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In Vitro and in Vivo Antimammary Tumor Activities and Mechanisms of the Apple Total Triterpenoids

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ABSTRACT: Consumption of apples has been linked to the prevention of various chronic diseases, including tumors and cardiovascular diseases. The apple total triterpenoid content (ATT) was extracted and concentrated from apple peels. The in vitro and in vivo antitumor activities, related antitumor mechanisms, were investigated. In vitro, ATT showed potent antiproliferative activities against human breast cancer (MCF-7, MDA-MB-231), human colon cancer (Caco-2), and human liver cancer (HepG2) cell lines. In vivo antitumor experiments showed that ATT could substantially reduce the occurrence and growth of mammary tumor with a good dose-dependent manner in a rat model. During the apoptosis in MDA-MB-231 cells induced by ATT, the caspase-independent pathway was involved in the process of apoptosis, and the mitochondrial transmembrane potential was markedly reduced. Also the PI3K/Akt/NF-κB pathway was activated. These results indicated that ATT-induced apoptosis of MDA-MB-231 cells may involve a mitochondrial-related pathway.

KEYWORDS: *apple, total triterpenoids, mammary tumor, antitumor activities, antitumor mechanisms, MDA-MB-231 cells*

■ INTRODUCTION

Apples (*Malus pumila*) play a very significant part in the diet of humans. Consumption of apples has been linked to the prevention of various chronic diseases.[1] Apple intake can reduce mammary tumor incidence and growth,[2] lung cancer incidence,[3,4] cardiovascular disease,[5] symptoms of chronic obstructive pulmonary disease,[6] and the risk of thrombotic stroke.[7] Meanwhile, apple extracts have been shown to have potent antioxidant and antiproliferative activity against human tumor cells.[8] Apple peels exhibited lipase-inhibitory activity, which may be linked to the ursenoic acid content. Furthermore, both triterpenoid content and lipase-inhibitory activity varied by cultivar.[9]

In our previous study, the bioactive compounds of apple peels were investigated, and it was found that the triterpenoids may be responsible for apple's anticancer activity.[10,11] There were 22 triterpenoids isolated and identified from the peels of red delicious apple. Ursanes and oleananes were the two classes of triterpenoids found in apple. Ursolic acid (UA) was the main and active constituent among the total triterpenoids.[10]

Apoptosis is a form of programmed cell death involving a biochemical cascade. It is also necessary for the destruction of cells considered a threat such as cells infected with viruses, cells with DNA damage, and cancerous cells. Impaired regulation of apoptosis leads to a variety of diseases. The progress of apoptosis is regulated by a series of signal cascades under certain circumstances: internal and external signals. Internal damage to a cell, such as oxidative damage, causes Bcl-2 to activate Bax, which creates holes in the mitochondrial membrane. These holes allow the entry of cytochrome *c* into the cytoplasm. Cytochrome *c* binds to Apaf-1 forming complexes that aggregate to form apoptosomes. The apoptosomes activate caspases. These executioner caspases activate a cascade of proteolytic activity that leads to the digestion of structural proteins, DNA degradation. For external signals, FasL and TNF-alpha/beta can bind to the cell membrane receptors Fas and TNF, respectively. Binding of the Fas or TNF ligands results in the activation of caspase-8, which unleashes a proteolytic cascade similar to that activated by caspase-9. Fas ligand (FasL) has been well characterized as a death factor. The death domain of Fas, FADD, and caspase-8 were required for NF-κB activation by FasL.[12] FasL is a critical downstream effector of p53-dependent apoptosis.[13,14] This cascade results in phagocytosis of the cell. However, recent evidence for FasL-independent activation of Fas suggests that the death receptor can also be activated intracellularly, in the absence of its ligand.[15] The caspase-cascade system plays vital roles in the induction, transduction and amplification of intracellular apoptotic signals.[16,17]

SIRT1 (sirtuin 1) is an NAD^+-dependent protein deacetylase with over two dozen known substrates that affect a wide variety of cellular processes, including metabolism, cell cycle, growth and differentiation, inflammation, senescence, apoptosis, stress response, and aging.[18] PI3K/Akt constitutes an important pathway regulating the signaling of multiple biological processes such as apoptosis, metabolism, cell proliferation, and cell growth.[19,20]

The family of NF-κB transcription factors play a key role in diverse biological processes, such as cell survival and tumor development. Members of the NF-κB family of dimeric transcription factors regulate expression of a large number of genes involved in immune responses, inflammation, cell survival, and cancer.[21]

In the present work, the total triterpenoids of apple (ATT) were extracted and concentrated from apple peels and its antitumor activities were investigated in vitro and in vivo.

