Co-administration of perifosine with paclitaxel synergistically induces apoptosis in ovarian cancer cells: More than just AKT inhibition

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Abstract

Here we report an oral alkylphospholipid perifosine dramatically sensitizes chemo-resistant ovarian cancer cells to paclitaxel induced cell death and apoptosis in vitro. We found that co-administration perifosine with paclitaxel in human ovarian cancer cells led to the inhibition of AKT/mTOR complex 1 (mTORC1), a marked increase in ceramide and reactive oxygen species (ROS) production, and a striking increase in the activation of pro-apoptosis pathways, including caspase 3, c-Jun N-terminal kinases (JNK) and AMP-activated protein kinase (AMPK). These signaling events together caused a marked increase of cancer cell apoptosis. Combining paclitaxel with perifosine may represent a novel anti-ovarian cancer strategy.

Keywords: Perifosine, Paclitaxel, Signal transduction, Ceramide, Chemotherapy, Ovarian cancer

1. Introduction

Ovarian cancer is one of the leading causes of death in women and accounts for nearly 15,000 deaths per year in the United States alone [1]. Because patient diagnosis often is delayed due to clinically silent symptoms, the prognosis for ovarian cancer is generally very poor, and the mortality rate is high [1]. Patients with ovarian cancer generally receive chemotherapy treatment with paclitaxel, platinum-based agents, or a combination of both [2,3]. Paclitaxel binds tubulin, promoting tubulin polymerization and preventing microtubule disassembly, thereby inhibiting the completion of mitosis, forming a G2–M block, and eventually causing cell apoptosis [4,5]. Unfortunately, development of recurrent chemo-resistance is a common outcome of the disease treatment, even in patients who have an initially positive clinical response to chemotherapy [6]. As such, developing a novel strategy against paclitaxel resistance is necessary and urgent.

Perifosine, the first oral alkylphospholipid, has been shown to interact with the cell membrane and affect signal transduction pathways [7–10], which eventually leads to a profound cytotoxicity effect in a number of human tumor cells with few side effects in clinical phase I trials [11,12]. Recently, multiple groups have reported on perifosine’s capacity to synergize with radiation [13] or certain anticancer drugs, including histone deacetylase inhibitor (HDACI) and anti-epidermal growth factor receptor (EGFR) antibody [14,15]. Perifosine has been shown to disrupt recruitment of AKT to the plasma membrane and inhibit AKT phosphorylation and activation [7]. Recent studies indicated that...
perifosine also inhibits extracellular signal-regulated kinase (ERK) 1/2 at a relative high dose and activates c-Jun NH2-terminal kinase (JNK) and p21, the latter leads to cell cycle arrest in G1 and G2. However, the potential role of perifosine in paclitaxel’s effect on ovarian cancer cells is not well studied. Here we found that perifosine sensitizes ovarian cell to paclitaxel induced cell death and apoptosis by modulating multiple signaling events, by inhibiting AKT/mTORC1, facilitating ceramide and ROS production, and promoting JNK and AMPK activation.

2. Materials and methods

2.1. Chemicals and reagents

Perifosine was obtained from Selleck Chemical LLC, Houston, TX; Paclitaxel (taxol), anti-tubulin, goat anti-rabbit IgG-HRP and goat anti-mouse IgG-HRP antibodies were purchased from Santa Cruz Biotechnology (Shanghai, China). Monoclonal mouse anti-β-actin, fumonisin B1, mevinolin and methyl-β-cyclodextrin (mβCD) were obtained from Sigma (St. Louis, MO). p-JNK1/2 (Thr 183/Tyr 185), JNK1/2, p-AKT (Ser 473), p-AKT (Thr 308), p-AMPK (Thr 172), p-LKB1 (Ser 428), p-Acetyl-CoA Carboxylase(ACC) (Ser79), AKT1, cleaved-caspase 3 (rabbit mAb) and cleaved-PARP (mouse mAb) antibodies were purchased from Cell Signaling Technology (Shanghai, China). SP600125, Compound C, 5-Aminoimidazole-4-carboxamide ribotide (AICAR), z-DEVDfmk and z-VADfmk were purchased from EMD Bioscience (Shanghai, China).

