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Gleevec-Mediated Inhibition of Rad51 Expression and Enhancement of Tumor Cell Radiosensitivity

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ABSTRACT

Rad51 is an essential component of the homologous DNA repair pathway and has been implicated as a determinant of cellular radiosensitivity. Gleevec is a relatively specific inhibitor of c-Abl, a tyrosine kinase that can play a role in the regulation Rad51. The aim of this study was to determine the effects of Gleevec on Rad51 levels and the radiosensitivity of two human glioma cell lines and a nonimmortalized normal human fibroblast cell line. Exposure of both glioma cell lines to radiation resulted in an increase in Rad51 expression; Gleevec treatment alone reduced Rad51 expression. When glioma cells were pretreated with Gleevec, radiation-induced Rad51 expression and nuclear foci formation were reduced. Accordingly, pretreatment of the glioma cells with Gleevec resulted in an enhancement in their radiosensitivity. These data indicate that Gleevec enhances radiation-induced tumor cell killing and suggest that the mechanism involves the reduction in Rad51 levels. In contrast to the glioma cell lines, radiation or Gleevec treatments had no effect on Rad51 expression or foci formation in the normal fibroblast cells. Consistent with these observations, Gleevec did not modify the radiosensitivity of the normal cell line. These results suggest that Rad51 expression is subject to different regulatory processes in the glioma and normal cell lines and further suggest that Rad51 may be an appropriate target for selectively enhancing the radiosensitivity of brain tumor cells.

INTRODUCTION

Attempts to develop clinically relevant radiosensitizers have traditionally used an empirical approach combining radiation with standard cytotoxic chemotherapeutic agents. Although often effective in experimental models, the results obtained when these combinations are applied in a clinical setting have been generally less than expected. Recently, arising from an increased understanding of the molecular mechanisms of radioresponse, attempts to increase tumor radiosensitivity have begun to use a target-based approach. Potential targets are often those molecules that participate in a process that contributes to cell survival. Among the events critical to the survival response after irradiation is DNA repair, specifically the repair of DSBs.[2] Although there are many molecules that participate in DNA repair, Rad51 would appear to be a reasonable candidate as a target for selective tumor cell radiosensitization.

Rad51 is a critical component of the DNA DSB repair pathway (1, 2). Exposure of cells to ionizing radiation induces the formation of Rad51 nuclear foci at sites of DSBs, which are then involved in homologous recombinational repair (3–5). A number of studies using genetic approaches have shown that modulating Rad51 levels affects the level of radiation-induced cell death. Vispe *et al.* (6) demonstrated that the plasmid-mediated overexpression of Rad51 in Chinese hamster ovary cells resulted in a decrease in radiosensitivity. Reduction in Rad51 levels using antisense oligonucleotides or a ribozyme increased the radiation sensitivity of glioma cells (7, 8) and prostate carcinoma cells (9), respectively. Moreover, a recent study (10) reported that Rad51 is expressed at higher levels in tumor cells as compared with normal cells, suggesting that targeting this repair protein has the potential for a tumor-selective effect on radiosensitivity.

Whereas genetic approaches are feasible, the ability to compromise Rad51 using a clinically applicable drug would aid considerably in the evaluation of this repair protein as a potential therapeutic target for radiosensitization. Although the molecular regulation of Rad51 levels and activity has not been completely defined, the tyrosine kinase c-Abl appears to play a significant role (11). Slupianek *et al.* (12) reported that the fusion tyrosine kinase BCR/Abl elevates Rad51 expression, stimulates DNA repair, and induces resistance of leukemic cells to cisplatin and mitomycin C. These investigators then showed that STI571 (Gleevec), which is a relatively specific inhibitor of Abl kinase, reduced the elevated Rad51 levels and enhanced the sensitivity of leukemic cells to these chemotherapeutic agents (13). Given the role of Rad51 in regulating radioresponse and based on these reports using leukemic cells, we have determined the effects of Gleevec on the radiosensitivity of two cell lines initiated from solid tumors. In these studies, exposure of U251 and SF539 glioma cells to Gleevec resulted in reduced Rad51 expression, suppressed formation of radiation-induced Rad51 foci and increased the level of radiation-induced cell death. In contrast, Gleevec had no effect on these parameters in the nonimmortalized normal human fibroblast cell line N1.

MATERIALS AND METHODS

Reagents. Gleevec, provided by Novartis (Basel, Switzerland), was dissolved in DMSO to a stock concentration of 10 mM and stored at –20°C. Antibodies to Rad51 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and antibodies to actin were obtained from Chemicon (Temecula, CA).

Cell Lines. The two human glioma tumor cell lines evaluated in this study (U251 and SF539) were obtained from American Type Culture Collection (Manassas, VA). Each cell line was grown in RPMI 1640 (Life Technologies, Inc., Rockville, MD) containing 5 mM glutamate and 5% FBS and maintained at 37°C in an atmosphere of 5% CO_2 and 95% room air. The nonimmortalized normal human fibroblast cell line N1 (provided by Dr. W. A. Brock; The University of Texas M. D. Anderson Cancer Center, Houston, TX) was grown in DMEM containing gentamicin (10 μg/ml), 20% fetal bovine serum, and glutamine (2 mM). This fibroblast cell line was initiated from normal skin and used for experiments in passages 1 and 6.

Irradiation. Cultures were irradiated using a Pantak (Solon, OH) X-ray source at a dose rate of 1.55 Gy/min.

Cell Cycle Analysis. Evaluation of cell cycle phase distribution was performed using FCM. After the designated treatment of actively proliferating cultures, floating and attached cells were collected for fixation, stained with propidium iodide, and analyzed using FCM (14) by the Clinical Services Program at National Cancer Institute-Frederick.

