Long-term cisplatin exposure impairs autophagy and causes cisplatin resistance in human lung cancer cells

Buntitabhon Sirichanchuen · Thitima Pengsuparp · Pithi Chanvorachote

Abstract Cisplatin-based chemotherapy frequently resulted in acquired resistance of cancer cells. The underlying mechanism of such resistance is not fully understood especially the involvement of autophagy and autophagic cell death. This study thus investigated whether an alteration in autophagy could be responsible for cisplatin resistance in the long-term exposure lung carcinoma cells. The cisplatin resistant clone (H460/cis) of H460 cells was established by exposing the cells with gradually increasing concentrations of cisplatin until chemoresistance acquisition was elucidated by MTT, Hoechst 33342 staining and comet assays. Degree of autophagosome formation and level of LC3 marker were evaluated by acridine orange and western blot analysis, respectively. H460/cis cells exhibited irregular shape with ∼3-fold resistant to cisplatin-induced apoptosis compared with H460 cells. Proteins analysis for LC3 indicated that the levels of LC3 in resistant cells were significantly lower than those in H460 cells. Moreover, autophagosome formation detected by acridine orange staining was dramatically reduced in the resistant cells, suggesting the role of autophagy in attenuating of cisplatin-induced cell death. Further, co-treatment of cisplatin with autophagy inducer, trifluorperazine, could resensitize H460/cis cells to cisplatin-induced cell death. Our findings reveal the novel mechanisms causing cisplatin resistance in lung carcinoma cells after long-term drug exposure regarding autophagy.

Keywords Cisplatin · Autophagy · Lung cancer · Cisplatin resistance · Apoptosis

B. Sirichanchuen · T. Pengsuparp · P. Chanvorachote
Biopharmaceutical Sciences Program, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand

T. Pengsuparp (✉)
Department of Pharmacy Practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand
e-mail: thitima.pe@chula.ac.th

P. Chanvorachote (✉)
Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Pathumwan, Bangkok 10330, Thailand
e-mail: pithi_chan@yahoo.com

Introduction

The widely prescribed cisplatin-based chemotherapy has been accepted to be one of the most effective therapeutic approaches for the treatment of many solid tumors; however, acquired drug resistance developed during the therapy has become important obstacle and reduced the survival rate of patients. Based on the concept that the better understanding in molecular basis of such resistance, the more specific and effective strategy could be obtained, researches investigating about the molecular alteration in resistant cancer cells have garnered increasing attention recently.

Likewise, the cellular self-eating machinery termed "autophagy" is in the great interest, and was reported to be associated with several cellular processes including cell death and detoxification. Role of autophagy in affecting cancer cell, live and death, is still in controversial [1–3]. There were some data supporting that autophagy induction could gain benefit for cancer treatment. For example, resveratrol could induce autophagy and resulted in tumor growth arrest in ovarian cancer [4], as well as the treatment of tamoxifen, which targets the estrogen receptor, also induced autophagic cell death in breast cancer cells [5]. However, there was a conflicting evidence indicating that

autophagy could promote tumor cell survival and restrict necrosis, inflammation, and could induce tumorigenesis in mouse kidney epithelial (iBMK) cells [6]. Somehow, autophagy also acts as a cytoprotective mechanism protecting DNA damaging effects of Temozolomide and Etoposide in several malignant glioma cell lines [7]. Thus, autophagic process affects differently depending on cell types and particular agents.

Long-term chemotherapeutic exposure is required for the effective cancer therapy. Cisplatin exposure has been long known to result in cisplatin resistance in many cancers. Although it is known that the chemotherapeutic resistance results from the blockage of apoptotic process, utilizing of alternative cell-death pathways in cancer treatment becomes the point of interest. Autophagy can cause cell death in subsequence of strong detrimental stimuli. Besides, the mechanism in regulation of cisplatin resistance regarding autophagy is still far from clear. This study thus investigated the molecular alteration of autophagy in that resistant H460/cis cells generated by long-term cisplatin exposure. We demonstrated herein for the first time that the cell exposure to cisplatin could adapt cell death machinery toward inhibition of autophagy and consequently reduced their sensitivity to cisplatin-induced apoptosis. As microtubule-associated protein 1 light chain 3 (MAP1LC3) or LC3 is recognized as the marker of autophagy, we also found that the ratio of LC3 type II to I also decreased in H460/cis cells. Moreover, addition of an autophagic inducer trifluorperazine (TFP) to cisplatin-treated H460/cis cells could sensitize the cells to cisplatin-mediated death. Thus, our results revealed that such adaptation during cisplatin exposure was regard to autophagy downregulation, which these findings should facilitate the strategy in resistant cancer treatment.

Materials and methods

Cell culture and reagents

Human non-small cell lung cancer cells H460 (NCI-H460) cells were obtained from the American Type Culture Collection (Manassas, VA, USA) was cultured in RPMI 1640 containing 10% fetal bovine (Hyclone, Cramlington, UK), 1% penicillin and streptomycin (Gibco, Carlsbad, CA, USA) in a humid atmosphere of 5% CO_2 at 37°C. H460 cells were grown, and cisplatin (Sigma, St. Louis, MO, USA) was then added to the medium at gradually increasing concentrations starting from 0.33 μM (0.1 μg/ml) until the concentration reached 4.67 μM (1.4 μg/ml). During continuous exposure to cisplatin, culture medium was replaced with freshly prepared medium containing cisplatin at indicated concentrations every 3 days and

maintained in that concentration for at least two passages [8, 9]. Cell population survived when concentration reach 4.67 μM was named H460/cis cells.

