Exp Oncol 2008
30, 3, 195–201

Experimental ONCOLOGY

EPOTHILONE B INDUCES GLIOBLASTOMA CELL DEATH VIA SURVIVIN DOWN-REGULATION

*Q.A. Quick**

Department of Biological Sciences, Grambling State University, Grambling, LA 71245, USA

Aim: **The clinical resistance of glioblastomas to chemotherapeutic agents can be attributed to drug efflux pumps, such as P-glycoprotein, which contributes to reduce drug efficacy. The present study examined the utility of epothilone B, which is not a substrate for P-glycoprotein, on glioblastoma cells.** ***Methods: In vitro*** **methods with glioblastoma cells varying in p53 status were used to assess the efficacy of epothilone B to induce anti-neoplastic responses. Immunofluorescence and ELISA procedures were used to examine levels of tubulin and survivin in epothilone B treated glioblastoma cells, while acridine orange labeling was used to detect the mode of epothilone B induced cell death.** ***Results:*** **A clinically achievable concentration of epothilone B induced a cytotoxic response in p53 mutant glioblastoma cells, as a consequence of survivin down-regulation and tubulin redistribution, while a cytostatic response was observed in p53 null glioblastoma cells with a modest increase in survivin expression post-epothilone B treatment. However, p53 wild-type glioblastoma cells did not sustain a positive anti-tumorigenic response to epothilone B.** ***Conclusion:*** **Epothilone B, induced positive differential responses in glioblastoma cells with abnormal p53 status, but not in p53 wild-type cells. This suggests that epothilone B is a potential alternative to classic microtubule inhibiting agents (ie vincristine, paclitaxel) used to treat clinical glioblastomas with p53 mutations.**

Key Words: **glioblastoma, epothilone B, survivin, p53.**

Gliomas comprise fewer than 2% of malignant neoplasms but are arguably the deadliest and most difficult types of cancers to treat with current chemotherapeutic agents used clinically. The limited effectiveness of chemotherapy during the treatment of gliomas, particularly glioblastomas, is largely due to permeability restrictions imposed by the blood brain barrier present in the brain as well as the activation of intracellular cytoprotective pathways that antagonize the effects of chemotherapeutic agents used to treat this disease. It has therefore become imperative to examine novel agents and approaches that might overcome the limitations of current chemotherapy regimens used to treat glioblastomas clinically.

Microtubules are cytoskeletal proteins formed from heterodimers of alpha (α) and beta (β) tubulin that primarily function to provide structure and stability in eucaryotic cells important for mitotic spindle formation during cell division and vesicular and organelle transport. These cellular processes are governed by the dynamic instability of microtubule polymerization and depolymerization, characterized by microtubule nucleation at the microtubule-organizing center and guanine triphosphate hydrolysis, respectively [1]. The kinetic changes of microtubules, between assembly and disassembly have made them primary targets in the experimental investigation and clinical treatment of many human cancers [2].

Clinically, three classes of microtubule inhibitory agents that induce positive anti-tumorigenic responses have primarily been used to treat human cancers, the vinca alkaloids, colchicines, and taxanes [1]. However, the clinical effectiveness of these compounds is accompanied by harmful side effects and is limited by the activity of P-glycoprotein (P-gp), an energy dependent drug efflux transporter protein that plays an intricate role in the resistance of cancer cells to chemotherapeutic agents [3]. These clinical caveats have made it necessary to investigate the utility of new microtubule inhibiting agents that circumvent mechanisms contributing to the resistance of human cancers.

In the current study, we investigated the anti-tumorigenic effects of the contemporary microtubule inhibiting agent, epothilone B in glioblastoma cells using clinically achievable concentrations. Epothilones are microtubule-stabilizing agents isolated from the myxobacterium *Sorangium cellulosum* [4] and have been shown to be effective microtubule inhibitory agents in clinical and experimental studies of human cancers such as breast, prostate, ovarian, and lung cancer [5–7]. Epothilones are proving to be practical alternatives to classic taxanes (ie paclitaxel) historically used as chemotherapeutic agents in the treatment of several human cancers. This is due in large part to the high water solubility of epothilones and their ability to inhibit the growth of cancer cells overexpressing the P-gp efflux pump [8]. Additionally, we assessed the involvement of the anti-apoptotic protein, survivin in epothilone B treated glioblastoma cells.

MATERIALS AND METHODS

Cells conditions and reagents. T98, LNZ, and U87 glioblastoma cells were purchased from the American Type Culture Collection (Manassas, VA-USA). All cell lines were maintained in Dulbecco's Modified Eagles Medium-DMEM (Invitrogen, Carlsbad, CA-USA) containing 10% Fetal Bovine Serum (Invitrogen), 2 mM L-glutamine (Invitrogen), 100 nM MEM non-essential amino acids (Invitrogen), and penicillin-streptomycin (Invitrogen) at 37 °C and 5% CO_2. Epothilone B was purchased from EMD-Calbiochem Biosciences (San Diego, CA-USA).

