

Research Article

Autoregulatory Feedback Mechanism of P38MAPK/Caspase-8 in Photodynamic Therapy-Hydrophilic/Lipophilic Tetra- α -(4-carboxyphenoxy) Phthalocyanine Zinc-Induced Apoptosis of Human Hepatocellular Carcinoma Bel-7402 Cells

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Photodynamic therapy (PDT) is a novel and promising antitumor treatment. Our previous study showed that hydrophilic/lipophilic tetra- α -(4-carboxyphenoxy) phthalocyanine zinc- (T α PcZn-) mediated PDT (T α PcZn-PDT) inhibits the proliferation of human hepatocellular carcinoma Bel-7402 cells by triggering apoptosis and arresting cell cycle. However, mechanisms of T α PcZn-PDT-induced apoptosis of Bel-7402 cells have not been fully clarified. In the present study, therefore, effect of T α PcZn-PDT on apoptosis, P38MAPK, p-P38MAPK, Caspase-8, Caspase-3, Bcl-2, Bid, Cytochrome c, and mitochondria membrane potential in Bel-7402 cells without or with P38MAPK inhibitor SB203580 or Caspase-8 inhibitor Ac-IEFD-CHO was investigated by haematoxylin and eosin (HE) staining assay, flow cytometry analysis of annexin V-FITC/propidium iodide (PI) double staining cells and 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide (JC-1), and immunoblot assay. We found that T α PcZn-PDT resulted in apoptosis induction, activation of P38MAPK, Caspase-8, Caspase-3, and Bid, downregulation of Bcl-2, release of Cytochrome c from mitochondria, and disruption of mitochondrial membrane potential in T α PcZn-PDT-treated Bel-7402 cells. In contrast, SB203580 or Ac-IEFD-CHO attenuated induction of apoptosis, activation of P38MAPK, Caspase-8, Caspase-3, and Bid, downregulation of Bcl-2, release of Cytochrome c from mitochondria, and disruption of mitochondrial membrane potential in T α PcZn-PDT-treated Bel-7402 cells. Taken together, we conclude that Caspase-3, Bcl-2, Bid, and mitochondria are involved in autoregulatory feedback of P38MAPK/Caspase-8 during T α PcZn-PDT-induced apoptosis of Bel-7402 cells.

1. Introduction

Photodynamic therapy (PDT), also known as photochemotherapy, is a developing strategy and has shown promising antitumor efficacy. It is based on the concept that photosensitizing drugs, when exposed to appropriate wavelength of light, are capable of resulting in photochemical destruction of tumors by yielding reactive oxygen species (ROS), such as singlet oxygen and free radicals [1–4]. Compared with traditional therapies, such as surgery and chemotherapy, the outstanding advantage of PDT is that it destroys tumor cells selectively because it is possible to expose the photosensitizer to light and to activate it specifically in the target tumor tissue.

Photosensitizers play a crucial role in PDT. Currently, although only a few photosensitizers (temoporfin, talaporfin, verteporfin, and 5-aminolevulinic acid), which are approved for antitumor treatment, have been proved to be effective in a wider spectrum of antitumor, they present various defects that stimulate the development of better photosensitizer candidates [5–7]. Phthalocyanines, consisting of a planar macrocycle with an 18 π -electron system, are one of the most potential photosensitizer candidates because of the desirable electronic absorption and photophysical properties [8–10]. In addition, phthalocyanines with hydrophilic/lipophilic structure may become promising candidates for selective photosensitizers inasmuch as hydrophilic group contributes to the

transport of drug in the body and lipophilic group redounds to the uptake of drug in cancer cells. Accumulating evidences have shown highly selective growth inhibitory effects of hydrophilic/lipophilic phthalocyanines-mediated PDT on a variety of cancer cells [11–13].

Apoptosis that plays a crucial role during development and maintenance of tissue homeostasis by eliminating old cells, unnecessary cells, and unhealthy cell is the process of programmed cell death that may occur in multicellular organisms. Apoptosis is characterized by morphologic and biochemical changes including chromatin condensation, cell shrinkage, nucleus and cytoplasm fragment, DNA fragmentation, and formation of membrane blebs and apoptotic bodies. Accumulating evidences clearly indicate that apoptosis is one key pathway in phthalocyanines-PDT process [11–13].

P38 mitogen-activated protein kinase (MAPK) is a member of the MAPK family of kinases that is responsive to stress stimuli (ionizing radiation, ultraviolet irradiation, oxidative stress, FAS ligand, and cytokines) and is involved in cell proliferation, differentiation, apoptosis, and autophagy. Nowadays, P38MAPK has been documented to play a crucial regulatory role in phthalocyanines-PDT-induced apoptosis of cancer cells [14, 15].

Caspases, a family of cysteine proteases, are central regulators of apoptosis signaling [16]. Initiator Caspase-8 is closely coupled to proapoptotic signals. Once activated, Caspase-8 cleaves and activates downstream effector Caspase-3, whose cleavage leads to the morphological and functional changes associated with apoptosis [17]. Nowadays, Caspase-8 and Caspase-3 have been documented to play a crucial regulatory role in phthalocyanines-PDT-induced apoptosis of cancer cells [18–21].

