Oleifolioside B-mediated autophagy promotes apoptosis in A549 human non-small cell lung cancer cells

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Abstract. The biochemical mechanisms of cell death by oleifolioside B (OB), a cycloartane-type triterpene glycoside isolated from *Dendropanax morbifera* Leveille, were investigated in A549 human lung carcinoma cells. Our data indicated that exposure to OB led to caspase activation and typical features of apoptosis; however, apoptotic cell death was not prevented by z-VAD-fmk, a pan-caspase inhibitor, demonstrating that OB-induced apoptosis was independent of caspase activation. Subsequently, we found that OB increased autophagy, as indicated by an increase in monodansylcadaverine fluorescent dye-labeled autophagosome formation and in the levels of the autophagic form of microtubule-associated protein 1 light chain 3 and Atg3, an autophagy-specific gene, which is associated with inhibiting phospho-nuclear factor erythroid 2-related factor 2 (Nrf2) expression. However, pretreatment with bafilomycin A1, an autophagy inhibitor, attenuated OB-induced apoptosis and dephosphorylation of Nrf2. The data suggest that OB-induced autophagy functions as a death mechanism in A549 cells and OB has potential as a novel anticancer agent capable of targeting apoptotic and autophagic cell death and the Nrf2 signaling pathway.

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Introduction

Programmed cell death (PCD) plays an essential role in regulating various biological processes, including morphogenesis, maintaining tissue homeostasis, and eliminating damaged and infected cells. Two major forms of PCD have been classified as apoptosis and autophagy (1-3). Apoptotic cell death (type I PCD) is characterized by membrane blebbing, chromosomal DNA fragmentation, cell shrinkage and the formation of apoptotic bodies (4-6). Autophagic cell death (type II PCD) is a catabolic process in which cytosolic macromolecules and damaged organelles are sequestered in double-membrane autophagosomes, which subsequently fuse with lysosomes for degradation by forming acidic autophagolysosomes (7,8). Different from apoptosis, autophagy may contribute to cell survival or death. Although apoptosis and autophagy are two distinct biological processes, crosstalk exists between them (9-11).

Lung cancer is the most common cause of cancer-related morbidity and mortality in men and women around the world, accounting for approximately 30% of all cancer deaths (12). Lung cancers are generally classified into two histological types, small cell lung cancer and non-small cell lung cancer

(NSCLC). NSCLC accounts for approximately 85% of all lung cancers. Most lung cancers are closely related to an advanced stage at diagnosis with a poor prognosis. Over the last few decades, chemotherapy has improved the outcome for patients with late stage NSCLC, but only slightly (13,14). Therefore, novel and more effective antitumor agents must be explored and developed.

Dendropanax morbifera Leveille has been used in traditional medicine to treat several diseases, such as headache, infectious diseases and skin diseases. Previous studies have shown that the components of this plant have many pharmacological activities, including anti-complement, anti-diabetic and anti-atherogenic properties (15-17). Recently, oleifolioside B (OB), a cycloartane-type glycoside, was isolated from the lower stem parts of *D. morbifera*, which has anti-plasmodial activity *in vitro* (18). However, little is known regarding the anticancer activity of OB or its signal molecular mechanisms in cancer cells. In this present study, we investigated for the first time the anticancer mechanisms by which OB induces apoptosis and autophagy in A549 NSCLC cells.

Materials and methods

Materials. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), bafilomycin A1, 4,6-diamidino-2-phenylindole (DAPI), monodansylcadaverine (MDC) and doxorubicin were purchased from the Sigma-Aldrich Chemical Co. (St. Louis, MO). Antibodies specific for actin, Bad, Bax, Bcl-2, Bcl-xL, Bid, caspase-3, caspase-8, caspase-9, cIAP-1, cIAP-2, death receptor (DR) 4, DR5, Fas, Fas legend (FasL), Fas-associated death domain (FADD), cellular FLICE-like inhibitory protein (c-FLIP), nuclear factor erythroid 2-related factor 2 (Nrf2), poly(ADP-ribose) polymerase (PARP), survivin, XIAP, heme oxygenase 1 (HO-1) and goat anti-rabbit IgG-FITC were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies specific for Atg3, Atg5, Atg7, Atg12, Beclin-1 and microtubule-associated protein 1 light chain 3 (LC3) were purchased from Cell Signaling (Beverly, MA). The antibody specific for phospho (p)-Nrf2 was purchased from Epitomics (Burlingame, CA). Peroxidase-labeled donkey anti-rabbit and sheep anti-mouse immunoglobulins and an enhanced chemiluminescence (ECL) kit were purchased from Amersham (Arlington Heights, IL). Caspase activity assay kits were purchased from R&D Systems (Minneapolis, MN). z-VAD-fmk was purchased from Calbiochem (San Diego, CA). OB was kindly provided by Professor Hyung-In Moon of Dong-A University (Busan, Republic of Korea) (18) and dissolved in dimethyl sulfoxide (DMSO) as a stock solution at 30 mM concentration. Dilutions were made in Dulbecco's modified Eagle's medium (DMEM, Gibco-BRL, Gaithersburg, MD).

