International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2016.19.27

Research Article

Paclitaxel-Induced G_2/M Arrest via Different Mechanism of Actions in Glioma Cell Lines with Differing p53 Mutational Status

Byeal-I Han and Michael Lee

Division of Life Sciences, College of Life Sciences and Bioengineering, Incheon National University, Incheon 406-772, Republic of Korea

Abstract

A major concern with regard to glioma treatment arises from the fact that high-grade gliomas are insensitive to the majority of anticancer therapies. The aim of the present study was to investigate anti-proliferation potential of the mitotic inhibitor paclitaxel in three glioma cells with different BRAF mutation status. The U-87-MG cells were found to be more resistant to paclitaxel than other two glioma cell lines T98G and DBTRG-05MG, suggesting that the response of glioma cells to paclitaxel is not affected by the BRAF genotype. In addition, despite the induction of both apoptosis and autophagy in all glioma cell lines tested, our study has not specifically addressed the correlation of apoptosis and autophagy induction with growth inhibition. Instead, we found that paclitaxel caused a remarkably significant G_2/M arrest in response to paclitaxel in T98G and DBTRG-05MG cells, whereas, less significant G_2/M arrest was detected after paclitaxel treatment in U-87-MG, which exhibited more resistant to paclitaxel than other two cell lines. It is observed that T98G cells with mutant p53 progress through G_0/G_1 checkpoint and greatly accumulated in the subsequent G_2/M phase. In case of DBTRG-05MG cells with wild type p53, paclitaxel-induced growth inhibition displayed characteristics of $p27^{Kip1}$-dependent G_2/M arrest. In this study data suggest that paclitaxel-induced growth inhibition of glioma cells is tightly correlated with the G_2/M arrest regardless of p53 mutation status.

Key words: Paclitaxel, BRAF mutation, glioma, G_2/M arrest, p53, $p27^{Kip1}$

Received: August 24, 2015 Accepted: December 02, 2015 Published: December 15, 2015

Citation: Byeal-I Han and Michael Lee, 2016. Paclitaxel-Induced G2/M Arrest via Different Mechanism of Actions in Glioma Cell Lines with Differing p53 Mutational Status. Int. J. Pharmacol., 12: 19-27.

Corresponding Author: Michael Lee, Division of Life Sciences, College of Life Sciences and Bioengineering, Incheon National University, 12-1 Songdo-dong, Yeonsu-gu, Incheon, 406-772, Republic of Korea Tel: 82-32-835-8247 Fax: 82-32-835-0763

Copyright: © 2016 Byeal-I Han *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

High-grade gliomas such as anaplastic astrocytoma and glioblastoma multiforme are lethal types of brain cancer (Wen and Kesari, 2008). In particular, glioblastoma (WHO: grade IV glioma), one of the most fatal solid cancers is characterized by an insensitivity to the majority of anticancer treatments (Chang *et al*., 2006). One major reason is the presence of expression P-glycoprotein and Multidrug Resistance Protein (MRP) which prevents chemotherapeutic drugs from reaching the brain (Decleves *et al*., 2002). Our previous reports also showed the expression of p-glycoprotein and MRPs in glioma cell lines (Ahn *et al*., 2014). On the other hand, no significant differences in expression of MDR proteins were detected between primary or recurrent gliomas, suggesting that glioma chemoresistance is mostly intrinsic.

Paclitaxel, used for the treatment of breast, ovarian, lung and colon cancers (Holmes *et al*., 1991; McGuire *et al*., 1989; Schiller *et al*., 2002) has been also found to exert significant cytotoxicity against malignant glioma cells *in vitro* (Liebmann *et al*., 1993). However, paclitaxel did not fare well in clinical trials because of its failure to reach an adequate concentration in the brain tumors (Glantz *et al*., 1995; Sanson *et al*., 2000), although it has been recently reported that brain-penetrating nanoparticles improve paclitaxel efficacy in malignant glioma (Nance *et al*., 2014; Zhao *et al*., 2010).

High prevalence of BRAF mutations in several tumor types including melanoma and thyroid cancer has led to the development of inhibitors of mutated BRAF (Ribas and Flaherty, 2011). Epithelioid glioblastomas also showed a high percentage of the BRAF-V600E mutation (Kleinschmidt-DeMasters *et al*., 2013). However, the duration of clinical response with selective BRAF inhibitors is short in many patients due to acquired resistance to oncogenic BRAF inhibitor (Flaherty *et al*., 2010). Our previous study suggested that, unlike resistance to paclitaxel, resistance to BRAF inhibitors was not attributable to P-glycoprotein overexpression (Ahn and Lee, 2013). Instead, re-activation of the MAPK pathway through RAF-1 activation has accepted as a mechanism that contributes to the mechanism of acquired resistance to BRAF inhibitors (Aplin *et al*., 2011). Paclitaxel-induced cytotoxicity was found to be inversely correlated with RAF-1 kinase activity (Rasouli-Nia *et al*., 1998). However, the role of RAF signaling pathway in paclitaxel-induced cytotoxicity are still controversial. Blagosklonny *et al*. (1997) reported that paclitaxel led to activation of RAF-1, resulting in apoptosis via inactivation of antiapoptotic proteins such as Bcl-2. Our previous report also showed that down-regulation of RAF-1 caused a significant decrease in cell susceptibility to paclitaxel (Ahn *et al*., 2009; Lee *et al*., 2003).

