Phosphatase and Tensin Homologue Deficiency in Glioblastoma Confers Resistance to Radiation and Temozolomide that Is Reversed by the Protease Inhibitor Nelfinavir

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Abstract

Glioblastomas are malignant brain tumors that are very difficult to cure, even with aggressive therapy consisting of surgery, chemotherapy, and radiation. Glioblastomas frequently have loss of the phosphatase and tensin homologue (PTEN), leading to the activation of the phosphoinositide-3-kinase (PI3K)/Akt pathway. We examined whether PTEN deficiency leads to radioresistance and whether this can be reversed by nelfinavir, a protease inhibitor that decreases Akt signaling. Nelfinavir decreased Akt phosphorylation and enhanced radiosensitization in U251MG and U87MG glioblastoma cells, both of which are PTEN deficient. In the derivative line U251MG-PTEN, induction of wild-type PTEN with doxycycline decreased P-Akt expression and increased radiosensitivity to a similar extent as nelfinavir. Combining these two approaches had no greater effect on radiosensitivity than either alone. This epistasis-type analysis suggests that the nelfinavir acts along the Akt pathway to radiosensitize cells. However, nelfinavir neither decreased Akt phosphorylation in immortalized human astrocytes nor radiosensitized them. Radiosensitization was also assessed in vivo using a tumor regrowth delay assay in nude mice implanted with U87MG xenografts. The mean time to reach 1,000 mm³ in the irradiation + nelfinavir group was 71 days, as compared with 41, 34, or 45 days for control, nelfinavir alone, or radiation alone groups, respectively. A significant synergistic effect on tumor regrowth was detected between radiation and nelfinavir. (P = 0.01). Nelfinavir also increased the sensitivity of U251MG cells to temozolomide. These results support the clinical investigation of nelfinavir in combination with radiation and temozolomide in future clinical trials for patients with glioblastomas. [Cancer Res 2007;67(9):4467–73]

Introduction

Glioblastoma multiforme is the most common primary adult brain tumor. Despite aggressive therapy, including surgery, radiotherapy, and temozolomide, patients with these tumors have a poor prognosis with a median survival of under 1 year (1, 2). The phosphoinositide-3-kinase (PI3K) signaling pathway is commonly activated in these tumors, often by virtue of phosphatase and tensin homologue gene (PTEN) mutation, but also possibly by epidermal growth factor receptor (EGFR) expression (3, 4). Chakravarti et al. (3) found significantly reduced survival times in patients whose tumors showed PI3K pathway activation. These patients were treated by a combination of surgery with postoperative radiation as the only adjuvant therapy, which suggested that this pathway might play an important role in radiation resistance. Activation of the Ras/PI3K/Akt pathway has been shown to lead to resistance to ionizing radiation in tumor cells (5, 6). Conversely, inhibition of the PI3K/Akt pathway has been shown to radiosensitize a variety of different cell types (5–12) including gliomas (13, 14). Kim et al. used Akt small interfering RNA (siRNA) to determine the role of this protein in tumor radiation survival; however, the remaining studies used the pharmacologic inhibitor LY294002 (5–10, 12). The finding that glioma cells can be radiosensitized by inhibition of Akt has recently been challenged by de la Penas et al. (15). These investigators found that the drug perifosine, which led to the down-regulation of Akt phosphorylation, failed to radiosensitize a number of glioma cell lines. Therefore, they concluded that Akt did not seem to be a relevant target for glioma cell radiosensitization. Furthermore, they pointed out that LY294002 can inhibit not only PI3K, but also other members of the phosphoinositide-3-kinase–related kinase (PIKK) family known to modulate radiation sensitivity such as ATM and DNA protein kinase (16). Because of this controversy, we decided to investigate this by applying genetic approaches to manipulate the PI3K/Akt pathway.

