Effects of 17-allylamino-17-demethoxygeldanamycin on the induction of apoptosis and cell cycle arrest in HCT-116 cells

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Received July 24, 2015; Accepted January 6, 2017

DOI: 10.3892/ol.2017.6442

Abstract. The present study investigated the effects of HSP90 inhibitor 17-allylamino-17-demethoxygeldanamycin (17-AAG) on apoptosis and the cell cycle of the HCT-116 human colon carcinoma cell line, with the aim of elucidating their underlying mechanisms. MTT was used to examine the inhibitory effects of 17-AAG on the proliferation of HCT-116 cells at various time points and doses. The cells were stained with Annexin V-fluorescein isothiocyanate/propidium iodide and evaluated by flow cytometry. The expression of signal transducer and activator of transcription (STAT)3, cyclin D1, cytochrome *c* (cyt-*c*), caspase 9 and caspase 3 at the mRNA and protein level was determined using reverse transcription-polymerase chain reaction and western blotting. Treatment with 17-AAG at a concentration of 1.25-20 mg/l for 24 and 48 h significantly inhibited the proliferation of HCT-116 cells in a time-dependent and concentration-dependent manner. Treatment with 17-AAG at concentrations of 1.25, 2.5 and 5 mg/l for 48 h significantly induced apoptosis and cell cycle arrest in HCT-116 cells. Exposure to 17-AAG at concentrations of 1.25, 2.5 and 5 mg/l for 48 h significantly downregulated the mRNA and protein expression of STAT3 and cyclin D1, but upregulated cyt-*c*, caspase 9 and caspase 3 in a concentration-dependent manner in HCT-116 cells. Therefore 17-AAG is able to inhibit cell proliferation, inducing apoptosis and G_1 stage cell cycle arrest by downregulating the expression of cyclin D1, and promoting the mitochondria apoptosis by downregulating STAT3 in HCT-116 cells.

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Key words: 17-allylamino-17-demethoxygeldanamycin, HCT-116 cells, apoptosis, cell cycle, signal transducer and activator of transcription 3

Introduction

Colon cancer is the third most prevalent type of human cancer, which is cured primarily via surgical excision and chemotherapy (1). Therapeutic methods utilizing molecular targets to alter cancer cell properties possess more powerful antitumor functions and fewer toxic effects towards healthy cells (2). As a result, small molecule target therapeutics for cancer has been the subject of numerous investigations (2). The heat shock protein (hsp) family, particularly hsp90, is associated with the stability of a number of key oncogenic target proteins, including receptor and non-receptor tyrosine kinases, and is therefore a promising molecular target for cancer treatment (3). Increased levels of hsp90 expression affects the early-stage of carcinogenesis and the morphology of cancer cells, which is associated with the therapeutic effects of hsp90 inhibitors (4). As a potent anti-cancer treatment, a number of hsp90 inhibitors have been the subjects of several studies (5,6). The overexpression of hsp90 has been demonstrated in colon cancer and is associated with the proliferation of colon cancer cells.

17-allylamino-17-demethoxygeldanamycin (17-AAG) is a derivative of geldanamycin (an hsp90 inhibitor), which specifically interacts with the N-terminal ATP binding domain of hsp90, inhibiting the intrinsic ATPase activity that is important for its chaperone function (7). Subsequently, hsp90 substrate proteins are degraded via the ubiquitin-proteasome pathway. The majority of these substrate proteins are signaling molecules involved in numerous signaling pathways that influence tumor development (8). The present study aimed to elucidate the effects of 17-AAG on the cell cycle, proliferation and apoptosis of HCT-116 cells, and investigate the molecular mechanisms underlying the effect of 17-AAG treatment on colon cancer cells.

Materials and methods

Cell culture. The HCT-116 human colon carcinoma cell line was provided by Professor Zheng Huachuan of The First Affiliated Hospital of Liaoning Medical University (Jinzhou, China). The cells were cultured in RPMI-1640 supplemented

