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Dihydroartemisinin potentiates the anticancer effect of cisplatin via mTOR inhibition in cisplatin-resistant ovarian cancer cells: involvement of apoptosis and autophagy

Xue Feng [a], Ling Li [b], Hong Jiang [a], Keping Jiang [a], Ye Jin [a], Jianhua Zheng [a,*]

[a] Department of Gynecology and Obstetrics, The First Affiliated Hospital of Harbin Medical University, Harbin 150001, China
[b] Department of Brain Cognition Computing Lab, University of Kent, Kent CT2 7NZ, UK

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ABSTRACT

Dihydroartemisinin (DHA) exhibits anticancer activity in tumor cells but its mechanism of action is unclear. Cisplatin (DDP) is currently the best known chemotherapeutic available for ovarian cancer. However, tumors return *de novo* with acquired resistance over time. Mammalian target of rapamycin (mTOR) is an important kinase that regulates cell apoptosis and autophagy, and its dysregulation has been observed in chemoresistant human cancers. Here, we show that compared with control ovarian cancer cells (SKOV3), mTOR phosphorylation was abnormally activated in cisplatin-resistant ovarian cancer cells (SKOV3/DDP) following cisplatin monotherapy. Treatment with cisplatin combined with DHA could enhance cisplatin-induced proliferation inhibition in SKOV3/DDP cells. This mechanism is at least partially due to DHA deactivation of mTOR kinase and promotion of apoptosis. Although autophagy was also induced by DHA, the reduced cell death was not found by suppressing autophagic flux by Bafilomycin A1 (BAF). Taken together, we conclude that inhibition of cisplatin-induced mTOR activation is one of the main mechanisms by which DHA dramatically promotes its anticancer effect in cisplatin-resistant ovarian cancer cells.

1. Introduction

It has been observed that dihydroartemisinin (DHA), a sesquiterpene lactone isolated from the traditional Chinese herb *Artemisia annua*, can be used as a primary anti-malarial drug with low toxicity [1,2]. Recently, studies have shown that DHA also has antitumorigenic properties in various cancers [3–5]. However, its mechanism of action has not been fully elucidated.

Ovarian cancer is currently the fifth leading cause of death in women [6]. As a result of late diagnosis and its highly aggressive behavior, the 5-year survival rate for ovarian cancer is approximately 45%. Although most patients will achieve a complete clinical response after maximal cytoreductive surgery together with chemotherapy at first, nearly 50% of patients will eventually develop recurrent disease [6]. It has been suggested the high mortality rate is partly due to the high rate of acquired chemoresistance [7].

Investigations are being carried out in the development of new therapeutics to deal with chemoresistance. Autophagy, an evolutionarily conserved process of lysosome-dependent cellular catabolic degradation, is characterized by the sequestration of bulk cytoplasm and organelles in double-membrane autophagic vesicles [8]. In addition, studies have demonstrated that cell apoptosis and autophagy share several similar signaling pathways and molecular mechanisms [9,10]. However, the question of whether the autophagic activity in cancer cells is the cause of death or promotes survival remains unclear.

Among many cellular metabolic mechanisms, the signaling pathway mediated by the mammalian target of rapamycin (mTOR) is always abnormally activated in breast [11], gastric [12], lung [13] and prostate cancer [14]. In ovarian cancer, persistent activation of mTOR creates a pro-survival status in cells, and several studies have concluded that downregulation of mTOR helps to sensitize these cells to chemotherapy [15–17]. Therefore, we propose DHA, a new mTOR deactivator, could be a "sensitizer" to be used in combination with cisplatin in the treatment of chemoresistant ovarian cancer.

In the present study, we investigated whether DHA could boost the anti-cancer effect of cisplatin and induce autophagy in chemoresistant human ovarian cancer SKOV3/DDP cells via inhibition of mTOR. Through proliferation, cell cycle, apoptosis, autophagy, and mTOR activity analyses, the results suggest that DHA enhances the sensitivity of cisplatin-resistant ovarian cancer cells to chemotherapy by inhibiting mTOR, resulting in the promotion of apoptosis. In addition, DHA induced autophagy may not directly involved in regulation of the cell death in this study.

* Corresponding author. Fax: +86 451 53603642.
E-mail address: zhengjianhua1115@126.com (J. Zheng).

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2. Materials and methods

2.1. Chemicals and reagents

DHA, Cisplatin and BAF were purchased from Sigma Chemical Co. (Sigma–Aldrich, St. Louis, MO, USA). CCK-8 cell counting kit (Beyotime Inst. Biotech, Haimen, China) was stored at −20 °C. GFP-LC3 plasmids were a gift from Professor Tianwei Xu (Harbin Medical University, Harbin, China). Annexin V-FITC and propidium iodide (PI) were purchased from BaoSai Biotechnology (BaoSai Biotechnology Co. Ltd., Beijing, China). Bafilomycin A1 was purchased from Sigma Chemical Co. (Sigma–Aldrich, St. Louis, MO, USA). Antibodies against mTOR, phospho-mTOR, LC3, BCL-2, BAX, C-MYC, cyclin A, cleaved caspase-3 and β-actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.2. Cell lines and culture

The human ovarian cancer cell line SKOV3 and SKOV3/DDP cells, a cell clone derived from SKOV3 cells chronically exposed to cisplatin, were obtained from The Cell Bank, Chinese Academy of Sciences (Beijing, China). Both lines were cultured in RPMI 1640 medium supplemented with 8% fetal bovine serum (FBS) and 100 U/mL streptomycin/penicillin at 37 °C in a humidified atmosphere containing 5% CO_2. SKOV3/DDP cells were additionally cultured with 2 μM/L cisplatin to maintain drug-resistance. All of the above reagents were purchased from HyClone China Ltd.

