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Original article

A newly synthesized platinum-based compound (PBC-II) increases chemosensitivity of HeLa ovarian cancer cells via inhibition of autophagy

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ABSTRACT

There are many mechanisms of resistance, chemoresistance of HeLa cells to anti-cancer agents seems to be autophagy-mediated. While using very effective anti-cancers such as Doxorubicin and cisplatin, cells overcome the cytotoxicity of these drugs through promotion of what so-called cytoprotective autophagy. Here in this study, we sought to introduce a novel platinum-based compound PBC-II that possesses anti-cancer activity. Our data showed that PBC-II is able to induce apoptosis at relatively low concentrations, with no detectable reactive oxygen species (ROS). However, further experiments demonstrated that exposure of HeLa cells to PBC-II did not promote autophagy; rather, it resulted in accumulation of p62 and decrease in LC3-II levels. Autophagy was then promoted in HeLa cells pharmacologically by Doxorubicin and genetically by siRNA IL-10. In order to confirm promotion of autophagy in our model, we performed acridine orange staining to assess for autophagy under microscope as well as via flow cytometry. We then measured protein level of autophagy markers p62 and LC3 by western blot. Our data indicated that PBC-II interferes with therapy-induced autophagy. We also determined PI3K activity while co-incubation of PBC-II with autophagy inducers. It was clear that PI3K activation decreased when PBC-II was co-administered with autophagy inducers. Collectively, PBC-II exerts unique anti-proliferative effects associated with inhibition of autophagy, which indicates that PBC-II is potentially a promising agent to be used in resistant ovarian tumors.

1. Introduction

Ovarian cancer is considered one of the major tumors that threaten women's lives worldwide (Ferlay et al., 2010; Sankaranarayanan and Ferlay, 2006). Although incidence of ovarian cancer is relatively low, its mortality rate is highest compared to other gynecological tumors (Razi et al., 2016; Beral et al., 2008). At early stages, ovarian cancer shows no clinical signs, leading patients to be presented in clinic when cancer is metastasizing and invading the surrounding organs at late stages. The five-year survival rate of ovarian cancer does not exceed 43% with over than 80% of patients will suffer from tumor relapse, making the disease prognosis very poor (Srivastava et al., 2017; Piccart et al., 2003). The current therapeutic approach for ovarian cancer is composed of surgical excision of tumor with six to eight courses of combination of paclitaxel and platinum-based compounds such as cisplatin (Piccart et al., 2003). Even though remission can reach 80%, relapse and resistance to ovarian cancer occur in 60% of women, causing the high mortality of disease (Luvero et al., 2014; Mantia-Smaldone et al., 2011; Tomao et al., 2017). Therefore, ovarian cancer is still a serious problem to be managed.

Here in this work, we sought to find a newly synthesized platinum-based drug that effectively promotes cell killing mechanisms in ovarian carcinoma cells to overcome resistance that limits the use of cisplatin. PBC-II is novel platinum (II) was recrystallized

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from dichloromethane/acetonitrile. This compound has shown a potential anticancer activity with fewer side effects (data under publication). While exposing HeLa cells to PBC-II, our results showed that PBC-II induces apoptosis at micromolar range. Surprisingly, our data showed that PBC-II inhibits autophagy with no evidence of generation of reactive oxygen species. We then used genetic and pharmacological approaches to promote autophagy in combination with PBC-II. Our data suggest that PBC-II potently inhibits drug-induced autophagy in HeLa ovarian carcinoma. In conclusion, PBC-II exerts a very potent effect on human ovarian carcinoma cells with novel characteristics in inhibition of autophagy, which comprises a mode of resistance in this particular type of tumors.

