Radiosensitization of Human Tumor Cells by the Phosphatidylinositol 3-Kinase Inhibitors Wortmannin and LY294002 Correlates with Inhibition of DNA-dependent Protein Kinase and Prolonged G2-M Delay

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*ABSTRACT*

Members of the phosphatidylinositol (PI) 3-kinase gene family, including the ataxia telangiectasia gene and the DNA-dependent protein kinase (DNA-PK), are involved in regulating cellular radiosensitivity. We have investigated two structurally unrelated PI 3-kinase inhibitors, wortmannin and LY294002, to determine whether they inhibit DNA-PK and increase cellular radiosensitivity. The PI 3-kinase inhibitors wortmannin and LY294002 were effective radiosensitizers of human tumor cells, with sensitization enhancement ratios (at 10% survival) of 2.8 and 1.9, respectively, in SW480 cells. Wortmannin and LY294002 inhibited the kinase activity of purified DNA-PK and inactivated cellular DNA-PK kinase activity. Inhibition of cellular DNA-PK activity occurred at the same concentrations of wortmannin that caused radiosensitization, and this correlation was found in a range of tumor cell lines. However, cells deficient in either DNA-PK (scid cells) or the ataxia telangiectasia protein were also partly sensitized to radiation by wortmannin, indicating the involvement of more than one protein kinase in the mechanism of action of wortmannin. Wortmannin also affected the G2-M checkpoint. SW480 cells had a reversible G2-M delay of 20 h following irradiation. However, wortmannin-treated SW480 cells had a prolonged G2-M delay; more than 75% of cells were arrested in G2 at 50 h postirradiation. This suggests the accumulation of significant unrepaired DNA damage following inhibition of PI 3-kinase family members. Therefore, PI 3-kinase inhibitors may represent a new class of radiosensitizers that inhibit the repair of DNA damage.

INTRODUCTION

Members of the PIK family gene family play a key role in regulating the cellular response to DNA damage (1, 2). PIK proteins are high molecular weight proteins that contain a conserved kinase domain at the COOH-terminus (2). PIK proteins have been identified in yeast and Drosophila as well as in mammalian cells, and include the human AT gene (3, 4), DNA-PK (5), and the yeast *tell* (6, 7) and *mecl* (8) genes. The *Saccharomyces cerevisiae* *tell* gene is a homolog of the human AT gene (6). *Tell* mutants have shortened telomeres and exhibit chromosome instability (6, 7). The yeast *mecl* gene is required for cell cycle checkpoints and recombination, and cells lacking *mecl* are sensitive to DNA-damaging agents (8). The *Schizosaccharomyces pombe* *rad3* gene is required for S and G2 arrest and for DNA repair (9). The *Drosophila* *mei-41* gene is required for DNA repair and chromosome stability, and *mei-41*-deficient cells are extremely radiosensitive (10). The PIK family also includes the genes mutated in the inherited human disease AT (3, 4) and the mouse SCID mutation (5). AT is characterized by immunodeficiency, cerebellar ataxia, and chromosomal instability (11, 12). AT cells are extremely sensitive to ionizing radiation, have shortened telomeres, and are defective in the repair of DNA damage (13–16). AT cells are also defective in the activation of p53 following DNA damage (17) and have an increased rate of apoptosis (18). SCID mice are deficient in the repair of dsDNA breaks, have faulty V(D)J recombination, and are extremely sensitive to radiation (19–21). Therefore, PIK family members appear to be involved in regulating the cell cycle, in activating checkpoints following DNA damage, and in controlling the cellular response to genotoxic agents.

