Original Paper

Plumbagin Triggers ER Stress-Mediated Apoptosis in Prostate Cancer Cells via Induction of ROS

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Key Words

Reactive oxygen species • Plumbagin • ER stress • Prostate cancer

Abstract

Background/Aims: Prostate cancer (PCa) is the second most frequently diagnosed cancer in men worldwide. Currently available therapies for hormone-refractory PCa are only marginally effective. Plumbagin (PLB), a natural naphthoquinone isolated from the traditional folk medicine *Plumbago zeylanica*, is known to selectively kill tumor cells. Nevertheless, antitumor mechanisms initiated by PLB in cancer cells have not been fully defined. **Methods:** MTT assay was used to evaluate the effect of PLB on the viability of cancer cells. Cell apoptosis and reactive oxygen species (ROS) production were determined by flow cytometry. Protein expression was detected by western blotting. *In vivo* anti-tumor effect was measured by using tumor xenograft model in nude mice. **Results:** In the present study, we found that PLB decreases cancer cell growth and induces apoptosis in DU145 and PC-3 cells. In addition, by increasing intracellular ROS levels, PLB induced a lethal endoplasmic reticulum stress response in PCa cells. Importantly, blockage of ROS production significantly reversed PLB-induced ER stress activation and cell apoptosis. *In vivo*, we found that PLB inhibits the growth of PCa xenografts without exhibiting toxicity. Treatment of mice bearing human PCa xenografts with PLB was also associated with induction of ER stress activation. **Conclusion:** Inducing ER stress by PLB thus discloses a previously unrecognized mechanism underlying the biological activity of PLB and provides an in-depth insight into the action of PLB in the treatment of hormone-refractory PCa.

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Introduction

Prostate cancer (PCa) is the second most frequently diagnosed cancer in men worldwide, with 1.1 million new cases estimated to have occurred in 2012 [1]. Current prostate cancer therapy includes surgery, hormone therapy, radiation, and chemotherapy [2]. Despite the recent advances in diagnostic methods and improvement in treatment

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strategies, the prognosis of hormone-refractory PCa remains largely unsatisfactory [3, 4]. PCa first manifests as an androgen-dependent disease and can be treated with androgen-deprivation therapy. However, androgen-deprivation therapy eventually fails, and progress into the hormone-refractory stage, which accounts for the majority of PCa patient deaths [5]. On the other hand, chemotherapy usually brings serious side effects and drug resistance in PCa patients. Therefore, new therapeutic agents that can against hormone-refractory PCa are urgently needed.

Natural products have historically been invaluable as a source of therapeutic agents, and served human kind in the treatment of various diseases for centuries [6, 7]. It is roughly estimated that half of modern marketed drugs originate from natural products [8]. Plumbagin (PLB), a natural naphthoquinone isolated from the traditional folk medicines *Plumbago zeylanica*, was recently identified as selectively toxic to cancer cells *in vitro* and *in vivo* [9-11]. The roots of *Plumbago zeylanica* have been used in Indian medicine for more than 2,500 years for treatments of various diseases [11]. PLB has also been shown to inhibit proliferation and induce apoptosis of PCa cells [11-13]. However, antitumor mechanisms initiated by PLB in PCa cells have not been fully defined.

In this study, we show that PLB significantly inhibits the growth of PCa cells *in vitro* and *in vivo*. Mechanistically, we present in this study for the first time that ER stress contributes to PLB-induced apoptosis in PCa cells. By increasing intracellular ROS levels, PLB induced a lethal endoplasmic reticulum stress response in PCa cells. Importantly, blockage of ROS production significantly reversed PLB-induced ER stress activation and cell apoptosis. *In vivo*, we found that PLB inhibits the growth of PCa xenografts without exhibiting toxicity. Treatment of mice bearing human PCa xenografts with PLB was also associated with induction of ER stress activation. Our study provides an in-depth insight into the action of PLB in the treatment of hormone-refractory PCa.