Immunoblot Analysis. Treated cells were scraped into PBS and centrifuged, and the cell pellet was resuspended in 3 volumes of extraction buffer [20

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[2] The abbreviations used are: DSB, double-strand break; FCM, flow cytometry; RT-PCR, reverse transcription-PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MID, mean inactivation dose; CML, chronic myelogenous leukemia.

mM HEPES (pH 7.9), 400 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP40, 1 mM DTT, 1 mM phenylmethylsulfonyl fluoride, 1 mg/ml aprotinin, 1 mg/ml leupeptin, 250 mg/ml benzamide, 50 mM NaF, and 1 mM NaO_3V_4]. Immunoblot analysis was then performed as described previously (15). Visualization and quantification were performed using the Typhoon scanner (Molecular Dynamics, Sunnyvale, CA).

mRNA Analysis (RT-PCR). RNA was extracted using the RNAeasy Mini Kit from Qiagen (Valencia, CA) following the manufacturer's instructions, and RNA quality was assessed spectrophotometrically. Ten ng of total RNA were then used for one-step real-time quantitative RT-PCR reactions using an ABI Prism 7700 (Applied Biosystems, Foster City, CA) thermal cycler. Oligonucleotides used for forward and reverse reactions and TaqMan Probes were acquired from the Assay-On-Demand Gene Expression Products from Applied Biosystems. Reactions contained 1× TaqMan Universal PCR Master Mix, 1× reverse transcriptase, 350 nM forward and reverse primers, and 100 nM TaqMan probe. The reverse transcription conditions involved an incubation of the sample at 48°C for 30 min. The amplification conditions comprised a 10-min polymerase activation at 95°C and 40 cycles at 95°C for 15 s and 60°C for 1 min. Relative expression of Rad51 was determined by normalization to both the GAPDH expression and to the Rad51/GAPDH ratio in the control sample using the comparative C_T method (16). All real-time quantitative RT-PCR experiments included a no template and no probe control and were performed in triplicate in three independent experiments.

Immunocytochemistry. Cells were grown and treated in chamber slides. At specified times after treatment with Gleevec and/or radiation, cells were fixed in 4% paraformaldehyde for 10 min at room temperature followed by a 1% NP40/PBS solution for 15 min and then washed twice in PBS. The primary antibody was added at a dilution of 1:100 in 1% BSA and incubated overnight at 4°C followed by rinsing in PBS and incubation in the dark with a FITC-labeled secondary antibody at a dilution of 1:100 in 1% BSA at room temperature for 1 h. After washing in PBS, cells were incubated in the dark with 4′,6-diamidino-2-phenylindole (1 μg/ml) for 30 min. Coverslips were mounted in an antifade solution (Dako Corp., Carpinteria, CA) and sealed with nail polish. Nuclear foci were evaluated using a Leica DMRXA fluorescent microscope (Wetzlar, Germany). Images were captured by a Photometrics Sensys charge-coupled device camera (Roper Scientific, Tucson, AZ) and imported into IP Labs image analysis software package (Scanalytics, Inc., Fairfax, VA). Nuclei were classified as positive for Rad51 foci formation when ≥5 foci were detected per nuclei. At least 100 cells selected at random were counted per experimental point.

Clonogenic Survival Analysis. Cultures were trypsinized to generate a single cell suspension, and a specified number of cells were seeded into each well of a 6-well tissue culture plate. After allowing cells time to attach overnight, Gleevec or DMSO (vehicle control) was added at specified concentrations, and plates were irradiated 6 h later. Twelve to 14 days after seeding, colonies were stained with crystal violet, the number of colonies containing at least 50 cells was determined, and surviving fractions were calculated. Radiosensitivity was quantified according to the MID, representing the area under the curve (17). The effects of Gleevec on radiosensitivity were expressed as the radiation enhancement ratio, defined as (MID of control group)/(MID of treatment group).

RESULTS

Gleevec had been reported to reduce Rad51 levels in leukemic cells (13). To ascertain whether a similar effect occurs in cells originating from solid tumors, the effects of Gleevec on Rad51 expression in two glioma cell lines were determined (Fig. 1). After 24 h of Gleevec exposure, Rad51 protein levels were reduced in the U251 cells at concentrations beginning at 5 μM and in SF539 cells at concentrations beginning at 2.5 μM (Fig. 1*A*). After addition of Gleevec (7.5 μM) to the culture media, Rad51 protein began to decrease after 6 h, reaching a significant reduction by 24 h in both cell lines (Fig. 1*B*). To determine whether Gleevec also resulted in suppression of Rad51 mRNA levels, quantitative real-time RT-PCR analysis was performed. Following a similar time course used for the reduction in protein, Rad51 mRNA levels in both glioma lines were decreased at

