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Ellipticine-induced apoptosis depends on Akt translocation and signaling in lung epithelial cancer cells

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ABSTRACT

Ellipticine and its analogues were reported as topois[illegible]se II inhibitors and promising antitumor agents. In this work, we showed that the [illegible] of human n[illegible]all-cell-lung-cancer (NSCLC) epithelial cells A549 can be inhibited by ellipt[illegible]e. The inhibitory effec[illegible]was reverted by PI3K inhibitors. The sub-G_1 phase cells after ellipticine tre[illegible]ment appeared at the expense of those that accumulated first at S- and G_2/M phases during the early [illegible]ge of treatm[illegible] We showed that the progression leading to cell death was impaired by wortmannin, [illegible]h reverted [illegible]ptosis by retaining cells at S- and G_2/M transition states. The characteristic apoptosis ma[illegible]53 acti[illegible]n after treatment appeared first followed by poly(ADP-ribose)polymeras[illegible]RP) fragmen[illegible]y disappeared upon co-treatment with wortmannin and the apoptotic phe[illegible]ssed. Furthermore, ellipticine regulated endogenous survival signaling by up-regulating pho[illegible]orylat[illegible]hat returned to its basal level later. Furthermore, ellipticine induced nucleus translocaliz[illegible]n o[illegible]kt and recruitment of autophagosomes. The autophagic-related cell death w[illegible]terfered [illegible]rtmannin and the suppressed growth reverted. The Akt-related cell death also occ[illegible]3-deficie[illegible]ells with stable expression of exogenous p53. The work showed that ellipticine-i[illegible]ed cyt[illegible]xicity in [illegible]LC cells was achieved through autophagy and apoptotic death as a result of [illegible]modu[illegible]. Being a topoisomerase II inhibitor, ellipticine proved a regulator in autophagy-related c[illegible]oration of p53 and Akt.

1. Introduction

Ellipticine (5,11-d[illegible]thyl-6[illegible]pyrido[4,3-*b*]carbazole), one of the naturally occu[illegible]alkal[illegible]s, was isolated from the leaves of the evergreen tree *Oc[illegible]iptica* L[illegible]l (Apocynaceae) found in Oceania. The [illegible]and it[illegible]logu[illegible]e known to inhibit topoisomerase II [illegible]vity [illegible]human[illegible] cells. Ellipticine analogues are active a[illegible]st brai[illegible]umor cel[illegible]nes [1] and proved potent against a pane[illegible]can[illegible]. The drug was promising in treating metastati[illegible]t cancer and brain tumors [3]. The anticancer activities of ellipti[illegible]and its derivatives, such as 9-methoxyellipticine, retelliptine, eli[illegible]iniums, 9-hydroxyellipticine and 9-chloro-2-methylellipticinium have also been reported effective against a panel of cancer cell lines [3–9]. Although ellipticine and their derivatives are known capable of intercalating DNA, generating cytotoxic free radicals and uncoupling oxidative phosphorylation [10–12], its efficacy in human lung cancer cells and the associated action mechanisms were not completely understood.

The ellipticine-mediated cytotoxicity is mediated by inhibiting topoisomerase II activity [13]. The strategy is commonly used as an effective anticancer strategy. In this work, we further described that the proliferation of human NSCLC cells A549 can be suppressed by ellipticine and the inclusion of PI3K inhibitors blocked the inhibitory effects. Being a topoisomerase II inhibitor, the drug restrained proliferation by arresting cells at S- and G_2/M transition states prior to induction of sub-G_1 cell populations. The initial drug-mediated phosphorylation of Akt-serine^{473} (Akt-Ser^{473}) helped in maintaining cell viabilities at the initial stage of drug treatment. However, the induced up-regulated p53 and the subsequent Akt dephosphorylation lead to poly(ADP-ribose)polymerase (PARP) fragmentation and final apoptosis. Further analysis revealed that ellipticine caused nucleus translocation of both p53 and phosphorylated Akt as well as the development of autophagic cells. The effects were attenuated by wortmannin, which blocked the apoptotic phenotype. The work demonstrated that ellipticine induced cytotoxicity in A549 cells by modulating signaling pathway and subcellular redistribution of Akt and p53. Thus, PI3K-regulated autophagic cell development by controlling cell growth provided a new dimension for topoisomerase II inhibitor, ellipticine.

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2. Materials and methods

2.1. Cell lines and monolayer growth determination of NSCLC cells in culture

Human lung cell carcinoma cell lines H322, H1437, H1299 and A549 were acquired from ATCC and grown in DMEM (Invitrogen, Grands Islands, NY). All cultured cells were supplemented with L-glutamine, sodium pyruvate, and supplemented with 7% heat-inactivated FCS in the humidified atmosphere of 5% CO_2 at 37 °C. All cell lines were examined and found to be free of mycoplasma contamination using a MycoTect kit (Invitrogen, Grands Islands, NY).

The H1299 cells transfected with cytomegalovirus promoter-driven pcDNA-p53 consisting of full-length wild-type p53 were established previously and maintained in serum-supplemented DMEM [14].

