Ailanthone induces G_2/M cell cycle arrest and apoptosis of SGC-7901 human gastric cancer cells

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Abstract. Ailanthone is a major quassinoid extracted from the Chinese medicinal herb *Ailanthus altissima*, which has been reported to exert antiproliferative effects on various cancer cells. The present study aimed to investigate the antitumor effects of ailanthone on SGC-7901 cells, and to analyze its underlying molecular mechanisms. Following treatment with ailanthone, Cell Counting kit-8 was used to detect the cytotoxic effects of ailanthone on SGC-7901 cells *in vitro*. The typical apoptotic morphology of SGC-7901 cells was observed by Hoechst 33258 staining. Cell cycle progression and apoptosis were measured by flow cytometry, and the protein and mRNA expression levels of Bcl-2 and Bax were analyzed by western blot analysis and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) respectively, in SGC-7901 cells. The results of the present study indicated that ailanthone inhibited the proliferation of SGC-7901 cells in a dose- and time-dependent manner *in vitro*, and also demonstrated that ailanthone induced G_2/M phase cell cycle arrest and apoptosis of SGC-7901 cells. Furthermore, analysis of the underlying molecular mechanisms revealed that ailanthone downregulated the expression levels of Bcl-2, whereas the expression levels of Bax were upregulated at the protein and mRNA levels. In conclusion, ailanthone may inhibit the proliferation of SGC-7901 cells by inducing G_2/M phase cell cycle arrest and apoptosis via altering the protein and mRNA expression levels of Bcl-2 and Bax in SGC-7901 cells.

Introduction

Gastric cancer (GC) is one of the most common malignant tumors worldwide. According to GLOBOCAN estimates,

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GC is the fourth most common cancer, with the third highest cancer-associated mortality rate in males worldwide (1). In addition, GC is more prevalent in Eastern Asian countries (2). At present, it is accepted that surgery is the only effective cure for GC; however, chemotherapy is the main treatment for patients with advanced GC, which can effectively improve the quality of life and maximize survival time of patients (3). However, chemotherapy can cause a series of problems, including drug resistance and adverse reactions; therefore, it is not considered ideal for the treatment of advanced GC. Hence, the development of effective antitumor drugs, which possess high efficiency and low toxicity, is required.

At present, the majority of antitumor drugs are natural products derived from plants, which have been used for the clinical treatment of cancer, including vincristine, paclitaxel and docetaxel (4,5). The Chinese medicinal herb *Ailanthus altissima* has long been used for treatment of colds and gastric diseases in traditional Chinese medicine (6). Ailanthone, which is extracted from *Ailanthus altissima* (7), is a type of quassinoid that has been reported to exert obvious antitumor effects. Previous studies have revealed that ailanthone possesses numerous biological activities, including antimalarial, antibacterial and anti-inflammatory activities (8,9). Previous studies have indicated that ailanthone exerts growth-inhibitory effects against HeLa, Jurkat, HepG2, Hep3B, R-HepG2, MCF-7, MDA-MB-231 and A549 tumor cells *in vitro* (10-13). In addition, ailanthone induces apoptosis of Huh-7 cells and significantly inhibits the growth of Huh-7 tumors in a nude mouse xenograft model, without exerting secondary adverse effects (14). Apoptosis is a type of programmed cell death, which is regulated at the gene level, and results in the elimination of damaged cells (15). Anti-apoptotic mechanisms serve an important role in the development and progression of cancer, and are considered hallmarks of cancer and a potential reason for the poor effects of cancer treatment (16,17). Promoting apoptosis has been identified as an effective means of cancer treatment; therefore, ailanthone may potentially be used to treat tumors in the future.

The effects of ailanthone have yet to be reported on GC cells. The present study aimed to investigate the inhibitory effects of ailanthone on the SGC-7901 human GC cell line and to elucidate its potential molecular mechanisms *in vitro*.

Materials and methods

Materials. Pure ailanthone (Fig. 1A) was extracted and isolated from *Ailanthus altissima*. The ailanthone sample (purity ≥98%) was provided by the Institute of Traditional Chinese Medicine and Natural Products, Jinan University (Guangzhou, China). Taxol was obtained from Beijing SL Pharmaceutical Co., Ltd. (Beijing, China). Dimethyl sulfoxide (DMSO) was purchased from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany). The Cell Counting Kit-8 (CCK-8) assay (cat no. KGA317) was obtained from Nanjing KeyGen Biotech Co., Ltd. (Nanjing, China). RPMI-1640 (cat no. 11875-093) and penicillin-streptomycin (PS; cat no. 15140-122) were purchased from Gibco; Thermo Fisher Scientific, Inc. (Waltham, MA, USA). Fetal bovine serum (FBS; cat no. 100-700) was obtained from Gemini Bio Products (West Sacramento, CA, USA). The antibodies for mouse monoclonal β-actin (cat no. BM0626), mouse monoclonal B-cell lymphoma 2 (Bcl-2; cat no. BM0200) and rabbit polyclonal Bcl-2-associated X protein (Bax; cat no. BA0315-2) were purchased from Wuhan Boster Biological Technology Ltd. (Wuhan, China). Horseradish peroxidase (HRP)-conjugated goat anti-mouse (cat no. 62-6520) and anti-rabbit (cat no. G-21234) immunoglobulin (Ig)G were obtained from Invitrogen; Thermo Fisher Scientific, Inc.