Determination of cisplatin cytotoxicity

H460 cells and H460/cis cells were harvested with trypsin and resuspended in a final concentration of 1×10^5 cells/ml. 200 μl of cell suspension was distributed evenly into 96-well cell culture plate. After 24 h of incubation, various concentrations of cisplatin were added to cells and further incubated for 24 h. After incubation period, cell viability is determined by MTT assay [10, 11]. In brief, 100 μl of 0.4 mg/ml MTT (Sigma, St. Louis, MO, USA) was added into each well and further incubated for 4 h. Formazan crystals were dissolved in 100 μl DMSO. The absorbance of formazan solution is quantified by Microplate reader at 570 nm. Concentration of cisplatin at IC_{50} was calculated by CurveExpert 1.3.

For apoptosis detection

Cells were stained with 10 μM Hoechst 33342 (Sigma, St. Louis, MO, USA), and the fluorescent dye stained in cells was visualized by a fluorescence microscope. Cells that displayed intensely condensed and/or fragment nuclei stained by Hoechst 33342 were considered as apoptotic cells.

Comet single cell gel electrophoresis systems

Cells were trypsinized and suspended in 1% low melting agarose. Then, they were spread on a microscope slide pre-coated with 0.8% of normal melting agarose. After letting the low melting agarose dry, slides were placed in a lysis solution (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris-base pH 10, 1% Triton X-100, and 10% DMSO) for 60 min at 4°C (protected from light). Then, slides were equilibrated in electrophoresis buffer (0.3 M NaOH, 1 mM EDTA, pH > 13), and were run in a horizontal electrophoresis chamber at 700 mA 25 V for 5 min. Slides were washed with Tris buffer pH 7.5 and fixed with absolute MeOH for 5 min [12, 13]. Finally, slides were stained with SYBRgreen. The pictures of unwind DNA taken under inverted fluorescence microscope were analyzed by CometScore v1.5 for tail length, %DNA in tail, tail moment, and olive moment parameters. More than 70% in each parameter, compared with untreated control, was considered as the cut-off point of toxicity and apoptosis cell death.

Qualitative and quantitative analysis of autophagosome formation

Cells were harvested, centrifuged at 1,200 rpm for 5 min, washed with PBS twice, resuspended in 1 μM acridine

orange (AO, Sigma, St. Louis, MO, USA), and incubated for 17 min [14, 15]. Autophagosome formation was qualitatively detected by fluorescence inverted-microscope (IX51 Olympus, Japan). Moreover, AO fluorescence intensity was also measured by flow cytometry (BD FACSCalibur, CA, USA).

Western blotting

Cells were harvested with trypsinization, and then washed twice with PBS. Cells were incubated with ice-cold lysis buffer (50 mM Tris pH 7.4, 100 mM NaCl, 2 mM EDTA, 1% sodium deoxycholate, 0.1% SDS, 1% Triton X-100, 2 mM Na_3VO_4 and complete mini-cocktail protease inhibitors) for 30 min at 4°C. After centrifugation at 15,000 rpm for 15 min, supernatants were collected [16]. Samples containing 30 μg of proteins were denatured, loaded, and separated on 12% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Protein bands were transferred to PVDF membranes, and blocked for 1 h at room temperature with 5% skim milk in TBST (10 mM Tris–HCl, pH 7.5, 100 mM NaCl, 0.01% tween20). The membranes were immunoblotted with rabbit polyclonal LC3 antibody (1:500, Abcam: Cambridge, MA, USA). To assure equivalent protein loaded, the membranes are simultaneously incubated with mouse monoclonal β-actin antibody (1:3,000, Abcam: Cambridge, MA, USA). Membranes were washed three times with TBST, incubated with HRP-conjugated rabbit IgG secondary antibody (1:3,000, Abcam: Cambridge, MA, USA) for 1 h at room temperature and washed extensively before detection. Protein bands were visualized using an enhanced chemiluminescence Western blot analysis system (Pierce, Rockford, IL, USA), and quantified the protein bands using ImageJ 1.43u software (NIH, USA).

Statistical analysis

All results from three or more independent experiments were expressed in mean ± SEM. Statistical differences between means were determined using analysis of variance (ANOVA) and post hoc test. $P < 0.05$ was selected to reflect significance.

Results

Exposure to gradually increasing concentrations of cisplatin rendered H460 cells resist to cisplatin-mediated death

Cisplatin exposure during chemotherapy is likely to force cancer cell adaptation for acquired drug resistance. Cisplatin resistant clone of lung carcinoma H460 cells was generated by continuous exposure to stepwise increasing concentrations of cisplatin starting from 0.33 μM until reaching 4.67 μM over a period of 12 months. After establishment of H460/cis cells, we characterized cell death in response to cisplatin treatment (0.1–1,000 μM) in these cells comparing with those parental H460 cells. Cell viability assay revealed that percentage of cell survival of H460 and H460/cis cells decreased in dose-dependent manner. At 100 μM of cisplatin, the percentage of cell viability in both cells showed significantly difference (Fig. 1a). H460/cis cells exhibited ∼2.4 times more resistant than H460 cells ($P < 0.001$) with the 50% inhibitory concentrations (IC_{50}) of cisplatin in H460 and H460/cis were 37.6 ± 0.7 μM and 90.7 ± 4.4 μM, respectively (Fig. 1b). It was noteworthy noting that under phase-contrast microscopy, H460 cells had an irregular shape and enlargement in size of the cells comparing to the parental H460 cells (Fig. 1c). Further, we evaluated mode of cell death in response to cisplatin. Both cisplatin resistant and sensitive cells were treated with cisplatin at the concentration of 40 μM, and Hoechst 33342 and propidium iodide (PI) staining were accessed. After 24 h of cisplatin treatment, H460 cells underwent apoptotic cell death ∼20% as determined by the increase in number of cells with intense blue fluorescence and chromatin condensation (Fig. 2a, b). Meanwhile, only 7% apoptosis could be detected in H460/cis cells (Fig. 2a, b). PI staining assay provided the information that less necrotic cells could be observed in both H460 and H460/cis cells (Data not shown). Further, our results obtained from comet assay indicated that, H460/cis had fewer apoptosis characteristics than those of H460 cells (Fig. 3a, b).