This study provide the evidence that glioma cell lines with different BRAF mutation status can be targeted using paclitaxel. Importantly, it is demonstrated that despite paclitaxel-induced apoptosis and autophagy in all glioma cell lines tested, differential growth inhibitory effect of paclitaxel on glioma cell lines might not depend on the autophagy and apoptosis induction. Furthermore, these results indicated that paclitaxel-induced growth inhibition of glioma cells was tightly correlated with the G_2/M arrest. Importantly, these findings as a rationale for the potential therapeutic application of paclitaxel in gliomas was discussed.

MATERIALS AND METHODS

Antibodies and reagents: Rabbit polyclonal anti-p21^{Cip1} and anti-p27^{Kip1} antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-LC3 antibody was obtained from Sigma (St. Louis, MO, USA). For the apoptosis assay, the FITC Annexin V Apoptosis Detection Kit was purchased from BD Biosciences Pharmingen (San Diego, CA, USA). Dulbecco's modified Eagle's medium, fetal calf serum and penicillin-streptomycin were purchased from Life Technologies (Grand Island, NY, USA). The reagents for sodium dodecyl sulfate-polyacrylamide gel electrophoresis were obtained from Bio-Rad (Hercules, CA, USA). Paclitaxel was obtained from Sigma.

Cell lines, cell culture and chemical treatment: The human grade 3-4 malignant glioma cell lines (T98G, U-87-MG and DBTRG-05MG) were obtained from the Korean Cell Line Bank (Seoul, Korea) or the American Type Culture Collection (Manassas, VA, USA). The DBTRG-05MG is heterozygous for the BRAF-V600E mutation, whereas T98G and U-87MG are homozygous for wild-type BRAF (Davies *et al*., 2002; Nicolaides *et al*., 2011). All cell lines were maintained at 37°C in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, penicillin-streptomycin and glutamine. For experimental purposes, cells were cultured in 60 mm tissue culture dishes until they reached ~80% confluency. Paclitaxel was dissolved in dimethyl sulfoxide (DMSO) and freshly diluted for each experiment. The DMSO concentration was less than 0.1% in all experiments.

Cell growth assay: The cells were plated in quadruplicate in 96 well microliter plates (Costar, Cambridge, MA, USA) at a

density of 5×10^3 cells per well and then treated with paclitaxel at 37°C in a humidified 5% CO_2/95% air incubator. On day 3, the cells were incubated with MTT at 37°C for 3 h. The absorbance of the samples against a background control (medium alone), which was used as a blank, was measured at 450 nm using a microliter plate (ELISA) reader (Molecular Devices, Sunnyvale, CA, USA).

Cell cycle assay: The cells were washed once with phosphate-buffered saline (PBS), trypsinized and collected by centrifugation at $400\times g$ for 5 min. The cells (10^6 cells per sample) were fixed with 70% ethanol and stained with 50 μg mL^{-1} Propidium Iodide (PI) for 5 min. Cell cycle distribution was examined by measuring the DNA content using a Gallios flow cytometer and the Kaluza analysis software (Beckman Coulter, Inc., Brea, CA, USA). A minimum of 10^4 cells per data point were examined.

Quantitation of autophagy: The cells were grown on chamber slides, washed with PBS and fixed in 10% formalin solution for 10 min. Cells were transiently transfected with the pEGFP-LC3 plasmid (Addgene, Cambridge, MA, USA) using lipofectamine 2000 (Life Technologies) in opti-minimal essential medium I (Life Technologies) according to the manufacturer's protocol. After 48 h, the transfected cells were treated with paclitaxel for the indicated times. Fixed cells were classified as cells with predominantly diffuse GFP-LC3 fluorescence or a punctate GFP-LC3 pattern using a zeiss axio scope. A1 epifluorescence microscope (Carl Zeiss MicroImaging, Inc., USA). To quantify the percentage of cells undergoing autophagy, 200 cells from each group were counted in two independent fields and the percentages of GFP-LC3-positive cells with GFP-LC3 dots were determined.

Annexin V-PI double staining: For apoptosis assay, the phosphatidylserine translocation to the outer leaflet of the plasma membrane was assessed through its reaction with annexin V-fluorescein isothiocyanate (FITC). After treatment with paclitaxel, 2×10^6 cells were harvested, washed with ice-cold PBS, resuspended in 200 μL binding buffer (10 mM HEPES/NaOH [pH 7.4], 140 mM NaCl and 2.5 mM $CaCl_2$) and incubated with 5 μL Annexin V conjugated with FITC for 10 min at room temperature in the dark. The samples were then washed with binding buffer, resuspended in PBS, counter stained with PI and analyzed with a Gallios flow cytometer and the Kaluza analysis software (Beckman Coulter, Inc.). Annexin V^-/PI^+ cells were considered necrotic, whereas Annexin V^+/PI^+ cells were considered late apoptotic. Annexin V^+/PI^- cells were identified as apoptotic cells.

