Growth inhibition of human malignant glioma cells induced by the PI3-K–specific inhibitor

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Object. The phosphatase and tensin homolog deleted from chromosome 10 (PTEN) functions as a tumor suppressor by negatively regulating the growth/survival signals of the phosphatidylinositol 3-kinase (PI3-K)/Akt pathway. The PI3-K/Akt pathway in PTEN-deficient tumors may be one of the key targets for anticancer therapy. The authors examined the effects of the PI3-K inhibitor 2-(4-morpholinyl)-8-phenylchromone (LY294002) on human malignant glioma cells, and compared these effects on PTEN-deficient cells with those on PTEN–wild-type (PTEN-wt) cells.

Methods. Using human malignant glioma cell lines, including the PTEN-deficient cells A172 and U87MG and the PTEN-wt cells LN18 and LN229, the effects of LY294002 on cell growth, apoptosis, and chemotherapeutic agent–induced cytotoxicity were evaluated. The LY294002 inhibited the growth of U87MG cells associated with reduced phosphatidylinositol 3,4,5-trisphosphate and phosphorylated Akt, and also induced growth inhibition in three other cell lines. Although LY294002 caused apoptosis in all four cell lines, apoptosis seemed to contribute to only a small portion of growth inhibition induced by LY294002. There was no link between the status of PTEN and the median inhibitory concentration values for LY294002 or between the gene status and the extent of LY294002-induced apoptosis. The LY294002 significantly augmented the cytotoxicity induced by etoposide in PTEN-deficient cells, but not in PTEN-wt cells. Enhancement of 1,3-bis(2-chloroethyl)-1-nitrosourea– and cisplatin-induced cytotoxicity by LY294002 was not linked to the status of PTEN. No marked difference in the amounts of phosphorylated Akt was found between PTEN-deficient and PTEN-wt cells.

Conclusions. The findings show that PI3-K is a possible target for therapy in patients with gliomas, and PI3-K inhibitors in combination with chemotherapeutic agents could be potent therapeutic modalities for patients with malignant gliomas.

KEY WORDS • phosphatidylinositol 3-kinase • PTEN • malignant glioma • 2-(4-morpholinyl)-8-phenylchromone • growth inhibition • apoptosis

Malignant gliomas, including anaplastic astrocytomas and GBMs, which are common primary tumors of the central nervous system, are characterized by aggressive growth and invasiveness. Invasion of malignant glioma cells into the adjacent normal brain parenchyma makes gross-total resection difficult. In addition, most gliomas eventually become resistant to drugs, resulting in the failure of chemotherapy. For these reasons, clinical outcomes of patients with malignant gliomas are poor. The tumorigenesis of malignant gliomas has been associated with a number of alterations in related genes.

The gene PTEN (also known as MMAC1/TEP1), which encodes a phosphoinositide phosphatase, is frequently mutated or deleted at chromosome 10q23 in malignant gliomas. It has been reported that PTEN mutations are associated with decreased survival time among patients with malignant gliomas and that expression of PTEN is associated with the survival time of patients with GBM. Deletion or mutation of the PTEN tumor suppressor gene has been detected also in a number of cancers such as prostate, breast, and endometrial carcinomas and melanoma in humans. Germ-line mutations in PTEN have been associated with Cowden disease and related syndromes characterized by hamartomas and a predisposition to tumors.