Cell culture and treatment. The SGC-7901 human GC cell line (cat. no. KG026) was obtained from Nanjing KeyGen Biotech Co., Ltd. The cells were cultured in RPMI-1640 medium supplemented with 10% FBS and 1% PS in a humidified incubator containing 5% CO_2 and 95% air at 37˚C for cell subculture and all experiments. Stock solutions of ailanthone were prepared in DMSO, and stored at -20˚C. Prior to use, stock solutions were immediately diluted to the required concentration with RPMI-1640 complete medium; the terminal concentration of DMSO in the culture medium was ≤0.1%. Control cells were treated with DMSO (0.1%), without ailanthone and taxol.

Cell viability assay. The CCK-8 assay was used to measure cell viability. Taxol was used in the positive control group. SGC-7901 cells in the exponential growth phase ($5x10^3$ cells/well) were seeded and cultured in 96-well plates for 24 h, and were then treated with 0.1% DMSO (control group), ailanthone (0.5, 1, 2, 4 and 8 μM) or taxol (1.25, 2.5, 5, 10 and 20 μM) for 24, 48 and 72 h at 37˚C, each group was analyzed four times. Subsequently, 10 μl CCK-8 solution was added to each well. After 3 h at 37˚C, the optical density was measured at a wavelength of 450 nm using a microplate reader (RT-6000; Rayto Life and Analytical Sciences Co., Ltd., Shenzhen, China). Relative cell viability was determined using the following formula: Relative cell viability = (mean A450 of experimental groups/mean A450 of control groups) x100%.

Hoechst 33258 staining. Hoechst 33258 staining was used to observe the apoptotic morphology of cells. Exponentially growing SGC-7901 cells were cultured on glass coverslips in 6-well plates ($3x10^5$ cells/well) for 24 h, and were then treated with 0.1% DMSO or ailanthone (1 and 2 μM) for 48 h at 37˚C. The cells were dried and were fixed in 4% paraformaldehyde for 30 min at room temperature. Subsequently, the cells were

washed three times with phosphate-buffered saline (PBS), and were stained with 10 μg/ml Hoechst 33258 (cat no. KGA211-10; Nanjing KeyGen Biotech Co., Ltd.) for 10 min at room temperature. The cells were washed a further two times with PBS and were mounted using antifade mounting medium (cat no. P0126; Beyotime Institute of Biotechnology, Shanghai, China). Finally, nuclear morphology was observed by fluorescence microscopy (Olympus BX43; Olympus Corporation, Tokyo, Japan).

Annexin V-allophycocyanain (APC)/7-amino-actinomycin D (ADD) apoptotic analysis. Exponentially growing SGC-7901 cells were seeded and cultured in 6-well plates ($3x10^5$ cells/well) for 24 h, and were then treated with 0.1% DMSO or ailanthone (1-4 μM) for 48 h at 37˚C. The cells were washed two times with cold PBS, and were then incubated with Annexin V APC/7-ADD (cat no. KGA1026; Nanjing KeyGen Biotech Co., Ltd.) for 15 min at room temperature in the dark according to the manufacturer's protocol. Subsequently, the cells were analyzed by flow cytometry. Fluorescence was measured using a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). The experiment was independently repeated three times, and the proportion of apoptotic cells was calculated using the FACSCalibur internal software system (BD Biosciences).

DNA content analysis. The SGC-7901 cells were seeded and cultured in 6-well plates ($3x10^5$ cells/well) for 24 h, and were then treated with 0.1% DMSO or ailanthone (1-4 μM) for 48 h at 37˚C. Cells were harvested, washed with PBS and were fixed in 70% ethanol at 4˚C overnight. Subsequently, cells were incubated with 1% RNase A at 37˚C for 30 min and with propidium iodide solution (cat no. KGA511; Nanjing KeyGen Biotech Co., Ltd.) at 4˚C for 30 min in the dark. The DNA content of cells was measured using a FACSCalibur flow cytometer (BD Biosciences). The experiment was independently repeated three times, and data were analyzed using the MultiCycle DNA content and cell cycle analysis software (FlowJo, version 7.6.5; FlowJo LLC, Ashland, OR, USA).