Crystal violet cell proliferation assay. Dose response experiments were performed using a modification of the crystal violet assay [20]. Cells were plated in 24 well plates for twenty-four hours, treated with 1 nM, 5 nM, and 10 nM epothilone B (vehicle controls were treated with DMSO (Sigma-Aldrich, St. Louis, MO-USA) and allowed to incubate for 48 h. Next tissue culture medium was

Recieved: June 11, 2008.
*Correspondence: Fax: 318-274-3741
E-mail: quickq@gram.edu
Abbreviations used: P-gp – P-glycoprotein.

removed; the cell monolayer was fixed with 100% methanol for 5 min and stained with 0.5% crystal violet in 25% methanol for 10 min. Cells were then washed three times five minutes each with distilled water to remove excess dye and allowed to dry overnight at room temperature. The incorporated dye was then solubilized in 0.1 M sodium citrate (Sigma-Aldrich) in 50% ethanol. Next, 100 µl of treated and control samples were transferred to 96 well plates and optical densities read at 540 nm using a Biorad 680 microplate reader (BioRad, Hercules, CA-USA).

Determination of viable cell number by trypan blue dye exclusion. Cells were plated for 24 h, treated with 10 nM epothilone B (EMD-Calbiochem) and allowed to incubate for 1, 3, 4, and 5 days at 37 °C. At the end of each time point, cells were trypsinized from a monolayer culture with 0.25% trypsin-EDTA (Invitrogen), centrifuged, and resuspended in media or PBS. Next, 50 µl of cell suspension were mixed with 50 µl of trypan blue and counted using a hemacytometer.

Western blotting. Cells were plated in DMEM containing 10% FBS for twenty-four hours, treated with 10 nM epothilone B (EMD-Calbiochem), and allowed to incubate for 24, 48, and 72 h. Cells were lysed in lysis buffer (pH 6.8) containing 60 mM Tris and 2% SDS. Protein concentrations were determined using the Bradford Method. Samples were electrophoresed in a 12% polyacrylamide gel, transferred to nitrocellulose membranes and immunoblotted with a polyclonal antibody against tubulin (Cell Signaling, Danvers, MA-USA). Signals were detected using a horseradish peroxidase conjugated secondary antibody and colorimetric detection system (BioRad).

ELISA. Cells were plated for twenty-four hours, treated with 10 nM epotholine B (EMD-Calbiochem, San Diego, CA-USA) and allowed to incubate two and four hours. Next cells were fixed in 4% paraformaldehyde for 5 min, rinsed with PBS, permeabilized in 0.075% triton X-100 /PBS for 5 minutes and rinsed again with PBS. Cells were then blocked in PBS containing 3% bovine serum albumin (Sigma) and 1.5% horse serum (VectorLabs, Burlingame, CA-USA) for one hour at room temperature. Samples were then incubated with a primary antibody against survivin (Cell Signaling) for twenty-four hours at 4 °C. Subsequently, cells were rinsed with PBS, incubated with a horseradish peroxidase conjugated secondary antibody for one hour, and rinsed again. Next, cells were treated with TMB peroxidase substrate (BioRad) for five minutes and optical densities read at 655 nm.

Clonogenic survival. Cells were plated for twenty-four hours, treated with 1 nM, 250 pM epothilone B (EMD-Calbiochem), or DMSO (vehicle) and allowed to incubate at 37 °C for 10–14 days. At the termination of the incubation period, cells were fixed with absolute methanol, stained with 1% crystal violet for 10 min, rinsed in tap water and allowed to dry. Colonies, consisting of > 50 cells, were then counted to determine surviving fraction. Surviving fraction (SF) was determined as follows: SF = number of colonies counted/number of cells plated x plating efficiency.

Acridine orange labeling. Cells were plated for twenty-four hours, treated with epothilone B (10 nM) or DMSO (vehicle) and allowed to incubate for 72 h. Following epothilone B treatment cells were incubated with media containing 250 ng/ml acridine orange (Molecular Probes–Invitrogen, Carlsbad, CA-USA) for 15 min. Acridine orange was removed and images were captured using an Olympus IX-51 (Olympus,Center Valley, PA-USA) fluorescent microscope.

Immunofluorescence labeling. Cells were plated and allowed to attach for 24 h in 12 well plates. Cells were then treated with epothilone B (10 nM and 30 nM) or vehicle (DMSO) for six hours, rinsed in PBS, and fixed in 4% paraformaldehyde for 5 min at room temperature. Cells were then rinsed with PBS post-fixation, permeabilized in 0.075% Triton-X 100/PBS for five minutes, rinsed again, and blocked with 3.0% bovine serum albumin, 1.5% horse serum (VectorLabs) in PBS for 1 h at room temperature. Cells were next incubated overnight at 4 °C with an α-tubulin (Molecular Probes-Invitrogen) antibody in 1% BSA/PBS. Samples were next rinsed three times with PBS, incubated with an Alexa 488 goat-anti-mouse conjugated secondary antibody (Molecular Probes-Invitrogen) for 1 h in the dark, rinsed again and examined with an Olympus IX-51 (Olympus) fluorescent microscope.

Statistical analysis. Student's (*t*) test and one way Analysis of Variance statistical analysis were performed to convey significance relating to cell number and protein levels.

RESULTS

Dose response of glioblastoma cells to epothilone B. To assess the efficacy of epothilone B, T98 (p53 mutant), U87 (p53 wild-type), and LNZ (p53 null) glioblastoma cells were treated with 1 nM, 5 nM, and 10 nM epothilone B for 48 h (Fig. 1). Data revealed a dose dependent decrease in cell proliferation of T98, U87, and LNZ glioblastoma cells treated with epothilone B. Exposure of cells to 10 nM epothilone B demonstrated a 35% and 34% reduction ($p < 0.05$) in cell proliferation of T98 (p53 mutant) and LNZ (p53 null) glioblastoma cells, respectively, as compared to vehicle treated controls (see Fig. 1). In contrast U87 (p53 wild type) glioblastoma cells exposed to epothilone B (10 nM) showed only a minor decrease that was not significant ($p > 0.05$), as determined by student (*t*) test.