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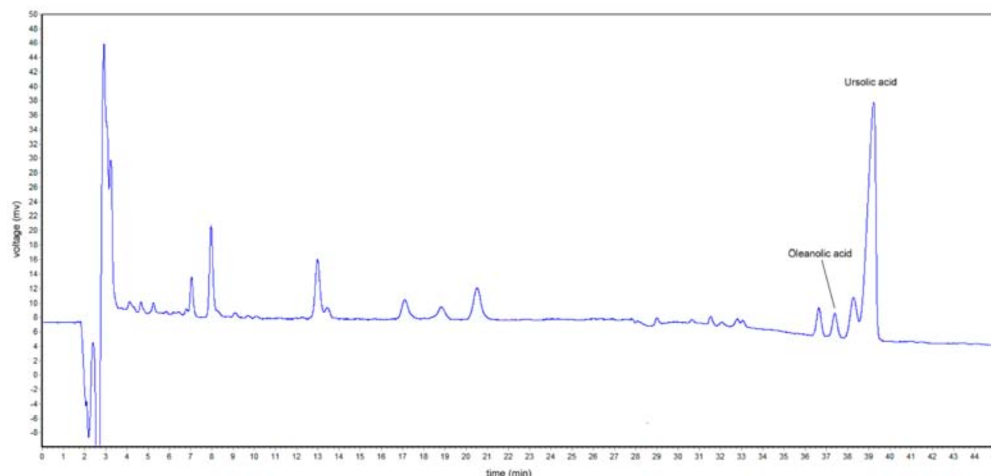

Figure 1. HPLC profile of ATT.

Table 1. IC_{50} Values for the Inhibition of Human Tumor Cells of AP, ATT, UA, and OA (Mean ± SD, n = 8)

	IC_{50} (mg/L)				
sample	MCF-7	MDA-MB-231	K562	Caco-2	HepG2
AP	25.6 ± 3.1	35.8 ± 3.7	62.5 ± 7.9	76.3 ± 6.6	30.1 ± 2.6
ATT	5.3 ± 0.8	4.7 ± 0.6	25.9 ± 3.6	21.6 ± 2.6	10.5 ± 1.7
UA	7.9 ± 1.1	11.2 ± 1.3	18.0 ± 2.2	20.1 ± 3.0	23.0 ± 2.5
OA	13.6 ± 2.1	16.3 ± 2.5	38.7 ± 4.6	22.6 ± 3.4	20.3 ± 2.8
cisplatin	3.1 ± 0.5	15.8 ± 1.9	21.6 ± 3.2	10.5 ± 1.8	5.1 ± 0.6

The related mechanisms of ATT-induced MDA-MB-231 cell apoptosis were investigated through caspase-independent mitochondria pathway and PI3K/Akt/NF-κB pathway.

■ MATERIALS AND METHODS

Plant Material. Apples of red Fuji variety were purchased from Wuhan Shahu Fruits Market (Wuhan, China). They were harvested in October 2009.

Reagents. All analytical and HPLC grade reagents were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ). 7,12-Dimethylbenz-[*a*]anthracene (DMBA) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). D101 macroporus resin was product of Xi'an Lanxiao Resin Corporation Ltd. (Xi'an, China).

Fetal bovine serum (FBS) and cell culture medium were purchased from Gibco Life Technologies (Grand Island, NY). Ac-YVAD-CMK, Z-IETD-FMK, and Z-LEHD-FMK were purchased from ICN (Aurora, OH). Z-DEVD-FMK and Z-VAD-FMK were purchased from Calbiochem (Gibbstown, NJ). Akt inhibitor, staurosporine, wortmannin, PDTC, MG132, H-7, Hoechst 33258, propidium iodide (PI), RNase A, proteinase K, and 3-(4,5-dimethylthiazol-2-yl)-2,5 -diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).

Extraction and Concentration of the Total Apple Triterpenoids (ATT) from Apple Peels. Fresh apples of red Fuji variety (50.0 kg) were peeled by an apple peeler. The fresh apple peels (5.5 kg) were dried under 60 °C and then crushed into small pieces, which were extracted twice using 10 volumes of 95% ethanol for 2 h at reflux. The 95% ethanol extract (AP) was evaporated under vacuum at 45 °C to remove ethanol. The residue was then resuspended in 1000 mL of water and subjected to a D101 macroporus resin column (50 × 1000 mm). The resin column was eluted with 3000 mL of 60% ethanol and then with 3000 mL of 95% ethanol, respectively. The 95% ethanol elution was concentrated in vacuum, yielding a white powder (21.0 g, 0.38% yield of fresh apple).