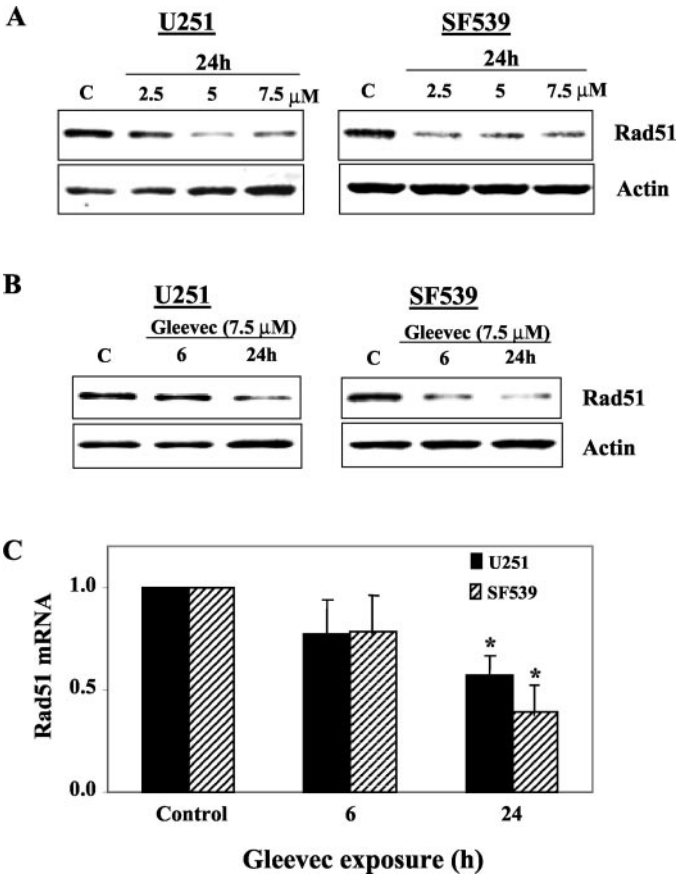

Fig. 1. The effect of Gleevec on Rad51 levels in tumor cells. *A*, Rad51 protein levels as a function of Gleevec concentration. Cells were exposed to the designated Gleevec concentration for 24 h and collected for immunoblot analysis. *B*, Rad51 protein levels as a function of time after Gleevec addition. Cells were exposed to Gleevec (7.5 μM) for the specified time period and collected for immunoblot analysis. The immunoblots shown are representative of at least two independent experiments with actin serving as a protein loading control. *C*, Rad51 mRNA levels after Gleevec exposure. Cells were exposed to Gleevec (7.5 μM) for the specified time period and collected for RT-PCR analysis of the Rad51/GAPDH mRNA ratios. Each value represents the mean ± SE for three independent experiments. Statistical analysis was performed using unpaired Student's *t* test. *, $P < 0.05$ as compared with control values. For each set of data, *Control* or *C* indicates cultures exposed to the vehicle control DMSO.

6 h and significantly reduced after 24 h of Gleevec (7.5 μM) exposure (Fig. 1*C*). These results indicate that the Gleevec-mediated decrease in Rad51 protein levels was, at least in part, the result of a reduction of Rad51 mRNA, suggesting inhibition of transcription.

Before combining Gleevec with radiation, it was first necessary to determine the effects of radiation on Rad51 expression in the glioma cell lines. Immunoblot analysis at 24 h after irradiation indicates that Rad51 protein levels increased in a dose-dependent manner in both the U251 and SF539 cell lines (Fig. 2*A*). In both cell lines, the maximum increase after 10 Gy was reached at 24 h after irradiation (Fig. 2*B*). To determine whether there was a radiation-induced increase in Rad51 mRNA levels, quantitative real-time RT-PCR analysis was performed. As shown in Fig. 2*C*, the Rad51 mRNA levels in both cell lines were maximally induced at 6 h and were returning to control levels by 24 h after irradiation. These results indicate that the radiation-induced increase in Rad51 protein occurs through an increase in mRNA, suggestive of an increase in transcription. To our knowledge, this is the first report of radiation inducing Rad51 expression in mammalian cells.

Experiments were then performed to determine whether Gleevec influences radiation-induced Rad51 expression in the glioma cell lines (Fig. 3). For analysis of Rad51 protein levels, cells were exposed to

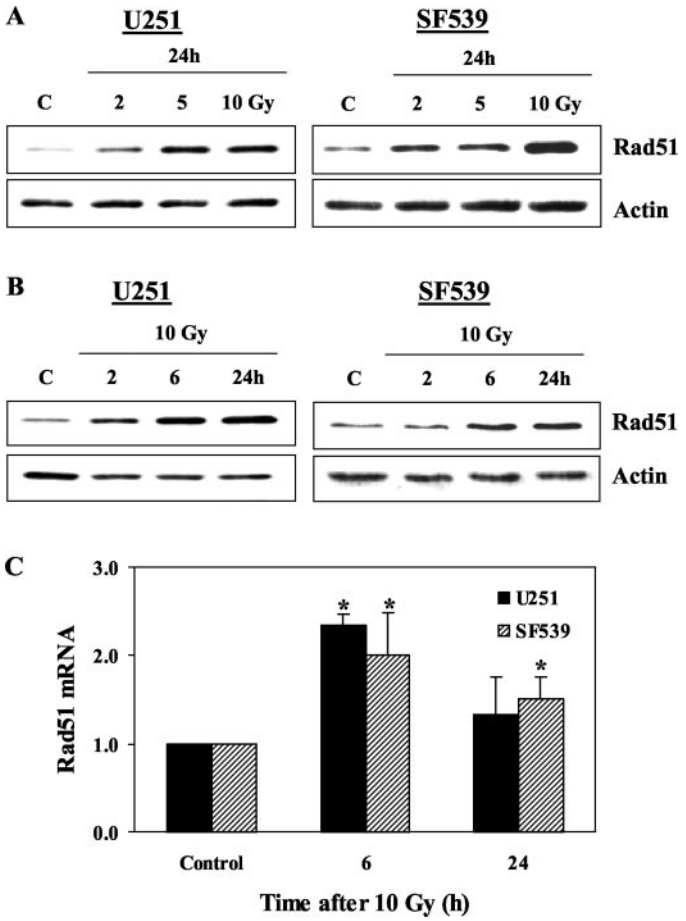

Fig. 2. The effect of radiation on Rad51 levels in tumor cells. *A*, Rad51 protein levels as a function of radiation dose. Cells were exposed to the designated dose of radiation and collected 24 h later for immunoblot analysis. *B*, Rad51 protein levels as a function of time after irradiation. Cells were exposed to 10 Gy and collected for immunoblot analysis at the designated time points. The immunoblots shown are representative of at least two independent experiments with actin serving as a protein loading control. *C*, Rad51 mRNA levels after irradiation. Cells were exposed to 10 Gy and collected at the specified times for RT-PCR analysis of the Rad51/GAPDH mRNA ratios. Each value represents the mean ± SE for three independent experiments. Statistical analysis was performed using unpaired Student's *t* test. *, $P < 0.05$ as compared with control values. For each set of data, *Control* or *C* indicates untreated cultures.