Western blot analysis. Following treatment with 0.1% DMSO or ailanthone (1-4 μM) for 48 h at 37˚C, SGC-7901 cells were washed twice with ice-cold PBS and suspended in radio immunoprecipitation assay lysis buffer (cat no. KGP702; Nanjing KeyGen Biotech Co., Ltd.) on ice for 30 min. The lysates were then cleared by centrifugation at 12,000 x g for 15 min at 4˚C. Subsequently, the bicinchoninic acid protein assay kit (cat no. KGP902; Nanjing KeyGen Biotech Co., Ltd.) was used to measure the total protein concentration of each sample according to the manufacturer's protocol. Protein samples (30 μg) from each group were separated by 15% SDS-PAGE and were then transferred onto polyvinylidene difluoride membranes (Pall Corporation, Port Washington, NY, USA). Membranes were blocked with 5% (w/v) non-fat dry milk dissolved in TBS containing 0.05% Tween-20 (TBST) at room temperature for 1 h, and were then washed three times with TBST. Subsequently, membranes were incubated with primary antibodies against Bcl-2 (1:200), Bax (1:200) and β-actin (1:400) overnight at 4˚C. After washing three times with TBST, the membranes were incubated with HRP-conjugated goat
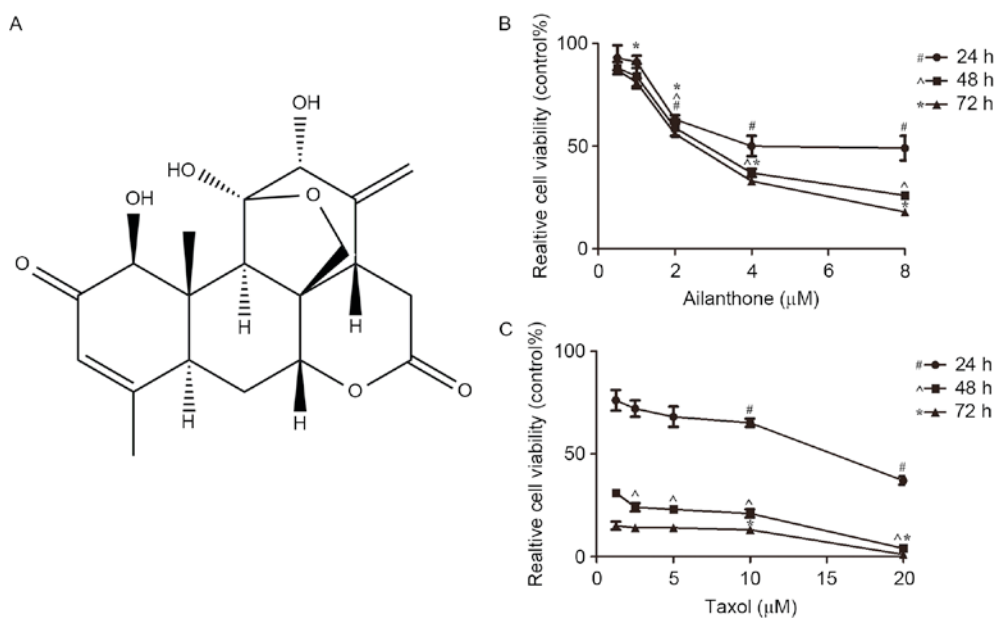
Figure 1. Structure of ailanthone, and growth-inhibitory effects of ailanthone and taxol on SGC-7901 cells. (A) Structure of ailanthone. The molecular formula of ailanthone is $C_{20}H_{24}O_7$. (B and C) Ailanthone induced dose- and time-dependent inhibitory effects on SGC-7901 cell viability. #$P<0.05$, ^$P<0.05$ and *$P<0.05$ vs. the 0.5 μM group. Taxol, as a positive control drug, also inhibited the viability of SGC-7901 cells in a dose- and time-dependent manner. #$P<0.05$, ^$P<0.05$ and *$P<0.05$ vs. the 1.25 μM group.

anti-mouse (1:2,000) or anti-rabbit (1:2,000) IgG secondary antibodies at room temperature for 1 h. The immunoreactive bands were visualized with enhanced chemiluminescent substrates (cat no. 34080; Thermo Fisher Scientific, Inc.) using an X-ray film processor (Kodak, Rochester, NY, USA). β-actin was used as a loading control. The experiment was independently repeated three times, and Quantity One software (version 4.6.2; Bio-Rad Laboratories, Inc., Hercules, CA, USA) was used to analyze the densitometry of each band.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). RT-qPCR analysis was used to analyze the mRNA expression levels of Bcl-2 and Bax in cells treated with 0.1% DMSO or ailanthone (1-4 μM) for 48 h at 37°C. Total RNA was extracted from the cells by TRIzol reagent (cat no. 15596-026; Invitrogen; Thermo Fisher Scientific, Inc.). Subsequently, first-strand cDNA synthesis was conducted using the TransScript® One-Step gDNA Removal and cDNA Synthesis SuperMix kit (cat no. AT311-02; Beijing TransGen Biotech Co., Ltd., Beijing, China) according to the manufacturer's protocol. RT-qPCR analysis was performed on the StepOne Plus Real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) in a 20-μl reaction mixture containing 10 μl AceQ® qPCR SYBR Green Master Mix (Low ROX Premixed) (cat no. Q131-02/03; Vazyme Biotech Co., Ltd., Nanjing, China), 0.8 μl primer mixture (forward and reverse, 10 μM), 1 μl cDNA and 8.2 μl DEPC water. According to the mix kit manufacturer's protocol, the thermocycling profile consisted of 95°C for 5 min, followed by 40 cycles at 95°C for 10 sec and 60°C for 30 sec. GAPDH was used as an endogenous control, and data were analyzed using the $2^{-\Delta\Delta Cq}$ method (18). The experiment was performed four times. The primer sequences were as follows: Bcl-2 forward, 5'-GGT GGGGTCATGTGTGTGG-3' and reverse, 5'-CGGTTCAGG