2.2. Cell culture

Human ovarian cancer cell lines CaOV3, SKOV3 and A2780 as well as human colon cancer cell line HT-29 were maintained in DMEM medium (Sigma, St. Louis, MO), supplemented with a 10% fetal bovine serum (FBS) (Sigma, St. Louis, MO), penicillin/streptomycin (1:100, Sigma, St. Louis, MO) and 5-Aminoimidazole-4-carboxamide ribotide (AICAR), z-DEVDfmk and z-VADfmk were purchased from EMD Bioscience (Shanghai, China).

2.2.1. Cell culture

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2.3. Western blot

As reported before [16], 40 μg of protein from each treatment was separated by 10% SDS-PAGE and transferred onto a PVDF membrane. After blocking with 10% instant nonfat dry milk, membranes were incubated with specific antibodies overnight at 4 °C, followed by incubation with the secondary antibody. Antibody binding was detected with the enhanced ECL detection system. Notable western blots results were quantified using Image J software (downloaded from NIH website) after normalizing to corresponding loading controls.

2.4. Cell viability assay (MTT dye assay)

Cell viability was measured by the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide (MTT) method as reported previously [16].

2.5. Assessment of the percentage of apoptotic cells

As reported before [16], to detect apoptotic cells, cells were stained with DNA binding dye Hoechst 33342 (Sigma, St. Louis, MO). Cells with the indicated treatment were fixed with 4% formaldehyde in phosphate buffered saline (PBS) for 10 min at 4 °C and then washed with PBS. Cells were incubated for 20 min with 5 μg/ml of Hoechst 33342 (Sigma, St. Louis, MO) to stain the nuclei. After washing with PBS, the apoptotic cells were observed under a Confocal Fluorescence microscope (Leica TCS SMD FCS, Leica, Germany) (magnification 1:100). Cells exhibiting condensed chromatin and fragmented nuclei (Hoechst 33342 stain, Blue) were scored as apoptotic cells. For each Hoechst experiment, at least 200 cells in five random scope fields were counted for apoptotic rate.

2.6. Measurement of ceramide generation

Cells were labeled with 5 μCi [3H]palmitate (Mandel Scientific, Guelph, ON, Canada) overnight for the detection of sphingomyelin. The radioactive medium was removed, and the cells were washed with non-radioactive medium. For the determination of ceramide generation, the cells were left in [3H]palmitate-containing medium (18). After 3 h of starvation in 1% FBS containing media, cell were given indicated treatments. The lipids were then extracted with chloroform/methanol. Cells were scraped into 1 ml of ice-cold methanol. One milliliter of chloroform and 0.9 ml of 2 M KCl + 0.2 M H3PO4 were added to each aliquot, and the chloroform phases were dried under nitrogen. Ceramide was separated by thin layer chromatography (TLC) using Silica Gel 60-coated glass plates (Fisher). Fifty percent of the lengths of these TLC plates were developed in chloroform/methanol/acetic acid (9:1:1) and then dried. The plates were re-developed in petroleum ether/diethyl ether/acetic acid (60:40:1) and then dried and stained with iodide vapor. The identity of the ceramide was standardized against authentic ceramide standards. Radioactive ceramide was then quantified once scraped from the TLC plates by liquid scintillation counting.

2.7. Measurement of reactive oxygen species (ROS) production

ROS generation was determined by concomitant increase in dichlorofluorescein fluorescence (DCFH-DA), according to protocol (Gene Research Lab, Taibei, China). After indicated treatments, ovarian cancer cells were stained with 10 μM of DCFH-DA for 30 min at 37 °C. Fluorescently stained cells were transferred to polystyrene tubes with cell-strainer caps (Falcon, Shanghai, China) and subjected to fluorescence-activated cell sorting (FACS; Beckton Dickinson FACScan, Taibei, China) using Cell Quest 3.2 (Beckton Dickinson software for acquisition and analysis. In each analysis, 10,000 events were recorded. Induction of ROS generation was expressed in arbitrary units (vs. Control).

2.8. Caspase 3 activity assay

To evaluate caspase-3 activity, cell lysates were prepared according to the kit (Promega, WI) after their
respective treatments. Assays were performed in 96-well microtitre plates by incubating 20 l g cell lysates in 100 l reaction buffer (1% NP-40, 20 mM Tris–HCl pH 7.5, 137 mM NaCl, 10% glycerol) containing the caspase 3 sub-
strate (DEVD-pNA) at 5 l M. Lysates were incubated at 37°C for 2 h. Thereafter, the absorbance at 405 nm was measured with a spectrophotometer.