2.3. Cell viability assay

SKOV3 (1×10^4) and SKOV3/DDP (6×10^3) cells were seeded into 96-well plates and cultured for 24 h. After that, cells were pretreated with or without 100 nM of BAF for 2 h [18], and then treatment with cisplatin, DHA or their combination for indicated concentrations and times. Before BAF, cisplatin, DHA added, cells were treated with DMSO only as the untreated control. At each time point, 10 μL CCK-8 kit were added to each well and cultured at 37 °C for about 1 h. Then, a microplate reader (Thermo) was used to calculate the absorbance at 450 nm. The survival rate of cells (%) = (experimental group OD value−blank group OD value)/(control group OD value−blank group OD value), and the inhibition rate of cell proliferation (%) equaled 100% minus the survival rate. To determine the concentration of cisplatin necessary to result in 50% inhibition (IC50), the data was calculated using the weighted linear regression method with SPSS 19.0 software.

2.4. Cell cycle analysis

SKOV3/DDP cells were seeded in 12-well plates and cultured overnight. After treatment with DHA at 0–80 μM for 48 h, the cells were harvested, washed with cold PBS, and subsequently fixed with 70% ethanol at 4 °C overnight. After removal of the ethanol, approximately 1×10^6 cells were washed with cold PBS, and then re-suspended in PBS containing PI and RNase A for 30 min in the dark. Flow cytometry (Epics Altra II, Beckman Coulter, USA) and ModFit LT software were used to determine the percentages of cells in G0/G1, S, or G2/M phase.

2.5. Transmission electron microscopy (TEM) analysis

After designated treatment, SKOV3/DDP cells were washed with 0.1 cacodylate buffer (pH 7.4) and fixed with 2% glutaraldehyde in PBS for 24 h at 4 °C. Subsequently, the rest of the procedure was conducted using the standard procedure. Zeiss transmission electron microscopy was used to examine the section samples. The quantitation of autophagic vacuoles in each cell was determined with 20 cells in each sample, respectively.

2.6. GFP-LC3 plasmid transfection and autophagic flux assay

SKOV3/DDP cells were seeded on sterile coverslips in tissue culture plates. After that, they were transfected with a GFP-LC3 expression plasmid at nearly 70–80% confluence using the lipofectamine transfection regent (Invitrogen, Carlsbad, CA, USA). 48 h later, the cells were pretreated with or without BAF. Then, after designated treatment, cells were fixed with 4% formaldehyde for 15 min and washed with cold PBS. The numbers of GFP-LC3 puncta were manually counted. For each group, 20 cells in randomly visual fields were selected for counting [19].

2.7. Apoptosis assay

After designated treatment, SKOV3/DDP cells were washed with ice-cold PBS, then harvested and counted. A total of 1×10^5 cells were suspended in 500 μL of binding buffer and incubated with 10 μL of Annexin V and 5 μL of PI for 20 min. The cell apoptosis rate (%) was measured by flow cytometry (Epics Altra II, Beckman Coulter, USA). A laser scanning confocal microscope (TCS SP5 II, Leica, Germany) was used to visualize cells that had undergone early or late phase apoptosis.

2.8. Western blot analysis

Cells were preincubated with DHA and/or cisplatin as previously described. Western blotting was performed as previously described [3]. Briefly, the cells were washed with ice-cold PBS and sonicated in RIPS buffer and homogenized, and then cellular debris was removed by centrifugation. Protein concentration was determined using the Bio-Rad DC protein assay (Bio-Rad Laboratories, Shanghai, China). The protein lysates were separated by 12% or 15% SDS–PAGE and transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% BSA (Beyotime Inst. Biotech, Haimen, China) and then incubated with primary antibodies (1:500 or 1:1000) overnight at 4 °C, and subsequently with horseradish peroxidase-conjugated secondary antibodies (1:1500 or 1:2000). The blots were then visualized by enhanced chemiluminescence (Millipore Corporation, Billerica, MA, USA). Blots were also stained with anti-β-actin as an internal control and to confirm that each sample contained a similar amount of protein.

2.9. Statistical analysis

All data and results were confirmed in at least three independent experiments. The data are expressed as mean values ± SD. Student *t*-test and the analysis of variance (ANOVA) test were used to test for statistically significant differences between two groups or among more groups, respectively. $P < 0.05$ was defined as statistical significance.

