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Enhanced Radiation-Induced Cell Killing and Prolongation of γH2AX Foci Expression by the Histone Deacetylase Inhibitor MS-275

Kevin Camphausen,[1] William Burgan,[2] Michael Cerra,[2] Kelli A. Oswald,[2] Jane B. Trepel,[3] Min-Jung Lee,[3] and Philip J. Tofilon[2]

[1]Radiation Oncology Branch and [2]Molecular Radiation Therapeutics Branch, Radiation Oncology Sciences Program, [3]Medicine Branch, National Cancer Institute, Bethesda, Maryland

ABSTRACT

Histone deacetylase (HDAC) inhibitors are undergoing clinical evaluation for cancer therapy. Because HDAC modulates chromatin structure and gene expression, parameters considered to influence radioresponse, we have investigated the effects of the HDAC inhibitor MS-275 on the radiosensitivity of two human tumor cell lines (DU145 prostate carcinoma and U251 glioma). Acetylation status of histones H3 and H4 was determined as a function of time after MS-275 addition to and removal from culture medium. Histone acetylation increased by 6 h after MS-275 addition, reaching a maximum between 24 and 48 h of exposure; providing fresh drug-free medium then resulted in a decrease in histone acetylation that began by 6 h and approached untreated levels by 16 h. Treatment of cells with MS-275 for 48 h followed by irradiation had little or no effect on radiation-induced cell death. However, exposure to MS-275 before and after irradiation resulted in an increase in radiosensitivity with dose enhancement factors of 1.9 and 1.3 for DU145 and U251 cells, respectively. This MS-275 treatment protocol did not result in a redistribution of the cells into a more radiosensitive phase of the cell cycle or in an increase in apoptosis. However, MS-275 did modify the time course of γH2AX expression in irradiated cells. Whereas there was no significant difference in radiation-induced γH2AX foci at 6 h, the number of cells expressing γH2AX foci was significantly greater in the MS-275-treated cells at 24 h after irradiation. These results indicate that MS-275 can enhance radiosensitivity and suggest that this effect may involve an inhibition of DNA repair.

INTRODUCTION

Histone acetylation plays a critical role in modulating chromatin structure and regulating gene expression. Because of the aberrant acetylation status of histone proteins in tumor cells, the processes that govern this form of chromatin modification have been considered targets for cancer chemotherapeutic agents. Histone acetylation is determined by the opposing actions of histone acetyltransferase and histone deacetylase (HDAC), with each containing a number of family members (1, 2). Whereas oncogenesis has been associated with histone acetyltransferase inactivation, it is aberrant HDAC activity leading to the transcriptional repression of tumor suppressor genes that is considered a common event contributing to tumor formation (3). Given this molecular insight, the inhibition of HDAC activity has generated considerable interest as a cancer therapy approach. HDAC inhibition has been reported to induce tumor cell differentiation, apoptosis, and/or growth arrest, depending on the experimental system (4). However, for most cell lines generated from solid tumors, the primary effect of HDAC inhibition is one of cytostasis, as is the case for most target-directed chemotherapeutic agents.

In addition to slowing tumor cell proliferation, the HDAC inhibitors sodium butyrate and trichostatin A also have been shown to enhance tumor cell radiosensitivity (4, 5). Early work done in the 1980s demonstrated that sodium butyrate at relatively nontoxic concentrations increased the radiosensitivity on human colon carcinoma cell lines (5). However, because of its short half-life and low achievable serum concentration, sodium butyrate has limited clinical applicability (6–9). Similarly, trichostatin A has been shown to produce a significant increase in the *in vitro* radiosensitivity of a human colon carcinoma cell line (4). However, trichostatin A has excessive cytotoxicity apparently because of actions not involving histone acetylation and is unstable under *in vivo* conditions (10–12). Moreover, the mechanisms mediating the radiosensitization induced by sodium butyrate and trichostatin A have not been defined nor has the histone acetylation status been correlated to radiosensitization (4, 5).