2.9. shRNA, cDNA plasmids transfection

shRNAs for AMPKα1 (sc-29673-SH) and mTOR (sc-35409-SH) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). A plasmid encoding a constitutively active AKT1 cDNA (Plasmid 16244) was purchased from addgene (Cambridge, MA). CaOV3 cells were seeded in a 6-well plate 2 days prior to transfection and cultured to 70% confluence the following day in 1% FBS medium. For transfection experiments, 3.6 l l PLUS™ Reagent (Invitrogen, Carlsbad, CA) was diluted in 90 l l of RNA dilution water (Santa Cruz, CA) for 5 min at room temperature. Then, 2 µg of either the indicated plasmid or scrambled plasmid were added to PLUS™ Reagent and left for 5 min at room temperature. 3.6 µl of Lipofectamine (Invitrogen, Carlsbad, CA) was then added to the complex. After a 30 min incuba-

Fig. 1. Cytotoxicity of human ovarian cancer cell lines in response to paclitaxel and perifosine. CaOV3 cells were treated for 48 h (a and c) with the indicated concentration of paclitaxel in the presence or absence of perifosine (5 µM) or (b) with indicated concentration of perifosine with or without paclitaxel (100 nM). Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay(a and b) or by direct live cell counts (c). SKOV3, A2780 cells (d) or HT-29 cells (e) were either left untreated or treated for 48 h with paclitaxel (100 nM), perifosine (5 µM) or both, cell viability were detected by MTT assay. CaOV3 cells were either left untreated or treated for 48 h with doxorubicin (150 nM), perifosine (5 µM) or both, cell viability were detected by MTT assay (f). The values in the figures are expressed as the means ± standard deviation (SD). *P < 0.05 vs. group without perifosine treatment. Experiments were repeated at least thrice to insure consistency of results.
tion period, the transfection complex was formed. Finally, the complex was added to wells containing 1 ml of medium. Successfully transfected cells were selected by puromycin for the shRNA plasmid. Western blots were performed to test transfection efficiency.

2.10. Statistical analysis

The values in the figures are expressed as the mean ± standard deviation (SD). The figures in this study were representative of more than three different experiments. Values of $P < 0.05$ were considered statistically significant (ANOVA) with a Newman–Keuls post test. All experiments were repeated at least three times and similar results were obtained.

3. Results

3.1. Cytotoxicity of human ovarian cancer cell lines in response to paclitaxel and perifosine

We first tested the potential effect of perifosine on paclitaxel induced ovarian cancer cell death by using a cell viability assay (MTT assay). The MTT assay is a standard colorimetric assay for measuring the activity of enzymes in mitochondria that reduce MTT. The assay therefore basically tests mitochondrial activity as an indicator of cell viability. The agents that lead to cell toxicity and mitochondrial dysfunction will cause a decreased purple color and OD reader in the assay. Cancer cell death therefore is indicated as reduced cell viability reflected by the OD number. As shown in Fig. 1a–e, in all three ovarian cancer cell lines including CaOV3, SKOV3 and A2780, as well as HT-29 colon cancer cells, while perifosine itself only slightly affected cancer cell viability, it dramatically enhanced paclitaxel induced ovarian cancer cell death (reduced cell viability) in a dose dependent manner. Further, as shown in Fig. 1f, perifosine also increased doxorubicin induced CaOV3 cell death. We then decided to set
the concentration of perifosine at the 5 µM for the following experiment. At this concentration, perifosine alone had almost no effect on cancer cell viability, while dramatically enhancing paclitaxel induced cell death (Fig. 1a and c).

3.2. Cell apoptosis of CaOV3 cells in response to paclitaxel and perifosine

Previous studies have indicated that paclitaxel-induced apoptosis is dependent on the caspase-3 driven mitochondrial apoptosis pathway [17]. Activation of caspase-3 by Src kinase inhibition enhanced paclitaxel induced ovarian cancer cell apoptosis [18]. We then tested the effect of perifosine on paclitaxel induced caspase-3 activity and cell apoptosis. As shown in Fig. 2a and c, the combination of paclitaxel and perifosine led to a marked increase of caspase-3 activity and up-regulation of cleaved caspase-3 and cleaved-PARP. Perifosine also dramatically sensitized CaOV3 cells to paclitaxel induced apoptosis as shown in Fig. 2b. A general caspase inhibitor z-VDADfmk and a caspase-3 specific inhibitor Z-DEVDfmk largely inhibited paclitaxel plus perifosine induced CaOV3 cell death (Fig. 2d). These data indicate that perifosine enhanced paclitaxel induced activation of caspase-3 and ovarian cancer cell apoptosis.

