Pancreatic cancer is the fifth leading cause of cancer death in the United States. The disease presents late in life (mean age, >60 years) and the incidence virtually equals mortality where median survival time is 4 to 6 months at diagnosis and the 5-year survival rate is only 1% to 3% (1). The American Cancer Society estimates that in 2005, ~32,180 people in the United States will be diagnosed with pancreatic cancer and ~31,800 will die of the disease. Despite our advances in the understanding of the molecular biology of pancreatic cancer, the treatment options are still unsatisfactory and there is at present no curative treatment, hence the high death rates (2). Chemotherapy, antihormonal treatment (3), radiotherapy (4), and anti–pancreatic cancer–specific antibodies (3) have not led to a significant improvement. Moreover, due to the disseminated nature of the disease at presentation, surgical resection of pancreatic ductal adenocarcinoma is only carried out in 15% to 20% of patients (5).

Pancreatic ductal adenocarcinoma is characterized by mutations and/or silencing of tumor suppressor genes, such as p53 and Smad4, the overexpression of mitogenic growth factors and their cognate high affinity tyrosine kinase receptors, and mutation of K-ras (6). There are also defects in cell cycle–regulating genes, such as the increased expression of cyclin D1 and mutation/silencing of p16, which contribute to the inactivation of the retinoblastoma protein (7). These alterations contribute to the excessive growth of pancreatic tumors and to the resistance of pancreatic cancers to chemotherapeutic agents (8).

Histone acetylation is a posttranslational modification of the nucleosomal histones that affects chromatin structure and regulates gene expression. The acetylation status of histones is modulated by histone acetyl transferases and histone deacetylases (HDAC). Histone acetyl transferases are generally considered to be transcriptional activators because histone acetylation is associated with transcriptionally active chromatin, whereas HDACs are considered as transcriptional inhibitors because histone deacetylation is associated with transcription repression (9, 10). Moreover, HDAC activity is increased in cancer cells and this increase has been shown to induce oncogenic transformation by modulating the function of transcription factors, such as p53 and nuclear factor-κB (11),

### Abstract

**Purpose:** Pancreatic cancer is an aggressive human malignancy that is generally refractory to chemotherapy. Histone deacetylase inhibitors are novel agents that modulate cell growth and survival. In this study, we sought to determine whether a relatively new histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), inhibits pancreatic cancer cell growth.

**Experimental Design:** The effects of SAHA on the growth of three pancreatic cancer cell lines (BxPC3, COLO-357, and PANC-1) were examined with respect to cell cycle progression, p21 induction and localization, and interactions with the nucleoside analogue gemcitabine.

**Results:** SAHA induced a G1 cell cycle arrest in BxPC-3 cells and COLO-357 cells but not in PANC-1 cells. This arrest was dependent, in part, on induction of p21 by SAHA, as p21 was not induced in PANC-1 cells, and knockdown of p21 using small interfering RNA oligonucleotides nearly completely suppressed the effects of SAHA on cell cycle arrest in COLO-357 and partly attenuated the effects of SAHA in BxPC-3. COLO-357 and BxPC-3 cells, but not PANC-1 cells, were also sensitive to gemcitabine. In the gemcitabine-resistant PANC-1 cells, a 48-h cotreatment with SAHA rendered the cells sensitive to the inhibitory and proapoptotic effects of gemcitabine. An additive effect on growth inhibition by SAHA and gemcitabine was observed in COLO-357 and BxPC-3 cells. Moreover, analysis of p21 distribution in COLO-357 cells revealed that SAHA induced the cytoplasmic localization of both p21 and phospho-p21.