Cell culture and cell viability assay. The A549 cell line was obtained from the American Type Culture Collection (Rockville, MD) and cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco-BRL). Cell viability was measured based on formation of blue formazan metabolized from colorless MTT by mitochondrial dehydrogenases, which are active only in live cells.

Flow cytometric analysis. To analyze the percentage of apoptotic cells, cells were collected, washed with cold phosphate-buffered saline (PBS), and fixed in 75% ethanol at 4˚C for 30 min. The DNA content of the cells was measured using a DNA staining kit (CycleTEST PLUS Kit, Becton-Dickinson, San Jose, CA). Propidium iodide (PI)-stained nuclear fractions were obtained by following the kit protocol. The cells were then filtered through 35-mm mesh, and DNA content fluorescence was determined using a FACSCalibur flow cytometer within 1 h. The cellular DNA content was analyzed with CellQuest software (Becton-Dickinson).

DNA fragmentation assay. After OB treatment, the cells were lysed in a buffer containing 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA and 0.5% Triton X-100 for 1 h at room temperature. The lysates were vortexed and cleared by centrifugation at 15,000 rpm for 10 min at 4˚C. The DNA in the supernatant was extracted using a 25:24:1 (v/v/v) equal volume of neutral phenol:chloroform:isoamyl alcohol. To assay the DNA fragmentation pattern, samples were loaded onto 1.0% agarose gel containing 0.1 μg/ml ethidium bromide (EtBr) and electrophoresis was carried out.

Caspase activity assay. The enzymatic activity of the caspases was assayed using a colorimetric assay kit according to the manufacturer's protocol. The cells were incubated in the absence and presence of OB for the indicated times. The cells were harvested and lysed in a lysis buffer for 30 min on an ice bath. Lysed cells were centrifuged at 14,000 rpm for 20 min, and equal amounts of protein (100 μg per 50 μl) were incubated with 50 μl reaction buffer and 5 μl colorimetric tetrapeptides, Asp-Glu-Val-Asp (DEVD)-p-nitroaniline (pNA) for caspase-3, Ile-Glu-Thr-Asp (IETD)-pNA for caspase-8, and Leu-Glu-His-Asp (LEHD)-pNA for caspase-9, at 37˚C for 2 h in the dark. Caspase activity was determined by measuring the changes in absorbance at 405 nm using an ELISA reader.

MDC staining. To observe autophagy formation, A549 cells were grown on glass coverslips for 24 h. The cells were incubated in the absence and presence of OB for the indicated times, and then the cells were treated with 0.05 mM MDC at 37˚C in 5% CO_2 for 1 h. The cells were then fixed with 4% paraformaldehyde in PBS for 10 min. The cellular changes were analyzed with a fluorescence microscope.

Protein extraction and western blot analysis. Whole-cell protein extracts from A549 cells were prepared with cell lysis buffer (20 mM sucrose, 1 mM EDTA, 20 μM Tris-HCl, pH 7.2, 1 mM DTT, 10 mM KCl, 1.5 mM $MgCl_2$ and 5 μg/ml aprotinin) for 30 min. Cells were disrupted by sonication and extracted at 4˚C for 30 min. The protein extracts were quantified using the Bio-Rad kit (Pierce Biotechnology, Rockford, IL). For western blot analysis, lysate proteins (30-50 μg) were resolved over sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and transferred onto nitrocellulose transfer membranes (Schleicher & Schuell, Keene, NH). Specific proteins were detected with an ECL Western blotting kit according to the recommended procedure. In a parallel experiment, cells were washed with ice-cold PBS and collected. Then cytoplasmic
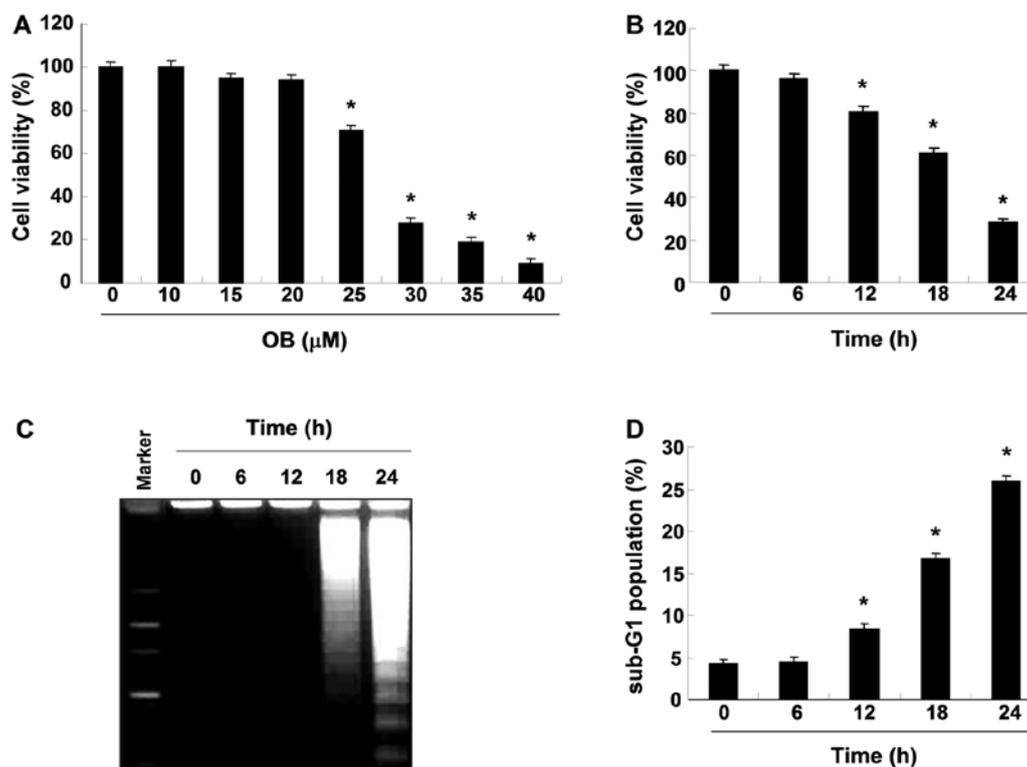
Figure 1. OB induces cell growth inhibition and apoptosis in A549 cells. (A and B) Cells were seeded at 2x10⁵ cells/ml for 24 h, and then treated with the indicated concentrations of OB for (A) 24 h or 40 μM OB for the indicated durations (B). Then cell viability was determined with MTT assay. (C) After the cells were treated with 30 μM OB for the indicated durations, the cells were stained with DAPI solution for 10 min and then photographed with a fluorescence microscope using a blue filter (x400). (C) For the DNA fragmentation analysis, genomic DNA was extracted from cells grown under the same conditions as (B), separated by 1.0% agarose gel electrophoresis, and visualized under UV light after staining with EtBr. The marker indicates a size marker the DNA ladder. (D) To quantify the degree of apoptosis induced by OB, cells were evaluated with a flow cytometer for sub-G1 DNA content, which represents the cells undergoing apoptotic DNA degradation. Each point represents the mean ± SD of three independent experiments. *Significantly different from control (p<0.05).

