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ORIGINAL ARTICLE

Dual role of reactive oxygen species in autophagy and apoptosis induced by compound PN in prostate cancer cells

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Abstract

Background *Pharbitis nil* (L.) Choisy (PN) is used as a traditional herb in East Asia and exhibits anti-parasitic, purgative, diuretic, anti-inflammatory, and anti-cancer activities. However, the molecular mechanisms underlying the anti-cancer activity are not well understood.

Objective This study aims to elucidate the effects of reactive oxygen species (ROS), generated after treatment with the compound PN, on the induction of apoptosis and autophagy, which are pathways that underly the mechanisms of cell death and cell survival in human prostate cancer cells.

Results The MTT assay and western blot analysis were used to assess the effects of compound PN on cell viability and the expression of apoptosis- and autophagy-related proteins in prostate cancer PC-3 cells. The effects of PN on apoptosis (via annexin V/propidium iodide staining), autophagy (via acridine orange staining), and ROS (via DCFH-DA staining) were investigated using flow cytometry. Compound PN induced the production of intracellular and mitochondrial ROS leading to increased apoptosis and autophagy in PC-3 cells. Interestingly, pretreatment with *N*-acetyl-L-cysteine (NAC), an intracellular ROS scavenger, enhanced compound PN-induced apoptosis, but reduced levels of autophagy. In contrast, pretreatment with diphenyleneiodonium (DPI), an inhibitor of mitochondrial ROS, reduced compound PN-induced apoptosis and enhanced autophagy. Inhibition of autophagy led to acceleration of apoptosis in a PN-induced ROS-dependent manner. Compound PN-induced ROS production from two different sources, an intracellular source and mitochondrial source. ROS production in these differing locations had different effects on apoptosis and autophagy. They acted either by promoting cell death or cell survival through regulating autophagy to either escape or enhance apoptotic cell death.

Conclusion This crosstalk between ROS-activated signals in apoptosis and autophagy induction by PN provides new insights into the molecular mechanisms of this compound and suggests that PN may be a potential therapy for prostate cancer treatment.

Keywords Apoptosis · Autophagy · Human prostate cancer · *Pharbitis nil* (L.) choisy · Reactive oxygen species

Introduction

Apoptosis and autophagy, processes involved in programmed cell death, have been the focus for cancer therapies, as autophagy promotes cell survival by suppressing apoptosis induction, but can lead to cell death (Yonekawa and Thorburn 2013). Reactive oxygen species (ROS) are associated with many diseases, including cancer, metabolic disease, Alzheimer's disease, and cardiovascular disease (Uttara et al. 2009; Srivastava and Kumar 2015). ROS produced from cellular oxygen metabolism play an important role in cellular signaling and homeostasis (Ray et al. 2012). ROS are also key molecules in the regulation of apoptosis and autophagy in cancer, and thus, affect cell death and survival (Poillet-Perez et al. 2015). Low levels of ROS usually stimulate cell proliferation and survival, whereas excessive levels induce cell death through diminished antioxidant capacity. Many studies have shown that oxidative stress induces two types of ROS, that is, endogenous and

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exogenous ROS (Panieri et al. 2016). Endogenous ROS are produced by the mitochondrial electron transport chain and nitric oxide synthase, which includes lipoxygenase, NADPH oxidase, and cytochrome c oxidase. Endogenous ROS play important roles in apoptosis through the regulation of mitochondrial membrane potentials, as loss of mitochondrial membrane potential can result in the release of cytochrome *c*. Exogenous ROS can be produced by external sources, such as radiation or drugs (Chen et al. 2017; Lee et al. 2013), and recent reports have suggested that increasing exogenous ROS levels with hydrogen peroxide induces apoptosis via inhibition of the antioxidant system (Liou and Storz 2010). Excessive oxidative stress disrupts the balance of intracellular antioxidant/oxidant levels, resulting in damage to biological molecules and cell death (Panieri and Santoro 2016).

Pharbitis nil (L.) Choisy (PN), commonly known as morning glory, is a plant that is native to most tropical countries. The seeds and roots of PN are traditionally used as herbal medicine in East Asia to treat cancer. PN has been shown to be cytotoxic to gastric cancer cells through regulating tumor suppressor genes (Ko et al. 2004; Ju et al. 2011; Kim et al. 2014). Recently, the extract of the seeds of *Pharbitis nil* were shown to have anti-inflammatory activity, anti-fungal activity, and analgesic effects on abdominal pain (Kim et al. 2014). PN contains several bioactive small molecules, such as, resin glycosides (eg.,pharbitin), gibberellins, flavonoids, chlorogenic acid derivatives, anthocyanins, diterpenoids, and triterpene saponins (Jung 2008; Kim et al. 2009). However, the mechanisms underlying the anticancer activity of PN are not clear. We hypothesized that PN regulates ROS production, leading to effects on autophagy and apoptosis. Therefore, we investigated the mechanisms responsible for the anticancer effects of compound PN, isolated from *Pharbitis nil,* in human prostate cancer PC-3 cells, with a focus on the relationship between apoptosis and autophagy mediated by ROS production.