With advances in drug discovery, there have been a number of new HDAC inhibitors developed with more favorable *in vivo* pharmacokinetic and toxicity profiles. However, given their disparate chemical structures and the lack of mechanistic information pertaining to the radiosensitization induced by sodium butyrate and trichostatin A, it cannot be assumed that these clinically applicable HDAC inhibitors will affect tumor cell radiosensitivity. One of the new HDAC inhibitors undergoing clinical investigation is the benzamide MS-275. It is a potent HDAC inhibitor and has been reported to have *in vivo* antitumor activity in a number of preclinical models (12). As an initial step in identifying a clinically applicable HDAC inhibitor for combination with radiotherapy, we have investigated the effects of MS-275 on the radiosensitivity of two human tumor cell lines. The data presented indicate that MS-275 enhances tumor cell radiosensitivity, which was correlated with changes in histone acetylation status. Furthermore, the MS-275-mediated radiosensitization was associated with a prolonged expression of γH2AX foci, suggesting a decrease in the repair of radiation-induced DNA double-strand breaks (DSBs).

MATERIALS AND METHODS

Cell Lines and Treatment. Two human tumor cell lines were evaluated: a glioma (U251) and a prostate carcinoma (DU145), which were obtained from American Type Culture Collection (Manassas, VA). Each cell line was grown in RPMI 1640 (Life Technologies, Inc., Rockville, MD) containing glutamate (5 mM) and 5% fetal bovine serum, and maintained at 37°C in an atmosphere of 5% CO_2 and 95% room air. MS-275, provided by the Developmental Therapeutics Program of the National Cancer Institute (Bethesda, MD), was dissolved in DMSO to a stock concentration of 10 mM and stored at −20°C. Cultures were irradiated using a Pantak (Solon, OH) X-ray source at a dose rate of 1.55 Gy/min.

Clonogenic Assay. To evaluate radiosensitivity, two treatment protocols were used. In the first, cells were irradiated as exponentially growing monolayer cultures in T25 tissue culture flasks. Immediately after irradiation, cultures were trypsinized into a single cell suspension, and a specified number of cells were seeded into the individual wells of a six-well tissue culture plate. In the second protocol, specified numbers of cells were first seeded into the wells of a six-well tissue culture plate, and radiation was delivered 6 h later. At this time, cells had attached but had not yet divided. In each protocol, plates were incubated for colony formation for 10–14 days. Colonies were stained with crystal violet, the number of colonies containing at least 50 cells was determined, and surviving fractions were calculated.

Cell Cycle Phase Analysis. Evaluation of cell cycle phase distribution was performed using flow cytometry. The treatment protocols were essentially the

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Requests for reprints: Philip J. Tofilon, Molecular Radiation Therapeutics Branch, Radiation Oncology Sciences Program, EPN/6015A, 6130 Executive Boulevard, MSC 7440, Rockville, MD 20892-7440. Phone: (301) 496-6336; E-mail: tofilonp@mail.nih.gov.

same as in the clonogenic survival experiments, except that the cells were seeded initially into 10-cm dishes. All of the cultures were subconfluent at the time of collection. Cultures were collected for fixation, stained with propidium iodide, and analyzed using flow cytometry as described previously by the Clinical Services Program at National Cancer Institute-Frederick (10).

Histone Acetylation Analysis. The acetylation status of histones H3 and H4 was determined by immunoblot analysis. After the specified MS-275 exposure, cells were scraped into PBS, washed, and resuspended in lysis buffer containing Tris [0.02 M (pH 7.4)], 1% Triton X-100, 0.02% 2-mercaptoethanol, and 2 ng/ml aprotinin. Proteins were solubilized by sonication and subjected to SDS-PAGE using Novex NuPage 4–12% gels and the NuPage MES buffer system according to the manufacturer's instructions (Invitrogen, San Diego, CA). After electrophoresis, the gel was electroblotted onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA). The nonspecific sites on the membrane were blocked at room temperature for 3 h with 5% nonfat milk in Tris-buffered saline supplemented with 0.1% Tween 20. Membranes were probed with antibodies to acetylated histone H3, acetylated histone H4 (Upstate Biotechnology, Lake Placid, NY), or actin (Chemicon International, Inc., Temecula, CA) diluted in blocking solution overnight at 4°C. Membranes then were washed three times in Tris-buffered saline supplemented with 0.1% Tween 20 and incubated with the appropriate FITC-conjugated secondary antibody at a 1:500 dilution in blocking solution for 1 h at room temperature. Membranes again were washed three times in Tris-buffered saline supplemented with 0.1% Tween 20 and incubated with an anti-FITC alkaline phosphatase-conjugated antibody (1:1000) for 1 h at room temperature. ECF Western blotting detection reagents (Amersham Biosciences, Piscataway, NJ) were used to detect the alkaline phosphatase tertiary antibody. Visualization and quantification were performed using the Typhoon scanner (Molecular Dynamics, Sunnyvale, CA).