Apoptosis regulator Bcl-2 is a family of evolutionarily related proteins. These proteins govern mitochondrial outer membrane permeabilization and comprise proapoptotic (Bax, Bid, Bak, etc.) and antiapoptotic (Bcl-2, Bcl-XL, Bcl-w, etc.) molecules. Recent studies have demonstrated that induction of proapoptotic proteins and inhibition of antiapoptotic proteins can be caused by phthalocyanines-PDT in apoptosis of cancer cells [11, 21, 22].

Mitochondrion is a membrane-enclosed organelle found in most eukaryotic cells. Cytochrome c is a small heme protein found loosely associated with the inner membrane of the mitochondrion. Membrane potential (also transmembrane potential or membrane voltage) is the difference in electrical potential between the interior and the exterior of a biological cell. Accumulated evidences have demonstrated that release of Cytochrome c from mitochondria and reduction of mitochondrial membrane potential appear to be key events in phthalocyanines-PDT-induced apoptosis of cancer cells [19, 20].

We found previously that tetra- α -(4-carboxyphenoxy) phthalocyanine- (T α PcZn-) mediated PDT (T α PcZn-PDT) (Figure 1) resulted in the inhibition of proliferation and induction of apoptosis in Bel-7402 cells [11], which is one of the most widely used experiment models for hepatocellular carcinoma. However, mechanisms of T α PcZn-PDT-induced apoptosis of Bel-7402 cells have not been fully clarified. In the present study, the objective of our study is to investigate the

autoregulatory feedback mechanism of P38MAPK/Caspase-8 in T α PcZn-PDT-induced apoptosis of Bel-7402 cells. Based on in vitro model, we found that Caspase-3, Bcl-2, Bid, and mitochondria were involved in autoregulatory feedback of P38MAPK/Caspase-8 during T α PcZn-PDT-induced apoptosis of Bel-7402 cells.

2. Materials and Methods

2.1. Materials. Anti-P38MAPK, anti-phos-P38MAPK, anti-Caspase-8, anti-Caspase-3, anti-Bcl-2, anti-Bid, and anti-Cytochrome c antibodies were purchased from Cell Signals (OH, USA). P38MAPK inhibitor SB203580 and Caspase-8 inhibitor Ac-IEFD-CHO were obtained from Merck Calbiochem (Darmstadt, Germany). JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) Staining Kit was purchased from Genmed Scientifics (MA, U.S.A). Annexin-V-FLUOS Staining Kit was purchased from Roche (Basel, Switzerland). DMSO was purchased from Sigma (MO, USA). DMEM medium was purchased from Gibco (CA, USA). Fetal bovine serum was purchased from PAA (Coelbe, Germany). Penicillin was purchased from Harbin Pharmaceutical Group (Heilongjiang, China). Streptomycin was purchased from Dalian Merro Pharmaceutical (Liaoning, China). Polyvinylidene difluoride membrane (PVDF) membrane was purchased from Amersham Pharmacia Biotech (NJ, USA). T α PcZn was synthesized as described in our previous report [23]. The T α PcZn stock solution was prepared in DMSO and stored at 4°C in the dark. When used, the stock solution was appropriately diluted to obtain the desired concentration with a final DMSO concentration of 0.1%. All other chemicals and reagents were of analytic grade.

2.2. Cell Culture and Treatment. Hepatocellular carcinoma Bel-7402 cells were obtained from Harbin Medical University, China, and cultured in DMEM medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/L streptomycin in a humidified atmosphere of 5% CO₂ at 37°C. Bel-7402 cells in logarithmic growth phase were seeded in culture plates and incubated for 24 h in a humidified atmosphere of 5% CO₂ at 37°C. After being rinsed with phosphate-buffered saline, the cells were treated with T α PcZn stock solution diluted in medium for 2.5 h at 37°C in the presence or absence of SB203580 or Ac-IEFD-CHO. Then the cells were irradiated with a SS-B instrument (Wuxi Holyglow Physiotherapy Instrument Co., Ltd., Jiangsu, China) emitting red light within the wavelength range of 600 to 700 nm. The light dose was about 53.7 J/cm². Thereafter, cells were harvested at 3 h. Control cells were treated with 0.1% DMSO under the same conditions.

2.3. Cell Morphology Assay. At 3 h posttreatment, Bel-7402 cells on a glass slide within 6-well culture plates were fixed with 10% formaldehyde for 15 min, washed with phosphate-buffered saline, and stained with haematoxylin and eosin (HE). Cells morphology was then observed under IX70 inverted microscope (Olympus, Tokyo, Japan).