3.3. Effects of perifosine on paclitaxel induced AKT/mTORC1 activation

A number of different groups have demonstrated that paclitaxel activates AKT and mTORC1 signaling, the latter serves as resistant factor and protects cancer cells from death/apoptosis [19–21]. Perifosine is known to inhibit AKT activation [7,22], so we next tested the effect of perifosine on paclitaxel induced AKT and downstream mTORC1 activation. CaOV3 cells were starved in warm PBS (37 °C) for 30 min to remove background AKT/mTORC1 activation before paclitaxel treatment. As shown in Fig. 3a, we confirmed that paclitaxel induced activation of AKT (Ser 473 and Thr 308 phosphorylation) and downstream mTORC1 (S6K and S6 phosphorylation), as co-administration of perifosine completely blocked paclitaxel induced AKT/mTORC1 activation (Fig. 3b). Interestingly, introducing a constitutively active AKT (CA-AKT) to restore AKT activation (Fig. 3c) reduced perifosine plus paclitaxel induced CaOV3 cell death (Fig. 3d) and apoptosis (Fig. 3e). These results indicate that inhibition of AKT is involved in perifosine's effect on paclitaxel induced CaOV3 death. However, since CA-AKT did not reverse paclitaxel + perifosine induced cell death/apoptosis completely, we speculate other factors may also contribute to cell death/apoptosis.

3.4. mTOR and lipid rafts are involved in paclitaxel induced AKT activation

To further investigate paclitaxel induced AKT activation, we then focused on mTOR and lipid rafts. There are at least two mTOR complexes, mTORC1 and mTORC2. While mTORC1 serves as upstream kinase for phosphorylation of S6K and 4E-BP1, mTORC2, the complex that is composed of Sin1, rictor, mLST8 and mTOR, is an upstream kinase for AKT phosphorylation at serine 473 [23]. Knocking down mTOR by shRNA decreased paclitaxel induced AKT phosphorylation at Ser 473 but not at Thr 308 (Fig. 4a). Interestingly, CaOV3 cells were more sensitive to paclitaxel induced cell death when the mTOR expression level was reduced due to shRNA knockdown (Fig. 4b). We thus suggest that mTORC2 might be involved in full activation of AKT by paclitaxel, serving as a pro-survival factor. Interestingly, disrupting lipid raft with the cholesterol biosynthesis...
inhibitor mevinolin or cholesterol extractor methyl-β-cyclodextrin (mβcdx) inhibited AKT activation (Fig. 4c), increased cell death (Fig. 4d) and caspase-3 activity (Fig. 4e), indicating that the cholesterol present in membrane microdomains and intact lipid raft is a prominent mediator of AKT activation by paclitaxel in CaOV3 cells.

3.5. Ceramide production of CaOV3 cells in response to paclitaxel and perifosine

It has been well established that paclitaxel induces ceramide generation, which regulates cell apoptosis [24,25]. Interestingly enough, a recent study also indicated that perifosine enhances ceramide production. We then tested the effect of perifosine on paclitaxel induced ceramide production. As shown in Fig. 5a, perifosine largely enhanced paclitaxel induced ceramide accumulation in CaOV3 cells. Fumonisin B1, a known ceramide synthase inhibitor that inhibits de novo ceramide production [25,26], dramatically inhibited ceramide accumulation and CaOV3 cell death in response to perifosine plus paclitaxel treatment (Fig. 5d and e). Notably, adding the exogenous cell permeable short chain C6-ceramide dramatically enhanced paclitaxel induced CaOV3 cell death and apoptosis (Fig. 5b and c). Based on these results, we conclude that perifosine increases paclitaxel induced ceramide production, which serves as a key mediator for cancer cell apoptosis.