2.2. MTT assay determination for proliferation rate and cell viability evaluation

The effect of cell proliferation by ellipticine (Sigma, St. Louis, MO) was measured by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay. Briefly, cells were plated in 96-well culture plates (5×10^3 cells/well). After 24 h incubation, the cells were treated with different concentrations of ellipticine. Fifty microliters of MTT test solution was added to each well. After 4 h incubation, the absorbance was measured on an ELISA plate reader (Multiskan EX, Labsystems) at a test wavelength of 492 nm and a reference wavelength of 690 nm. The viability of untreated ce the presence of diluted vehicle control was regarded as 100%. works were conducted in three different experiments.

2.3. Flow cytometry of cell cycle analysis by propidi staining

To determine phase distribution of DNA c prop iodide (PI) staining was performed. Br , 3×10 ls collected were washed once and fixed in 70% nol overnig fter centrifugation at 700 rpm for 5 min a C, pellet was st ed with 5 μg/ml PI (Sigma, St. Louis, MO) plus 0. /ml RNaseA in PBS buffer for 15 min at room te erature in the d The analysis was performed with FACScan v cyto eter (Becto ickinson, Mansfield, MA). Cell cycle di outio ere analyzed by Cell-Quest and Modfit software (Becto on, Ma field, MA). The statistics of cell distributio were c lated f three individual experiments.

To test t nhibi y effect, cells were treated with wortmannin (a, St MO) for 24 h prior to addition of fresh media and d ed concentrations.

2.4. Green fluores protein-tagged light-chain 3 plasmid transfection

A549 cells were grown on sterile histologic slides, and, after 24 h the cells, were transfected with green fluorescent protein (GFP)-tagged light-chain 3 (LC3) plasmid (a gift from Dr. Wei-Pang Huang, Department of Life Science, National Taiwan University, Taipei, Taiwan) using a mixture of LipofectAMINE (Invitrogen) and GFP-LC3 plasmid in Opti-MEM medium (Invitrogen) at a ratio of 5 μl LipofectAMINE per milliliter of medium per 1 μl plasmid. After 6 h of incubation, cells were placed in regular complete medium and cultured for 1 day. After cells were treated with ellipticine and wortmannin or vehicle control for 2 days, the medium was changed, and cells were further incubated for 24 h at 37 °C. The slides were then washed with PBS, and cells fixed in cold methanol. Cells were then washed in PBS twice, and coverslips were mounted with glycerol/PBS (3:1) solution. Slides were examined under a fluorescent microscope (Leica).

2.5. Western blot analysis

Cells cultured in 0.5% serum-supplemented media were treated with ellipticine were washed with PBS and scraped in lysate buffer containing 1% triton X-100, 150 mM NaCl, 5 mM EDTA, 1% aprotonin, 5 mM PMSF and 10 μg/ml leupeptin in 20 mM sodium phosphate. Protein concentration was d by the BCA assay (Pierce Biotechnology, Rockfor) and 2 of total protein was performed for Western b analysis. P in samples were electrophoresed on SDS-p lyacr ide gels, nsferred to nitrocellulose filters). The h s were t inc ed in fresh blocking solution and pr d for h wit 00 dilution of p53, pAkt-Ser^{473}, Akt, C β, pC β-Ser^{21} a PARP antibodies (Cell Signaling Tech logy s, MA) spectively. Blots were washed twice in P and th ubat with a 1:4000 dilution of peroxidase-co ed secon dy (Kirkegaard and Perry Laboratories ., hersburg, M h PBS-T for 1 h at 22 °C. Blots were again washed t for 10 min in PBS-T and then detected by ECL illu tion syste ersham).

2.6. Immunofluorescence analysis

cells gr on coverslips were fixed for 10 min in PBS con g 3.7% rmaldehyde. The fixed coverslips were washed in PBS ng 0.1% Triton X-100 for 10 min, washed twice in (5 min), and incubated in a blocking buffer (PBS containing e serum albumin) for 30 min. The cells were then incu ated in the blocking buffer containing the primary antibody for 1 h and washed three times in PBS (5 min) before incubation with the appropriate TRITC-conjugated secondary antibody plus DAPI (Molecular Probes Inc.) for further 30 min. The cells were washed three times in PBS (5 min) and washed in water. The stained cells were mounted on glass slides and examined for fluorescence under a fluorescent microscope (Leica).

3. Results

3.1. Growth inhibition induced by ellipticine was neutralized by wortmannin

To evaluate the effects of ellipticine on cell growth, the viabilities in human lung cancer cells with various *p53* genotypes were determined. Compared to H322 (p53 mutant p53 (R248L)) and H1437 (mutant p53 (R267P)), cell viabilities of ellipticine-treated epithelial NSCLC cells A549 (wild-typed p53) were reduced to less than 30% with increased concentrations of the drug using MTT assay (Fig. 1A). Compared with cells treated with vehicle control alone, less than 50% of the cells were left after 48 h after treating with 5 μM ellipticine. The viable cells were further reduced to less than 10% after 96 h. The induced growth inhibition in A549 cells was abrogated progressively by increasing wortmannin concentrations from 100 to 200 nM and by LY294002. Similar to vehicle control, treatment with wortmannin alone exerted no effect on cell growth at all in culture (Fig. 1B).