Materials and Methods

Reagents and cell culture

Plumbagin (PLB), N-acetylcysteine (NAC), catalase were purchased from Sigma (St. Louis, MO). Antibodies including anti-GAPDH, goat anti-mouse IgG-HRP and donkey anti-rabbit IgG-HRP were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies including anti-CHOP, anti-ATF4, anti-p-eIF2 α and anti-eIF2 α were purchased from Cell Signaling Technology (Danvers, MA). FITC Annexin V apoptosis Detection Kit I and Propidium Iodide (PI) were purchased from BD Pharmingen (Franklin Lakes, NJ). Human prostate cancer cell lines DU145 and PC-3 were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The cells were maintained in RPMI 1640 medium (Gibco, Eggenstein, Germany) containing 10% fetal bovine serum (FBS), 100 units/mL penicillin, and 100 μ g/mL streptomycin in a humidified cell incubator with an atmosphere of 5% CO₂ at 37°C.

Cell viability assay

Cells were seeded into 96-well plates at a density of 8×10^3 per well and allowed to attach overnight in RPMI 1640 containing 10% FBS. PLB was dissolved in DMSO and diluted with 1640 medium to final concentrations of 1, 2, 4, 6, 8, 10, 15 and 20 μ M. The tumor cells were incubated with PLB for 24 h or 48 h before the MTT assay.

Cell apoptosis analysis

Apoptosis of prostate cancer cells treated with PLB was analyzed using a fluorescein isothiocyanate (FITC) Annexin V apoptosis detection kit. The cells (2×10^5 cells) were seeded on 6-well plates and incubated for 24 h in medium until cells reached 70% confluency. Then, cells were treated with PLB in a dose-dependent manner for 24 h. Cells were then harvested, washed twice with ice-cold PBS, and evaluated for apoptosis by double staining with FITC conjugated Annexin V and Propidium Iodide (PI) in binding buffer for 30 min using a FACSCalibur flow cytometer.

Colony formation assay

Cells were seeded in six-well plates at a density of 1000 cells per well and allowed to attach overnight in RPMI 1640 containing 10% FBS. Cells were treated with PLB for 24 h after which the chemical was removed by extensive washing and incubation was continued for an additional 7 days. The plates were washed twice with PBS and fixed with 4% paraformaldehyde for 15 min and then stained with 1% crystal violet for 5 min. All statistical measurements were acquired from three independent experiments.

Western blotting analysis

Cells or tumor tissues were homogenized in protein lysate buffer, and debris was removed by centrifugation at 12,000 g for 10 min at 4°C. Concentrations of protein in whole-cell extracts were determined using the Bradford protein assay (Bio-Rad, Hercules, CA) with BSA as the standard. After addition of sample loading buffer, protein samples were electrophoresed and then transferred to poly-vinylidene difluoride transfer membranes. The blots were blocked for 2 h at room temperature with fresh 5% nonfat milk in TBST and then incubated with specific primary antibody in TBST overnight at 4°C. Following three washes with TBST, the blots were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h, and the immunoreactive bands were visualized by using ECL kit (Bio-Rad, Hercules, CA). The density of the immunoreactive bands was analyzed using Image J computer software (National Institute of Health, MD).

Quantitative RT-PCR

Cells were homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA) for extraction of RNA according to the manufacturer's protocol. Both reverse transcription and quantitative PCR were carried out using a two-step M-MLV Platinum SYBR Green qPCR SuperMix-UDG Kit (Invitrogen, Carlsbad, CA). Eppendorf mastercycler eprealplex detection system (Eppendorf, Hamburg, Germany) was used for q-PCR analysis. The following gene-specific primer pairs were used: CHOP: (F) 5'-atggcagctgagtcattgccttc-3', (R) 5'-agaagcaggggtcaagagtggtgaa-3'. β -actin: (F) 5'-ttcctgggcatggagtcct-3', (R) 5'-aggaggagcaatgatcttgatc-3'. Gene expressions were analyzed with the comparative threshold cycle (Ct) method after normalizing to the housekeeping gene β -actin.