and nuclear proteins were prepared using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce Biotechnology).

Immunocytochemistry. Cells were grown on coverslips and treated as indicated. Cells were washed twice in PBS, fixed with 4% paraformaldehyde in PBS at room temperature for 30 min, and then permabilized with 0.25% Triton X-100 solution for 10 min. The cells were subsequently incubated in a blocking solution of 1% bovine serum albumin (BSA) and incubated with primary antibodies at room temperature for 1 h. After the incubation period, the samples were rinsed four times with PBS and then incubated with the secondary anti-rabbit-FITC diluted 1:200 in buffer for 1 h at room temperature. Nuclei were stained with 1 μg/ml DAPI, and then captured using a Zeiss LSM 510 laser scanning confocal device (Carl Zeiss).

Statistical analysis. All data are expressed as mean ± SD. The significant differences between the groups were determined using an unpaired Student's t-test. A value of p<0.05 was considered significant. All figures shown represent results from at least two independent experiments with a similar pattern.

Results

Induction of apoptosis by OB in A549 cells. To investigate the effects of OB on cell viability in A549 cells, cells were

treated with OB and subjected to an MTT assay. As shown in Fig. 1A and B, treatment with OB significantly reduced cell viability in a concentration- and time-dependent manner. Subsequently, to examine whether OB inhibits the proliferation of A549 cells by inducing apoptosis, genomic DNA was extracted from cells and agarose gel electrophoresis were assessed. As indicated Fig. 1C, treatment with OB time-dependently induced DNA fragmentation, a hallmark of apoptosis, in a time-dependent manner. In addition, flow cytometric analysis also revealed that treatment with OB increased the accumulation of cells at the apoptotic sub-G1 phase in a time-dependent manner (Fig. 1D). Taken together, these results indicate that the cytotoxic effects observed in response to OB are associated with the induction of apoptotic cell death in A549 cells.

Modulation of apoptosis regulatory proteins by OB in A549 cells. To determine which pathway was involved in the apoptosis induction of OB-treated A549 cells, the expression levels of death receptor-related proteins and the Bcl-2 and IAP family of proteins were determined with western blot analysis to measure the expression of the proteins. As shown in Fig. 2, exposure to OB led to a significant reduction in the anti-apoptotic protein cIAP-2, survivin and c-FLIP in a time-dependent fashion. However, OB treatment resulted in a time-dependent increase in the level of the pro-apoptotic FasL proteins. Under these conditions, although we did not
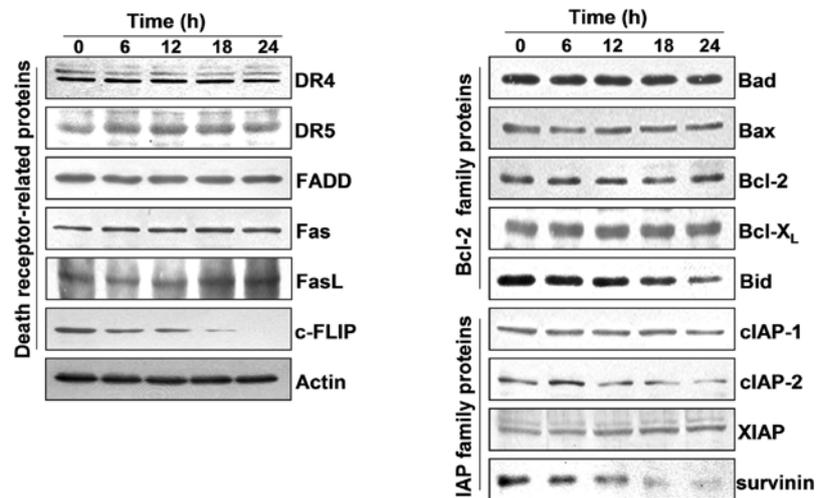
Figure 2. Modulation of apoptosis-related protein expression by OB in A549 cells. A549 cells were treated with 30 μM OB for the indicated durations. The cells were lysed, and then the cellular proteins were separated with SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with the indicated antibodies. Proteins were visualized using an ECL detection system. Actin was used as an internal control.