Materials and methods

Reagents

Compound PN was purified from the methanol extract of seeds of *Pharbitis nil*, as previously described (Choi 2017). 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2′, 7′-dichlorofluorescein diacetate (DCFH-DA), diphenyleneiodonium (DPI), 3-methyadenine (3-MA), and acridine orange (AO) were purchased from Sigma Chemical (St. Louis, MO, USA). A FITC Annexin V Apoptosis Detection Kit from BD Biosciences (San Jose, CA, USA) was used. Antibodies for microtubule-associated protein 1 light chain 3 (LC3), caspase-3, poly (ADP-ribose) polymerase (PARP), and β–actin were purchased from Cell Signaling Technology (Beverly, MS, USA) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. Goat-anti-mouse and goat-anti-rabbit IgG secondary antibodies were obtained from Enzo Life Science (Farmingdale, NY, USA) and ECL Western kit from iNtRON Biotechnology (Seongnam, Korea).

Cell culture and viability

Human prostate cancer PC-3 cells were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle's Medium (DMEM, WelGENE Inc., gyeongsan, Korea) supplemented with 10% fetal bovine serum (FBS, WelGENE Inc.) and 100 units/ml of penicillin and streptomycin (WelGENE Inc.) in a humidified atmosphere containing 5% CO_2 at 37 °C. PC-3 cells were seeded at a density of 1×10 (Ray et al. 2012) cells/well and treated with various concentrations of compound PN for 24 h. The MTT assay was performed to determine cell viability, as previously described (Kim et al. 2017a).

Measurement of apoptosis

PC-3 cells were seeded at a density of 1×10^4 cells/well and then treated with various concentrations of compound PN for 24 h. After treatment, cells were collected and stained using the FITC Annexin V Apoptosis Detection Kit (BD Biosciences), according to the manufacturer's instructions. Apoptotic cells were counted by flow cytometry (FACSCalibur, Becton Dickinson Co. Franklin Lakes, NJ, USA) and data were analyzed using Cell Quest software (Becton Dickinson Co.).

Intracellular and mitochondrial reactive oxygen species (ROS) production

Intracellular and mitochondrial ROS production were assessed by DCFH-DA or MitoSOX staining, respectively (Kim et al. 2017a, c). PC-3 cells were cultured at a density of 1×10^4 cells/well, treated with various concentrations of compound PN for 24 h, and stained with 10 μM of DCFH-DA or 5 μM MitoSOX at 37 °C for 15 min in the dark. ROS production was measured using flow cytometry (FACSCalibur, Becton Dickinson Co.) and analyzed using Cell Quest software (Becton Dickinson Co.). Mitochondrial ROS images were obtained using a confocal microscope (Olympus, Tokyo, Japan).

Detection of acidic vesicular organelles (AVOs)

To quantify the number of AVOs, PC-3 cells were cultured at a density of 1×10^4 cells/well, treated with various

concentrations of compound PN for 24 h, and stained with 1 μM acridine orange (AO) at 37 °C for 20 min in the dark. The number of AVOs was determined using flow cytometry (FACSCalibur, Becton Dickinson Co.) and analyzed using the Cell Quest software (Becton Dickinson Co.).

Western blot analysis

Cells were lysed with lysis buffer [20 mg/ml aprotinin, 1 mM benzamdine, 5 mM EDTA (pH 8.0), 50 μl/ml leuptin, 1 mg/ml pepstatin, 1 mM PMSF, 150 mM NaCl, 10 mM Tris (pH 7.4), 1% Triton X-100]. Cell lysates were separated on 8–15% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to polyvinylidene fluoride (PVDF) membrane. After blocking membranes with 5% skim milk in TBS-T buffer [20 mM Tris (pH 7.4), 150 mM NaCl, 0.1% Tween 20] at room temperature for 2 h, and the membranes were incubated with specific primary antibodies at 4 °C overnight and washed three times with TBS-T buffer for 10 min. Finally, the membranes were incubated with secondary antibody at room temperature for 2 h and rewashed. Band densities were determined using a fluorescence scanner (LAS 3000, Fuji Film, Tokyo, Japan) and data were analyzed using Multi Gauge V3.0 software (Fuji Film).

Statistical analysis

Statistical comparisons were made using ANOVA. Results are presented as the means ± SDs of experiments performed in triplicate. P values < 0.001 were considered statistically significant.

Results

Compound PN induces caspase-3-dependent apoptosis in prostate cancer PC-3 cells

In a previous study by our group, we demonstrated that compound PN significantly inhibited cell proliferation in a concentration-dependent manner and induced cell cycle arrest and apoptosis in PC-3 cells in a dose-dependent manner (Choi 2017). Therefore, we examined the effect of PN treatment on the expression of apoptosis-related proteins, Bax, Bcl-2, caspase-3, and PARP. Compound PN did not affect the expression of Bax but led to reduced Bcl-2 expression, thus, significantly increasing the Bax/Bcl-2 ratio (Fig. 1a). Compound PN also reduced pro-caspase-3 expression and markedly increased levels of the cleaved forms of PARP and caspase-3 substrates (Fig. 1a). In addition, pretreatment with Z-DEVD-FMK, an inhibitor of caspase-3, significantly suppressed compound PN-induced apoptosis (Fig. 1b) and prevented the cleavage of caspase-3 and PARP (Fig. 1c). These results indicate that compound PN-induced apoptosis is associated with Bcl-2 family and caspase-3-dependent pathways in PC-3 cells.