Quantitation of Total Triterpenoids, Ursolic Acid, and Oleanolic Acid in ATT. The total triterpenoid content in ATT was determined by colorimetric methods using the reported protocol.[22]

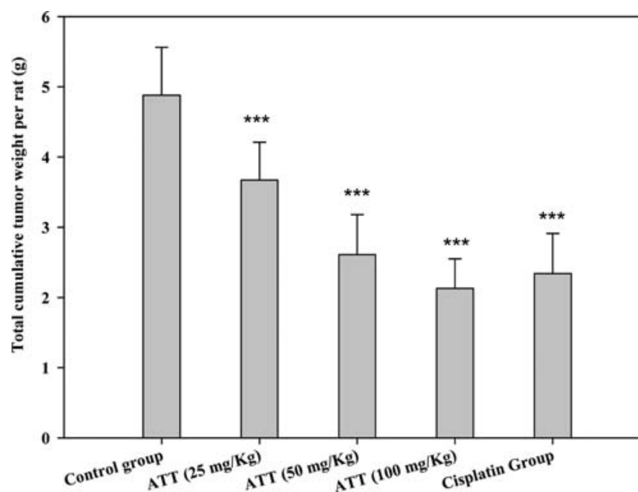

Figure 2. The total cumulative weight of observable mammary tumors of each group at the 20th day of the first treatment (mean ± SD, n = 10).

Briefly, UA standards (80−400 μg/mL) were prepared fresh in methanol before use. 1 mL of sample or UA standard solution was added into each test tube (15 × 150 mm) and then taken to dryness under nitrogen gas. To each tube was added 0.3 mL of 5% vanillin−glacial acetic acid solution and 1.0 mL of perchloric acid, which was reacted in a 60 °C water bath for 20 min. Then, 5 mL of glacial acetic acid was added and mixed well. The absorbance was measured at 550 nm using a Microplate Reader (Kehua Technologies, Inc., Shanghai, China). Total triterpenoid content was expressed as mg of UA equivalent per gram of sample. Data were reported as mean ± SD for at least three replicates.

Ursolic acid (UA) and oleanolic acid (OA) were determined by HPLC using a Waters 600 instrument equipped with a PDA detector (Milford, MA). The chromatographic conditions were as follows: column, LiChrospher 100 RP-100e 5 μm (4.6 × 250 mm); detection

Table 2. Change in Body Weight of Rats after Different Treatments between the 1st and 20th Days of Treatment (Mean ± SD, n = 10)

group	change in body weight (g)
control	6.81 ± 1.92
ATT (50 mg/kg)	5.36 ± 1.21
ATT (25 mg/kg)	5.76 ± 1.52
ATT (12.5 mg/kg)	6.42 ± 1.58
cisplatin (10 mg/kg)	−25.63 ± 5.31**[a]

[a]**p < 0.001 vs control group.

wavelength, 210 nm; mobile phase, $CH_3CN-H_2O-CF_3COOH$ (800:200:1); flow rate, 0.8 mL/min; column temperature, 40 °C.

Measurement of Inhibitory Activity on Tumor Cell Proliferation. Human breast cancer (MCF-7, MDA-MB-231), human colon cancer (Caco-2), and human liver cancer (HepG2) cell lines were purchased from American Type Culture Collection (ATCC, Rockville, MD). They were used for bioactivity evaluation of ATT, UA, and OA based on the established protocols.[10] Briefly, cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (Gibco Life Technologies), 10 mM HEPES, 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamicin and were maintained at 37 °C in 5% CO_2. A total of 2.5×10^4 cells in growth medium were placed in each well of a 96-well flat-bottom plate. After 12 h of incubation, the growth medium was replaced by medium containing different concentrations of the test samples. After 48 h of incubation, cell proliferation was determined by colorimetric MTT assay. Cell proliferation (percent) was determined at 48 h from the MTT absorbance (490 nm) reading for each concentration compared to the control. At least three replications for each sample were used to determine the cell proliferation.

Antimammary Tumors in Rat Model. All protocols involving animal experiments were approved by the ethics committee of Wuhan University, China. The DMBA was dissolved in olive oil at a concentration of 25 mg/mL. Female Sprague−Dawley rats at the age of 50 days weighing 160−180 g were gavaged with 60 mg of DMBA/kg body weight, a dose sufficient to cause 100% tumor incidence over the course of study.[23]

After 12 weeks of DMBA administration, the mammary carcinomas of rats were confirmed by histological examination and breast palpation for continuing experiments. The tumor bearing rats were divided initially into five groups, namely, control group, ATT groups (25, 50, 100 mg/kg), and cisplatin group (10 mg/kg). Each group had 10 rats. ATT was dissolved in glycerin (5.0 mg/mL). Cisplatin was dissolved in normal saline for injection at 2.0 mg/mL. The control group received normal saline. The ATT groups were gavaged with ATT at 25, 50, and 100 mg/kg, respectively. The cisplatin group was