Cisplatin exposure suppressed autophagosome formation in lung cancer cells

Autophagy has garnered increasing attention recently since autophagy plays a key role in either cellular detoxify mechanism or cell death determination [5]. We thus tested whether the reduction of cell viability in response to cisplatin in H460/cis cells involved autophagy. Since autophagy associated with cellular machinery in autophagosome formation, AO dye, a specific autophagosome indicator [14, 15, 17] was used. H460 and H460/cis cells were stained with 1 μM of AO for 17 min, and autophagosome was visualized under fluorescence inverted microscope. As shown in Fig. 4a, H460/cis cells exhibited lower in both AO intensity and a number of intracellular AO-positive spots, than those of H460 cells. We further quantified the presence of autophagosome by subjecting the stained cell to flow cytometer with FACS analysis. The consistent results were obtained and indicated that the

Fig. 1 The characteristics of cisplatin resistant H460/cis cells compared with their parental H460 cells. H460/cis cells were established by culturing in the gradually increasing concentration of cisplatin, starting from 0.33 μM (0.1 μg/ml) until reaching the concentration of 4.67 μM (1.4 μg/ml). For determination of cisplatin cytotoxicity, cells were treated with various concentrations of cisplatin and incubated for 24 h. Then, cell viability was determined by MTT assay and calculated as the percentage of cell viability compared with the control untreated cells (**a**). Each value is the mean ± SEM of four independent experiments and each was experimented in five wells. The differences between groups were determined by analysis of variance (ANOVA) and post hoc test. *$P < 0.05$ compared with the same concentration of cisplatin in H460 cells. The IC_{50} values of cisplatin in both cells were also calculated by CurveExpert 1.3 (**b**). Each value is the mean ± SEM of four independent experiments. **$P < 0.001$ as determined by Student's unpaired two-tailed *t* test compared with the IC_{50} value in H460 cells. Moreover, phase-contrast microscopy showed the enlargement of cell size of H460/cis cells compared with H460 cells (**c**). *Scale bars* were 100 μm
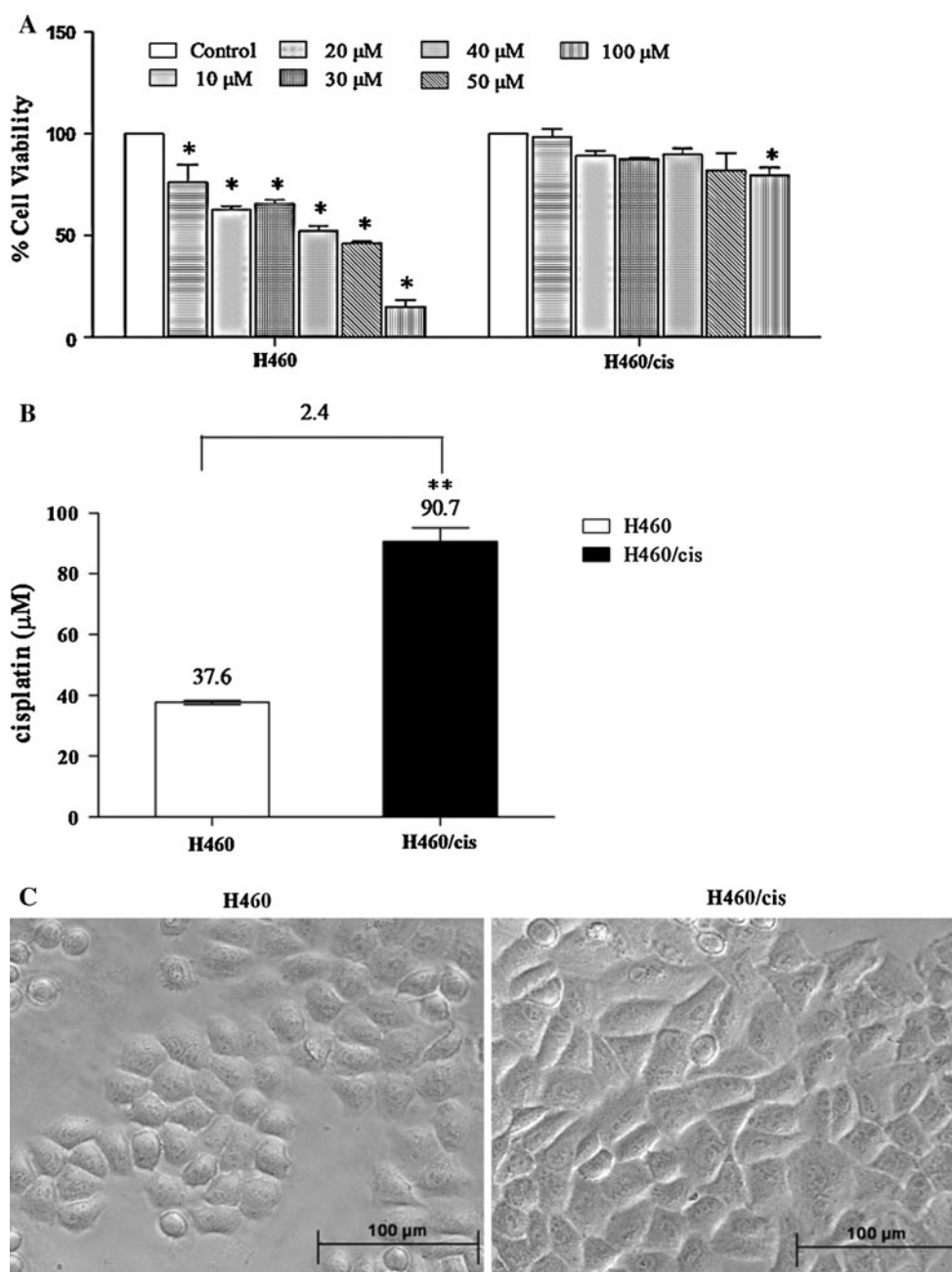
degree of autophagy in H460/cis cells were noticeably diminished that in H460 cells (Fig. 4b, c).