Gleevec for 6 or 24 h, irradiated (10 Gy), and then collected for analysis 24 h after irradiation. The 24 h collection point after irradiation was chosen because the maximum increase in Rad51 protein levels, as shown in Fig. 2*B*, is reached by this time. Therefore, the total Gleevec exposure time for these studies was 30 and 48 h for the 6 and 24 h time points, respectively. For U251 cells (Fig. 3*A*), exposure to Gleevec alone resulted in significant decreases in Rad51 protein levels at each time point examined as compared with untreated control cells; radiation alone increased Rad51 levels, consistent with the results shown in Figs. 1 and 2. When Gleevec-pretreated cells were irradiated, Rad51 levels were reduced as compared with the irradiation alone control. However, for both the 6 and 24 h Gleevec pretreatments, Rad51 remained elevated after irradiation as compared with the unirradiated control cells. Therefore, Gleevec reduced the radiation-stimulated increase in Rad51 protein levels. The effects of Gleevec on radiation-induced Rad51 mRNA in U251 cells are shown in Fig. 3*B*. After irradiation alone, Rad51 mRNA levels reached a maximum increase at 6 h (see Fig. 2*C*); thus the effects of a 6- and 24-h Gleevec pretreatment on Rad51 mRNA levels were determined at 6 h after exposure to 10 Gy. Gleevec reduced Rad51 mRNA levels in nonirradiated cells. However, with the combined treatment, Rad51 mRNA expression remained above that of unirradiated, vehicle-treated control cells. Similar patterns of response of both Rad51 protein levels and Rad51 mRNA levels were obtained for the SF539 cells (Figs. 3, *C* and *D*). Although Gleevec alone suppressed the

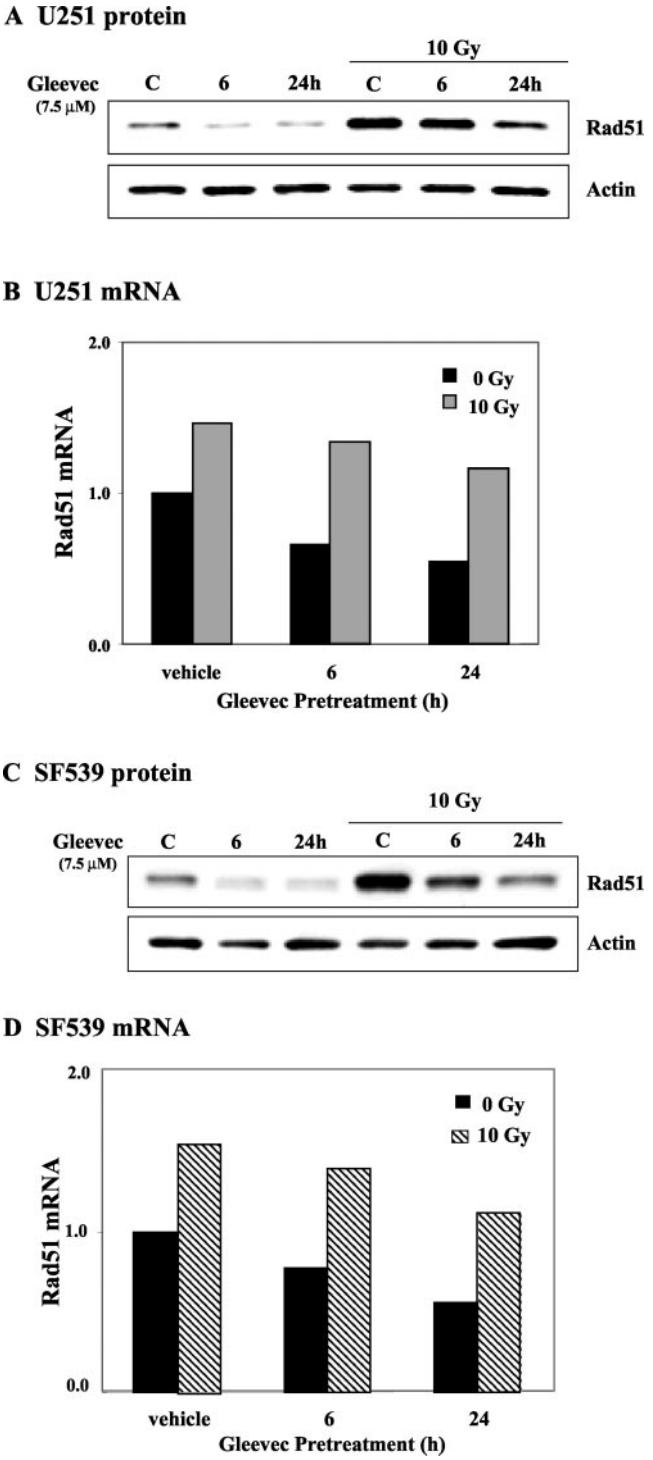

Fig. 3. Rad51 expression after exposure of glioma cells to the combination of Gleevec and radiation. *A*, U251 Rad51 protein levels after pretreatment with Gleevec (7.5 μM) for 6 or 24 h before irradiation with 10 Gy. Cells were then collected for immunoblot analysis at 24 h after irradiation. *C* indicates cells treated with vehicle control DMSO. *B*, U251 Rad51 mRNA levels after pretreatment with Gleevec (7.5 μM) for 6 or 24 h before irradiation with 10 Gy. Cells were collected for RT-PCR analysis of the Rad51/GAPDH mRNA ratios at 6 h after 10 Gy. *C*, SF539 Rad51 protein levels after the same treatment protocol as described for U251 in *A*. *D*, SF539 Rad51 mRNA levels after the same protocol and procedures as described for U251 in *B*. Immunoblots and mRNA expression values are representative of at least two independent experiments.