Preparation of cell lysates and immunoblot analysis: Whole cell lysates were prepared as follows. Cells were washed twice with ice-cold PBS and harvested by scraping into RIPA lysis. The cell lysates were clarified by centrifugation at $15,000\times g$ and 4°C for 10 min and the protein concentration of the lysates was determined with a BCA protein assay reagent kit. For immunoblotting, whole cell lysates were denatured in Laemmli sample buffer and resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were transferred onto nitrocellulose membranes and immunoblot analysis was performed using the appropriate primary antibodies. The immune complexes on the nitrocellulose membranes were detected using the ECL-Plus chemiluminescent system (Amersham Pharmacia Biotech, Piscataway, NJ, USA). Fluorescent images were captured using the KODAK Image Station 4000R (Carestream Health, Inc., Rochester, NY, USA). Band intensities were quantified using the Kodak Molecular Imaging software (version 4.5.0; Carestream Health, Inc.).

RESULTS

Growth inhibitory effect of paclitaxel on glioma cells with different BRAF mutation status: The inhibitory concentrations of paclitaxel in the three glioma cell lines with different BRAF mutation status were determined using MTT assays. The cell line DBTRG-05MG contains the BRAF-V600E mutation, whereas the cell lines T98G and U-87-MG are homozygous for wild-type (WT) BRAF. Of the two cell lines harboring WT BRAF, U-87-MG cells were more strongly resistant to paclitaxel than T98G cells (Fig. 1). More interestingly, paclitaxel induced similar levels of growth inhibition in T98G cells and DBTRG-05MG cells, regardless of the BRAF mutation status, although the inhibitory effect of paclitaxel on DBTRG-05MG cells was slightly greater than its effect on T98G cells. These results suggest that the response of glioma cells to paclitaxel is not affected by the BRAF genotype.

Apoptosis induction after G_2/M-phase arrest with paclitaxel: In addition, it has been reported that chemotherapeutic drug resistance in glioma cells is mainly due to high resistance to apoptotic cell death (Steinbach and Weller, 2004). In order to characterize the precise mechanism by which paclitaxel induces differential growth inhibition in three glioma cell lines, the effect of paclitaxel on cell cycle distribution was determined by flow cytometry. Paclitaxel caused a large increase in the number of cells in the G_2/M phase in all cell lines tested with a concomitant

decrease in the number of cells in the G_0/G_1 phase (Fig. 2a). The effect of paclitaxel on cell cycle was most prominent in T98G cells. However, interestingly, we found

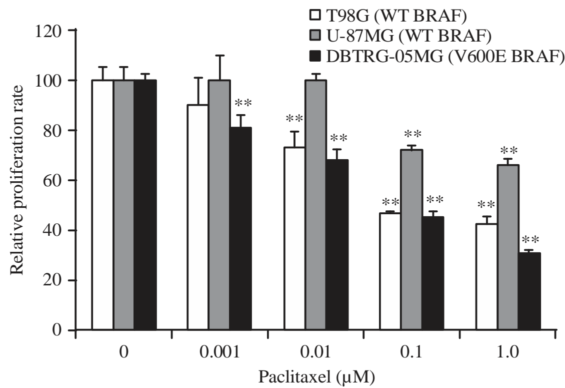

Fig. 1: The growth inhibitory effect of paclitaxel on BRAF-WT and BRAF-V600E glioma cells. (A) Three glioma cell lines (T98G, U-87MG and DBTRG-05MG) were treated with paclitaxel (0.001-1 μM) for 3 days. Glioma cells were BRAF homozygous or heterozygous for wild-type or V600E mutation. The cell growth in response to paclitaxel was then evaluated using the MTT assay. The relative proliferation rate of the cells treated with vehicle alone was regarded as 100%. The values represent the Means±SD of quadruplicate determinants from one of three representative experiments. **$p<0.01$ as determined by Dunnett's T-test compared to vehicle control group

less significant G_2/M arrest in DBTRG-05MG cells, which exhibited more sensitive to paclitaxel than T98G cells. We also investigated the effect of paclitaxel on the expression levels of the cyclin-dependent kinase inhibitors $p21^{Cip1}$ and $p27^{Kip1}$, which contribute to the regulation of cell cycle progression. Immunoblot analysis showed that paclitaxel had little effect on the induction level of expression of $p21^{Cip1}$ in all cell lines tested (Fig. 2b). Very interestingly, it is found that $p21^{Cip1}$ was expressed at very low levels in T98G cells, which have been found to possess mutant p53 (a mutation at codon 237) (Yin *et al*., 2005). It has been known that cancer cells with p53 mutations express low levels of $p21^{Cip1}$ mRNA (Mousses *et al*., 2001). On the other hand, a significant upregulation of $p27^{Kip1}$ was observed in DBTRG-05MG cells. However, no detectable increase of $p27^{Kip1}$ was observed in T98G and U-87-MG cells.

In G_2/M phase, paclitaxel has been known to be able to cause G_2/M arrest and apoptosis through microtubule disorganization (Horwitz, 1994). Thus, apoptotic cell death was assessed by staining with fluorescein isothiocyanate-labeled annexin V and PI. Unexpectedly, paclitaxel treatment resulted in an emergence of apoptotic cells in all glioma cell lines tested regardless of sensitivity level to paclitaxel cytotoxicity (Fig. 3). In particular, the percentage of apoptotic cells in U-87-MG cells, which are strongly resistant to paclitaxel, were similar to that observed in T98G and DBTRG-05MG cells (Fig. 3). These results imply that the differential inhibitory effects in three glioma cell lines are unlikely to result from the difference in apoptosis.