To date, the transformation of microtubule-associated protein 1 light chain 3 (MAP1LC3 or LC3) protein from the native cytosolic form of LC3 (LC3-I) to membrane-bound lapidated (LC3-II) is recognized as an important process of autophagosome formation [16, 17]. The proportion of LC3-II conversion is used as an indicator of autophagy activity. We confirmed the decrease of autophagosome formation during the process of H460/cis cells establishment by western blot analysis detecting LC3-I and LC3-II levels. As shown in Fig. 4d, the ratio of LC3-II conversion was dramatically decreased in a time-dependent

manner. The degree of autophagy in H460/cis cells was ∼47% compared with that of parental H460 cells. Together our results indicated for the first time that the adaptation of lung carcinoma cells in response to cisplatin exposure involved alteration in cellular autophagy level.

Reduction of autophagosome was responsible for cisplatin resistance in H460 cells

Since H460/cis cells exhibited significantly less autophagic activity, we further tested whether such reduction was associated with cisplatin resistance. TFP, a known autophagic inducer, was used to induce autophagosome

Fig. 2 The apoptotic nucleus morphology stained with Hoechst 33342 dye. The cells were treated with 40 μM of cisplatin for 24 h. Then cells were washed twice with PBS and added new media with 10 μM of Hoechst 33342. The nucleus morphology of H460 cells and H460/cis cells in cisplatin treatment and control were taken under fluorescence microscope (**a**). The pictures shown were one each from five independent experiments. The living cells showed the normal bright blue nuclei and the apoptotic cells showed the condensed intensive bright blue nuclei. *Scale bars* were 200 μm. The percentage of apoptotic cell death was determined (**b**). Each value is the mean ± SEM of five independent experiments. At least two hundred of cells were counted for one experiment. *$P < 0.05$, **$P < 0.001$ as determined by Student's unpaired two-tailed t test compared with control each cell. #$P < 0.05$ as determined by Student's unpaired two-tailed t test compared with apoptotic cell death in H460 cells
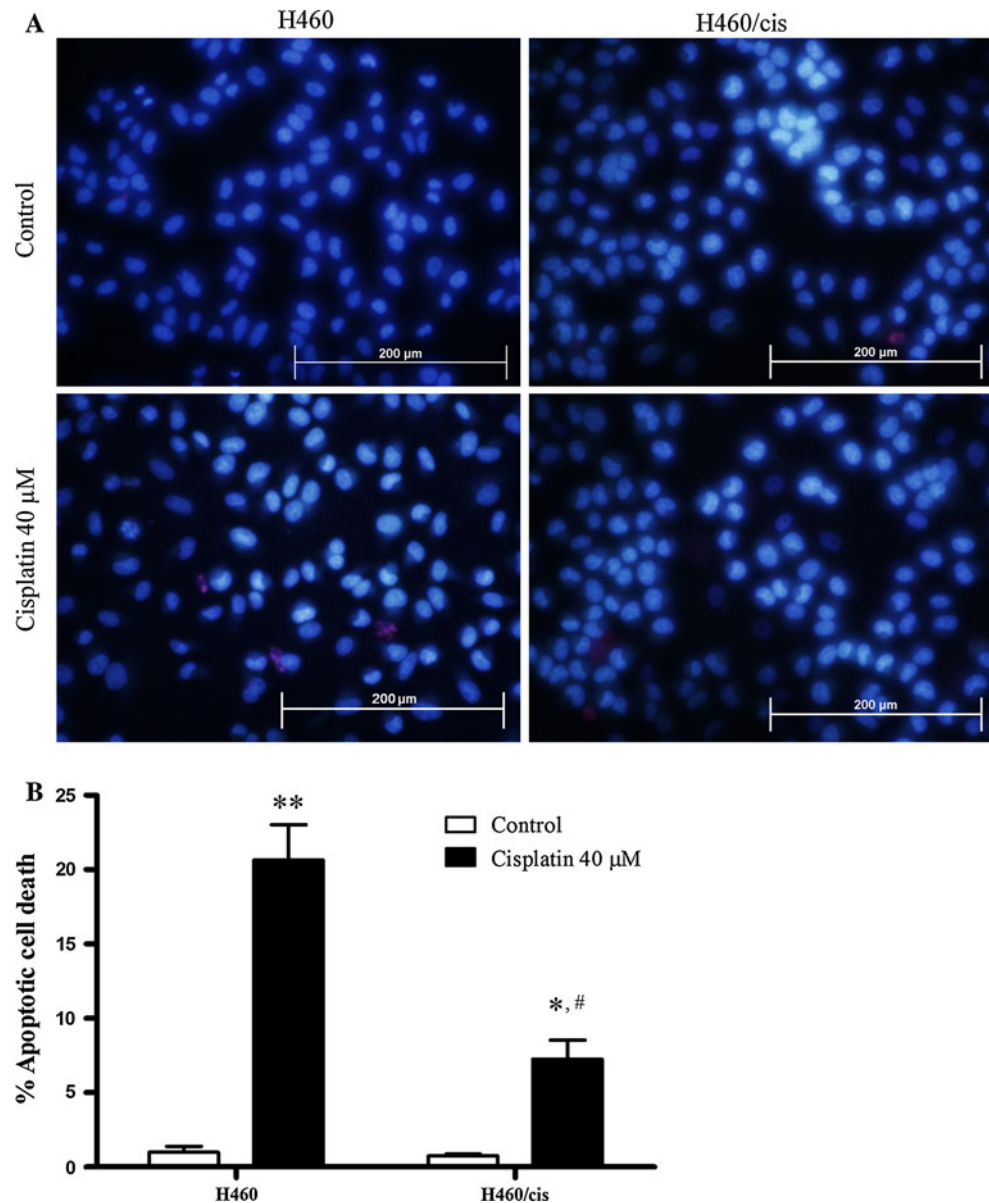
formation in these cells. H460 and H460/cis cells were co-treated with TFP at the concentration of 5 μM and cisplatin at the concentration of 40 μM, and then cell viability was evaluated by MTT assay. When treatment of cells with TFP alone showed neither toxic nor proliferative effect on these cells (data not shown), the co-treatment of cisplatin and TFP in H460/cis cells exhibited significantly sensitized H460/cis cells to cisplatin-induced cytotoxicity compared with cisplatin-treated control (Fig. 5). On the contrary, the additional TFP in cisplatin treatment had no augmentative effect in H460 cells.