detect the truncated form of the pro-apoptotic protein Bid, a BH3-only pro-apoptotic member of the Bcl-2 family, our results indicate that OB treatment caused time-dependent downregulation of the whole form of the Bid proteins, which reflects Bid cleavage and activation.

Caspase activation by OB in A549 cells. To investigate whether OB-induced apoptosis in A549 cells involves the caspase cascade pathway, the caspase expression levels and activity were determined. As shown in Fig. 3A, western blot analyses indicated that the active forms of caspase-3 and -8 increased and the expression of pro-caspase-9 decreased in a time-dependent manner following OB treatment. For further quantification of the proteolytic activation of caspases, protein in the lysates of cells treated with OB was normalized and then assayed for *in vitro* activities using fluorogenic substrates. As indicated in Fig. 3B, treatment with OB resulted in a time-dependent increase in caspase activity (-3, -8 and -9) compared with the control cells, which was associated with the progressive proteolytic cleavage products of PARP, an activated caspase-3 substrate protein. To further investigate the significance of caspase activation in OB-induced apoptosis, A549 cells were pretreated with z-VAD-fmk, a broad-spectrum caspase inhibitor, for 1 h, followed by treatment with 30 μM OB for 24 h. Interestingly, pretreatment with z-VAD-fmk did not restore cell viability compared with control (Fig. 4A), and failed to suppress the OB-induced apoptosis (Fig. 4B). However, under the same condition, z-VAD-fmk significantly suppressed doxorubicin-induced growth inhibition and apoptosis (Fig. 4). Taken together, these results suggest that OB-induced apoptosis was independent of caspase activation in A549 cells.

Induction of autophagy by OB in A549 cells. As shown in Fig. 5A, based on our finding that extensive cytoplasm vacuolization compared with control cells appeared in cytosol after OB treatment, we hypothesized that the vacuoles might be associated with autophagy. For this study, we used MDC,
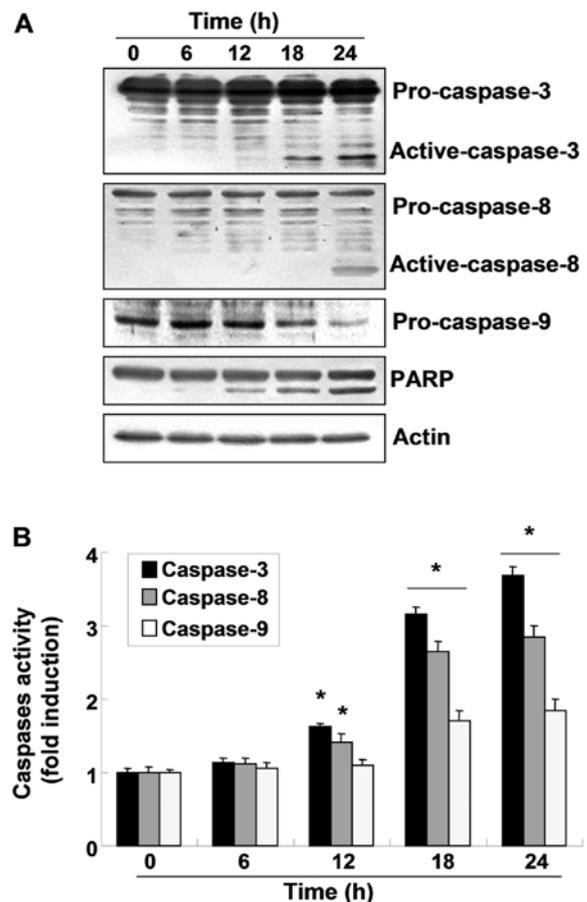