Immunofluorescent Staining for γH2AX. Cells were grown and treated in chamber slides. At specified times, medium was aspirated, and cells were fixed in 4% paraformaldehyde for 10 min at room temperature. Paraformaldehyde was aspirated, and the cells were treated with a 0.2% NP40/PBS solution for 15 min. Cells then were washed in PBS twice, and the anti-γH2AX antibody (Upstate Biotechnology) was added at a dilution of 1:500 in 1% BSA and incubated overnight at 4°C. Cells again were washed twice in PBS before incubating in the dark with an FITC-labeled secondary antibody at a dilution of 1:100 in 1% BSA for 1 h. The secondary antibody solution then was aspirated, and the cells were washed twice in PBS. Cells then were incubated in the dark with 4′,6-diamidino-2-phenylindole (1 μg/ml) in PBS for 30 min and washed twice, and coverslips were mounted with an antifade solution (Dako Corp., Carpinteria, CA). Slides were examined on a Leica DMRXA fluorescent microscope (Wetzlar, Germany). Images were captured by a Photometrics Sensys CCD camera (Roper Scientific, Tucson, AZ) and imported into IP Labs image analysis software package (Scanalytics, Inc., Fairfax, VA) running on a Macintosh G3 computer (Apple, Cupertino, CA). For each treatment condition, γH2AX foci were determined in at least 50 cells. To account for the γH2AX foci appearing in unirradiated S phase cells, cells were classified as positive (*i.e.*, containing radiation-induced γH2AX foci) when more than five foci were detected (13).

RESULTS

Two human tumor cell lines of different histologic origins were used in these investigations: DU145 (prostate carcinoma) and U251 (glioma). These studies were based on the hypothesis that the HDAC inhibitory action of MS-275 modifies tumor cell radiosensitivity. Therefore, as an indicator of HDAC inhibition, initial experiments determined the effects of MS-275 on histone acetylation status in each cell line. These analyses used an MS-275 concentration of 300 nM, which results in surviving fractions of ~0.50 for both cell lines (see below), an appropriate survival range for subsequent combination experiments with radiation. Cells were exposed to MS-275 for 6–48 h, and the presence of acetylated histones H3 and H4 was determined by immunoblot analysis (Fig. 1*A*). In both cell lines, an increase in the levels of acetylated histones H3 and H4 was detected by 6 h after the addition of MS-275, reaching a maximum from 24 h to 48 h. To determine the dependence of the elevated acetylation levels on the continued presence of MS-275, cultures were first exposed to the HDAC inhibitor for 48 h to induce maximal acetylation. The medium then was removed, the cultures were rinsed in PBS, and fresh MS-275-free medium was added. Following this protocol, H3 and H4 acetylation levels began to decrease by 6 h after MS-275 removal, approaching control levels by 16–24 h (Fig. 1*B*). These results indicate that the hyperacetylation of histones H3 and H4 depends on the continuous exposure to MS-275.