Moreover, we also inhibited autophagic inductive effects of TFP in H460/cis cells with 3-methyladenine (3-MA), the specific phosphatidylinositol-3 kinase (PI3K) class III which regulated autophagic activity [17]. The

introduction of 3-MA at the concentration of 200 μM could reverse sensitizing effect of autophagic inducer and cell viability in response to the treatment was increased to approximately the same level of that in cisplatin-treated alone as shown in Fig. 5. These suggested that the acquired resistance of H460/cis cells resulting from long-term exposure to cisplatin at least partly involved the machinery of cell in suppression of autophagy.

Discussions

Acquired resistance to chemotherapy is a major hindrance to the success of cancer treatment. Understanding the underlying mechanisms by which tumors become resistant

Fig. 3 The nucleus morphology in comet single cell gel electrophoresis. Cells were treated with 40 μM of cisplatin for 24 h, and then were harvested by trypsinization and embedded in low-melting agarose on normal melting agarose-precoated microscope slide. The nucleus membrane were lysed and run in electrophoresis for 5 min. The nuclear DNA was stained with SYBRgreen and visualized under fluorescence microscope (**a**). The DNA fragmentation was analyzed by CometScore v1.5 for tail length, % DNA in tail, tail moment, and olive moment parameters (**b**). Values are mean ± SEM of three independent experiments. *$P < 0.05$ compared with control of each cell
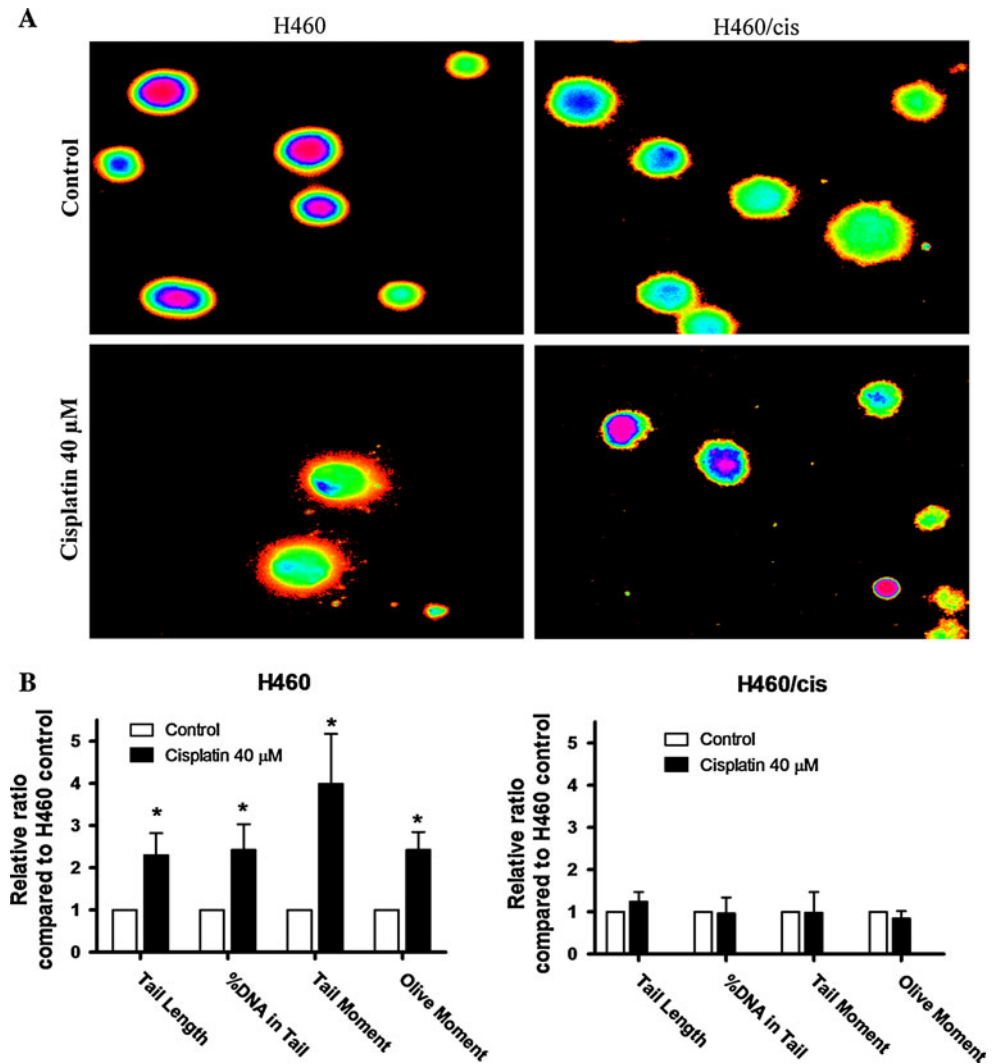
to a particular agent is key to identifying new drugs or combination regimens. For cisplatin, an effective agent, using worldwide for the treatment of various cancers, including lung cancer. For the best treatment outcome, it has to be administrated repetitively. However, its therapeutic efficacy is limited due to the initiation of acquired resistance. The mechanisms of cisplatin resistance appear to be multifactorial and believed to process through 4 main pathways which are a change of drug transportation, an increase of drug detoxifying systems, a modification in DNA repairing machinery, and alteration in the apoptotic cell death pathways [18–21].