The COOH-terminus of PIK proteins contains a region with homology to the kinase domain of the mammalian p110-PI 3-kinase protein (1–5, 22), suggesting that PIK family members may function as protein kinases. The gene involved in the SCID mutation encodes a protein kinase identified previously as DNA-PK (21, 23–26). DNA-PK has a subunit of M₈₆,000 that binds specifically to the ends of DNA (23, 24), causing activation of the ser/thr kinase activity of the catalytic domain of M₈₆,000 (24, 26). In SCID cells, the catalytic subunit of DNA-PK is absent (21), and the loss of DNA-PK activity may account for the increased sensitivity to radiation and the inability to repair dsDNA breaks seen in Scid cells (19, 21, 25). The

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The abbreviations used are: PIK, phosphatidylinositol 3-kinase; AT, ataxia telangiectasia; SCID, severe combined immunodeficiency; DNA-PK, DNA-dependent protein kinase; SER, sensitization enhancement ratio; SF2Gy, surviving fraction of cells at 2 Gy; dsDNA, double-stranded DNA.
Inhibition of DNA-PK Increases Radiosensitivity

AT protein has recently been demonstrated to possess kinase activity (27), suggesting that the kinase activity of PIK family members is central to their ability to function in the regulation of the response to DNA damage. As such, they make ideal targets for pharmacological intervention in the clinic. Compounds that block the function of the AT or DNA-PK proteins would greatly enhance the efficacy of agents that cause DNA damage, such as radiation therapy, and would allow for the treatment of tumors that are particularly radioresistant. The microbial metabolite wortmannin is a specific inhibitor of the p110-PI 3-kinase (28, 29). Wortmannin forms a covalent adduct with Lys-802 of p110-PI 3-kinase, inactivating the kinase activity (30). Lys-802 is located upstream of the kinase domain of p110-PI 3-kinase (30), and this lysine residue is conserved in the kinase domain of both the DNA-PK and AT proteins (4, 5). This suggests that PIK proteins, including DNA-PK and the AT protein, should also be inhibited by wortmannin.

We have demonstrated previously that wortmannin is a radiosensitizer of human tumor cells (31). These results indicated that a wortmannin-sensitive pathway, possibly involving PIK family members, may regulate the response of the cell to DNA damage. Here, we have extended these studies to determine whether PI 3-kinase inhibitors inhibit PIK family members, leading to increased sensitivity of human tumor cells to radiation. Three compounds were tested: wortmannin (28); LY294002, a chemically unrelated PI 3-kinase inhibitor (32); and rapamycin, a specific inhibitor of the mammalian FRAP protein (33). FRAP, a member of the mammalian PIK family, is involved in the regulation of S6 kinase and cell cycle progression (33, 34). LY294002 and wortmannin caused significant radiosensitization, whereas rapamycin was ineffective, indicating that FRAP is not involved in regulating radiosensitivity. Both wortmannin and LY294002, but not rapamycin, inhibited DNA-PK kinase activity. Radiosensitization by wortmannin correlated closely with inhibition of DNA-PK kinase in a wide range of tumor cells. Irradiation of wortmannin-treated cells caused significant cell cycle defects, including prolonged G2 arrest. PI 3-kinase inhibitors may regulate cellular radiosensitivity through inhibition of DNA-PK.

**MATERIALS AND METHODS**

**Cells.** SW480, Du145, PC-3, and HeLa cells were obtained from the American Type Culture Collection (Rockville, MD). AT cells (ATSBHA) were obtained from the National Institute of General Medical Sciences (Camden, NJ). scSV3 Scid cells were obtained from Dr. D. Weaver (Dana-Farber Cancer Institute, Boston, MA). Cells were cultured in: Ham's F-12 medium/10% FCS (SW480 and HeLa); RPMI-1640/10% FCS (Du145 and PC3); Ham's F-12 medium/15% FCS/4 μg/ml hydrocortisone (SC61 and SQ2OB); MEM/15% FCS (AT cells); and DMEM/10% FCS (Scid cells). Irradiation was carried out using a 250Kvp X-ray machine at a dose rate of 1.2 Gy/min at 37°C. Stock solutions of wortmannin (Sigma Chemical Co., St. Louis, MO), LY294002 (Biomol, Plymouth Meeting, PA), or rapamycin (Biomol) were dissolved in 100 mM DMSO. For cell survival, cells were plated onto 60-mm dishes and allowed to attach for 24 h (35). The number of cells per dish was chosen to ensure that 50–100 colonies would survive a particular treatment. Cells were pretreated with wortmannin or DMSO (solvent) for 20 min, and then irradiated at the indicated dose. At 16 h after irradiation, the cells were washed in three complete changes of medium to remove the wortmannin. Cells were fixed 10 days later in methanol/acetic acid (75/25%) and stained with crystal violet (35, 36). Colonies were examined under a low-power microscope and scored if they contained more than 50 cells. Cell survival after irradiation was normalized to the level of unirradiated controls exposed to the same concentration of DMSO or wortmannin. The plating efficiencies of the cells were 57–65%. SERs were calculated at 10% survival using software from CricketGraph (Islandia, NY) to determine the slope of the line. For scid cells in Fig. 4, SER was calculated by extrapolating to 10% survival using the software package. SF2Gy was calculated from the actual data.