**Conclusions:** These data indicate that SAHA exerts proapoptotic effects in pancreatic cancer cells, in part, by up-regulating p21 and sequestering it in the cytoplasm, raising the possibility that SAHA may have therapeutic potential in the treatment of pancreatic cancer.
resulting in altered gene transcription and increased proliferation (12). HDAC inhibitors (HDACI) represent a new class of targeted anticancer agents, and several structural classes of HDACI have been developed and are in clinical trials. Second-generation, novel compounds that inhibit class I/II HDAC are promising new anticancer agents due to their low toxicity and high tolerance in cancer treatment (13). These compounds have been shown to inhibit growth, induce differentiation, and decrease the survival of transformed/cancer cells but not normal cells (14, 15). One such compound, suberoylanilide hydroxamic acid (SAHA), induces differentiation, growth arrest, or apoptosis of transformed human cells in culture at micromolar concentrations (16). SAHA was originally identified based on its ability to induce differentiation of murine erythroleukemia cells (17). Subsequently, it was found to induce differentiation of human breast adenocarcinoma cells (18) and growth arrest in human prostate carcinoma (19, 20) and bladder transitional cell carcinoma cells (21). SAHA induces apoptosis in transformed hematopoietic cells, including Jurkat, CEM, and ARP-1 cells (22–24).

In the present study, we examined the effects of SAHA on three human pancreatic cancer cell lines. We report that BxPC3 and COLO-357 cells are sensitive to the growth-suppressive effects of SAHA. We further show that SAHA can be used to sensitize pancreatic cancer cells to the apoptosis-inducing effects of gemcitabine. These results indicate that SAHA may have a therapeutic potential in pancreatic cancer.

Materials and Methods

Cell culture. The human pancreatic cancer cell lines PANC-1 and BxPC-3 were purchased from American Type Culture Collection (Manassas, VA). BxPC-3 was maintained in RPMI 1640 supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin (Irvine Scientific, Irvine, CA) in 5% CO2. COLO-357 cells (a gift from R.S. Metzger, Duke University, Durham, NC) and PANC-1 cells were maintained in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin in 5% CO2. For all experiments, the cell lines were treated in serum-free medium (0.1% bovine serum albumin, 5 μg/ml transferrin, and 5 ng/ml sodium selenite and antibiotics). SAHA was purchased from BioMol (Plymouth Meeting, PA), and gemcitabine was purchased from Ely Lilly.

Immunoblotting. Cells were washed and lysed in lysis buffer in the presence of a protease inhibitor solution consisting of 1 mmol/L phenylmethysulfonyl fluoride, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 μg/ml pepstatin. Lysates were then subjected to SDS-PAGE, transferred to nitrocellulose membranes (25 μg/lane), and incubated with the indicated antibodies. The anti–poly(ADP-ribose) polymerase (PARP) antibody was purchased from Cell Signaling Technologies (Beverly, MA). Anti-p21 and anti–acetylated lysine-specific histone H3 antibodies were purchased from Upstate Biotechnologies (Waltham MA). Membranes were then stripped and probed with an anti–extracellular signal-regulated kinase 2 (ERK2) antibody from Santa Cruz Biotechnology (Santa Cruz, CA) to normalize for protein loading (25).

Flow cytometry. Cells were plated in six-well plates at a density of 100,000 per well and allowed to adhere overnight. Cells were treated with 10 μmol/L SAHA in serum-free medium for 48 h. Cells were then trypsinized and fixed in ice cold 70% ethanol and stained with propidium iodide in PBS. Flow cytometric data were acquired using a FACScan analysis system equipped with a FACStation, MAC PowerPC computer and CellQuest Acquisition software from Becton Dickinson (San Jose, CA). Data were analyzed using ModFit LT 2.0 software from Verity Software House (Topsham, ME) as reported previously (26).