3.6. JNK activation in CaOV3 cells in response to paclitaxel and perifosine

Activation of JNK has been known to be an important contributor in paclitaxel induced cancer cell apoptosis [27,28]. We then tested effect of perifosine on paclitaxel induced JNK activation. As shown in Fig. 6a, perifosine increased paclitaxel induced JNK and downstream c-Jun phosphorylation in CaOV3 cells, and SP600125, the JNK inhibitor [29], reduced JNK activation in paclitaxel plus perifosine treated CaOV3 cells (Fig. 6c), while exogenous C6-ceramide enhanced JNK activation in response to paclitaxel (Fig. 6d). Based on these results, we conclude that perifosine increases ceramide production by paclitaxel, leading to enhanced JNK activation and increased cell death/apoptosis.

3.7. AMPK activation in CaOV3 cells in response to paclitaxel and perifosine

A recent study indicated that AMP-activated protein kinase (AMPK) activation mediates doxorubicin induced cell apoptosis in multiple cancer cell lines [30]. Increasing ceramide accumulation by directly adding C6 ceramide dramatically enhanced AMPK activation and cell apoptosis in a reactive oxygen species (ROS) dependent manner [30]. Since we here demonstrated that perifosine increased ceramide production in CaOV3
cells, we then tested the potential effect of paclitaxel and perifosine on AMPK activation. As shown in Fig. 7a, we found that paclitaxel activated AMPK, as indicated by AMPKα (Thr 172) and downstream Acetyl-CoA Carboxylase (ACC, Ser 79) phosphorylation in CaOV3 cells. Importantly, adding perifosine dramatically enhanced AMPK activation by paclitaxel (Fig. 7a). Knocking-down AMPKα with shRNA inhibited paclitaxel plus perifosine induced AMPK activation, cell death and cell apoptosis (Fig. 7d–f). Further, an AMPK inhibitor compound C decreased paclitaxel plus perifosine induced CaOV3 cell death, while AMPK activator 5-Amino-imidazole-4-carboxamide ribotide (AICAR) increased it (Fig. 7g). Interestingly, perifosine also facilitated paclitaxel induced ROS production (Fig. 7b), and the superoxide dismutase mimetic MnTBAP strongly reduced paclitaxel induced AMPK activation (Fig. 7c). We then conclude that perifosine enhanced paclitaxel induced ROS production and subsequent AMPK activation, which is also involved in cancer cell apoptosis.

4. Discussion

Here we found that co-administration of perifosine with paclitaxel in human ovarian cancer cells leads to AKT/mTORC1 inhibition, a marked increase in ceramide and ROS production, and a striking increase in activation of pro-apoptosis pathway JNK and AMPK. These signaling events together lead to a marked increase of cancer cell apoptosis (Fig. 7h). The enhancement of paclitaxel induced cytotoxicity and apoptosis by perifosine in ovarian cancer cells involves activation of caspase-3. Co-administration of perifosine and paclitaxel resulted in a large accumulation of cleaved caspase-3, cleavage of poly-ADP ribose polymerase (PARP), and increased caspase-3 activity.

Ceramide belongs to the group of sphingosine-based lipid second messenger molecules and is involved in the regulation of different cellular responses to various exogenous stimuli [31,32]. Ceramide action and regulation of its production have recently attracted broad attention due to the emerging understanding of the role of ceramide as an intracellular mediator of apoptosis [25,30,33–36]. Previous studies have demonstrated the very interesting relationship between ceramides and apoptosis in tumor cells, and this suggested that agents or chemotherapy treatments that can increase intracellular ceramide production or accumulation would provide favorable pro-apoptotic outcomes [37,38]. It has also been well established that paclitaxel induces ceramide generation, which also mediates cell apoptosis [24,25]. Both fumonisin B1, a ceramide synthase inhibitor, and L-cycloserine, a serine palmitoyltransferase inhibitor, inhibited paclitaxel-induced ceramide generation and cancer cell death [25]. Previous studies also indicated the potential of exogenous cell permeable ceramides (C6 ceramide) to enhance the pro-cancer cell death effect of paclitaxel [39,40]. As a matter of fact, it has been demonstrated that cancer cells were 20-fold more sensitive to paclitaxel when C6-ceramide was added as a medium supplement [25]. Here we demonstrated that perifosine dramatically enhanced paclitaxel induced ceramide production in CaOV3 cells, which may underscore one of the key mechanisms promoting cancer cell apoptosis.