One approach to manipulate the PI3K/Akt pathway was to use glioblastoma cell lines in which wild-type PTEN was inducible. PTEN is a tumor suppressor gene that encodes a phosphatase that dephosphorylates the D-3 position of phosphoinositide phosphates such as PI(3,4,5)P3 to convert them to PI(4,5)P2. Inactivation of PTEN leads to increased levels of PI(3,4,5)P3 and increased Akt activation (17, 18). In one study of human glioblastoma cell, loss of PTEN strongly correlated with Akt activation (4). PTEN mutations have been found in 25% to 44% of glioblastomas (19–23). Another study found PTEN mutations in 37% of glioblastomas but in only 18% of anaplastic astrocytomas (24). Most human glioblastoma cells in culture, including U251MG and U87MG, are PTEN deficient (25). We used derivatives of these cell lines that have been engineered so that wild-type PTEN is induced in response to doxycycline, leading to a decrease in phosphorylated Akt (26). By inducing PTEN in these cell lines, we showed that down-regulating Akt activity sensitized cells to killing by radiation. We then showed that nelfinavir, a drug that inhibits Akt phosphorylation and radiosensitizes other human cell lines (27), also sensitized glioblastoma cells. Both induction of PTEN expression and treatment with...
nelfinavir enhanced sensitivity to temozolomide, a chemotherapeutic agent that is now routinely used in the treatment of patients with glioblastomas.

**Materials and Methods**

**Cells.** U87MG, a malignant glioma cell line that is PTEN deficient but contains wild-type p53, was obtained from the American Type Culture Collection. U251MG, a human malignant glioma cell line that is PTEN deficient but contains mutant p53, and LN229 cells, which contain wild-type PTEN, were both obtained from the Brain Tumor Research Center Tissue Bank at the University of California San Francisco. NHA (a gift from H. Shu, Emory University School of Medicine, Atlanta, GA) were normal human astrocytes immortalized as described previously (13). The derivative cell lines U251-wtPTEN, U251-C124Smut, U87-wtPTEN, U87-C124Smut have been described previously (26). All cell lines were grown in DMEM (4,500 mg/L glucose, Life Technologies, subsidiary of Invitrogen) supplemented with 10% fetal bovine serum (Atlanta Biologicals), penicillin, and streptomycin. Cells were grown in a humidified incubator containing 8% CO2, 5% CO2, and 95% air at 37°C.

**Drugs.** Nelfinavir (Viracept; Pfizer) was purchased for research use from the hospital inpatient pharmacy of the Hospital of the University of Pennsylvania. Nelfinavir came as solid caplets and was ground into a fine powder and subsequently dissolved in 100% ethanol.

**Protein extraction and Western blot analysis.** For protein isolation, cells were trypsinized and washed once in PBS. The pellets were then solubilized in 0.3 to 0.5 mL of 1× sample buffer (1% Triton X-100, 20 mmol/L Tris (pH 7.6), 150 mmol/L NaCl, 2 mmol/L EDTA, 10% glycerol, 1 mmol/L DTT, 1 mmol/L sodium orthovanadate supplemented with complete protease inhibitors (Roche)), boiled for 5 min and passed repeatedly through a 26-gauge needle. Samples were centrifuged at 10,000 g, and the supernatants were retained. Protein concentrations were determined using a BCA Protein Assay kit (Pierce).

**For Western blotting, equal amounts of total protein were run in each lane of an SDS-PAGE gel (12% acrylamide).** Each protein sample was mixed with an equal volume of 2× Laemmli buffer and boiled at 95°C for 5 min before loading onto the gel. After completion of gel electrophoresis, protein was transferred to a Hybond nitrocellulose membrane (Amersham-Pharmacia) over 1 h using a blotting apparatus. The following antibodies were used: rabbit monoclonal anti–phospho-Akt (S473; Cell Signaling, 193H12; 1:1,000 dilution), rabbit polyclonal anti–phospho-FoxO1 (Cell Signaling, 244F9; 1:1,000 dilution) and beta-actin (1:1,000 dilution).