Compound PN triggers intracellular and mitochondrial ROS generation in prostate cancer PC-3 cells

To determine whether apoptosis induced by compound PN in PC-3 cells is associated with ROS production, we examined levels of ROS status treatment with PN using DCHF-DA and MitoSOX staining. As shown in Fig. 2a, intracellular ROS levels were significantly increased from 6.72 to 40.62% after treatment with PN. The levels of ROS increased in a dose-dependent manner when treated with 2, 4, 6, and 8 μg/ml PN. Conversely, pretreatment with ROS inhibitors, either 5 mM NAC or 0.5 μM DPI, significantly prevented intracellular ROS production induced by compound PN (Fig. 2b). Mitochondrial-specific ROS production was increased with PN treatment, and was dramatically reduced by pretreatment with a general ROS scavenger, NAC, and by a mitochondrial ROS inhibitor, DPI, which inhibits NADPH oxidase (Fig. 2c). Confocal microscopy was used to confirm the location of mitochondrial ROS production. Compound PN significantly increased mitochondrial ROS, and these increases were suppressed by pretreatment with NAC or DPI (Fig. 2d). These results indicate that compound PN induces both intracellular and mitochondrial ROS production.

Pretreatment with NAC or DPI differentially regulates apoptosis through ROS production induced by compound PN in prostate cancer PC-3 cells

Since we observed both increases in ROS production and apoptosis after treatment with PN, we aimed to examine the relationship between ROS production and compound PN-induced apoptosis using NAC and DPI. As shown in Fig. 3a, pretreatment with NAC significantly increased induction of apoptosis from 54.13 to 89.03%, as compared with treatment with compound PN alone (Fig. 3a), as indicated by the significantly enhanced levels of cleavage of pro-caspase-3 and PARP (Fig. 3b). Previous reports have shown that pretreatment with NAC significantly increases anticancer agent-induced apoptosis (Qanungo et al. 2004). However, upon pretreatment with DPI, we found that induction of apoptosis was attenuated (Fig. 3c) and that there was suppression of compound PN-induced cleavage of pro-caspase-3 and PARP (Fig. 3d). These results suggest that the effects of ROS on apoptosis induced by compound PN depend on the source of the ROS production.

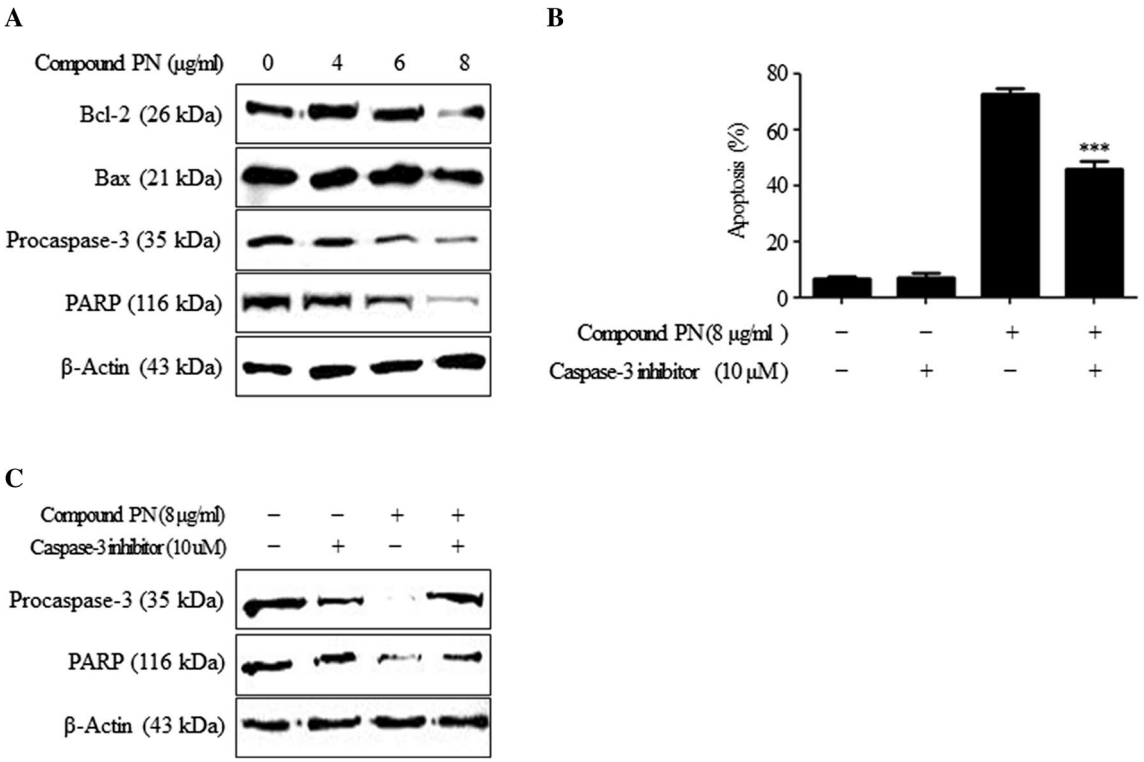

Fig. 1 Compound PN-induced caspase-3-dependent apoptosis in prostate cancer PC-3 cells. **a** Expression of apoptosis-related proteins. **b** Effects of Z-DEVD-FMK on apoptosis. **c** Effects of Z-DEVD-FMK on expression of apoptosis-related proteins. Cells were treated with the indicated concentrations of compound PN for 24 h in the presence or absence of pretreatment with 10 μM Z-DEVD-FMK. Apoptosis and apoptosis-related proteins were analyzed using annexin V/PI staining and western blotting, respectively. All data represent at least three independent experiments. Data represent the mean ± SD, ***$p < 0.001$