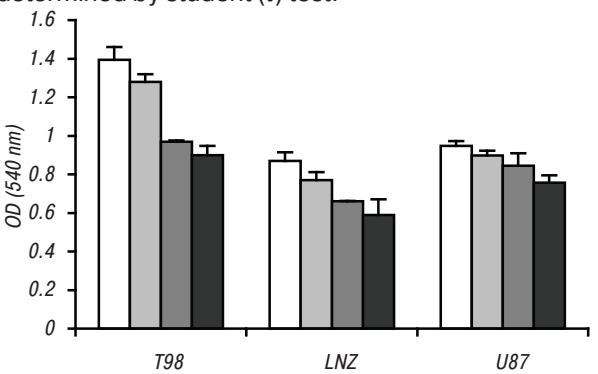

Fig. 1. Dose response experiments of epothilone B treated glioblastoma (T98, LNZ, U87) cells. Vehicle treated control cells (solid white bars); 1 nM epothilone B (solid black bars); 5 nM epothilone B (hatched white bars); 10 nM epothilone B (speckled black bars). Data shown are representative of three independent experiments (means ± SE) performed in duplicate showing similar results

Time course analysis. To further examine the utility of epothilone B, time course experiments were performed over a five day time period on glioblastoma cells treated with 10 nM epothilone B (Fig. 2). Time course analysis showed a reduction in total cell number of T98 (p53 mutant) cells, in response to epotholine B (10 nM) exposure, at each time point examined as compared to vehicle treated control cells at the same time point over the five day time course, as determined by trypan blue dye exclusion. Additionally, we observed a significant ($p < 0.05$) decline in epothilone B (10 nM) treated T98 (p53 mutant) cells over the five day time period as determined by ANOVA analysis, with a 79% reduction in total cell number observed between day 0 and day 5 (see Fig. 2). These data parallel several experimental studies in prostate [6, 9], breast [5], and ovarian [10] cancer that also demonstrated the cytotoxic effects of epothilone B.

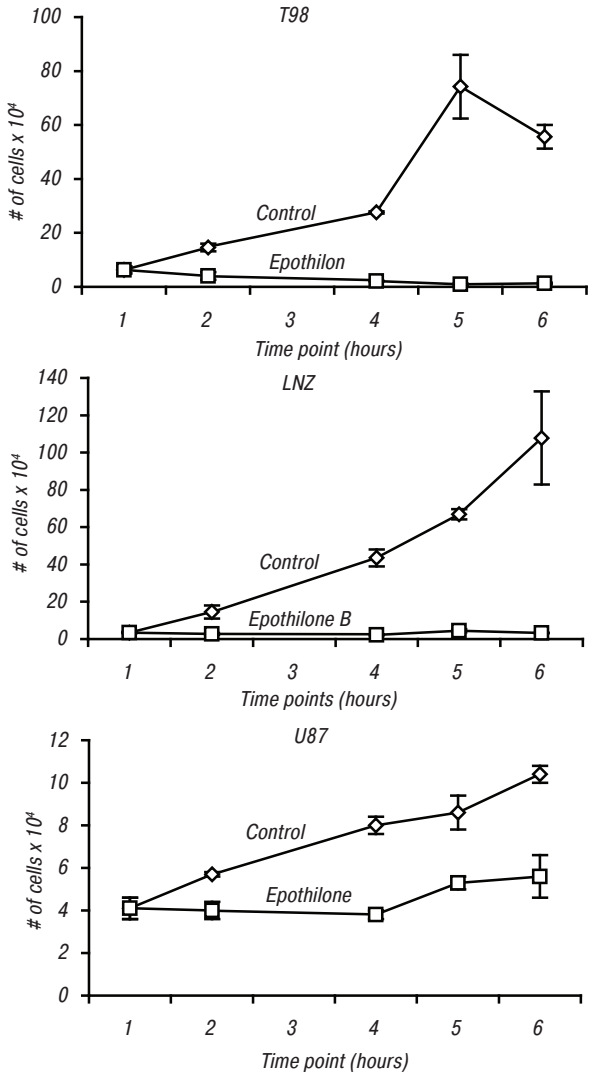

Fig. 2. Time course analysis of glioblastoma cells treated with epothilone B (10 nM) or DMSO (vehicle control). Top: T98 (p53 mutant) glioblastoma cells Middle: LNZ (p53 null) glioblastoma cells. Bottom: U87 (p53 wild type) glioblastoma cells. Data shown are representative of three independent experiments (means ± SE) performed in duplicate displaying similar results

Consistent with time course analysis in T98 (p53 mutant) glioblastoma cells, epothilone B (10 nM) also inhibited proliferative capacity in LNZ (p53 null) glioblastoma cells, due to a prolonged growth arrest, over the five day time period as compared to vehicle treated control cells (see Fig. 2). However, in U87 (p53 wild type) glioblastoma cells time course data displayed a transient growth arrest post-epothilone B treatment, followed by a pronounced proliferative recovery, where a 29% increase in total cell number was observed on day 4 as compared to day 0 (see Fig. 2). Taken together these data suggest that epothilone B induced p53 independent cytotoxic and cytostatic responses in glioblastoma cells, supporting studies conducted by Vikhanskaya et al. (1998) [11] and Bacus et al. (2001) [12] who showed microtubule inhibiting agents induce p53-independent anti-tumorigenic effects in ovarian and breast cancer cells, respectively.