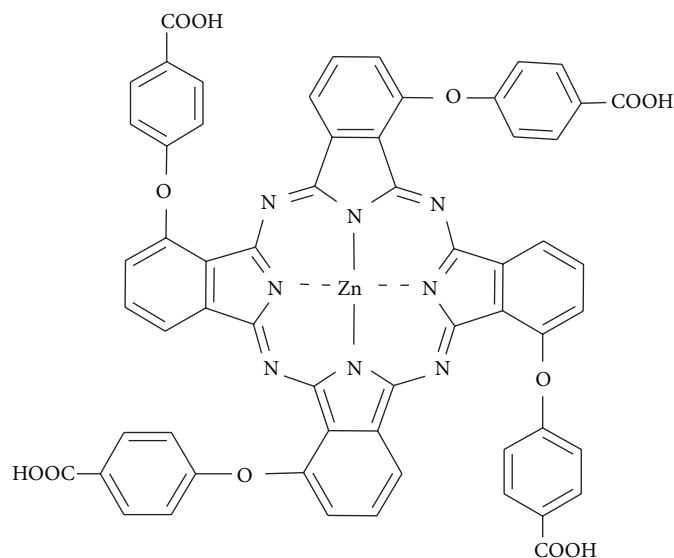
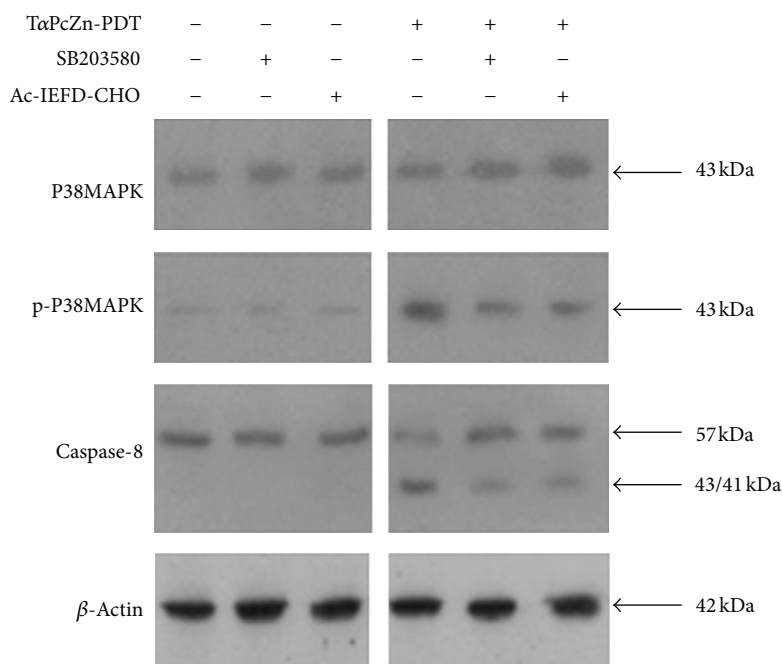
FIGURE 1: Chemical structure of T α PcZn [11].

FIGURE 2: Effect of T α PcZn-PDT on P38MAPK, p-P38MAPK, and Caspase-8, respectively, without or with SB203580 or Ac-IEFD-CHO in T α PcZn-PDT-induced apoptosis of Bel-7402 cells analyzed by immunoblot assay. Bel-7402 cells were pretreated with T α PcZn (54 μ M) in the absence or presence of SB203580 (10 μ M) or Ac-IEFD-CHO (10 μ M) for 2.5 h, exposed to red-light irradiation (53.7 J/cm²), and then incubated for 3 h. Expression of P38MAPK, p-P38MAPK, and Caspase-8 was analyzed by immunoblot assay.

2.4. Flow Cytometry Analysis Of Annexin V-FITC/PI Double Stained Cells for Apoptosis. Confirmation of apoptosis was determined by measurement of externalized phosphatidylserine residues as detected using annexin V-FITC. The harvested Bel-7402 cells were collected and washed with ice-cold phosphate-buffered saline and then suspended in 500 μ L of annexin V binding buffer A. 100 μ L aliquot was taken, 2 μ L of annexin V-FITC and 2 μ L of propidium iodide (PI) were added, and the mixture was incubated for 5 min at

room temperature in the dark. After the addition of 400 μ L of binding buffer, 1×10^4 cells were analyzed on a FACSCAN flow cytometer (Becton Dickinson, CA, USA) by using CellQuest software. The results are shown as a dot plot graph. In each graph, the percentages of viable cells, early apoptotic cells, and late apoptotic cells are, respectively, indicated in lower-left, lower-right, and upper-right quadrant; the y -axis corresponds to relative PI staining; the x -axis corresponds to the log of the FITC signal.