Compound PN induces autophagy in prostate cancer PC-3 cells

To investigate whether compound PN induces autophagy in PC-3 cells, we examined the formation of AVOs, a marker for autophagosome formation, by flow cytometry, using AO dye. As shown in Fig. 4a, the number of AVO-positive cells dramatically increased in a dose-dependent manner after treatment with compound PN for 24 h at differing concentrations. The conversion of LC3-I to LC3-II, a marker of autophagy, also increased in a dose-dependent manner. However, the intracellular levels of beclin-1, another marker of autophagy, were not altered (Fig. 4b). Pretreatment with 3-MA, a general autophagy inhibitor, reduced the number of AVO-positive cells (Fig. 4c) and inhibited the conversion of LC3-I to LC3-II (Fig. 4d), as compared with treatment of compound PN alone. These observations suggest that compound PN induces autophagy and is involved in the conversion of LC3-I to LC3-II and autophagosome formation.

Inhibition of autophagy enhances compound PN-induced apoptosis by increasing mitochondrial ROS production in prostate cancer PC-3 cells

To evaluate the relationship between autophagy and apoptosis, we assessed apoptosis induction after pretreatment with 3-MA in PC-3 cells. Pretreatment with 3-MA enhanced compound PN-induced apoptosis (from 60.52 to 89.29%), caspase-3 activation, and PARP cleavage (Fig. 5a, b). Previous studies have suggested that that ROS are key regulators of autophagy induction (Poillet-Perez et al. 2015). To examine whether ROS production regulates compound PN-induced autophagy, we measured autophagy induction after pretreatment with NAC or DPI. We found inhibition of intracellular ROS production via treatment with NAC decreased AVO formation from 57.68 to 21.58% and reduced the conversion of LC3-I to LC3-II, as compared with treatment with compound PN alone (Fig. 5c, d). However, inhibition of mitochondrial ROS production by treatment with DPI increased AVO formation and the conversion of LC3-I to