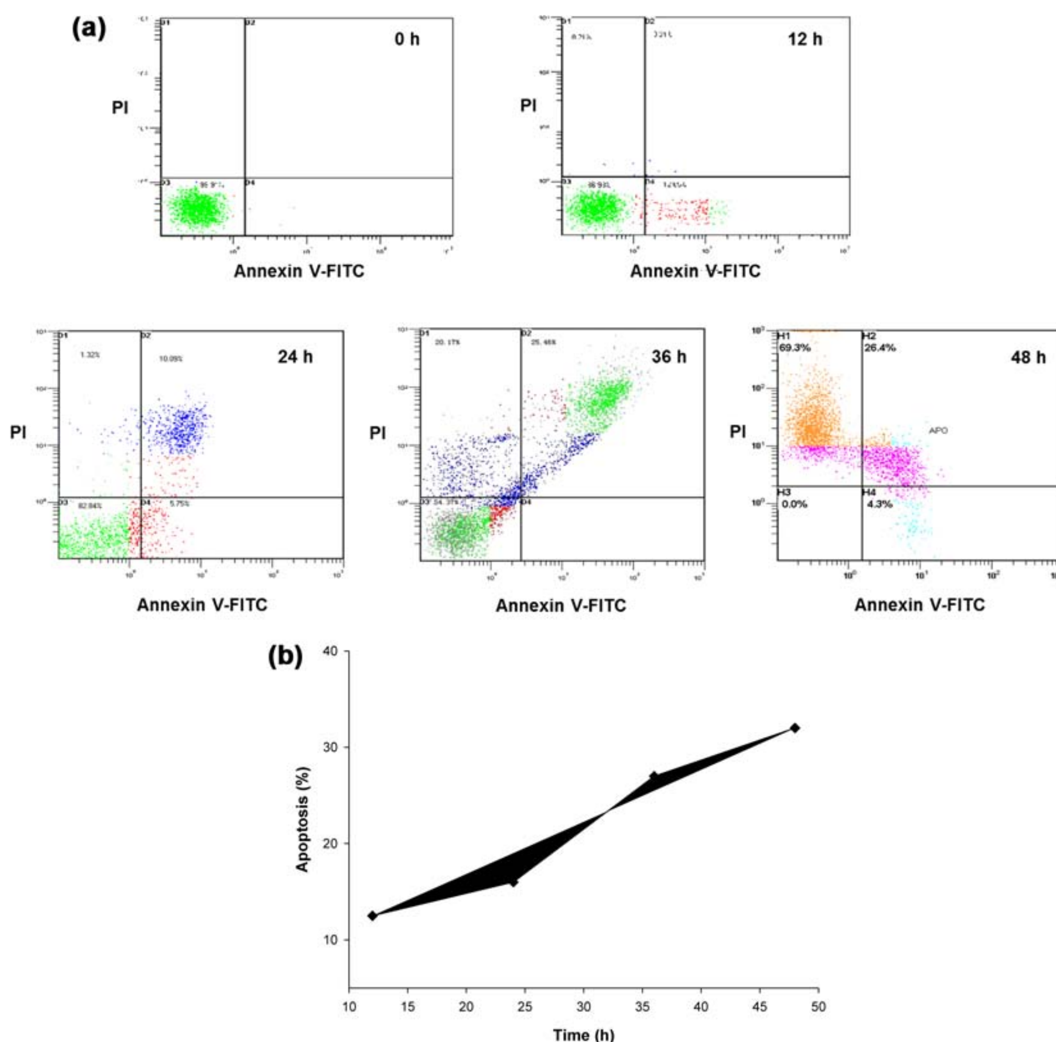

Figure 3. Effect of the ATT on cell cycle kinetics and quantitative percentage of apoptotic cells toward MDA-MB-231 cells. (A) After MDA-MB-231 cells triggered by 5 mg·L^{-1} ATT at the indicated times, the cells were stained with Annexin V-FITC/PI and subsequently analyzed by flow cytometry. (B) Percentage of apoptosis.

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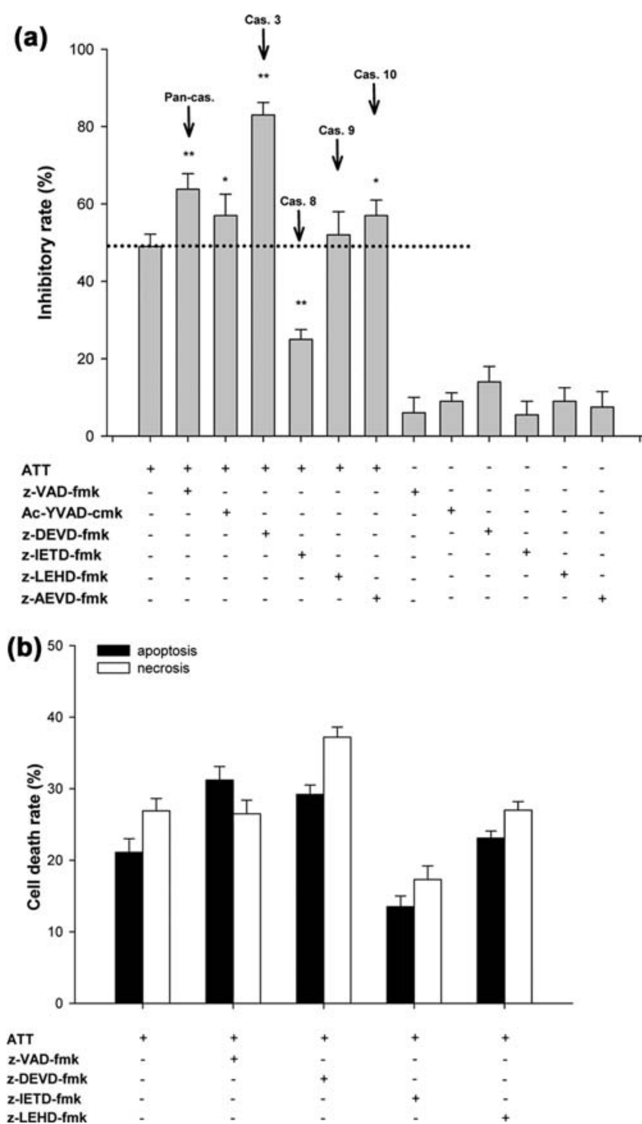