2. Materials and methods

2.1. Cell culture

HeLa ovarian carcinoma cells were obtained from ATCC and kept in 10% DMSO (Sigma Chemical, St. Louis, MO) with Fetal Bovine Serum (FBS) (GIBCO Life Technologies, Gaithersburg, MD) and stored frozen in liquid nitrogen until day of use. Cells were thawed off and cultured in a T75 flask (Cellstar) in RPMI 1640 medium with 5% fetal bovine serum, 5% bovine calf serum, 2 mM L-glutamine, and penicillin/streptomycin 0.5 mL/100 mL medium (10,000 units/mL penicillin and 10 mg/mL streptomycin (GIBCO Life Technologies, Gaithersburg, MD) and incubated at 37 °C, 5% CO2, in a moisturized environment. Once cells reached 80% confluency, cells were washed with 1X PBS (GIBCO) and harvested by 0.25% trypsin-EDTA (GIBCO) (incubation for 5 min). Trypsin then was deactivated by addition of 5 mL of serum-containing RPMI 1640, cells were collected and centrifuged at 1,500 rpm for 5 min. Media and trypsin were removed and 5 mL of new sterile medium was added to the pellet; cells were resuspended and 500 μL of suspension was placed into 96 mm^3 plate filled with 10 mL of RPMI 1640 medium. In every experiment, cells were cultured under identical conditions and incubated overnight to allow for adherence before treatment with continuous drug exposure. PBC-II agent was generously provided by our collaborator Dr. Isab, Department of Chemistry, College of Sciences, King Fahad University of Petroleum and Minerals.

2.2. IL-10 and SiRNA transfection

Transient transfection of siRNA was achieved using lipofectamine transfection 3000 reagent (Invitrogen, Carlsbad, CA), as described before (Alotaibi et al., 2018). In brief, ovarian carcinoma (HeLa) cells were plated in triplicates with cells density of 20,000 per well in 12-well plates for 16 h until cells reached approximately 70% confluence. One hour before transfection the cells were cultured in antibiotic-free medium. The cells were incubated with transfection mixtures containing 20 nM of GAPDH- or IL-10-siRNA (Sense: ACAUACUGCUAACCGACUCdTdT, Antisense: GAGUCGGUUAGCAGUAUGUdTdT) for 8 h, and then the medium was replaced with full culture medium and the cells incubated for 48 h. Control cells (un-transfected cells) were transfected with lipofectamine 3000 reagent only. The RNA was extracted via TRIzol (Invitrogen, Carlsbad, CA), reverse transcriptase polymerase chain reaction (RT-PCR) assay and the real time RT-PCR assay were done to confirm silencing as previously described (Alotaibi et al., 2018).

2.3. Annexin V-PI staining for apoptosis

In cells undergoing early apoptosis, phosphatidyl serine (PS), which is originally located in the inner cell membrane, transfers to the outer cell membrane. Translocated phosphatidyl serine (PS) can be bound with V attached to a fluorescent chromophore (FITC) to identify early apoptosis. Propium iodide (PI) penetrates non-intact cellular membranes and stains cells, serving as a marker of late apoptosis or necrosis. In this experiment, cells were plated overnight to permit cells to adhere. On the following day, cells were treated with the drug. At several time points, adherent and non-adherent cells were harvested by 0.25% trypsin and centrifuged at 2,000 rpm for 5 min. Cells were then resuspended in 1 mL of 1X PBS, transferred to 2 mL tubes, and centrifuged at 2,000 rpm for 5 min. PBS was removed and 100 μL of 1X binding buffer (BD Biosciences) was added to pellets per tube. 5 μL of Annexin V-FITC (BD Biosciences) and 5 μL of PI at 10 μg/mL (BD Biosciences) were then added per tube. In the dark, cells were softly vortexed and incubated for 15 min at room temperature. Before anaysis by flow cytometry, 400 μL of 1X binding buffer was added per tube and samples were analyzed by flow cytometry technique as soon as possible to measure the fluorescence at 530 nM.

2.4. Evaluation of autophagy by acridine orange staining

200,000 cells were seeded in 6-well plates and permitted to adhere overnight and drug-treated the next day. At various time points, drug was removed and cells washed once with 1X PBS. Acridine orange dye was diluted in PBS in a ratio of 1:10000 (prepared in the dark) and added to cells for staining, and incubated for 15 min. Dye then was aspired and plates were washed with 1X PBS and fresh medium was added. Photographs were taken with an Olympus 1X 70 microscope and an Olympus SC 35 camera.

Flow analysis was utilized to count the cell population positively stained with acridine orange. Treated cells were harvested, collected, and centrifuged at speed of 1500 rpm. Supernatant was removed and pellets were resuspended in 1 mL of fresh medium. Cell suspension was filtered through standard flow cytometry 40 μm filter (BD falcon). A 10:1000 dilution of acridine orange in 1X PBS was prepared (in the dark) and protected from light until ready for use. At flow cytometry, 10 μL of acridine orange solution was added to per sample to make the dilution of 1:10000. When analyzed by flow cytometry using the FC500 Flow cytometer with CXP software (Beckman Coulter, USA), the acridine orange dye is excited at wavelength 525 nM for green fluorescence and 620 nM for red fluorescence.