**Small interfering RNA.** Small interfering RNA (siRNA) was prepared by Dharmacon Research. The Akt1 siRNA sequence was 5’-GGAGGGUUGG-GUGGCACAAA-3’. Nontargeting siRNA 2 from Dharmacon was used as a negative control. siRNA were transfected into cells by OligofectAMINE (Invitrogen) according to the manufacturer’s instructions. For siRNA, 600 pmol per 60-mm dish was used.

**Figure 1.** Down-regulation of the Akt pathway in glioblastoma cells increases radiosensitivity. A, U251MG cells engineered to be inducible for either wild-type PTEN or PTEN/C124S (a phosphatase-dead mutant) were exposed to doxycycline (1 μg/mL). After varying lengths of time, samples from replicate dishes were harvested for protein. Western blotting was done using antibodies as indicated. Numbers at the bottom of the gel, relative P-Akt/Ser473 (ratio of P-Akt density to total Akt density), with the lane with the darkest band normalized to 1.0. B, in vitro clonogenic survival assay for U251-wtPTEN and U251-C124Smut cells. Cells were treated with doxycycline or carrier (control). Twenty-four hours later, they were trypsinized and seeded from a single cell suspension into dishes containing doxycycline-free media. After cells had attached, they were irradiated. Survival fraction is plotted on the Y-axis versus the dose of radiation on the X-axis. Plating efficiency for U251-wtPTEN cells (with 0 Gy radiation) was 48% for both control and doxycycline-treated cells.

For U251-C124Smut cells, the plating efficiencies (0 Gy) were 55% and 49%, respectively, for control and doxycycline-treated cells. All surviving fractions with radiation were normalized to these baseline plating efficiencies. C, U87MG cells were exposed to Akt1 siRNA, control siRNA, or no siRNA. Twenty-four hours later, they were trypsinized and seeded into dishes. After cells attached, they were irradiated with different doses. Plating efficiencies for U87MG cells (0 Gy) were 57%, 32%, and 18%, respectively, for control, control siRNA, and Akt1 siRNA. D, samples were harvested for protein in the experiment described in (C). Numbers at the bottom of the gel, relative Akt1 level (ratio of Akt1 density to total Akt density), with the lane with the most intense band normalized to 1.0.
(Thr24)/FoxO3 (Thr32) (Cell Signaling; 1:1,000 dilution), mouse monoclonal anti-PTEN (Cell Signaling, 26H9; 1:1,000), anti-β-actin (Sigma-Aldrich; 1:1,000), rabbit polyclonal anti–pan-Akt (Cell Signaling, 62A8; 1:2,000), mouse monoclonal anti-Akt1 (Cell Signaling, 2H10), rabbit monoclonal anti-Akt2 (Cell Signaling), rabbit monoclonal anti-Akt3 (Cell Signaling). Goat anti-mouse or goat anti-rabbit secondary antibodies (Amersham-Pharmacia) used were at a dilution of 1:2,000.

Radiation survival determination. Cells in exponential growth phase were trypsinized to create a single cell suspension. Cells were then seeded into 60-mm dishes at defined densities and irradiated at a dose rate of 1.6 Gy/min using a Mark I cesium irradiator (J.L. Shepherd). Before irradiation, cells were treated as described in figure legends (doxycycline or nelfinavir added, Akt1 siRNA, etc.) Colonies were stained and counted 10 to 14 days after irradiation. A colony by definition had >50 cells. The surviving fraction was calculated by dividing the number of colonies formed by the number of cells plated times plating efficiency. Each point on the survival curve represents the mean surviving fraction from at least three replicates.