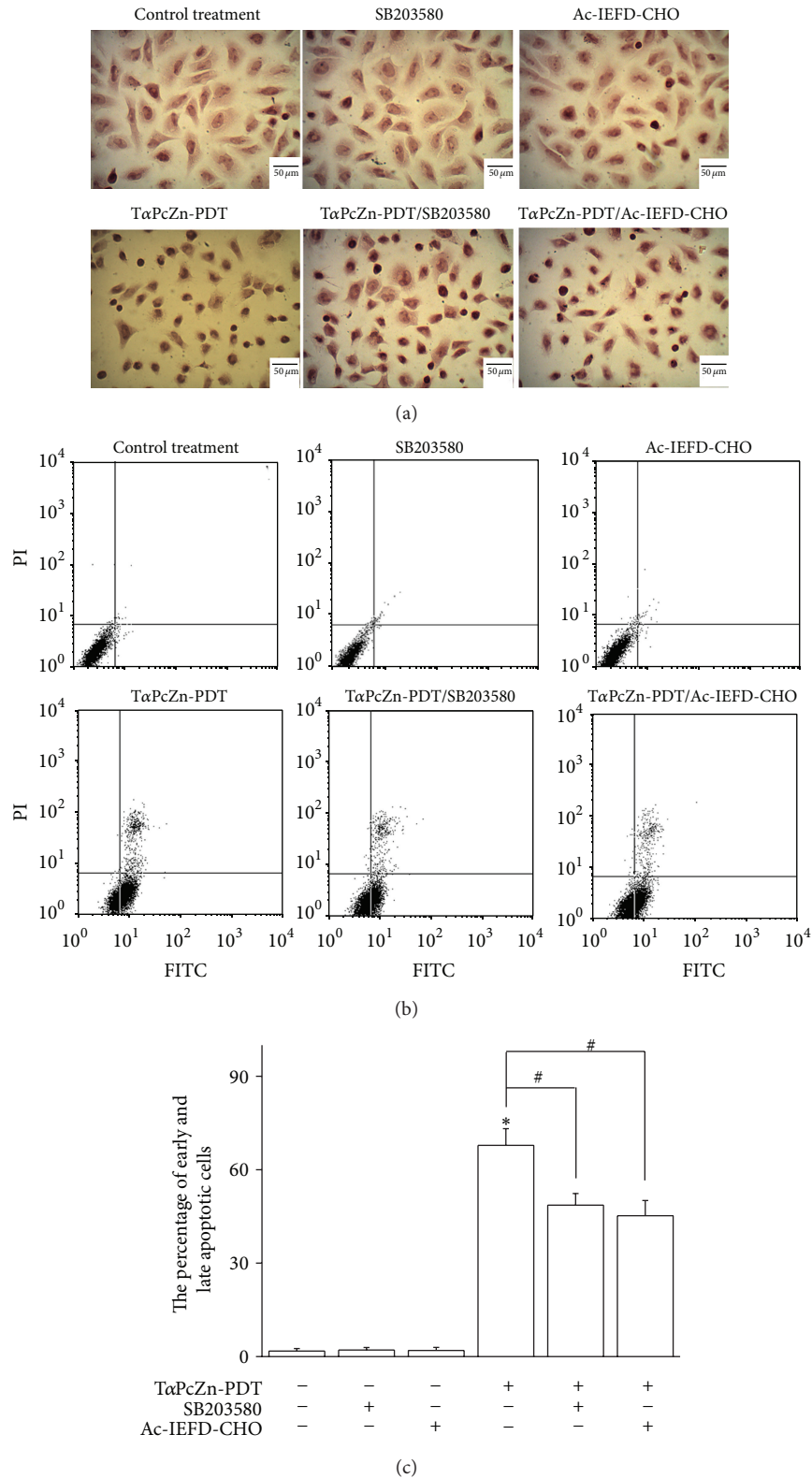


FIGURE 3: Effect of TαPcZn-PDT on apoptosis of Bel-7402 cells in the absence or presence of SB203580 or Ac-IEFD-CHO. Bel-7402 cells were pretreated with TαPcZn (54 μM) in the absence or presence of SB203580 (10 μM) or Ac-IEFD-CHO (10 μM) for 2.5 h, exposed to red-light irradiation (53.7 J/cm²), and then incubated for 3 h. The morphology of apoptotic cells was analyzed by HE staining assay (a), and the percentage of apoptotic cells was assayed by flow cytometry analysis of annexin V-FITC/PI double stained cells (b and c). Bars under each panel represent 50 μm. Values presented are representative of three independent experiments (means ± S.D.; *P < 0.05, compared with control treatment; #P < 0.05, compared with TαPcZn-PDT treatment).

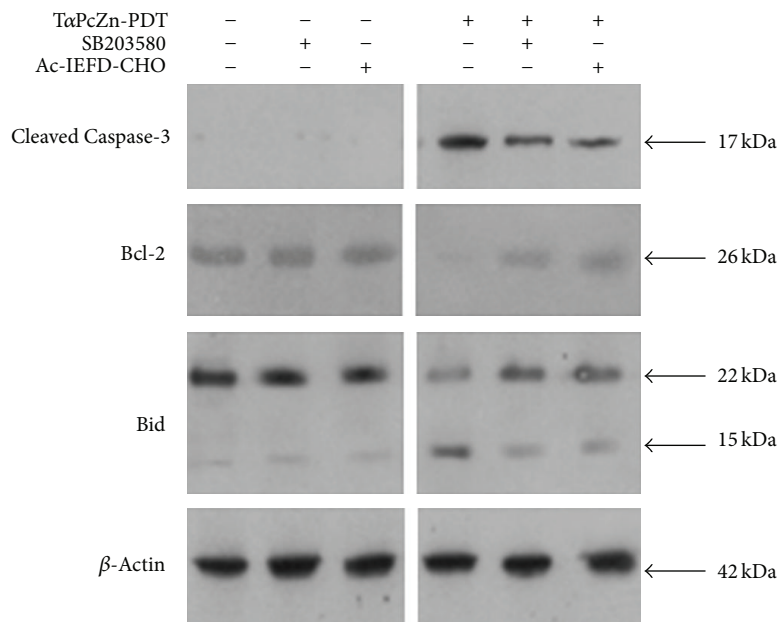


FIGURE 4: Effect of TαPcZn-PDT on Caspase-3, Bcl-2, and Bid, respectively, without or with SB203580 or Ac-IEFD-CHO in TαPcZn-PDT-induced apoptosis of Bel-7402 cells analyzed by immunoblot assay. Bel-7402 cells were pretreated with TαPcZn (54 μM) in the absence or presence of SB203580 (10 μM) or Ac-IEFD-CHO (10 μM) for 2.5 h, exposed to red-light irradiation (53.7 J/cm²), and then incubated for 3 h. Expression of cleaved Caspase-3, Bcl-2, and Bid was analyzed by immunoblot assay.