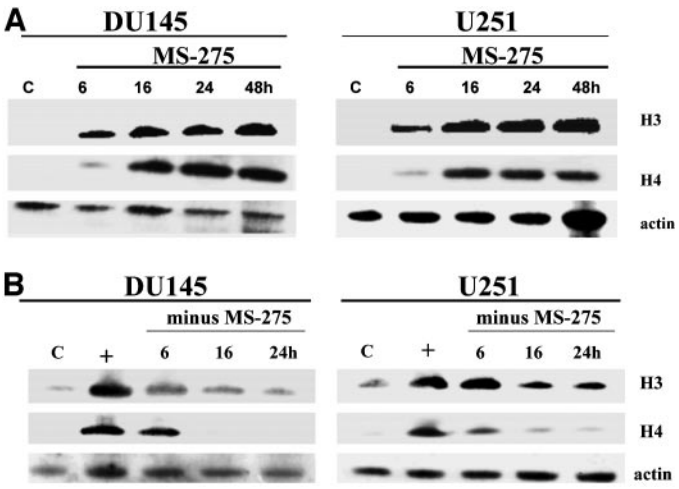

Fig. 1. Histone acetylation status determined after exposure to and removal of MS-275. *A*, cells were exposed for the designated period to MS-275 (300 nM) and collected for immunoblot analysis of acetylated histones H3 and H4. *B*, cultures were exposed for 48 h to MS-275 (300 nM), rinsed in PBS, and fed fresh MS-275-free medium. At the specified times, cells were collected for immunoblot analysis of acetylated histones H3 and H4. In these blots, the "+" refers to cells exposed for 48 h to MS-275 and collected for analysis. Each blot is representative of two independent experiments with actin used as a loading control. *C* refers to cultures exposed to the vehicle only (DMSO).

As an initial evaluation of the effects of MS-275 on tumor cell radiosensitivity, DU145 and U251 cultures were exposed to MS-275 (300 nM) for 48 h (time of maximum histone acetylation), irradiated, trypsinized into single cell suspensions, and plated into MS-275-free medium for determination of colony-forming efficiency. As illustrated by the survival curves shown in Fig. 2*A*, this MS-275 treatment protocol resulted in a minor increase in DU145 cell radiosensitivity and had no effect on the radioresponse of U251 cells. The surviving fractions after MS-275 exposure were only 0.79 ± 0.07 and 0.68 ± 0.03 for DU145 and U251 cells, respectively. Whereas histone acetylation has been associated with radiosensitization, the specific mechanisms involved have not been defined. Therefore, it was possible that an enhancement in radiation-induced cell killing required the hyperacetylation to be maintained after irradiation. As shown in Fig. 1*B*, histone acetylation begins to decrease by 6 h after removal of MS-275. To maintain acetylation levels after irradiation, cultures were exposed to MS-275 for 48 h, trypsinized into single cell suspensions, and seeded into MS-275-containing medium (300 nM). After allowing 6 h for cell attachment (but no division), cells then were irradiated. This somewhat nontraditional protocol was followed in an attempt to eliminate effects of trypsinization on postirradiation signaling/recovery processes and yet provide a continual exposure to MS-275, and thus continual histone hyperacetylation, before and after irradiation. Moreover, this protocol allows for the irradiation of single cells and not microcolonies, which eliminates the confounding parameter of multiplicity and its effects on apparent radiosensitivity. This contin-