The SAPK/JNK pathway, which is mainly activated by cellular stress, plays an important role in mediating the induction of apoptosis [28]. It has been well established...
that SAPK/JNK cascade is ceramide’s major downstream signaling event in regulating cell apoptosis [36,41]. Direct elevation of intracellular ceramide levels by treatment of cells with ceramide analogs or exogenous sphingomyelinase markedly activated the SAPK/JNK cascade in a manner similar to stress [36,41]. Furthermore, disruption of activation of the SAPK/JNK pathway abrogated TNF-, stress-, and ceramide-induced apoptosis [36,41,42]. Since we showed that perifosine facilitates ceramide production in response to paclitaxel in CaOV3 cells, it is not surprising to see an elevated JNK activation after co-administration. To confirm this, we found that fumonisin B1, which reduced ceramide production after perifosine plus paclitaxel co-administration, also dramatically inhibited JNK and c-Jun phosphorylation [36,41]. Although AMPK activation has been shown to mediate its pro-cell apoptosis effects primarily through modulation of multiple downstream signaling events by regulating JNK [53,54,57], p53 [48,58] and inhibiting mTORC1 [46,49,52,59]. For example, it has been reported that sustained AMPK activation leads to JNK activation which is required for the induction of apoptosis in liver cells [54]. Further, activation of AMPK is involved in vincristine-induced cell apoptosis in B16 melanoma cells through mTORC1 inhibition and p53 induction [60]. Additionally, it has also been shown that AMPK-dependent transcriptional induction and phosphorylation of p53 play a crucial role in the induction of apoptosis under carbon source depletion [48]. Recent studies also indicate that AMPK activation contributes to doxorubicin induced myocardial H9c2 cell apoptosis [61]. Interestingly, a recent study indicated that doxorubicin induced ceramide production activates AMPK in a ROS-dependent manner, and

![Fig. 6. JNK activation in CaOV3 cells in response to paclitaxel and perifosine. CaOV3 cells were treated with paclitaxel (100 nM) in the presence or absence of perifosine (5 μM) for 3 and 6 h, JNK and c-Jun phosphorylation were measured by Western blots. Effects of JNK inhibitor SP600125 (10 μM) on paclitaxel (100 nM) plus perifosine (5 μM) induced CaOV3 cell death were measured by MTT assay (b), effect of ceramide synthase inhibitor fumonisin B1 (25 μM) on perifosine (5 μM) plus paclitaxel (100 nM) induced JNK and c-Jun phosphorylation were measured in (c), while effect of C6 ceramide (2.5 μg/ml) on paclitaxel (100 nM) induced JNK/c-Jun phosphorylation were detected in (d). JNK and Jun phosphorylation in c and d was quantified by using Image J software after normalized to JNK1 and indicated as fold change (set ‘#’ marked lane as fold ‘1’). The values in the figures are expressed as the means ± standard deviation (SD). *P < 0.05. Experiments were repeated at least thrice to insure consistency of results.]

In addition to balancing cellular energy, the other important outcome of AMPK activation is regulation of cell apoptosis in response to various stimulations [43–56]. AMPK activation has been shown to inhibit pro-apoptotic effects primarily through modulation of multiple downstream signaling events by regulating JNK [53,54,57], p53 [48,58] and inhibiting mTORC1 [46,49,52,59]. For example, it has been reported that sustained AMPK activation leads to JNK activation which is required for the induction of apoptosis in liver cells [54]. Further, activation of AMPK is involved in vincristine-induced cell apoptosis in B16 melanoma cells through mTORC1 inhibition and p53 induction [60]. Additionally, it has also been shown that AMPK-dependent transcriptional induction and phosphorylation of p53 play a crucial role in the induction of apoptosis under carbon source depletion [48]. Recent studies also indicate that AMPK activation contributes to doxorubicin induced myocardial H9c2 cell apoptosis [61]. Interestingly, a recent study indicated that doxorubicin induced ceramide production activates AMPK in a ROS-dependent manner, and
direct addition C6 ceramide to doxorubicin dramatically increased AMPK activation and cell apoptosis [30]. Here, we also demonstrated that co-administration paclitaxel and perifosine strongly induces ROS production and activates AMPK in CaOV3 cells, which might be another key contributor to cancer cell apoptosis.

In conclusion, our data from the signal transduction studies and in vitro cell culture experiments strongly suggest that perifosine enhances the antitumor activity of paclitaxel in ovarian cancer cells by modulating multiple signal events beyond simple AKT inhibition.

Conflict of interest

None declare.

References