2.5. JC-1 Assay for Mitochondrial Membrane Potential. Mitochondrial membrane potential was detected using JC-1 fluorescent dye by flow cytometry. The harvested cells were incubated with fresh culture medium containing JC-1 dye (2.5 ug/mL) for 20 min at 37°C in the dark, and then about 1×10^4 cells were analyzed on a flow cytometer by using CellQuest software. The results are shown as a dot plot graph. In each graph, the *y*-axis corresponds to JC-1 oligomer-associated red fluorescence, the *x*-axis corresponds to JC-1 monomer-associated green fluorescence, and the shift down of fluorescence from red to green indicates the collapse of mitochondrial membrane potential.

2.6. Immunoblot Assay. All immunoblots were performed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) Bis-Tris gel electrophoresis as outlined by the supplier. For total cellular protein, Bel-7402 cells were lysed in buffer containing 25 mM HEPES, pH 7.5, 0.3 M NaCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 0.1% Triton X-100, 20 mM β-glycerophosphate, 0.5 mM DTT, 1 mM sodium orthovanadate, 0.1 μM okadaic acid, and 1 mM phenylmethylsulfonyl fluoride. Equal amounts of lysate protein ran on 12% SDS-PAGE and electrophoretically transferred to PVDF membrane. After blocking, the blots were incubated with specific primary antibodies (anti-P38MAPK, anti-phospho-P38MAPK, anti-Caspase-3, anti-Caspase-8, anti-Bcl-2, and anti-Bid antibodies) overnight at 4°C and further incubated for 1 h with horseradish peroxidase-conjugated respective secondary antibody. Bound antibodies were detected by enhanced chemiluminescence kit with Lumino Image Analyzer (Founder, Beijing, China). The mitochondria and

cytosolic fractions isolated from the cells were collected for immunoblot assay of Cytochrome c as previously described [24]. The Cytochrome c protein was assayed using an anti-Cytochrome c antibody.

2.7. Statistical Analysis. Values are means ± S.D. of three independent experiments. Statistical significance was determined using Student's unpaired two-tailed *t*-test. *P* value less than 0.05 was considered significant in all cases.

3. Results

3.1. Autoregulatory Feedback of P38MAPK/Caspase-8 Is Essential for TαPcZn-PDT-Induced Apoptosis of Bel-7402 Cells. Our previous study showed that TαPcZn-PDT apparently induced apoptosis of Bel-7402 cells [11]. To evaluate whether P38MAPK and Caspase-8 are involved in TαPcZn-PDT-induced apoptosis of Bel-7402 cells, effect of TαPcZn-PDT on P38MAPK, p-P38MAPK, and Caspase-8, respectively, was investigated by immunoblot assay in the present study. Compared with the control treatment, TαPcZn-PDT resulted in little effect on P38MAPK, upregulation of p-P38MAPK, and activation of active Caspase-8 (Figure 2), suggesting that active P38MAPK and Caspase-8 might modulate TαPcZn-PDT-induced apoptosis of Bel-7402 cells.

To determine whether attenuation of P38MAPK, p-P38MAPK, and Caspase-8 influences Bel-7402 cells in TαPcZn-PDT-induced apoptosis, we used the P38MAPK inhibitor SB203580 or Caspase-8 inhibitor Ac-IEFD-CHO. Compared with TαPcZn-PDT treatment, SB203580 resulted