with 10% fetal bovine serum (FBS) and 100 U/ml penicillin (all from Hyclone; GE Healthcare Life Sciences, Shanghai, China) and incubated at 37˚C with 5% CO_2. Cells were monitored daily and the medium was replaced every 2-3 days as required. Cells in the logarithmic growth phase were selected and transferred to 96-well plates ($5x10^4$ cells/ml). Upon cell adherence, five concentrations of 17-AAG (1.25, 2.5, 1.5, 1.10 and 1.20 mg/l) were added to the experimental groups and an untreated control group was included. The control group was only cultured with RPMI-1640 supplemented with 10% FBS and 10 μl RPMI-1640 was added when 10 μl 17-AAG was added to the other groups. MTT solution was added to the wells after 24 or 48 h, and the medium was discarded after 4 h. Subsequently, 150 μl/well dimethyl sulfoxide was added and the plate was agitated for 15 min. Optical density (OD) was evaluated at a wavelength of 490 nm using a microplate reader (ELX808; BioTek, China, Beijing, China). Finally, the growth inhibition rates at various doses of 17-AAG were calculated using the equation: Growth inhibition rate = [(OD control group) - (OD17 - AAG group)]/(OD control group - OD blank group).

Cell cycle and apoptosis assays. HCT-116 cells ($5x10^4$ cells/ml) in the logarithmic phase of growth were added to petri dishes to establish a control group (RPMI-1640, 10% FBS) and experimental groups with various concentrations (1.25, 2.5 and 5 mg/l) of 17-AAG. Following 48 h the cells were collected by centrifugation at 1,000 x g at 4˚C for 5 min. Subsequently, 2 ml PBS was added to each group to resuspend the cells in order to wash them. Cells for the apoptosis assay were then resuspended in 500 μl binding buffer (Hangzhou Biosci Biotech Co., Ltd., Hangzhou, China) and stained with 5 μl propidium iodide (PI) and 5 μl Annexin V (Hangzhou Biosci Biotech Co., Ltd.) for 15 min at room temperature in dark. Subsequently, the cells were analyzed by Flow cytometry (BD FACSVerse; BD Biosciences, Franklin Lakes, NJ, USA). The cells for cell cycle assay were collected after 48 h and incubated in 70% ethanol for 12 h at -20˚C. Subsequently, the cells (10^6) were resuspended in 500 μl binding buffer (Hangzhou Biosci Biotech Co., Ltd.) following centrifugation at 1,000 x g and 4˚C for 5 min. Ribonuclease A (125 mg/ml, 1 μl; Beyotime Institute of Biotechnology, Jiangsu, China) was added to the buffer and the cell suspension was incubated at 37˚C for 30 min. Following incubation, 5 μl PI (Hangzhou Biosci Biotech Co., Ltd.) was added to each cell sample and incubated for 30 min at 20˚C in dark. Flow cytometry (BD FACSVerse; BD Biosciences) was used to detect the cell cycle stage of HCT-116 cells and apoptotic cells.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-PCR) analysis. HCT-116 cells at the logarithmic phase of growth were added to petri dishes to establish a control group (RPMI-1640, 10% FBS) and experimental groups with various concentrations of 17-AAG (1.25, 2.5 and 5 mg/l). All the cells were collected after 48 h and added to Eppendorf tubes, following which 1 ml TRIzol® (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was added and tubes were incubated on ice for 10 min. Subsequently, 200 μl chloroform was added, and the cells were placed in a shaker for 15 sec at room temperature and centrifuged for 15 min at 12,000 x g and 4˚C. Following centrifugation, the upper layer of supernatant (~200 μl) was collected and ~200 μl isopropyl alcohol was added to each tube. The tubes were kept on ice for 10 min and centrifuged for 15 min at 12,000 x g and 4˚C. Subsequently, all the liquid was discarded and cells were washed with 70% ethanol diluted with diethylpyrocarbonate-treated water, and the RNA concentration of each sample was evaluated using an ultraviolet spectrophotometer.

RT-PCR was performed using the TaKaRa RNA PCR (avian myeloblastosis virus) kit (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's protocol (9). Primer sequences for signal transducer and activator of transcription (STAT)3, cyclin D1, cyt-*c*, caspase 9 and caspase 3 are provided in Table I, and were used for DNA amplification following RT.