Growth assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide growth assay (27), which in pancreatic cancer cells correlates well with results obtained by cell counting with a hemocytometer and by monitoring 1H]thymidine incorporation (28, 29), was used to monitor cell growth. Cells were plated at a density of 10,000 per well, using 96-well microtiter plates (Corning, Acton, MA), and allowed to adhere overnight before SAHA addition for 48 h. Colorimetric changes were measured using a microtiter plate reader with a 570 nm filter.

p21 Small interfering RNA. BxPC-3 and COLO-357 cells were transfected with 20 mmol/L SMARTpool p21 small interfering RNA (siRNA; Dharmacon, Lafayette, CO) and jetPEI using the recommended fast protocol (Qbiogene, Solon, OH). Cells were plated to 60-mm dishes and allowed to adhere overnight. The following day, the cells were split to 96-well plates for a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay or 60-mm dishes for Western blot analysis. The cells were then treated with 10 μmol/L SAHA for 48 h. Cell proliferation was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (described above), and p21 expression was determined by Western blotting.

Measurement of caspase-3 activity. Caspase-3 activity was measured using Colorimetric Assay kits from R&D Systems (Minneapolis, MN). In brief, cells were scraped into PBS, pelleted at low speed, and resuspended in lysis buffer for 10 min at 4°C. Cell lysates were cleared
by centrifugation and assayed for caspase-3 activity using a DEVDpNA peptide substrate and incubated for 6 h at 37°C. The activities were quantified spectrophotometrically at a wavelength of 405 nm. Caspase activity was calculated as the change in absorbance at 405 nm and divided by total protein concentration.

Confocal microscopy. Cells were seeded overnight on Lab-Tek chamber slides (Nalge Nunc, Rochester, NY) followed by a 48-h incubation in the absence or presence of SAHA. Cells were then washed twice with PBS and fixed for 10 min with 2% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) in PBS, washed with PBS, and incubated with 50 mmol/L NH4Cl in PBS for 10 min. After washing with PBS, cells were permeabilized for 15 min with 0.1% Triton X-100/2% bovine serum albumin in PBS and incubated with anti-p21 or anti–phospho-p21 antibody (1:500 dilution) for 1 h at 23°C. Cells were then washed twice with PBS and incubated for 30 min at 23°C with 5 μg/mL Alexa Fluor 555 goat anti-rabbit IgG (H+L). Nuclei were stained with 0.2 μg/mL Hoechst 33258 pentahydrate (Molecular Probes, Eugene, OR). After three washes with PBS and one with water, slides were mounted with PermaFluor (Thermo Electron Corp., Pittsburgh, PA). To block phosphatase activity, slides were incubated for 15 min with 20 mmol/L NaF/2 mmol/L sodium orthovanadate/PBS before fixation and fixed with paraformaldehyde containing the same phosphatase inhibitors. All images were captured using Zeiss LSM 510 META confocal system and LSM release 3.2 software (Carl Zeiss, Inc., Thornwood, NY) as reported previously (26).

Results

SAHA inhibits the growth of pancreatic cancer cell lines. HDACIs have been shown to induce cell cycle arrest, differentiation, and apoptosis of tumor-derived cell lines (30, 31). To elucidate the effects of the HDACi SAHA in pancreatic cancer cells, three pancreatic cancer cell lines, BxPC-3, COLO-357, and PANC-1, were treated with increasing concentrations of SAHA and cell proliferation was assessed by 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyloxetrazolium bromide assay. Treatment with SAHA caused a dose-dependent decrease in the proliferation of BxPC-3 and COLO-357 cells (Fig. 1A). At its maximally effective concentration (25 μmol/L), SAHA inhibited the growth of BxPC-3 and COLO-357 cells by 42% and 50%, respectively. This decrease in cell proliferation was not due to SAHA-induced apoptosis as SAHA did not induce cleavage of PARP or increase Annexin 5 staining (data not shown). By contrast, the growth of PANC-1 cells was not inhibited by SAHA (Fig. 1A).