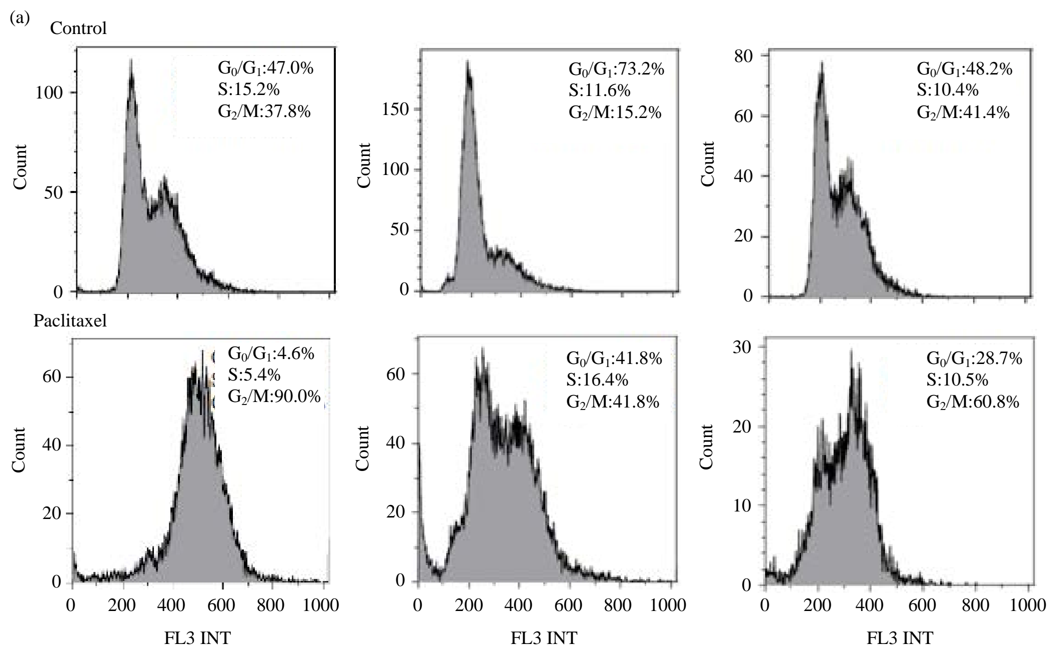

Fig. 2: Continue

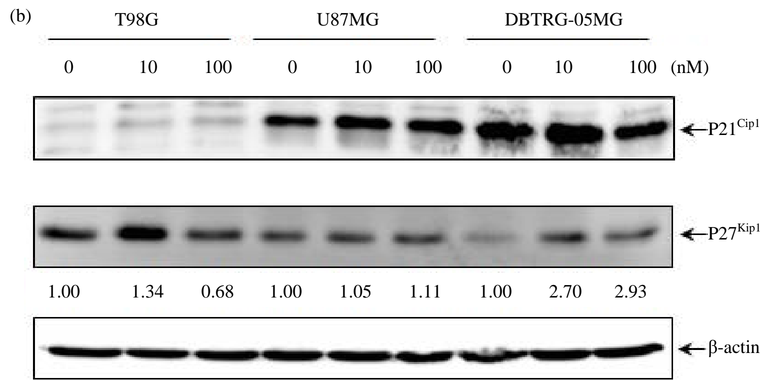

Fig. 2(a-b): Detection of G_2/M arrest in cells treated with paclitaxel, (a) After treatment with paclitaxel (100 nM) for 24 h, cell cycle progression was assessed by staining fixed cells with propidium iodide to estimate the percentage of cells in the G_0/G_1 (2N DNA content), G_2/M (4N DNA content) and S phases (2 to 4N DNA content). The percentage of cells in each phase of the cell cycle was determined by flow cytometry and quantitated using the Cell Quest Pro software and (b) Whole cell extracts were prepared after 24 h of paclitaxel treatment. The expression levels of p21^{Cip1} and p27^{Kip1} were assessed by immunoblotting. β-actin expression was assessed as protein loading control. The presented results are representative of at least three independent experiments