3. Results

3.1. Cisplatin-sensitivity and mTOR activity in human ovarian cancer SKOV3 and cisplatin-resistant phenotype SKOV3/DDP cell lines

SKOV3 and SKOV3/DDP cells were exposed to various doses of cisplatin for 24, 48 or 72 h. Cell viability was measured by CCK-8 assay. As shown in Fig. 1A, cisplatin reduced the cell numbers in a time- and dose-dependent manner. After 48 or 72 h treatment, compared with lower doses, at least 50% of the cell growth was suppressed by 40 μM of cisplatin. The IC50 values of cisplatin in

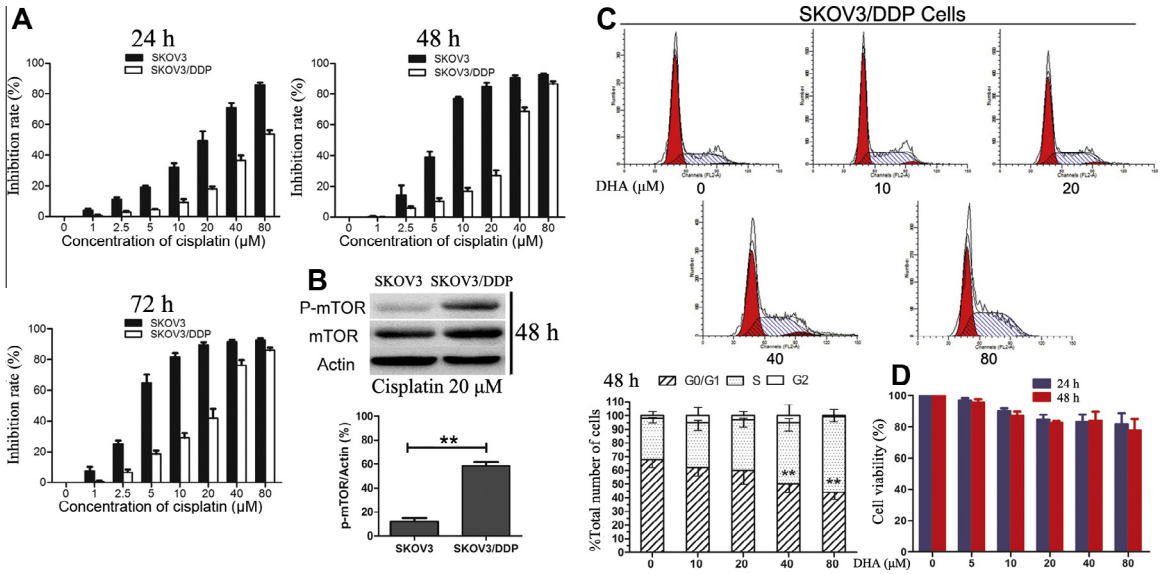

Fig. 1. Characteristics of human ovarian cancer SKOV3 and cisplatin-resistant SKOV3/DDP cell lines. (A) Two cell lines were treated with different concentrations of cisplatin for 24–72 h. Cell viability was determined by CCK-8 assays. (B) SKOV3/DDP or SKOV3 cells were treated with cisplatin (20 μM) for 48 h. The expression of p-mTOR and mTOR proteins were detected by Western blot assay and the level of each protein was normalized to that of β-actin. (C) Cells were treated with different concentrations of DHA for 48 h followed by cell cycle distribution assay. (D) Cells were treated with DHA for 24–48 h. Cell viability was determined by CCK-8 assays. Each value was expressed as means ± SD. $^{**}p < 0.01$ versus control.

SKOV3 and SKOV3/DDP cells were 5.92 ± 1.21 and 28.00 ± 1.32 μM after 48 h exposure, respectively, implying that compared with SKOV3 cells, SKOV3/DDP cells had significant cisplatin-resistance. Taken the above results, we then use 20 μM of cisplatin for subsequent experiments. By Western blot analysis (Fig. 1B), we found the expression of phospho-mTOR was upregulated in SKOV3/DDP cells at 20 μM cisplatin treatment for 48 h. These data indicate that the cisplatin-resistant properties of SKOV3/DDP cells under lower dosages might be relevant to the activity of mTOR phosphorylation.

3.2. DHA induced cell cycle arrest and autophagy in SKOV3/DDP cells

To understand the efficacy of DHA to SKOV3/DDP cells, CCK-8 assay, cell cycle analysis, GFP-LC3 transfection assay, TEM analysis and Western blotting were used. As shown in Fig. 1D, various doses of DHA for 24 or 48 h could not induce significant cell death. However, cell cycle analysis showed that DHA inhibits cell proliferation by inducing cell cycle arrest at S phase in a concentration-dependent manner (Fig. 1C). DHA could also induce autophagy in SKOV3/DDP cells. Western blot results showed that 40 μM of DHA remarkably increased the expression of LC3-II protein (Fig. 2C). After autophagosome-specific GFP-LC3 transfection assay, the 40 μM DHA-treated group showed higher fluorescent density and more GFP-labeled vacuoles (green fluorescence dots) compared with the control group (Fig. 2A). Then, TEM scanning revealed a large number of structures which resembled autophagosomes (black arrow marks) (Fig. 2B). These results suggest that DHA could arrest the cell cycle in SKOV3/DDP cells, and induction of cell autophagy might be a response to DHA stimulation. However, no significant apoptosis was observed following mono-DHA treatment in SKOV3/DDP cells (Fig. 3B).

