Vorinostat, a histone deacetylase inhibitor, enhances the response of human tumor cells to ionizing radiation through prolongation of γ-H2AX foci

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Abstract

Vorinostat (suberoylanilide hydroxamic acid) is the prototype of a family of hybrid polar compounds that can induce growth arrest in transformed cells and shows promise for the treatment of cancer. Vorinostat specifically binds to and inhibits the activity of histone deacetylases resulting in acetylation of nucleosomal histones and an activation of gene transcription. Because histone deacetylases modulate chromatin structure and gene expression, both of which can influence radioresponse, this study was designed to examine the capacity of Vorinostat to influence radiation response in human tumor cells and investigate the mechanism underlying these interactions. Vorinostat induced hyperacetylation of histone H4 in a dose-dependent manner. We tested its ability to radiosensitize three human tumor cell lines (A375, MeWo, and A549) using clonogenic cell survival assays. Clonogenic cell survival assay showed that Vorinostat significantly radiosensitized all three tumor cell lines, substantially reducing the surviving fraction at 2 Gy. We examined potential mechanisms that may contribute to the enhanced radiation response induced by Vorinostat. Vorinostat and radiation alone did not induce apoptosis in the melanoma cell line. However, enhanced apoptosis was observed when cells were exposed to both Vorinostat and radiation, suggesting that Vorinostat renders tumor cells more susceptible to radiation-induced apoptosis. Results from DNA damage repair analysis in cultured A375 cells showed that Vorinostat had a strong inhibitory effect on the nonhomologous end joining pathway after radiation. A detailed examination of the involvement of the DNA repair pathway following Vorinostat treatment showed that Vorinostat reduced the expression of the repair-related genes *Ku70, Ku80*, and *Rad50* in A375 cells as detected by Western blot analysis. We also examined γ-H2AX phosphorylation as a predictive marker of radiotherapy response to Vorinostat and observed that the combination of Vorinostat and radiation caused a prolongation of expression of DNA repair proteins such as γ-H2AX. Overall, we conclude that Vorinostat enhances tumor radioresponse by multiple mechanisms that may involve antiproliferative growth inhibition and effects on DNA repair after exposure to radiation. [Mol Cancer Ther 2006;5(8):1967–74]

Introduction

The regulation of acetylation of lysine residues on the NH_2-terminal tails of histones is a major mechanism controlling cellular differentiation and the biological behavior of cancer cells. Histone acetylation modulates nucleosome and chromatin structure and regulates transcription factor accessibility and function and, in general, chromatin composed of nucleosomes with hypoacetylated histones is transcriptionally silent (1, 2). The turnover of histone acetylation is regulated by the opposing enzymatic activities of histone acetyltransferases and histone deacetylases (HDAC; ref. 3). Importantly, dysregulated HDAC activity has been found in certain human cancers and such tumor cells are unable to undergo normal cellular differentiation possibly contributing to their neoplastic transformation (3, 4). Thus, targeting HDACs with small-molecule inhibitors is currently being tested as a therapeutic strategy for treating human malignancies.

Several compounds, such as butyrates, the anticonvulsant valproic acid, and the antifungal agent trichostatin A, have been shown to act as HDAC inhibitors, but their clinical effectiveness has been limited by low potency, high toxicity, or poor stability (5–7). A class of novel synthetic hybrid polar compounds with potent inhibitory effect on HDAC activity has been described. The prototype of this class of compounds, hydroxamic acid–based suberoylanilide hydroxamic acid (also known as Vorinostat), causes accumulation of acetylated histones in cultured cells, induces differentiation and/or apoptosis of transformed cells in culture, and inhibits the growth of tumors in animals (8–10). The inhibition of HDACs by Vorinostat occurs through a direct interaction with the catalytic site

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of the enzyme, as shown by X-ray crystallography studies, and leads to the accumulation of acetylated histones H2a, H2b, H3, and H4 (11). The result of HDAC inhibition is believed not to have a generalized effect on the genome but rather only affects the transcription of a small subset of genes. Vorinostat has been shown to selectively induce the expression of a specific set of genes (12).

