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# Autophagy inhibition promotes paclitaxel-induced apoptosis in cancer cells

Guangmin Xi [a], Xiaoyan Hu [a], Baolin Wu [b], Hanming Jiang [a], Charles Y.F. Young [c] Yingxin Pang [a], Huiqing Yuan [a,*]

[a] Department of Biochemistry and Molecular Biology, Shandong University School of Medicine, Jinan 250012, China
[b] Department of Biochemistry and Chemistry, The Ohio State University, Columbus, OH 43210, USA
[c] Department of Urology, Mayo Clinical College of Medicine, Mayo Clinic, Rochester, MN 55905, USA

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ABSTRACT

Paclitaxel has been demonstrated to be an effective mitotic inhibitor and apoptosis inducer to treat aggressive malignancies. In this paper, we have provided a line of evidence that promotion of apoptotic cell death by paclitaxel was accompanied with induction of autophagy in A549 cells. Paclitaxel treatment could lead to the formation of acidic vesicular organelles (AVOs), the induction of Atg5, Beclin 1 and microtubule-associated protein 1 light chain 3 (LC3) expressions, and the increase of punctate fluorescent signals in A549 cells pre-transfected with green fluorescent protein (GFP)-tagged LC3. Interestingly, paclitaxel-mediated apoptotic cell death was further potentiated by pretreatment with autophagy inhibitor 3-methyladenine (3-MA) or small interfering RNA against the autophagic gene *beclin 1*. These findings suggest that paclitaxel-elicited autophagic response plays a protective role that impedes the eventual cell death, and inhibition of autophagy could be an adjunctive strategy for enhancing chemotherapeutic effect of paclitaxel as an antitumor agent.

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## 1. Introduction

Chemotherapy is an important option in curing or controlling various cancers including lung cancer. Paclitaxel, which stabilizes microtubule and causes apoptosis, offers both symptomatic and survival benefits for lung adenocarcinoma. The paclitaxel-based combination therapies are standard treatments for nearly all patients diagnosed with non-small cell lung carcinoma (NSCLC) [1]. However, clinical treatment with paclitaxel often encounters a number of undesirable side effects as occurred using other anticancer agents. The dose increment of systemic administration of paclitaxel would generate unacceptable levels of toxicity to normal cells, especially of bone marrow, gastrointestinal tract, and the hair follicles [2]. Therefore, many attempts have been made to enhance its therapeutic effectiveness, simultaneously reducing its toxicity. In an effort to search for strategies that could enhance cancer cell killing mediated by paclitaxel, we have investigated possible pro-survival pathways that are activated in response to paclitaxel. Herein, we report the induction of autophagy by paclitaxel.

Autophagy is an evolutionary conserved process in which cell engulfs cytoplasmic constituents within a double-membrane vacuole (named autophagosome) and delivers them to the lysosome for degradation [3].

*Abbreviations*: AVOs, acidic vesicular organelles; LC3, microtubule-associated protein 1 light chain 3; 3-MA, 3-methyladenine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide.

* Corresponding author. Address: Department of Biochemistry and Molecular Biology, Shandong University School of Medicine, 44 Wenhua Xi Road, Jinan 250012, China. Tel.: +86 531 88382092; fax: +86 531 88382019.

*E-mail address:* lyuanhq@sdu.edu.cn (H. Yuan).

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Autophagy contributes to maintaining cellular homeostasis as a result of quality control of both proteins and organelles. In addition to its basic role in the turnover of proteins and organelles, autophagy has multiple physiological and pathophysiological functions [4,5]. When cells encounter environmental stressors such as nutrient starvation and pathogen infection, autophagy is induced to provide nutrients and energy required for cell survival. So autophagy is recognized as a cytoprotective process against environmental stress [6,7]. Meanwhile, autophagy is also an alternative route of programmed cell death, called type-2 programmed cell death or autophagic cell death [8]. In tumor cells, the role of autophagy may depend on the type of tumor, the stage of tumorigenesis, and the nature and extent of the insult. Appropriate modification of autophagy, that is, inhibition of cytoprotective autophagy or promotion of cyto-killing autophagy could augment cytotoxicity caused by anticancer therapy in tumor cells [9–11]. Thus, in addition to apoptotic response, it would be very useful to determine if an antitumor agent can induce autophagy and what type of autophagies it is. In this study, we found that paclitaxel-induced autophagy in cancer cells, and inhibition of autophagy could lead to enhancement of paclitaxel-mediated cytotoxicity through increasing apoptosis.