2.5. Senescence by β-Galactosidase staining

Detection of senescence was by β-Galactosidase staining after exposure to the drug treatment. Cells were washed once with 1X PBS and fixed with 2% formaldehyde/ 0.2% glutaraldehyde for 5 min. Fixing solution was aspired and PBS was used again to wash and a staining solution composed of 1 mg/mL 5-bromo-4-chloro-3-inolyl-β-galactosidase in dimethylformamide (20 mg/mL stock), 5 mM potassium ferricyanide, 150 mM NaCl, 40 mM citric acid/-sodium phosphate, 2 mM MgCl2, at pH 6.0 was added and the cells were incubated overnight at 37 °C. The following day, cells were washed twice with PBS and pictures were taken.

2.6. Detection of reactive oxygen species

Cells were plated and treated with indicated concentrations for 24 h. On experiment day, cells were collected and centrifuged at 1500 rpm and mixed with 1X assay buffer (Muse Oxidative Stress Kit MCH100111, millipore). Then, oxidative stress reagent (Muse Oxidative Stress Kit MCH100111, millipore) was diluted in 1X buffer (1:100) to make intermediate solution. After that, intermediate solution was diluted again in 1X buffer (1:80) to make working

Muse Oxidative Stress working solution. Cells were then mixed with working solution and incubated for 30 min at 37 °C. Samples were read using Muse Cell Analyzer (0500–3115), millipore.

2.7. Determination of PI3K activation

Cells were plated and treated with indicated concentrations for 24 h. On experiment day, cells were collected and centrifuged at 1500 rpm, and washed with 1X PBS. Cells were then fixed with fixation buffer (Muse PI3K Activation Dual Detection Kit, MCH200103) followed by washing step. Cells were then permeabilized by permeabilization buffer for 5 min on ice. Mixture of antibody cocktail with 1X assay buffer was made and incubated at room temperature for 3 min in dark. Cells were then resuspended in the mixture and run through Muse Cell Analyzer (0500-3115), millipore.

2.8. Western blotting

In order to determine whether some proteins were induced, degraded, or downregulated, we conducted a specific assay to analyze protein levels. After the indicated time points, we collect viable and non-viable cells in a pellet. These collected cells were mixed with 100 to 200 μL of 1X Tris lysis buffer (1 M Tris (pH 6.8), 10% SDS, and dH2O) containing protease inhibitors and boiled for 5 min. Protein concentration was measured using the Bradford protein assay (BioRad) and 40 μg of total cell lysate was separated in a 12% gel using SDS-PAGE. Proteins were transferred onto nitrocellulose membrane for 1.5 h and washed 3 times with PBS containing 0.01% Tween for 5 min in each time. Membrane was blocked for one hour with TBS-Tween 20 buffer containing 5% nonfat dry milk. The proteins were then blocked with the primary antibody and left overnight in 4 °C. In the following day, the primary antibody was washed out and the immunoflourescent secondary antibody was added for an hour at room temperature. Membrane was washed three times and bands were detected using enhanced chemiluminescence detection reagents from Pierce (Rockford, IL).

2.9. Statistical analysis

Statistics were performed with GraphPad Prism (GraphPad Software, Inc., La Jolla, United States) using ANOVA followed by post-hoc analysis. The significance of group values was determined based on a p-value of $p < 0.05$.

3. Results

3.1. PBC-II induces apoptosis in HeLa ovarian carcinoma cells

In order to investigate whether PBC-II promotes cell death in our model of interest, we exposed HeLa cells to different concentrations of the indicated drug to 24 h. First, we screened for apoptosis as one of the most desired effects in treatment of cancer in an intent to kill tumor cells. Our data shows that PBC-II significantly enhances apoptosis in concentrations as low as 1 uM. Percentage of apoptotic cells increased in a dose-dependent manner (Fig. 1A). To understand whether apoptosis was accompanied with elevated levels of reactive oxygen species, we tested this certain question based on dihydroethidium (DHE) reagent. The results showed that PBC-II does not generate reactive oxygen species (ROS), indicating that apoptosis was induced independently of oxidative stress (Fig. 1B).