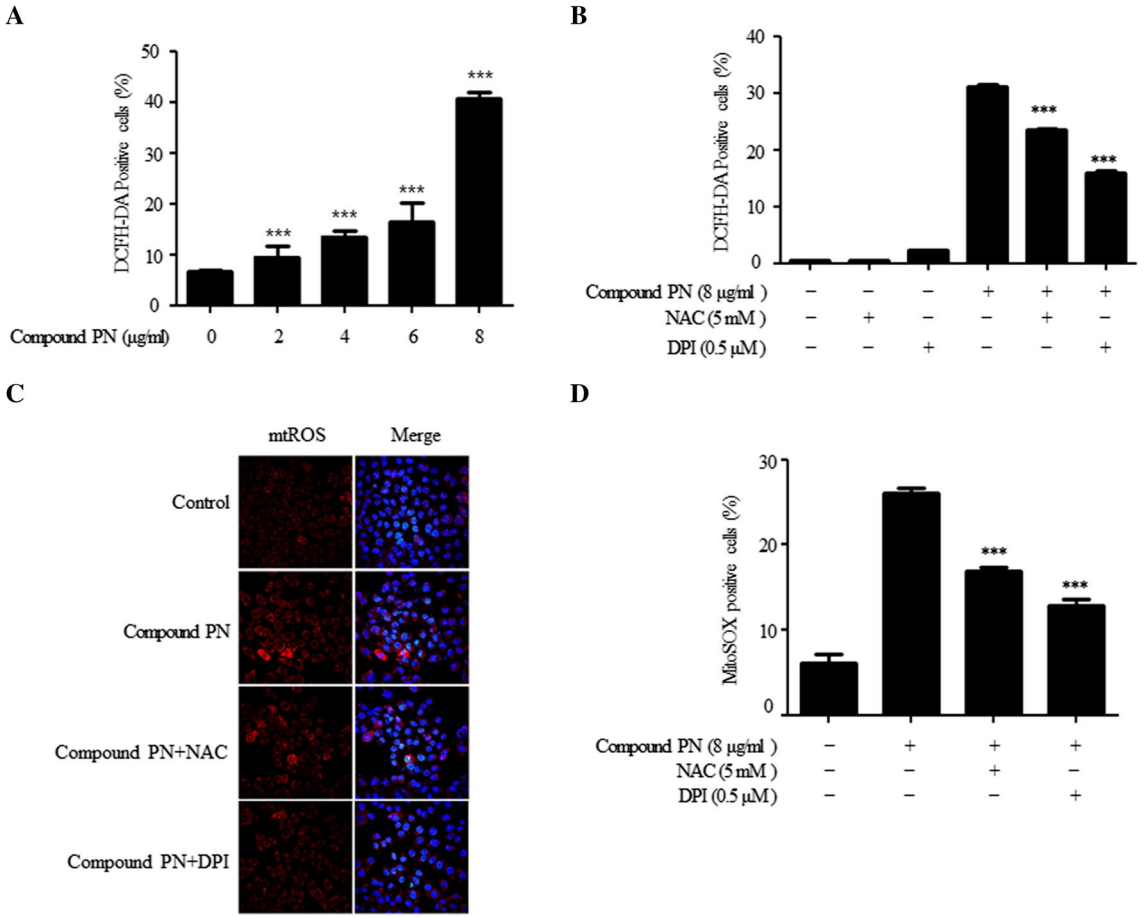

Fig. 2 Compound PN-induced production of both intracellular and mitochondrial reactive oxygen species (ROS) in prostate cancer PC-3 cells. **a** Intracellular ROS production. **b** Effects of NAC and DPI on intracellular ROS production. **c** Confocal microscopic images of mitochondrial ROS. **d** Effects of NAC and DPI on mitochondrial ROS production. Cells were pretreated with 5 mM NAC or 0.5 μM DPI for 1 h before treatment with indicated concentration of compound PN for 24 h. Cells were stained with 10 μM DCFH-DA or 5 μM MitoSOX. The fluorescence intensities were detected for intracellular or mitochondrial ROS production, respectively, using flow cytometry. Mitochondrial ROS images were obtained using a confocal microscope. All data represent at least three independent experiments. Data represent the mean ± SD, ***$p < 0.001$

LC3-II (Fig. 5e, f). These results suggest that ROS produced in different subcellular locations have differential effects on autophagy induced by treatment with compound PN, depending on the source of the ROS. Autophagy appears to be inversely proportional to apoptosis induced by intracellular ROS production and a reduction in mitochondrial ROS accumulation is responsible for enhanced autophagy, resulting in increased cell survival.

Discussion

PN is a bioactive compound isolated from the seeds of *Pharbitis nil* (L.) Choisy using Diaion HP-20, C18 reverse phase and Sephadex LH-20 column chromatography (Choi 2017). Recent reports have shown that lignans from *Pharbitis nil* lead to cell death in breast cancer cells (Ju et al. 2011; Kim et al. 2014). However, the molecular mechanisms underlying this process, including the mechanisms through which ROS, apoptosis, and autophagy are linked, have not been elucidated. The present study, aimed to assess the role of ROS in PN-induced autophagy and apoptosis in prostate cancer cells. Compound PN-induced apoptosis was confirmed through detection of the expression of the apoptosis-related proteins, Bax, Bcl-2, caspase-3, and PARP, indicating caspase-3-dependent activation. Our results demonstrated that ROS produced in different subcellular locations had differential effects on PN-induced autophagy and apoptosis. Compound PN-induced autophagy, as we observed increased formation of AVOs and LC3-II conversion, which were suppressed by 3-MA, an inhibitor of autophagy. Inhibition of autophagy by 3-MA also enhanced PN-induced apoptosis.

ROS are produced from different subcellular locations, including the mitochondria, endoplasmic reticulum, and