3.2. Ellipticine arrested A549 cells at S- and G_2/M transition states prior to inducing sub-G_1 phase cells and the effect can be reverted by wortmannin

Cell cycle distributions were determined by flow cytometry. Compared with A549 cells with vehicle control alone, ellipticine

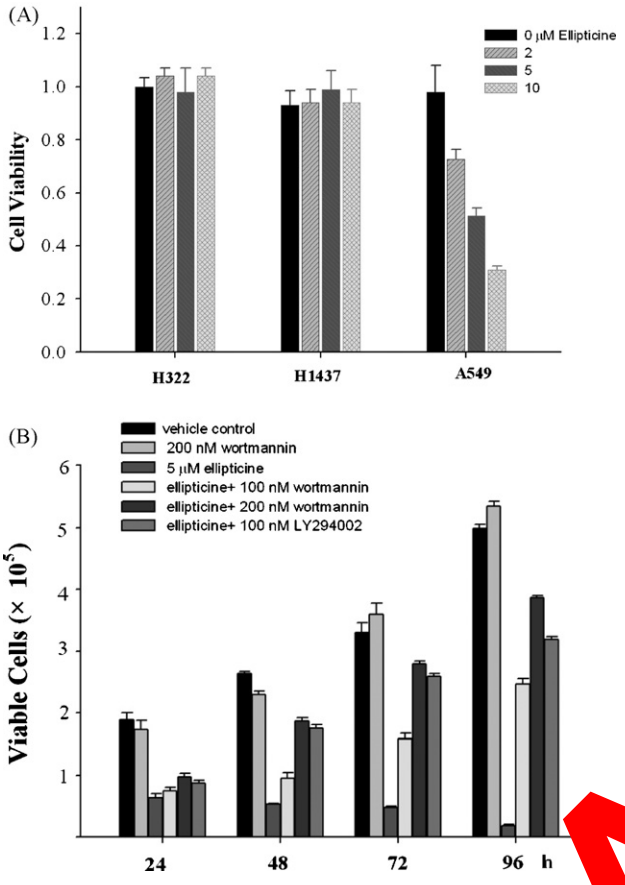

Fig. 1. (A) Cell viabilities of H322, H1437 and A549 cells with various concentrations of ellipticine Cells were seeded into 6-well plates (5 /well). After 24 h for complete adherence, the cells were incubated vehicle 0.1% DMSO and different concentrations of ellipticine (0, 2, 5 and μM, respectively). After 48 h of treatment, 50 μl of MTT solution were added the cultures. After incubation, the absorbance was measured on an ELISA plate reader test wavelength of 492 nm with a reference wavelength of 6 nm. The cell viabilities were determined as percentages of vehicles control. Growth curves of A549 cells with ellipticine affected by various PI3K inhibitors. A549 cells were cultured with ellipticine together with wortmannin or LY 002 for hours (h) specified. Cells were trypsinized and the viable cells counted by Trypan exclusion assay. The error bars represented standard error of three independent experiments conducted.

arrested cells at S- G_2 transition states beginning on the first day (Fig. 2). Cells of G_1 phase appeared 3 days later; while those at G_2 were decreased simultaneously. As wortmannin concentration was increased from 100 to 200 nM, cells at sub-G_1 state disappeared proportionally and most of them remained at S- and G/M The results implied that the onset of ellipticine-induced cell death involves Akt signaling and wortmannin blocked cell progression mostly at S- and G_2/M phases, whereas cells of sub-G_1 state were reduced. The cell population distribution by wortmannin alone is not affected and similar to that of vehicle control.

3.3. Ellipticine phosphorylates Akt at serine-473 first before being dephosphorylated in A549 cells that can be associated with the appearance of apoptotic signals

PARP is a key participant in DNA base excision repair and in maintaining genome integrity [15]. The appearance of proteolytic cleavage of the precursor PARP marked the final commitment of apoptosis. In drug-treated A549 cells, the intense 89-kDa fragment of PARP in Western blot serves as a hallmark of apoptosis (Fig. 3). The induced p53 was no longer observed 48 h after treatment. The induced MDM2 at 48 h was associated with degradation of transiently activated p53. The effect was suppressed by wortmannin and the induced apoptosis reverted.

The enhanced phosphorylation serine-473 of Akt was detected a day after ellipticine treatment as indicated by Western blot (Fig. 4A). The intensity of phosphorylated Akt was increased by more than 2-fold before final dephosphorylation (Fig. 4B). The effect was blocked by wortmannin and proved reproducible. On the other hand, Akt-dependent phosphorylation on serine-21 of glycogen synthase kinase-3β (GSK3β), a downstream target specific for Akt, was increased and then returned to basal level, while the intensities of GSK3β were unchanged. The addition of wortmannin suppressed the transient phosphorylation.

3.4. Ellipticine induced nuclear translocation of Akt in A549 cells

By analyzing protein extracted from cytoplasmic and nuclear fractions, Akt was found migrated into nucleus following ellipticine treatment. Both cytoplasmic and nuclear Akt was found phosphorylated after treatment as revealed from Akt-Ser^{473}-specific antibody (Fig. 5A). On the other hand, the phosphorylated Akt was found only in cytoplasmic fraction after wortmannin treatment. Besides, in immunostaining experiments, phosphorylated Akt located in both nucleus and cytoplasm after ellipticine treatment, was retained in cytoplasm by wortmannin (Fig. 5B). In addition, nuclear translocation of p53 was also assisted by ellipticine, and the movement was blocked when cultured together with wortmannin as shown in p53 antibody-stained fluorescence experiments.