Tumor generation in mice and drug treatment. Pathogen-free female Ncr-nu/nu mice were obtained from Taconic Industries and housed in the animal facilities of University Laboratory Animal Resources and the

Figure 2. Nelfinavir decreases Akt phosphorylation in selected glioblastoma cells. Three glioblastoma cell lines (U251MG, U87MG, LN229) or NHA (immortalized normal human astrocytes) were treated with varying doses of nelfinavir for 24 or 48 h as indicated. Protein was harvested, and Western blotting was done using antibodies as shown. A and B, numbers at the bottom of the gel, relative P-Akt(Ser473) level (ratio of P-Akt density to total Akt density) with the first lane normalized to 1.0. For (C) and (D), no numbers are provided because the level of P-Akt was constant across all the lanes.

Figure 3. Nelfinavir increases radiosensitivity in cell lines in which Akt phosphorylation is decreased. Cells in exponential growth were trypsinized and seeded from a single cell suspension. After cells had attached, nelfinavir or control solvent was added. One hour later, cells were irradiated with different doses of radiation. A, U251MG cells were treated with either 10 or 20 μmol/L of nelfinavir as indicated. The plating efficiencies of U251MG cells with and without 20 nmol/L nelfinavir (with 0 Gy radiation) were 44% and 60%, respectively. B–D, 20 μmol/L of nelfinavir was used. B, the plating efficiencies of U87MG cells with and without nelfinavir (0 Gy) were 28% and 48%, respectively. C, the plating efficiencies of NHA cells with and without nelfinavir (0 Gy) were 37% and 61%, respectively. D, the plating efficiencies of LN229 cells with and without nelfinavir (0 Gy) were 26% and 48%, respectively.
Institute for Human Gene Therapy of the University of Pennsylvania. All experiments were carried out in accordance with the University Institutional Animal Care and Use Committee guidelines. When the mice were 5 to 7 weeks of age, tumors were initiated in the flank by s.c. injection of 1 × 10^6 U87MG cells suspended in 100 μl Matrigel (BD Collaborative Research). Tumors were palpable between 5 and 7 days postinoculation. Each mouse was given 4 g of feed (transgenic dough diet, Bioserve) daily, which contained 1.58 mg of nelfinavir. Based on an average weight of 20 g for a mouse, this worked out to 79 mg nelfinavir/kg/day.

Densitometry. Gels were scanned on an Epson 2450 Perfection Photoscanner using Adobe Photoshop 7.0. Bands on the gels were quantitated using NIH Image J software.

Statistical analysis. A regression model was fit to time to tumor volume of 1,000 mm^3 data that included terms to estimate the individual (main) effects of radiation and nelfinavir (NLF) and the interaction of these two treatments on the tumor growth. The linear model took the form

\[ Y = \beta_0 + \beta_1 (\text{radiation}) + \beta_2 (\text{NLF}) + \beta_3 (\text{radiation} \times \text{NLF}) \]

where \( Y \) is the number of days to reach a volume of 1,000 mm^3 during the observation period, radiation and NLF are indicators for the treatment received (1 = yes, 0 = no), and radiation \times NLF is an interaction term. A single animal in the radiation + NLF group did not reach a tumor volume of 1,000 mm^3 before sacrifice on day 79. This observation was treated as an event on day 79, and linear regression modeling was employed to test for synergy. This approach produces a slightly conservative estimate of effect. The test of synergy between radiation and NLF was conducted on the interaction term using a one-sided Wald statistic to determine whether \( \beta_3 \) > 0, indicating synergy. \( P < 0.05 \) was considered to be significant.

A two-sided Student's \( t \) test was employed to compare the mean survival fraction values between control and either doxycycline- or nelfinavir-treated groups. A log_{10} transformation was applied to survival fractions (because these are fractions of surviving cells in 10^6 to 10^4 cells plated) before statistical comparison. All analyses were done in SPSS 12.0 (SPSS, Inc.).