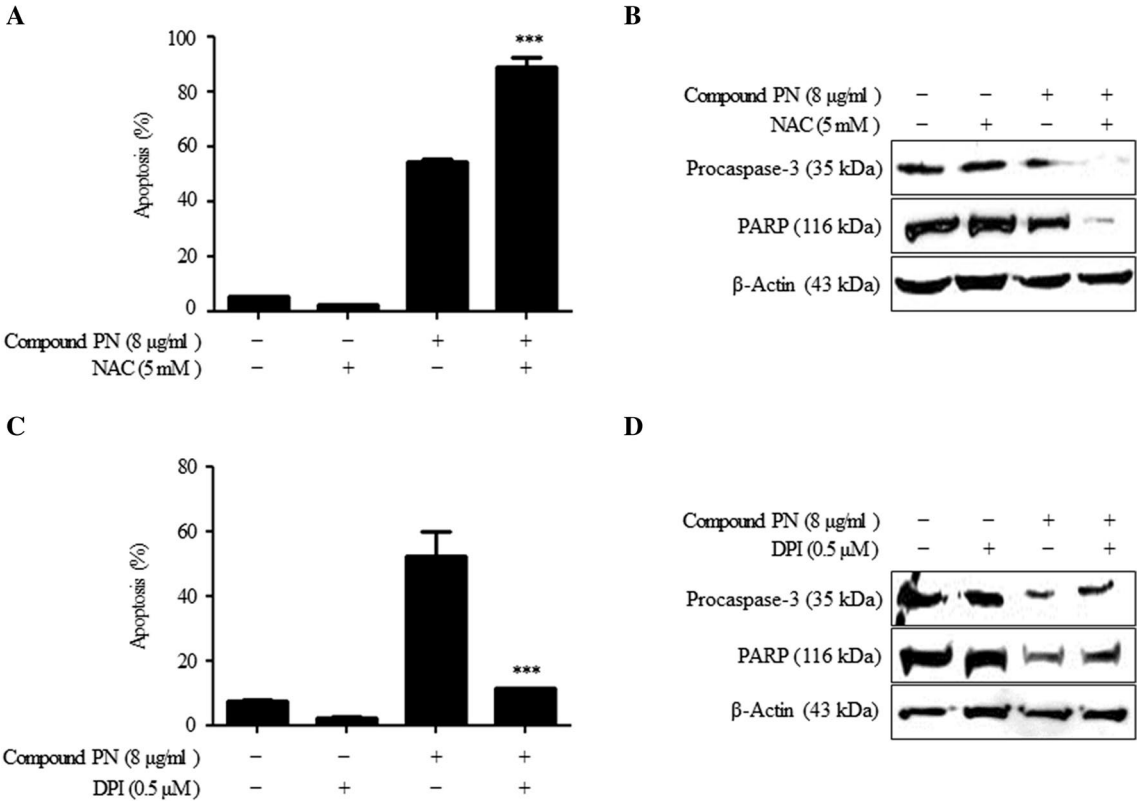

Fig. 3 Intracellular and mitochondrial ROS differentially regulate compound PN-induced apoptosis in prostate cancer PC-3 cells. **a** Effects of NAC on apoptosis. **b** Effects of NAC on expression of apoptosis-related proteins. **c** Effects of DPI on apoptosis. **d** Effects of DPI on expression of apoptosis-related proteins. Cells were pretreated with 5 mM NAC or 0.5 μM DPI for 1 h before treatment with the indicated concentration of compound PN for 24 h. Apoptosis and apoptosis-related proteins were analyzed using annexin V/PI staining and western blotting, respectively. All data are representative of at least three independent experiments. Data are shown as the mean ± SD, ***$p < 0.001$

peroxisomes and by many enzymes, such as, xanthine oxidase, lipoxygenase, cytochrome P450 monooxygenase, cyclooxygenase, NADPH oxidase, and nitric oxide synthase (Kim et al. 2007, 2017c). Oxidative stress has become an increasingly important therapeutic target for the treatment of cancer (Nicco and Batteux 2017) and several anticancer drugs have been designed to target ROS-mediated pathways in different cancer types (Kim et al. 2007, 2013). In the present study, inhibition of intercellular ROS production by NAC, a general ROS scavenger, considerably enhanced compound PN-induced apoptosis, but reduced its effect on autophagy. In contrast, inhibition of mitochondrial ROS generation using DPI, a mitochondrial ROS inhibitor, significantly reduced compound PN-induced apoptosis, but increased its effect on autophagy. These results suggest that compound PN induces the production of ROS from different subcellular locations, both from intracellular molecules and the mitochondria. Our findings suggest that ROS produced in these differing locations have different effects on PN-induced apoptosis and autophagy. We also found that the ROS produced in different locations had differing effects on cell death and survival. These findings are consistent with previous reports that autophagy is associated with the protection of damaged organelles (Daido 2005; Levine 2007; Song 2009) and cancer cell death (Kim et al. 2016, 2017b, c; Quillet-Mary 1997).

In summary, compound PN was found to induce ROS production from two sources, both intracellularly and from the mitochondria. ROS production in these locations differentially influenced apoptosis and autophagy, which either resulted in cell death or promoted cell survival through the regulation of autophagy to escape or enhance apoptotic cell death. Targeting the crosstalk between ROS-activated signals in induction of autophagy and apoptosis by treatment with compound PN represents a new strategy for prostate cancer treatment.

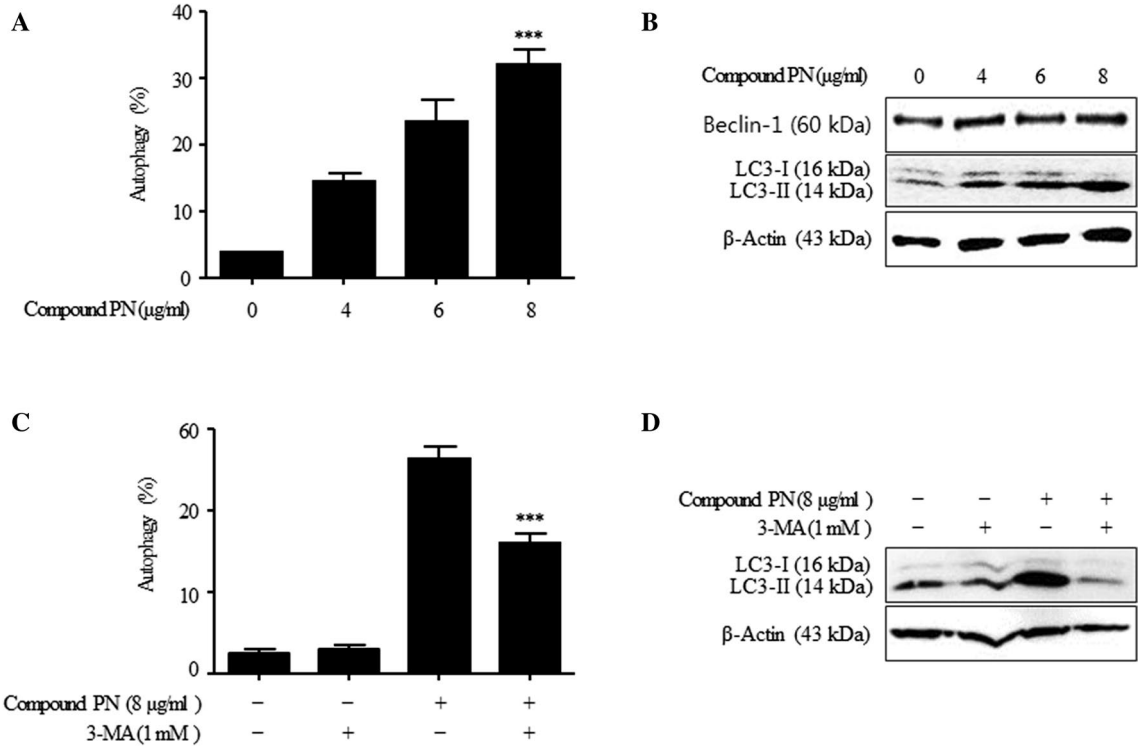

Fig. 4 Compound PN induces autophagy in prostate cancer PC-3 cells. **a** Autophagy induction. **b** Expression of beclin-1 and LC-3B. **c** Effects of 3-MA on autophagy. **d** Effects of 3-MA on expression of LC-3B. Cells were treated with different concentrations of compound PN for 24 h in the presence or absence of pretreatment with 1 mM 3-MA for 1 h. Autophagy and autophagy-related proteins were analyzed using acridine orange (AO) staining and western blotting, respectively. All data are representative of at least three independent experiments. Data are shown as the mean ± SD, $***p < 0.001$