3.5. Increased autophagy is associated with ellipticine-induced apoptosis in A549 cells

Autophagy has gained increasing attention as an alternative route toward cell death. Microtubule-associated light chain LC3 is an important component during autophagosome formation. The use of the GFP-tagged LC3 plasmid has become an effective marker for the autophagy progression. A549 cells were transfected with GFP-LC3 plasmid and the distribution of GFP can be detected. As shown in Fig. 6A, diffuse cytoplasmic localization of GFP-LC3 was observed in untreated A549 cells, whereas ellipticine-treated cells acquired increased punctate fluorescence that overlapped with Lysotracker positive spots after 48 h, which suggested the presence of autophagic cells relevant to apoptosis. The increases in punctate fluorescence by ellipticine can be neutralized in cells treated with 3-methyladenine (3-MA). In addition, the viability was observed increased in cells treated with increasing concentrations of 3-MA compared to those with ellipticine alone, indicating that the autophagy attributed to apoptotic cell death due to ellipticine (Fig. 6B). Thus, autophagic cells are closely related to the onset of apoptotic tumor cell killing, suggesting the importance of autophagy in ellipticine-mediated growth inhibition.

3.6. Ellipticine-induced Akt nuclear translocation and apoptosis is dependent on p53

Both H1299 cells lacking p53 and the stable clone H1299-p53 with exogenous p53 were tested with ellipticine. Compared to H1299 cells, cell viabilities of ellipticine-treated H1299-p53 cells were decreased in dose-dependent manner (Fig. 7A). The decreased viability was recovered by wortmannin. In Western blot analysis, p53 level and phosphorylated Akt were elevated in H1299-p53 clone in the presence of ellipticine and the increased expression blocked by wortmannin (Fig. 7B). On the other hand, wortmannin

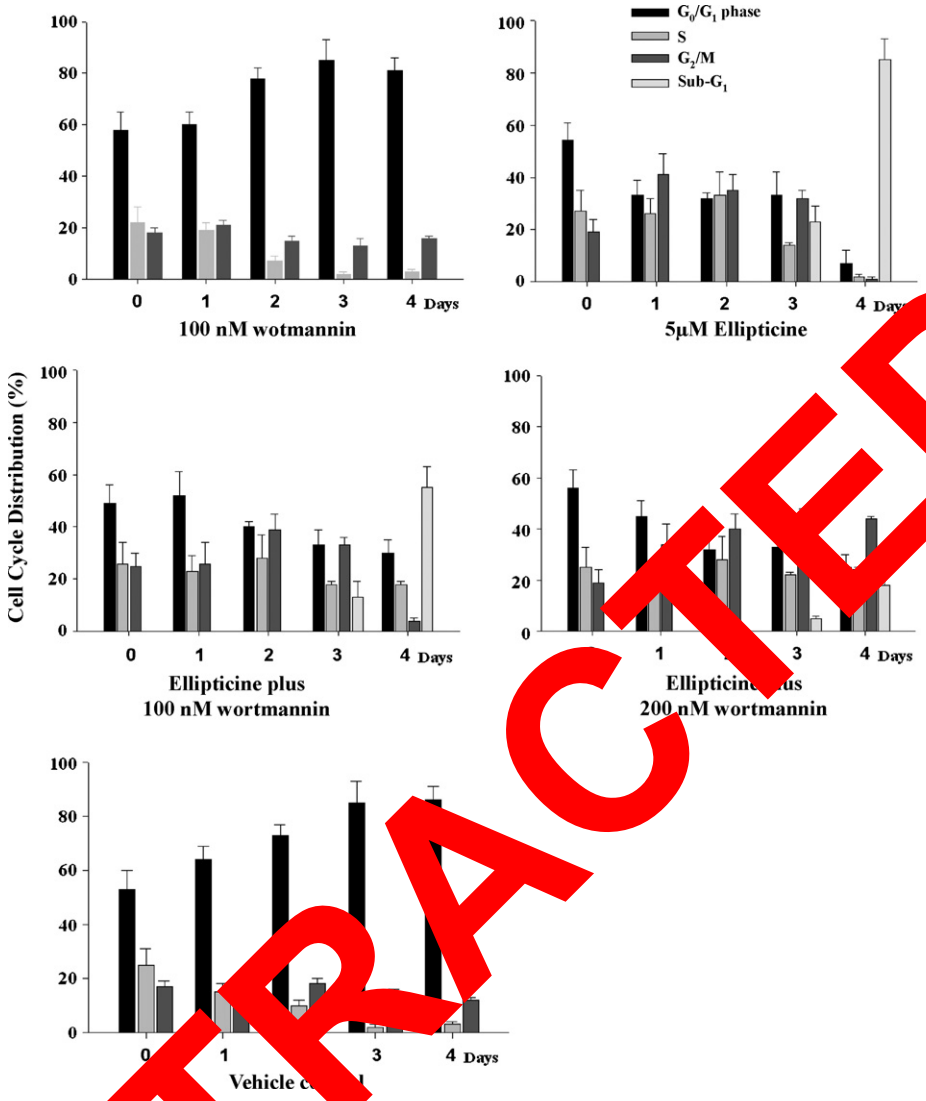

Fig. 2. Cell cycle histograms analysis of ellipticine-t A549 cells with or without wortmannin. Exponentially growing 3×10^5 A549 cells were treated with wortmannin alone or with ellipticine (5 μM) alon ogether with d 200 nM of wortmannin for the time points as indicated. The trypsinized cells were analyzed by flow cytometry. The percentage distribution of ce cle phases was det ed by FACS analysis following PI staining. The error bars represented standard errors in three independent experiments conducted.