Epothilone B effects on reproductive capacity. In addition to time course analysis, clonogenic survival experiments were performed to assess the ability of epothilone B to inhibit reproductive capacity of glioblastoma cells exposed to a non-toxic concentration (Fig. 3). We observed 47% and 88% fewer colonies ($p < 0.05$) in epothilone B (250 pM) treated T98 and LNZ glioblastoma cells, respectively, as compared to vehicle treated controls. Clonogenic survival data also revealed, like T98 and LNZ cells, U87 glioblastoma cells had a reduced reproductive capacity in response to epothilone B as compared to vehicle controls. However no significant ($p > 0.05$) difference was observed between untreated U87 cells (data not shown) and U87 cells treated with epothilone B, in contrast to T98 and LNZ cells (data not shown). Furthermore, crystal violet staining (Fig. 4) displayed a complete abrogation of T98 and LNZ glioblastoma cell colony formation when treated with 1 nM epothilone B, as compared to untreated and vehicle treated control cells. In contrast treatment of U87 glioblastoma cells with 1 nM epothilone B did not completely inhibit colony formation (see Fig. 4).

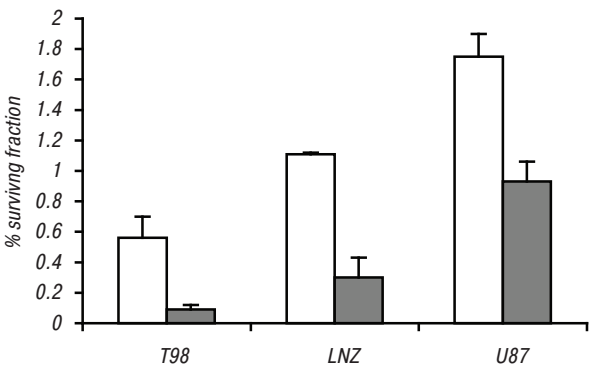

Fig. 3. Clonogenic survival of glioblastoma cells treated with epothilone B for 72 h. Vehicle control (white bars); 250 pM epothilone B (black bars). Data shown are representative of at least three independent experiments (means ± SE) with comparable results

Additionally, in effort to determine the mode of glioblastoma cell death as a consequence of epothilone B exposure we used acridine orange labeling to assess autophagy or type II cell death. Fluorescent labeling experiments with acridine orange did not detect cytoplasmic autophagic vacuoles characteristic of autophagic cell death. Instead acridine orange labeling revealed the presence of fragmented DNA and apoptotic cell bodies in T98, LNZ, and U87

glioblastoma cells (Fig. 5), 72 h post-treatment with 20 nM epothilone B. Consistent with results seen here Mooberry *et al.* (1999) [13] also observed induced DNA fragmentation in aortic smooth muscle cells in response to treatment with the contemporary microtubule stabilizing agent, laulimalide [13].

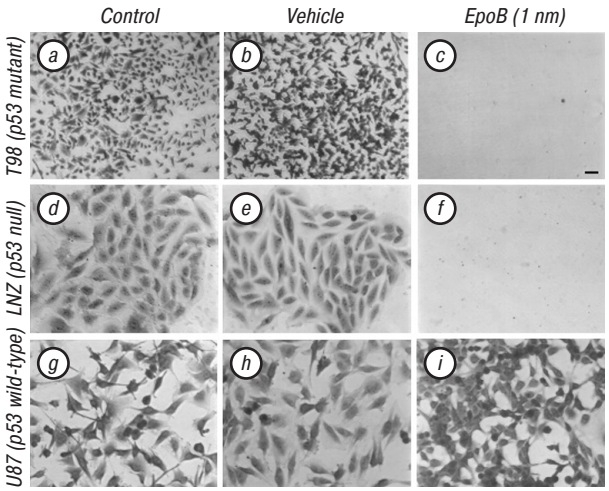

Fig. 4. Crystal violet staining of T98 (*a–c*), LNZ (*d–f*), and U87 (*g–i*) glioblastoma cells treated with 1 nM epothilone B. Cells shown are of a single colony from a clonogenic survival experiment performed in duplicate that is representative of three independent experiments showing similar results (scale bar = 80 μm)

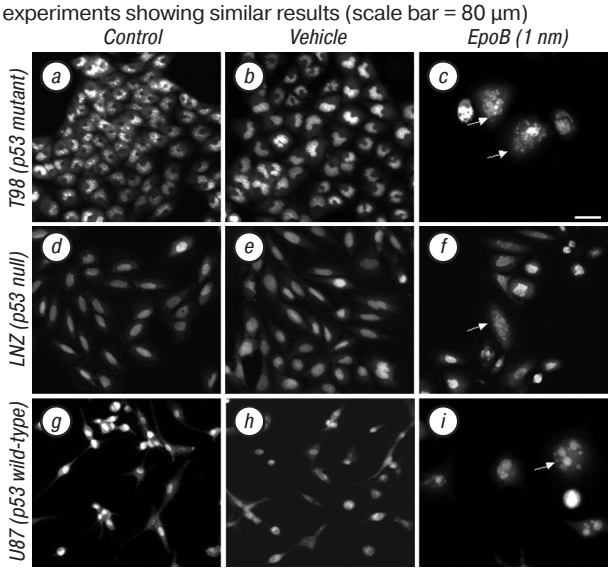

Fig. 5. Acridine orange labeling in glioblastoma cells treated with 10 nM epothilone B for 72 h. *a–c:* T98; *d–f:* LNZ; *g–i:* U87. Data shown are representative of four independent experiments displaying comparable results (scale bar = 160 μm)