SAHA induces accumulation of acetylated histone H3. To confirm that the concentrations of SAHA used in the proliferation assay were inhibiting the activity of HDACs, the acetylation status of histone H3 was examined by Western blot analysis using an antibody specific for the acetylated lysine residues of histone H3. In the basal state, COLO-357 cells exhibited the lowest levels of acetylated histone H3, BxPC3 cells exhibited intermediate levels, whereas PANC-1 cells exhibited the highest levels of acetylated histone H3 (Fig. 1B). SAHA (10 μmol/L) caused a marked increase in histone H3 acetylation in BxPC-3 and COLO-357, indicating that SAHA inhibits the deacetylation activity of the HDACs in these cell lines (Fig. 1B). By contrast, histone H3 was only slightly increased in PANC-1 cells compared with control, indicating that SAHA may be less effective in inhibiting HDAC activity in these cells.
SAHA induces a G₁ cell cycle arrest. SAHA treatment of cancer cell lines has been shown to induce a G₁ cell cycle block by up-regulating the cyclin-dependent kinase inhibitor p21 (21, 32). Because SAHA decreased the proliferation of BxPC-3 and COLO-357 cells, the effects of SAHA on cell cycle arrest and p21 regulation were examined next by fluorescence-activated cell sorting analysis and p21 immunoblotting. After the treatment with 10 μmol/L SAHA for 48 h, BxPC-3 and COLO-357 cells exhibited a shift into G₁ arrest by 15% to 30%, whereas PANC-1 cells were unaffected (Fig. 2). Analysis of p21 protein levels showed that SAHA treatment induced an increase in p21 expression in BxPC-3 and COLO-357, whereas PANC-1 cells exhibited minimal p21 induction (Fig. 3A). To further determine whether p21 up-regulation was necessary for SAHA-induced growth arrest, knockdown of p21 protein levels using a siRNA strategy was done next. BxPC-3 and COLO-357 cells were transfected with p21 double-stranded siRNA oligonucleotides or nontargeting double-stranded siRNA oligonucleotides. After 48 h of SAHA treatment, the presence of p21 siRNA blocked the SAHA-mediated up-regulation of p21 protein in both cell lines and this effect was especially dramatic for SAHA-induced growth arrest.
in COLO-357 cells (Fig. 3B). Moreover, in COLO-357 cells, suppression of p21 nearly completely reversed SAHA-mediated growth inhibition (Fig. 3C). In contrast, p21 suppression only partially decreased SAHA-induced growth inhibition in BxPC-3 cells. These findings indicate that up-regulation of p21 is necessary for SAHA-induced cell cycle arrest in COLO-357 cells but not in BxPC-3 cells.

SAHA enhances gemcitabine-induced apoptosis. The nucleoside analogue gemcitabine is commonly used in pancreatic cancer treatment regimens. However, gemcitabine is not curative in pancreatic cancer and treatment only increases patient survival by a few months (33). Previous studies have shown that SAHA increases the sensitivity of some cancer cells to chemotherapeutic drugs (34, 35). To determine if SAHA increased the sensitivity of pancreatic cancer cells to the growth-inhibitory/apoptotic effect of gemcitabine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide proliferation assays were done. First, to test the sensitivity of the cell lines to gemcitabine, BxPC-3, COLO-357, and PANC-1 cells were treated with increasing concentrations of gemcitabine (1-25 μmol/L) for 24 h. Gemcitabine treatment decreased the proliferation of BxPC3 and COLO-357 cells by 85% and 75%, respectively (Fig. 5). SAHA-mediated potentiation of gemcitabine-induced apoptosis. A, proliferation. PANC-1 cells were plated at a density of 8,000 per well to 96-well microtiter plates, allowed to adhere overnight, and incubated for 48 h in the absence (white columns) or presence of 10 μmol/L SAHA (black column), 10 μmol/L gemcitabine (gray column), both agents at these concentrations (stipled column), 25 μmol/L gemcitabine (column with horizontal lines), or SAHA with 25 μmol/L gemcitabine (hatched column). Growth was assessed as described in Materials and Methods. Columns, mean of three experiments; bars, SE.

