A platycoside-rich fraction from the root of *Platycodon grandiflorum* enhances cell death in A549 human lung carcinoma cells via mainly AMPK/mTOR/AKT signal-mediated autophagy induction

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**A R T I C L E   I N F O**

Chemical compounds studied in this article:
Platycodon D (PubChem CID: 162859)
Drapr-plateycon D (PubChem CID: 70698266)
Polygalacin D (PubChem CID: 102004664)
Polygalacin D2 (PubChem CID: 53325781)
Platycoside J (PubChem CID: 11528185)

Keywords:
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Autophagy
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**A B S T R A C T**

**Ethnopharmacological relevance:** The root of *Platycodon grandiflorum* (PG), commonly known as Kilkyong in Korea, Jiegeng in China, and Kikyo in Japan, has been extensively used as a traditional anti-inflammatory medicine in Asia for the treatment of respiratory conditions, such as bronchitis, asthma, and tonsillitis. Platycosides isolated from PG are especially well-known for their anti-cancer effects.

**Aim of the study:** We investigated the involvement of autophagic cell death and other potential molecular mechanisms induced by the platycoside-containing butanol fraction of PG (PGB) in human lung carcinoma cells.

**Materials and methods:** PGB-induced growth inhibition and cell death were measured using a 5-diphenyl-tetrazolium bromide (MTT) assay. The effects of PGB on autophagy were determined by observing microtubule-associated protein 1 light chain 3 (LC3) redistribution with confocal microscopy. The PGB-mediated regulation of autophagy-associated proteins was investigated using Western blotting analysis. Furthermore, the anti-cancer mechanism of PGB was confirmed using chemical inhibitors. A high-performance liquid chromatography (HPLC)-DAD system was used to analyze the platycosides in PGB.

**Results:** In A549 cells, PGB induced significant autophagic cell death. Specifically, PGB upregulated LC3-II in a time- and dose-dependent manner, and it redistributed LC3 via autophagosome formation in the cytoplasm. PGB treatment increased the phosphorylation of AMP-activated protein kinase (AMPK) and subsequently suppressed the AKT/mammalian target of the rapamycin (mTOR) pathway. Furthermore, PGB inhibited cell proliferation by regulating the mitogen-activated protein kinase (MAPK) pathways. In this study, six types of platycosides were identified in the PGB using HPLC.

**Conclusions:** PGB efficiently induced cancer cell death via autophagy and the modulation of the AMPK/mTOR/ AKT and MAPK signaling pathways in A549 cells. Therefore, PGB may be an efficacious herbal anti-cancer therapy.

**1. Introduction**

Lung cancer is currently the most prevalent malignant tumor, the leading cause of cancer-related deaths, and impacts the death rate more than colon, breast, and prostate cancers. Chemotherapy with platinum compounds or platinum-based combinations with other anti-cancer drugs are currently the optimal treatment in lung cancer patients; however, the prognosis for lung cancer is typically inadequate because of various drug side effects, including resistance and toxicity (Ferlay et al., 2007; Siegel et al., 2012). Therefore, the research and development of new drugs and therapeutic targets is crucial to advance the treatment of lung cancer patients.

The root of *Platycodon grandiflorum* (PG), commonly known as Kilkyong in Korea, Jiegeng in China, and Kikyo in Japan, has been extensively used as a traditional anti-inflammatory medicine in Asia for the treatment of respiratory conditions, such as bronchitis, asthma, and tonsillitis (Zhang et al., 2015). In addition, PG was reported to have various pharmacological activities in hyperlipidemia (Han et al., 2002), inflammatory disease (Wang et al., 2004), cancer (Kim et al., 2005), and the immune responses (Kim et al., 2006). PG is composed of several phytochemical constituents, including polysaccharides, saponins, flavonoids, polyphenolic, and polyacetylenes. Notably, triterpenoid saponins isolated from PG, i.e., platycosides, were reported as the major class of bioactive compounds (Zhang et al., 2015). These PG...
saponins show activity against several types of cancer cells, such as leukemia such as leukemia (Kim et al., 2008), breast (Yu and Kim, 2012), lung (Zhao et al., 2015), and gastric cancer (Chun et al., 2013) by inducing programmed cell death.