Epothilone B induced effects on tubulin. Epothilone B is a microtubule-stabilizing agent that exerts its effects by binding to tubulin, an α/β heterodimer complex of microtubules. We therefore assessed tubulin protein levels in glioblastoma cells treated with 10 nM epothilone B over seventy-two hours. Time course protein analysis using immunoblotting procedures revealed no change in the overall levels of tubulin as a consequence of epothilone B treatment (Fig. 6). To further assess the effects of epothilone B on tubulin in glioblastoma cells immunofluorescent experiments were conducted. Immunolabeling studies revealed the presence of an extended filamentous array of α-tubulin in control glioblastoma cells, while a more perinuclear localization and considerable reduction of filamentous α-tubulin was observed in epothilone B treated T98, LNZ, and U87 glioblastoma cells (Fig. 7). These changes in the organization of α-tubulin parallel those seen by Dietzmann *et al.* (2003) [14] in LN405 glioma cells treated with epothilone D.

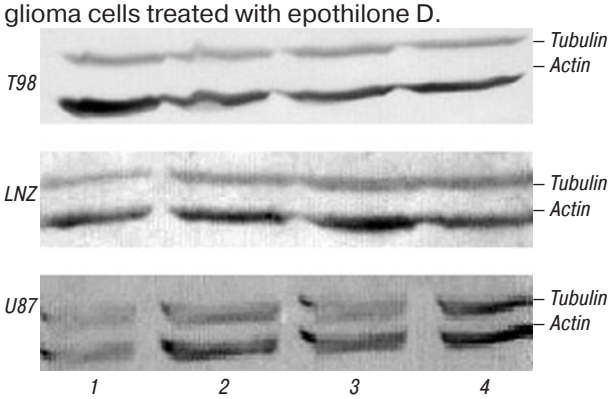

Fig. 6. Time course analysis of tubulin expression in glioblastoma cells exposed to 10 nM epothilone B. Lane: (1) vehicle control, (2) 24 h, (3) 48 h, (4) 72 h. Results shown are representative of three independent experiments that displayed similar results

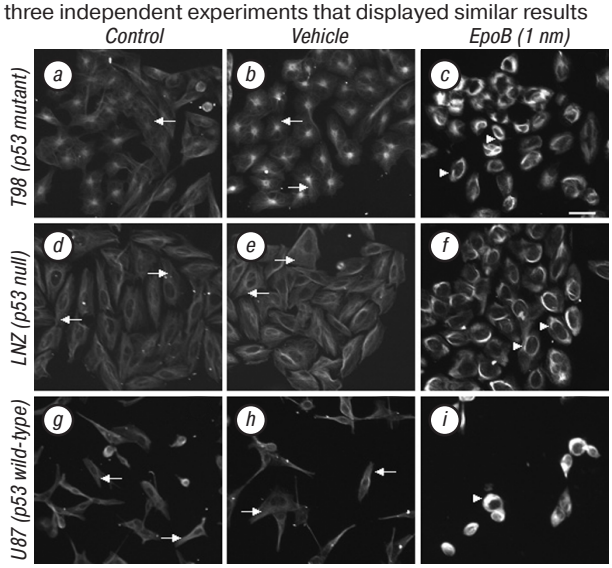

Fig. 7. Immunofluorescence labeling of α-tubulin in epothilone B (20 nM) treated glioblastoma cells at six hours. T98 cells (*a–c*); LNZ cells (*d–f*); U87 (*g–i*). Arrows: cytoplasmic expression of α-tubulin; Arrowheads: α-tubulin expressed at the periphery of the nucleus. Shown are tubulin expressing cells observed from at least three areas of an experiment representative of at least three independent studies exhibiting similar results (scale bar = 160 μm)

Survivin expression in response to epothilone B. In a further effort to assess the mechanistic basis underlying cellular responses in epothilone B treated glioblastoma cells (T98, LNZ, U87), we examined survivin levels, a known mitotic regulator and anti-apoptotic protein [15], in glioblastomas [16]. ELISA experiments revealed a time dependent decrease of survivin in T98 cells post-epothilone B (10 nM) exposure as compared to vehicle treated control cells, with a 51% ($p < 0.01$) decrease observed at 6 h (Fig. 8). However LNZ cells showed a 60% and 32% ($p < 0.05$) increase of survivin at 2 and 4 h, respectively, post-epothilone B (10 nM) treatment as compared to vehicle treated controls. Epothilone B treated U87 cells displayed no significant ($p > 0.05$) changes in survivin levels when compared to vehicle

controls (Fig. 8). These data are consistent with studies performed by Griffin *et al.* (2003) [10] and Wang *et al.* (2005) [17], who similarly showed a reduction of survivin in ovarian cancer cells after treatment with the microtubule inhibiting agents BMS-247550, an epothilone B analogue, and taxol, respectively.