Measurement of reactive oxygen species generation

Cellular ROS contents were measured by flow cytometry (BD Bioscience, CA). Briefly, 2×10^5 cells were plated on 6-well plates, allowed to attach overnight, and exposed to PLB for the indicated times. Cells were stained with 10 μ M DCFH-DA (Sigma, St. Louis, MO) at 37°C for 30 min. Cells were collected and the fluorescence was analyzed using a FACSCalibur flow cytometer. In some experiments, cells were pretreated with 5 mM NAC for 2 h prior exposure to compounds and analysis of ROS generation.

Transient transfection of small interfering RNA (siRNA)

The siRNA duplexes used in this study were purchased from Invitrogen (Carlsbad, CA, USA) and have the following sequences: CHOP (5'-GAGCUCUGAUUGACCGAAUGGUGAA-3'). Negative Universal Control (Invitrogen) was used as the control. DU145 cells (2×10^5 /well) were seeded into 6-well plates and cultured for 24 h, and then were transfected with siRNA duplexes against human CHOP (100 nM) or control siRNA by lipofectamine 2000 (Invitrogen) according to manufacturer's protocol. Cells were further incubated for 48 h before harvest for detection of CHOP expression by Western blot.

Determination of caspase-3/9 activity

Caspase-3/9 activity in cell lysates was determined using a Caspase-3/9 activity kit (Beyotime Institute of Biotechnology, Nantong, China) according to the manufacturer's protocol. The caspase-3/9 activity was normalized by the protein concentration of the corresponding cell lysate and expressed as percentage of treated cells to that of control.

In vivo antitumor study

All animal experiments were complied with the Wenzhou Medical University's Policy on the Care and Use of Laboratory Animals. Five-week-old athymic BALB/cA nu/nu female mice (18-22 g) purchased from Vital River Laboratories (Beijing, China) were used for *in vivo* experiments. Animals were housed at a constant room temperature with a 12 h light/12 h dark cycle and fed a standard rodent diet and water.

DU145 cells were harvested and injected subcutaneously (5×10^6 cells in 100 μ L of PBS) into the right flank of mice. Mice were treated with PLB at the dose of 2 mg/kg body weight by intraperitoneal (i.p.) injection once every other day of induction. At the end of experiment, the animals were sacrificed and the tumors were removed and weighed for use in proteins expression studies. The tumor volumes were determined by measuring length (l), width (w) and calculating volume ($V = 0.5 \times l \times w^2$) at the indicated time points.

MDA assay

Tumors were harvested after all mice were sacrificed. The tissue samples were homogenized and sonicated in RIPA buffer on ice. Tissue lysates were then centrifuged at 12,000 g for 10 min at 4 °C to collect the supernatant. The total protein content was determined by using the Bradford protein assay kit (Bio-Rad, Hercules, CA). Tumor tissue proteins were normalized according to their concentrations and subjected to MDA assay as described in the Lipid Peroxidation MDA assay kit (Beyotime Institute of Biotechnology, Nantong, China). MDA levels were detected using multimode microplate readers (SpectraMax M5, Molecular Devices, USA) at 532 nm.

Statistical analysis

All experiments were assayed in three independent experiments ($n = 3$). Data are expressed as means $\pm$ SEM. All statistical analyses were performed using GraphPad Prism version 5.0 (GraphPad, San Diego, CA). Student's t-test and two-way ANOVA were employed to analyze the differences between sets of data. A p value <0.05 was considered statistically significant.