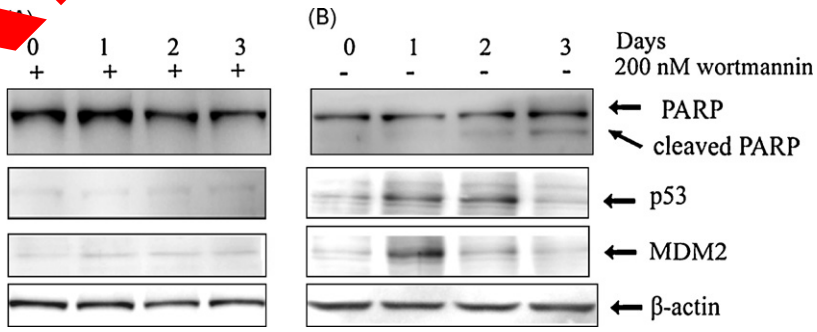

Fig. 3. Western blot analysis. The cultured A549 cells were treated with 5 μM of ellipticine in the presence (+) or absence (−) of 200 nM of wortmannin for the days as specified. Cell pellets were resuspended in lysis buffer and an equal amount of protein was separated by SDS-PAGE separating gel and electroblotted. (A) The blots were then incubated in fresh blocking solution and probed for 1 h with 1:3000 dilution of PARP, MDM2, β-actin or p53 antibody, followed by incubating with a 1:4000 dilution of horseradish peroxidase-conjugated secondary antibody and then developed by ECL detection system. (B) The blots were incubated in fresh blocking solution and probed for 1 h with 1:3000 dilution of Akt, pAkt-Ser473, GSK3β or pGSK3β antibody, followed by incubating with the corresponding secondary antibodies before ECL detection.

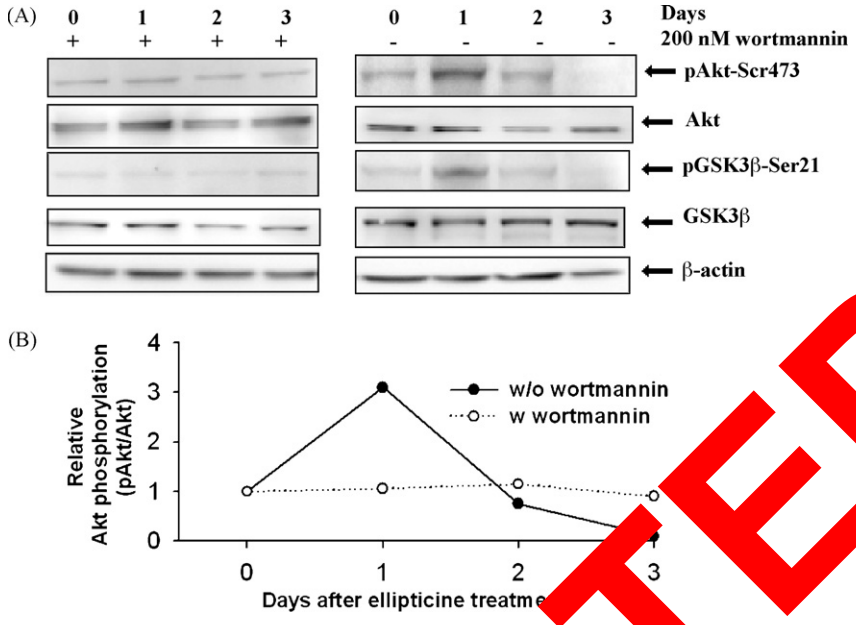

Fig. 4. Western blot analysis. A549cells were treated with to 5 μM of ellipticine in the presence (+) or absence (−) of 200 nM of wortmannin for the days as specified. Cell pellets were resuspended in lysis buffer and an equal amount of protein was separated by SDS-PAGE separating gel and electroblotted. (A) The blots were incubated in blocking solution and probed for 1 h with 1:3000 dilution of Akt, pAkt-Ser^{473}, GSK3β or pGSK3β-Ser^{21} antibody followed by incubating with a 1:4000 dilution of horseradish peroxidase-conjugated secondary antibody and then developed by ECL detection system. (B) Ratios of phosphor-Akt (pAkt) to total Akt are graphically depicted as a function of days after ellipticine incubation in the presence (solid line) or absence (dash line) of wortmannin.