Results

U87MG and U251MG cell lines both contain PTEN mutations, resulting in a high level of Akt activation. To manipulate the PTEN level and consequently the phospho-Akt levels in U251MG cells, we used a derivative cell line containing a tetracycline-inducible wild-type PTEN transgene (26). We confirmed that the addition of doxycycline to this cell line increased expression of PTEN and decreased the level of both phospho-Akt(S473) and phospho-Akt(T308), as expected (Fig. 1A). An isogenic control cell line was also used in which a mutant, phosphatase-dead form of PTEN (124S) was induced with doxycycline, which did not result in a decrease in phospho-Akt(S473) expression (Fig. 1A). U251-wtPTEN cells were treated with doxycycline and irradiated, and clonogenic survival was measured. Induction of wild-type PTEN in these cells led to radiosensitization, whereas no such effect was seen in U251-C124Smut, the control counterpart cell line in which mutant PTEN was induced (Fig. 1B).

We did similar studies using U87MG cells engineered so that wild-type PTEN would be induced by the addition of doxycycline (26). These cells also showed radiosensitization when wild-type PTEN was induced; however, induction of phosphatase-dead PTEN in the control cell line did not have this effect (data not shown). These experiments established that the mutation of PTEN in the glioblastoma cell lines is associated with decreased radiosensitivity that can be reversed by restoring wild-type PTEN expression.

\[ P-Akt_{(S473)} = \beta_0 + \beta_1 (\text{radiation}) + \beta_2 (\text{NLF}) + \beta_3 (\text{radiation} \times \text{NLF}) \]

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\[ P-Akt_{(S473)} = \beta_0 + \beta_1 (\text{radiation}) + \beta_2 (\text{NLF}) + \beta_3 (\text{radiation} \times \text{NLF}) \]
Loss of PTEN leads to Akt1 activation. To show that Akt1 itself was associated with radioresistance, we used siRNA to knock down the Akt1 protein level. Figure 1C shows that U87MG cells treated with Akt1 siRNA showed radiosensitization compared with cells exposed to control siRNA. Protein lysates harvested from parallel plates in this experiment confirmed that the level of Akt1 was decreased by the siRNA (Fig. 1D). This siRNA was specific for Akt1 because the levels of Akt2 and Akt3 were not altered.

HIV protease inhibitors have been recently shown to decrease Akt phosphorylation in cells (27). We titrated doses of the inhibitor nelfinavir and found that 20 μmol/L led to a decrease in phospho-Akt in both U251MG and U87MG cells (Fig. 2A and B, respectively). This dose of nelfinavir also led to a decrease in phospho-S6, a downstream target of Akt (data not shown). However, not all glioblastoma cell lines respond to nelfinavir. LN229 glioblastoma cells did not show a decrease in phospho-Akt levels, even with 30 μmol/L (Fig. 2C). Likewise, 30 μmol/L nelfinavir failed to decrease phospho-Akt expression in immortalized normal human astrocytes (NHA; Fig. 2D).

The ability of nelfinavir to decrease phospho-Akt expression correlated with its ability to radiosensitize. U251MG cells showed a decrease in phospho-Akt expression with 20 μmol/L nelfinavir, but not with 10 μmol/L (Fig. 2A). Consistent with this, 20 μmol/L of drug led to radiosensitization, whereas 10 μmol/L did not (Fig. 3A). Similarly, U87MG cells, in which 20 μmol/L of nelfinavir decreased P-Akt levels, showed radiosensitization with this dose (Fig. 3B), but not with 10 μmol/L (data not shown). In contrast, LN229 and NHA cells, which showed no decrease in P-Akt with 20 μmol/L of nelfinavir, were not radiosensitized by this concentration of drug (Fig. 3C and D, respectively).