TACTCAGTCATCC-3'; Bax forward, 5'-CCCGAGAGGTCT TTTTCCGAG-3' and reverse, 5'-CCAGCCCATGATGGT TCTGAT-3'; and GAPDH forward, 5'-GGAGCGAGATCC CTCCAAAAT-3' and reverse, 5'-GGCTGTTGTCATACT TCTCATGG-3'.

Statistical analysis. Data are presented as the mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Data were analyzed by one-way analysis of variance followed by Tukey's test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Ailanthone induces antiproliferatice effects on SGC-7901 cells. The CCK-8 assay was used to determine the effects of ailanthone on the growth of SGC-7901 cells treated with 0.1% DMSO (control group), ailanthone (0.5-8 μM) or taxol (1.25-20 μM) for 24, 48 and 72 h at 37°C. Ailanthone inhibited the viability of SGC-7901 cells in a dose- and time-dependent manner (Fig. 1B). The half maximal inhibitory concentration (IC_{50}) values of ailanthone in SGC-7901 cells at 24, 48 and 72 h were 5.473, 2.906 and 2.47 μM, respectively. Cells treated with taxol were considered the positive control group, taxol also inhibited the growth of SGC-7901 cells in a dose- and time-dependent manner (Fig. 1C). The IC_{50} value of taxol in SGC-7901 cells was 14.47 μM at 24 h.

Ailanthone induces apoptosis of SGC-7901 cells. A Hoechst 33258 staining assay was used to observe the apoptotic morphology of SGC-7901 cells treated with ailanthone (1 and 2 μM) or 0.1% DMSO for 48 h at 37°C. Characteristic apoptotic morphology, including nuclear shrinkage and chromatin
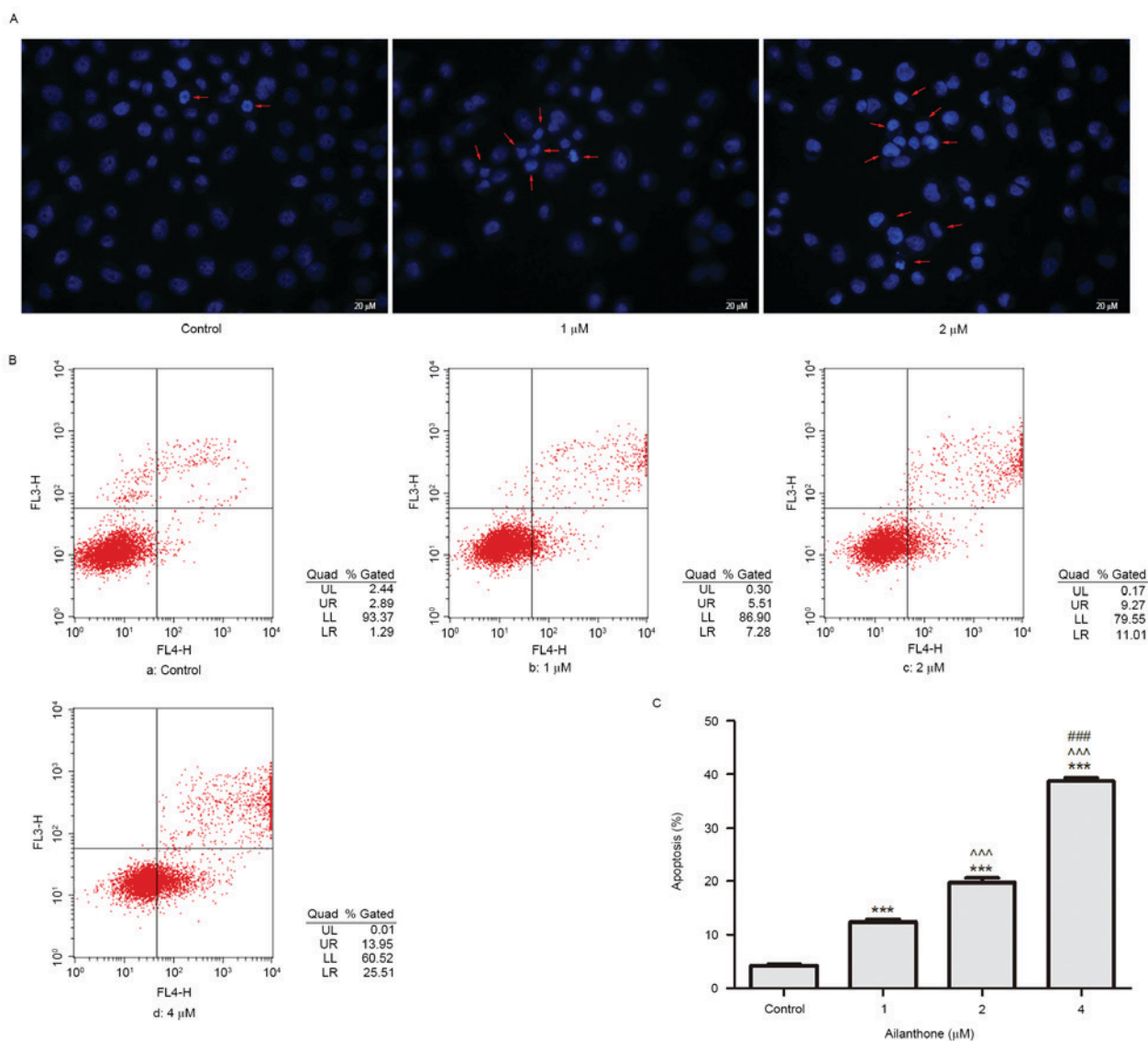
Figure 2. Ailanthone induces apoptosis of SGC-7901 cells. (A) Following treatment with 0.1% DMSO or ailanthone (1 and 2 μM) for 48 h at 37˚C, Hoechst 33258 staining was used to determine the morphological alterations of cells. Red arrows indicate characteristic apoptotic morphology. Scale bar, 20 μm. (B) Detection of apoptosis in SGC-7901 cells treated with ailanthone for 48 h at 37˚C. Early (LR quadrant) and late (UR quadrant) apoptotic cells were detected by flow cytometry. (a) Control cells; (b-d) cells treated with 1, 2 and 4 μM ailanthone, respectively. The experiment was repeated three times, and representative results are presented. (C) Percentage of apoptotic cells. Data are presented as the mean ± standard deviation, n=3. ***P<0.001 vs. the control group; ^^^P<0.001 vs. the 1 μM group; ###P<0.001 vs. the 2 μM group. LL, lower left; LR, lower right; UL, upper left; UR, upper right.