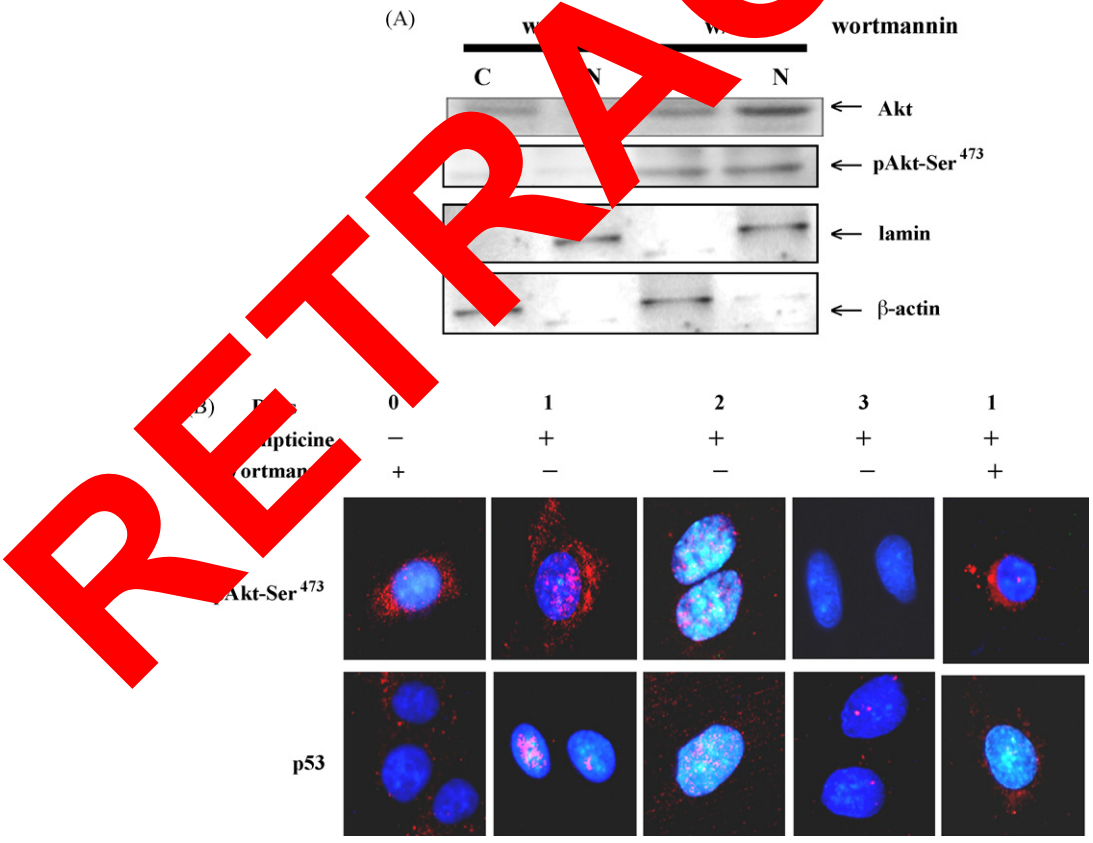

Fig. 5. Western blot and immunofluorescence analysis. The cultured A549 cells were treated with 5 μM of ellipticine in the presence (+) or absence (−) of 200 nM of wortmannin for the days as specified. (A) The nuclear protein (N) and cytoplasmic (C) fractions in cell extracts after a day of ellipticine treatment were analyzed and separated with SDS-PAGE gels. The blot after transfer was probed with antibodies against Akt, pAkt-Ser^{473}, β-actin or lamin, followed by incubating with horseradish peroxidase-conjugated secondary antibody and then developed by ECL detection system. (B) Cells plated on coverslips in 6-well plates were treated with ellipticine in the presence (+) or absence (−) of wortmannin for the days as specified. The cells were fixed and stained with anti-pAkt-Ser^{473} and p53 antibody (red), respectively. The cells were counterstained with DAPI to visualize the nuclei (blue). The presence of pAkt-Ser^{473} was found mainly in the nucleus in 100% of the cells a day after ellipticine treatment, when more than 100 cells were observed under each condition.