**Cell Cycle Distributions.** SW480 cells were harvested by trypsinization, collected by centrifugation, and then fixed in 70% ethanol. Cells were stained with 0.01% propidium iodide, treated with RNase (Sigma) and then analyzed on a Becton Dickinson flow cytometer using CellFIT cell cycle-analysis software and the SOBR model (35).

**DNA-PK Kinase Assays.** Extracts were prepared by the methods of Finnie et al. (37) and Lees-Miller et al. (38). Cells (10⁶) were resuspended in 100 μl of extraction buffer (50 mM NaF; 20 mM HEPES, pH 7.8; 450 mM NaCl; 25% glycerol v/v; 0.2 mM EDTA; 0.5 mM DTT; and 0.5 mM phenylmethylsulfonyl fluoride) and subjected to three freeze/thaw cycles (−80°C/30°C). Extracts were centrifuged (15 kg for 10 min at 4°C), and supernatants were stored at −80°C. Phosphorylation reactions were performed according to Finnie et al. (37) and Lees-Miller et al. (38) using a synthetic peptide substrate (Glu-Pro-Pro-Leu-Ser-Glu-Ala-Pha-Ala-Asp-Leu-Trp-Lys-Lys-Promega, Madison, WI). Five μg of extract were incubated in 40 μl (final volume) of kinase buffer (25 mM HEPES, pH 7.5; 50 mM KCl; 10 mM MgCl₂; 20% glycerol v/v; 0.1% NP40; 1 mM DTT; 0.5 μM ATP; and 5 μM [γ-³²P]ATP) in the presence of 0.2 μM peptide and 0.2 μg of calf thymus DNA as indicated. Incubations were carried out at 25°C for 10 min. The reactions were stopped by the addition of 40 μl of 30% acetic acid and spotted onto 2.5 cm circles of P-81 paper (Whatman). The P-81 paper was washed four times for 5 min each in 15% acetic acid and transferred to scintillation vials containing 3.5 ml of water, and bound radioactivity was determined in a scintillation counter. 1 unit of DNA-PK activity equals 1 pmol PO₄ transferred/μg protein/10 min of reaction time. Purified DNA-PK kinase assays were carried out as follows: 0.5 Sp₁ units of purified DNA-PK protein (Promega) were incubated with wortmannin or DMSO (solvent) for 12 min at 25°C in 10 μl of sample buffer (25 mM HEPES, pH 7.5; 50 mM KCl; 10 mM MgCl₂; 20% v/v glycerol; 0.1% NP40; 20 μM ZnCl₂; and 1 mM DTT). 10 μl of reaction mix (0.2 mM peptide, 0.2 μg calf thymus DNA, 100 μM ATP, 5 μM [γ-³²P]ATP, 13 mM spermidine, and 4 mM MgCl₂) were added, and the reaction was allowed to incubate for 10 min at 25°C. Reactions were terminated by the addition of 21 μl of 30% acetic acid and analyzed as described above.
RESULTS