## 2. Materials and methods

### 2.1. Cell lines and cell culture

A549, PC-3, and HT-29 cell lines were obtained from The Cell Bank of Chinese Academy of Sciences (Shanghai). Human glioma cancer cell line U87 which stably expresses GFP-LC3 protein was kindly gifted by Yan bing (Shan Dong university). A549 cells were cultured with F12 medium supplemented with 10% foetal bovine serum and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin). PC-3, HT-29 and GFP-LC3 transfected U87 cells were maintained in DMEM medium supplemented with 10% foetal bovine serum and antibiotics. Cultures were incubated in a humidified atmosphere of 95% air and 5% $CO_2$ at 37 °C.

### 2.2. Cell viability measurement

Cell viability was determined via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazoliumbromide (MTT) assay as described previously [12]. Cells were seeded in 96-well plates at a density of $1 \times 10^4$ cells and incubated for 24 h. After treatment with paclitaxel (10, 20, 40 nM, respectively) in the presence or absence of 3-MA (5 mmol/L), 10 μL of MTT (5 mg/mL) was added to each well and incubated in a humidified 5% $CO_2$ atmosphere at 37 °C for 4 h. Crystals were dissolved in 100 μL of DMSO. The absorbance of the solution was read spectrophotometrically at 570 nm using a microtitre plate reader (Bio-Rad). Cell viability was calculated according to the following formula: cell viability (%) = A570 (tested group-blank group)/A570 (vehicle-treated control group-blank group) × 100. At least three replicates were performed for each treatment.

### 2.3. DAPI staining

Morphological changes of apoptosis were determined by DAPI staining as described previously [12]. A549 cells were treated with vehicle or desired concentrations of paclitaxel for 24 h. After washing with PBS, cells were fixed with methanol/acetone (1:1) for 5 min at room temperature. The fixed cells were then washed with PBS and permeabilized with 0.1% TritonX-100 for 10 min prior to staining with DAPI (1:2000 dilution, in 1x PBS) for 10 min. The cells were washed with PBS and mounted. Images of DAPI fluorescence were collected using a Nikon phase-fluorescence microscope. Moderately fluorescent and round nuclei were considered normal. Bright and condensed/fragmented nuclei were regarded as apoptotic.

### 2.4. Immunoblotting assay

After treatment with paclitaxel at desired concentrations, cells were lysed with a solution containing Tris–HCl (50 mmol/L, pH 6.8), SDS (2% w/v), glycerol (10%), and dithiothreitol (10 mmol/L), supplemented with protease inhibitor mix (Thermo Fisher). Cell lysates were centrifuged at 12,000*g* for 30 min. Equal amounts of the protein

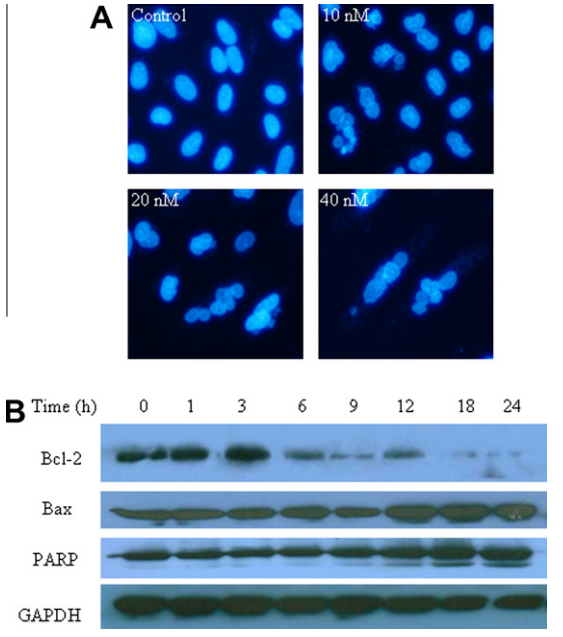


**Fig. 1.** Paclitaxel treatment induces apoptosis in A549 cells. (A) A549 cells were treated with 10, 20, and 40 nM paclitaxel for 24 h. After fixation, cells were stained with DAPI and cell morphological characterization was analyzed using fluorescence microscope. (B) The expression levels of Bax, Bcl-2 and PARP in A549 cells after treatment with 40 nM paclitaxel for 1, 3, 6, 9, 12, 18 and 24 h. Total cell proteins (50 μg) were separated by SDS–PAGE, and then transferred to nitrocellulose membrane. The membranes were immunoblotted with desired antibody. Immunoreactive proteins were visualized using an ECL. GAPDH was used as a loading control. Results shown are representative of at least two independent experiments.

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