The primary effect of HDAC inhibition is one of cytostasis in most solid tumor cell lines, suggesting that HDAC inhibitors will have only limited success as single modalities for most solid tumors. Because HDAC modulates chromatin structure and gene expression, variables considered to influence radioresponse, we investigated the effects of Vorinostat on the radiosensitivity of three human tumor cell lines growing *in vitro*: A375 and MeWo (human melanoma) and A549 (non–small-cell lung cancer). Exposure of these cells to Vorinostat, 24 hours before irradiation, resulted in a significant increase in radiosensitivity. Using A375 cells as a model system, we have shown that Vorinostat has the ability to down-regulate several key proteins involved in the nonhomologous end joining pathway, which is critical for repairing radiation-induced DNA double-strand breaks. In addition, treatment with Vorinostat prolonged the appearance of repair foci identified by phosphorylated H2AX (γ-H2AX). Based on these findings, we believe that the radiosensitizing effect of Vorinostat is due to its ability to inhibit the repair of radiation-induced lesions in DNA.

Materials and Methods

Cell Lines

The human melanoma cell lines A375 and MeWo were obtained from the American Type Culture Collection (Manassas, VA) and routinely maintained in RPMI 1640 supplemented with 10% fetal bovine serum, 10,000 units/mL of penicillin-streptomycin, and 2 mmol/L L-glutamine. The human non–small-cell lung cancer cell line A549 was obtained from American Type Culture Collection and routinely maintained in F-12/Ham medium supplemented with 10% fetal bovine serum, 10,000 units/mL of penicillin-streptomycin, and 2 mmol/L L-glutamine.

Chemicals

Vorinostat (suberoylanilide hydroxamic acid) was obtained from ATON Pharma (Tarrytown, NY). A 10 mmol/L stock was prepared in DMSO and stored at −70°C in aliquots until further use.

Cell Cycle Analysis

Cell cycle arrest was assessed by propidium iodide staining and fluorescence-activated cell sorting analysis. Cells were harvested after 24 hours of treatment with Vorinostat, pelleted by centrifugation, and resuspended in PBS containing 50 μg/mL propidium iodide, 0.1% Triton X-100, and 0.1% sodium citrate. Samples were stored at 4°C for 16 hours and vortexed before fluorescence-activated cell sorting analysis (BD PharMingen FACScan, FL-3 channel; BD PharMingen, San Diego, CA). The cell cycle calculations were done with the MultiCycle Program from Phoenix Flow Systems (San Diego, CA).

DNA Fragmentation

Apoptosis-specific DNA fragmentation was measured by a modification of a published procedure (13). The assay is based on the solubility of low molecular weight DNA in solutions of low salt concentration. Briefly, cells labeled by [^{14}C]TdR incorporation for one cycle were treated with Vorinostat for 24 hours. The cells were irradiated and incubated for an additional 4 hours to allow apoptosis. Cells were harvested after treatment, washed with PBS, and then lysed with 0.5 mL of lysis buffer [10 mmol/L Tris, 1 mmol/L EDTA, 0.2% Triton X-100 (pH 7.5)] on ice for 20 minutes. The chromatin was pelleted by centrifugation at 14,000 × *g* for 10 minutes. The supernatant (fragmented DNA) was removed and the chromatin pellet was solubilized in 1 mL of Soluene (Packard, Meriden, CT). Radioactivity was determined with a liquid scintillation counter (Packard Instruments, Downers Grove, IL). DNA fragmentation was expressed as the percentage of radioactivity found in the supernatant fraction compared with the total radioactivity (pellet plus supernatant).

Clonogenic Survival

The effectiveness of the combination of Vorinostat and ionizing radiation was assessed by clonogenic assays. Briefly, the human tumor cells were treated with the vehicle control (DMSO) or Vorinostat at the indicated concentration for 24 hours and then irradiated with a high dose-rate ^{137}Cs unit (4.5 Gy/min) at room temperature. Following treatment, cells were trypsinized and counted. Known numbers were then replated in 100-mm tissue culture dishes and returned to the incubator to allow macroscopic colony development. Colonies were counted after ~14 days and the percent plating efficiency and fraction surviving a given treatment were calculated based on the survival of nonirradiated cells treated with the vehicle or Vorinostat.