A number of growth/survival factors exert their effects through receptor tyrosine kinases that induce activation of PI3-K and lead to increases in cellular PIP3 levels. Phosphatidylinositol 3,4,5-trisphosphate then binds to two protein kinases, Akt (protein kinase B), the product of the cellular oncogene c-Akt, and phosphoinositide-dependent kinases to bring these kinases together. As a result of phos-
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phorylation by phosphoinositide-dependent kinases, the oncogenic protein kinase Akt is activated and inhibits apoptosis by phosphorylation of the downstream proapoptotic proteins BAD, caspase 9, and Forkhead transcription factors.3,7,10 This Akt also promotes cell proliferation by stabilizing cyclin D by inhibition of glycogen synthase kinase 3.19 On the other hand, the tumor suppressor protein PTEN dephosphorylates PIP2 and, thus, decreases cellular levels of PIP3.35 The decrease in the amount of PIP2, in turn, suppresses proliferative and antiapoptotic effects of Akt, leading to the inhibition of cell growth and survival.10 In PTEN-deficient cells, increased PIP2 levels participate in the tumorigenesis and resistance to chemotherapy through activation of the Akt-mediated cell growth and survival pathway.2 The exogenous expression of PTEN (the negative regulator of cell growth and survival) in PTEN-deficient cells reduces levels of both PIP2 and phosphorylated Akt.30,37 In PTEN-deficient human malignant glioma cells, exogenous PTEN expression induces G1 cell-cycle arrest or augments the susceptibility of cells to apoptotic stimuli, in association with a reduction in activated Akt.8,22,45 Based on these studies, we expected that, besides overexpression of PTEN, suppression of PI3-K by a specific inhibitor would also reduce both PIP2 and phosphorylated Akt levels, and thus preferentially prevent growth in PTEN-deficient glioma cells. The PI3-K would be a target to develop a new strategy for therapy in patients with PTEN-deficient gliomas. A specific inhibitor of PI3-K, 2-(4-morpholino)-8-phenylchromone, also known as LY294002, has been reported to induce growth inhibition through apoptosis and/or G1 cell-cycle arrest in vitro and in vivo in cells derived from a variety of tumors such as prostate, ovarian, and lung carcinomas.4,6,16,29,41 In addition, LY294002 has been shown to augment the susceptibility of tumor cells to several anticancer drugs.3,10 Nonetheless, the effects of LY294002 on cell growth or anticancer drug–induced cytotoxicity have not been documented in glioma cells, whereas the PI3-K inhibitor has been reported to suppress attachment, migration, or invasiveness in these cells.21,25 We examined the effects of LY294002 on cell growth and apoptosis in human malignant glioma cells, and we compared the effects of the inhibitor on PTEN-deficient and PTEN-wt cells.

Materials and Methods

Antibodies and Reagents

The following antibodies were used in this study: anti-Akt and anti–phospho-Akt (Ser473; New England Biolabs, Beverly, MA), and anti-PTEN antibody (NeoMarkers, Fremont, CA). The reagents included the following: [32P]orthophosphate (New England Nuclear Life Science Products, Boston, MA); bisbenzimide H33342 fluorochrome trihydrochloride (Hoechst 33342; Nacalai Tesque, Kyoto, Japan); LY294002, BCNU, and cisplatin (Sigma Chemical Co., St. Louis, MO); and etoposide (Calbiochem, Nottingham, UK). These reagents were dissolved in dimethyl sulfoxide. The final concentrations of dimethyl sulfoxide in the culture media prepared for glioma cells did not exceed 0.1%.

Cell Lines

Cell lines used in this study included the human malignant glioma cell lines U87MG and A172 (American Type Culture Collection, Rockville, MD) and the human GBM cell lines LN18 and LN229 (kind gifts from Dr. de Tribolet at the University Hospital, Lausanne, Switzerland).11 The cells were maintained in DMEM containing 10% FBS, 50 U/ml penicillin G, and 250 µg/ml streptomycin in a humidified atmosphere containing 5% CO2 at 37°C.

Phospholipid Analysis

After 24 hours in culture, the U87MG cells were washed with labeling medium containing 10 mM 2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid–NaOH (pH 7.4), 136 mM NaCl, 4.9 mM KCl, 1 mM CaCl2, and 5.5 mM glucose, and were incubated with [32P]orthophosphate (1.85 MBq/ml) in labeling medium for 2 hours at 37°C. The cells were washed twice with labeling medium and then incubated with DMEM containing 10% FBS with the indicated concentrations of LY294002 for 10 minutes. Phospholipid extraction and thin-layer chromatography were performed as described previously.42

Immunoblot Analysis

Cultured cells were washed with phosphate-buffered saline, lysed in 50 mM Tris-HCl (pH 7.5) containing 150 mM NaCl, 1% Triton X-100, 1 mM ethylenediamine tetraacetic acid, 1 mM NaVO4, and 1 mM phenylmethanesulfonyl fluoride, and were then centrifuged at 12,000 G at 4°C for 10 minutes. The supernatant was subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis on a 10% polyacrylamide gel and transferred to a nitrocellulose membrane. The membrane was incubated with the indicated primary antibodies followed by incubation with species-appropriate horseradish peroxidase–conjugated secondary antibodies. The bands were visualized using the enhanced chemiluminescence system (Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer’s instructions.39