Figure 3. Activation of caspases and degradation of PARP by OB in A549 cells. (A) Cells treated with 30 μM OB for the indicated durations were lysed. Then the cellular proteins were separated with SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with the indicated antibodies. Proteins were visualized using an ECL detection system. Actin was used as an internal control. (B) Cells grown under the same conditions as (A) were lysed, and aliquots (50 μg protein) were assayed for *in vitro* caspase-3, -8 and -9 activity using DEVD-pNA, IETD-pNA and LEHD-pNA as the substrate, respectively. The released fluorescent products were measured. Data are expressed as the mean $\pm$ SD of three independent experiments. The significance was determined with Student's t-test (*p<0.05 vs. untreated control).
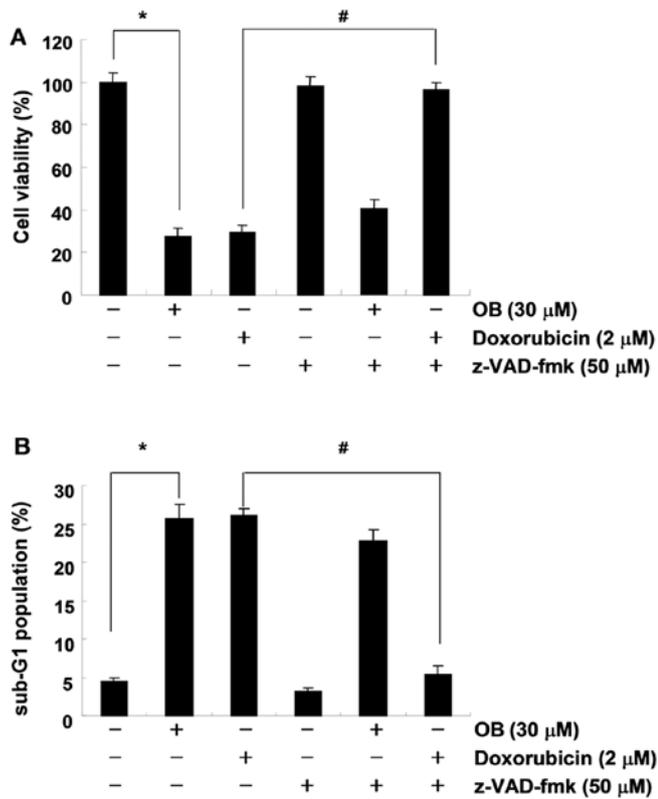
Figure 4. Effects of a pan-caspase inhibitor, z-VAD-fmk, on OB-induced apoptosis in A549 cells. (A) Cells were pretreated 50 μM z-VAD-fmk for 1 h, and then treated with 30 μM OB or 2 μM doxorubicin as the positive control for 24 h. Cell viability was determined with MTT assay. (B) A549 cells grown under the same conditions as (A) were evaluated with a flow cytometer for sub-G1 DNA content. Each point represents the mean ± SD of three independent experiments. *Significantly different from control (p<0.05). #Significantly different from doxorubicin-treated cells (p<0.05).

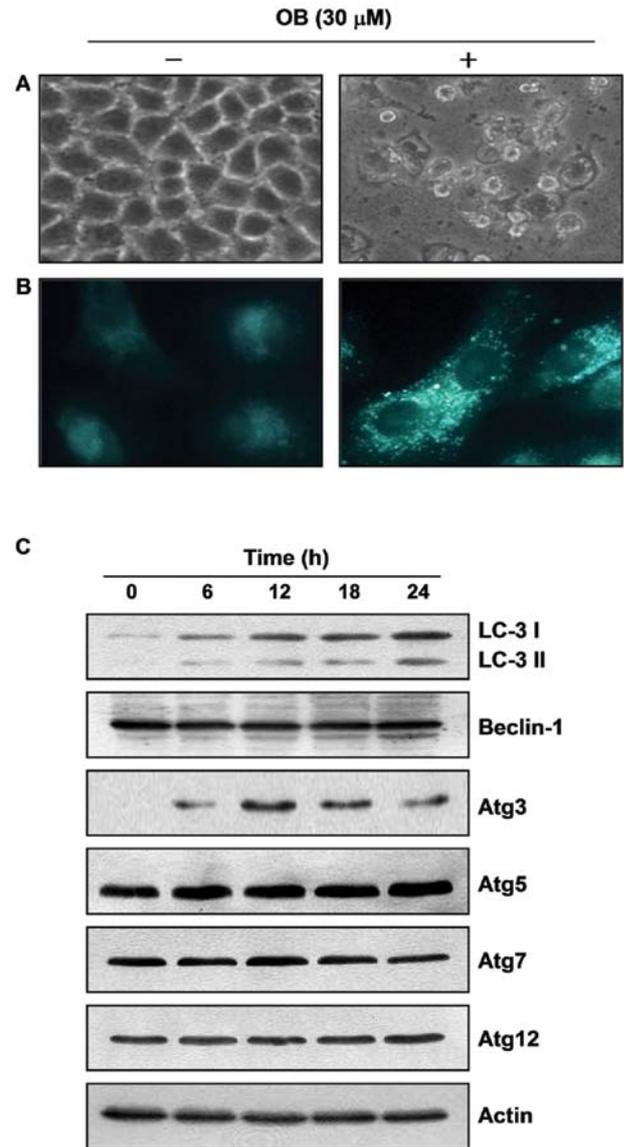
Figure 5. Induction of autophagy by OB in A549 cells. (A and B) Cells were incubated without or with 30 μM OB for 24 h. Then the cells were observed using an inverted microscope (A) or stained with MDC solution for 1 h, and then photographed with a fluorescence microscope using a blue filter (B, x400). (C) The cells were treated with 30 μM OB for the indicated durations, lysed, and then cellular proteins were separated with SDS-polyacrylamide gels and transferred onto nitrocellulose membranes. The membranes were probed with the indicated antibodies. Proteins were visualized using an ECL detection system. Actin was used as an internal control.