Western Blot Analysis

Cells were harvested after treatment with various doses of Vorinostat for 24 hours at 37°C, rinsed in ice-cold PBS, and lysed in lysis buffer containing 50 mmol/L HEPES (pH 7.9), 0.4 mol/L NaCl, 1 mmol/L EDTA, 2 μg/mL leupeptin, 2 μg/mL aprotinin, 5 μg/mL benzamidine, 0.5 mmol/L phenylmethylsulfonylfluoride, and 1% NP40. The lysates were centrifuged at 14,000 rpm to remove any cellular debris. Protein concentrations of the lysates were determined by the Bio-Rad Dc protein assay system (Hercules, CA). Equal amounts of protein were separated by 12% SDS-PAGE, transferred to Immobilon (Millipore, Bedford, MA), and blocked with 5% nonfat dry milk in TBS-Tween 20 (0.05%, v/v) for 1 hour at room temperature. The membrane was incubated with primary antibody overnight. Antibodies to histone H4 and acetylated histone H4 were obtained from Upstate Biotechnology (Lake Placid, NY); Ku70, Rad50, and Bax were from Santa Cruz Biotechnology (Santa Cruz, CA); Ku86 was from Sigma Chemicals (St. Louis, MO); p21 was from Oncogene Sciences (Uniondale, New York, NY); and actin was from Chemicon (Temecula, CA). After washing, the membrane was incubated with the appropriate horseradish peroxidase

secondary antibody (diluted 1:2,000; Amersham Pharmacia Biotech, Arlington Heights, IL) for 1 hour. Following several washes, the blots were developed by enhanced chemiluminescence (Amersham).

Immunofluorescent Staining for γ-H2AX

Cells were grown and treated with 2.5 μmol/L Vorinostat for 24 hours on coverslips placed in 35-mm dishes. At specified times, medium was aspirated and cells were fixed in 1% paraformaldehyde for 10 minutes at room temperature. Paraformaldehyde was aspirated and the cells were fixed in 70% ethanol for 10 minutes at room temperature, followed by treatment with 0.1% NP40 in PBS for 20 minutes. Cells were then washed in PBS twice and then blocked with 5% bovine serum albumin in PBS for 30 minutes, following which anti-γ-H2AX antibody (Trevigen, Gaithersburg, MD) was added at a dilution of 1:300 in 5% bovine serum albumin in PBS and incubated overnight at 4°C with gentle shaking. Cells were then washed thrice in PBS before incubating in the dark with a FITC-labeled secondary antibody at a dilution of 1:300 in 5% bovine serum albumin in PBS for 30 minutes. The secondary antibody solution was then aspirated and the cells were washed four times in PBS. Cells then were incubated in the dark with 4′,6-diamidino-2-phenylindole (1 μg/mL) in PBS for 5 minutes and coverslips were mounted with an antifade solution (Molecular Probes, Eugene, OR). Slides were then examined on a Leica fluorescent microscope. Images were captured by a charge-coupled device camera and imported into Advanced Spot Image analysis software package for storage purposes. For each treatment condition, γ-H2AX foci were counted by eye in at least 50 cells from the stored images.

Statistical Analysis

Most analyses were done using *t* test (two-sample assuming unequal variances) and described as mean ± SE (Sigma Plot 5.02v, Richmond, CA). At each time point examined, ANOVA was used to test whether the average number of γ-H2AX foci per cell after combined Vorinostat/radiation treatment was greater than expected from the sum of the foci produced by each agent alone.[4] A difference was regarded as significant if $P < 0.05$.

Results

Vorinostat Treatment Leads to Acetylation of Histones

We determined the degree of histone acetylation in A375 cells after culture with Vorinostat. Western blot analysis showed that before incubation with Vorinostat, the levels of acetylated histone H4 were low and did not increase remarkably with 1 μmol/L Vorinostat for 24 hours. However, incubation with 2.5 μmol/L Vorinostat for 24 hours resulted in the maximal accumulation of acetylated histone H4 in the A375 cells (Fig. 1). The levels of accumulated acetylated histones remained unchanged with a further increase in Vorinostat. Nonacetylated histone H4 was included as a control (Fig. 1). The effect of Vorinostat on p21 and Bax protein levels was also determined by Western blot analysis. The expression levels of p21 and the proapoptotic protein Bax have previously been shown to increase significantly with Vorinostat treatment (14, 15). There was a dose-dependent accumulation of p21 as well as Bax following exposure to Vorinostat for 24 hours (Fig. 1).

Vorinostat Induces Cell Cycle Arrest and Apoptosis in Melanoma Cells

To assess the effects of Vorinostat on cell cycle arrest, cells were treated with 2.5 μmol/L Vorinostat for 24 hours. Cell cycle distributions of irradiated cells were compared with controls and, as shown in Fig. 2A, Vorinostat alone caused an increase in cells primarily in the G_1 (67.5%) phase; 12.1% of cells were in the G_2 phase and 20.4% in the S phase. A significant G_2-M-phase block was observed in response to radiation; 48% versus 25% in the untreated controls. After combined drug and radiation treatment, cells accumulated in the G_2 phase (42.8%) with a concomitant reduction in the G_1-phase cells (31.6%; Fig. 2A).