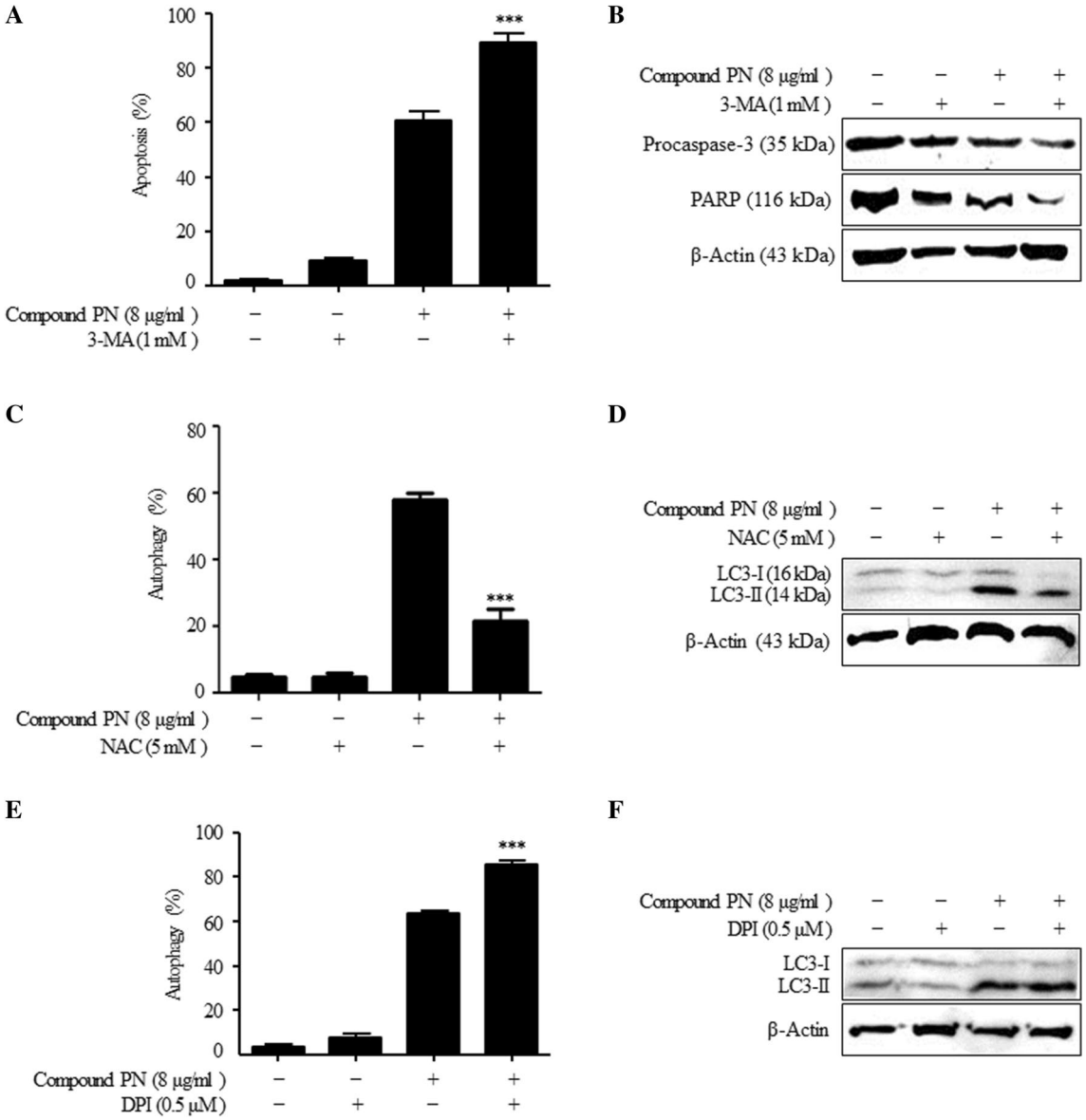

Fig. 5 Inhibition of autophagy enhanced compound PN-induced apoptosis by regulation of mitochondrial ROS production in prostate cancer PC-3 cells. **a** Effects of 3-MA on apoptosis. **b** Effects of 3-MA on expression of apoptosis-related proteins. **c** Effects of NAC on autophagy. **d** Effects of NAC on expression of LC-3B. **e** Effects of DPI on autophagy. **f** Effects of DPI on expression of LC-3B. Cells were pretreated with 5 mM NAC or 0.5 μM DPI for 1 h before treatment with the indicated concentrations of compound PN for 24 h. Apoptosis and apoptosis-related proteins were analyzed using annexin V/PI staining and western blotting, respectively. Autophagy and autophagy-related proteins were analyzed using acridine orange (AO) staining and western blotting, respectively. All data are representative of at least three independent experiments. Data are shown as the mean ± SD, $***p < 0.001$

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Author contributions HD Choi, KY Kim, and KI Park conducted research and experiments, designed research, and wrote the paper. SH Kim, SG Park, SN Yu and YW Kim conducted research and experiments. DS Kim, KT Chung and SC Ahn reviewed the literature, revised the manuscript and coordinated the study. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Human and animal rights The experimental procedure followed the actual law of animal protection that was approved by the Institutional Animal Care and Use Committee of Korea Institute of Oriental Medicine, Korea.