The data above show a relationship between nelfinavir’s ability to both down-regulate Akt phosphorylation and radiosensitize cells. To examine whether nelfinavir’s radiosensitizing effect lies along the same pathway as Akt1, we did an epistasis-type analysis. Either induction of PTEN expression in U251MG cells or nelfinavir treatment caused a similar decrease in Akt phosphorylation, but the two together did not have a greater effect than either alone (Fig. 4A). Likewise, induction of PTEN expression in U251MG cells showed similar radiosensitization (Fig. 1B) as did treatment with nelfinavir, but combining the two did not increase the effect over either alone (Fig. 4B). Additional experiments were done with U87MG cells. Akt1 siRNA or nelfinavir decreased P-Akt to a similar extent, but the combination did not yield a greater effect (Fig. 4C). Similarly, Akt1 siRNA increased radiosensitivity in U87MG cells to a similar extent as nelfinavir exposure; but combining the two had no greater effect (Fig. 4D). Therefore, nelfinavir exposure and down-regulation of the Akt pathway through either PTEN induction or Akt1 knockdown are epistatic for radiosensitization, suggesting that they function along the same pathway.

Radiosensitization was also assessed in vivo using the tumor regrowth delay assay. Mice bearing U87MG tumors were randomly assigned to one of four treatment arms (radiation plus drug,
radiation alone, drug alone, or mock treatment). Mice were treated with nelfinavir for 5 days before irradiation. A single fraction radiation dose of 6 Gy was chosen to yield a growth delay without being curative. W confirmed that this treatment led to a decrease in the level of P-Akt(S473) within the tumors (Fig. 5B). The mean time to tumor volume of 1,000 mm³ was 41 days in the control group, 34 days in the nelfinavir group, and 45 days in the radiation group (Fig. 5C). The mean time increased in the radiation + nelfinavir group (71 days). By linear regression modeling, a significant synergistic effect on tumor regrowth was detected between radiation and nelfinavir ($P = 0.01$; Fig. 5C).

Because temozolomide is now used routinely in the treatment of patients with glioblastomas, we investigated whether inhibiting PI3K/Akt pathway activity potentiated temozolomide sensitivity. A series of in vitro assays were done to test this interaction. Decreasing PI3K/Akt pathway activity by inducing wild-type PTEN in U251-wtPTEN cells with doxycycline led to a statistically significant increase in sensitivity to a range of temozolomide doses from 10 to 50 μmol/L (Fig. 6A). Interestingly, at the same time, each temozolomide doses nelfinavir led to highly significant ($P < 0.001$) increases in cell killing (Fig. 6B). We also determined that temozolomide pretreatment did not interfere with nelfinavir radiosensitization (Fig. 6C).

**Discussion**

Glioblastomas carry a very poor prognosis. Even when extremely high radiation doses have been delivered with brachytherapy or stereotactic radiosurgery in adults with glioblastomas, local relapses are common. In one study in which 90 Gy was delivered using a three-dimensional conformal boost, 21 out of 23 patients failed in the high-dose region (28). The dose that can be delivered to a glioblastoma is limited by toxicity to normal brain tissue. In a study of patients with gliomas, the actuarial incidence of brain necrosis was 2.5% for patients receiving 50 Gy and 5% for patients who received 64.8 Gy (29). Therefore, understanding the molecular mechanisms by which tumors resist killing by radiation treatment and finding a means of circumventing these might prove useful in improving treatment outcome for these tumors.

Our results clearly show that decreasing activity of the PI3K/Akt pathway in human glioblastoma cells with mutant PTEN, either by expressing wild-type PTEN or decreasing Akt1 with siRNA, may increase their sensitivity to radiation. It has been reported that PTEN can increase p53 activity and the expression of p53 target genes thereby increasing sensitivity to chemotherapeutic agents (30). This is unlikely to be the mechanism by which PTEN expression increased radiosensitivity in our system because increased radiosensitivity was imparted by PTEN in both U251MG, which contains mutant p53, and U87MG, which contains wild-type p53.

Therefore, Akt seems to be a valid target for glioma cell radiosensitization. Our conclusions are very different from those of de la Pena et al. (15), who found that the drug perifosine, which led to down-regulation of Akt phosphorylation, failed to sensitize a number of glioma cells to radiation. This led them to conclude that in glioma cells, Akt was not a suitable target for radiosensitization.