Figure 4. Effects of caspases on ATT induced MDA-MB-231 cell death. (A) Effects of caspase inhibitors on 5 mg·L^{-1} ATT induced MDA-MB-231 cell death. The cells were cultured in the absence or presence of z-VAD-fmk(20 μmol·L^{-1}), Ac-YVAD-cmk(20 μmol·L^{-1}), z-DEVD-fmk(20 μmol·L^{-1}), z-IETD-fmk(20 μmol·L^{-1}), z-LEHD-fmk(20 μmol·L^{-1}), and z-AEVD-fmk(10 μmol·L^{-1}), 1 h prior to the addition of ATT, and then incubated for 24 h. $n = 5$. Mean ± SD. *$p < 0.05$, **$p < 0.01$ vs 5 mg·L^{-1} ATT group. (B) The characteristics of cell death were determined by LDH activity-based assays. MDA-MB-231 cells were cultured in the absence or presence of z-VAD-fmk-(20 μmol·L^{-1}), z-DEVD-fmk(20 μmol·L^{-1}), z-IETD-fmk(20 μmol·L^{-1}), and z-LEHD-fmk(20 μmol·L^{-1}), 1 h prior to the addition of ATT, and then incubated for 24 h. $n = 3$, mean ± SD.

treated with 10 mg/kg cisplatin by intraperitoneal injection. The rats of all groups were treated with the corresponding samples for 2 weeks.

The body weights were measured before the rats were sacrificed at the 20th day. The total weight of all observable tumors in each rat was calculated for analysis.

DNA Cell Cycle Analysis. MDA-MB-231 cells were treated with ATT in culture medium as described above for different time intervals (0, 12, 24, 36, or 48 h). The cells were then harvested, washed with cold phosphate buffered saline (PBS), and processed for cell cycle analysis.[24] Briefly, the cells were centrifuged, washed in PBS, and fixed in cold 70% ethanol for at least 24 h, and then they were stained with a PI solution (20 mg/L propidium iodide and 20 mg/L RNase in PBS). The sample was read on a Coulter Epics XL flow cytometer (Beckman-Coulter, Inc., Indianapolis, IN).

Flow Cytometric Analysis Using Annexin V and PI. Externalization of phosphatidylserine (PS) on the plasma membrane of apoptotic cells was detected using Annexin V-FITC.[25] Briefly, cells were collected by trypsinization and allowed to recover for 30 min in growth medium. Cells were washed twice with cold PBS and then resuspended in 1 time binding buffer at a cell density of 1×10^6 cells/mL. 100 μL of the solution (1×10^5 cells) was transferred to a 5 mL culture tube, and 5 μL of Annexin V-FITC and 5 μL of PI were added. The cells were gently vortexed and incubated for 15 min at rt (25 °C) in the dark. 400 μL of 1× binding buffer was added to each tube. The cells were analyzed by flow cytometry within one hour.

Measurement of Mitochondrial Transmembrane Potential ($_\Delta\Psi_m$). To measure the mitochondrial transmembrane potential ($_\Delta\Psi_m$), the MDA-MB-231 cells were treated with 5 mg·L^{-1} ATT at the indicated time, washed with PBS, and then incubated with PBS containing 1 μg/mL rhodamine 123 at room temperature for 30 min. After two washes and final resuspension in PBS, the fluorescence of cells was measured by flow cytometry.[26]

Statistical Analysis. Experimental values are expressed as mean ± SD. Comparison of mean values between various groups was performed by one-way analysis of variance followed by multiple comparisons using the Tukey test. P value <0.05 is considered to be significant.