3.3. DHA sensitized SKOV3/DDP cells to cisplatin-induced apoptosis by inhibiting mTOR pathway

To determine whether DHA could enhance the cytotoxicity of cisplatin, SKOV3/DDP cells were co-treated with 20 μM of cisplatin and various doses of DHA for 24, 48 or 72 h. The results of CCK-8 assay suggested that DHA from 40 μM exhibited a significant synergistic activity in reducing cell growth rate by 24 or 48 h treatment (Fig. 3A). Next, the cells were stained with Annexin V-FITC and PI, then subjected to flow cytometry and observed by the laser scanning confocal microscopy. As shown in (Fig. 3B) compared with mono-treatment, cisplatin combined with DHA produced significantly more remarkable apoptosis. In addition, this phenomenon was also confirmed by laser scanning confocal microscopy, which demonstrated more early (green marks) and late (red marks) apoptotic cells in the combined treatment group (Fig. 3D). By inverted microscopy, significant morphological changes could also be observed in the combined treatment group (Fig. 3C). We next examined the expression of apoptosis relevant proteins in each treatment group. Western blot analysis suggested, compared with SKOV3 cells, SKOV3/DDP cells exhibited constitutive phospho-mTOR activity following mono-cisplatin treatment. However, DHA significantly abrogated the effect of cisplatin on mTOR activation, which correlated with the inhibition of cell cycle and augmentation of apoptosis by the combined treatment. As shown in Fig. 3E, DHA alone or its combination with cisplatin significantly decreased the expression of C-MYC and cyclin A. Moreover, combined group not only further enhanced the expression of BAX and the activity of cleaved caspase-3, but also decreased the expression of BCL-2. These results confirm that in SKOV3/DDP cells, DHA with cisplatin co-treatment *in vitro* abrogates cisplatin-induced mTOR activity, which is believed to be responsible for the enhanced apoptosis observed.

3.4. Blocking autophagic flux by BAF was independent of cisplatin-induced apoptosis

In order to determine whether the enhanced autophagy by DHA contributes to the anticancer effect of cisplatin, BAF was applied as an autophagic flux inhibitor. GFP-LC3 plasmids were used to detect the autophagosome fusion in autophagic flux. We found compared with control and non BAF treatment group, BAF plus group showed significantly more GFP-LC3 puncta (Fig. 2D). BAF inhibits fusion of