The A375 melanoma cell line was also tested for its susceptibility to Vorinostat-induced apoptosis with a DNA fragmentation assay. The DNA fragmentation assay showed that there was a dose-dependent induction of apoptosis with Vorinostat in the A375 cells (Fig. 2B). Apoptosis increased from 0.1% in the control cells to 4.5%, 8.5%, and 10.8% following a 24-hour incubation with 2.5, 5, and 10 μmol/L dose of Vorinostat respectively. A 5-Gy dose of ionizing radiation alone did not induce apoptosis but, when given at the end of the 24-hour treatment with 2.5 μmol/L Vorinostat, apoptosis was significantly enhanced in the A375 cells when compared with 2.5 μmol/L Vorinostat alone ($P = 0.01$). Similarly, ionizing radiation in combination with 5 and 10 μmol/L Vorinostat significantly enhanced apoptosis when compared with Vorinostat alone at the same dose ($P = 0.01$).

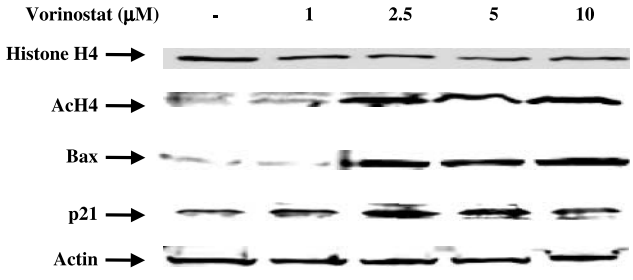

Figure 1. Vorinostat leads to accumulation of acetylated histone H4, p21, and Bax. A375 melanoma cells were treated with indicated concentrations of Vorinostat for 24 h. Protein was extracted and analyzed by Western blot. Nonacetylated histone H4 was used as a control for acetylated histone H4. Actin was used as a loading control for p21 and Bax. Representative of at least two independent experiments.

[4] StataCorp. Stata statistical software: release 8.0. College Station (TX): Stata Corporation; 2004.

Treatment with Vorinostat Enhances Radiosensitivity of Human Tumor Cells in an *In vitro* Clonogenic Survival Assay

We determined the survival of human tumor cells exposed to combinations of Vorinostat and ionizing radiation using clonogenic assays. MeWo, A375, and A549 cells were pretreated with 2.5 μmol/L Vorinostat for 24 hours, following which the cells were irradiated and plated for clonogenic cell survival. Figure 3 shows that Vorinostat suppressed the clonogenic survival of all three tumor cell lines, MeWo, A375, and A549. Survival at 2 Gy was reduced from 44 ± 0.6% in the control cells to 26 ± 0.7% in the Vorinostat-treated A375 cells (P = 0.0047; Fig. 3A). Similar results were obtained on exposure of MeWo cells to Vorinostat, with surviving fraction at 2 Gy being reduced from 52 ± 0.6% in the control MeWo cells to 22.6 ± 0.5% in Vorinostat-treated MeWo cells (P = 0.001; Fig. 3B). In the A549 cells, surviving fraction at 2 Gy decreased from 61.25 ± 0.61% in control cells to 22.6 ±