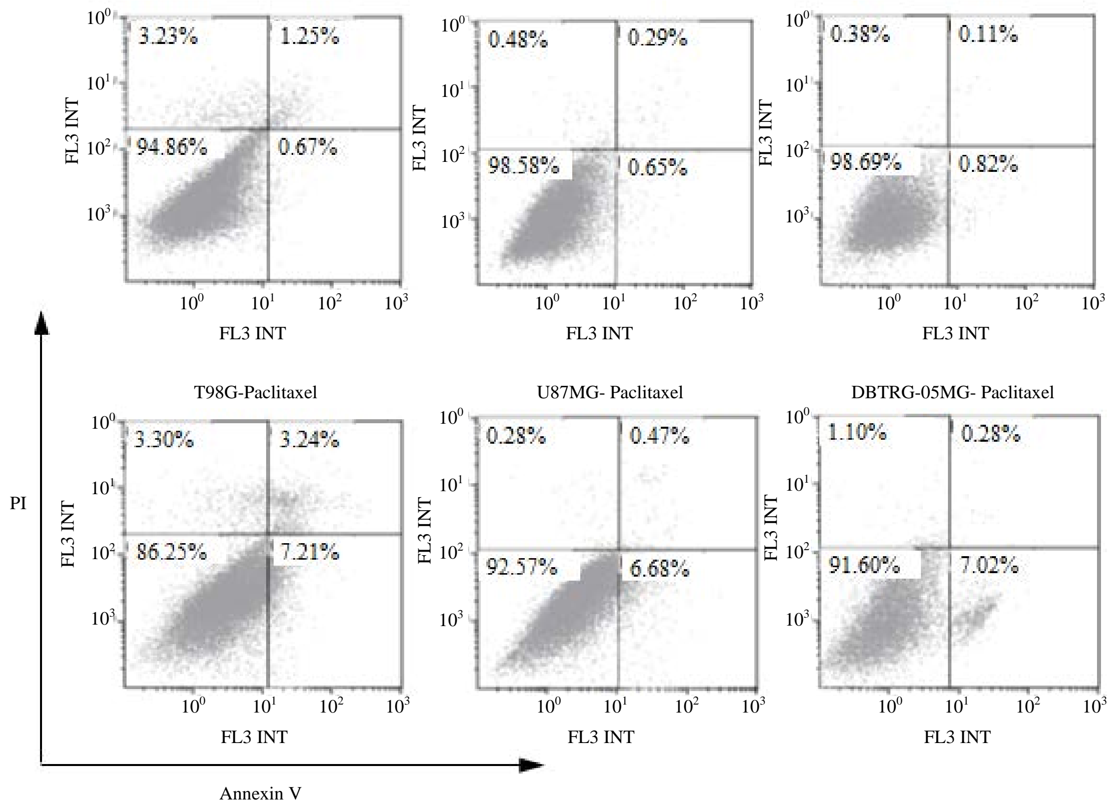

Fig. 3: Effect of paclitaxel treatment on apoptosis induction. Apoptosis was evaluated after treating cells with 100 nM of paclitaxel and staining with Annexin-V after 24 h. The flow cytometry profile represents Annexin V-fluorescein isothiocyanate staining on the x-axis and propidium iodide on the y-axis. The numbers represent the percentage of cells in each quadrant. The presented results are representative of at least three independent experiments

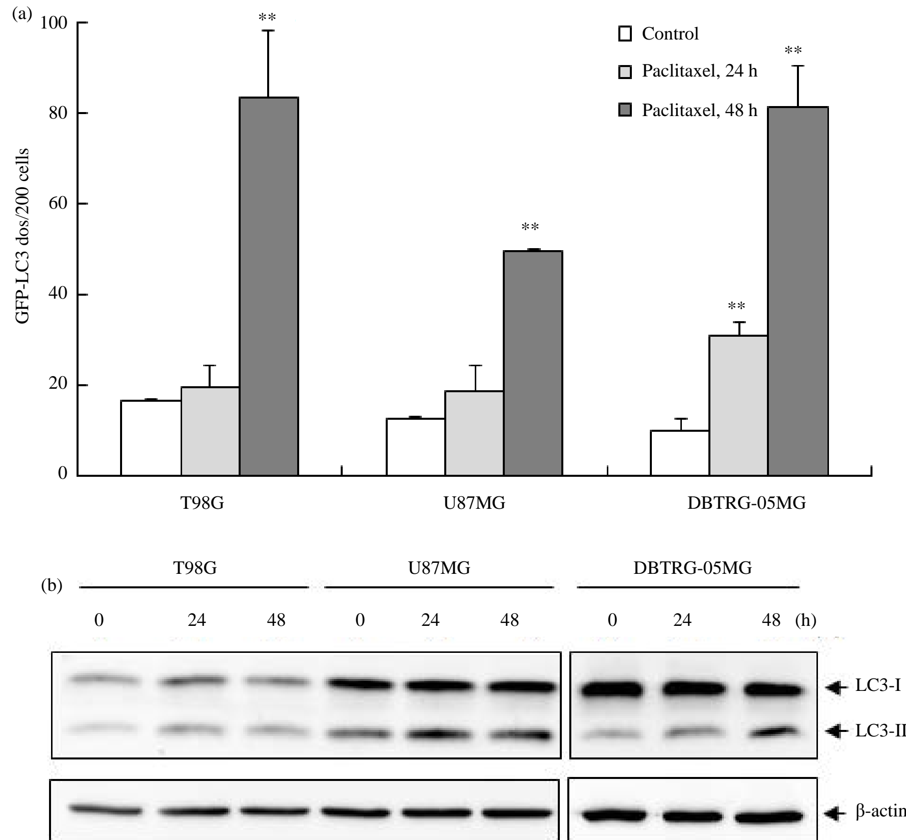

Fig. 4(a-b): The role of autophagy in paclitaxel-induced growth inhibition, (a) At 48 h after transfection with the plasmid pEGFP-LC3, cells were incubated with 100 nM of paclitaxel for the indicated times at 37°C and immediately analyzed by fluorescence microscopy. The percentage of cells showing autophagy was quantified (Mean±SD) by counting the number of cells expressing a punctate pattern of LC3-GFP among 200 GFP-positive cells. The presented results are representative of at least three independent experiments. **p<0.01 as determined by two-tailed Student's t-test compared to vehicle control in each cell line and (b) Glioma cells were treated with paclitaxel for the indicated times. The change in the electrophoretic mobility of LC3 from a non-autophagic (LC3-I) form to an autophagic membrane-recruited (LC3-II) form was determined by immunoblotting. β-actin expression was assessed as protein loading control. The presented results are representative of at least three independent experiments