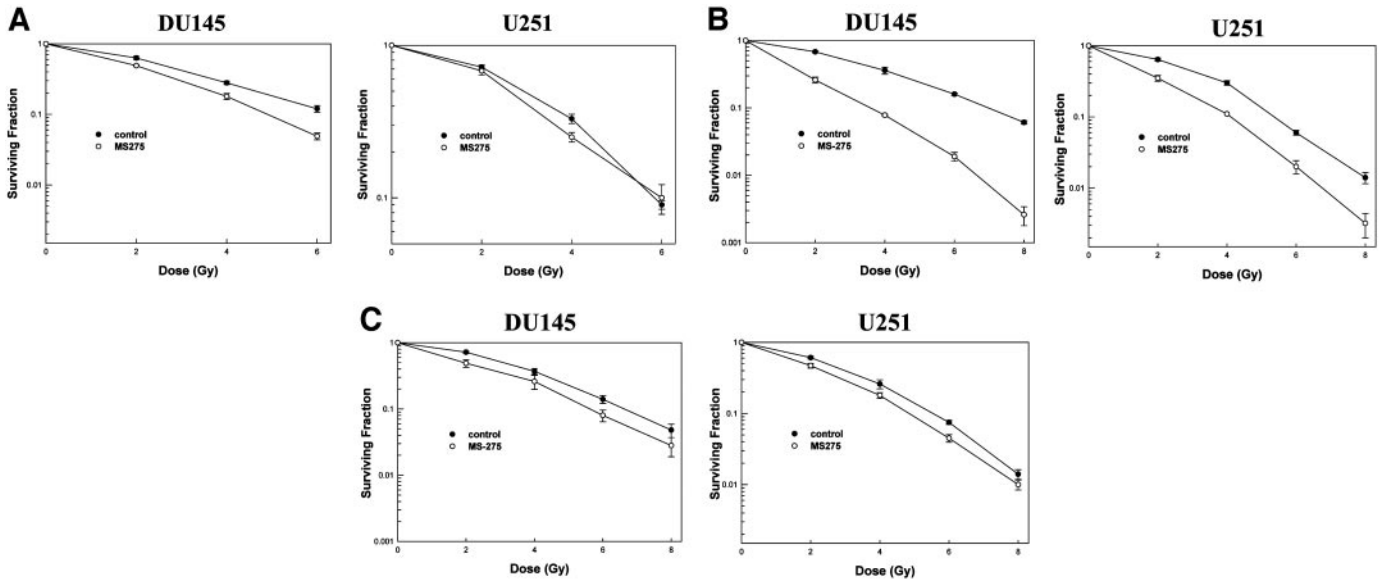

Fig. 2. The effects of MS-275 on tumor cell radiosensitivity. *A*, cultures were exposed for 48 h to MS-275 (300 nM), irradiated with graded doses of X-rays, trypsinized, and plated as single cells in MS-275-free medium for determination of colony-forming efficiency. *B*, cultures were treated for 48 h to MS-275 (300 nM), trypsinized, and plated as single cells in MS-275-containing medium (300 nM). After allowing 6 h for attachment, cells were irradiated with graded doses of X-rays, and colony-forming efficiency was determined in the continued presence of MS-275. *C*, 6 h after the plating, cells were irradiated with graded doses of X-rays, MS-275 (300 nM) was added immediately, and colony-forming efficiency was determined in the continued presence of MS-275. In each case, colonies were determined 10–14 days later, and survival curves were generated after normalizing for cell killing by MS-275 alone. Values represent the mean from three or four independent experiments; *bars*, ± SE. Control refers to exposure to vehicle (DMSO) only.

uous MS-275 exposure protocol resulted in a significant enhancement in the radiosensitivity of DU145 and U251 cells (Fig. 2*B*). The dose enhancement factors at a surviving fraction of 0.1 were 1.9 and 1.3 for DU145 and U251, respectively. The surviving fractions for MS-275 exposure only in this protocol were 0.51 ± 0.04 and 0.37 ± 0.09 for DU145 and U251 cells, respectively. The results shown in Fig. 2, *A* and *B*, suggested that simply the addition of MS-275 immediately after irradiation could be sufficient to enhance radiation-induced cell killing. However, when MS-275 was added only after irradiation, without a preirradiation exposure, there were only minor increases in radiosensitivity for both cell lines (Fig. 2*C*). For this protocol, the surviving fractions for MS-275 exposure only were 0.49 ± 0.04 and 0.69 ± 0.03 for DU145 and U251 cells, respectively. These data indicate that MS-275 exposure is required before and after irradiation to obtain maximal enhancement in radiosensitivity.

MS-275 has been reported to modify cell cycle distribution by decreasing the number of cells in S phase (12). Moreover, exposure to another HDAC inhibitor trichostatin A can result in the accumulation of cells in mitosis (11). Because such effects can impact radiosensitivity, flow cytometry was used to determine the cell cycle phase distribution of DU145 and U251 cells after MS-275 treatment according to the continuous exposure protocol (Fig. 2*B*). DU145 and U251 cells were exposed for 48 h to MS-275, trypsinized, and seeded into MS-275-containing medium. Six h after seeding, the time of irradiation in Fig. 2*B*, cells were collected for flow cytometric analysis. As shown in Table 1, there was essentially no difference in cell cycle phase distribution patterns for vehicle and MS-275-treated DU145 or U251 cells. These results indicate that redistribution of cells into a radiosensitive phase of the cell cycle does not account for the MS-275-mediated enhancement in radiation-induced cell killing. It should be noted that the MS-275 concentration used to enhance radiosensitivity in this study was 300 nM, whereas the concentration that affected the cell cycle distribution of a human ovarian carcinoma cell line was 1 μM (12).

MS-275 has been reported to induce apoptosis in some tumor cells lines but not in others (14). To determine whether apoptosis is involved in the radiosensitization shown in Fig. 2, flow cytometry was used to quantify the percentage of cells with a sub-G_1 DNA content. DU145 and U251 cells were exposed to MS-275 for 48 h, seeded into medium containing MS-275 (continuous exposure and maximum sensitization; Fig. 2*B*), and 6 h later irradiated with 4 Gy. Cells were collected for flow cytometric analyses at 24 and 48 h after irradiation. Individual treatments with MS-275 or radiation resulted in <10% apoptosis, and MS-275 did not enhance the level of radiation-induced apoptosis (data not shown).