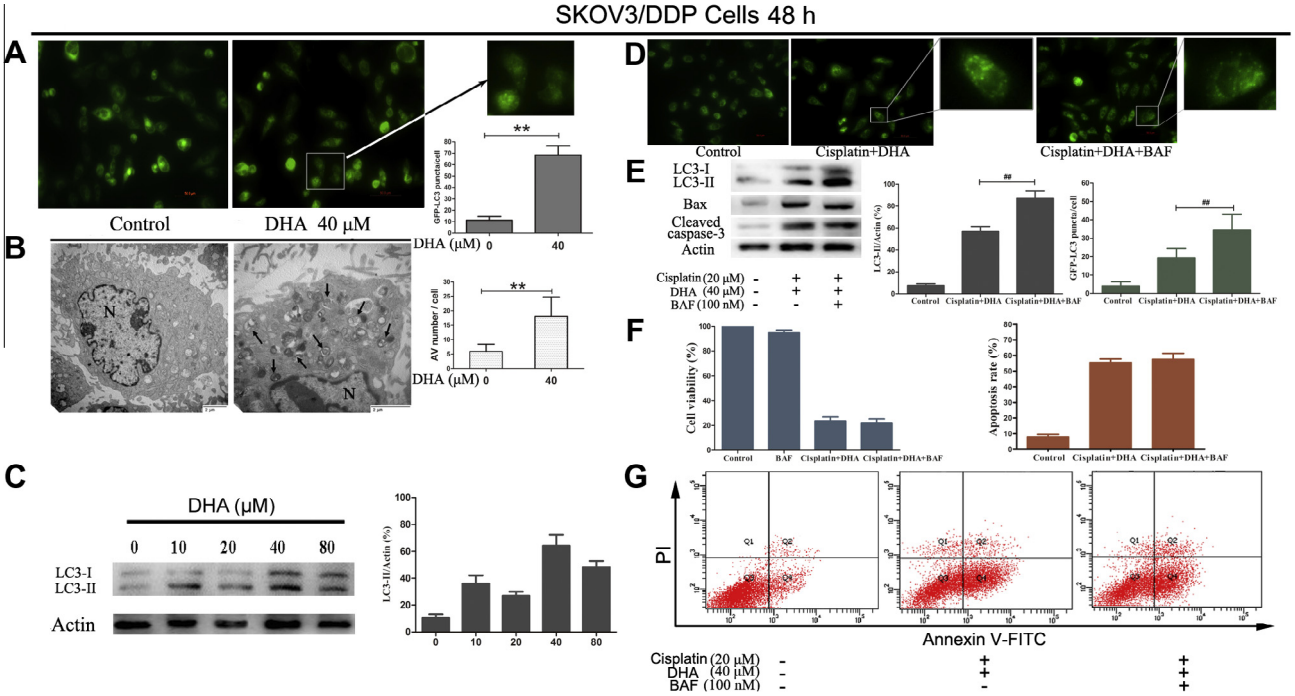

Fig. 2. Autophagy activity and effects of autophagic flux inhibition in SKOV3/DDP cells. (A and D) Autophagosome-specific GFP-labeled LC3 vacuoles were examined by fluorescence microscopy (magnification, ×400). Boxed areas were further enlarged image. Bar chart represents the percentage of cells with GFP-LC3. (B) Formation of autophagic vacuoles were observed under TEM (magnification, ×15,000). Autophagic vacuoles were indicated by arrowheads. Bar chart represents the quantitation of autophagic vacuoles (AV) in each cell. (C and E) Cells were treated with 40 μM of DHA and/or other regents. The expression of LC3-I, LC3-II and/or Bax, Cleaved-caspase3 proteins were detected by Western blot assay and the level of each protein was normalized to that of β-actin. (F) Cell viability in different group was determined by CCK-8 assays. (G) Flow cytometry was performed to measure the total percentage of early and late apoptotic cells. Each value was expressed as means ± SD. $^{**}p < 0.01$ versus control. $^{\#\#}p < 0.01$ versus non-BAF group.

lysosomes with autophagosomes and LC3-II protein is involved in the formation of autophagosomes [18]. Thus, LC3-II levels was further assessed by Western blot, and it resulted in significant increase by adding BAF. However, the level of Bax and caspase-3 have no significant changes (Fig. 2E). Finally, Cell viability and apoptosis rate were monitored. The results of CCK-8 and apoptosis assay indicated mono use or combined apply with other reagents, BAF treatment has no significant effect on cell viability and apoptosis (Fig. 2F and G). These findings suggest under the exist of apoptosis inducer, such as cisplatin, inhibition of autophagy is not involved in the SKOV3/DDP cells death process.

4. Discussion

Ovarian cancer is among the most aggressive malignancies in women, primarily due to asymptomatic presentation of the disease and late diagnosis [6]. Besides maximal cytoreductive surgery, cisplatin and its platinum derivatives have been accepted to be the first-line chemotherapeutic agents [20]. DHA, a derivative of artemisinin, can induce apoptosis in various types of cancer cells [3–5]. Current studies have implicated that the anticancer molecular mechanisms of DHA are varied. For instance, hypoxia inducible factor-1 alpha (HIF-1α) [21], extracellular signal-regulated protein kinases (ERK) [22], NF-κB and its targeted gene products [3], focal adhesion kinase (FAK) phosphorylation and matrix metalloproteinase-2 (MMP-2) [23] are all target genes of DHA in different cancer cells. However, the anticancer activity of DHA in cisplatin-resistant ovarian cancer cells is largely unclear.

In the present study, compared with normal SKOV3 cells, SKOV3/DDP cells were resistant to mono-cisplatin treatment (Fig. 1A), and mTOR phosphorylation was simultaneously activated in SKOV3/DDP cells (Fig. 1B). Induction of apoptosis is a primary mechanism by which cisplatin works [20]. In contrast, phosphorylation of mTOR enhances survival signals that inhibit apoptosis as well as promote cancer cell proliferation [11,12,24]. mTOR kinase lies downstream of the phosphatidylinositol 3′ kinase and type I insulin-like growth factor receptor [11]. This kinase was involved in the regulation of cyclins, C-MYC, some apoptosis factors and NF-κB [13,25,26]. Therefore, the abnormal activity of mTOR could be reasonable for the cisplatin-resistance of SKOV3/DDP cells, which prompt us to study whether DHA exerts its anticancer activity by inhibiting mTOR.

Although mono-DHA treatment could not induce significant cell death in SKOV3/DDP cells (Fig. 1D), cell cycle arrest was evident (Fig. 1C). By experiments, we found DHA could arrest the cell cycle in S phase. Cyclin A plays a critical role in regulating the transition from S phase to G2/M [27]. Then, as an oncogene, over expression of C-MYC results in coordinated changes in increased cancer cell proliferation. Hence as demonstrated by Western blotting, DHA arrest cell cycle could be the result of cyclin A and c-myc inhibition via downregulation of mTOR phosphorylation (Fig. 3E).