Results

PLB suppresses cells growth and induces apoptosis in prostate cancer cells

We first determined the effects of PLB on cell viability of two human prostate cancer cell lines, DU145 and PC-3, by MTT assay. As shown in Fig. 1B-1C, treated with PLB for 24 h or 48 h significantly decreases the viability of DU145 and PC-3 cells, and these decreases were dose dependent. To elucidate the mechanisms of PLB treatment induced growth inhibition, we then examined the pro-apoptotic effects of PLB on DU145 and PC-3 cells. As shown in Fig. 1D-1G, treatment with PLB for 24 h dose dependently increases the proportion of apoptotic cells in both DU145 and PC-3 cells. We confirmed these findings by caspase activity assay and found that PLB markedly increases caspase 3 and caspase 9 activation, suggesting the activation of apoptosis pathway (Fig. 1H-1I). To further determine the effects of PLB on the growth of prostate cancer cells, we conducted colony formation assay in DU145 cell line. As shown in Fig. 1J-1K, PLB significantly inhibits colony formation ability of DU145 cells, indicating that PLB has potentially inhibitory effects on prostate cancer cell growth.

PLB induces oxidative stress in prostate cancer cells

ROS generation has been reported to play an important role in the pro-apoptotic effects of PLB in some tumor cell lines [14, 15]. Therefore, we examined the levels of intracellular ROS in PLB-treated and untreated cells by flow cytometry. As shown in Fig. 2A-2B, PLB treatment caused a time-dependent increase in ROS levels in DU145 and PC-3 cells. In addition, treatment with PLB for 1 h in DU145 cells caused a dose-dependent increase in ROS levels (Fig. 2C). To identify the role of ROS in mediating PLB's anti-cancer effects, ROS scavenger NAC was used. As shown in Fig. 2D, pre-treatment with NAC markedly reversed the PLB-induced increase in ROS levels. The MTT results revealed that scavenging of ROS significantly attenuated PLB-induced cell growth inhibition against DU145 cells (Fig. 2E). Similar results were observed in PC-3 cells (Fig. 2F-2H). To further determine the role of ROS in mediating PLB's anti-cancer effects, other antioxidant catalase was used. As shown in Fig. 2I, pretreatment with catalase (2000 U/mL) for 2 h markedly reversed the PLB-induced increase in ROS levels. The MTT results revealed that pretreatment with catalase (2000 U/mL) for 2 h significantly attenuated PLB-induced cell growth inhibition against DU145 cells (Fig. 2J).

Fig. 1. PLB suppresses cells growth and induces apoptosis in prostate cancer cells. (A) The chemical structure of PLB. (B-C) DU145 or PC-3 cells were incubated with increasing doses of PLB (1–20 μM) for 24 h or 48 h respectively. Cell viability was determined by MTT assay. (D-G) DU145 or PC-3 cells were incubated with PLB for 24 h, percentage of cell apoptosis was determined by Annexin-V/PI staining and flow cytometry. (H-I) Cells were incubated with PLB for 20 h, caspase 3 and caspase 9 activity in the cell extracts were determined by an assay kit using specific substrate. (J-K) PLB inhibited the colony formation ability of DU145 cells. The results shown are representative of at least three independent experiments. The p value were obtained based on student t-test. * $p < 0.05$ versus DMSO treatment.

Fig. 2. PLB induces oxidative stress in prostate cancer cells. (A-B) Intracellular ROS generation induced by PLB was measured in DU145 or PC-3 cells by staining with DCFH-DA (10 μM) and flow cytometry analysis. (C and F) Intracellular ROS generation induced by increasing doses of PLB was measured in DU145 or PC-3 cells by staining with DCFH-DA (10 μM) and flow cytometry analysis. (D and G) Cells were pre-incubated with 5 mM GSH for 2 h before exposure to PLB. Intracellular ROS generation was measured by flow cytometry. (E and H) Cells were pre-incubated with or without 5 mM NAC for 2 h before exposure to PLB for 24 h. Cell viability was determined by MTT assay. (I) Cells were pre-incubated with 2000 U/mL catalase for 2 h before exposure to PLB. Intracellular ROS generation was measured by flow cytometry. (J) Cells were pre-incubated with or without 2000 U/mL catalase for 2 h before exposure to PLB for 24 h. Cell viability was determined by MTT assay. * $p < 0.05$ versus DMSO treatment. # $p < 0.05$ versus PLB treatment.