a commonly used autophagic dye, as a more specific marker for autophagy. The results indicated that untreated control cells presented diffused staining, but OB treatment clearly resulted in an extensive punctuate MDC staining pattern (Fig. 5B). However, LC3, another typical marker of autophagy, exists in the cytosol and is called LC3-I, after its C-terminal region, which is cleaved through post-translational modification. When autophagosomes are formed, LC3-I changes to LC3-II through conjugation with phosphatidylethanolamine and is recruited into the autophagosome membrane. That is why LC3-II accumulation is a critical marker of autophagy (19). As shown in Fig. 6C, OB treatment induced an increase in LC3-II expression. In addition, treatment with OB strongly increased the essential autophagosome-regulatory gene expression of Atg3, and slightly increased the gene expression of Atg5, but not Atg7 and Atg12. We also found an accumulation of LC3 puncta following OB treatment through fluorescence microscopic observation (Fig. 7A). Taken together, these results indicate that OB-induced cytoplasmic vacuoles are related to autophagy induction.

Modulation of Nrf2 in OB-treated A549 cells. Because many recent studies have reported that Nrf2 is involved in cancer cell survival and in the acquisition of drug resistance

against anticancer therapies, we investigated whether the Nrf2 signaling pathway is involved in OB-caused cell death. As shown in Fig. 6, although exposure of cells to OB led to dephosphorylation of Nrf2 proteins without altering their total levels, Nrf2 and p-Nrf2, the active form of Nrf2, were translocated to the nucleus in response to OB treatment, which was associated with decreased expression of HO-1 whose transcription is regulated by Nrf2. Therefore, OB-induced cell death appeared to be responsible for nuclear translocation of p-Nrf2.

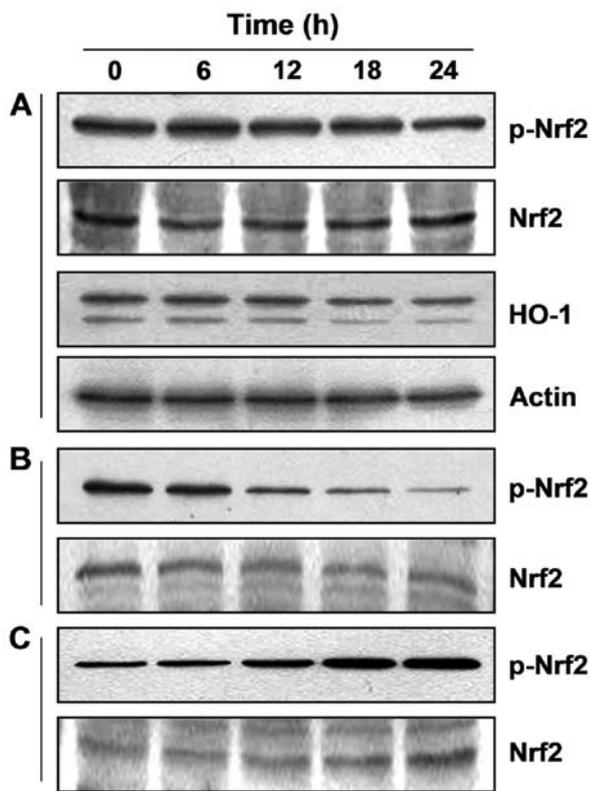*OB-induced autophagy as a death mechanism in A549 cells.* To elucidate the molecular mechanism controlling the rela-

Figure 6. Effects of OB on the Nrf2 levels in A549 cells. Cells were treated with 30 μM OB for the indicated time period. (A) Total cell lysate, (B) cytosolic, and (C) nuclear proteins were extracted, and then equal amounts of the proteins were resolved on SDS-polyacrylamide gels and transferred to the nitrocellulose membranes. Proteins were detected with anti-p-Nrf2, Nrf2 and HO-1 antibodies and ECL detection. Actin was used as an internal control.

tionship between OB-induced apoptosis and autophagy, we next investigated whether OB-induced autophagy functioned as a survival mechanism or a death mechanism. As shown in Fig. 7A, pretreatment with bafilomycin A1, a well-known inhibitor of autophagosomal lysosome degradation, blocked the formation of OB-induced LC3 puncta, representing recruited LC3-II in the cytosol. Western blot analysis also showed that PARP cleavage, caspase-3 activation, and p-Nrf2 translocation were restored to levels seen in the control cells in response to bafilomycin A1 pretreatment, which was connected with significant blockage of OB-induced apoptosis and growth inhibition (Fig. 7C and D). Taken together, these results clearly show that OB treatment induces autophagy as a cell death mechanism in A549 cells.