RESULTS AND DISCUSSION

Quantitation of Total Triterpenoids, UA, and OA in ATT. After concentration using a D101 macroporus resin column, the total triterpenoid content in ATT was 93.0 ± 4.2% as measured by the colorimetric method. In ATT, the contents of ursolic acid (UA) and oleanolic acid (OA) were 43.5 ± 1.3% and 6.5 ± 1.5%, respectively, determined by HPLC (the HPLC profile was shown in Figure 1). The apple total triterpenoids (ATT) was used for bioactivity study.

Antiproliferative Activity. The antiproliferative activity of apple peel 95% ethanol extract (AP), apple total triterpenoids (ATT), ursolic acid (UA), and oleanolic acid (OA) was evaluated by MTT assay. Their antiproliferative activities are given in Table 1. AP, ATT, UA, and OA showed potent antiproliferative activities against all four cancer cells, especially to human breast cancer cell lines (MCF-7 and MDA-MB-231). ATT showed the highest antiproliferative activity toward MCF-7 and MDA-MB-231 cells, for which the IC_{50} values were 5.3 ± 0.8 and 4.7 ± 0.6 mg/L, respectively. Generally, ATT showed the highest antiproliferative activity against the four cells, compared to AP and its two main active constituents, UA and OA.

In Vivo Antimammary Tumor Activity. ATT was evaluated for in vivo antimammary tumor activity, since it showed significant antiproliferative activity against breast tumor cell lines (MCF-7 and MDA-MB-231) in vitro. The in vivo antimammary tumor results are given in Figure 2. It is shown that ATT could reduce mammary tumor in a rat model. The total cumulative weight of observable mammary tumors in the group of ATT was substantially lower compared to control group ($p < 0.001$), which also showed a strong dose−response trend. Meanwhile, the body weights of rats had a slight change after the treatment with ATT, which was quite different from the cisplatin group (Table 2).

Effect of ATT on Cell Cycle Kinetics and Quantitative Percentage of Apoptotic Cells on MDA-MB-231 Cell. Based on morphologic observation, it was suggested that ATT induced MDA-MB-231 cell death involved in a mechanism of apoptosis. The cell cycle distributions of MDA-MB-231 cells were investigated by flow cytometry after treatment with ATT.

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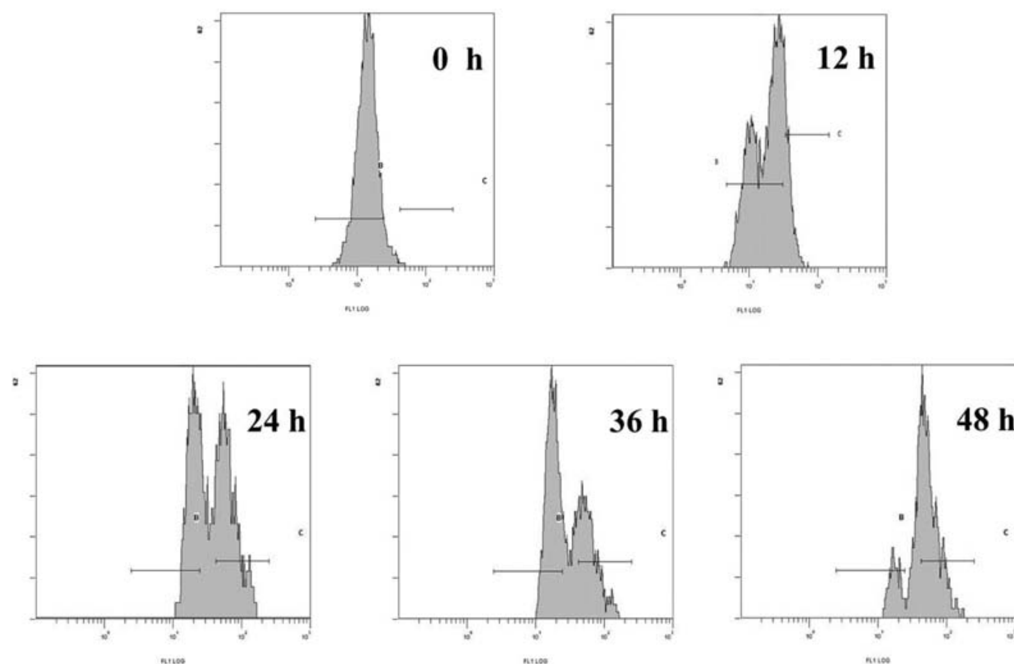

Figure 5. Effect of ATT on the loss mitochondrial membrane potential in MDA-MB-231 cells. The cells were incubated with 5 mg·L^{-1} ATT at indicated times and then were stained with rhodamine 123 and analyzed by FCM.