Fig. 3. PLB activates ER stress pathway in prostate cancer cells. (A-C) DU145 cells were treated with PLB (10 μM) for the indicated times, the protein levels of p-eIF2α and ATF4 were determined by western blot. (D-F) DU145 cells were treated with PLB for 3 h, the protein levels of p-eIF2α and ATF4 were determined by western blot. (G) DU145 cells were treated with PLB for 6 h. The mRNA expression of CHOP was analyzed by qRT-PCR. (H) DU145 cells were treated with PLB (10 μM) for the indicated times, the protein level of CHOP was determined by western blot. (I) DU145 cells were treated with PLB for 12 h, the protein level of CHOP was determined by western blot. *p<0.05 versus DMSO treatment.

ER stress contributes to PLB-induced apoptosis in prostate cancer cells

The next step is to investigate the underlying mechanisms of the anti-cancer effects of PLB. DU145 cells were used for the subsequent studies. Increased ROS levels and perturbation in the intracellular redox status increase the levels of unfolded proteins in the ER and induce ER stress response [16, 17]. Therefore, we next examined the expressions of ER stress-related proteins, such as p-eIF2α and ATF4 in PLB-treated DU145 cells. The time-course result indicated that PLB (10 μM) could significantly activate ER stress. The expression levels of p-eIF2α and ATF4 reached the peak at 3 h after treatment (Fig. 3A-3C). Treatment with PLB also dose-dependently increased the expression of p-eIF2α and ATF4 in the DU145 cells (Fig. 3D-3F). CHOP induction is probably the most sensitive to ER

Fig. 4. ER stress contributes to PLB-induced apoptosis in prostate cancer cells. (A) DU145 cells were infected with CHOP siRNA or control siRNA, the mRNA expression of CHOP was analyzed by qRT-PCR after stimulation with PLB (10 μ M) for 6 h. (B) DU145 cells transfected with CHOP siRNA or control siRNA were treated with PLB (10 μ M) for 12 h, the protein level of CHOP was determined by western blot. (C-D) DU145 cells transfected with CHOP siRNA or control siRNA were treated with PLB (10 μ M) for 24 h, percentage of cell apoptosis was determined by Annexin-V/PI staining and flow cytometry. (E-F) DU145 cells transfected with CHOP siRNA or control siRNA were treated with PLB (10 μ M) for 20 h, caspase 3 and caspase 9 activity in the cell extracts were determined by an assay kit using specific substrate. * $p < 0.05$ versus DMSO treatment. # $p < 0.05$ versus PLB treatment.

stress response, and CHOP is considered as a marker of commitment of ER stress-induced apoptosis. We found that PLB treatment resulted in significant increases in the mRNA and protein levels of CHOP (Fig. 3G-3I).

To further investigate whether ER stress was involved in the anti-tumor effects of PLB. We next examined the effect of siRNA-mediated depletion of CHOP in DU145 cells. Knockdown of CHOP by siRNA, markedly attenuated CHOP expression in the mRNA and protein levels (Fig. 4A-4B). This was associated with an appreciable reduction in PLB-induced apoptosis, caspase 3 and caspase 9 activation in DU145 cells (Fig. 4C-4F). These findings demonstrate that PLB-induced cell apoptosis is at least partly mediated by ER stress pathway.