The term of "autophagy" becomes a great interest in cancer research field nowadays. In order to comprehend the involvement of autophagy on cisplatin resistance in non-small cell lung carcinoma, we established the resistant non-small cell lung carcinoma H460 cells by exposing the cells with continuous administration of cisplatin. As the results of cell viability assay, H460/cis cells could not undergo

cell death in cisplatin treatment at IC_{50} concentration. This means that apoptotic cell death pathway or type I programmed cell death was blocked in these cells (Figs. 1, 2, 3). Autophagy is the process of cell components degradation itself through the machinery of lysosome, which turns to be the cause of cell death [22–24]. However, in certain conditions autophagy resulted in an increasing survival of cells in response to death stimuli and the different outcomes are likely to depend on cell types, agents used, and condition of treatment [1–7]. The protective role of autophagy was presented in several previous studies. When autophagy was induced by death stimuli, its function was most likely trended to facilitate cell survival. On the contrary, we found that treatment of the cells with barely toxic dose of cisplatin for relatively long time could result in a decrease of autophagy that plays an opposite role on cell survival. Importantly, such reductions of autophagy in H460/cis cells detected by AO-cell staining and western blot analysis of LC3 (Fig. 4d) was concomitant with the

Fig. 4 The degree of autophagosome formation in H460 cells and H460/cis cells. The cell was staining with acridine orange (AO) for 17 min and the fluorescent intensity was visualized by fluorescence inverted microscopy (a) and by flow cytometer (b). The degree of autophagy was determined by the geometric means of AO fluorescence intensity in H460 cells and H460/cis cells (c). Values are mean ± SEM of five independent experiments. **$P < 0.001$ as determined by Student's unpaired two-tailed t test, compared with control H460 cells. As the lipidated LC3-II protein is the marker of autophagy, the levels of LC3-I and II protein were quantified by western blotting (d). H460/cis cells were collected during transformation process at 1, 2, 4, 6, and 12 months. Cell lysate was separated on 12% SDS-PAGE. The results were represented of one of three independent experiments. The relative ratio of LC3-II conversion normalized with β-actin