Cell Proliferation

Tumor cells that had been trypsinized and suspended in DMEM, with or without 10% FBS, were seeded at 5 × 104 cells/well in 96-well flat-bottomed plates (Becton Dickinson Labware, Bedford, MA) and allowed to attach to the plates overnight. Subsequently, the medium was removed and replaced with DMEM, with or without 10% FBS, in the presence or absence of the reagents under examination, and the cells were incubated at 37°C for 72 hours. Ratios of the numbers of viable cells treated with reagents to numbers of vehicle-treated viable cells were determined using MTT (Nacalai Tesque) dye conversion as described elsewhere.15 The absorbance was measured at 595 nm with background subtraction at 655 nm. Cytotoxicity was calculated as follows: cytotoxicity = (control − sample)/(control − blank), in which control is the absorbance for vehicle-treated cells, sample is that for drug-treated cells, and blank is that for the medium to which MTT was added. All determinations were made in the range at which absorbance correlated linearly with cell number. These experiments were performed at least three times in triplicate assays.

Apoptosis Assays

Tumor cells treated under the indicated conditions were subjected to morphological examination and detection of DNA fragmentation. For the morphological studies, the nuclei of the cells were stained with 100 µg/ml Hoechst 33342 and counted in three fields by using a fluorescence microscope. The ratio of dead cells with features of apoptosis, such as chromatin condensation and nuclear fragmentation, to total cells (percentage of apoptosis) was determined. The apoptosis index was the ratio of the percentage of apoptosis in cells treated with LY294002 to that in cells treated with vehicle. Morphological studies performed using Hoechst 33342 were conducted at least twice in duplicate assays. A DNA fragmentation assay was performed in the manner described previously.17

Statistical Analysis

The unpaired Student t-test was used for a comparison between two groups and a one-factor analysis of variance for a comparison of four groups. Differences were considered to be statistically significant when the probability value was less than 0.05.
Results

Effects of LY294002 on the PI3-K/Akt Pathway in U87MG PTEN-Deficient Human Malignant Glioma Cells

To confirm that LY294002, the PI3-K–specific inhibitor, really suppresses the PI3-K/Akt signaling pathway in PTEN-deficient glioma cells, we examined the effects of the PI3-K inhibitor on the accumulation of PIP3, which is the product of the PI3-K catalyzing reaction, and Akt phosphorylation, which is the downstream event of PIP3 accumulation.19 Phospholipids were extracted from 32P-labeled U87MG PTEN-deficient glioma cells incubated with or without LY294002 and were analyzed using thin-layer chromatography.13 The density of the spot corresponding to PIP3 from cells treated with LY294002 showed a dose-dependent decrease, and 10 μM LY294002 almost completely inhibited the production of PIP3 (Fig. 1A). To detect phosphorylated Akt, lysates from cells treated with LY294002 were subjected to immunoblotting with anti-Akt and anti–phospho-Akt antibodies. There was a decrease in the signal intensity of phosphorylated Akt from cells treated with LY294002, whereas the signal intensity of total Akt was not affected by treatment with the reagent (Fig. 1B). Thus, LY294002 inhibits the PI3-K/Akt signaling pathway in U87MG PTEN-deficient human malignant glioma cells.

Inhibitory Effect of LY294002 on the Growth of Human Malignant Glioma Cells

We examined the effects of LY294002 on the growth of human malignant glioma cells to clarify the potential of a PI3-K inhibitor as a cytotoxic agent. In addition to U87MG cells, the PTEN-deficient human malignant glioma cell line A172 and the PTEN-wt human malignant glioma cell lines LN18 and LN229 were treated with LY294002 for 72 hours, after which the relative number of viable cells was assessed using MTT.13,18 The LY294002 reduced the number of viable cells in a dose-dependent manner (Fig. 2). The IC50 values for LY294002 in all four glioma cells were within a relatively narrow range (16–30.9 μM; Table 1). We also examined the growth-inhibiting effects of three anticancer drugs, etoposide, BCNU, and cisplatin, which are often used as therapy in patients with tumors, including gliomas. The IC50 values for the anticancer drugs obtained with the MTT assay in our experiments were comparable to those determined using a clonogenic method.44 Under our conditions, the IC50 values for LY294002 were comparable to the IC50 values for these three anticancer drugs. When the IC50 values were compared among the four cell lines, no link was found between the PTEN status of the cells and IC50 values for either LY294002 or the anticancer drugs in the presence of 10% FBS (Table 1). Because trophic factors present in serum could activate survival pathways other than PI3-K/Akt to reduce the cytotoxicity of LY294002 in these cells, we also evaluated the IC50 values of glioma cells for LY294002 in the absence of serum. We found no association of IC50 values with PTEN status (data not shown).