Based on characteristic cell morphology changes, programmed cell death can be classified into three distinct groups: apoptosis, autophagy, and necrosis (Hague and Verkhratsky, 2009). Autophagy and apoptosis are often targets for cancer therapies because these processes can be used to eliminate injured or aged cells and organelles. Specifically, persistent and excessive autophagy contributes to cancer cell death and affects the proliferative and survival mechanisms of cancer cells (Hague and Verkhratsky, 2009; Yang and Kionsky, 2009, 2010). Furthermore, autophagy involves the sequestration of proteins and organelles in autophagosomes, which then induces lysosomal cell death better known as autophagic cell death (Sridharan et al., 2011). During autophagosome formation, microtubule-associated protein-1 light chain-3 (LC3) is recruited to the autophagosome, and conjugated LC3-II localizes on autophagosomes and autolysosomes. Thus, autophagic activity is measured by quantifying LC3-II using biochemical methods (Mizushima, 2007).

AMP-activated protein kinase (AMPK) is a key energy sensor protein that regulates the cellular metabolism of sugar and fat to maintain the energy homeostasis of cells (Meley et al., 2006). In terms of autophagy induction, AMPK stimulates autophagy by inhibiting mTOR complex 1 (mTORC1), a major negative regulator of autophagy, at the level of tuberous sclerosis complex 2 (TSC2) and raptor (Gwinn et al., 2008; Yang and Kionsky, 2010). Furthermore, AMPK directly affects Unc-51-like kinase 1 (ULK1) and Beclin-1, which contributes to autophagy induction (Lee et al., 2010).

Recent studies demonstrated that platycodin D triggers autophagy via the mitogen-activated protein kinase (MAPK) signaling pathway in human hepatocellular and lung carcinoma cells (Li et al., 2013; Zhao et al., 2015). MAPK cascades, including extracellular signal-regulated kinase (ERK), p38, and c-jun N-terminal kinase (JNK), are involved in cell death and/or survival. Thus, these cascades are chemotherapeutic targets of the autophagy signaling pathway in cancer cells (Wada and Penninger, 2004).

We investigated if PGB, a platycoside-enriched butanol fraction of P. grandi-florum, was associated with the autophagy signaling pathway via MAPK signaling pathway activation, thus enhancing cancer cell apoptosis in A549 cells. Furthermore, we demonstrated that the regulation of MAPK signaling pathways by PGB mediates autophagy; however, this PGB-induced regulation was atypical.

2. Materials and methods

2.1. Materials and reagents

Dulbecco’s modified Eagle’s medium (DMEM) for cell cultivation was obtained from Lonza (Walkersville, MD, USA). Fetal bovine serum (FBS), penicillin and streptomycin, and Trypsin-EDTA were purchased from Hyclone (Logan, UT, USA). Bovine serum albumin (BSA), 5-diphenyl-tetrazolium bromide (MTT), and 3-methyladenine (3-MA), an autophagy inhibitor, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Inhibitors for identifying various protein expressions, such as rapamycin and AKT inhibitor were purchased from Calbiochem® (Merck Millipore, Darmstadt, Germany).

2.2. Herb materials and preparation of PGB

The root of P. grandi-florum (PG, 3 kg) was purchased from the Korea Medicine Herbs Association (Yeongcheon, Korea) and identified by Prof. KiHwan Bae, Chungnam National University, Korea. PG was refluxed with methanol for 3 h (3×6 L). After boiling, the extract was filtered out using standard testing sieves (pore size, 150 μm) (Retsch, Haan, Germany) and evaporated by a rotary evaporator N-1000 (EYELA, Japan), fractionated with each solvent system (ethylacetate and butanol) using a separatory funnel. After that, each solvent fraction was prepared in the form of powder by freeze-drying without residual solvent for cell test.