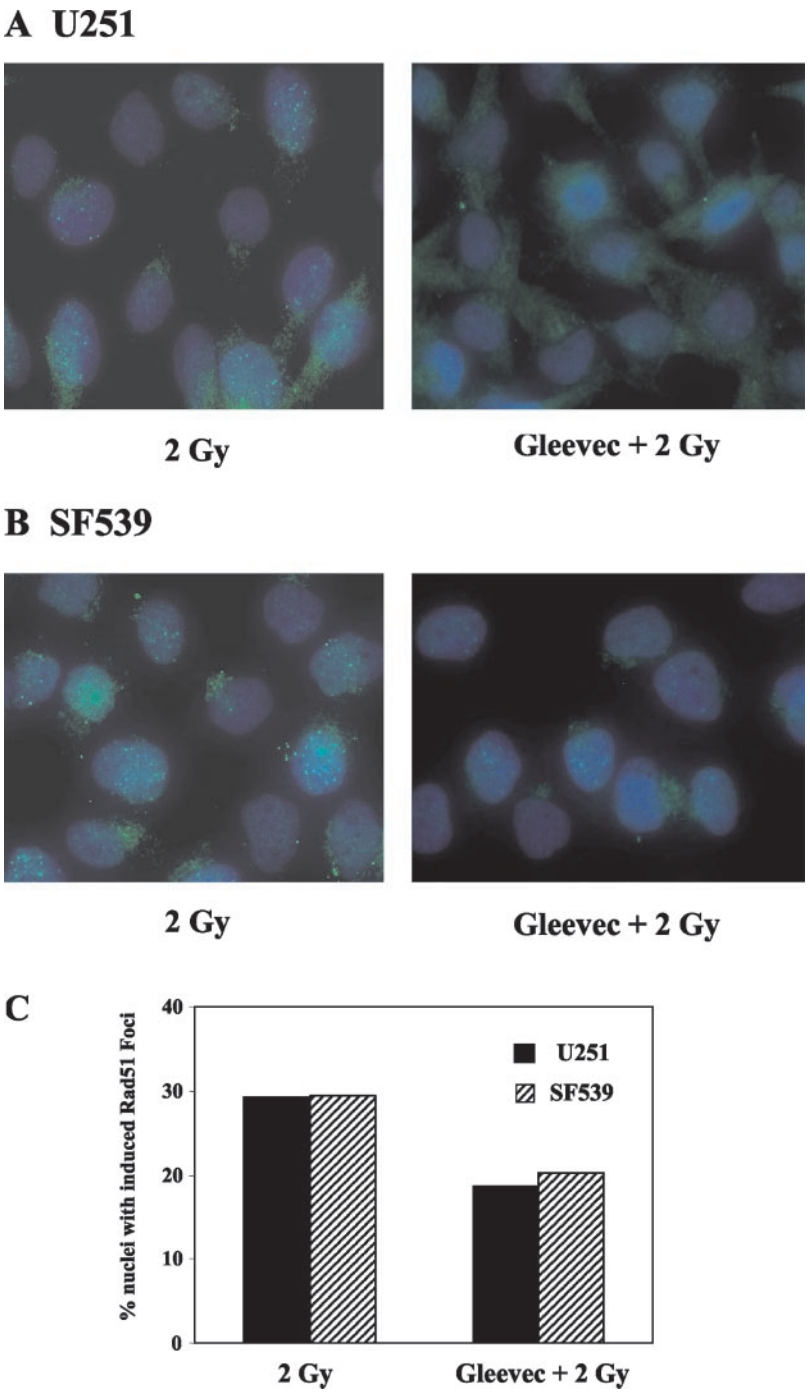

Fig. 4. Radiation-induced Rad51 foci in Gleevec treated glioma cells. Glioma cell cultures cells were exposed to Gleevec (7.5 μM) at 6 h before irradiation with 2 Gy and fixed for foci evaluation 6 h later. Representative micrographs are shown for U251 (*A*) and SF539 (*B*) cells. *C*, quantification of Rad51 foci in U251 and SF539 cells exposed to 2 Gy or the Gleevec/radiation combination exemplified in *A* and *B*. Values are representative of two independent experiments in which 100 nuclei were evaluated per treatment group.

radiation-induced increase in Rad51 protein levels in SF539 cells, for each pretreatment exposure time, Rad51 levels remained equal to or above those in vehicle-treated cells (Fig. 3*C*). Evaluation of Rad51 mRNA levels in SF539 cells at 6 h after 10 Gy showed that Gleevec pretreatment slightly suppressed the induction, with Rad51 mRNA levels in the irradiated cells, remaining equal to or greater than those in vehicle-treated cells (Fig. 3*D*). These protein and mRNA measurements suggest that although Gleevec reduces the basal level of Rad51 expression in these glioma cell lines, it has little effect on the radiation-induced expression of Rad51.

Radiation induces the formation of Rad51 foci, which are assumed to directly participate in the DNA repair process (18). The maximum Rad51 foci induction after irradiation of U251 and SF539 cells occurred at 6 h and decreased to control levels over the next 48 h (data not shown). The micrographs shown in Fig. 4, *A* and *B*, are representative of the Rad51 foci detected in U251 and SF539 cells at 6 h after 2 Gy and in cells exposed to Gleevec for 6 h before irradiation. In control cells (exposed to the vehicle DMSO) and in Gleevec-reated cells, Rad51 foci were detected in $\leq$10% of the cells (data not shown). The actual numbers of cells containing radiation-induced Rad51 foci were determined for each treatment, and data are presented in Fig. 4*C*. Gleevec pretreatment reduced the number of cells expressing radiation-induced Rad51 foci in both glioma cell lines. Thus, in addition to inhibiting the expression of Rad51, Gleevec pretreatment reduced the formation of Rad51 foci in irradiated cells.

To determine whether Gleevec actually enhances the sensitivity of these glioma cell lines to radiation-induced death, a clonogenic assay was performed. Because the function of Rad51 in DSB repair is

dependent on foci formation, and Gleevec delivered 6 h before irradiation reduced Rad51 foci (Fig. 4), for clonogenic survival analyses cells were irradiated 6 h after Gleevec was added to the culture media. Radiation survival curves were generated for each cell line after normalization for the level of cell killing induced by Gleevec alone; the surviving fractions for U251 and SF539 cells exposed to Gleevec (7.5 μM) only were 0.50 ± 0.14 and 0.33 ± 0.05, respectively. As shown in Fig. 5, Gleevec enhanced the radiosensitivity of the U251 and SF539 cell lines with radiation enhancement ratios of 1.3 and 1.5, respectively.