Resistance to apoptosis inducers, including cisplatin, is a major cause of treatment failure. By defining the cytotoxicity of combined treatment DHA with cisplatin, it is hoped that this research will contribute to improvement of this therapeutic area. In this study, we found that even at lower dosage, cisplatin, when combined with DHA, produced greater proliferation inhibition and apoptosis in SKOV3/DDP cells than when applied alone (Fig. 3). These results confirmed that DHA could reverse the drug resistance of SKOV3/DDP cells and increase the efficacy of chemotherapy in these cells. BCL-2, BAX and caspase-3 are involved in regulating cell apoptosis. BCL-2 functions as a survival signal that inhibits apoptosis, and this protein is frequently overexpressed in drug-resistant cancer cells [28]. In this study, high BCL-2 protein expression was observed in SKOV3/DDP cells following single cisplatin

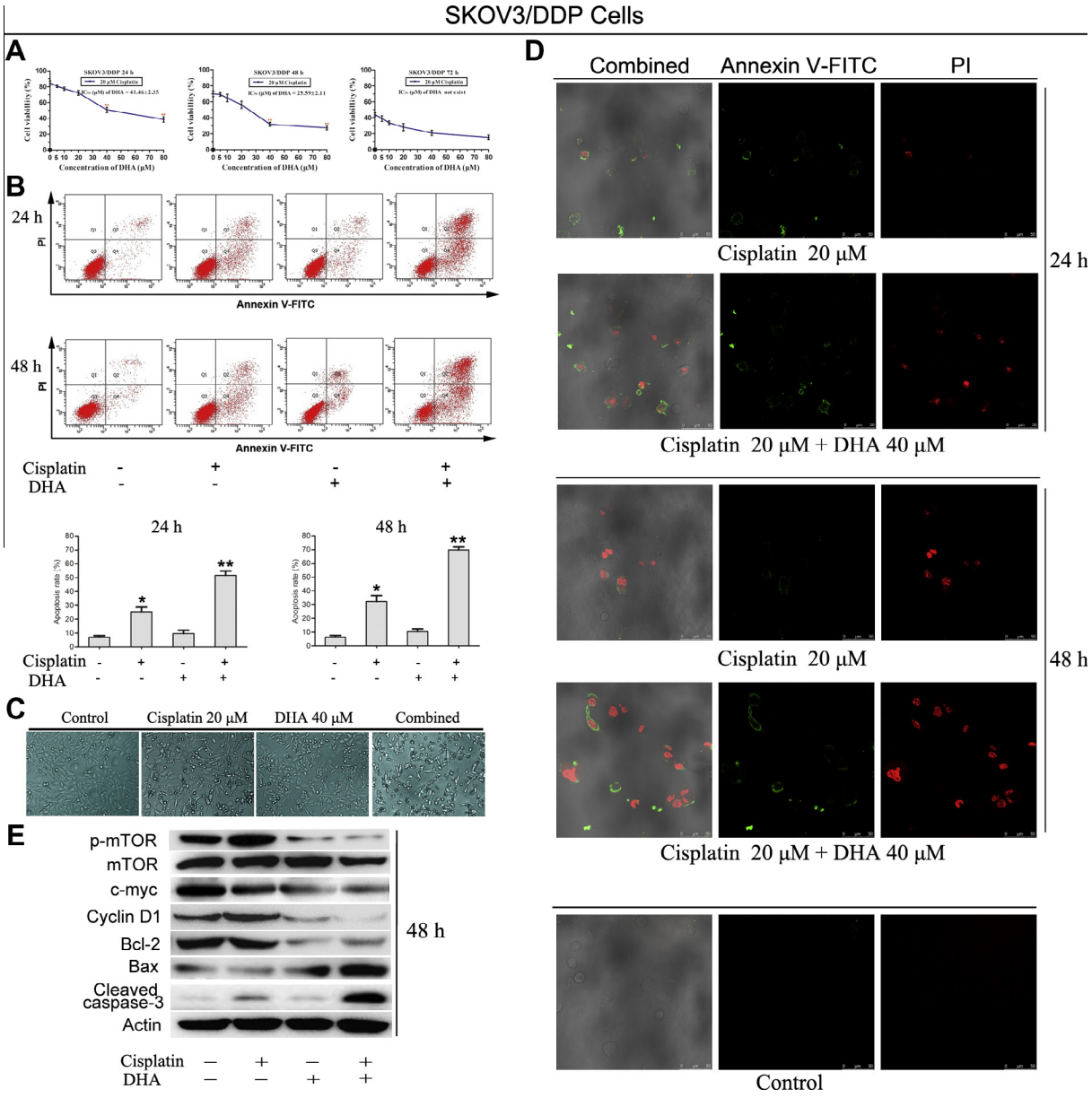

Fig. 3. DHA sensitized SKOV3/DDP cells to cisplatin-induced apoptosis. (A) The cells were co-treated with 20 μM of cisplatin and various doses of DHA for 24, 48 or 72 h. Cell viability was determined by CCK-8 assays. (B) The cells were treated with cisplatin (20 μM) and/or DHA (40 μM). Flow cytometry was performed to measure the total percentage of early and late apoptotic cells. (C) After treatment for 48 h, cell density was observed by phase-contrast microscopy (magnification, ×200). (D) Representative images of either treatment group for 24 or 48 h were taken by laser scanning confocal microscopy; apoptosis cells were labeled with green fluorescence (early-stage apoptosis) and red fluorescence (late-stage apoptosis) (magnification, ×630). (E) Western blot analysis of the expression of proteins under different conditions and the level of each protein was normalized to that of β-actin. Each value was expressed as means ± SD. $^{*}p < 0.05$ versus control; $^{**}p < 0.01$ versus control. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

treatment, but combined treatment significantly downregulated BCL-2 (Fig. 3E). In contrast, BAX and cleaved caspase-3 were the main proteins that induced pathologic apoptosis and are useful markers of the effectiveness of anticancer drugs [3]. Our results suggest combined treatment significantly upregulated the expression of cleaved caspase-3 and BAX compared to single agent treatment. Moreover, previous studies have also suggested that these molecules are also directly or indirectly regulated by mTOR [29].