2.3. Characterization of phytochemicals

For reference standards, platycodon D, platycodon D3, Daepi-platycodon D, and polygalacin D were purchased from Natural Products Bank (NPBANK) in Korea Promotion Institute for Traditional Medicine Industry (KOTMIN; gyeongsan, Korea). Polygalacin D2 and platycoside J isolated from PGB were provided from Prof. Chun Liang, Xixixi Medical University. HPLC grade solutions including water, acetonitrile and methanol were purchased from J.T. Baker (Austin, TX, USA) and Trifluoroacetic acid for analysis reagent was purchased from DAE JUNG Chemical & Materials Co. (Siheung, Korea). To determine phytochemical profile and chemical components of PGB, HPLC analysis was performed on a Hitachi Elite Lachrom (Hitachi, Japan) with an Alltech 3300 ELSD (GRACE, USA) and Agilent C18 column (50 mm×4.60 mm i.d., 5 μm). HPLC conditions including ELSD (70 °C, 1.5 mL/min) were performed using the method reported by Ha et al. (2006). The sample injection volume was 10 μL. Peaks were assigned by comparing their retention times with that of each standard eluted in parallel with a series of mobile phases.

2.4. Cell viability assay

Two kinds of human lung non-small cell lung cancer cells, A549 and NCI-H1299, were purchased from the Korean Cell Line Bank (KCLB, Seoul, Korea), which were respectively cultured in DMEM supplemented with 10% FBS and 1% antibiotics contained 100 U/mL penicillin G and 100 μg/mL streptomycin. WI-38 human normal lung fibroblast cells were cultured in MEM supplemented with 10% FBS and 1% antibiotics contained 100 U/mL penicillin G and 100 μg/mL streptomycin. Cells were incubated in a humidified 5% CO2 atmosphere at 37 °C. Cells (4×106 per 1 mL) were inoculated in a 96-well plate and treated with extract of the root of P. grandi-florum or its solvent fractions. After incubation, cell viability was determined using MTT colorimetric assay based on the reduction of tetrazolium salt to its insoluble formazan. For incubation study, cells were pretreated inhibitors for 1 h and treated with PGB for 24 h. After incubation, cell viability was determined using MTT assay.

2.5. Western blot analysis

The cell treated with PGB were lysed in RIPA buffer contained include protease and phosphatase inhibitors (Roche, Basel, Switzerland). The preparation of lysates and Western blot analysis were performed as previously described (Kim et al., 2013). Proteins were detected using primary antibodies specific for LC3 I/II, beclin-1, bcl-2, phosphor-ULK1 (p-ULK1), ULK1, bax, caspase-3, PARP, p-AMPK, AMPK, p-AKT, AKT, p-mTOR, mTOR, Raptor, Rictor, p-ERK, p-p38, p-JNK, and GAPDH. All primary and secondary antibodies in this study were purchased from Cell signaling Technology, Inc. (Boston, MA, USA) except for LC3 (Novus Biologicals, Littleton, CO, USA). These were followed by incubation with HRP-conjugated secondary antibodies for 1 h at room temperature. The each specific protein was detected using a SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL, USA) and an ImageQuant LAS 4000 Mini (GE Healthcare, Piscataway, NJ, USA). Band intensities were determined using ImageJ software (National Institute of Health, USA).
2.6. Immunocytochemistry

Cells (7×10^4 per 1 mL) were inoculated in confocal dish (cover-glass-bottom dish) and treated with PGB for 24 h. After that, cells were blocked with TBS/0.1% Triton X-100/3% goat serum (Gibco, Grand Island, NY, USA) and incubated with primary antibody specific for LC3 (Novus Biologicals, Littleton, CO, USA) overnight at 4 °C. They were washed with TBS and incubated for 3 h in presence of anti-rabbit IgG labeled with Alexa Fluor-568 (Life technologies, Inc.). Before finishing last washed out, cells were stained with DAPI for 30 min and immediately washed with TBS. Images were acquired using a FV10i FLUOVIEW Confocal Microscope (Olympus, Tokyo, Japan).

2.7. Statistical analysis

Data are presented as means ± SD. Student’s t-test was employed to assess the statistical significance of differences between the control and PGB-treated groups. Values of p < 0.05 and < 0.01 were considered to indicate statistical significance.