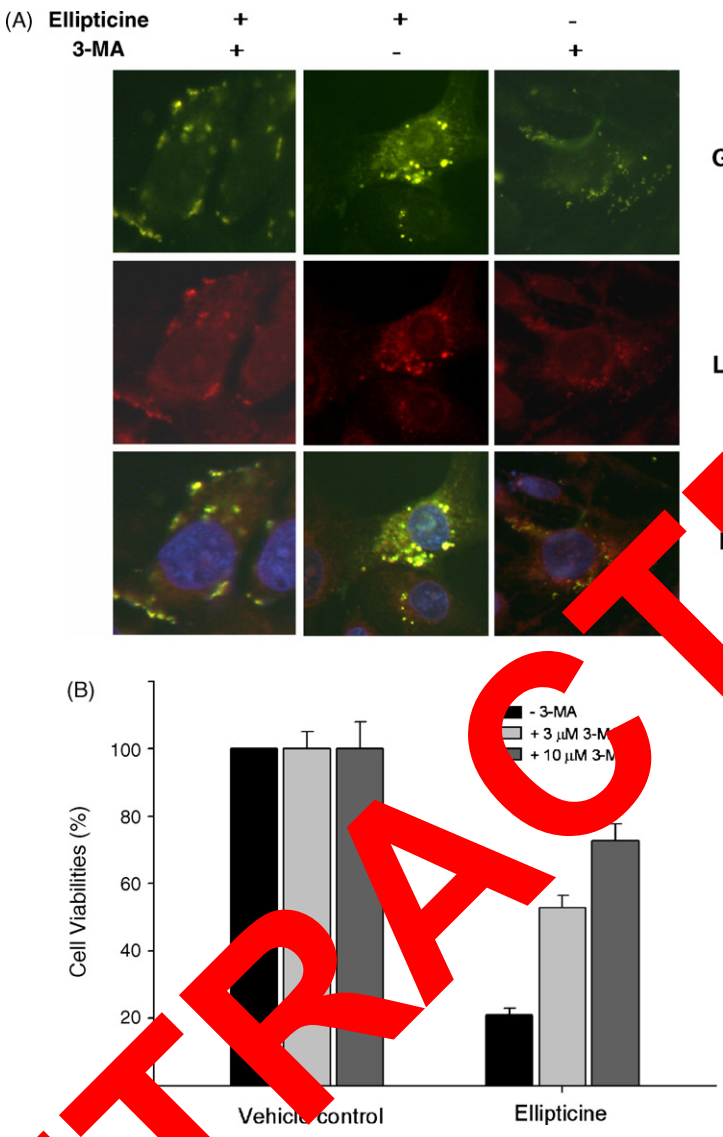

Fig. 6. (A) Autophagosome formati visualized in G -expressing A549 cells by ellipticine. A549 cells transfected with GFP-LC plasmid were incubated with vehicle control DMSO, 5 μM of ellipticin 0 μM 3-aminoadenin MA) for 48 h, and observed under the fluorescence microscope for GFP (green), Lysotracker (red) and counter-stained with DAPI (blue). Pu te fluor nce in GFP-LC3 transfected cells was detected overlapped with Lysotracker in ellipticine-treated A549 cells. (B) The effect of 3-MA on ellipticine-induce death 49 cells. Relative cell viability was determined 48 h after treatment with 5 μM of ellipticine for 48 h in the presence and absence of 3 or 10 μM of 3-MA, respec g untreate lls in the absence of 3-MA as 100%. Bars, standard errors.

inhibited el icine- uced nu translocation of Akt in H1299-p53 cells the ef not detected at all in the parental cells H1299 (Fig.

4. Discussion

More detailed mechanisms on the drug action concerning ellipticine-induced cell death were gradually uncovered. Previous studies reported that p53 and Fas/Fas ligand death receptor are involved in ellipticine-mediated cell growth inhibition [5,7]. More work indicated that ellipticine induced endoplasmic reticulum stress that contributed to the drug cytotoxicity [1]. Ellipticine is capable of activating p53 downstream responsive elements and, therefore, causing apoptotic cell death in cells in a panel of cancer cells as an effective growth inhibitor [2,4,16]. As an effective topoisomerase II inhibitor that specifically acted in A549 lung cancer cells, ellipticine arrested cells first at S- and G_2/M check points before final commitment to apoptotic death. The final growth inhibition was blocked by wortmannin by suppressing sub-G_1 cell population and the viable cells increased proportionally.

DNA damage in cells causes either irreversible senescence or apoptosis in tumor cells [14,17–19]. The characteristic marker for apoptosis, cleaved PARP in response to environmental stress, appeared on the third day after treatment. The late appearance of intense 89-kDa fragment of nuclear polymerase PARP involved in DNA repair marked delayed apoptotic cell death following prolonged cell arrest. In this work, we showed that ellipticine inhibited the growth of A549 cells by first activating p53 accompanied with cleavage of 116-kDa precursor, PARP, into 89-kDa fragment. The breakdown of the induced p53 and MDM2 began on the third day and the effect was suppressed by wortmannin. The delayed response to topoisomerase inhibitor in carcinoma cells with wild-type p53 can be characterized by prolonged cell arrest without apparent apoptosis [20,21]. For instance, the growth of NSCLC cells was blocked at G_2/M phase and exhibited senescence-like state