The mechanism by which wortmannin increases cellular radiosensitivity is not known. However, mammalian PI3-kinase family members, including FRAP (33), DNA-PK (5), the AT protein (3, 4), and p110-PI 3-kinase (22), are all potential targets. In this study, we have compared two PI3-kinase inhibitors, LY294002 (32) and rapamycin (33), to wortmannin. The synthetic compound LY294002 is a specific inhibitor of the mammalian p110-PI 3-kinase, which is structurally unrelated to wortmannin (32). The immunosuppressant rapamycin is a specific inhibitor of FRAP, a PI3-kinase family member involved in the activation of S6 kinase (34). Rapamycin binds to the FKBP12 protein, preventing FKBP12 from binding to and activating the FRAP protein. To determine whether these compounds displayed significant cytotoxicity, SW480 cells (a human colorectal adenocarcinoma) were exposed to either 20 μM wortmannin, 50 μM LY294002, or 10 μM rapamycin and the surviving fraction of cells was determined. Wortmannin and LY294002 displayed minimal cytotoxicity, with 73 and 80% of SW480 cells surviving this treatment. Rapamycin was more toxic, decreasing the surviving fraction to 16%. In Fig. 1, the effect of wortmannin, LY294002, and rapamycin on the clonogenic cell survival of SW480 cells exposed to increasing doses of ionizing radiation was assessed. Both 20 μM wortmannin (Fig. 1, ●) and 50 μM LY294002 (Fig. 1, △) were able to sensitize SW480 cells to X-ray irradiation at all doses of radiation used. The SER at 10% survival was 2.8 for 20 μM wortmannin and 1.9 for 50 μM LY294002 (Fig. 1). In contrast, 10 μM rapamycin did not significantly sensitize SW480 cells to radiation (Fig. 1, ■). Inhibition of FRAP by rapamycin occurs in the nanomolar range (34). Varying the concentration of rapamycin from 100 nM to 10 μM did not result in significant radiosensitization. This indicates that the PI3-kinase family member FRAP is not involved in regulating the radiosensitivity of the cell.

Previous studies have shown that wortmannin can inhibit the kinase activity of purified DNA-PK (5). In the experiment shown in Fig. 2, we examined the effect of wortmannin, LY294002, and rapamycin on the activity of DNA-PK in cellular extracts. SW480 cells were incubated with either solvent (control), wortmannin, LY294002, or rapamycin for 2 h, and cellular extracts were prepared and assayed for DNA-PK activity as described in “Materials and Methods.” Minimal phosphorylation was detected using non-specific control peptides (data not shown). In the absence of exogenous DNA, there was minimal DNA-PK kinase activity in control extracts. Addition of dsDNA greatly stimulated the activity of DNA-PK (Fig. 2, control). Extracts from wortmannin-treated SW480 cells had no detectable DNA-PK activity, and there was no increase following DNA addition. Similar results were seen with the PI3-kinase inhibitor LY294002. Rapamycin did not affect the activity of DNA-PK in cellular extracts (Fig. 2), and incubation of cell extracts from untreated cells with 10 μM rapamycin did not alter DNA-PK activity (control, 100 ± 3.7% of maximal activity; rapamycin, 100 ± 3.9% of maximal activity). Therefore, both wortmannin and LY294002 can inhibit DNA-PK and increase radiosensitivity.

\[ \text{Unpublished observation.} \]
Previous studies have indicated that wortmannin inhibits purified DNA-PK at 0.5–1 μM (5). In Fig. 3A, the concentration of wortmannin required to inhibit purified DNA-PK was measured. Maximal inhibition of purified DNA-PK was seen at 2 μM wortmannin (Fig. 3A, inset); 50% inhibition was seen at 0.3 μM. In Fig. 3A, SW480 cells were exposed to wortmannin as indicated, and cell extracts were assayed for DNA-PK activity. DNA-PK was inhibited at 20 μM in cell extracts; 50% inhibition occurred at 7 μM (Fig. 3A). Complete inhibition of DNA-PK in the cell requires a 10-fold higher concentration of wortmannin than that required for inhibition of purified DNA-PK. Previous studies indicate that inhibition of cellular p110-PI 3-kinase activity requires 10–20-fold higher concentrations of wortmannin than those required to inhibit purified p110-PI 3-kinase (39–41). This difference has been attributed to the instability of wortmannin in tissue-culture media (29, 39). In Fig. 3B, SW480 cells were irradiated in the presence of increasing concentrations of wortmannin, and the SF2Gy was determined. After irradiation, the SF2Gy was decreased from 0.6 without wortmannin to 0.08 in the presence of 20 μM wortmannin. Maximal radiosensitivity was seen at 20 μM and half-maximal at 8 μM. Thus, inhibition of DNA-PK by wortmannin in SW480 cells correlates closely with the observed increase in radiosensitivity.