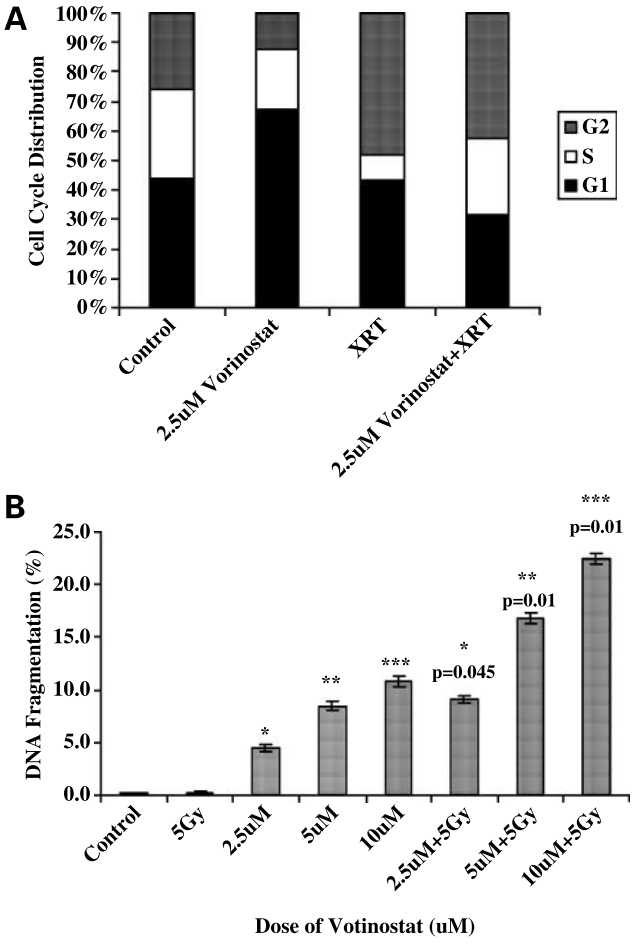

Figure 2. Vorinostat alters the cell cycle profile in human A375 melanoma cells and induces apoptosis. **A,** cell cycle analysis of A375 cells. Cells were pretreated with 2.5 μmol/L Vorinostat for 24 h, following which they were irradiated at 5 Gy and harvested for cell cycle analysis 4 h later. Control cells were exposed to vehicle (DMSO) and subjected to the same protocol. **B,** apoptosis induction in the A375 cells as determined by DNA fragmentation assay. Cells were exposed to increasing concentrations of Vorinostat for 24 h, following which they were irradiated at 5 Gy and harvested for DNA fragmentation 4 h later. *Columns,* average of two independent experiments; *bars,* SE.

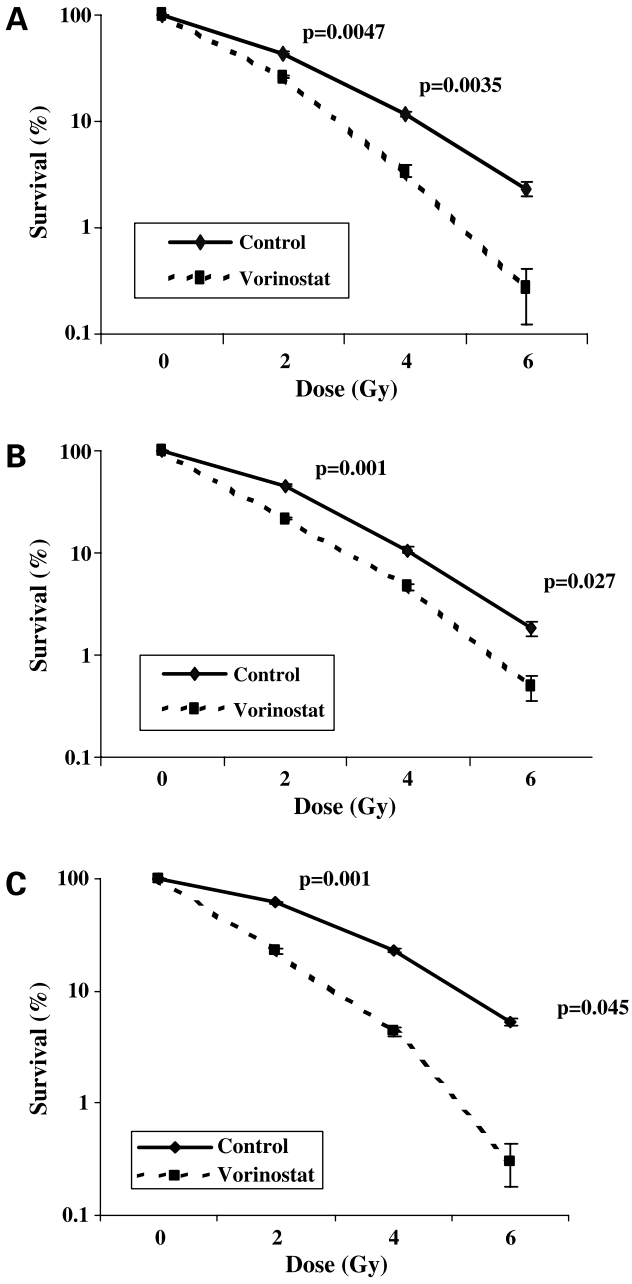

Figure 3. Treatment with Vorinostat sensitizes human tumor cells to ionizing radiation. Radiosensitization by Vorinostat was assessed on the basis of clonogenic cell survival assays. A375 (**A**), MeWo (**B**) and A549 (**C**) cells were pretreated with 2.5 μmol/L Vorinostat for 24 h, following which the drug was washed off and cells were irradiated with various doses of radiation and plated for cell survival. *Points,* average of three independent experiments each plated in triplicate; *bars,* SE.