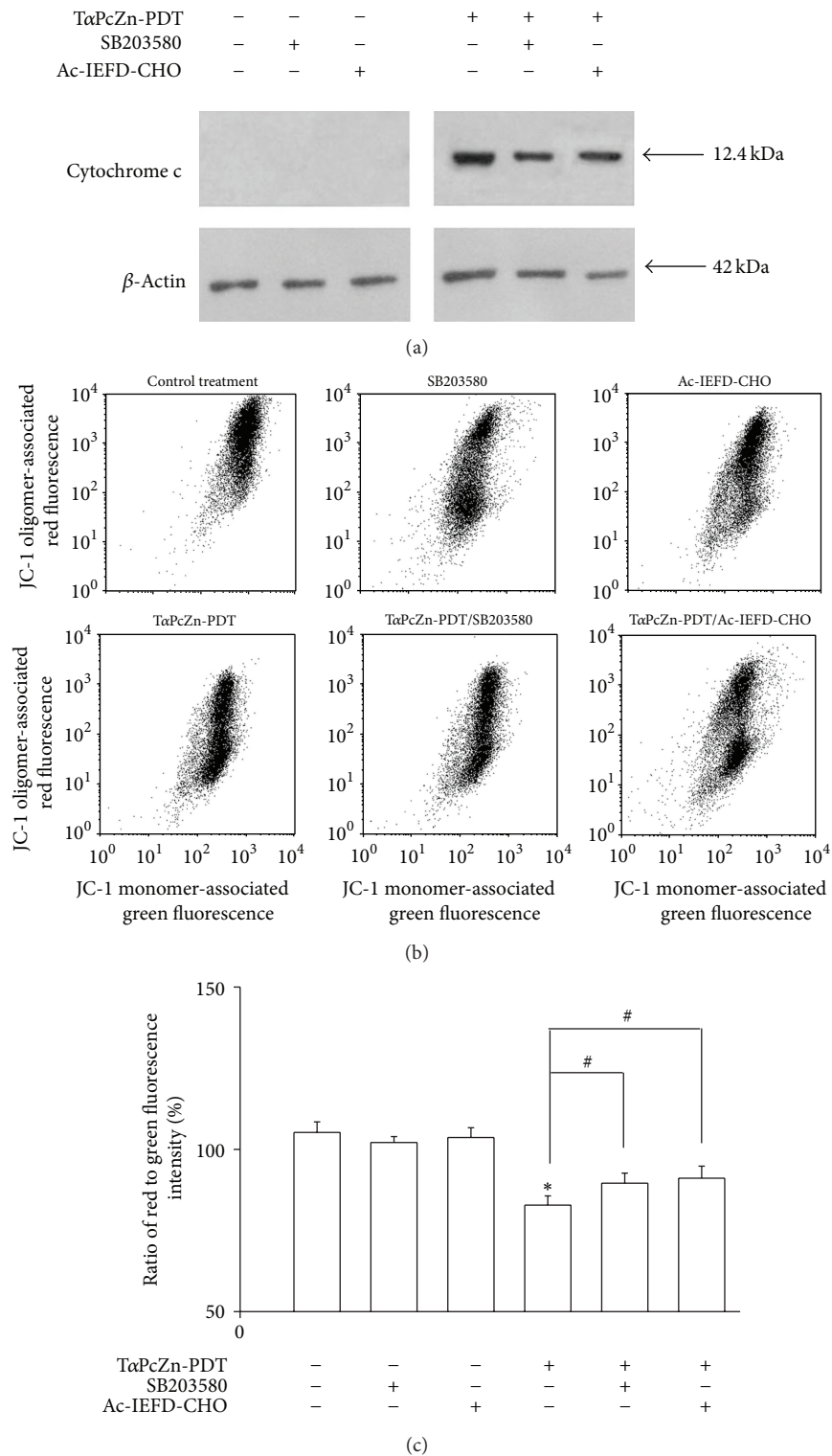


FIGURE 5: Effect of TαPcZn-PDT on mitochondria of Bel-7402 cells in the absence or presence of SB203580 or Ac-IEFD-CHO. Bel-7402 cells were pretreated with TαPcZn (54 μM) in the absence or presence of SB203580 (10 μM) or Ac-IEFD-CHO (10 μM) for 2.5 h, exposed to red-light irradiation (53.7 J/cm²), and then incubated for 3 h. Expression of Cytochrome c was analyzed by immunoblot assay (a), and mitochondria membrane potential (b) and ratio of red to green fluorescence intensity (c) in Bel-7402 cells were determined by JC-1 assay of flow cytometry, respectively. Values presented are representative of three independent experiments (means ± S.D.; * *P* < 0.05, compared with control treatment; # *P* < 0.05, compared with TαPcZn-PDT treatment).

in little effect on P38MAPK and downregulation of p-P38MAPK, and Ac-IEFD-CHO led to downregulation of 43/41-kDa subunit of active Caspase-8 in $\text{T}\alpha\text{PcZn}$ -PDT-treated Bel-7402 cells (Figure 2), suggesting that attenuation of active P38MAPK and Caspase-8 might be involved in $\text{T}\alpha\text{PcZn}$ -PDT-induced apoptosis of Bel-7402 cells.

To confirm whether Caspase-8 or P38MAPK regulates apoptosis in $\text{T}\alpha\text{PcZn}$ -PDT-treated Bel-7402 cells, effect of SB203580 or Ac-IEFD-CHO on apoptosis, respectively, was investigated by several ways in the present study. Firstly, the morphological characteristics of apoptotic cells were assessed by HE staining assay. Compared with control cells, cells treated with $\text{T}\alpha\text{PcZn}$ -PDT apparently exhibited morphological characteristics of apoptotic cells, such as cell shrinkage, chromatin condensation, nucleus and cytoplasm fragment, and membrane blebs formation (Figure 3(a)). However, compared with $\text{T}\alpha\text{PcZn}$ -PDT treatment, SB203580 or Ac-IEFD-CHO resulted in less morphological characteristics of apoptosis in $\text{T}\alpha\text{PcZn}$ -PDT-treated cells (Figure 3(a)). Furthermore, apoptosis of cells was quantitatively evaluated by flow cytometry analysis of annexin V-FITC/PI double staining cells. Compared with control treatment, $\text{T}\alpha\text{PcZn}$ -PDT apparently induced cells apoptosis (Figures 3(b) and 3(c)). However, compared with $\text{T}\alpha\text{PcZn}$ -PDT treatment, SB203580 or Ac-IEFD-CHO resulted in less effect of apoptosis on $\text{T}\alpha\text{PcZn}$ -PDT-treated cells (Figures 3(b) and 3(c)). These results indicate that active P38MAPK and Caspase-8 are essential for $\text{T}\alpha\text{PcZn}$ -PDT-induced apoptosis of Bel-7402 cells.