In the experiment shown in Table 1, the relationship between DNA-PK and radiosensitivity was explored in a range of tumor cell lines. The human tumor cell lines SCC61 and SQ20B (squamous cell carcinoma), PC-3 and DU-145 (prostate adenocarcinoma), HeLa (cervical carcinoma), and SW480 (colon adenocarcinoma) were examined. These were selected to cover a range of radiosensitivities, including radiosensitive (SCC61) and radioresistant (SQ20B) cell lines. The cells in Table 1 are ranked in order of increasing radiosensitivity. No correlation was observed between the SF2Gy and the absolute levels of DNA-PK activity (Table 1), as has been noted before (42). Exposure of SQ20B, DU-145, SW480, PC-3, HeLa, or SCC61 cells to wortmannin decreased the SF2Gy after irradiation (Table 1) and increased the radiosensitivity at 2 Gy 2–5-fold. Again, there was no strict correlation between intrinsic radiosensitivity and sensitivity to wortmannin. When DNA-PK activity was measured, inhibition of cellular DNA-PK activity correlated closely with increased radiosensitivity in all the cell lines tested. The data in Table 1 indicate that there is a strong correlation between the ability of wortmannin to act as a radiosensitizer and the inhibition of DNA-PK.

The ability of wortmannin to act as a radiosensitizer of cell lines that are deficient in either the AT protein (AT5VIA cells) or DNA-PK (scSV3 SCID cells) was examined. Wortsmanin sensitized both AT cells (Fig. 4, left) and Scid cells (Fig. 4, right) to radiation. AT5VIA cells were not very sensitive to wortmannin, with a SER at 10% survival of only 1.6 (Fig. 4), suggesting that the AT protein may be the main target of wortmannin. The scSV3 Scid cells, with a SER of 2.0, were more sensitive to wortmannin. For comparison, SW480 cells had a SER of 2.8 (Fig. 1). These results suggest that wortmannin may inhibit both DNA-PK and the AT protein and that the AT protein may be the main contributor to radiosensitivity in the presence of wortmannin. However, the results do not exclude the possibility that a third, uncharacterized kinase may be the real target for wortmannin.

Cells in which PIK family members have been inactivated, such as AT and SCID cells, display prolonged G1 arrest and cell cycle aberrations (14–16). The effect of wortmannin on cell cycle kinetics of exponentially growing SW480 cells was analyzed by fluorescence-activated cell sorting. Untreated cells
Wortmannin sensitizes AT and SCID cells to radiation. AT5VIBA (AT, left) and scSV3 (ScID, right) cells were exposed to 20 μM wortmannin for 10 min, irradiated at the indicated dose, and assessed for colony formation as described in “Materials and Methods” 12 days later. C and O, solvent (DMSO); □ and ○, 20 μM wortmannin. Data points, results (n = 4); bars, SE.

Table 1  Inhibition of DNA-PK and radiosensitization of human tumor cells by wortmannin

<table>
<thead>
<tr>
<th>Cell line</th>
<th>SF2Gy DNA-PK activitya</th>
<th>DNA-PK activityb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>SQ20B</td>
<td>0.80 ± 0.11</td>
<td>0.38 ± 0.09</td>
</tr>
<tr>
<td>DU145</td>
<td>0.76 ± 0.09</td>
<td>0.27 ± 0.02</td>
</tr>
<tr>
<td>SW480</td>
<td>0.66 ± 0.15</td>
<td>0.15 ± 0.06</td>
</tr>
<tr>
<td>PC-3</td>
<td>0.57 ± 0.08</td>
<td>0.21 ± 0.05</td>
</tr>
<tr>
<td>HeLa</td>
<td>0.43 ± 0.08</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>SCC61</td>
<td>0.35 ± 0.07</td>
<td>0.06 ± 0.03</td>
</tr>
</tbody>
</table>

a  Cells were irradiated (2 Gy) in the absence (−) or presence (+) of 20 μM wortmannin, and the SF2Gy was calculated as described in “Materials and Methods.” Results ± SE, n = 4 replicates.
b  Cells were incubated for 2 h in the absence (−) or presence (+) of 20 μM wortmannin, and DNA-PK activity in cellular extracts was measured as described in “Materials and Methods.” Results are expressed as units of activity ± SE, n = 3 replicates.