1.24% with Vorinostat. The plating efficiencies for the cell lines were reduced by 2.5 μmol/L Vorinostat alone from 54% to 28% for A375, 46% to 23% for MeWo, and 58% to 35.5% for A549. Dose enhancement factors were calculated at 10% cell survival by dividing the dose of radiation from the radiation-only survival curve with the corresponding dose from the Vorinostat plus radiation curve. Dose enhancement factors were 1.34 for the MeWo cells, 1.4 for A375, and 1.7 for A549.

Modulation of DNA-Repair Gene Expression by Vorinostat

As an initial investigation into the mechanism responsible for Vorinostat-mediated radiosensitization, we examined the effect of Vorinostat treatment on the expression of proteins known to be involved in the repair of radiation-induced double-strand breaks. It has been reported that suppressed levels of these proteins enhance the radiosensitivity of human tumor cells. Western blot analysis for Ku70, Ku86, and Rad50 was done on whole-cell extracts harvested from Vorinostat-treated cells (Fig. 4). The levels of all these proteins decreased following Vorinostat treatment in the A375 melanoma cell line. There was a dose-dependent decrease in the levels of Ku70, Ku86, and Rad50 proteins following treatment with Vorinostat in both irradiated and nonirradiated cells. Decreases in Ku70 and Ku86 levels were evident at the 2.5 μmol/L dose of Vorinostat, with maximum decrease observed at the 10 μmol/L concentration. Ionizing radiation alone did not change Ku70, Ku86, or Rad50 protein levels at 4 hours postirradiation compared with untreated controls (Fig. 4).

Vorinostat Prolongs the Expression of γ-H2AX Foci

To test the hypothesis that Vorinostat impairs the repair of damaged DNA, γ-H2AX foci were assessed as indicators of DNA damage. As shown by the micrographs in Fig. 5A, γ-H2AX foci could be clearly distinguished after irradiation (2 Gy) of A375 cells. The average number of γ-H2AX foci per cell was counted in the micrographs and the results are presented in Fig. 5B. Treatment with Vorinostat alone had a slight effect on γ-H2AX foci that was not statistically significant compared with untreated controls. We also tested the significance of the increase in foci per cell seen for the Vorinostat plus radiation time points compared with the foci produced by radiation alone and those produced by Vorinostat alone. These tests indicated that the foci per cell for the Vorinostat plus radiation were significantly increased at 30 minutes ($P = 0.02$), 1 hour ($P = 0.005$), and 2 hours ($P = 0.008$) compared with the foci produced by Vorinostat alone. A similar increase in foci per cell for Vorinostat and radiation was seen when compared with radiation alone at 30 minutes ($P = 0.06$), 1 hour ($P = 0.008$), and 2 hours ($P = 1.006$). We also tested the significance of the increase in foci per cell seen for the Vorinostat plus radiation time points compared with the sum of foci produced radiation and Vorinostat when used as single agents. These tests indicated that the foci per cell Vorinostat plus radiation were significantly increased at 30 minutes ($P < 0.001$), 1 hour ($P < 0.001$), 2 hours ($P < 0.001$), and 24 hours ($P < 0.03$). This prolongation of γ-H2AX foci levels following the combination is consistent with an inhibition of the repair of DNA damage by Vorinostat.

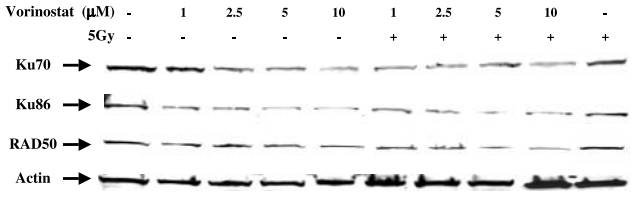

Figure 4. Involvement of DNA repair proteins in Vorinostat-mediated radiosensitization process. A375 cells were exposed to the designated concentration of Vorinostat for 24 h, following which they were irradiated with 5 Gy and incubated for an additional 4 h. At the end of the treatment, cells were collected for immunoblot analysis for Ku70, Ku86, and Rad50. Actin was used as a loading control.