As an initial investigation into the mechanism responsible for MS-275-mediated radiosensitization, γH2AX foci were used as an indicator of DNA damage (15–17). As shown by the micrographs in Fig. 3, γH2AX foci could be clearly distinguished after irradiation (5 Gy) of DU145 and U251 cells. In both cell lines, the number of cells expressing γH2AX foci remained constant from 1 to 6 h after irradiation (data not shown); by 24 h the percentage of positive cells began to decrease (Fig. 4). In cultures exposed to MS-275 before and after irradiation, the percentage of cells expressing foci was essentially the same at the 6-h time point as for 5 Gy only. However, at the 24-h time

Table 1 *Influence of MS-275 on cell cycle phase distribution*

Cells (DU145 and U251) were exposed to MS-275 (300 nM) for 48 h, trypsinized, and plated into MS-275-containing medium: Six h later, cells were collected for flow cytometric analysis of cell cycle phase distribution. Control refers to cultures exposed to vehicle (DMSO) following the same protocol. Data shown are representative from two independent experiments.

Treatment	$\%G_1$	%S	$\%G_2$/M
DU145			
Control	62.0	14.6	22.9
MS-275	61.1	10.7	26.0
U251			
Control	74.0	10.1	8.2
MS-275	68.2	9.0	11.1

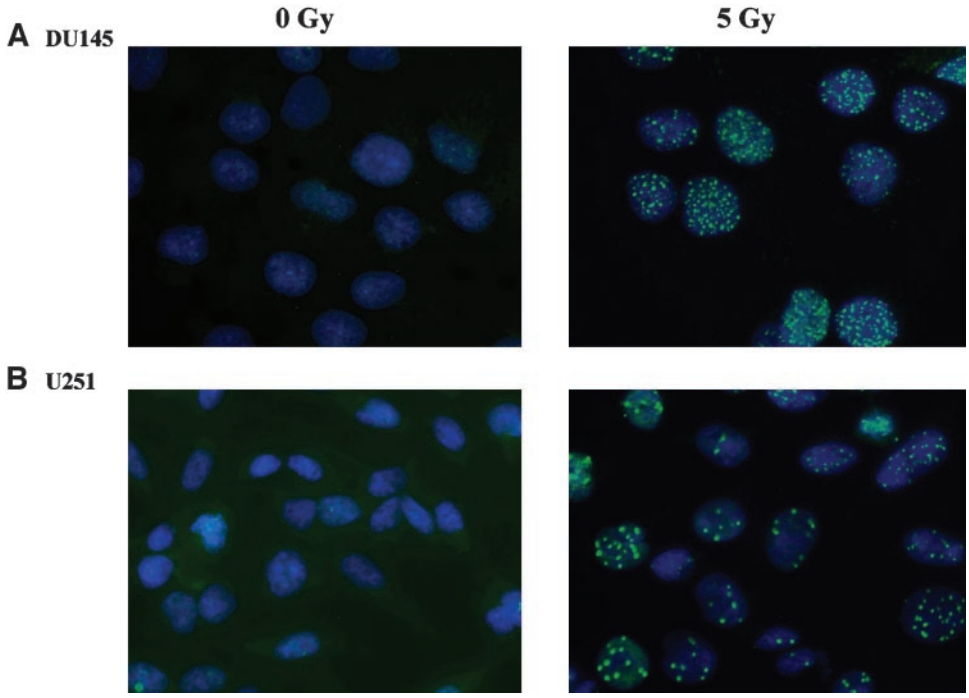

Fig. 3. Radiation-induced γH2AX foci. Micrographs obtained from control cells and cells that had received radiation (5 Gy) 6 h earlier, (*A*) DU145 and (*B*) U251.

point, the number of cells expressing γH2AX foci in cultures receiving the combined MS-275/radiation treatment was significantly greater as compared with the radiation-only group. Treatment with MS-275 alone had no significant effect on γH2AX foci. The maintenance of γH2AX foci levels suggests that the MS-275-mediated radiosensitization involves the inhibition of the repair of DNA damage (17).