Fig. 5. Induction of ER stress activation and cancer cell apoptosis by PLB is dependent on ROS production. (A-B) DU145 cells were pre-incubated with or without 5 mM NAC for 2 h before exposure to PLB for 3 h. The protein levels of p-eIF2 α and ATF4 were determined by western blot. (C) DU145 cells were pre-incubated with or without 5 mM NAC for 2 h before exposure to PLB for 6 h. The mRNA expression of CHOP was analyzed by qRT-PCR. (D) DU145 cells were pre-incubated with or without 2000 U/mL catalase for 2 h before exposure to PLB for 6 h. The mRNA expression of CHOP was analyzed by qRT-PCR. (E) DU145 cells were pre-incubated with or without 5 mM NAC for 2 h before exposure to PLB for 12 h. The protein level of CHOP was determined by western blot. (F-G) DU145 cells were pre-incubated with or without 5 mM NAC for 2 h before exposure to PLB for 24 h, percentage of cell apoptosis was determined by Annexin-V/PI staining and flow cytometry.

Fig. 6. PLB inhibits DU145 xenograft tumor growth in vivo, accompanied with increased ROS level and ER stress activation. (A-B) PLB treatment significantly inhibited tumor volume (A) and tumor weight (B) of DU145 human prostate cancer xenografts in nude mice, but did not affect body weight (C) of mice. (D) HE staining of the major organs, magnification 20x. (E) The levels of MDA in the tumor tissues. (F) The mRNA expression of CHOP in the tumor tissues. (G-H) Western blot analysis on the expression of CHOP from respective tumor tissue lysates. GAPDH was used as protein loading control.

Induction of ER stress activation and cancer cell apoptosis by PLB is dependent on ROS production

We next tested the effects of ROS inhibition on PLB-induced ER stress activation. As shown in Fig. 5A-5B, PLB-induced ER stress activation, including the expression of p-eIF2 α and ATF4 was significantly blocked by NAC pretreatment in DU145 cells. In addition, the PLB-induced effects on CHOP expression in the mRNA and protein levels were attenuated by pretreatment with NAC or catalase (Fig. 5C-5E). Consistent with the abolishment of ER stress activation, NAC pretreatment fully reversed PLB-induced cell apoptosis in DU145 cells (Fig. 5F-5G). These results suggest that ROS production may be the primary and up-stream mechanism in mediating PLB's anti-cancer activity.

PLB inhibits DU145 xenograft tumor growth in vivo, accompanied with increased ROS level and ER stress activation

To evaluate the *in vivo* impact of PLB treatment, we used a subcutaneous xenograft model of DU145 cells in immunodeficient mice. Intraperitoneal administration of PL at dose of 2 mg/kg significantly reduced DU145 tumor volume and weight versus vehicle control (Fig. 6A-6B). Importantly, PLB treatment was well tolerated, without significant weight loss (Fig. 6C). Histopathological analyses of vital organs (liver and kidney) also revealed that PLB treatment did not result in the toxicity (Fig. 6D). Mechanistically, we found that PLB treatment significantly increased the level of lipid peroxidation product (MDA), a marker of ROS, in tumor tissues (Fig. 6E). In addition, PLB treatment increased the expression of CHOP in the mRNA and protein levels (Fig. 6F-6H).

Discussion

PCa is the most common type of cancer in american men and ranks second to lung cancer in cancer-related deaths [1]. Hormone-refractory invasive PCa is the end stage and accounts for the majority of PCa patient deaths [2]. At present, there is no effective treatment for androgen independent metastatic PCa [18]. Therefore, there is an urgent need for novel agents that can be effective and selective in the treatment of hormone-refractory PCa. We present here that PLB, a quinoid constituent isolated from the roots of medicinal plant *Plumbago zeylanica*, inhibits the growth and induces apoptosis of hormone-refractory PCa cells. PLB stimulated a rapid increase in reactive oxygen species (ROS) production in PCa cells. By increasing intracellular ROS levels, PLB increased expression of some ER stress regulatory proteins in a dose dependent manner in DU145 cells. Importantly, blockage of ROS production significantly reversed PLB-induced ER stress activation and cell apoptosis. *In vivo*, administration of PLB (2 mg/kg), beginning 15 days after ectopic implantation of hormone-refractory DU145 PCa cells, reduces both tumor weight and volume by 70%. Treatment of mice with PLB was also associated with induces of ER stress activation. These findings imply that PLB induces apoptosis of PCa cells through ROS-mediated ER stress pathway as illustrated in Fig. 7.