Interestingly, in this study we also have found cell autophagy was activated by DHA treatment. As a process of consuming cellular components and generating energy, autophagy has become of a great interest in cancer research. In autophagy, LC3 is vital protein for autophagosome formation and activation [30]. On the one hand, some scholars believe that autophagy could help the cancer cells adapt to starvation and metabolic stress [31,32]. Others, however, suggest that unrestrained autophagy could potentially result in progressive consumption of cellular components and subsequently induce cancer cell death [8,33,34]. In this study, we investigated the autophagy levels by GFP-LC3 plasmid transfection assay, TEM and Western blotting. The results suggest DHA could increase the expression of LC3-II protein significantly (Fig. 2). It is suggested that mTOR acts as negative regulator of autophagy [35], thus we speculated that when mTOR was blocked by DHA, autophagy was subsequently activated. Further, we detected the autophagic flux in the absence and presence of autophage inhibitor BAF. The results indicate that although BAF could block the autophagic flux induced by DHA (Fig. 2D and E), it has no significant effect on cell apoptosis induced by cisplatin (Fig. 2F and G). As

for this phenomenon, some researchers suggested that, among several forms of programmed cell death, apoptotic cell death is the fastest, and autophagic cell death only becomes visible when apoptosis pathway is inhibited [36–38]. Therefore, we think that autophagy inhibition may not affect the SKOV3/DDP cells' survival in the presence of cisplatin. And it is still worth further investigating the interaction between apoptosis and autophagy as they can exhibit either synergistic or antagonistic effects depending on the cancer cell type and/or stimuli.

Disclosure of potential conflicts of interest

The authors declare that they have no conflict of interest.