DISCUSSION

Previous studies have shown that sodium butyrate and trichostatin A, two structurally disparate compounds with the common action of inhibiting HDAC, enhanced the *in vitro* sensitivity of tumor cells to radiation (4, 5, 18). Those reports suggested that HDACs might serve as a target for radiosensitizers. However, a significant impediment to the targeting of HDAC has been the lack of a clinically applicable HDAC inhibitor. Although sodium butyrate has shown some anti-

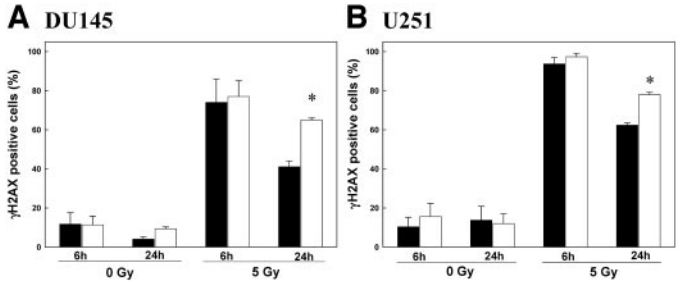

Fig. 4. Influence of MS-275 on radiation-induced γH2AX foci. DU145 (*A*) and U251 (*B*) cells growing in chamber slides were exposed to MS-275 (300 nM) for 24 h, irradiated (5 Gy), and fixed at the specified times for immunocytochemical analysis of nuclear γH2AX foci. ■, data from vehicle-treated cells; □, data from cells exposed to MS-275. Foci were evaluated in 50 nuclei per treatment per experiment; the data represent the mean of three independent experiments; *bars*, ± SE. Cells with more than five foci per nucleus were classified as positive for radiation-induced γH2AX. *, $P < 0.01$ according to Student's *t* test (5 Gy *versus* MS-275/5 Gy).

cancer activity in case reports and small studies managing leukemia, the short half-life and low achievable concentration in serum have prevented its effective use as an anticancer agent (6–9). A number of HDAC inhibitors with *in vivo* activity have been developed recently. One such agent is the benzamide MS-275. This compound has potent HDAC inhibitory activity and has been reported to inhibit tumor growth in a number of experimental *in vivo* tumor models (12, 14). MS-275 has entered recently into Phase I clinical trials. The combination of the clinical applicability of MS-275 and previous HDAC inhibitors having a radiation enhancement effect was the stimulus for investigating the combination of MS-275 and radiation.

As shown in Fig. 2, MS-275 can enhance significantly the radiosensitivity of human tumor cell lines. However, unlike sodium butyrate and trichostatin, there was a requirement for MS-275 to be present before and after irradiation to achieve the maximal increase in radiosensitivity (4, 5). The requirement for different treatment protocols for MS-275 *versus* sodium butyrate and trichostatin A suggests that different mechanisms are involved. Given that the chemical formulas of clinically applicable HDAC inhibitors range from the benzamides (MS-275) to hydroxamates to aliphatic acids to cyclic tetrapeptides, it is likely that the process of HDAC inhibition will vary between compounds and, furthermore, that each compound will have an additional set of cellular activities (19). Therefore, it will be difficult to generalize the radiosensitizing potential among HDAC inhibitors, underscoring the need to investigate the individual compounds.

A possible explanation for the requirement of preirradiation and postirradiation exposure to MS-275 may lie in the time course of histone hyperacetylation. Whereas maximal hyperacetylation was achieved by 24–48 h of exposure, the degree of acetylation rapidly decreased by 6 h after removal of MS-275, approaching control levels by 16–24 h. The failure of MS-275 administered only before or only after irradiation to significantly enhance radiosensitivity suggests that the hyperacetylation must be present at the time of and for at least 6 h after radiation exposure. Whereas sodium butyrate and trichostatin A

have been reported to enhance radiosensitivity, the sensitizing effect of these compounds has not been correlated directly to histone acetylation status (4, 5). Butyrate-induced sensitization was attributed to cell differentiation; whether histone acetylation status was affected at the radiosensitizing dose or over the treatment time in the cell models examined was not addressed (4). Trichostatin A-induced radiosensitization was attributed to chromatin compaction and correlated with histone phosphorylation (5). Moreover, trichostatin A exposure causes cells to accumulate in mitosis, a radiosensitive phase of the cell cycle, which complicates the mechanistic interpretation of the observed radiosensitization (11). To our knowledge, the results presented here, although far from establishing a causal relationship, are the first to demonstrate a correlation between MS-275-induced hyperacetylation and the enhancement of radiosensitivity.

