|-------|-------|-------|-------|---|-------|-------|-------|-------|-----|-------|-------|-------|-------|-----|-------|-------|-------|
| Control      | 25 | 19    | 36    | 50    | 59    | 50 | 37    | 32    | 13    | 28    | 25 | 17    | 3     | 4     | 3     | 1    |
| 0.4 μmol/L SAHA | 30 | 32    | 39    | 27    | 23    | 26 | 33    | 33    | 24    | 10    | 12 | 11    | 11    | 11    | 11    | 11    |
| 0.6 μmol/L SAHA | 24 | 22    | 20    | 18    | 15    | 23 | 47    | 41    | 36    | 11    | 22 | 21    | 11    | 11    | 11    | 11    |
| 0.8 μmol/L SAHA | 14 | 14    | 11    | 11    | 11    | 9  | 60    | 43    | 45    | 15    | 32 | 35    | 15    | 32    | 35    | 35    |
| 1 μmol/L SAHA | 17 | 14    | 11    | 7     | 4     | 6  | 65    | 59    | 47    | 11    | 23 | 35    | 11    | 23    | 35    | 35    |

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*The values represent the average and SD from three independent experiments and the difference between the HCT116 p53+/+ and p53−/− cell lines is significant (P < 0.001).
smaller in size than the majority of the nondividing cells (Fig. 3C-E). These findings were consistent with the conclusion that SAHA induced large polyploid cells that lost the capacity to divide.

**SAHA-induced polyploid cells have low clonogenicity.** To evaluate the clonogenicity of polyploid cells, HCT116 p21−/− cells cultured with 0.8 μmol/L SAHA, and after 3 days of culture with the HDAC inhibitor, cells were collected, and sorted by size to populations of relatively small and large cells. Cells from culture without SAHA and the “small” and “large” cells sorted after culture with SAHA were plated in soft agar without SAHA and incubated for 14 days. The large cells sorted from culture with SAHA grew significantly fewer colonies than either the control or the small cell populations ($P < 0.05$; Fig. 4).

**SAHA-induced polyploid cells undergo senescence.** We then determined if the polyploid cells undergo senescence. HCT116 p21+/+ and p21−/− cells were cultured without or with 0.8 μmol/L cells for up to 5 days and then cultured without SAHA for another 5 days, and cells were stained for SA-β-gal, which is a marker for senescent cells (39). Cultures with SAHA contained many polyploid cells (Fig. 5B and D). On day 3 of culture with SAHA, few polyploid cells were SA-β-gal-positive (Fig. 5C), whereas ~60% polyploid cells were SA-β-gal-positive on day 5 (Fig. 5F), and virtually all of the polyploid cells became SA-β-gal-positive when cells were cultured with SAHA for 5 days and then cultured with SAHA-free medium for an additional 3 days (Fig. 5E). The senescent polyploid cells became larger with more nuclei compared with the polyploid

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**Figure 2.** HCT116 cells treated with SAHA replicated DNA without cell division and became polyploid cells with features of mitotic abnormalities and cytokinesis failures. A, SAHA induced a transient mitotic arrest. Percentage of MPM-2-positive cells in HCT116 cell lines cultured with or without SAHA at indicated concentrations for indicated time. B, morphology of HCT116 p21−/− cells (original amplification ×200). The size and shape of cells and nuclei were relatively monomorphic. C, HCT116 p21−/− cultured with 0.8 μmol/L SAHA for 3 days (original amplification ×200). The cells were polymorphic with many large cells, which contained giant and/or multiple varied size nuclei, including micronuclei. D-G, few HCT116 p21+/+ (D), p21−/− (E), p53+/+ (F), and p53−/− (G) cells cultured without SAHA spontaneously became polyploid cells. H-K, HCT116 p21+/+ (H) and p21−/− (I) cells cultured with 0.8 μmol/L SAHA, p53+/+ cells cultured with 2 μmol/L SAHA (J) and p53−/− cells cultured with 1 μmol/L SAHA (K) for 2 days continued to replicate DNA, as indicated by BrdUrd incorporation, without cell division and formed polyploid cells.
cells in culture with SAHA for day 3 (Fig. 5B and D), suggesting that the polyploid cells kept replicating DNA without cell division, namely endoduplication, before senescence. The senescent cells were characterized by flattening and spreading (40). Similar results were observed in HCT116 p53+/+ and p53−/− cells cultured with 2 μmol/L SAHA for 5 days and in fresh medium for another 5 days.

We next analyzed whether p21−/− and p53−/− affect the development of senescence in SAHA-induced polyploid cells. The proportion of the polyploid cells that are SAH−gal-positive cells in HCT116 p21−/− or HCT116 p53−/− cell culture was not significantly different from that of their parental cells at day 5 of culture with SAHA (P > 0.05; Fig. 5F).