To determine whether P38MAPK regulates Caspase-8 and whether Caspase-8 regulates P38MAPK, effect of SB203580 on Caspase-8 and effect of Ac-IEFD-CHO on P38MAPK in $\text{T}\alpha\text{PcZn}$ -PDT-treated Bel-7402 cells were, respectively, investigated by immunoblot assay in the present study. Compared with $\text{T}\alpha\text{PcZn}$ -PDT treatment, SB203580 downregulated 43/41-kDa subunit of active Caspase-8 and Ac-IEFD-CHO downregulated p-P38MAPK in $\text{T}\alpha\text{PcZn}$ -PDT-treated Bel-7402 cells (Figure 2), suggesting that autoregulatory feedback of P38MAPK/Caspase-8 is involved in $\text{T}\alpha\text{PcZn}$ -PDT-induced apoptosis of Bel-7402 cells. All of the above results suggest that autoregulatory feedback of P38MAPK/Caspase-8 is essential for $\text{T}\alpha\text{PcZn}$ -PDT-induced apoptosis of Bel-7402 cells.

3.2. Caspase-3 and Bcl-2 Family Proteins Are Involved in Autoregulatory Feedback of P38MAPK/Caspase-8 in $\text{T}\alpha\text{PcZn}$ -PDT-Induced Apoptosis of Bel-7402 Cells. To determine whether Caspase-3 and Bcl-2 family are involved in autoregulatory feedback of P38MAPK/Caspase-8 during $\text{T}\alpha\text{PcZn}$ -PDT-induced apoptosis of Bel-7402 cells, effect of $\text{T}\alpha\text{PcZn}$ -PDT on Caspase-3, Bcl-2, and Bid, respectively, in the absence or presence of SB203580 or Ac-IEFD-CHO was investigated by immunoblot assay in the present study. Compared with the control treatment, $\text{T}\alpha\text{PcZn}$ -PDT resulted in upregulation of 17-kDa subunit of active Caspase-3, downregulation of Bcl-2, and upregulation of 15-kDa subunit of active Bid (Figure 4), suggesting that Caspase-3, Bcl-2, and Bid are involved in

$\text{T}\alpha\text{PcZn}$ -PDT-induced apoptosis of Bel-7402 cells. Furthermore, compared with the $\text{T}\alpha\text{PcZn}$ -PDT treatment, SB203580 or Ac-IEFD-CHO resulted in downregulation of 17-kDa subunit of active Caspase-3, upregulation of Bcl-2, and downregulation of 15-kDa subunit of active Bid (Figure 4), suggesting that autoregulatory feedback of P38MAPK/Caspase-8 might regulate Caspase-3, Bcl-2, and Bid in $\text{T}\alpha\text{PcZn}$ -PDT-induced apoptosis of Bel-7402 cells.

3.3. Mitochondrion Is Involved in Autoregulatory Feedback of P38MAPK/Caspase-8 in $\text{T}\alpha\text{PcZn}$ -PDT-Induced Apoptosis of Bel-7402 Cells. To determine whether mitochondrion is involved in autoregulatory feedback of P38MAPK/Caspase-8 during $\text{T}\alpha\text{PcZn}$ -PDT-induced apoptosis of Bel-7402 cells, effect of $\text{T}\alpha\text{PcZn}$ -PDT on Cytochrome c and mitochondria membrane potential, respectively, in the absence or presence of SB203580 or Ac-IEFD-CHO was investigated by immunoblot assay and JC-1 assay of flow cytometry in the present study. Compared with the control treatment, $\text{T}\alpha\text{PcZn}$ -PDT resulted in upregulation of Cytochrome c and a decrease in ratio of red to green fluorescence intensity, suggesting that release of Cytochrome c from mitochondria and disruption of mitochondrial membrane potential are involved in $\text{T}\alpha\text{PcZn}$ -PDT-induced apoptosis of Bel-7402 cells (Figure 5). Furthermore, compared with the $\text{T}\alpha\text{PcZn}$ -PDT treatment, SB203580 or Ac-IEFD-CHO resulted in downregulation of Cytochrome c and an increase in ratio of red to green fluorescence intensity (Figure 5), suggesting that autoregulatory feedback of P38MAPK/Caspase-8 might regulate mitochondria in $\text{T}\alpha\text{PcZn}$ -PDT-induced apoptosis of Bel-7402 cells.