Our results also directly contradict those of Lee et al. (31), who showed that targeted deletion of both PTEN alleles in HCT116 colon carcinoma cells resulted in increased Akt phosphorylation and increased radiosensitivity. We cannot reconcile our results with this group’s findings; however, we show unequivocally that decreasing P-Akt by expressing wild-type PTEN or by using Akt1 siRNA in glioblastoma cells increases radiosensitivity.

The clinical development of drugs that inhibit the PI3K/Akt pathway has been a challenge. The two commonly used inhibitors in the laboratory, LY294002 andwortmannin, were found to be too toxic for clinical use (32). Therefore, we chose to evaluate nelfinavir, where safety profile is well established in HIV patients and has

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**Figure 6.** Effect of PI3K/Akt pathway activation and nelfinavir on the sensitivity to temozolomide. **A.** U251-PTEN cells were treated with doxycycline. Twenty-four hours later, cells were trypsinized, seeded as a single cell suspension, and allowed to attach. Temozolomide (TMZ) (10, 20, or 50 100 μmol/L) was added, then 4 h later, media were replaced with temozolomide/doxycycline-free media. Plating efficiencies (without temozolomide) with and without doxycycline were 57% and 41%, respectively. At each temozolomide dose, mean survival fractions between untreated control and doxycycline-treated groups were compared by two-sided Student’s t test applied to log_{10} transformed data with statistical significance indicated by $P$ value. **B.** U251MG cells were treated with nelfinavir (20 μmol/L) for 48 h, cells were then trypsinized, seeded as a single cell suspension, and allowed to attach for 4 h. Temozolomide was added, then 4 h later, the media were replaced with temozolomide-free media. Plating efficiency (no temozolomide) was 10% either with or without nelfinavir. Mean survival fractions were compared by two-sided Student’s $t$ test. C. same protocol was followed as in (B), but after media were replaced with temozolomide-free media, cells were irradiated. For (A–C), colonies were counted after 10 to 14 d.
been shown to decrease levels of Akt phosphorylation. This drug clearly radiosensitized U251MG and U87MG cells in vitro and, when combined with radiation therapy in vivo, exhibited synergy in delaying tumor regrowth of U87MG xenografts. Although the in vivo effects of nelfinavir might be explained solely by a direct effect on the intrinsic radiosensitivity of glioblastoma cells, nelfinavir might be having additional effects that could increase radiosensitization. Results from our lab indicate that nelfinavir may also have effects on tumor oxygenation that could increase killing by radiation in vivo (33).

Because the PI3K/Akt pathway is often constitutively activated in many cancer cells but not in most normal cells, in theory, its inhibition might increase the therapeutic ratio by enhancing tumor cell killing while sparing normal tissues surrounding the tumor. In fact, we found that even high doses of nelfinavir failed to decrease P-Akt levels in two cell lines with comparatively low levels of P-Akt: normal human astrocytes (NHA) and LN229, a glioblastoma cell line with wild-type PTEN. The reason for this is not obvious, but may have to do with the level of P-Akt and/or how its expression is deregulated in a particular cell.

Down-regulating the Akt pathway by inducing PTEN also increases the sensitivity of U251MG cells to temozolomide (Fig. 6A).

Hirose et al. (34) have shown the converse, that increasing Akt activity in U87MG cells leads to increased resistance to temozolomide. We have clearly shown that in U251MG cells, nelfinavir enhances the cytotoxic effects of temozolomide. Furthermore, temozolomide did not interfere with nelfinavir’s radiosensitizing effect. Therefore, these results offer a rationale for the use of nelfinavir in patients with glioblastomas receiving radiation and temozolomide. Nelfinavir has been used to treat patients with HIV for over a decade. It has a good safety profile, although it can cause insulin resistance and diabetes with long-term use (35). Our results support the clinical evaluation of this drug in combination with radiation in future clinical trials for patients with glioblastomas.

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References


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