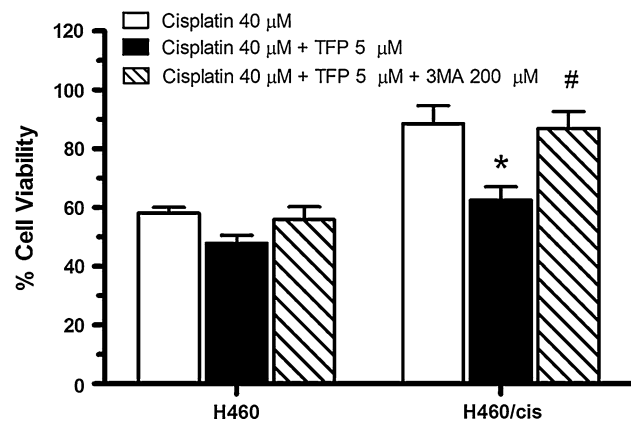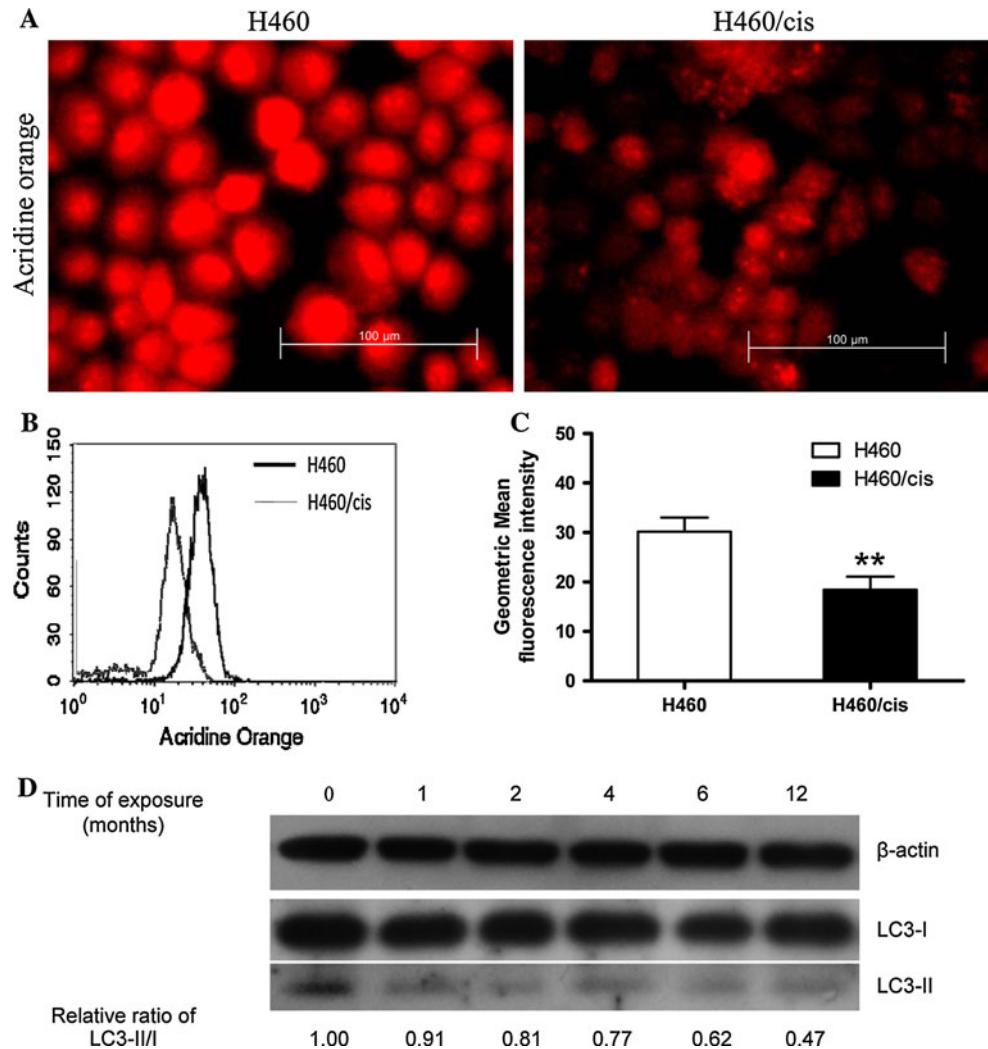

Fig. 5 The reverse effects of autophagic inducer and inhibitor in cisplatin treatment in H460/cis cells. Either sub-toxic concentration trifluoperazine at the concentration of 5 μM or 3-MA at the concentration of 200 μM was co-incubated with cisplatin 40 μM. Cytotoxicity of co-treatment was analyzed by MTT assay. Values are mean ± SEM of three independent experiments. *$P < 0.05$ compared with their cisplatin-treated cells. #$P < 0.05$ compared to their cisplatin and trifluoperazine co-treated cells

decrease response to cisplatin-mediated cells death implying that the autophagy could be responsible for cisplatin-induced resistance in these cells.

Previous reports have indicated that long-term cisplatin treatment also resulted in Bcl-2 overexpression [9, 25–29]. Moreover, Bcl-2 protein has been shown to act as a negative inhibitor of autophagy [27–32] via its interaction with Beclin 1 [33]. Consistent with above mentions, our results showed that H460/cis cells exhibited higher level of Bcl-2 than that of parental H460 cells (data not shown) with reduced autophagy. Taken together, not only did the present study indicate for the first time that long-term exposure of lung carcinoma cells to cisplatin resulted in autophagic reduction, but also supported the previous observation that Bcl-2 protein acted as a negative regulator for autophagy as well as apoptosis. Furthermore, we found that addition of autophagic inducer TFP to H460/cis cells could reverse the resistance of the cells to cisplatin-mediated apoptosis (Fig. 5). Co-treatment of cisplatin and TFP could reduce percentage of H460/cis cell viability back to the level of

parental H460 cells. This finding has strengthened the possibility that long-term cisplatin exposure renders cells resisting to the drug via autophagy-dependent mechanism.

In summary, the present study reported herein that long-term exposure to cisplatin led to cisplatin resistance in lung carcinoma H460 cells and lowered autophagy level. These observations inferred that long-term exposure to cisplatin caused acquired drug resistance through the mechanism involving autophagy reduction. Therefore, upregulation of autophagy may be a useful adjuvant approach for treatment cancer to prevent resistance in further therapy.