The results showed that subdiploid peak was increased in a time-dependent manner, which suggested that ATT induced a classical apoptosis. To further confirm and quantify apoptosis in MDA-MB-231 cells triggered by ATT, cells were stained with Annexin V-FITC/PI and subsequently analyzed by flow cytometry. The percentage of apoptotic cells expressed as Annexin V positive cells investigated. After treatment with ATT at different time intervals, apoptosis increased from 12.5% (t = 0 h) to 32.0% (t = 48 h) (Figure 3A, Figure 3B). All the results above suggested that ATT could promote MDA-MB-231 cell apoptosis. Therefore, the apoptotic mechanisms were further investigated.

Caspase-Independent Signaling Involved in ATT Induced MDA-MB-231 Cell Death. Caspases are a family of 14 cysteine proteases that specifically cleave proteins at the C-terminal position of an aspartate residue, which can be activated by different apoptotic signaling to trigger apoptosis. To determine whether caspases contribute to the progress, the different caspase inhibitors were tested. The z-VAD-fmk, the z-DEVD-fmk, and the z-AEVD-fmk substantially promote the cell death, and the z-IETD-fmk effectively enhanced cell viability induced by ATT, but the z-LEHD-fmk had almost no effect on MDA-MB-231 cell viability (Figure 4A). In order to further confirm the characteristic cell death impacted by caspase inhibitors, MDA-MB-231 cells treated by caspase inhibitors were assessed by LDH activity-based assays second. As shown in Figure 4B, both apoptosis and necrosis were increased in z-VAD and z-DEVD-fmk treated cells compared with ATT treated cells, and the morphological changes give similar results.

Effect on the Mitochondrial Transmembrane Potential ($_\Delta\Psi_m$) by ATT Treatment. The loss of mitochondrial transmembrane potential is a hallmark for apoptosis. It is an early event preceding phosphatidylserine externalization and coinciding with caspase activation.[27] Kinetic data indicate that mitochondria undergo major changes in membrane integrity before classical signs of apoptosis become manifest. These changes concern both the inner and the outer mitochondrial membranes, leading to a disruption of the inner transmembrane potential ($_\Delta\Psi_m$) and the release of intermembrane proteins through the outer membrane. In this study, mitochondrial transmembrane potential ($_\Delta\Psi_m$) was further assessed using rhodamine 123, a specific fluorescent probe for the analysis of mitochondrial transmembrane potential. The mitochondrial transmembrane potential decreased in a time-dependent manner, with the major decrease occurring at 36 h (Figure 5), suggesting that ATT induced MDA-MB-231 cell apoptosis through the mitochondrial pathway.

PI3K/Akt and NF-κB Involved in ATT-Treated MDA-MB-231 Cells. Generally the PI3K/Akt pathway exerts its action in cell proliferation. Whether or not it is involved in ATT-treated MDA-MB-231 cells remains unknown, therefore the different inhibitors were tested. The PI3K family inhibitor (wortmannin), Akt inhibitor, PKC inhibitor (staurosporine), and PKCα inhibitor (H7) significantly promoted cell death (Figure 6A). As shown in Figure 6B, both NF-κB inhibitor (PDTC) and the proteasome inhibitor (MG-132) promoted cell death.

The total triterpenoids of apple (ATT) were extracted and concentrated from apple peels. UA and OA were the main bioactive constituents of ATT. The extracts of apple peels and flesh demonstrated significant bioactivities, such as antioxidant and antimammary tumors.[2] These provide solid evidence that consuming apple has many health benefits. In the in vitro antiproliferative assay, AP, ATT, UA, and OA exhibited potent inhibitory action against the tested cell lines, especially to human breast tumor cell lines. Among them, ATT exhibited the strongest antiproliferative activity.

The ability of cancer cells to avert the apoptotic program has been identified as one of the major mechanisms for the development of cancers. The present studies demonstrated that ATT inhibited the proliferation of MDA-MB-231 cells dramatically. Based on morphologic observation, it is suggested that ATT induced MDA-MB-231 cell death involved in a mechanism of apoptosis. DNA histograms used for cell cycle analysis consistently showed the presence of apoptotic

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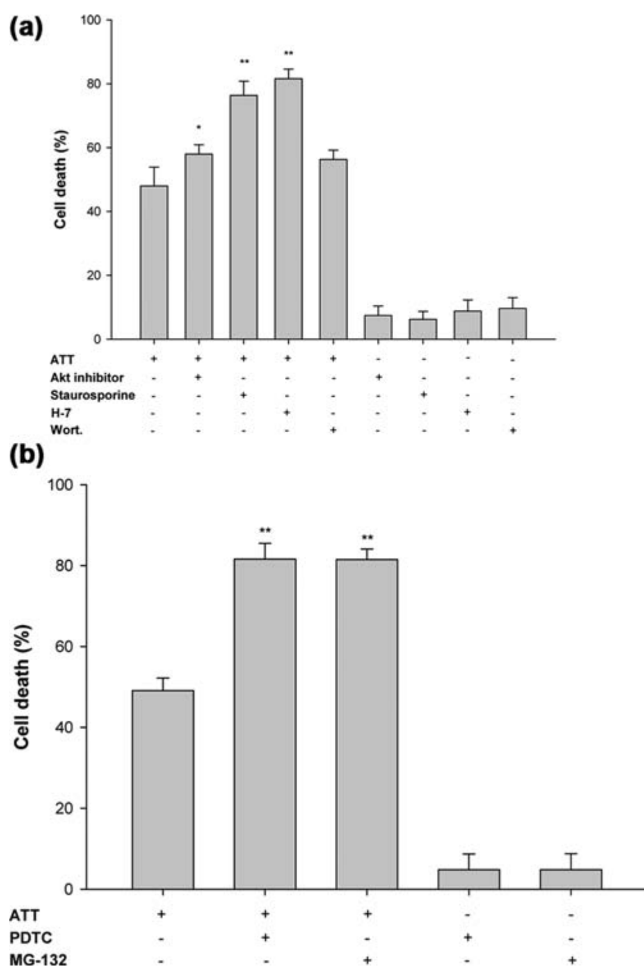