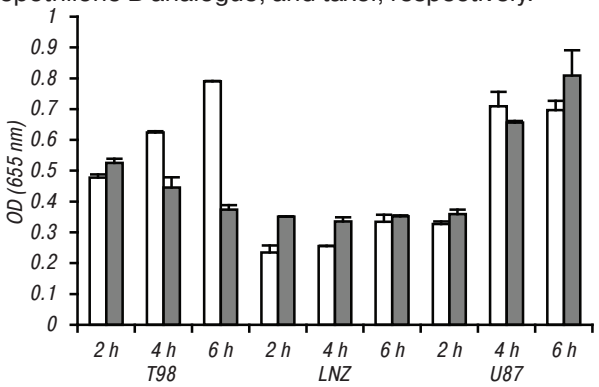

Fig. 8. Survivin expression in glioblastoma cells treated with 10 nM epothilone B. Vehicle controls (white bars); 10 nM epothilone B (black bars). Data shown are representative of three independent experiments (means ± SE) performed in duplicate displaying comparable results

DISCUSSION

The clinical resistance of glioblastomas to chemotherapeutic drugs, particularly classic microtubule inhibiting agents (vincristine, paclitaxel), is well documented [18]. The present study was undertaken to assess the efficacy of the contemporary microtubule stabilizing agent epothilone B on glioblastoma cells. Data here has shown that a clinically relevant concentration of epothilone B induced growth arrest in glioblastoma cells, a result consistent with the primary mode of action of microtubule targeting agents. Additionally, we observed the promotion of glioblastoma cell death with epothilone B, a response often observed as a subsequent event to mitotic growth arrest in tumor cells exposed to microtubule inhibiting agents. These findings parallel previous preclinical investigations by Yoshida *et al.* (2000) [19] and Dietzmann *et al.* (2003) [14] that also demonstrated anti-neoplastic effects with the microtubule targeting agents estramustine and epothilone D on glioblastoma cells, respectively. Furthermore, data here extends previous observations of microtubule inhibiting agents on glioblastomas by providing evidence on the involvement of survivin and p53 in the tumor cell response of this neoplasm.

Microtubule inhibiting agents exert their anti-tumorigenic effects on cancer cells by inhibiting microtubule dynamics and function. We therefore examined changes in alpha-tubulin, a component of the microtubule heterodimeric complex. We observed reduced filametous alpha-tubulin, representative of a disrupted microtubule network, in epothilone B treated glioblastoma cells. These data provide evidence that epothilone B induced changes of tubulin in glioblastoma cells are involved in our observed cytostatic response. Furthermore these data suggest that changes in the microtubule network play a role in the cytotoxic response of glioblastoma cells treated with epothilone B, changes similarly seen in aortic smooth muscle cells, lung cancer cells, and leukemia cells that also underwent cell death in response to microtubule targeting agents [20, 21].

Due to our observation that epothilone B induced glioblastoma cell death was not preceded by mitotic growth arrest and the lack of experimental evidence regarding a direct role of tubulin in drug induced cell death we examined the role of the anti-apoptotic protein, survivin. In addition to survivin's role as an anti-apoptotic protein, it is also a mitotic regulator of cell division as a component of the chromosome passenger complex (Aurora B and Inner Centromere Protein) at kinetochores, which functions to ensure proper chromosome and microtubule spindle association [15]. Survivin has been shown to co-localize with microtubules [15, 22] and inhibiting its function has been shown to promote malformations in microtubule assembly [22]. Additionally, it has been shown that glioblastomas express survivin and contributes to the clinical resistance of this neoplasm to therapy [16]. In light of survivin's expression in glioblastomas and its role as a pro-survival protein and mitotic regulator we examined survivin levels in epothilone B treated glioblastoma cells in the current study. We observed a down-regulation of survivin in epothilone B treated p53 mutant glioblastoma cells; paralleling results in ovarian cancer cells treated with the epothilone B derivative, BMS 247550 [11] and taxol [17]. Furthermore, we observed an increase of survivin expression in p53 null glioblastoma cells that underwent growth arrest post-epothilone B exposure, a response also seen in taxol treated breast and cervical carcinoma cells [25, 26]. Collectively these data suggest that epothilone B induces glioblastoma cell death via apoptosis. This was further supported in the present study by the presence of fragmented DNA and apoptotic cells bodies with a marker for autophagy or type II cell death. In support of our findings Li *et al.* (2007) [27] also detected fragmented chromatin and apoptotic cell bodies with the autophagic marker, acridine orange, in epidermoid carcinoma cells treated with oridonin. The identification of apoptotic cells with a marker for autophagy in the present study can in part be explained by an established relationship between autophagy and apoptosis. Although the mechanism underlying the synergistic relationship between autophagy and apoptosis are not clear it is established that autophagy precedes apoptosis during this cooperative mode of cell death [28]. This sequential mode of cell death has been described experimentally in oridonin treated breast cancer cells [29] and lysosome asscociated membrane protein (LAMP) depleted Hela cells [30]. The detection of fragmented DNA and apoptotic cell bodies with acridine orange labeling described here suggests that this sequential mode of cell death is occurring during epothilone B induced glioblastoma cell death. It should also be stated that the presence of fragmented chromatin and apoptotic cell bodies in p53 null and wild-type glioblastomas cells suggest that a small subpoplation of these cells were sensitive to

drug treatments. Taken together these data support a pro-survival role for survivin in glioblastoma cells, which is likely due to survivin's function in microtubule and chromosome stability. However, it should also be mentioned that studies in breast tumor cells have identified cytoprotective functions of mitochondria localized survivin [25], a pool of survivin that may also be contributing to epothilone B treated glioblastoma cell responses observed here.