* P = 0.004, by comparison with respective control. B, PARP cleavage. Cells were incubated for 48 h in the absence or presence of 25 μmol/L gemcitabine and 25 μmol/L SAHA. Cell lysates were subjected to immunoblotting with an antibody that recognizes the cleaved form of PARP (89 kDa). ERK2 immunoblotting confirmed equal loading of lanes. C, caspase activation. Cells were incubated for 48 h in the absence (white column) or presence of 25 μmol/L gemcitabine and 25 μmol/L SAHA (black column), 25 μmol/L gemcitabine (column with horizontal lines), or both SAHA and gemcitabine (hatched column). Cell lysates were assayed for caspase-3 activity as described in Materials and Methods. Columns, mean of three experiments; bars, SE. * P = 0.012, by comparison with control.

SAHA enhances gemcitabine-induced apoptosis. The nucleoside analogue gemcitabine is commonly used in pancreatic cancer treatment regimens. However, gemcitabine is not curative in pancreatic cancer and treatment only increases patient survival by a few months (33). Previous studies have shown that SAHA increases the sensitivity of some cancer cells to chemotherapeutic drugs (34, 35). To determine if SAHA increased the sensitivity of pancreatic cancer cells to the growth-inhibitory/apoptotic effect of gemcitabine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide proliferation assays were done. First, to test the sensitivity of the cell lines to gemcitabine, BxPC-3, COLO-357, and PANC-1 cells were treated with increasing concentrations of gemcitabine (1-25 μmol/L) for 24 h. Gemcitabine treatment decreased the proliferation of BxPC3 and COLO-357 cells by 85% and 75%, respectively (Fig. 5).
respectively (Fig. 4A), and this effect was associated with induction of apoptosis as evidenced by an increase in PARP cleavage (Fig. 4B). By contrast, PANC-1 cells were inhibited by only 20% at all concentrations tested (Fig. 4A) and this effect was not associated with PARP cleavage (Fig. 4B). Gemcitabine also caused an increase in the activity of caspase-3 in BxPC3 and COLO-357 but not in PANC-1 cells (Fig. 4C).

Next, to determine whether SAHA potentiates the effects of gemcitabine, the cells were incubated with 10 μmol/L SAHA with or without 1 to 25 μmol/L gemcitabine for 24 h. At these concentrations, the combination of SAHA and gemcitabine caused an additional but slight decrease in the proliferation of BxPC-3 and COLO-357 cells by comparison with either agent alone (Fig. 5A) and this decrease was associated with a slight increase in apoptosis as evidenced by an increase in PARP cleavage (Fig. 5B) and caspase-3 activity (Fig. 5C). Gemcitabine and SAHA treatment also had a slightly additive inhibitory effect on the proliferation of PANC-1 cells, decreasing proliferation by 35% in the presence of 10 μmol/L gemcitabine (Fig. 5A). However, this decrease was not due to induction of apoptosis because it was not associated with increased PARP cleavage (Fig. 5B) or caspase-3 activation (Fig. 5C).

In view of the resistance of PANC-1 to gemcitabine-induced apoptosis, we next sought to determine whether prolonging the incubation of SAHA and gemcitabine for 48 h would alter either the proliferation or the apoptosis of PANC-1 cells. Gemcitabine alone decreased cell proliferation by 15% to 22% (Fig. 6A). By contrast, following concomitant incubation with SAHA for 48 h, gemcitabine inhibited proliferation by 40% to 50% and this decrease was associated with enhanced apoptosis as evidenced by increased PARP cleavage (Fig. 6B) and caspase-3 activity (Fig. 6C). Taken together, these results suggest that SAHA can increase the sensitivity of pancreatic cancer cell lines to gemcitabine and this effect is mediated, in part, by an enhancement of the proapoptotic actions of gemcitabine.