3. Results

3.1. PGB and its butanol fraction induce formation of cytoplasmic vacuoles in A549 human lung carcinoma cells

To investigate whether the changes in cell morphology following PGB treatment were related to cell proliferation in A549 and NCI-H1299 cells, we completed a solvent fractionation of PG using ethyl acetate (PGE) and butanol (PGB). As shown in Fig. 1A, in A549 cells, the growth rates were 53.5%, 69.7%, and 32.4% of control cells for PG, PGE, and PGB, respectively. PGB inhibited cell viability more than twice as strongly as PGE in A549 cells. In addition, in NCI-H1299 cells, the growth rates were 44.7%, 72.6%, and 65.4% of control cells for PG, PGE, and PGB, respectively. The potential cytotoxic effect of PG, PGE, and PGB on normal cells was assessed using WI-38 human lung fibroblasts. WI-38 cells were unaffected by PGE and PGB under the same conditions that were cytotoxic to lung cancer cells. Instead, PG inhibited the viability of WI-38 cells about 33.5% under the same conditions. Therefore, subsequent tests focused on A549 cells to further define the inhibitory action of PG, PGE, and PGB. As a result of determining the morphological change by treatment with PG, PGE and PGB on A549 cells, PG and PGB caused the formation of vacuoles in the cytoplasm of A549 cells, whereas PGE did not (Fig. 1B). Furthermore, in MTT assay, the IC_{50} values of PGB were about 107.3 ± 4.45 μg/mL in A549 cells (data not shown). Based on these results, we used PGB within a concentration range of 50–200 μg/mL and incubation time of 24 h for further experiments.

3.2. PGB triggers autophagy in A549 cells

To clarify if the formation of vacuoles in the cytoplasm following treatment with PGB was associated with autophagy, the expression of autophagy-related proteins was determined using western blotting. As shown in Fig. 2A, PGB dose-dependently increased the ratio of LC3-II to LC3-I. Moreover, the protein level of Beclin-1 increased after PGB treatment. Bcl-2 expression was inhibited by PGB, whereas Bax expression was slightly enhanced. To confirm the induction of autophagy by PGB, we examined the intracellular distribution of LC3 in response to PGB treatment in A549 cells using immunocytochemistry and confocal microscopy. There were significant changes in the levels of autophagy-related proteins following treatment with 100 μg/mL PGB for 24 h. PGB-treated cells showed an intense and punctate LC3
fluorescence pattern when compared with untreated cells (Fig. 2B). Using 3-MA, a common autophagy inhibitor, we assessed if PGB-induced cell death was caused by autophagy induction in A549 cells. As shown in Fig. 2C, cell viability in PGB-treated cells was increased by approximately 67% after treatment with non-cytotoxic concentrations. Under the same conditions, 3-MA reduced PGB-induced LC3 II expression (Fig. 2D).

3.3. PGB induces autophagy by suppressing the AKT/mTOR pathway and activating AMPK signals in A549 cells

To investigate the molecular mechanisms of PGB-mediated autophagy, AMPK, AKT, and mTOR were measured in PGB-treated A549 cells. As shown in Fig. 3A, the conversion of LC3 I/II increased in a time-dependent manner after treatment with 100 µg/mL PGB. The phosphorylation of AMPK was also affected by PGB treatment. PGB reduced the phosphorylation of AKT and mTOR. Furthermore, PGB
decreased raptor and rictor expression, which are comprised of mTOR complexes. These changes directly affected the viability of A549 cells. We pre-incubated cells with or without inhibitors of AMPK (Compound C, 2.5 µm), mTOR (rapamycin, 10 µm), and AKT (AKTi, 10 µm) for 1 h, and then treated cells with 100 µg/mL PGB for 24 h. As shown in Fig. 3B, Compound C and AKTi mildly increased cell death by approximately 7.87% and 7.10%, respectively, when compared with PGB. In contrast, rapamycin reduced cell viability by up to 47.69%. Our western blot analysis demonstrated that pre-incubation with rapamycin and AKTi markedly increased LC3-II expression. In contrast, co-treatment with Compound C, an inhibitor of AMPK, decreased LC3-II levels (Fig. 3C).