Furthermore, we have shown here that epotholine B invokes an anti-tumorigenic response in glioblastoma cells with aberrant p53 expression with limited utility in p53 wild-type glioblastoma cells. These findings are consistent with the well established utility of the classic microtubule stabilizing agent, taxol, which has been shown in several studies to efficiently induce cell kill in tumor cells with deficient p53 [31, 32]. Taken together data from this study provide preclinical evidence for the use of epothilone B and its analogues in the treatment of primary and secondary glioblastomas with p53 mutations present in approximately 30% and 65%, respectively, of these high-grade gliomas [33, 34]. Additionally, therapeutic agents specifically targeting tumor cells, particularly glioblastomas, with abnormal p53 have strong clinical promise due to their ability to spare normal cells. Future studies from our laboratory will examine further the relationship of p53 and survivin in glioblastomas treated with novel microtubule inhibiting agents, as the functional loss of p53 has been associated with survivin up-regulation [35]. Epotholine B, is a viable alternative to current taxanes and vinca alkaloids used in adjuvant and neoadjuvant approaches for the clinical treatment of glioblastomas.

ACKNOWLEDGMENTS

Support for this research was provided by Research Initiative for Scientific Enhancement (National Institute of General Medical Sciences-5R25GM066714-04) and Career Opportunities in Research (National Institute of Mental Health-5T34MH017102-25) training grants from the National Institutes of Health (USA).

REFERENCES

1. **Mollinedo F, Gajate C.** Micortubles, microtubule-interfering agents and apoptosis. Apoptosis 2003; **8**: 413–50.

2. **Zhou J, Giannakakou P.** Targeting microtubules for cancer chemotherapy. Curr Med Chem Anticancer Agents 2005; **5**: 65–71.

3. **Fojo AT, Menefee M.** Microtubule targeting agents: basic mechanisms of multidrug resistance (MDR). Semin Oncol 2005; **32** (6 Suppl 7): S3–8.

4. **Nicolaou KC, Ritzén A, Namoto K.** Recent developments in the chemistry, biology and medicine of the epothilones. Chem Commun (Camb) 2001; **17**: 1523–35.

5. **Yamaguchi H, Paranawithana SR, Lee MW, *et al.*** Epothilone B analogue (BMS-247550)-mediated cytotoxicity through induction of Bax conformational change in human breast cancer cells. Cancer Res 2002; **62**: 466–71.

6. **Sepp-Lorenzino L, Balog A, Su DS, *et al.*** The microtubule-stabilizing agents epothilones A and B and their desoxy-derivatives induce mitotic arrest and apoptosis in human prostate cancer cells. Prostate Cancer Prostatic Dis. 1999; **2**: 41–52.

7. **Uyar D, Takigawa N, Mekhail T, *et al.*** Apoptotic pathways of epothilone BMS 310705. Gynecol Oncol 2003; **91**: 173–8.

8. **Wartmann M, Altmann KH.** The biology and medicinal chemistry of epothilones. Curr Med Chem Anticancer Agents 2002; **2**: 123–48.

9. **Ioffe ML, White E, Nelson DA, *et al.*** Epothilone induced cytotoxicity is dependent on p53 status in prostate cells. Prostate 2004; **61**: 243–7.

10. **Griffin D, Wittmann S, Guo F, *et al.*** Molecular determinants of epothilone B derivative (BMS 247550) and Apo-2L/TRAIL-induced apoptosis of human ovarian cancer cells. Gynecol Oncol 2003; **89**: 37–47.

11. **Vikhanskaya F, Vignati S, Beccaglia P, *et al.*** Inactivation of p53 in a human ovarian cancer cell line increases the sensitivity to paclitaxel by inducing G2/M arrest and apoptosis. Exp Cell Res 1998; **241**: 96–101.

12. **Bacus SS, Gudkov AV, Lowe M, *et al.*** Taxol-induced apoptosis depends on MAP kinase pathways (ERK and p38) and is independent of p53. Oncogene 2001; **20**: 147–55.

13. **Mooberry SL, Tien G, Hernandez AH, *et al.*** Laulimalide and isolaulimalide, new paclitaxel-like microtubule-stabilizing agents. Cancer Res 1999; **59**: 653–60

14. **Dietzmann A, Kanakis D, Kirches E, *et al.*** Nanomolar concentrations of epothilone D inhibit the proliferation of glioma cells and severely affect their tubulin cytoskeleton. J Neurooncol 2003; **65**: 99–106.

15. **Altieri DC.** Targeted therapy by disabling crossroad signaling networks: the survivin paradigm. Mol Cancer Ther 2006; **5**: 478–82.

16. **Chakravarti A, Noll E, Black PM, *et al.*** Quantitatively determined survivin expression levels are of prognostic value in human gliomas. J Clin Oncol 2002; **20**: 1063–8.

17. **Wang Z, Xie Y, Wang H.** Changes in survivin messenger RNA level during chemotherapy treatment in ovarian cancer cells. Cancer Biol Ther 2005; **4**: 716–9.

18. **Haroun RI, Clatterbuck RE, Gibbons MC, *et al.*** Extreme drug resistance in primary brain tumors: in vitro analysis of 64 resection specimens. J Neurooncol 2002; **58**: 115–23.

19. **Yoshida D, Hoshino S, Shimura T, *et al.*** Drug-induced apoptosis by anti-microtubule agent, estramustine phosphate on human malignant glioma cell line, U87MG; in vitro study. J Neurooncol 2000; **47**: 133–40.

20. **Grzanka A, Grzanka D, Orlikowska M.** Cytoskeletal reorganization during process of apoptosis induced by cytostatic drugs in K-562 and HL-60 leukemia cell lines. Biochem Pharmacol 2003; **66**: 1611–7.