condensation, was observed in the ailanthone groups; however, apoptotic morphology was hardly detected in the control group (Fig. 2A). In order to further confirm the occurrence of apoptosis, cells were stained with Annexin V-APC/7-ADD and were analyzed by flow cytometry. The apoptotic rate of cells was significantly increased with concentration of ailanthone (1-4 μM) from 12.44 to 38.71%. Representative results are presented in Fig. 2B. As shown in Fig. 2C, following treatment with 1, 2 and 4 μM ailanthone for 48 h at 37˚C, the percentage of apoptotic cells was 12.44±0.43, 19.77±0.83 and 38.71±0.7% respectively, which was significantly higher than in the control group (4.31±0.2%; P<0.001). These results indicated that ailanthone induced apoptosis of SGC-7901 cells in a dose-dependent manner.

Ailanthone induces cell cycle arrest of SGC-7901 cells. Flow cytometry was used to analyze the distribution of SGC-7901 cells within the cell cycle following treatment with ailanthone (1-4 μM) or 0.1% DMSO for 48 h at 37˚C. G_2/M phase cell cycle arrest was observed in cells exposed to ailanthone compared with the control group; representative results are presented in Fig. 3A. As shown in Fig. 3B, ailanthone induced a G_2/M phase cell cycle arrest of SGC-7901 cells in a dose-dependent manner; the percentage of cells in G_2/M phase was 14.13±0.48, 18.10±0.56 and 20.56±0.33% in the 1, 2 and 4 μM groups, respectively. Ailanthone significantly increased the percentage of cells in G_2/M phase compared with the control group (12.63±0.78%; P<0.05).