The LY294002-Induced Apoptosis in Malignant Glioma Cells

To determine if LY294002 causes apoptosis in human malignant glioma cells, DNA fragmentation and nuclear structure were examined after treatment of cells with LY294002. The PI3-K inhibitor (20 μM) caused oligonucleosomal DNA fragmentation in LN229 cells (Fig. 3, lane 8). The LY294002 (50 μM) also induced DNA fragmenta-
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**TABLE 1**

*Drug-induced growth inhibition in human malignant glioma cells in four treatment groups*

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>PTEN</th>
<th>LY294002</th>
<th>Etoposide</th>
<th>BCNU</th>
<th>Cisplatin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC₅₀ (µM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U87MG</td>
<td>mt</td>
<td>16.0 ± 2.5</td>
<td>2.4 ± 0.8</td>
<td>48.2 ± 10.0</td>
<td>4.0 ± 1.8</td>
</tr>
<tr>
<td>A172</td>
<td>mt</td>
<td>26.0 ± 2.8</td>
<td>29.9 ± 11.8</td>
<td>119.9 ± 17.8</td>
<td>3.9 ± 0.8</td>
</tr>
<tr>
<td>LN18</td>
<td>wt</td>
<td>19.5 ± 6.3</td>
<td>4.9 ± 1.5</td>
<td>153.9 ± 72.4</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>LN229</td>
<td>wt</td>
<td>30.9 ± 3.5</td>
<td>1.5 ± 0.4</td>
<td>30.0 ± 9.0</td>
<td>3.6 ± 0.9</td>
</tr>
</tbody>
</table>

* Tumor cells were treated with vehicle or drug for 72 hours and the relative number of viable cells was determined using MTT. Data are expressed as the mean IC₅₀ values ± SDs. Values represent those of three independent experiments performed at least three times. Abbreviations: mt = mutant; wt = wild-type.

**FIG. 3.** Gels showing LY294002-induced oligonucleosomal DNA fragmentation in malignant glioma cells. A DNA fragmentation assay was performed after treatment of A172 (lanes 1–3), LN18 (lanes 4–6), and LN229 (lanes 7 and 8) cells with 0 µM (lanes 1, 4, and 7), 20 µM (lane 8), or 50 µM (lanes 2, 3, 5, and 6) LY294002 in the absence (lanes 1, 2, 4, and 5) or presence (lanes 3 and 6–8) of 10% FBS for 24 to 72 hours.

**Enhancement of Anticancer Drug–Induced Cytotoxicity by LY294002**

Questioning whether LY294002 would enhance chemotherapeutic agent–induced cytotoxicity, we compared the LY294002-enhanced cytotoxicity in PTEN-deficient cells (the U87MG and A172 cell lines) with that in PTEN-wt cells (the LN18 and LN229 cell lines). The LY294002 significantly enhanced the cytotoxicity induced by each of chemotherapeutic agents examined in the PTEN-deficient cells (Fig. 4A and B). Although LY294002 significantly augmented BCNU- and cisplatin-induced cytotoxicity in PTEN-wt cells and LN18 cells, respectively, enhancement of etoposide-induced cytotoxicity was not significant in either of the PTEN-wt cells (Fig. 4C and D). These results indicate that the increases in BCNU- or cisplatin-induced cytotoxicity attributed to LY294002 are not linked to PTEN status in the glioma cells, whereas the PI3-K inhibitor could enhance etoposide-induced cytotoxicity more effectively in PTEN-deficient glioma cells than in PTEN-wt cells.

The LY294002 did not significantly augment apoptosis induced by either chemotherapeutic agent examined in the four glioma cell lines, although the increases in etoposide-induced apoptosis by LY294002 tended to be larger in PTEN-deficient than in PTEN-wt cells (data not shown).