The small cells that survive from SAHA treatment are less sensitive to SAHA. We next determined if the small cells which were not polyploid and remained viable in culture with SAHA are different from the unselected control in sensitivity to the HDAC inhibitor. We sorted the small cell population from HCT116 p21−/− cells cultured with 0.8 μmol/L SAHA for 3 days and grew the small cells in SAHA-free medium for 5 days to let them recover from SAHA treatment. We found that the small cells that survived SAHA treatment were less sensitive to SAHA, that is, 1.2 μmol/L SAHA was required to inhibit cell growth by 50% of the small cells by 48 hours compared with 0.7 μmol/L SAHA for the unselected population of HCT116 p21−/− cells.

**Discussion**

HDAC inhibitors have been shown to induce G1 and G2-M cell cycle arrest (12, 41), caspase-dependent apoptosis (37, 41–43), and cell death independent of caspase activation (28, 30, 31, 44). In this study, we showed that SAHA can induce polyploid cells which lose proliferating capacity and become committed to senescence.

Studies have shown that HDAC inhibitors can cause aberrant acetylation of histones in the centromere domain of chromosome, disrupt the function of kinetochore, and cause mitotic defects (18–22). The mitotic defects result in cell death either by apoptosis or by mitotic death/catastrophe (25, 26). Mitotic catastrophe is a
form of cell death that results from aberrant mitosis, and is characterized by the formation of large nonviable cells with multiple micronuclei containing uncondensed chromosome (40). The present study showed that cells cultured with SAHA could exit from mitosis without cell division and survive with continuing DNA replication and become polyploid cells, and the polyploid cells can then proceed to senescence.

We found that SAHA can induce polyploidy in transformed cells, although not to the same extent in all transformed cell lines, but not in normal cells. It has been reported that HDAC inhibitors can trigger a G2 checkpoint in normal cells, which is defective in tumor cells (25). Although the property of this checkpoint is not understood, the present findings indicate that normal cells are resistant to HDAC inhibitor–induced polyploidy and cell death compared with transformed cells. This difference may confer selectivity of the HDAC inhibitor consistent with its use as an anticancer agent with a potentially good therapeutic index.

The transformed cells without functioning p53 or p21 cultured with SAHA developed a higher proportion of abnormal mitosis, failure of cytokinesis, and polyploidy than parental wild-type cells. This is consistent with the function of p21 and p53 to maintain genomic integrity (33, 45).

Senescence is a physiologic process that limits the proliferative span of normal cells. The senescence phenotype can be induced in transformed tumor cells by overexpression of p53, pRb, p16, or p21 tumor suppressor genes (46–49), or by antitumor reagents such as cisplatin (50), doxorubicin (38), retinoid (51), and radiation (38). HDAC inhibitor sodium butyrate induced senescence in gynecologic cancer cells (52) and sodium butyrate, sodium dibutyrate and trichostatin A induced senescence in human fibroblasts (53, 54). We found that SAHA induced characteristics of senescence in the polyploid cells which accumulated in the HDAC inhibitor–treated HCT116 cells lines.

Induction of senescence has been shown to contribute to the treatment outcome of chemotherapy (55). The induction of senescence may also contribute to the antitumor efficacy of HDAC inhibitors. In this regard, HDAC inhibitor may have advantage over certain chemotherapy reagents. Unlike the chemotherapy drugs which are genotoxic and induce DNA damage and whose antitumor effects depend on p53, p21, and p16 for senescence induction (38, 55), HDAC inhibitor–induced senescence in tumors could be independent of p53, p21, and p16 functions (52). Because inactivation of p53 and p16 are common defects in human tumors (56), application of HDAC inhibitors alone or in combination with chemotherapeutic drugs may achieve a better outcome.

An interesting finding was that the small cells in HCT116 p21−/− cells that survived from SAHA treatment are less sensitive to SAHA-induced cell growth arrest. Because cancer cells have intrinsic genomic instability and heterogeneity, whether cells that survive from SAHA treatment in other tumors could become less sensitive to SAHA than the original cells remains to be determined.

In summary, the induction of polyploid cells is an antitumor mechanism of HDAC inhibitor. The polyploid cells induced by SAHA may commit to senescence. Lack of p21 or p53 enhances this effect with increased polyploid formation. Because p53 abnormalities are common in human tumors, this pathway may play an important role in the antitumor mechanisms of SAHA.

Acknowledgments

Received 12/28/2004; revised 5/13/2005; accepted 6/22/2005.

Figure 5. SAHA-induced polyploid cells underwent senescence. A, HCT116 p21−/− cells cultured without 0.8 μmol/L SAHA for 3 days were negative for SA-β-gal. B–C, most HCT116 p21−/− cells cultured with 0.8 μmol/L SAHA for 3 days became polyploid cells (B), but only few of them were positive for SA-β-gal (C). D–E, most HCT116 p21−/− cells cultured with 0.8 μmol/L SAHA for 5 days and without SAHA for another 3 days became polyploid cells with more nuclei, large size and wide and fat cytoplasm (D), and virtually all polyploid cells were positive for SA-β-gal (E). F, percentage of SA-β-gal-positive polyploid cells in HCT116 p21+/+ and p21−/− cells cultured with 0.8 μmol/L SAHA, and p53+/+ and p53−/− cells cultured with 2 μmol/L SAHA for 5 days.
References

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