3.4. MAPKs signaling pathways contribute to induction of autophagic cell death by PGB in A549 cells

To identify the relationship between the MAPK pathways and autophagy induction, we analyzed the levels of phosphorylated MAPKs after PGB treatment using western blotting. As shown in Fig. 4A, p-p38 and p-JNK were downregulated after 6 h of treatment with 100 µg/mL PGB. The phosphorylation of JNK was affected after just 30 min of PGB exposure. The level of p-ERK showed a transient drop after a 1 h exposure to 100 µg/mL PGB, followed by a sharp upregulation after 6 h. To confirm that MAPKs were required for autophagy induction and subsequent cell death, A549 cells were pretreated with MAPK inhibitors, including PD98059 (which inhibits ERK1/2), SB203580 (p38), and SP600125 (JNK), for 1 h and then exposed to with 100 µg/mL PGB for 24 h (Fig. 4B). PD98059 significantly protected cells from PGB-treated cell death by approximately 20%, whereas SB203580 and SP600125 markedly enhanced cell death up to 25.29% and 59.83%, respectively, when compared with PGB alone. Under the same conditions, SB203580 and SP600125 increased PGB-induced LC3-II expression, whereas PD98059 decreased LC3-II expression when compared with PGB alone (Fig. 4C). The formation of cytoplasmic vacuoles caused by PGB was accelerated by SB203580 and SP600125, whereas PD98059 nearly completely prevented vacuole formation.

3.5. Autophagy induction by PGB is attributed to platycosides from PGB in A549 cells

We identified the platycosides derived from PG that were present in PGB using HPLC fingerprint analysis (Fig. 5). Mixed standards revealed six single representative platycosides peaks: platycodin D3 (1, tR: 45.34); platycoside J (2, tR: 53.47); deapi-platycodin D (3, tR: 54.48); polygalacin D2 (4, tR: 56.06); platycodin D (5, tR: 56.36); and polygalacin D (6, tR: 56.87) (Fig. 5A). These platycosides were identified in the PGB samples at similar retention times (1, tR: 45.21; 2, tR: 53.33; 3, tR: 54.31; 4, tR: 55.91; 5, tR: 56.26; and 6, tR: 56.77) (Fig. 5B). To determine if the autophagic effects of PGB were due to the actions of these platycosides, we treated A549 cells with each of the respective platycosides and assessed their anti-cancer effects. As shown in Fig. 6A, four platycosides showed anti-proliferative effects. At a concentration of 10 µg/mL, platycodin D, polygalacin D2, and polygalacin D strongly inhibited the growth of A549 cells by approximately 49.6%, 47.9%, and 48.1%, respectively, when compared with
control cells. This result was confirmed by changes in protein expression. At concentrations of 10 and 20 μg/mL, the majority of platycosides increased LC3-II levels and caused PARP cleavage, with the exception of platycoside J. Among these compounds, platycodin D, polygalacin D2, and polygalacin D affected cell death by inducing LC3-I/II conversion and PARP cleavage (Fig. 6B).

4. Discussion

The anti-cancer properties of PGB involve the induction of the AMPK/AKT/mTOR signaling pathway and the regulation of the MAPK signaling pathways in A549 human lung cancer cells. Furthermore, the platycosides within PGB exhibited anti-cancer effects by inducing autophagy and apoptosis in A549 cells.

Autophagy generates vacuoles called autophagosomes in the cytoplasm, which can be estimated by detecting the levels of LC3, a marker of autophagosome formation. LC3 consists of two forms, LC3-I and LC3-II, and the LC3-II/I ratio directly correlates with autophagosome formation (Chen and White, 2011). PGB-treated A549 cells showed a time- and dose-dependent increase LC3-II levels, suggesting that the autophagy induced by PGB inhibits cell viability.

Various signaling pathways are involved in the regulation of autophagy in cancer cells, including the Beclin-1 and Bcl-2 family pathways. The exogenous expression of Beclin-1 in cancer cells does not endogenously generate Beclin-1-initiated autophagy or cell death. In addition, Bcl-2 can negatively regulate the ability of Beclin-1 to induce autophagy by binding to its BH3 domain (Wang et al., 2010). In this study, PGB activated autophagy by releasing Beclin-1 from its interaction with Bcl-2 via the inhibition of Bcl-2 expression. Furthermore, treatment with the same PGB concentration that inhibited Bcl-2 expression also showed apoptotic effects via caspase-3 activation and PARP cleavage. Therefore, we suggest that PGB inhibits Bcl-2 activity and leads to autophagic cell death and apoptosis in human lung cancer cells.