**Phosphorylation of Akt in PTEN-Mutant or PTEN-wt Human Malignant Glioma Cells**

Because no relationship was observed between the sensitivity of human malignant glioma cells to LY294002 and the status of PTEN, we judged that the PTEN status in these cells may not correlate with the activity of the PI3-K/Akt pathway. Thus, we examined phosphorylation of Akt in the presence or absence of serum and the effects of LY294002 on this phosphorylation together with the amount of PTEN protein in human malignant glioma cells. Phosphorylated Akt was all but undetectable after serum deprivation in either PTEN-deficient or PTEN-wt malignant glioma cells (Fig. 5A). One hour after serum stimulation, increases in the amounts of phosphorylated Akt were observed in PTEN-deficient cells, but also in PTEN-wt cells (Fig. 5B). The difference in quantities of phosphorylated Akt between PTEN-deficient and PTEN-wt cells was not apparent. The total Akt in each cell line was unaffected by supplementation or deprivation of FBS. Treatment with 20 µM
etoposide (ET), 100 ng/ml caused apoptosis in all four human malignant glioma cell lines in response to ET treatment in the absence of serum. According to the comparison of apoptosis statuses, or by exogenous PTEN expression, which may also suppress Akt activity in these malignant glioma cells.

Discussion

Phosphatidylinositol 3,4,5-trisphosphate increases phosphorylation and activation of the protein kinase Akt, a promoter of cell survival and cell-cycle progression, and stimulates activity in the PI3-K/Akt pathway, which has been implicated in tumorigenesis.2,19 The PTEN protein inhibits PIP₃ accumulation to prevent Akt activation and functions as a tumor suppressor by interrupting the PI3-K/Akt signaling pathway.10,15 The elevated Akt activation in PTEN-deficient cells, which was caused by accumulation of PIP₃, was suppressed by overexpression of PTEN in association with growth inhibition accompanied by apoptosis and/or cell cycle arrest in the G₁ phase and with sensitization of the cells to apoptosis-inducing stimuli.8,12,22,37,45 To determine whether the PI3-K inhibitor LY294002 could mimic PTEN overexpression, we examined the effects of LY294002 on cell growth and susceptibility to anticancer drugs in vitro by using human malignant glioma cell lines with different PTEN statuses.

Effects of a PI3-K Inhibitor on Human Glioma Cells

Apparently this is the first report on the effects of a PI3-K inhibitor on the growth and apoptosis of human malignant glioma cells in which an LY294002-induced decrease in cellular PIP₃, levels has been demonstrated in association with a reduction in Akt phosphorylation in these cells. We have shown that LY294002 inhibited the growth of all four human glioma cell lines in a dose-dependent manner, and that the IC₅₀ values were comparable to those of known anticancer drugs.34 These results indicate that PI3-K inhibitors efficiently induce growth inhibition in human malignant glioma cells. It has been proposed that LY294002 suppresses cell growth through two different mechanisms, induction of apoptosis and G₁ cell-cycle arrest in several types of cells.4,6,29 Although LY294002 has been reported to induce G₁ cell-cycle arrest in U87MG cells, no previous study has shown that this compound can lead to growth suppression accompanied by apoptosis in malignant glioma cells.22 In our experiments, apoptosis occurred in all four malignant glioma cell lines treated with LY294002, even in the presence of serum. According to the comparison of apoptosis and growth inhibition, however, we assume that it is not apoptosis, but rather growth arrest that is predominantly responsible for LY294002-induced growth inhibition in malignant glioma cells in the presence of serum.

Although LY294002 has been reported to sensitize certain human tumor cells to apoptosis induced by anticancer drugs, no previous study has clarified whether the PI3-K inhibitor could augment cytotoxicity induced by chemotherapeutic agents in human glioma cells.31,40 Our evidence shows that LY294002 enhanced the cytotoxicity induced by etoposide, BCNU, and cisplatin in human malignant glioma cells. The effect, however, seems cumulative rather than sensitizing, which is consistent with data reported by Wick, et al.,45 that PTEN gene transfer does not sensitize malignant glioma cells to anticancer drugs. In contrast, Tachibana, et al.,36 showed that PI3-K inhibition by the dominant negative form of the enzyme enhances cisplatin-induced apoptosis in T98G glioblastoma cells. The discrepancy between the findings of these studies might be due to differences in the activities of the Ras/extracellular signal–regulated kinase (ERK) pathway caused by different serum concentrations, or by exogenous PTEN expression, which may also serve as a protein tyrosine phosphatase to affect the pathway.14,20