21. **Grzanka A, Grzanka D, Orlikkowska M, *et al.*** Estimation of taxol influence on changes in tubulin and vimentin systems in K-562 and HL-60 cell lines by immunofluorescence microscopy. Neoplasma 2005; **52**: 193–8.

22. **Fortugno P, Wall NR, Giodini A, *et al.*** Survivin exists in immunochemically distinct subcellular pools and is involved in spindle microtubule function. J Cell Sci 2002; **115**: 575–85.

23. **Blum R, Jacob-Hirsch J, Rechavi G, Kloog Y.** Suppression of survivin expression in glioblastoma cells by the Ras inhibitor farnesylthiosalicylic acid promotes caspase-dependent apoptosis. Mol Cancer Ther 2006; **5**: 2337–47.

24. **McLaughlin N, Annabi B, Bouzeghrane M, *et al.*** The Survivin-mediated radioresistant phenotype of glioblastomas is regulated by RhoA and inhibited by the green tea polyphenol (-)-epigallocatechin-3-gallate. Brain Res 2006; **1071**: 1–9.

25. **O'Connor DS, Wall NR, Porter AC, Altieri DC.** A p34(cdc2) survival checkpoint in cancer. Cancer Cell 2002; **2**: 43–54.

26. **Dohi T, Beltrami E, Wall NR, *et al.*** Mitochondrial survivin inhibits apoptosis and promotes tumorigenesis. J Clin Invest 2004; **114**: 1117–27.

27. **Li D, Cui Q, Chen SG, *et al.*** Inactivation of ras and changes of mitochondrial membrane potential contribute to oridonin-induced autophagy in A431 cells. J Pharmacol Sci 2007; **105**: 22–33.

28. **Gozuacik D, Kimchi A.** Autophagy as a cell death and tumor suppressor mechanism. Oncogene 2004; **23**: 2891–906.

29. **Cui Q, Tashiro S, Onodera S, *et al.*** Autophagy preceded apoptosis in oridonin-treated human breast cancer MCF-7 cells. Biol Pharm Bull 2007; **30**: 859–64.

30. **González-Polo RA, Boya P, Pauleau AL, *et al.*** The apoptosis/autophagy paradox: autophagic vacuolization before apoptotic death. J Cell Sci 2005; **118**: 3091–102.

31. **Woods CM, Zhu J, McQueney PA, *et al.*** Taxol-induced mitotic block triggers rapid onset of a p53-independent apoptotic pathway. Mol Med 1995; **1**: 506–26.

32. **Vikhanskaya F, Vignati S, Beccaglia P, *et al.*** Inactivation of p53 in a human ovarian cancer cell line increases the sensitivity to paclitaxel by inducing G2/M arrest and apoptosis. Exp Cell Res 1998; **241**: 96–101.

33. **Nozaki M, Tada M, Kobayashi H, *et al.*** Roles of the functional loss of p53 and other genes in astrocytoma tumorigenesis and progression. Neuro Oncol 1999; **1**: 124–37.

34. **Ohgaki H, Kleihues P.** Genetic pathways to primary and secondary glioblastoma. Am J Pathol 2007; **170**: 1445–53.

35. **Sah NK, Khan Z, Khan GJ, Bisen PS.** Structural, functional and therapeutic biology of survivin. Cancer Lett 2006; **244**: 164–71.

ЭПОТИЛОН В ВЫЗЫВАЕТ ГИБЕЛЬ КЛЕТОК ГЛИОБЛАСТОМЫ ПУТЕМ СНИЖЕНИЯ УРОВНЯ ЭКСПРЕССИИ СУРВИВИНА

Клиническая резистентность глиобластом к химиотерапии может быть объяснена действием насосов, откачивающих препараты, подобные Р-гликопротеину, которые играют важную роль в снижении эффективности лекарственных средств. *Цель:* изучить возможность применения эпотилона В, не являющегося субстратом для Р-гликопротеина, для воздействия на глиобластомные клетки. *Методы:* для оценки эффективности эпотилона В как антинеопластического препарата в работе *in vitro* использованы клеточные линии глиобластомы, отличающиеся по статусу p53. Для оценки уровня экспрессии тубулина и сурвивина в клетках, обработанных эпотилоном В, были использованы методы иммунофлуоресценции и ELISA. Окрашивание акридиновым оранжевым применяли для детекции погибших клеток после обработки эпотилоном В. *Результаты:* клинически достижимые концентрации эпотилона В оказывали цитотоксический ответ на клетки глиобластомы с мутантным p53 в результате снижения уровня сурвивина и перераспределения тубулина. В то же время цитостатический эффект наблюдали и на клетках глиобластомы, не экспрессирующих p53, что сопровождалось небольшим повышнием экпрессии сурвивина после обработки клеток эпотилоном В. Однако на клетках глиобластомы с p53 дикого типа не было выявлено антиопухолевого ответа на эпотилон В. *Выводы:* эпотилон В вызывает различный противоопухолевый ответ в клетках глиобластомы, экспрессирующих мутантный p53, либо отрицательных по p53. Это позволяет предположить, что эпотилон В является альтернативой классическим препаратам, ингибирующим микротрубочки (например винкристину, паклитакселу), которые используют в клинической практике при лечении глиобластом с мутантным белком p53.

Ключевые слова: **глиобластома, эпотилон В, сурвивин, белок p53.**