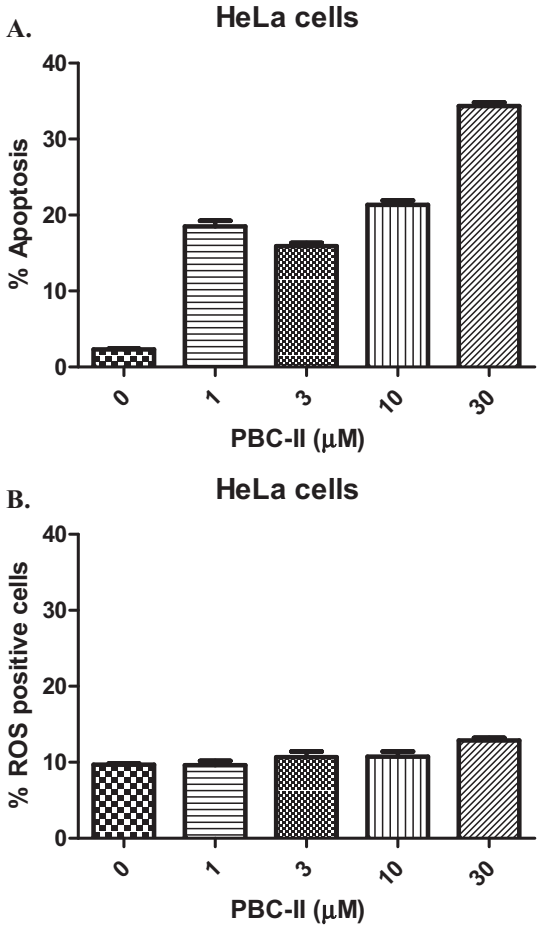

Fig. 1. PBC-II induces apoptosis with minimal generation of oxidative stress in HeLa ovarian carcinoma cells. A. HeLa cells were treated with 0, 1, 3, 10, 30 μM of PBC-II for 24 h. HeLa cells were then stained with Annexin V to assess for apoptosis. B. HeLa cells were treated with 0, 1, 3, 10, 30 μM of PBC-II for 24 h. HeLa cells were then stained with DHE as a marker of oxidative stress. In both assays, we calculated the positively stained cells. This experiment was done in triplicate (*$p < 0.05$ compared to control).

3.2. Lack of autophagy and senescence promotion by PBC-II

Autophagy (Macro-autophagy) is a self-induced degradative process of eukaryotic cells that leads to breakdown of unnecessary intracellular material within lysosomes or macro-autophagosomes (Galluzzi et al., 2017). Several studies reported that autophagy is triggered and upregulated in cancer cells during exposure to anticancer drugs (Gewirtz, 2014). Here in this study, we investigated whether autophagy is promoted in HeLa cells in response to PBC-II. During induction of autophagy, autophagosomes fuse with lysosomes to form acidic vacuoles, which are stainable by acridine orange dye (François et al., 1976). While exposure of HeLa cells to PBC-II, acridine orange staining did not present evidence of accumulation of acidic vacuoles, even at high concentrations of PBC-II (Fig. 2A). In addition, we also monitored other markers of autophagy such as LC3-II protein and p62 protein. LC3-I is a cytoplasmic protein that converts to LC3-II when lipidated and binds to autophagosomal membranes during autophagy (Scherz-Shouval et al., 2007). While treating HeLa cells with PBC-II, we found that LC3-II protein level decreases as drug concentration increases. Furthermore, autophagic flux was measured via p62 protein degradation. Consistent with LC3-I, p62 protein clearly increases in response to PBC-II at dose-dependent manner, suggesting that autophagy was not promoted in response to PBC-II (Fig. 2B). More-