Lack of Association Between PTEN Status and Sensitivity to a PI3-K Inhibitor

Based on the presumed activation of the PI3-K/Akt cell-survival pathway in PTEN-deficient cells, we anticipated that PTEN-deficient malignant glioma cells would be more sensitive to LY294002 than PTEN cells. Breast cancer cells deficient in PTEN have been reported to be more sensitive to the growth-inhibiting effect of LY294002 than cells expressing wild-type PTEN.28 In our experiments, however, there was no link between the status of PTEN and the effects of LY294002, either in the presence or absence of se-
Thus, we examined whether the presumption is valid in these glioma cells, and realized that regardless of the absence or presence of serum, the ratio of phosphorylated Akt in PTEN-deficient cells was not higher than that in PTEN-wt cells. From these results, we judged that PTEN does not solely determine amounts of phosphorylated Akt, namely, PTEN status is not a unique parameter to define activity of the PI3-K/Akt pathway in human glioma cells. This notion may support data that PTEN status has no correlation with the survival of patients with malignant glioma.

Recent studies focusing on the PI3-K/Akt pathway including PTEN functions have revealed critical factors that regulate the activity of the pathway. Lipid phosphatases, such as Src homology 2 domain-containing inositol phosphatase 2 (SHIP-2), dephosphorylate PIP3, and, consequently, decrease Akt activity. Alternatively, an Akt-binding protein carboxyl-terminal modulator protein (CTMP) binds to Akt and inhibits phosphorylation of the protein to suppress kinase activation. The phosphoinositide phosphatase activity of PTEN is regulated through phosphorylation of the PTEN tail. In addition, another tumor suppressor protein, p53, binds to the PTEN promoter and activates transcription of PTEN in response to apoptotic stimuli. Whether the significant increases in etoposide-induced cytotoxicity induced by LY294002, which was limited to PTEN-deficient cells, actually reflect the importance of PTEN status is uncertain. In addition to the proteins described earlier that directly affect the PI3-K/Akt pathway, we need to consider many other proteins that involve cell growth or apoptosis. Insofar as we have examined, expressions of the antiapoptotic proteins Bcl-XL, X-IAP, and c-IAP, and the proapoptotic protein Smac were equivalent among the glioma cells we used (data not shown). Extensive examination of the expression patterns of large numbers of genes involved in cell survival or apoptosis will also be required to characterize the responses of these cells to combinations of anticancer drugs and the PI3-K inhibitor.

Potential of a PI3-K Inhibitor in Therapy for Malignant Glioma

Our study indicates that PTEN does not exclusively determine the activity of the PI3-K/Akt pathway in these human glioma cells. The observation explains, at least in part, the failure to predict the efficiency of the PI3-K inhibitor LY294002 on growth inhibition or apoptosis accord-

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**FIG. 5.** Immunoblots showing Akt phosphorylation and PTEN in human malignant glioma cells. The cells were serum-starved for 2 days (3 days for LN18; A) and then stimulated with 10% FBS for 1 hour (B). Cells cultured with serum were treated with 20 μM LY294002 for 1 hour (C). Lysates of these cells were analyzed by immunoblotting with anti-phospho-Akt and anti-Akt antibodies. Lysates from tumor cells cultured for 3 days in DMEM containing serum were analyzed by immunoblotting with the anti-PTEN antibody (D). U87 = U87MG cells.
ing to the PTEN status in these tumor cells. Nevertheless, the results of our study also indicate that PI3-K inhibitors can efficiently inhibit cell growth and that these inhibitors, in combination with certain chemotherapeutic agents, can effectively induce growth inhibition in glioma cells, even in the presence of serum. Although in vivo studies are needed to verify the efficiency of growth inhibition in the PI3-K/Akt pathway, PI3-K is a possible target for therapy in patients with gliomas, and PI3-K inhibitors in combination with anticancer drugs could be potent therapeutic modalities for patients with malignant gliomas. Based on this idea, we will continue to test the cytotoxicity of LY294002 in combination with various chemotherapeutic agents.

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