continued to divide throughout the 50-h time course of the experiment: the percentage of cells in G1 (48%), S-phase (39%), and G2-M (13%) was essentially unchanged over 50 h. Wortmannin alone increased the number of cells in G1 over the first 30 h (Fig. 5A); a corresponding decline was seen in S-phase cells. Cells did not accumulate in either S phase or G2, indicating that wortmannin does not block either DNA synthesis or mitosis. The accumulation of SW480 cells in G1 may occur through inhibition of p110-PI 3-kinase by wortmannin (22); p110-PI 3-kinase is intimately involved in linking growth-factor receptors to intracellular signaling pathways and is required for progression through G1 (40, 43). Wortmannin-induced growth arrest is reversed between 20 and 30 h, and cells reenter the cell cycle, which can be seen as a decline in the number of G1 cells and an increase in the number of S-phase cells (Fig. 5A). This reversal in growth arrest with time is a reflection of the instability of wortmannin in aqueous solutions, as reported previously (29, 39, 40). Preliminary studies indicate that wortmannin has a half-life of less than 5 h in tissue culture medium.4 Irradiation of SW480 cells activates the G2 checkpoint, and the cells accumulate in G2 for the first 20 h postirradiation (Fig. 5B). SW480 cells have a mtp53 phenotype, and hence have no G1 checkpoint (17). Between 20 and 30 h, the G2 block is reversed, and cells exit into G1 and S-phase; a normal cell cycle distribution is restored by 50 h postirradiation (Fig. 4B). However, irradiation of wortmannin-treated cells leads to a very different cell cycle distribution. The G2 checkpoint is still activated, accompanied by an increasing accumulation of SW480 cells in G2 over the first 20 h (Fig. 5C) and a decline in G1 and S-phase cells. However, the G2 block in the presence of wortmannin does not reverse at 20 h, as was seen with radiation alone (Fig. 5B). Instead, cells continue to accumulate in G2 for up to 50 h, until approximately 70% of the SW480 cells are arrested in G2. The increase in G2 cells is mirrored by a progressive decline in both G1 and S-phase cells (Fig. 5C). Wortmannin is unstable and would be inactivated by 20 h postirradiation. In addition, the prolonged G2 arrest seen in irradiated wortmannin-treated cells is not reversed by the addition of fresh culture media at 15 h.4 Wortmannin may therefore inhibit an early step in the response of the cell to DNA damage, leading to a prolonged G2 block in the absence of wortmannin. If the irradiated, wortmannin-treated cells were left to recover for longer than 50 h, the cells

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Inhibition of DNA-PK Increases Radiosensitivity