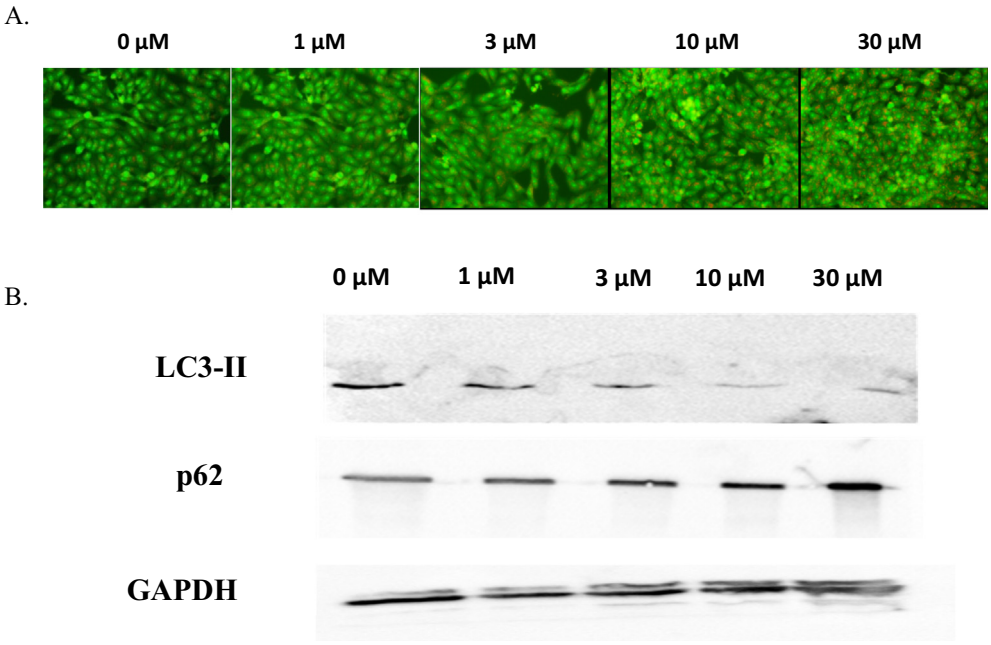

Fig. 2. Lack of autophagy induction by PBC-II in HeLa ovarian carcinoma cells. A. Cells were plated and treated with 0, 1, 3, 10, 30 μM of PBC-II for 24 h. At indicated point, acridine orange (1:10000) diluted in medium was added to cells. Images were taken under a fluorescent microscope at a magnification power of 20X. B. Conversion of LC-3I to LC3-II and completion of autophagy process was confirmed via measurement the LC3-I expression and the degradation of p62 by western blotting. HeLa ovarian cancer cells were treated with 0, 1, 3, 10, 30 μM of PBC-II for 24 h and levels of LC3-I and p62 protein was measured. There was no clear conversion of LC3-I neither degradation of p62 in response to indicated concentrations of PBC-II.

over, it has been already shown that induction of autophagy is closely accompanied by promotion of senescence in cancer cells (Alotaibi et al., 2016). We have stained HeLa cells 24 h post-treatment with β-galactosidase staining, a remarkable marker of senescence. Consistent with our observation to autophagy markers, we failed to determine positively stained cells upon exposure to PBC-II (data not shown). Taken together, autophagy and senescence seem to be not responsible for the cytotoxicity of PBC-II, on the contrary, these astonishing data have added up another aspect of PBC-II action as a novel inhibitor of autophagy in addition to being a potent apoptosis inducer.