In the autophagic-mediated signaling pathway, AMPK acts as a negative regulator of mTOR and contributes to the induction of autophagy by directly affecting ULK1 and Beclin-1 (Kim et al., 2011). AMPK is recognized as a sensor of cellular energy status and responds by phosphorylating several target proteins related to energy metabolism (Hardie, 2007). In the mammalian autophagy process, AMPK controls mTOR. Specifically, AMPK regulates the Ulk1 complex by suppressing the activation of raptor, a member of mTOR complex 1 (mTORC1) (Kim et al., 2011).

As a regulator of many cellular processes, including cellular proliferation and nutrient sensing, mTOR is critical for the regulation of autophagy (An et al., 2014). mTOR can exist in two complexes: mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 is composed of mTOR, Raptor, GβL, and DEPTOR, and this complex is inhibited by rapamycin. mTORC2 is composed of mTOR, rictor, and GβL, and this complex promotes cellular survival by activating AKT (Dodson et al., 2013). In our current study, PGB-induced autophagy occurs mainly through the inhibition of mTOR.
activity by regulating proteins in the mTOR signaling pathway, such as raptor or rictor. Rictor, a member of mTORC2, was strongly affected after a relatively short period of treatment with PGB. In addition, PGB treatment inhibited AKT activation and reduced the phosphorylation of mTOR. Therefore, we suggest that PGB suppresses cellular growth by modulating the phosphorylation of mTOR substrates. We used rapamycin to show that the inhibition of mTOR signaling by PGB caused the upregulation of autophagy and elevated the suppression of cell proliferation. This result indicates that co-treatment with an inhibitor of mTOR signaling and PGB may function as an anti-cancer therapy in A549 cells. In our current study, rapamycin showed an improvement in the autophagy induction of PGB and thus an increase in A549 cell viability by regulating proteins in the mTOR signaling pathway, such as raptor or rictor. Rictor, a member of mTORC2, was strongly affected after a relatively short period of treatment with PGB. In addition, PGB treatment inhibited AKT activation and reduced the phosphorylation of mTOR. Therefore, we suggest that PGB suppresses cellular growth by modulating the phosphorylation of mTOR substrates. We used rapamycin to show that the inhibition of mTOR signaling by PGB caused the upregulation of autophagy and elevated the suppression of cell proliferation. This result indicates that co-treatment with an inhibitor of mTOR signaling and PGB may function as an anti-cancer therapy in A549 cells. In our current study, rapamycin showed an improvement in the autophagy induction of PGB and thus an increase in A549 cell viability by regulating proteins in the mTOR signaling pathway, such as raptor or rictor. Rictor, a member of mTORC2, was strongly affected after a relatively short period of treatment with PGB. In addition, PGB treatment inhibited AKT activation and reduced the phosphorylation of mTOR. Therefore, we suggest that PGB suppresses cellular growth by modulating the phosphorylation of mTOR substrates. We used rapamycin to show that the inhibition of mTOR signaling by PGB caused the upregulation of autophagy and elevated the suppression of cell proliferation. This result indicates that co-treatment with an inhibitor of mTOR signaling and PGB may function as an anti-cancer therapy in A549 cells. In our current study, rapamycin showed an improvement in the autophagy induction of PGB and thus an increase in A549 cell viability by regulating proteins in the mTOR signaling pathway, such as raptor or rictor. Rictor, a member of mTORC2, was strongly affected after a relatively short period of treatment with PGB. In addition, PGB treatment inhibited AKT activation and reduced the phosphorylation of mTOR. Therefore, we suggest that PGB suppresses cellular growth by modulating the phosphorylation of mTOR substrates. We used rapamycin to show that the inhibition of mTOR signaling by PGB caused the upregulation of autophagy and elevated the suppression of cell proliferation. This result indicates that co-treatment with an inhibitor of mTOR signaling and PGB may function as an anti-cancer therapy in A549 cells. In our current study, rapamycin showed an improvement in the autophagy induction of PGB and thus an increase in A549 cell viability by regulating proteins in the mTOR signaling pathway, such as raptor or rictor. Rictor, a member of mTORC2, was strongly affected after a relatively short period of treatment with PGB. In addition, PGB treatment inhibited AKT activation and reduced the phosphorylation of mTOR. Therefore, we suggest that PGB suppresses cellular growth by modulating the phosphorylation of mTOR substrates. We used rapamycin to show that the inhibition of mTOR signaling by PGB caused the upregulation of autophagy and elevated the suppression of cell proliferation. This result indicates that co-treatment with an inhibitor of mTOR signaling and PGB may function as an anti-cancer therapy in A549 cells. In our current study, rapamycin showed an improvement in the autophagy induction of PGB and thus an increase in A549 cell
death. AKT treatment also increased the autophagy induction and cell death caused by PGB. In contrast, Compound C, an inhibitor of AMPK, nearly blocked the induction of autophagy by PGB. Taken together, our results demonstrate that PGB-activated AMPK is involved in autophagy induction, which affects mTOR/AKT signaling and cellular growth.