To determine whether the Gleevec-mediated enhancement in tumor cell radiosensitivity was the result of cell synchronization into a radiosensitive phase of the cell cycle, FCM was used to determine cell cycle phase distribution in each line 6 h after Gleevec exposure. Gleevec (7.5 μM) had no effect on the cell cycle phase distribution of each line (data not shown), indicating that accumulation of cells in a radiosensitive phase of the cycle dose was not involved in the radiosensitization induced by Gleevec. FCM was also performed at 24 and 48 h after irradiation (6 Gy) with and without Gleevec pretreatment. Gleevec did not alter progression through the cell cycle after irradiation (data not shown). In addition, Gleevec pretreatment did not result in the appearance of a sub-G_1 population in either cell line (data not shown). These data suggest that Gleevec-mediated increase in radiation-induced cell killing was not because of enhanced apoptotic cell death.

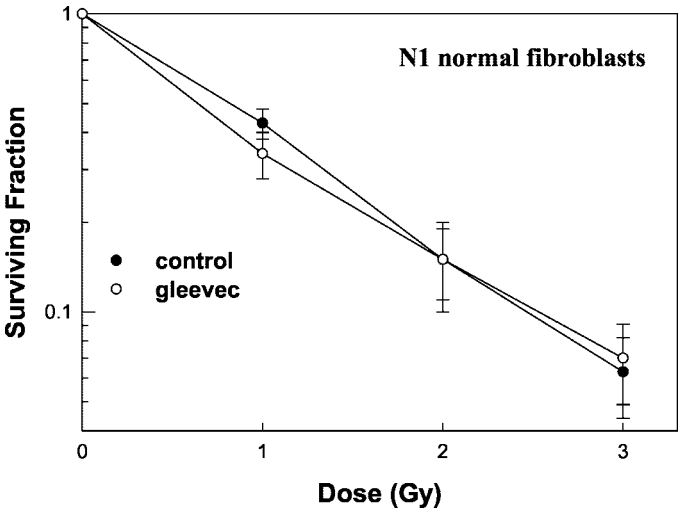

Fig. 6. The effects of Gleevec on the radiosensitivity of the normal human fibroblast cell line N1. Cells were exposed to Gleevec (7.5 μM) or vehicle (DMSO) for 6 h and irradiated with graded doses of X-rays. Colony-forming efficiency was determined 14 days later, and survival were curves generated after normalizing for cell killing by Gleevec alone. Each value represents the mean ± SE for three independent experiments.

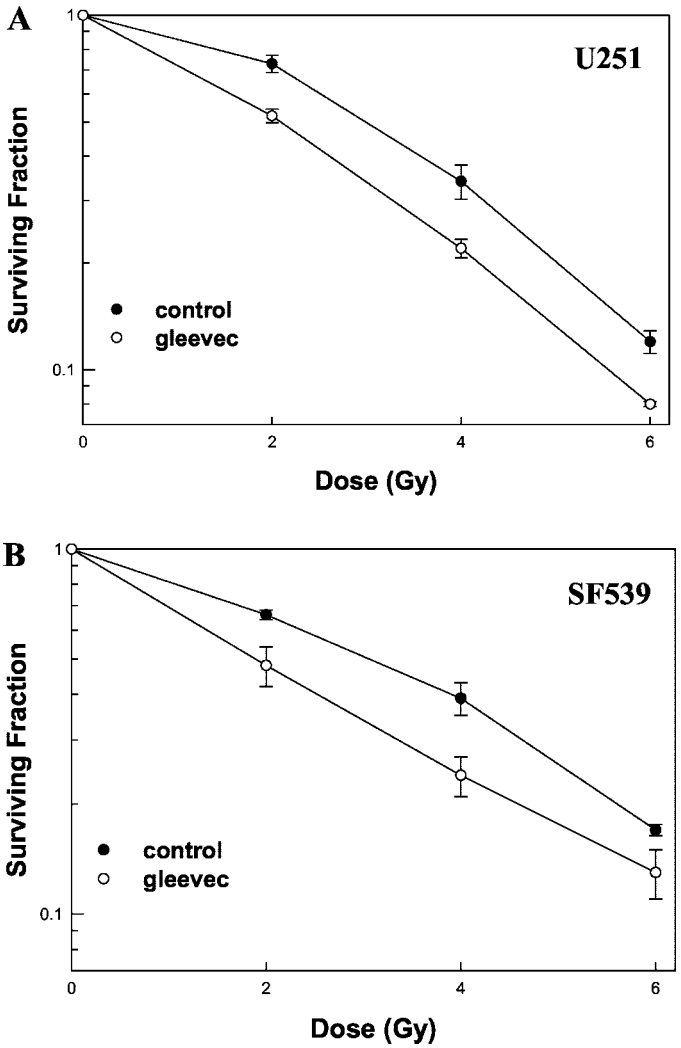

Fig. 5. The effects of Gleevec on tumor cell radiosensitivity. *A*, U251 human glioma cell line; *B*, SF539 human glioma cell line. Cells were exposed to Gleevec (7.5 μM) or vehicle control (DMSO) for 6 h and irradiated with graded doses of X-rays. Colony-forming efficiency was determined 10–14 days later, and survival curves were generated after normalizing for cell killing by Gleevec alone. Each value represents the mean ± SE for three independent experiments.

The therapeutic potential of Gleevec as a radiation modifier will ultimately depend on a selective increase in the radiosensitivity of tumor cells over normal cells. To begin to address this potential differential, we evaluated the effects of Gleevec on the radiosensitivity of the nonimmortalized, normal human diploid fibroblast cell line N1. The treatment protocol was the same as that used for the tumor cell lines. As shown in Fig. 6, a 6-h pretreatment with 7.5 μM Gleevec had no significant effect on N1 cell survival after irradiation; the surviving fraction for Gleevec treatment alone was 0.55 ± 0.07, which is similar to the glioma cell response. It should be noted that the radiosensitivity of N1 cells is considerably greater than that of the two tumor cell lines. This enhanced sensitivity of N1 (or resistance of the tumor cell lines) is consistent with a previous report showing that nonimmortalized, normal human fibroblast cell lines are generally more sensitive than tumor cell lines (19).