Identification of autophagy induction by paclitaxel in glioma cells: Autophagy causes a type of programmed cell death that is distinct from apoptosis (Okada and Mak, 2004). Thus, it is determined that whether autophagy plays an essential death-promoting role in paclitaxel-mediated growth inhibition of glioma cells. The percentage of GFP-LC3-positive cells was used to quantify autophagy. Figure 4a shows the percentage of cells that underwent autophagy significantly increased after 48 h of treatment with paclitaxel in all cell lines tested. The induction of autophagy by paclitaxel was confirmed by immunoblotting analysis, which indicated the conversion of non-autophagic LC3-I to autophagic LC3-II in response to 100 nM paclitaxel (Fig. 4b). Therefore, these observations imply that the differential growth inhibitory effect of paclitaxel on three glioma cell lines may not depend on the autophagy induction.

DISCUSSION

It has been known that despite the low incidence of mutation, RAS/RAF/ERK signaling is up-regulated in most of glioma case and high ERK activation correlates with poor prognosis for high-grade glioma patients (Mawrin *et al.*, 2003). Notably, up to 60% of pleomorphic xanthoastrocytomas (PXAs) have been shown to harbor the V600E mutation in WHO grade II samples (Dias-Santagata *et al.*, 2011). In this study, it is tested that whether glioma cell lines with different BRAF mutation status can be targeted using paclitaxel.

Mechanistically, paclitaxel blocks cells at the G_2/M junction of the cell cycle by interfering with normal microtubule breakdown during cell division (Zhou and Giannakakou, 2005). Our unpublished results revealed that paclitaxel might be an effective anticancer agent through regulating the expression of $p21^{Cip1}$ for the treatment of BRAF mutant melanoma cells resistant to BRAF inhibitors. However, this study revealed that no significant difference between mutant and wild-type BRAF glioma cell lines was observed when comparing the relative proliferation rate obtained by MTT assay.

The malignant gliomas are characterized by an intrinsic resistance to apoptosis (Ziegler *et al*., 2008). In fact, high-grade glioma cells showed only partial sensitivity to apoptosis-inducing therapies (Lefranc *et al*., 2007; Ziegler *et al*., 2008). The present study also showed that all glioma cells tested were relatively resistant to paclitaxel-mediated apoptosis. Paclitaxel induced similar weak levels of the increase in the apoptotic fraction in all glioma cell lines, regardless of the level of growth inhibition. These results indicate that the difference in sensitivity to paclitaxel in three glioma cell lines cannot be explained by differences in apoptosis induction. On the other hand, it has been reported that autophagy is required for the induction of necrotic cell death in cells that are unable to undergo apoptosis (Ullman *et al*., 2008). Although, there is an ongoing debate as to whether induction of autophagy is cytoprotective or cytotoxic. It is also demonstrated in this study that the induction of autophagy might offer an effective therapeutic strategy for v-Ha-ras-transformed cells (Eum and Lee, 2010, 2011). However, we found that the percentage of cells that underwent autophagy similarly increased after paclitaxel treatment in all glioma cell lines tested, implying that the differential inhibitory effects in three glioma cell lines are unlikely to result from the difference in autophagy.

Although, it was not observed significant differences among three glioma cell lines in induction of both apoptosis and autophagy, U-87-MG cells were found to be more resistant to paclitaxel than other two glioma cell lines T98G and DBTRG-05MG. We further found that paclitaxel caused a remarkably significant G_2/M arrest in response to paclitaxel in T98G and DBTRG-05MG cells, whereas less significant G_2/M arrest was detected after paclitaxel treatment in U-87-MG, which exhibited more resistant to paclitaxel than other two cell lines. In G_2/M phase, paclitaxel is able to cause G_2/M arrest through microtubule disorganization (Zhou and Giannakakou, 2005). These results imply that the differential growth inhibitory effect of paclitaxel on three glioma cell lines may be associated with the cell cycle arrest. In particular, the effect of paclitaxel on cell cycle was most prominent in T98G cells, which have been found to possess mutant p53 (a mutation at codon 237) (Yin *et al*., 2005). A functional p53 pathway can arrest growth by holding the cell cycle at the G_1/S regulation point in response to many chemotherapeutic drugs (Bartek and Lukas, 2001). Conversely, the cells with mutant p53 progress through G_0/G_1 checkpoint and greatly accumulated in the subsequent G_2/M phase. Thus, it is possible that paclitaxel caused much greater G_2/M arrest in T98G cells than that observed in other two glioma cells containing wild type p53. Consistent with our results, Wahl *et al*. (1996) reported that a loss of normal p53 function conferred sensitization to paclitaxel by increasing G_2/M arrest. On the other hand, among two glioma cells with wild type p53, a significant upregulation of $p27^{Kip1}$ was observed in DBTRG-05MG cells. Conversely, no detectable increase of $p27^{Kip1}$ was observed in U-87-MG cells, which exhibited a relatively strong resistance to paclitaxel. One line of evidence revealed that $p27^{Kip1}$ deficiency impaired G_2/M arrest in response to DNA damage (Payne *et al*., 2008). Thus, these results imply that paclitaxel might express its effects through the up-regulation of $p27^{Kip1}$ expression in glioma cell lines with wild type p53.