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References

[1] D. Sriram, V.S. Rao, K.V. Chandrasekhara, P. Yogeeswari, Progress in the research of artemisinin and its analogues as antimalarials: an update, Pharm. World Sci. 16 (2004) 169–180.
[2] V. Dhingra, K. Vishweshwar Rao, M. Lakshmi Narasu, Current status of artemisinin and its derivatives as antimalarial drugs, Life Sci. 66 (2000) 279–300.
[3] S.J. Wang, Y. Gao, H. Chen, et al., Dihydroartemisinin inactivates NF-kB and potentiates the anti-tumor effect of gemcitabine on pancreatic cancer both in vitro and in vivo, Cancer Lett. 293 (2010) 99–108.
[4] Y. Ji, Y.C. Zhang, L.B. Pei, et al., Anti-tumor effects of dihydroartemisinin on human osteosarcoma, Mol. Cell Biochem. 351 (2011) 99–108.
[5] X. Gao, Z. Luo, T. Xiang, et al., Dihydroartemisinin induces endoplasmic reticulum stress-mediated apoptosis in HepG2 human hepatoma cells, Tumori 97 (2011) 771–780.
[6] R. Siegel, E. Ward, O. Brawley, A. Jemal, Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths, CA Cancer J. Clin. 61 (2011) 212–236.
[7] D.K. Armstrong, Relapsed ovarian cancer: challenges and management strategies for a chronic disease, Oncologist 7 (2002) 20–28.
[8] D. Gozuacik, A. Kimchi, Autophagy as a cell death and tumor suppressor mechanism, Oncogene 23 (2004) 2891–2906.
[9] D. Tormo, A. Checińska, D. Alonso-Curbelo, et al., Targeted activation of innate immunity for therapeutic induction of autophagy and apoptosis in melanoma cells, Cancer Cell. 16 (2009) 103–114.
[10] R. Kang, H.J. Zeh, M.T. Lotze, D. Tang, The Beclin 1 network regulates autophagy and apoptosis, Cell Death Differ. 18 (2011) 571–580.
[11] J. Cidado, B.H. Park, Targeting the PI3K/Akt/mTOR pathway for breast cancer therapy, J. Mammary Gland Biol. Neoplasia 17 (2012) 205–216.
[12] S.E. Al-Batran, M. Ducreux, A. Ohtsu, et al., MTOR as a therapeutic target in patients with gastric cancer, Int. J. Cancer 130 (2012) 491–496.
[13] H.S. Choi, E.H. Jeong, T.G. Lee, et al., Autophagy inhibition with monensin enhances cell cycle arrest and apoptosis induced by mTOR or epidermal growth factor receptor inhibitors in lung cancer cells, Tuberc. Respir. Dis. 75 (2013) 9–17.
[14] V.M. Adhami, D.N. Syed, N. Khan, H. Mukhtar, Dietary flavonoid fisetin: a novel dual inhibitor of PI3K/Akt and mTOR for prostate cancer management, Biochem. Pharmacol. 84 (2012) 1277–1281.
[15] H. Itamochi, J. Kigawa, Clinical trials and future potential of targeted therapy for ovarian cancer, Int. J. Clin. Oncol. 17 (2012) 430–440.
[16] S. Mabuchi, T. Hisamatsu, T. Kimura, Targeting mTOR signaling pathway in ovarian cancer, Curr. Med. Chem. 18 (2011) 2960–2968.
[17] X.B. Trinh, P.A. van Dam, L.Y. Dirix, et al., The rationale for mTOR inhibition in epithelial ovarian cancer, Expert Opin. Invest. Drugs 18 (2009) 1885–1891.
[18] A. Yamamoto, Y. Tagawa, T. Yoshimori, et al., Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells, Cell Struct. Funct. 23 (1998) 33–42.
[19] G. Chen, Z. Ke, M. Xu, et al., Autophagy is a protective response to ethanol neurotoxicity, Autophagy 8 (2012) 1577–1589.
[20] C. Marchetti, C. Pisano, G. Facchini, et al., First-line treatment of advanced ovarian cancer: current research and perspectives, Expert Rev. Anticancer Ther. 10 (2010) 47–60.
[21] X.J. Huang, Z.Q. Ma, W.P. Zhang, et al., Dihydroartemisinin exerts cytotoxic effects and inhibits hypoxia inducible factor-1alpha activation in C6 glioma cells, J. Pharm. Pharmacol. 59 (2007) 849–856.
[22] N. Gao, A. Budhraja, S. Cheng, et al., Interruption of the MEK/ERK signaling cascade promotes dihydroartemisinin-induced apoptosis in vitro and in vivo, Apoptosis 16 (2011) 511–523.
[23] B. Wu, K. Hu, S. Li, et al., Dihydroartiminisin inhibits the growth and metastasis of epithelial ovarian cancer, Oncol. Rep. 27 (2012) 101–108.
[24] M. Laplante, D.M. Sabatini, mTOR signaling in growth control and disease, Cell 149 (2012) 274–293.
[25] J.F. Gera, I.K. Mellinghoff, Y. Shi, et al., AKT activity determines sensitivity to mammalian target of rapamycin (mTOR) inhibitors by regulating cyclin D1 and c-myc expression, J. Biol. Chem. 279 (2004) 2737–2746.
[26] H.C. Dan, M.J. Cooper, P.C. Cogswell, et al., Akt-dependent regulation of NF-{kappa}B is controlled by mTOR and Raptor in association with IKK, Genes Dev. 22 (2008) 1490–1500.
[27] C.H. Yam, T.K. Fung, R.Y. Poon, Cyclin A in cell cycle control and cancer, Cell Mol. Life Sci. 59 (2002) 1317–1326.
[28] R.T. Marquez, L. Xu, Bcl-2:Beclin 1 complex: multiple, mechanisms regulating autophagy/apoptosis toggle switch, Am. J. Cancer Res. 2 (2012) 214–221.
[29] X. Li, J. Zhang, Z. Chen, et al., Both PI3K- and mTOR-signaling pathways take part in CVB3-induced apoptosis of Hela cells, DNA Cell Biol. 32 (2013) 359–370.
[30] N. Mizushima, T. Yoshimori, B. Levine, Methods in mammalian autophagy research, Cell 140 (2010) 313–326.
[31] W.L. Sun, J. Chen, Y.P. Wang, H. Zheng, Autophagy protects breast cancer cells from epirubicin-induced apoptosis and facilitates epirubicin-resistance development, Autophagy 7 (2011) 1035–1044.
[32] T.R. O'Donovan, G.C. O'Sullivan, S.L. McKenna, Induction of autophagy by drug-resistant esophageal cancer cells promotes their survival and recovery following treatment with chemotherapeutics, Autophagy 7 (2011) 509–524.
[33] S. Jin, R.S. DiPaola, R. Mathew, E. White, Metabolic catastrophe as a means to cancer cell death, J. Cell Sci. 120 (2007) 379–383.
[34] R. Mathew, C.M. Karp, B. Beaudoin, et al., Autophagy suppresses tumorigenesis through elimination of p62, Cell 37 (2009) 1062–1075.
[35] C.H. Jung, S.H. Ro, J. Cao, et al., MTOR regulation of autophagy, FEBS Lett. 584 (2010) 1287–1295.
[36] M. Los, M. Mozoluk, D. Ferrari, et al., Activation and caspase mediated inhibition of PARP: a molecular switch between fibroblast necrosis and apoptosis in death receptor signaling, Mol. Biol. Cell 13 (2002) 978–988.
[37] H. Kim, J. Choi, J. Ryu, et al., Activation of autophagy during glutamate-induced HT22 cell death, Biochem. Biophys. Res. Commun. 388 (2009) 339–344.
[38] W. Chaabane, S.D. User, M. El-Gazzah, et al., Autophagy, apoptosis, mitoptosis and necrosis: interdependence between those pathways and effects on cancer, Arch. Immunol. Ther. Exp. (Warsz) 61 (2013) 43–58.