As an initial investigation into the mechanisms responsible for the selective effect of Gleevec on the radiosensitivity of the tumor cell lines *versus* that of normal cells, immunoblot analysis was performed to compare relative levels of Rad51 expression between the cell lines. As shown in Fig. 7*A*, Rad51 is expressed at significantly higher levels in both tumor lines as compared with the N1 fibroblasts. In addition to the relatively lower levels, Rad51 in the normal cells responded differently to Gleevec and radiation. Gleevec had no significant effect of Rad51 protein levels in N1 cells (Fig. 7*B*). Moreover, irradiation of N1 cells did not induce Rad51 protein expression (Fig. 7*C*). Finally, whereas 2 Gy resulted in a similar level of Rad51 foci formation in N1 as in the tumor cells, Gleevec had no effect on radiation-induced foci in N1 cells (Fig. 8). These results suggest that, in contrast to the glioma cell lines, the regulation of Rad51 in N1 cells is not susceptible to modulation by Gleevec or radiation. It would then appear that the inability of Gleevec to reduce Rad51 levels in these cells accounts for the lack of an enhancement in radiosensitivity.

DISCUSSION

Gleevec is an effective treatment for CML and, in general terms, has established the paradigm for a molecularly targeted drug (20). The

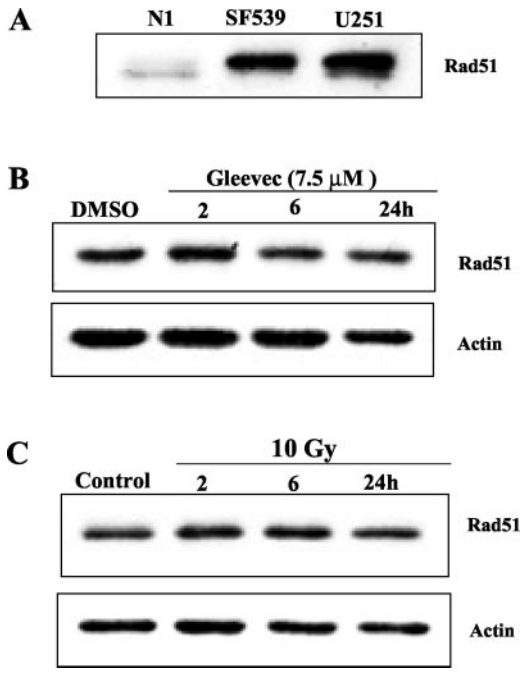

Fig. 7. Rad51 protein levels in the normal fibroblast cell line N1. *A*, comparison of Rad51 protein level in the N1 cell line with those in the glioma cell lines U251 and SF539. Immunoblots were generated from actively proliferating cultures; for each cell line, 20 μg of protein were evaluated. *B*, the effect of Gleevec on Rad51 protein levels in N1 cells. Cells were exposed to Gleevec (7.5 μM) for the specified time period and collected for immunoblot analysis. *C*, the effect of radiation on Rad51 protein levels in N1 cells. Cells were exposed to 10 Gy and collected for immunoblot analysis at the designated time points. The immunoblots shown are representative of at least two independent experiments.

target for Gleevec in CML is the tyrosine kinase c-Abl, which increases Rad51 levels, stimulating resistance to DNA-damaging chemotherapeutic agents (12). Because DNA DSBs are the critical lesion in radiation-induced cell death and Rad51 plays an important role in DSB repair, it was hypothesized that Gleevec modulates radiation sensitivity (21). Whereas this hypothesis was based on studies of the chemosensitivity of leukemic cells, the data presented here indicate that Gleevec can enhance the radiosensitivity of cells originating from solid tumors. Although a direct causal relationship was not established, the enhanced radiosensitivity of the glioma lines would appear to be related to the effects of Gleevec on Rad51. Haaf *et al.* (4) reported that after irradiation of human cells, Rad51 foci formation was suppressed in the presence of transcriptional inhibitors. Similarly, after exposure of glioma cells to Gleevec, we observed a decrease of Rad51 mRNA and protein levels, which is then likely responsible for the reduction in radiation-induced Rad51 foci. Because the generation of Rad51 foci after irradiation is assumed to directly contribute to the repair of DSBs, the reduction in foci formation is likely responsible for the enhanced radiosensitivity of the glioma cells.

Although not determining actual survival, Topaly *et al.* (22) demonstrated that STI-571 (Gleevec) enhanced the radiation-induced growth delay of CML cell lines, consistent with the data presented here. However, it should be noted that according to clonogenic survival analysis, Gleevec did not to enhance the radiosensitivity of a normal murine hematopoietic cell line or a human leukemic cell line (23). Rad51 levels were not determined in those cell lines, and it is unclear whether they were reduced by the Gleevec treatment protocol used in those studies. As described below, the failure of Gleevec to