Effects of ailanthone on the protein expression levels of Bcl-2 and Bax in SGC-7901 cells. Bcl-2 is considered an anti-apoptotic protein, whereas Bax is considered proapoptotic. In order to detect the protein expression levels of Bcl-2 and Bax, western blot analysis was performed. Following
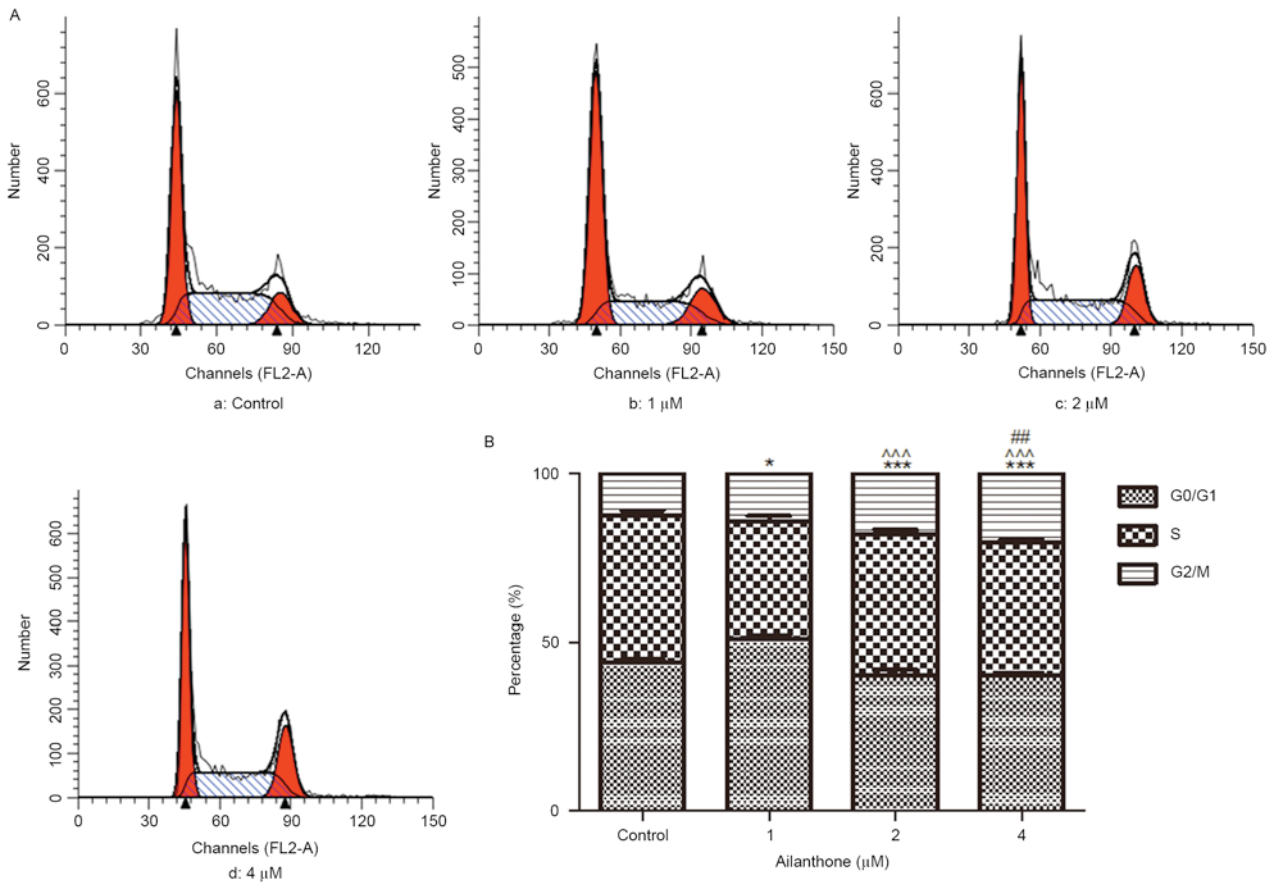
Figure 3. Ailanthone induces cell cycle arrest of SGC-7901 cells. (A) Flow cytometry was used to determine the DNA content of SGC-7901 cells treated with ailanthone for 48 h at 37˚C. (a) Control cells; (b-d) cells treated with 1, 2 and 4 μM ailanthone, respectively. The experiment was repeated three times, and representative results are presented. (B) Percentage of cells in each cell cycle phase. Data are presented as the mean ± standard deviation, n=3. Comparison of the percentage of cells in G_2/M phase: *P<0.05, ***P<0.001 vs. the control group; ^^^P<0.001 vs. the 1 μM group; ##P<0.01 vs. the 2 μM group.
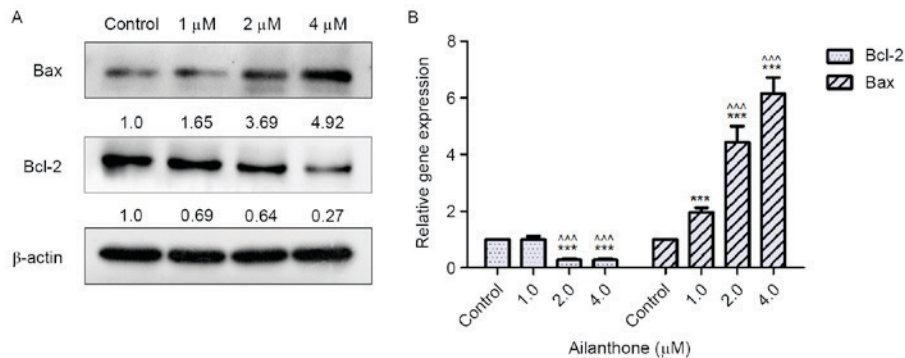