MAPKs, including ERK, p38, and JNK, play an important role in autophagy, which is linked to cell death and/or survival (Chen and White, 2011; Choi et al., 2010). Activated p38 and JNK participate in platycodon n-induced autophagy, whereas ERK activation is inhibited in A549 cells (Zhao et al., 2015). In contrast, platycodon D upregulates ERK phosphorylation in HepG2 human hepatocellular carcinoma cell (Li et al., 2015). In our study, the regulation of autophagy by PGB is also intimately connected to the MAPK signaling pathways. Notably, the phosphorylation of p38 and JNK but not ERK was reduced by PGB. Specifically, p-JNK sharply decreased after treatment with PGB. We investigated the cross-talk between the MAPK signaling pathways and autophagy induced by PGB using specific MAPK inhibitors. Our results showed that PGB-induced cell death largely depended on p38 and JNK inhibition. Under the same conditions, autophagy was increased by inhibiting the activation of p38 and JNK, whereas ERK inhibition decreased as a result of PGB-mediated autophagy induction. These findings suggest that the platycosides found in PGB play a critical role in the regulation of MAPK signaling pathways in A549 cells.

We assessed the anti-cancer effects mediated by PGB platycosides. Among the six identified platycosides, platycodon D, polygalacin D2, and polygalacin D strongly induced autophagy and apoptosis by increasing LC3 II expression and PARP cleavage in A549 cells. A recent study reported that platycodon D inhibits the phosphorylation of AKT and its downstream signals, such as p70S6K and 4EBP-1, in two types of non-small cell lung cancer cells (Zhao et al., 2015). In our study, platycodon D showed anti-proliferative effects and induced apoptosis and autophagy in A549 human lung cancer cells. Thus, the anti-cancer effects of polygalacin D and D2 involve the induction of apoptosis and autophagy. We did not determine the effects of active platycosides on AMPK/mTOR signaling pathways. However, we will investigate the autophagic mechanisms induced by these platycosides in cancer cells in future studies.

5. Conclusion

Our results demonstrate the induction of autophagic cell death by PGB in A549 human lung cancer cells. PGB-induced autophagic cell death occurred via AMPK activation and the inhibition of mTOR and AKT activity. These effects were reversed by autophagy-related inhibitors. We also determined that the anti-proliferative effects of PGB involve the MAPK signaling pathway and induction of autophagy in cancer cells. Although this study did not focus on apoptosis, the possible correlation between PGB-induced autophagy and apoptosis cannot be excluded. Several platycosides within PGB showed anti-cancer effects by inducing apoptosis and autophagy in A549 cells. Thus, our results indicate that PGB-induced autophagic cell death may occur via complex signaling mechanisms rather than through a single signaling mechanism. In conclusion, PGB shows potential as a herbal anti-cancer therapy.

Conflict of interests

All authors have no conflict of interests.

Author contributions

Nam-Hui Yim and Jin Yeul Ma conceived and designed the experiments; Nam-Hui Yim and Youn–Hwan Hwang performed the experiments; Chun Liang contributed polygalacin D2 and platycoside J; Nam-Hui Yim wrote the paper.

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