Figure 6. The effect s of PI3K/Akt and NF-κB on ATT-treated MDA-MB-231 cells. (A) The cells were cultured in the absence or presence of Akt inhibitor, staurosporine, H7, or wortmannin separately, 1 h prior to the addition of ATT, and then incubated for 24 h. $n = 5$. Mean ± SD. *$p < 0.05$, **$p < 0.01$ vs 5 mg·L^{-1} ATT group. (B) The cells were cultured in the absence or presence of PDTC or MG132 separately, 1 h prior to the addition of ATT, and then incubated for 24 h. $n = 5$. Mean ± SD. *$p < 0.05$, **$p < 0.01$ vs 5 mg·L^{-1} ATT group.

populations among cells treated with ATT. To ascertain that the mode of cell death was by apoptosis, Annexin V-FITC/PI-flow cytometry analysis was carried out. Apoptosis is characterized by distinct biochemical features, in which activation of catabolic processes and enzymes occurs before cytolysis, thereby facilitating cell morphological changes, such as PS externalization to the cell surface, mitochondrial alterations, etc. Early apoptotic cells tend to exhibit PS on the outer cell membrane, which is normally positioned across the inner membrane,[28] and it has a strong binding affinity to Annexin V.[29] Accordingly, early apoptotic and some late apoptotic cells (commonly known as secondary necrotic cells) can be quantified by flow cytometry assay with fluoresceinated Annexin V (Annexin V-FITC) and DNA-binding fluorochrome (PI).[30] The percentage of apoptotic cells expressed as Annexin V positive cells increased in a time-dependent manner.

Caspases are a family of cysteine proteases, which cleave protein substrates after their Asp residues. They appear to be involved in regulating the activation of apoptotic signal transmission, but it has become apparent that cells frequently die even when caspase function is blocked.[31] The result of this study showed that pretreatment with the caspase inhibitors z-VAD-fmk, z-DEVD-fmk, and z-AEVD-fmk significantly enhanced ATT-induced cell death, the z-IETD-fmk reduced the cell death, but the z-LEAD-fmk had almost no effect on MDA-MB-231 cell viability, therefore it implied that caspase-independent apoptosis happened. In order to further confirm the characteristic cell death affected by caspase inhibitors, MDA-MB-231 cells treated by caspase inhibitors were assessed by LDH activity-based assays. Both apoptosis and necrosis were increased in the Z-VAD and Z-DEVD treated group compared with the ATT treated group, and the morphological changes gave similar results. It has been reported that reactive oxygen species are involved in caspase-independent cell death,[32] but ROS generation was not detected in ATT treated MDA-MB-231 cell, coinciding with Kengo's result.[33] All these results suggest that the caspase-independent cascade happened in ATT-mediated MDA-MB-231 cell apoptosis.

Activation of the PI3K/Akt pathway is associated with tumorigenesis and resistance to apoptosis. Akt activation protects cells from a variety of apoptotic stimuli, including UV radiation and different classes of chemotherapeutic drugs and DNA damaging agents. Akt can also signal through to p53 by phosphorylating MDM2, resulting in its transport to the nucleus where it binds to p53 and promotes its degradation. Moreover, Akt inhibits apoptosis through the inhibition of Bcl-X_L by the activation of NF-κB,[34] and NF-κB thus appears to be a target of the antiapoptotic Ras/PI3K/Akt pathway.[35,36]

These results indicate that ATT-induced apoptosis of MDA-MB-231 cells may involve a mitochondrial-related pathway. Furthermore, other pathways regulating apoptosis should be further investigated.

This study demonstrated that the total apple triterpenoids (ATT) could effectively inhibit tumor growth in vitro and in vivo. Also, apple peels contains much higher triterpenoids (including UA and OA) than apple flesh.[10,37] Therefore, consumption of whole apples may be an effective strategy for cancer protection.

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Notes

The authors declare no competing financial interest.

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