Figure 4. Effects of ailanthone on the protein and mRNA expression levels of Bcl-2 and Bax in SGC-7901 cells treated with ailanthone for 48 h at 37˚C. (A) Western blot analysis of the effects of ailanthone on Bcl-2 and Bax protein expression; this figure is representative of three separate experiments with similar results. β-actin was regarded as a loading control. The numbers under the blots are the mean value of each band's relative densitometry analysis. (B) Reverse transcription-quantitative polymerase chain reaction analyses of Bcl-2 and Bax mRNA expression. The experiment was performed four times, and GAPDH was regarded as a loading control. The expression levels were normalized to the control group. Data are presented as the mean ± standard deviation, n=4. ***P<0.001 vs. the control group; ^^^P<0.001 vs. the 1 μM group. Bax, B-cell lymphoma 2-associated X protein; Bcl-2, B-cell lymphoma 2.

treatment with ailanthone (1-4 μM) or 0.1% DMSO for 48 h at 37˚C, the protein expression levels of Bcl-2 were decreased, whereas the protein expression levels of Bax were increased, compared with the control group (Fig. 4A). The downregulation of Bcl-2 and the enhanced expression of Bax were relative to ailanthone concentration. As shown in Fig. 5, treatment with 2 and 4 μM ailanthone significantly downregulated the protein

expression levels of Bcl-2 compared with the control group (P<0.05, P<0.001), also significantly upregulated the protein expression of Bax compared with the control group (P<0.01, P<0.001).

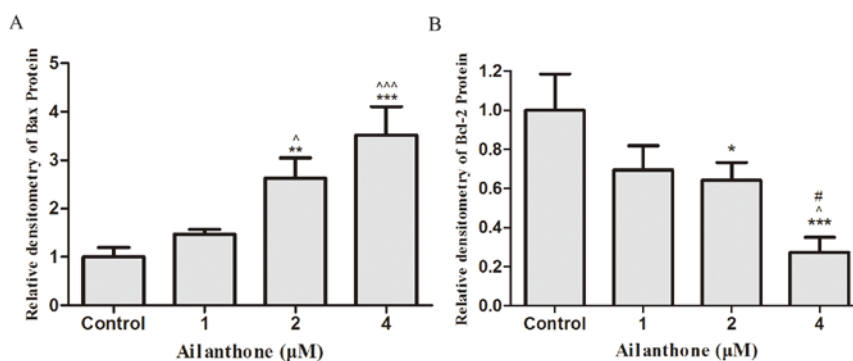*Effects of ailanthone on the mRNA expression levels of Bcl-2 and Bax in SGC-7901 cells.* As shown in Fig. 4B, the

Figure 5. The relative densitometry of (A) Bax and (B) Bcl-2 protein expression. Standardized to the control group, data are showed as mean ± standard deviation, n=3. *P<0.05, **P<0.01 and ***P<0.001 vs. the control group. ^P<0.05 and ^^^P<0.001 vs. the 1 μM group. #P<0.05 vs. the 2 μM group. Bax, B-cell lymphoma 2-associated X protein; Bcl-2, B-cell lymphoma 2.

results of RT-qPCR demonstrated that ailanthone altered the mRNA expression levels of Bcl-2 and Bax in SGC-7901 cells compared with in the control group. Notably, the mRNA expression levels of Bax were significantly upregulated in the ailanthone groups compared with the control group (P<0.001). Conversely, treatment with 2 and 4 μM ailanthone significantly reduced the mRNA expression levels of Bcl-2 compared with the control group (P<0.001). However, 1 μM ailanthone had no effect on the mRNA expression levels of Bcl-2 compared with the control group (P>0.05).

Discussion

Compounds derived from natural products have garnered much attention in cancer research, due to reasons of high efficiency and low toxicity. Ailanthone, which is a potent quassinoid, has been suggested as a potential tumor treatment in previous studies (13,14); however, the anticancer effects and potential mechanisms of ailanthone are unclear in SGC-7901 cells. The present study initially investigated the anti-proliferative activity of ailanthone against SGC-7901 cells, and demonstrated that ailanthone significantly inhibited SGC-7901 cell viability at low concentrations *in vitro*. The IC_{50} value for ailanthone in SGC-7901 cells at 48 h was 2.906 μM.

The present study further analyzed the mechanisms underlying the antiproliferative effects of ailanthone on SGC-7901 cells. Characteristic apoptotic morphology (cell nuclear shrinkage or chromatin condensation) was observed in SGC-7901 cells treated with ailanthone for 48 h using Hoechst 33258 staining. In addition, the rate of apoptosis was increased in a dose-dependent manner in ailanthone-treated SGC-7901 cells. Cancer is regarded as a disease associated with uncontrolled cell proliferation, and anti-apoptotic mechanisms are considered features of cancer that may result in this uncontrolled cell proliferation (16). Apoptosis is a physiological process that does not induce additional damage to normal cells and surrounding tissues when cancer cells are killed in an apoptotic manner (19). Therefore, enhanced apoptosis is considered an effective method for cancer treatment (20). The results of the present study demonstrated that the anticancer effects of ailanthone on SGC-7901 cells were partly due to the induction of apoptosis.