3.3. PBC-II inhibits genetically- and pharmacologically-induced autophagy in HeLa ovarian carcinoma cells

To determine how potent PBC-II inhibits autophagy, we sought to induce autophagy using pharmacological and genetic approaches. In our work, we utilized siRNA IL-10 and doxorubicin as autophagy inducers in HeLa ovarian carcinoma cells. IL-10 is an immunomodulatory cytokine that maintains immune homeostasis (Iyer and Cheng, 2012). Although the relationship between IL and 10 and autophagy seems to be controversial in literature (Wu et al., 2016), there is quite significant evidence that IL-10 may inhibit autophagy (Park et al., 2011; Wang et al., 2014; Santarelli et al., 2014). Therefore, silencing IL-10 might become an acceptable approach to induce autophagy in HeLa ovarian carcinoma cells. Also, the topoisomerase II inhibitor, doxorubicin, is a well-known drug that highly promote autophagy in many cancer cell lines including HeLa cells (Goehe et al., 2012; Di et al., 2009; Fang et al., 2017). Therefore, we applied both approaches as strategies in order to ensure whether promotion of autophagy in HeLa cells by different means is inhibitable by PBC-II. We exposed HeLa cells to 1 μM doxorubicin and siRNA for 24 h and stained them by acridine orange. Upon treatment, a significant fraction of the treated cell populations apparently demonstrated stained vacuoles per cell in treated groups compared to control (Fig. 3A). In addition, cells exposed to either siRNA IL-10 or doxorubicin became enlarged in comparison with control cells, demonstrating a morphological sign of autophagy. An additional technique was performed using flow cytometry to evaluate autophagy in cells based on the intensity of acridine orange staining. In this experiment, we found that cells treated with either siRNA IL-10 or doxorubicin showed a significant increase in the percentage of the cell population falling into the area of red-fluorescing cells, indicating these cells undergo autophagy when treated either with siRNA IL-10 or doxorubicin (Fig. 3B). Then, we investigated whether PBC-II is able to interfere with therapy-induced autophagy in HeLa ovarian carcinoma cells. We treated cells with autophagy inducers along with 30 μM PBC-II and assessed autophagy via LC3-I and p62 proteins. Our western blot data demonstrated that both autophagic flux and LC3-I conversion were drastically inhibited by PBC-II, indicating that inhibition of autophagy occurs at early steps. Therefore, we measured the activity of PI3 kinase as an early player of process of autophagy. While exposure of HeLa ovarian carcinoma cells to autophagy inducers, activated PI3 kinase levels became significantly elevated. Interestingly, co-treatment with PBC-II restored the activated PI3 kinase levels as low as control level (Fig. 3C). Collectively, PBC-II obviously targets therapy-induced autophagy at early steps.

4. Discussion

Progression of autophagy after exposure to chemotherapy in cancer treatment is a debatable topic. Many reports have indicated that autophagy play a significant role in proliferation, survival, and resistance (Liu et al., 2016; Peng et al., 2016; Lee et al., 2015). On the other hand, different studies in several laboratories demonstrated that autophagy can be also defined as type II-programmed cell death (Sharma et al., 2014; Shimizu et al., 2014; Biggers et al., 2013). Presumably, autophagy function differs between treatment models due to variations in cell types, used drugs, and duration of therapy. Since cisplatin is the drug of choice in ovarian cancers, cisplatin-induced autophagy seems to be