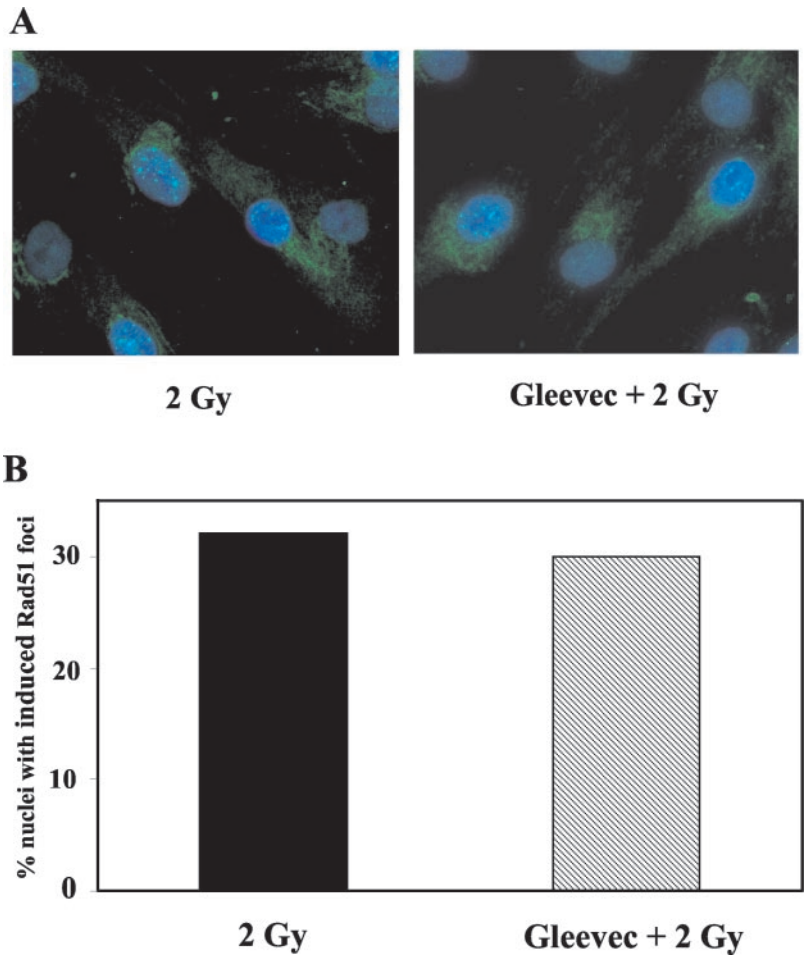

Fig. 8. Effects of Gleevec on radiation-induced Rad51 foci in the normal fibroblast cell line N1. Cells were exposed to Gleevec (7.5 μM) for 6 h before irradiation (2 Gy) and fixed for immunocytochemical analysis of Rad51 foci 6 h later. *A*, representative micrographs. *B*, quantification of Rad51 foci in N1 cells exposed to 2 Gy or the Gleevec/radiation combination exemplified in *A*. Values are representative of two independent experiments in which 100 nuclei were evaluated per treatment group.

enhance the radiosensitivity of those cell lines may also be consistent with the cell type-dependent effect observed in the studies reported here.

Whereas the irradiation of yeast results in an induction of Rad51, it is generally considered that radiation has no effect on Rad51 expression in mammalian cells (5, 24–26). However, as shown at the protein and mRNA levels, radiation did induce the expression of Rad51 in U251 and SF539 cells. This induction is not apparent at the protein level until 6 h after irradiation, which may account for it not being detected in a previous report (26). Moreover, the induction appears to be cell type dependent in that it is detected in both glioma cell lines but not in the normal fibroblast cell line. Whether Rad51 induction is a frequent event in irradiated tumor cells and whether it takes place *in vivo* remain to be determined. Moreover, it is unclear whether the increase in Rad51 protein beginning at approximately 6 h after irradiation participates in the radioresponse, *i.e.*, enhances DNA repair capacity, after a single dose of radiation. However, given the ability of increased Rad51 levels to reduce radiosensitivity (6), radiation-induced expression of Rad51 within a tumor cell may reduce the cell's sensitivity to subsequent irradiations. If this occurs, it could play a role in the ultimate tumor response to fractionated radiotherapy. Clearly, such speculation requires further investigation.

The difference in Rad51 levels detected between the glioma cells and the normal fibroblast cell line is consistent with a report indicating that in general Rad51 is expressed at higher levels in tumor cells as compared with normal cells (10). The data presented here extend those previous results to suggest that in addition to differences in basal levels, Rad51 expression in tumor cells is more susceptible to modulation. Whereas in the two tumor cell lines radiation induced Rad51 expression and Gleevec inhibited Rad51 expression, these agents had no effect on the low basal level of Rad51 in the normal N1 cell line. It has been suggested that it is the oncogenic activation of the c-Abl tyrosine kinase that is responsible for the elevated Rad51 level in leukemic and lymphoma cells (12). A similar situation in the glioma lines would indeed account for the ability of Gleevec to reduce Rad51 in these tumor cells and not in the normal N1 cells. However, Gleevec was unable to completely eliminate the radiation-induced increase in Rad51 levels in the glioma cells (Fig. 3). This suggests that there is an additional signaling process (*i.e.*, not present in normal cells) involved in the radiation-mediated increase in Rad51 levels in the tumor cell lines that is Gleevec resistant. Taken together, these data suggest that there are multiple signaling events contributing to the regulation of Rad51 expression in tumor cells that do not appear to be operative in normal cells.

The potential for differential expression in tumor *versus* normal cells along with a role in the repair of DNA damage suggests that targeting Rad51 may be an effective strategy for selectively enhancing tumor cell radiosensitivity. Indeed, the data presented here suggest that the Gleevec-mediated enhancement in radiosensitivity was the result of a selective reduction in Rad51 levels in tumor but not normal cells. However, although able to reduce constitutive Rad51 levels in the glioma cells, Gleevec did not effectively prevent Rad51 induction by radiation. It is unclear at this time whether the induction of Rad51 plays a role in the response to a single dose of radiation or to subsequent irradiations. However, delineating the signaling pathway responsible for the radiation-induced expression of Rad51 may identify additional molecules, such as c-Abl, that may serve as targets for radiation sensitizers. The data presented here indicate that Gleevec enhances the *in vitro* radiosensitivity of tumor cells but not normal fibroblasts. Clearly, the *in vitro* radioresponse of normal fibroblasts may not reflect that of normal brain tissue. Whether Gleevec actually improves therapeutic ratio by increasing glioma radiosensitivity although not enhancing normal brain toxicity awaits *in vivo* studies using an orthotopic brain tumor model.

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