Discussion

In the present study, we demonstrated that the growth inhibitory effects of OB, a cycloartane-type triterpene glycoside isolated from *D. morbifera*, were associated with induction of apoptosis and autophagy in A549 NSCLC cells. Our data also suggested that OB-induced autophagy functioned as a death mechanism, which was connected to the translocation of p-Nrf2 from cytoplasm to the nucleus.

Apoptotic cell death, type I PCD, is an important process that allows a cell to self-degrade to eliminate damaged cells (4,5). There are two main apoptotic pathways: the intrinsic mitochondrial pathway and the extrinsic death receptor pathway. The intrinsic pathway, which is triggered by various extracellular and intracellular stresses, produces mitochondrial-mediated signals, causing the mitochondrial permeability transition pore and the release of pro-apoptotic proteins such as cytochrome *c*. The release of cytochrome *c* is essential for caspase-9 activation. Activated caspase-9 in turn activates the downstream effector caspase-3 and -7, which rapidly cleave intracellular substrates (4-6). The extrinsic signaling pathway initiates apoptosis through transmembrane receptor-mediated interactions. The best characterized cytoplasmic death receptors are Fas, DR4 and DR5. After the respective ligands bind to the receptors, an adapter protein called Fas-associated death domain (FADD) or TRAIL-associated death domain (TRADD) is recruited to the death receptor, forming DISC to activate pro-caspase-8. The activation of caspase-8 is antagonized by c-FLIP, an enzymatically-inactive relative of caspase-8 that binds to DISC. Therefore, the knockdown of c-FLIP augments DISC recruitment, activation and processing of caspase-8, thus enhancing effector-caspase stimulation and apoptosis (20,21). Several pro-apoptotic and anti-apoptotic proteins are involved in the upstream and downstream of this process, such as IAP and Bcl-2 member proteins, as well as the expression of death receptors (22,23). In our study, among the Bcl-2 family proteins, OB treatment markedly downregulated the whole form of the Bid proteins in a time-dependent manner reflecting Bid cleavage and activation. OB also significantly inhibited the expression of c-FLIP without altering the DR4, DR4, FADD, Fas and FasL levels. Caspases are integral components of the apoptotic pathway. Although many reports have shown that chemotherapeutic agents induced apoptosis through the caspase-dependent pathway in cancer cells, apoptotic cell death can occur in a caspase-independent manner (24-26). In our case, OB induced activation of initiator caspases (caspase-8 and -9) and an effector caspase (caspase-3), but did not restore cell viability and failed to suppress the OB-induced apoptotic cell deaths by treatment with z-VAD-fmk, a broad-spectrum caspase inhibitor. The results indicate that treatment with OB-induced apoptosis was caspase-independent of the death receptor signaling and mitochondria pathways in A549 cells.