DNA content analysis revealed that cells exposed to ailanthone exhibited increased cell cycle arrest at G_2/M phase

compared with in the control group. In cancer cells, the genetic control of cell division is altered, leading to unlimited cell proliferation. Dysregulation of cell cycle progression is also considered a common characteristic of cancer. The cell cycle process is separated into four sequential phases: G_1, S, G_2 and M phases, and is regulated at numerous positions, known as checkpoints, by a series of proteins, including cyclin-dependent kinases (CDKs) and cyclins (21). Cells are held at cell cycle checkpoint for numerous reasons, including DNA damage, after which the cell cycle process is terminated and the cells undergo apoptosis (22). In the present study, SGC-7901 cells were arrested at G_2/M phase following treatment with ailanthone for 48 h, as determined by flow cytometry. In addition, the cell cycle progression of SGC-7901 cells was hindered by ailanthone, leading to inhibited cell proliferation. Notably, in a previous study, ailanthone significantly induced apoptosis, and arrested cells at G_1/S phase, via upregulated expression of p21 and p27, and downregulated expression of cyclins D and E and CDKs 2, 4 and 6, in Huh-7 hepatocellular carcinoma cells (14). It should be noted that the present study only analyzed the effects of ailanthone on cell cycle distribution without further analyzing its underlying mechanism in SGC-7901 cells. Combined with the previous study, it may be hypothesized that ailanthone affects different phases of the cell cycle in order to serve an antitumor role in various cancer cell lines. Our future studies aim to analyze the effects and underlying mechanisms of ailanthone on cell cycle progression in various cancer cell lines. Similarly, the growth-inhibitory effects of other natural products were partly associated with G_2/M phase arrest of SGC-7901 cells (23,24). Therefore, these data indicated that ailanthone may inhibit the proliferation of SGC-7901 cells partially via G_2/M phase arrest.

In principle, signals that induce cell death can be blocked by upregulation of anti-apoptotic molecules and/or downregulation of proapoptotic proteins (25). The balance between proapoptotic and anti-apoptotic molecules is considered a key point in the regulation of apoptosis, which decides whether cells will be killed by apoptosis. In the majority of eukaryotic cells, there are two major apoptotic pathways: The death receptor pathway and the mitochondrial pathway (26,27); the mitochondrial apoptotic pathway serves an important role in the apoptosis of eukaryotic cells. Furthermore, by affecting the permeability of the mitochondrial membrane, the Bcl-2 protein family is believed to be a switch that

controls the mitochondrial apoptotic pathway (28). Bcl-2 protein family members are usually divided into three subgroups: Anti-apoptotic proteins [Bcl-2, Bcl-extra large (xl), Bcl-2-like protein 2, Mcl-1, Bcl-2-like protein 10 and Bcl-2-related protein A1], proapoptotic proteins [Bax, Bcl-2 homologous antagonist/killer (Bak) and Bcl-2 related ovarian killer] and BH3-only proteins (Bcl-2-associated agonist of cell death, BH3 interacting-domain death agonist and Bcl-2-interacting killer) (29). The anti-apoptotic proteins, including Bcl-2 and Bcl-xl, suppress Bax and Bak, which serve a critical role in the induction of mitochondrial outer membrane permeability resulting in the release of cytochrome *c*, which consequently leads to caspase activation and apoptosis (30,31). Therefore, the ratio of pro- to anti-apoptotic Bcl-2 proteins may regulate the sensitivity of cells to apoptosis (24). An increase in the Bax/Bcl-2 ratio is generally believed to be a critical factor in the activation of programmed cell death (32). To further investigate the underlying molecular mechanisms of ailanthone-induced apoptosis, the protein and mRNA expression levels of Bcl-2 and Bax were detected in SGC-7901 cells treated with ailanthone. The results revealed that ailanthone significantly downregulated the expression of Bcl-2 and upregulated the expression of Bax at the protein and mRNA levels. Therefore, the present study demonstrated that ailanthone induced apoptosis of SGC-7901 cells by altering the expression levels of Bcl-2 and Bax.

In conclusion, the present study is the first, to the best of our knowledge, to reveal that the anticancer effects of ailanthone were partially due to G_2/M phase cell cycle arrest and apoptosis via modulation of Bcl-2 and Bax expression in SGC-7901 cells. Further studies are required to explore the effects of ailanthone on various tumor cell lines, and to evaluate the antitumor and adverse effects of ailanthone in animal models; however, the results of the present study indicated that ailanthone may act as a potential antitumor agent for the treatment of GC, and provided an experimental basis for future drug development.

Acknowledgements

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