Discussion

We have previously reported on the ability of HDAC inhibitors: sodium butyrate, phenylbutyrate, tributyrin, and trichostatin A (TSA) to restore radiosensitivity to human melanoma cells (16). Although sodium butyrate and phenylbutyrate synergistically interact with radiation, their short half-life *in vivo* and the requirement of high concentrations (millimolar) to achieve inhibition of HDAC activity make them unsuitable for clinical use (6). Similarly, use of trichostatin A is limited due to the excessive cytotoxicity associated with its mode of action. For that reason, HDAC inhibitors with longer half-life, lower toxicity, and greater stability have been developed, including Vorinostat (suberoylanilide hydroxamic acid), which is the prototype for a family of hybrid polar compounds that induce growth arrest in transformed cells (11, 17). Vorinostat induces differentiation and/or apoptosis in certain transformed cells in culture and is a potent inhibitor of HDACs (8–11). In addition, it has shown great promise in clinical trials (18). In the present study, we have shown that Vorinostat sensitizes a panel of human tumor cell lines of various origins to ionizing radiation. Our results are consistent with and confirm a recent report showing the ability of Vorinostat to synergistically enhance radioresponse of human tumor cells (19). Chinnaiyan et al. (19) have shown in their study the ability of Vorinostat to abrogate key DNA damage repair processes and attenuate prosurvival signals, thereby conferring radiosensitivity to human prostate cancer and glioma cells (19).

However, in spite of a growing interest in combining HDAC inhibitors with radiation as a clinical strategy for treating cancers, the exact molecular mechanism by which Vorinostat mediates its radiosensitizing effect is not known. Inhibition of HDAC activity facilitates chromatin relaxation and modifies gene transcription, both processes having