Because BxPC3 and COLO-357 cells were very sensitive to high concentrations of gemcitabine, we next examined the effects of relatively low concentrations of gemcitabine on SAHA-mediated growth inhibition. Accordingly, cells were incubated in the absence or presence of 10 μmol/L SAHA and either 500 nmol/L (BxPC3) or 10 nmol/L (COLO-357) gemcitabine for 48 h. In both cell lines, SAHA alone inhibited cell growth by 20% (P < 0.004), whereas gemcitabine alone inhibited the growth of BxPC3 and COLO-357 cells by 35% (P < 0.0006) and 49% (P < 0.0001), respectively. Moreover, at these concentrations, the combination of SAHA and gemcitabine was more effective than either agent alone, decreasing growth by 56% in BxPC3 cells (P < 0.002) and by 63% in COLO-357 cells (P < 0.015; Fig. 7).

SAHA enhances the cytoplasmic localization of p21. To determine whether SAHA altered the cellular distribution of p21 and phospho-p21, confocal microscopy was done next. In the absence of SAHA, both p21 and phospho-p21 localized to both the cytoplasm and the nucleus (Figs. 8 and 9). By contrast, when the cells were incubated for 48 h in the presence of 10 μmol/L SAHA, both p21 and phospho-p21 were found almost exclusively in the cytoplasm (Figs. 8 and 9).

Discussion

HDACIs constitute a new group of cancer treatment agents that generally exhibit minimal toxicity in patients (36). Second-generation inhibitors, such as valproic acid, LAQ824, SAHA, and depsipeptide, have shown variable degrees of promise in clinical trials for a variety of cancers, including hematologic malignancies, non–small cell lung cancer, breast cancer, and thyroid cancer (37). Due to their relative specificity toward cancer cells, these newer inhibitors may be useful in pancreatic cancer treatment when used alone or in combination with current chemotherapeutic drugs as they have been shown to sensitize cancer cells to multiple chemotherapeutic agents (38–40). In support of this possibility, it has been shown recently that trichostatin A when used in combination with chemotherapeutic agents, such as gemcitabine, gefitinib (Iressa), and irinotecan (CPT11), significantly sensitized pancreatic cancer cell lines to the growth-inhibitory effects of the agents when compared with trichostatin A alone (41). However, trichostatin A has a relatively low stability and exhibits toxicity in patients (42, 43).

In this study, we tested the effects of SAHA on three distinct pancreatic cancer cell lines BxPC-3, COLO-357, and PANC-1, which harbor mutations in K-ras, p53, and Smad4 genes, alterations that are commonly found in pancreatic cancers. SAHA (10 μmol/L) significantly decreased the proliferation of BxPC3 and COLO-357, which, by fluorescence-activated cell sorting analysis, was associated with a G1 cell cycle arrest. By
contrast, PANC-1 cells were resistant to the growth-inhibitory effects of SAHA at all concentrations tested. PANC-1 cells also exhibited a weak increase in the acetylation of histone H3, indicating that the histone deactylase activity was not decreased by SAHA in this cell line.

It has been shown previously that SAHA induces the up-regulation of the cyclin-dependent kinase inhibitor p21WAF1 by changing the association of proteins on the p21 promoter (22, 45), indicating that p21 is a direct target of HDACI. In BxPC3 and COLO-357 cells, but not in PANC-1 cells, there was marked up-regulation of p21 by SAHA. When the induction of p21 was prevented by using a p21 siRNA knockdown strategy, COLO-357 cells were no longer sensitive to SAHA-mediated growth inhibition. By contrast, in BxPC3 cells, SAHA-mediated growth inhibition was only partly attenuated. These observations suggest that a major mechanism in SAHA-induced growth arrest in some pancreatic cancer cells is dependent on p21 up-regulation. In support of this conclusion, trichostatin A induces the expression of p21 in pancreatic cancer cell lines (46). Furthermore, in the present study, SAHA did not induce the up-regulation of p21 in PANC-1 cells, which were resistant to its growth-inhibitory effects.