4. Discussion

Accumulated evidences have suggested that P38MAPK and Caspase-8 are involved in phthalocyanines-PDT-induced apoptosis of cancer cells [14, 15, 18]. Our previous study has showed that $\text{T}\alpha\text{PcZn}$ -PDT can apparently induce apoptosis of Bel-7402 cells [11]. However, it is unclear whether autoregulatory feedback of P38MAPK/Caspase-8 is essential for $\text{T}\alpha\text{PcZn}$ -PDT-induced apoptosis of Bel-7402 cells. Therefore, effect of $\text{T}\alpha\text{PcZn}$ -PDT on P38MAPK, p-P38MAPK, and Caspase-8, respectively, in $\text{T}\alpha\text{PcZn}$ -PDT-induced apoptosis of Bel-7402 cells without or with SB203580 or Ac-IEFD-CHO was firstly investigated in the present study. The result showed that $\text{T}\alpha\text{PcZn}$ -PDT caused activation of P38MAPK and Caspase-8, but SB203580 or Ac-IEFD-CHO, respectively, resulted in downregulation of active P38MAPK and Caspase-8, suggesting that activation of P38MAPK and Caspase-8 might be involved in $\text{T}\alpha\text{PcZn}$ -PDT-induced apoptosis of Bel-7402 cells. Furthermore, to confirm whether Caspase-8 and P38MAPK regulate apoptosis in $\text{T}\alpha\text{PcZn}$ -PDT-treated Bel-7402 cells, effect of SB203580 or Ac-IEFD-CHO on apoptosis, respectively, was investigated in the present study. The results showed that $\text{T}\alpha\text{PcZn}$ -PDT apparently induced apoptosis, but SB203580 or Ac-IEFD-CHO attenuated apoptosis of Bel-7402 cells in $\text{T}\alpha\text{PcZn}$ -PDT-treated cells, suggesting that activated P38MAPK and

Caspase-8 are essential for T α PcZn-PDT-induced apoptosis of Bel-7402 cells. Accumulated evidences have suggested that Caspase-8 regulates P38MAPK or P38MAPK regulates Caspase-8 in drugs-induced apoptosis of cancer cells [25–27]. However, it is unclear whether autoregulatory feedback of P38MAPK/Caspase-8 is involved in T α PcZn-PDT-induced apoptosis of Bel-7402 cells. Therefore, effect of SB203580 on Caspase-8 and effect of Ac-IEFD-CHO on P38MAPK were further investigated in the present study. The results showed that SB203580 attenuated activation of Caspase-8 and that Ac-IEFD-CHO attenuated activation of P38MAPK in T α PcZn-PDT-induced apoptosis of Bel-7402 cells, suggesting that autoregulatory feedback of P38MAPK/Caspase-8 might modulate T α PcZn-PDT-induced apoptosis of Bel-7402 cells. All of the above results suggest that autoregulatory feedback of P38MAPK/Caspase-8 is essential for T α PcZn-PDT-induced apoptosis of Bel-7402 cells.

Several studies have demonstrated that Caspase-3, Bcl-2 family proteins, and mitochondria are involved in phthalocyanines-PDT-induced apoptosis of cancer cells [18–22]. Our previous study has showed that Bcl-2 is involved in T α PcZn-PDT-induced apoptosis of Bel-7402 cells [11]. However, it is not clear whether Caspase-3, Bid, and mitochondria were involved in T α PcZn-PDT-induced apoptosis of Bel-7402 cells. Therefore, effect of T α PcZn-PDT on Caspase-3, Bid, Cytochrome c, and mitochondria membrane potential, respectively, in T α PcZn-PDT-induced apoptosis of Bel-7402 cells was firstly investigated in the present study. The result showed that T α PcZn-PDT apparently resulted in activation of Caspase-3 and Bid, release of Cytochrome c from mitochondria, and disruption of mitochondrial membrane potential, suggesting that Caspase-3, Bid, and mitochondria might modulate T α PcZn-PDT-induced apoptosis of Bel-7402 cells. Accumulated evidences have suggested that Caspase-3, Bcl-2 family proteins, and mitochondria are downstream of P38MAPK [27–30] or Caspase-8 [31–33]. However, it is unclear whether autoregulatory feedback of P38MAPK/Caspase-8 can regulate Caspase-3, Bcl-2, Bid, and mitochondria in T α PcZn-PDT-induced apoptosis of Bel-7402 cells. Therefore, effect of T α PcZn-PDT on Caspase-3, Bcl-2, Cytochrome c, and mitochondrial membrane potential, respectively, in the presence of SB203580 or Ac-IEFD-CHO was investigated in the present study. The results showed that SB203580 or Ac-IEFD-CHO attenuated activation of Caspase-3 and Bid, downregulation of Bcl-2, release of Cytochrome c from mitochondria, and disruption of mitochondrial membrane potential, suggesting that Caspase-3, Bcl-2 family proteins, and mitochondria are involved in autoregulatory feedback of P38MAPK/Caspase-8 during T α PcZn-PDT-induced apoptosis of Bel-7402 cells.

Taken together, our findings demonstrate that Caspase-3, Bcl-2, Bid, and mitochondria are involved in autoregulatory feedback of P38MAPK/Caspase-8 during T α PcZn-PDT-induced apoptosis of Bel-7402 cells.

Conflict of Interests

The authors do not have any conflict of interests with the content of the paper.


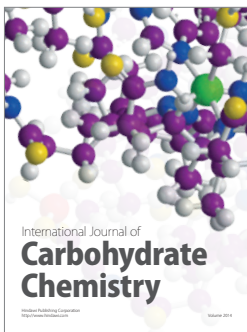
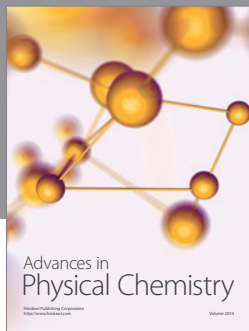
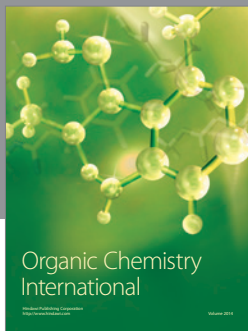
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