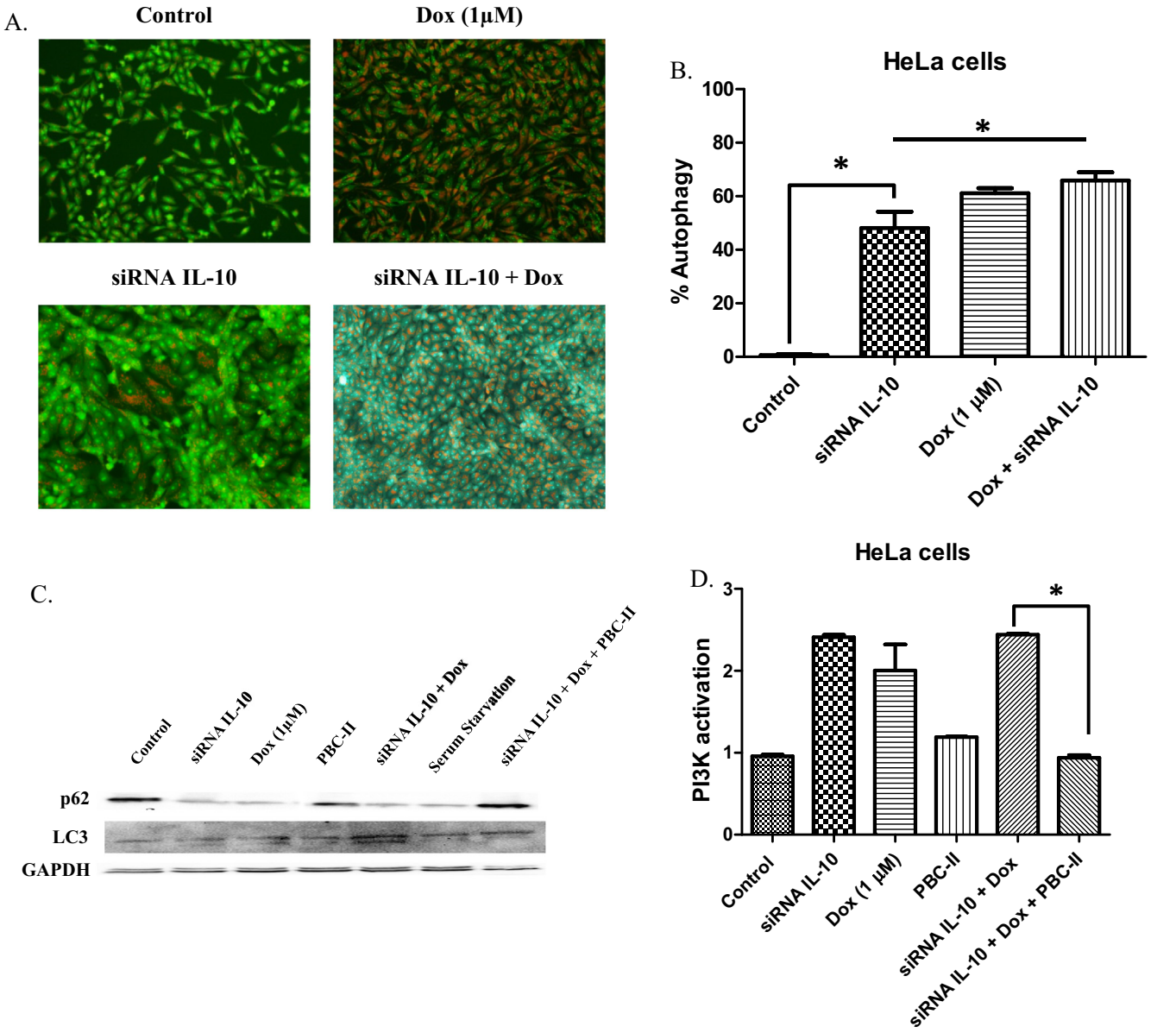

Fig. 3. PBC-II inhibits therapy-induced autophagy in HeLa ovarian carcinoma cells. A. HeLa cells were treated with siRNA IL-10 and 1 μM doxorubicin for 1 day. Cells were then harvested and centrifuged at 1500 rpm. Acridine orange was diluted in PBS (1:10000) and was then added to the cells for staining. The extent of autophagy was counted based on the number of cell population in quadrants Q2 and Q4 from our raw data. This experiment was performed three times (*p < 0.05 compared to control). B. Cells were plated and treated with siRNA IL-10 and 1 μM doxorubicin. A day post-treatment, acridine orange (1:10000) diluted in medium was added to cells. During autophagy, acidic vacuoles are stained with acridine orange dye. Images were taken under a fluorescent microscope at amagnification power of 20X. Three different experiments were performed to confirm the result. C. Conversion of LC-3I to LC3-II and completion of autophagy process in combination was assessed via measurement the LC3-I expression and the degradation of p62 by western blotting. HeLa ovarian cancer cells were treated with 1 μM doxorubicin, siRNA IL-10, 10 μM PBC-II, and combination for 24 h, and levels of LC3-I and p62 protein was measured. A clear inhibition of therapy-induced autophagy via PBC-II was observed in HeLa cells. D. Activation of PI3K was evaluated by flow cytometry. HeLa ovarian cancer cells were treated with 1 μM doxorubicin, siRNA IL-10, 10 μM PBC-II, and combination for 24 h, and levels of LC3-I and active PI3K level was measured. Data demonstrate that activation of PI3K during autophagy was clearly inhibited by PBC-II in HeLa cells (*p < 0.05 compared to control).

cytoprotective in ovarian carcinoma according to many reports (Ma et al., 2019; Long et al., 2018; Wang and Wu, 2014). Hence, utilizing drugs that induce autophagy such as cisplatin in ovarian carcinoma is not a wise decision. Therefore, we need to introduce innovative autophagy inhibitors that possess anticancer properties. We examined PBC-II effect on HeLa ovarian carcinoma. Expectedly, PBC-II alone induced significant apoptotic populations at micromolar range, with no clear markers of autophagy and senescence. Thus, it may be beneficial to implement this drug in treatment of ovarian carcinoma, which is addicted to autophagy as a chemoresistance mode.