The experiments (28, 29, 32) indicate that wortmannin can inhibit DNA-PK, which radiosensitization occurs. Also, LY294002 and wortmannin are structurally dissimilar (28, 32), but both inhibit DNA-PK and cause radiosensitization. Second, the inhibition of cellular DNA-PK activity by wortmannin and the increase in radiosensitivity had a similar concentration dependence (Fig. 3), and this relationship was observed in a number of tumor cell lines of targets for wortmannin. However, not all PIK family members are involved in regulating radiosensitivity. The cellular activity of p110-PI 3-kinase is inhibited at 5–100 nm wortmannin (28–30), but no increase in radiosensitivity is seen below 2 μM wortmannin (Fig. 3), indicating that p110-PI 3-kinase inhibition can occur without altering radiosensitivity. FRAP autophosphorylation is inhibited by rapamycin (33, 34). However, rapamycin does not sensitize cells to radiation (Fig. 1), indicating that FRAP is not involved in the regulation of cellular radiosensitivity. Wortmannin sensitized both AT and Scid cells to radiation, indicating that both DNA-PK and the AT protein may be inhibited by wortmannin. The AT cells were less sensitive to wortmannin (SER, 1.6; Fig. 4) than Scid cells (SER, 2.0), suggesting that the AT protein may be a more important determinant of wortmannin sensitivity than DNA-PK. Therefore, wortmannin and LY294002 may increase radiosensitivity by blocking the activity of both DNA-PK and the AT protein. This conclusion is supported by three observations. First, wortmannin and LY294002 are relatively specific inhibitors of PIK proteins. Concentrations of wortmannin that cause radiosensitization do not inhibit other cellular kinases, including mitogen-activated protein kinase, protein kinase A, and c-src kinase (28, 29, 32). Inhibitors of protein kinase C can act as radiosensitizers (44), but neither wortmannin nor LY294002 display any inhibition of protein kinase C at the concentrations used in these experiments (28, 29, 32). Wortmannin can inhibit myosin light chain kinase (29), but this is unlikely to be the mechanism by which radiosensitization occurs. Also, LY294002 and wortmannin are structurally dissimilar (28, 32), but both inhibit DNA-PK and cause radiosensitization. Second, the inhibition of cellular DNA-PK activity by wortmannin and the increase in radiosensitivity had a similar concentration dependence (Fig. 3), and this relationship was observed in a number of tumor cell lines of targets for wortmannin.
differing radiosensitivity (Table 1). Third, loss of DNA-PK activity is known to increase the sensitivity of cells to radiation (19, 21, 25). This suggests that one of the targets for wortmannin and LY294002 may be DNA-PK, although it is likely that the AT protein is also inhibited by these compounds. However, this is a phenomenological observation, and there are alternative mechanisms to explain the observed increase in radiosensitivity. For example, wortmannin may inhibit PIK proteins, as well as additional, uncharacterized kinases involved in regulating radiosensitivity.

Irradiation of wortmannin-treated cells caused an increase in the length of G₂ arrest (Fig. 4) compared to cells exposed only to radiation. This G₂ arrest appeared to be irreversible up to 50 h postirradiation and did not require the presence of wortmannin to maintain it. At later time points (>50 h), G₂ arrest was reversed and many of the cells entered G₁. However, exit from G₂ arrest was not associated with an increase in apoptosis, and we could find no evidence that irradiated SW480 cells had an increase in the rate of apoptosis following exposure to wortmannin. This suggests that wortmannin increases radiosensitivity by increasing classic clonogenic cell death in SW480 cells.

In both AT cells and in Chinese hamster ovary cells lacking functional DNA-PK activity, irradiation also causes a prolonged G₂ arrest compared to normal cells (14–16). This is consistent with the hypothesis that wortmannin inhibits the function of the AT protein and DNA-PK. The AT and DNA-PK proteins are involved in the recognition and repair of DNA damage, including the repair of dsDNA breaks and in regulating cell cycle progression (1, 2). Wortmannin can block the rejoining of strand breaks in Xenopus egg extracts (43), suggesting a direct effect of wortmannin on repair processes. Inhibition of DNA-PK and/or the AT protein by wortmannin may block the recognition of DNA damage and prevent the recruitment of repair capacity to the site of damage. Continued progression through G₂ and S-phase would mean DNA lesions are carried through into G₂, without repair or processing, including dsDNA breaks. This accumulation of DNA damage may arrest the cells in G₂.

In conclusion, we have shown that inhibition of the kinase activity of DNA-PK correlates closely with an increase in cellular radiosensitivity. This suggests that the kinase activity of the PIK family may be essential to their ability to regulate the cellular response to DNA damage. PIK family members are involved in regulating cellular radiosensitivity and represent excellent targets for pharmacological intervention in the clinic. The use of compounds that block the function of the AT gene or DNA-PK would greatly enhance the lethality of agents that cause DNA damage, such as radiation therapy. The combination of radiation with inhibitors of PIK family members could greatly increase the efficacy of radiation therapy and allow for the treatment of tumors that are particularly radiosensitive.

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Radiosensitization of human tumor cells by the phosphatidylinositol3-kinase inhibitors wortmannin and LY294002 correlates with inhibition of DNA-dependent protein kinase and prolonged G2-M delay.

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