CONCLUSION

This study has not specifically addressed the correlation of apoptosis and autophagy induction with growth inhibition. Instead, we observed that the cells with mutant p53 progress through G_0/G_1 checkpoint and greatly accumulated in the subsequent G_2/M phase. In case of glioma cells with wild type p53, paclitaxel-induced growth inhibition displayed characteristics of $p27^{Kip1}$-dependent G_2/M arrest. Thus, it is noteworthy that paclitaxel-induced growth inhibition of glioma cells is tightly correlated with the G_2/M arrest regardless of p53 mutation status.

ACKNOWLEDGMENT

This study was supported by the Incheon National University Research Grant in 2015.

REFERENCES

Ahn, J.H., K.H. Eum and M. Lee, 2009. The enhancement of Raf-1 kinase activity by knockdown of Spry2 is associated with high sensitivity to paclitaxel in v-Ha-*ras*-transformed NIH 3T3 fibroblasts. Mol. Cell. Biochem., 332: 189-197.

Ahn, J.H. and M. Lee, 2013. Autophagy-dependent survival of mutant B-Raf melanoma cells selected for resistance to apoptosis induced by inhibitors against oncogenic B-Raf. Biomol. Ther., 21: 114-120.

Ahn, J.H., Y.W. Lee, S.K. Ahn and M. Lee, 2014. Oncogenic BRAF inhibitor UAI-201 induces cell cycle arrest and autophagy in BRAF mutant glioma cells. Life Sci., 104: 38-46.

Aplin, A.E., F.M. Kaplan and Y. Shao, 2011. Mechanisms of resistance to RAF inhibitors in melanoma. J. Invest. Dermatol., 131: 1817-1820.

Bartek, J. and J. Lukas, 2001. Mammalian G1- and S-phase checkpoints in response to DNA damage. Curr. Opin. Cell Biol., 13: 738-747.

Blagosklonny, M.V., P. Giannakakou, W.S. El-Deiry, D.G.I. Kingston, P.I. Higgs, L. Neckers and T. Fojo, 1997. Raf-1/bcl-2 phosphorylation: A step from microtubule damage to cell death. Cancer Res., 57: 130-135.

Chang, S.M., N.A. Butowski, P.K. Sneed and I.V. Garner, 2006. Standard treatment and experimental targeted drug therapy for recurrent glioblastoma multiforme. Neurosurg. Focus, Vol. 20.

Davies, H., G.R. Bignell, C. Cox, P. Stephens and S. Edkins *et al*., 2002. Mutations of the BRAF gene in human cancer. Nature, 417: 949-954.

Decleves, X., A. Fajac, J. Lehmann-Che, M. Tardy and C. Mercier *et al*., 2002. Molecular and functional MDR1-Pgp and MRPs expression in human glioblastoma multiforme cell lines. Int. J. Cancer, 98: 173-180.

Dias-Santagata, D., Q. Lam, K. Vernovsky, N. Vena and J.K. Lennerz *et al*., 2011. BRAF V600E mutations are common in pleomorphic xanthoastrocytoma: Diagnostic and therapeutic implications. PLoS One, Vol. 6. 10.1371/journal.pone.0017948

Eum, K.H. and M. Lee, 2010. Targeting the autophagy pathway using ectopic expression of Beclin 1 in combination with rapamycin in drug-resistant v-Ha-*ras*-transformed NIH 3T3 cells. Mol. Cells, 31: 231-238.

Eum, K.H. and M. Lee, 2011. Crosstalk between autophagy and apoptosis in the regulation of paclitaxel-induced cell death in v-Ha-*ras*-transformed fibroblasts. Mol. Cell. Biochem., 348: 61-68.

Flaherty, K.T., I. Puzanov, K.B. Kim, A. Ribas and G.A. McArthur *et al*., 2010. Inhibition of mutated, activated BRAF in metastatic melanoma. N. Engl. J. Med., 363: 809-819.

Glantz, M.J., H. Choy, C.M. Kearns, P.C. Mills and L.U. Wahlberg *et al*., 1995. Paclitaxel disposition in plasma and central nervous systems of humans and rats with brain tumors. J. Natl. Cancer Inst., 87: 1077-1081.

Holmes, F.A., R.S. Walters, R.L. Theriault, A.U. Buzdar and D.K. Frye *et al*., 1991. Phase II trial of taxol, an active drug in the treatment of metastatic breast cancer. J. Natl. Cancer Inst., 83: 1797-1805.

Horwitz, S.B., 1994. Taxol (paclitaxel): Mechanisms of action. Ann. Oncol.: Official J. Eur. Soc. Med. Oncol., 5: S3-S6.

Kleinschmidt-DeMasters, B.K., D.L. Aisner, D.K. Birks and N.K. Foreman, 2013. Epithelioid GBMs show a high percentage of BRAF V600E mutation. Am. J. Surg. Pathol., 37: 685-698.