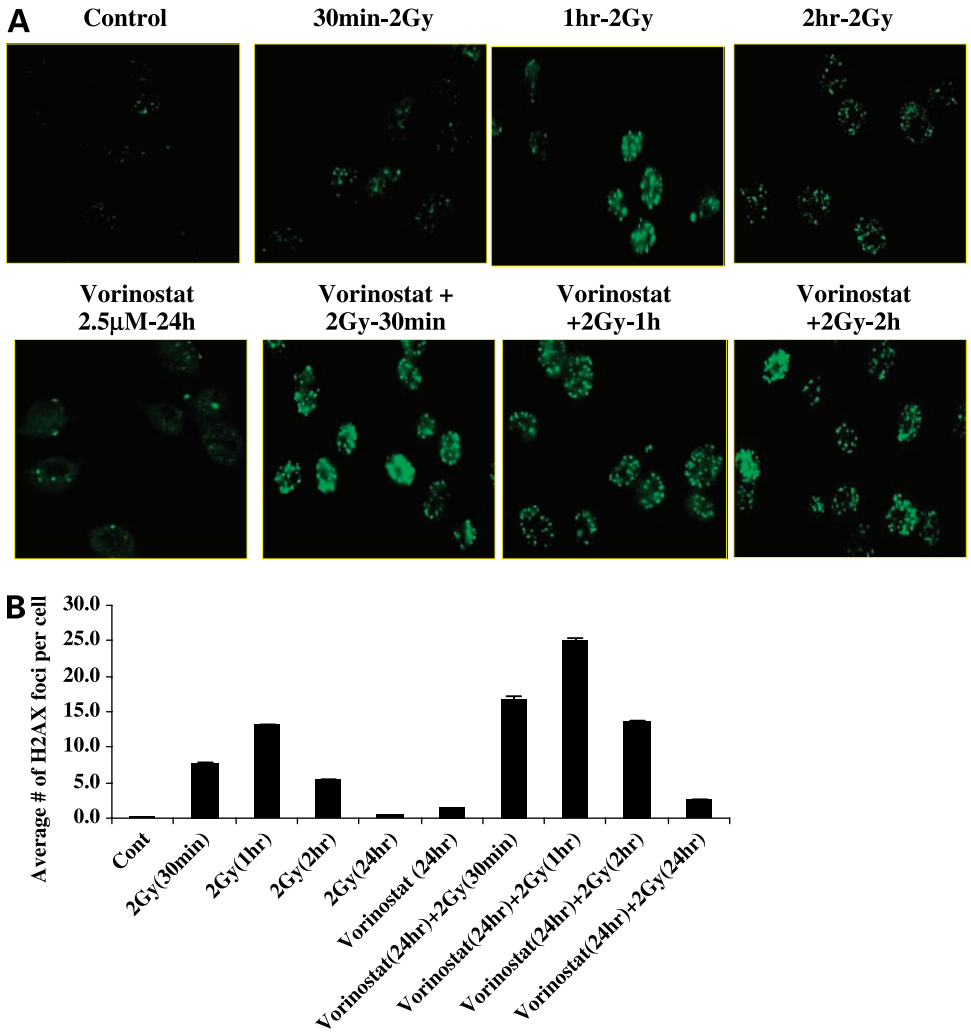

Figure 5. Influence of Vorinostat on radiation-induced γ-H2AX foci. A375 cells growing on slides in 35-mm dishes were pretreated with 2.5 μmol/L Vorinostat for 24 h, irradiated (2 Gy), and fixed at the specified times for immunocytochemical analysis of nuclear γ-H2AX foci. **A,** micrographs obtained from cells exposed to Vorinostat. **B,** quantitative analysis of foci present in the cells following various treatments. *Columns,* mean of three independent experiments.

implications in the regulation of radiosensitivity. In addition, actively transcribing genes have been shown to be generally more sensitive to the DNA damage produced by ionizing radiation thereby producing a favorable antitumor interaction between HDAC inhibitors and radiation. One explanation for this enhanced radioresponse following treatment with HDAC inhibitors could be their effect on DNA repair processes. Generally, radiosensitivity is governed by the capacity of the cell for efficient repair of radiation-induced lesions in the DNA, mainly the repair of double-strand breaks (20–22). There have been reports in the literature that HDAC inhibitors may radiosensitize by partly suppressing the DNA repair pathways (16, 19, 23–27). Notable in this regard are the reports that Vorinostat (19, 25, 26, 28), MS-275 (19, 25, 26, 28), and valproic acid (19, 25, 26, 28) suppress the expression of DNA repair–related genes following irradiation, and that Vorinostat reduces the levels of DNA-dependent protein kinase and Rad51 in human glioma and prostate cancer cells tested (19, 25, 26, 28).

Based on these observations, we examined the effect of Vorinostat on the levels of Ku70 and Ku86 proteins by Western blot analysis. Ku70, Ku86, and DNA-dependent protein kinase catalytic subunit are key proteins that participate in nonhomologus end joining pathway, which is especially important for repairing the radiation-induced double-strand breaks that are responsible for loss of clonogenic cell survival (20–22). Down-regulation of these key DNA repair proteins has been linked with an enhanced radioresponse (29, 30). In our study, levels of Ku70 and Ku86 proteins were suppressed in A375 cells treated with Vorinostat in a dose-dependent manner. Previous investigations have also shown that reduction in Rad50 levels increased the radiation sensitivity of tumor cell lines (31, 32). Therefore, we examined the effect of Vorinostat on the levels of Rad50 protein and found a dose-dependent decrease in Rad50 levels in the A375 cells following treatment. A second approach used by us to evaluate the involvement of DNA repair was to see if treatment with Vorinostat prolonged expression of phosphorylated H2AX

foci. At sites of radiation-induced DNA double-strand breaks, the histone H2AX becomes rapidly phosphorylated (γ-H2AX), forming nuclear foci that can be visualized by immunofluorescence microscopy (33, 34). Although the specific role of γ-H2AX in the repair of double-strand breaks is not well defined, a quantitative similarity between the induction and repair of DNA double-strand breaks and the formation and disappearance of γ-H2AX foci has been found (35–38). Thus, to assess involvement of DNA repair in Vorinostat-mediated radiosensitization, we tested whether Vorinostat treatment caused a prolonged expression of γ-H2AX foci indicating a decrease in the rate of repair of radiation-induced DNA double-strand breaks. Our results show that the number of radiation-induced γ-H2AX foci is higher in Vorinostat-treated cells compared with controls. This is evident at times up to 1 hour following irradiation, the time frame where the majority of double-strand breaks are repaired. Thus, this persistence of foci in the Vorinostat-treated cells is interpreted as an inhibition of the double-strand break repair pathway. Recent reports indicate that persistence of repair foci correlates with enhanced radiosensitivity (16, 28, 37). We conclude that Vorinostat radiosensitizes human tumor cells by suppressing the cellular capacity for repairing radiation-induced double-strand breaks.

In summary, we have shown that Vorinostat suppresses the cellular DNA repair capacity of human tumor cells and enhances their radioresponsiveness. Inhibition of DNA repair by Vorinostat may be due to a general suppression of the levels of proteins required for this process. In addition, Vorinostat restores radiation-induced apoptosis. Our observations reported here underscore the need for continued development of strategies for sensitizing human tumor cells to cancer therapies that kill cells by inducing DNA damage and/or apoptosis.

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