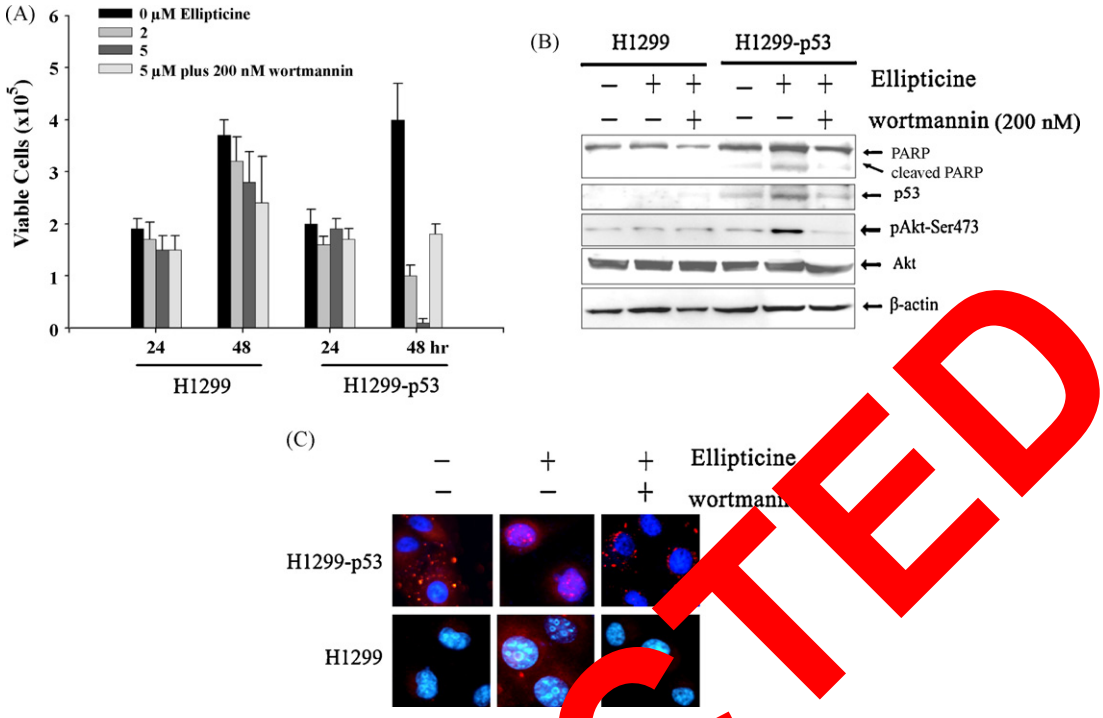

Fig. 7. (A) Cell growth determination of H1299 and H1299-p53 cells affected by ellipticine. Both H1299 and H1299-p53 cells were cultured with ellipticine with or without wortmannin for the time duration specified. Cells were trypsinized and the viable cells counted by Trypan blue exclusion assay. (B) Western blot analysis. Both H1299 and H1299-p53 cells were treated with 2 μM of ellipticine in the presence (+) or absence (−) of 200 nM of wortmannin for 2 days. Cell pellets were resuspended in lysis buffer and an equal amount of protein was separated by SDS-PAGE separating gel and electroblotted. The membrane was then incubated in blocking solution and probed for 1 h with 1:3000 dilution of PARP, Akt, pAkt-Ser^{473}, p53 and β-actin antibody, separately, followed by 1:4000 dilution of horseradish peroxidase-conjugated secondary antibody and then developed by ECL detection system. (C) Immunofluorescence analysis. Both H1299 and H1299-p53 cells plated on coverslips in 60-cm plates were treated with ellipticine in the presence (+) or absence (−) of wortmannin for 2 days as specified. The cells were stained with Akt antibody (red) and counterstained with DAPI to visualize the nuclei (blue).

prior to apoptosis [17]. Previous report indicated that treatment with cisplatin, etoposide, or vincristine to murine hematopoietic cells led to initial up-regulation of phosphorylated Akt, followed by rapid dephosphorylation to its basal level and the drug resistance was increased without sustaining cell growth [22]. Our work demonstrated that ellipticine arrested A549 cells first at G_2/M phase by modulating Akt phosphorylation before final commitment to cell death. However, cells were retained at G_2/M phase along with transient Akt phosphorylation is not completely understood.

Akt phosphorylation by ellipticine was terminated by wortmannin (Fig. 4B), and the effect attenuated ellipticine-induced cell death. Since functional Akt was reportedly active in A549 cells [23], the ellipticine induced dephosphorylation of Akt on serine-473 could be attributed to PTEN activation. In some cases, treatment with wortmannin alone is sufficient to inhibit Akt activity and cell proliferation thereby promoting apoptosis [24,25]. In this work, when wortmannin is included in culture, the ellipticine-mediated phosphorylation became inactivated and nucleus translocation of Akt blocked. These results further suggested that, despite brief Akt activation, ellipticine eventually acts as a specific inhibitor of the Akt-dependent signaling pathway, thereby making it an effective drug in treating lung cancer.

Akt activation promotes survival of NSCLC cells [23]. The activated Akt can be located to cell membrane in response to stimulation by growth factors such as insulin-like growth factor 1 (IGF-1). After mitogenic stimulation, Akt phosphorylates multiple substrates related to cell cycle progression and lead to reduction of p53transactivation [26]. The pleckstrin homology (PH) domain of Akt was reportedly a membrane-targeting module. After IGF-1stimulation, the PH domain of Akt is required for nucleus translocation and the movement terminated by wortmannin [27]. Since there is no nuclear signal sequence, how Akt is introduced into nucleus, phosphorylated and affects downstream regulators in NSCLC cells including apoptosis signals awaits further investigation. It will be of great value to determine how ellipticine interacts with Akt and assists in its nuclear translocalization. While ellipticine promoted Akt nucleus translocation and phosphorylation, the activation may stabilize p53 without binding to Mdm2 and therefore slow down the ubiquitination and degradation of p53 [28]. The activation of p53 also might block the anti-apoptotic effects of Akt, thereby leading to apoptosis [29]. The present work was conducted in A549 cells and in the stable clones with exogenous p53. Whether the effect is applicable in other cells remains to be seen. Unlike A549 cells, an enhanced Akt phosphorylation at serine-473 in both H322 and H1437 cells carrying mutated p53 was not detected (data not shown) and treatment with ellipticine did not affect their growth rate. Thus, it is likely that wild-type p53 and Akt act together in leading cell death by ellipticine. However, our results do not rule out the possibility that Akt directly promotes p53 stabilization and final degradation through different regulatory mechanisms.

Tumor cells may undergo both apoptosis and autophagy in response to some anticancer drugs, and the two pathways may occur separately or simultaneously. The disruption of the PI3K/Akt signaling pathway enhanced autophagy and induced apoptosis that contributed to the drug effectiveness in malignant glioma cells [30]. Modulation of Akt activity through pharmacological approaches promises good implication toward therapeutic modalities [24]. It has been shown that tumor suppressor PTEN mediates autophagy [31]. The deficiencies in suppression of autophagy can lead to malignant transformation [32]. Previous reports indicated that Akt inhibitor exerted anticancer effect as a result of radiosen-

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sitization by inducing autophagy [33]. Strategies in developing Akt inhibitions have been demonstrated effective in modulating cell proliferation and/or apoptosis *in vitro* and *in vivo* in cancer cells [34]. Despite the potential values of Akt inhibitors as useful cancer therapy, only few of them have been reported. Our work provides a new dimension of ellipticine as an effective Akt modulator that is closely associated with the onset of apoptosis for controlling the growth of NSCLC cells.

Conflict of interest statement

None declared.

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