Lee, M., W.S. Koh and S.S. Han, 2003. Down-regulation of Raf-1 kinase is associated with paclitaxel resistance in human breast cancer MCF-7/Adr cells. Cancer Lett., 193: 57-64.

Lefranc, F., V. Facchini and R. Kiss, 2007. Proautophagic drugs: A novel means to combat apoptosis-resistant cancers, with a special emphasis on glioblastomas. Oncologist, 12: 1395-1403.

Liebmann, J.E., J.A. Cook, C. Lipschultz, D. Teague, J. Fisher and J.B. Mitchell, 1993. Cytotoxic studies of paclitaxel (Taxol) in human tumour cell lines. Br. J. Cancer, 68: 1104-1109.

Mawrin, C., S. Diete, T. Treuheit, S. Kropf and C.K. Vorwerk *et al*., 2003. Prognostic relevance of MAPK expression in glioblastoma multiforme. Int. J. Oncol., 23: 641-648.

McGuire, E.P., E.K. Rowinsky, N.B. Rosenshein, F.C. Grumbine, D.S. Ettinger, D.K. Armstrong and R.C. Donehower, 1989. Taxol: A unique antineoplastic agent with significant activity in advanced ovarian epithelial neoplasms. Ann. Int. Med., 11: 273-279.

Mousses, S., N. Gokgoz, J.S. Wunder, H. Ozcelik, S. Bull, R.S. Bell and I.L. Andrulis, 2001. p53 missense but not truncation mutations are associated with low levels of *p21*$^{CIP1/WAF1}$ mRNA expression in primary human sarcomas. Br. J. Cancer, 84: 1635-1639.

Nance, E., C. Zhang, T.Y. Shih, Q. Xu, B.S. Schuster and J. Hanes, 2014. Brain-penetrating nanoparticles improve paclitaxel efficacy in malignant glioma following local administration. ACS Nano, 8: 10655-10664.

Nicolaides, T.P., H. Li, D.A. Solomon, S. Hariono and R. Hashizume *et al*., 2011. Targeted therapy for BRAF^{V600E} malignant astrocytoma. Clin. Cancer Res., 17: 7595-7604.

Okada, H. and T.W. Mak, 2004. Pathways of apoptotic and non-apoptotic death in tumour cells. Nat. Rev. Cancer, 4: 592-603.

Payne, S.R., S. Zhang, K. Tsuchiya, R. Moser and K.E. Gurley *et al*., 2008. p27^{kip1} deficiency impairs G_2/M arrest in response to DNA damage, leading to an increase in genetic instability. Mol. Cell. Biol., 28: 258-268.

Rasouli-Nia, A., D. Liu, S. Perdue and R.A. Britten, 1998. High Raf-1 kinase activity protects human tumor cells against paclitaxel-induced cytotoxicity. Clin. Cancer Res., 4: 1111-1116.

Ribas, A. and K.T. Flaherty, 2011. BRAF targeted therapy changes the treatment paradigm in melanoma. Nat. Rev. Clin. Oncol., 8: 426-433.

Sanson, M., M. Napolitano, R. Yaya, F. Keime-Guibert, P. Broet, K. Hoang-Xuan and J.Y. Delattre, 2000. Second line chemotherapy with docetaxel in patients with recurrent malignant glioma: A phase II study. J. Neurooncol., 50: 245-249.

Schiller, J.H., D. Harrington, C.P. Belani, C. Langer and A. Sandler *et al*., 2002. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N. Eng. J. Med., 346: 92-98.

Steinbach, J.P. and M. Weller, 2004. Apoptosis in gliomas: Molecular mechanisms and therapeutic implications. J. Neurooncol., 70: 245-254.

Ullman, E., Y. Fan, M. Stawowczyk, H.M. Chen, Z. Yue and W.X. Zong, 2008. Autophagy promotes necrosis in apoptosis-deficient cells in response to ER stress. Cell Death Differ., 15: 422-425.

Wahl, A.F., K.L. Donaldson, C. Faircnild, F.Y.F. Lee, S.A. Foster, G.W. Demers and D.A. Galloway, 1996. Loss of normal p53 function confers sensitization to Taxol by increasing G2/M arrest and apoptosis. Nat. Med., 2: 72-79.

Wen, P.Y. and S. Kesari, 2 008. Malignant gliomas in adults. N. Eng. J. Med., 359: 492-507.

Yin, D., H. Zhou, T. Kumagai, G. Liu, J.M. Ong, K.L. Black and H.P. Koeffler, 2005. Proteasome inhibitor PS-341 causes cell growth arrest and apoptosis in human glioblastoma multiforme (GBM). Oncogene, 24: 344-354.

Zhao, M., C. Liang, A. Li, J. Chang and H. Wang *et al*., 2010. Magnetic paclitaxel nanoparticles inhibit glioma growth and improve the survival of rats bearing glioma xenografts. Anticancer Res., 30: 2217-2223.

Zhou, J. and P. Giannakakou, 2005. Targeting microtubules for cancer chemotherapy. Curr. Med. Chem. Anticancer Agents, 5: 65-71.

Ziegler, D.S., A.L. Kung and M.W. Kieran, 2008. Anti-apoptosis mechanisms in malignant gliomas. J. Clin. Oncol., 26: 493-500.