References

Chen YF et al (2017) The roles of reactive oxygen species (ROS) and autophagy in the survival and death of leukemia cells. Crit Rev Oncol Hematol 112:21–30

Choi HD et al (2017) Biological activities of Pharbitis nil and partial purification of anticancer agent from its extract. J Life Sci 27:225–232

Daido S et al (2005) Inhibition of the DNA-dependent protein kinase catalytic subunit radiosensitizes malignant glioma cells by inducing autophagy. Cancer Res 65:4368–4375. https://doi.org/10.1158/0008-5472.CAN-04-4202, https://www.ncbi.nlm.nih.gov/pubmed/15899829

Ju JH et al (2011) Induction of apoptotic cell death by *Pharbitis nil* extract in HER2-overexpressing MCF-7 cells. J Ethnopharmacol 133:126–131

Jung DY et al (2008) *Triterpenoid saponins* from the seeds of *Pharbitis nil*. Chem Pharm Bull (Tokyo) 56:203–206

Kim YA et al (2007) Mitochondria-mediated apoptosis by diallyl trisulfide in human prostate cancer cells is associated with generation of reactive oxygen species and regulated by Bax/Bak. Mol Cancer Ther 6:1599–1609

Kim KY et al (2013) Interplay of reactive oxygen species, intracellular Ca^{2+} and mitochondrial homeostasis in the apoptosis of prostate cancer cells by deoxypodophyllotoxin. J Cell Biochem 114:1124–1134

Kim KH et al (2014) Identification of antitumor lignans from the seeds of morning glory (*Pharbitis nil*). J Agric Food Chem 62:7746–7752

Kim SH et al (2016) Salinomycin simultaneously induces apoptosis and autophagy through generation of reactive oxygen species in osteosarcoma U2OS cells. Biochem Biophys Res Commun 473:607–613

Kim KY et al (2017a) Inhibition of autophagy promotes salinomycin-induced apoptosis via reactive oxygen species-mediated PI3K/AKT/mTOR and ERK/p38 MAPK-dependent signaling in human prostate cancer cells. Int J Mol Sci 18

Kim KY et al (2017b) Salinomycin induces reactive oxygen species and apoptosis in aggressive breast cancer cells as mediated with regulation of autophagy. Anticancer Res 37:1747–1758

Kim SH et al (2017c) Mitochondrial ROS activates ERK/autophagy pathway as a protected mechanism against deoxypodophyllotoxin-induced apoptosis. Oncotarget 8(67):111581–111596

Kim KH, Choi SU, Lee KR (2009) Diterpene glycosides from the seeds of *Pharbitis nil*. J Nat Prod 72:1121–1127

Ko SG et al (2004) Induction of apoptosis by *Saussurea lappa* and *Pharbitis nil* on AGS gastric cancer cells. Biol Pharm Bull 27:1604–1610

Lee WL, Huang JY, Shyur LF (2013) Phytoagents for cancer management: regulation of nucleic acid oxidation, ROS, and related mechanisms. Oxid Med Cell Longev 2013:925804

Levine B (2007) Cell biology: autophagy and cancer. Nature 446:745–747

Liou GY, Storz P (2010) Reactive oxygen species in cancer. Free Radic Res 44:479–496

Nicco C, Batteux F (2017) ROS Modulator Molecules with therapeutic potential in cancers treatments. Molecules 23(1):84

Panieri E, Santoro MM (2016) ROS homeostasis and metabolism: a dangerous liason in cancer cells. Cell Death Dis 7:e2253

Poillet-Perez L, Despouy G, Delage-Mourroux R, Boyer-Guittaut M (2015) Interplay between ROS and autophagy in cancer cells, from tumor initiation to cancer therapy. Redox Biol 4:184–192

Qanungo S, Wang M, Nieminen AL (2004) *N*-acetyl-L-cysteine enhances apoptosis through inhibition of nuclear factor-κB in hypoxic murine embryonic fibroblasts. J Biol Chem 279:50455–50464

Quillet-Mary A et al (1997) Implication of mitochondrial hydrogen peroxide generation in ceramide-induced apoptosis. J Biol Chem 272:21388–21395

Ray PD, Huang BW, Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. Cell Signal 24:981–990

Song J et al (2009) Hypoxia-induced autophagy contributes to the chemoresistance of hepatocellular carcinoma cells. Autophagy 5:1131–1144

Srivastava KK, Kumar R (2015) Stress, oxidative injury and disease. Indian J Clin Biochem 30:3–10

Uttara B, Singh AV, Zamboni P, Mahajan RT (2009) Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Curr Neuropharmacol 7:65–74

Yonekawa T, Thorburn A (2013) Autophagy and cell death. Essays Biochem 55:105–117

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