In addition to caspase-dependent cell death, some cell death processes occur in a caspase-independent manner. Autophagy (type II PCD) is a cellular process characterized by sequestration of part of the cell cytoplasm, including long-lived protein organelles, in autophagic vesicles and their delivery to and subsequent degradation following fusion with the cellular lysosomes throughout the lifecycle of an organism in a caspase-independent manner (8,27). Autophagy thus maintains cellular homeostasis throughout the lifecycle of an organism. Recently, many reports have shown that chemotherapeutic agent-induced autophagy was associated with upregulation and processing of LC3/Beclin-1 coupled with induction of Atgs, and its recruitment to the autophagosomes (28,29). In the present study, OB did not alter the expression level of Beclin-1; however, the Atg3 and Atg5 levels, and the autophagic form of LC3 were increased after OB treatment indicating that OB also induced autophagy in A549 cells.
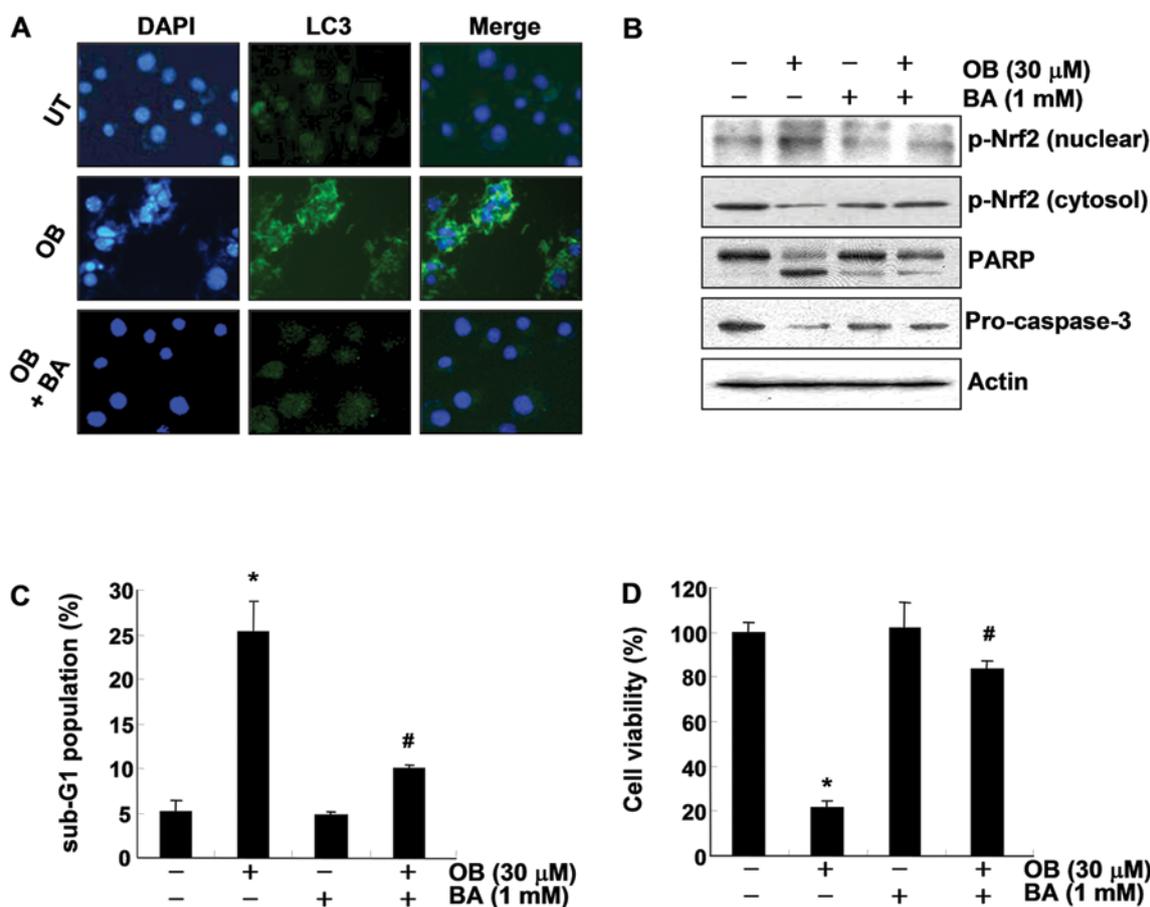
Figure 7. Effects of an autophagy inhibitor, bafilomycin A1, on OB-induced apoptosis in A549 cells. (A) Cells were preincubated with 1 μM bafilomycin A1 (BA) for 2 h and then treated with 30 μM OB for 24 h. The cells were fixed and stained with DAPI, anti-LC3 antibody, and then FITC-conjugated anti-rabbit IgG. Images were taken with a laser scanning confocal microscope. (B) The cells were pretreated with 1 μM BA for 2 h, and then treated with 30 μM OB for 24 h and lysed. Equal amounts of proteins were resolved by SDS-polyacrylamide gels, transferred to nitrocellulose and probed with the indicated antibodies. Actin was used as an internal control. (C and D) The cells grown under the same conditions as (B) were collected. Then the percentage of the (C) sub-G1 population and the (D) cell viability were evaluated with a flow cytometer and MTT assay, respectively. Each point represents the mean ± SD of three independent experiments. The significance was determined with Student's t-test (*p<0.05 vs. untreated control; $^#$p<0.05 present vs. absent BA).

Nrf2, an essential member of basic leucine zipper transcription factors, by binding to antioxidant response element (ARE) plays a key physiological role in regulating oxidative stress. Previous studies have reported that activation of the Nrf2-ARE/HO-1 pathway by chemotherapeutic agents has been correlated with preventing inflammatory diseases and cancer (30,31). Recently, Ansari *et al* (32) also reported that activation of the Nrf2-ARE/HO-1 pathway by chemotherapeutic agents caused protection against hydrogen peroxide-induced cell death. Interestingly, our results show that treatment with OB led to a significant reduction in the phosphorylation levels of Nrf2 proteins and expression of HO-1 proteins, but did not affect the total levels of Nrf2 expression. Furthermore, treatment with OB resulted in the translocation of p-Nrf2 from cytosol to the nucleus in A549 cells. Although further studies are needed, these results indicate that OB-induced cell death is involved in the translocation of p-Nrf2 into the nucleus in A549 cells.

Apoptosis and autophagy are two distinct forms of programmed cell death; however, apoptosis is always associated with cell death, while autophagy normally contributes either to cell survival or to cell death. In some cases, apoptosis and autophagy are interconnected positively or negatively (11,33-35). Thus, the molecular mechanism controlling the relationship between apoptosis and autophagy during cancer cell death by certain chemotherapeutic agents must be elucidated. Interestingly, in our study, OB-induced autophagy and OB-induced cell death and growth inhibition were significantly attenuated by pretreatment with an autophagy inhibitor bafilomycin A1. Furthermore, pretreatment with bafilomycin A1 had an impact in suppressing OB-induced downregulation of p-Nrf2. Taken together, the present results suggest that OB-induced autophagy functioned as a death mechanism and the Nrf2 signaling pathway is involved in this process in A549 cells.

In conclusion, the present results demonstrated that OB-induced cell death occurs through induction of autophagy as cell death mechanisms and caspase-dependent apoptosis. Although the role of the Nrf2-ARE/HO-1 pathway in OB-induced autophagy remains unknown, promoting autophagy could be an effective strategy for enhancing the antitumor activity of OB in A549 NSCLC cells.

Acknowledgements

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