BxPC-3 and COLO-357 exhibited a slight increase in apoptosis when incubated with both SAHA and gemcitabine, by comparison with their response to gemcitabine alone. However, when the cells were incubated for 48 h with submaximal concentrations

![Fig. 8. Immunofluorescence analysis of p21. COLO 357 cells were grown on glass slides and incubated for 48 h at 37°C in the absence (A) or presence (B) of 10 μmol/L SAHA. Cells were then subjected to immunofluorescent staining, using an anti-p21 antibody (1). Nuclei were stained with Hoechst 33258 dye (2), and regions were merged to assess signal colocalization (3). Original magnification, ×400.](image)

![Fig. 9. Immunofluorescence analysis of phospho-p21. COLO 357 cells were grown on glass slides and incubated for 48 h at 37°C in the absence (A) or presence (B) of 10 μmol/L SAHA. Cells were then subjected to immunofluorescent staining, using an anti-phospho-p21 antibody (1). Nuclei were stained with Hoechst 33258 dye (2), and regions were merged to assess signal colocalization (3). Original magnification, ×400.](image)
of gemcitabine, the addition of SAHA resulted in a significant increase in growth inhibition that was greater than that observed with either agent alone. It is possible, therefore, that the concentration of gemcitabine could be decreased in clinical protocols with SAHA cotreatment, as suggested previously for multiple myeloma (47). Similarly, PANC-1 cells, which were resistant to gemcitabine alone, exhibited a marked increase in sensitivity when treated with both gemcitabine and SAHA for 48 h. Moreover, this effect was associated with a proapoptotic action, as evidenced by PARP cleavage and caspase-3 activation. Thus, a pancreatic cancer cell line that is resistant to the growth-inhibitory and proapoptotic effects of gemcitabine can be rendered responsive to this nucleoside analogue by treatment with SAHA.

A surprising finding in the present study was the SAHA-mediated cytoplasmic localization of p21 and phospho-p21. It is generally accepted that p21 is located in the nucleus, where it interacts with cyclin-dependent kinase 4/6 complexes, thereby inhibiting their activity. However, several studies have reported on the cytoplasmic localization of p21 and indicated that when found in the cytoplasm p21 may promote cell proliferation (48, 49) and may contribute to p21-mediated resistance to apoptosis (50, 51). Other studies have suggested that the cytoplasmic accumulation of p21 abrogates the inhibitory effect of p21 (52). By contrast, in the present study, we determined that SAHA caused the cytoplasmic localization of both p21 and phospho-p21 in COLO-357 cells and that suppression of p21 levels in these cells completely eliminated the growth-inhibitory actions of SAHA. These observations suggest that in this cell line, the SAHA-induced cytoplasmic localization of p21 and phospho-p21 impedes cell cycle progression and facilitates apoptosis. In support of this conclusion, p21 has been reported to enhance cell cycle progression by acting as a nuclear import factor for cyclin D1 and its target kinases, cyclin-dependent kinase 4/6 (53), and to promote apoptosis (54–57).

It was shown recently that HDACIs can enhance the proapoptotic actions of bortezomib, a proteosome inhibitor, in pancreatic cancer cells (58). Given the low toxicity of SAHA, the recent findings with bortezomib, and our current findings, it is possible that SAHA and related compounds may ultimately be useful in various combination treatment strategies for pancreatic cancer.

References


The Histone Deacetylase Inhibitor Suberoylanilide Hydroxamic Acid Induces Growth Inhibition and Enhances Gemcitabine-Induced Cell Death in Pancreatic Cancer

Nichole Boyer Arnold, Nohea Arkus, Jason Gunn, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/13/1/18

Cited articles
This article cites 57 articles, 20 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/13/1/18.full.html#ref-list-1

Citing articles
This article has been cited by 6 HighWire-hosted articles. Access the articles at:
/content/13/1/18.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.