References

1. Edinger AL, Thompson CB (2003) Defective autophagy leads to cancer. Cancer Cell 4:422–424
2. Gozuacik D, Kimchi A (2004) Autophagy as a cell death and tumor suppressor mechanism. Oncogene 23:2891–2906
3. Levine B, Klionsky DJ (2004) Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev Cell 6:463–477
4. Opipari AW, Tan L, Boitano AE, Sorenson AR, Aurora A, Liu JR (2004) Resveratrol-induced autophagocytosis in ovarian cancer cells. Cancer Res 64:696–703
5. Kondo Y, Kanzawa T, Sawaya R, Kondo S (2005) The role of autophagy in cancer development and response to therapy. Nat Rev Cancer 5:726–734
6. Degenhardt K, Mathew R, Beaudoin B, Bray K, Anderson D, Chen G, Mukherjee C, Shi Y, Gélinas C, Fan Y, Nelson DA, Jin S, White E (2006) Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 10:51–64
7. Katayama M, Kawaguchi T, Berger MS, Pieper RO (2007) DNA damaging agent-induced autophagy produces a cytoprotective adenosine triphosphate surge in malignant glioma cells. Cell Death Differ 14:548–558
8. Mese H, Sasaki A, Alcalde RE, Nakayama S, Matsumura T (1998) Establishment and characterization of cisplatin-resistant human epidermoid carcinoma cell line, A431 cell. Chemotherapy 44:414–420
9. Yoon SS, Ahn KS, Kim SH, Shim YM, Kim J (2001) In vitro establishment of cis-diammine-dichloroplatinum(II) resistant lung cancer cell line and modulation of apoptotic gene expression as a mechanism of resistant phenotype. Lung Cancer 33:221–228
10. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB (1987) Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. Cancer Res 47:936–942
11. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63
12. Singh NP, McCoy MT, Tice RR, Schneider EL (1988) A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 175:184–191

13. Olive PL, Durand RE, Banáth JP, Johnston PJ (2001) Analysis of DNA damage in individual cells. Methods Cell Biol 64:235–249
14. Kanzawa T, Kondo Y, Ito H, Kondo S, Germano I (2003) Induction of autophagic cell death in malignant glioma cells by arsenic trioxide. Cancer Res 63:2103–2108
15. Paglin S, Hollister T, Delohery T, Hackett N, McMahill M, Sphicas E, Domingo D, Yahalom J (2001) A novel response of cancer cells to radiation involves autophagy and formation of acidic vesicles. Cancer Res 61:439–444
16. Egami Y, Kiryu-Seo S, Yoshimori T, Kiyama H (2005) Induced expressions of Rab24 GTPase and LC3 in nerve-injured motor neurons. Biochem Biophys Res Commun 337:1206–1213
17. Mizushima N, Yoshimori T, Levine B (2010) Methods in mammalian autophagy research. Cell 140:313–326
18. Köberle B, Tomicic MT, Usanova S, Kaina B (2010) Cisplatin resistance: preclinical findings and clinical implications. Biochim Biophys Acta 1806:172–182
19. Gottesman MM (2002) Mechanisms of cancer drug resistance. Annu Rev Med 53:615–627
20. Siddik ZH (2003) Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 22:7265–7279
21. Rabik CA, Dolan ME (2007) Molecular mechanisms of resistance and toxicity associated with platinating agents. Cancer Treat Rev 33:9–23
22. Wang CW, Klionsky DJ (2003) The molecular mechanism of autophagy. Mol Med 9:65–76
23. Inoue K, Kuwana H, Shimamura Y, Ogata K, Taniguchi Y, Kagawa T, Horino T, Takao T, Morita T, Sasaki S, Mizushima N, Terada Y (2010) Cisplatin-induced macroautophagy occurs prior to apoptosis in proximal tubules in vivo. Clin Exp Nephrol 14:112–122
24. Eum KH, Lee M (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
25. Sartorius UA, Krammer PH (2002) Upregulation of Bcl-2 is involved in the mediation of chemotherapy resistance in human small cell lung cancer cell lines. Int J Cancer 97:584–592
26. Waggoner SE, Baunoch DA, Anderson SA, Leigh F, Zagaja VG (1998) Bcl-2 protein expression associated with resistance to apoptosis in clear cell adenocarcinomas of the vagina and cervix expressing wild-type p53. Ann Surg Oncol 5:544–547
27. Muilenburg DJ, Coates JM, Virudachalam S, Bold RJ (2010) Targeting Bcl-2-mediated cell death as a novel therapy in pancreatic cancer. J Surg Res 2:276–281
28. Cho HJ, Kim JK, Kim KD, Yoon HK, Cho MY, Park YP, Jeon JH, Lee ES, Byun SS, Lim HM, Song EY, Lim JS, Yoon DY, Lee HG, Choe YK (2006) Upregulation of Bcl-2 is associated with cisplatin-resistance via inhibition of bax translocation in human bladder cancer cells. Cancer Lett 237:56–66
29. Wesarg E, Hoffarth S, Wiewrodt R, Kröll M, Biesterfeld S, Huber C, Schuler M (2007) Targeting BCL-2 family proteins to overcome drug resistance in non-small cell lung cancer. Int J Cancer 11:2387–2394
30. Saeki K, Yuo A, Okuma E, Yazaki Y, Susin SA, Kroemer G, Takaku F (2007) Bcl-2 down-regulation causes autophagy in a caspase-independent manner in human leukemic HL60 cells. Cell Death Differ 7:1263–1269
31. Lam D, Levraud JP, Luciani MF, Golstein P (2007) Autophagic or necrotic cell death in the absence of caspase and bcl-2 family members. Biochem Biophys Res Commun 363:536–541
32. Thorburn A (2008) Apoptosis and autophagy: regulatory connections between two supposedly different processes. Apoptosis 13:1–9
33. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, Levine B (2005) Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 122:927–939