Reactive oxygen species (ROS) are by-products of aerobic metabolism. ROS act as secondary messengers in cell signaling and are required for various biological processes in normal cells [19]. At low levels, ROS act as signaling molecules to activate proliferation and survival pathways. At moderately increased levels, ROS damage DNA and promote mutagenesis in cells. High ROS levels, however, exert an oxidative stress on the cell that can ultimately cause cell senescence or death [20]. Compared with normal cells, many types of cancer cell have increased levels of ROS [17, 20]. Therefore, it might be possible to selectively kill cancer cells by pharmacological ROS insults [21-23]. PLB occurs naturally in the medicinal herb *Plumbago zeylanica*, which has been safely used for centuries in oriental medicine for treatment of various ailments, including microbial infections and allergic reactions. PLB has also been found in *Juglans cinerea* (whitenut) and *Juglans nigra* (blacknut) [24]. Recently, PLB was found to be a promising anticancer compound [10, 25]. Some previous studies have shown that PLB can induce oxidative stress in various cell lines, and induction of ROS production is involved in the biological functions of PLB [14, 26, 27]. In accordance with previous studies, results of the present study indicated that PLB induced a rapid increase in ROS production in DU145 and PC-3 cells. It has recently been shown that PLB interacts with thioredoxin reductase 1 (TrxR1) to induce ROS in HL-60 cells [28]. Although not tested in our study, it is possible that PLB induces ROS in prostate cancer cells though similar mechanisms involving TrxR1 or GSH. Elucidating these mechanisms is a pressing issue and a focus of future studies.

Endoplasmic reticulum is also well known to regulate cellular responses to stress [29]. Aberrant accumulation of misfolded/unfolded proteins and lipids or sudden changes of the endoplasmic reticulum Ca^{2+} homeostasis leads to a cellular adaptive response known as

endoplasmic reticulum stress (ER stress) [29, 30]. Accumulation of misfolded proteins in ER can cause ER stress and ultimately lead to apoptosis [30]. ER stress-induced cancer cell apoptosis becomes an important signaling target for development of cancer therapeutic drugs. The inductions of cancer cell apoptosis by some anti-cancer agents such as auranofin and arsenic trioxide have been reported to be mediated by ER stress [31, 32]. It has recently been shown that metformin could induce ER stress-dependent apoptosis in prostate cancer cells [33]. Therefore, the therapeutic modulation of the proapoptotic ER stress could be a potential therapeutic strategy for hormone-refractory PCa treatment [33, 34]. However, PLB's effects on pro-apoptotic-ER stress in hormone-refractory PCa cells is unknown. By inducing ROS levels and oxidative stress, PLB treatment concomitantly induces ER stress response, which is highlighted by elevated levels of p-eIF2 α , and ATF4, as well as increase in the levels of CHOP. CHOP is considered as a marker of commitment of ER stress-induced apoptosis. Findings presented here demonstrated that the siRNA-mediated knockdown of CHOP significantly inhibits PLB-induced apoptosis in DU145 cells. Moreover, our findings showed that blockage of ROS production by NAC fully reversed PLB-induced ER stress and cell apoptosis, suggesting ER stress activation and cancer cell apoptosis induced by PLB is dependent on ROS production.

Fig. 7. Schematic illustration of the underlying mechanism of PLB's anti-cancer activity.

Conclusion

In summary, we investigated and reported the anti-tumor effects of PLB and the potential underlying mechanisms. We found that PLB reduced the growth of PCa cells through increased production of ROS, and activation of ER stress pathway. These results indicate that PLB possesses great potential as a promising candidate for the treatment of PCa. In addition, our results indicate that ROS production and ER stress could be targeted for the development of new anti-cancer drugs.

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Disclosure Statement

The authors disclose no potential conflict of interest.

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