Resistance of ovarian cancer to cisplatin, the first-line treatment of ovarian cancer, is an obstacle and probably results in treatment failure (Damia and Broggini, 2019). Several reports have indicated that inhibition of autophagy resulted in increased sensitivity of ovarian cancer cells to cisplatin as well as other chemotherapeutic agents (Liu et al., 2018; Qiu et al., 2017; He et al., 2015; Liang et al., 2016). In addition, studies have revealed that NF-E2-related factor 2 (Nrf2) enhanced cisplatin resistance via activating autophagy in ovarian carcinoma (Bao et al., 2014). Furthermore, another study showed that the oncoprotein, YAP, enhanced resistance of ovarian carcinoma through increasing the levels of autophagy (Xiao et al.,

2016). Therefore, the resistance to cisplatin is thought to be attributed to promotion of autophagy. The underlying mechanism by which autophagy suppresses cisplatin effect is still undetermined, but a report by Wang et al suggested that promotion of autophagy might be due to activation of ERK pathway (Wang and Wu, 2014). Similarly, resistance to paclitaxel was also found to be associated with increased levels of autophagy in human ovarian carcinoma, whereas inhibition of autophagy via downregulation of Beclin-1 resulted in cytotoxicity (Zhang et al., 2015). Taken together, autophagy seems to play a cytoprotective role against cisplatin and paclitaxel, which are the most commonly used drugs in human ovarian carcinoma.

Induction of autophagy was monitored by microtubule associated protein light chain LC3 expression in HeLa cells. LC3-II, a marker of autophagy, is produced by proteolytic cleavage of LC3-I and required for the formation of autophagosomes. We found that PBC-II increased the expression of LC3-I with accumulation of p62 i.e. inhibition of autophagic flux, indicating that autophagy has not been induced. These findings support the idea that autophagy in HeLa cells is not initiated upon exposure to PBC-II, especially that no significant reactive oxygen species were generated when cells exposed to PBC-II alone. We then confirmed autophagy via different approaches using genetic silencing of IL-10 and doxorubicin. Consistent with data previously shown in literature, we have found that HeLa cells incubated with doxorubicin as well as siRNA IL-10 demonstrated an increase in accumulation of acidic vacuoles as shown with acridine orange staining with no evidence of apoptosis. These results strongly suggest that both doxorubicin and siRNA IL-10 promote autophagy without induction of cell death processes.

Then, we tested the effect of combination of PBC-II with autophagy inducers on PI3 kinase activity on ovarian cancer cells. In addition to the role of PI3 kinase in autophagy regulation, PI3 kinase plays a pivotal role in proliferation, growth, and survival of several cancer models including ovarian carcinoma (Lee et al., 2005). Published studies have shown that interference with Akt, a downstream target of PI3 kinase, along with autophagy inhibition results in reduced cell viability of ascites-derived ovarian cancer (Correa et al., 2014; Noske et al., 2007). Furthermore, targeting PI3 kinase with siRNA in OVCAR-3 cells led to reduction in proliferation, invasion, and migration (Meng et al., 2006; Huang et al., 2011). Thus, it was worthy to investigate whether PBC-II affects PI3 kinase activity. Our data clearly indicated that PI3 kinase activity was extremely decreased by combination of PBC-II along with doxorubicin and IL-10. In addition, this reduction in PI3 kinase activity was accompanied with inhibition of autophagy based on classic markers of autophagy such as LC3 and p62.

It appears that action of PBC-II differs from cisplatin in modulation of PI3K activity. Although cisplatin, a well-known platinum-based drug, enhances PI3K activity in several cancer models (Wang et al., 2004; Cheng et al., 2002; Corti et al., 2019; Houédé and Pourquier, 2015), it was an interesting observation that PI3K activation levels were decreased when PBC-II was added to the treatment. However, the ability of PBC-II to reduce PI3K activation might also explain why apoptosis was significantly induced at relatively low concentrations. Therefore, it would be worth studying whether PBC-II could be used in addition to the promising PI3K inhibitors as synergistic regimen.

In summary, PBC-II was shown to induce apoptosis in HeLa ovarian carcinoma cells, as well as powerful effect in inhibition of autophagy. We have also confirmed that this compound may work as a chemo-sensitizing agent when used along with other treatment modalities by interference with autophagy. Interestingly, we found that this molecule can contribute to antitumor actions by reducing the activity of PI3 kinase that is necessary for chemoresistance, autophagy progression, and tumor proliferation. These findings build upon our previous work that supports the potential utility of developing PBC-II as a promising agent for cancer therapy.

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