Although histone hyperacetylation is presumed to be involved, the downstream events responsible for the MS-275-mediated enhancement in radiosensitivity remain to be defined. In general, HDAC inhibition relaxes chromatin structure, which in turn affects gene transcription. Each of these processes individually has been implicated in the regulation of radiosensitivity. In addition, HDACs can affect the acetylation of nonhistone proteins such as p53, E2F, and tubulin (11, 20). Thus, delineating the specific molecular pathways through which HDACs affect radiosensitivity is likely to be complex. However, we have begun to address the general process that may be involved in the enhancement of radiation-induced cell killing. The data presented indicate that accumulation of cells in a radiosensitive phase of the cell cycle or an increase in apoptotic cells death is not responsible for the radiosensitization induced by MS-275.

A critical event in determining radiosensitivity is the repair of DNA DSBs. γH2AX expression has been established recently as a sensitive indicator of DSBs induced by clinically relevant doses of ionizing radiation (15). At sites of radiation-induced DNA DSBs, the histone H2AX becomes phosphorylated rapidly (γH2AX), forming readily visible nuclear foci (15, 16). Although the specific role of γH2AX in the repair of DSBs has not been defined, recent reports indicate the dephosphorylation of γH2AX and dispersal of γH2AX foci in irradiated cells correlate with the repair of DNA DSBs (17, 21, 22). Moreover, Macphail *et al.* (23) in their study of 10 cell lines reported that the loss of γH2AX correlates with clonogenic survival after irradiation. The results presented here in which the expression of γH2AX in cells treated with the MS-275/radiation combination was similar to radiation exposure only at 6 h, but significantly greater at 24 h, are thus suggestive of an inhibition of DNA repair. This effect is consistent with results obtained after irradiation of G_0 lymphocytes exposed to the HDAC inhibitor sodium butyrate (24). In those studies, Stoilov *et al.* (24) showed that although sodium butyrate had no effect on the initial level of radiation-induced DNA damage or on the repair of single-strand breaks or alkali labile sites, it did inhibit the repair of chromosome breaks as detected by the premature chromosome condensation technique, suggestive of an inhibition of DNA DSBs.

H2AX appeared particularly relevant to this HDAC inhibitor study because this H2A histone variant, as most other histones, is acetylated at specific lysine residues (25). At this time, it is unclear whether MS-275 exposure affects the acetylation status of histone H2AX. Given the varying susceptibilities of histones to acetylation (26), the apparent selectivity of some HDAC inhibitors (1), and the lack of an available antibody to acetylated H2AX, this possibility requires a considerably more in-depth evaluation. However, if this were the case, the modulation of acetylation status of H2AX might directly influence its putative role in the response to DNA damage. Alternatively, it is also possible that the maintenance of radiation-induced γH2AX foci in MS-275-treated cells serves as merely a general indicator of unrepaired DSBs. Clearly, additional investigations are required to define the molecular processes behind these observations. However, these results do suggest that enhanced radiosensitivity induced by MS-275 involves an inhibition of the repair of DNA damage.

The use of molecularly targeted agents has received considerable attention as a cancer therapy strategy; to date, most of these agents are only cytostatic against solid tumors. However, in some cases, these agents are directed against molecules that also can affect radiosensitivity, suggesting that advantages could be obtained through the combination with radiotherapy. Such a situation appears to exist for MS-275 and its ability to inhibit HDACs. The data presented here indicate that MS-275 enhances the radiation-induced cell killing in two human tumor cell lines of different histologic origins, suggesting that the effect is not tumor-type specific and may serve as a general strategy for enhancing tumor cell radiosensitivity. The requirement for MS-275 exposure before and after irradiation established by these *in vitro* studies would appear important for the design and evaluation of *in vivo* MS-275/radiation combination protocols. Moreover, these results suggest that histone acetylation status can be used as a marker for a radiosensitizing concentration of MS-275, which would further aid in the design of effective *in vivo* combination protocols. The goal of this study was to evaluate the combination of MS-275 and radiation as a potential cancer therapy strategy. Whether the results can be extended to other clinically relevant HDAC inhibitors remains to be determined.

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