Western blot analysis. HCT-116 cells at the logarithmic phase of growth were added to petri dishes, including a control group (RPMI-1640, 10% FBS) and experimental groups with various concentrations of 17-AAG (1.25 and 2.5 mg/l). The cells were collected after 48 h. Lysis buffer (M-PER™ Mammalian Protein Extraction reagent; Thermo Fisher Scientific, Inc.) was added, and the cells were incubated on ice for 20 min. Subsequently, the cells were centrifuged for 20 min at 1,000 x g and 4˚C. The upper layer of supernatant was collected, and the protein concentration was evaluated by using the Pierce™ BCA Protein Assay kit (Thermo Fisher Scientific, Inc). Total cell lysates (30 μg) were separated by 12% SDS-PAGE and transferred to a polyvinylidene fluoride membrane (Merck Millipore, Darmstadt, Germany), and the blots were blocked with 5% skimmed milk. The membranes were washed three times by using 1 ml phosphate-buffered saline (PBS; Thermo Fisher Scientific, Inc.) for 5 min. The blots were probed with antibodies against STAT3, cyclin D1, cyclin B1, cyt-*c*, caspase 9 and caspase 3. Antibodies (1 ml) against STAT3 against STAT3 (cat. no. ab119352), cyclin D1 (cat. no. ab134175), cyclin B1 (cat. no. ab32053), cyt-*c* (cat. no. ab13575), caspase 9 (cat. no. ab32539) and caspase 3 (cat. no. ab32042) were added to the membranes at dilutions of 1:1,000. The membranes were placed on a shaker for 1 h at 20˚C. The membranes were subsequently washed three times using 1 ml PBS for 5 min. The secondary antibodies horseradish peroxidase (HRP)-conjugated anti-mouse immunoglobulin G (IgG; cat. no. ab131368) and HRP-conjugated anti-rabbit IgG variable domain of heavy chain single domain (cat. no. ab191866) were added to the membranes. All primary and secondary antibodies were purchased from Abcam (Shanghai, China). The membranes were placed in a shaker with the secondary antibody for 1 h at 20˚C, and subsequently washed 3 times with PBS. Pierce™ enhanced chemiluminescence western blotting substrate (Thermo Fisher Scientific, Inc.) was added to the membranes for 3 min, and the membranes were captured with the ChemiDoc XRS system (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Immunofluorescence assay. HCT-116 cells at the logarithmic growth phase were added to 6-well plates on a cover glass to form a control group (RPMI-1640, 10% FBS) and experimental groups with various concentrations of 17-AAG (1.25, 2.5 and 5 mg/l). The cells were collected after 48 h and

Table I. Primer sequences.

Gene name	Sequence, 5'-3'	Annealing temperature, ˚C	Number of cycles	Product size, bp
STAT3	F: CCTCCTCAGCATCTTATCCG R: CAGCCTGGGCATCCTTG	59	30	499
Caspase 3	F: TGGACCTGTTGACCTGA R: CACAAAGCGACTGGATG	57	30	269
Caspase 9	F: TGGACCTGTTGACCTGA R: CACAAAGCGACTGGATG	57	30	885
Cyt-*c*	F: GTCCGGTTGCGCTTTCCTT R: CGCAGTTTCCTCAAATTCTTTCTTC	60	40	156
Cyclin D1	F: TGTCCTACTACCGCCTCACACGCTTCCTCTCCG R: TCCTCTTCCTCCTCCTCGGCGGCCTTG	63	35	160
GAPDH	F: CAATGACCCCTTCATTGACC R: TGGAAGATGGTGATGGGATT	60	30	135

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; STAT3, signal transducer and activator of transcription; cyt-*c*, cytochrome *c*; bp, base pairs; F, forward; R, reverse.

washed once with PBS. Subsequently, 4% paraformaldehyde was added to the wells, and the cells were incubated at room temperature for 15 min prior to 3 washes with PBS. The cells were subsequently incubated with 1% Triton X-100 for 20 min at 20˚C and washed with PBS three times. Bovine serum albumin (1%; Beyotime Institute of Biotechnology) was added to the wells, which were then incubated for 30 min at room temperature. STAT3 primary antibody (1:200) was added to the wells and incubated overnight at 4˚C. The secondary antibody goat anti-mouse IgG (heavy chain and light chain; 1:400; cat. no. ab96879; Abcam) was added to the wells and incubated for 2 h at room temperature. The cells were washed three times with PBS. Following washing, DAPI was added to the wells and incubated for 5 min in the dark. The cells were observed under a fluorescence microscope and images were captured.

Statistical analysis. Statistical analysis was performed with SPSS (version 19.0; IBM SPSS, Armonk, NY, USA). The data were presented as the mean ± standard deviation. Data comparisons among groups were performed using one-way analysis of variance, and Turdey post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

HCT-116 cell proliferation is inhibited by 17-AAG treatment. The MTT assay results revealed that 1.25-20 mg/l of 17-AAG exhibited significant inhibitory effects ($P<0.01$) on the proliferation of HCT-116 cells in a concentration-dependent manner. The cell numbers in the 17-AAG treated groups were significantly reduced ($P<0.01$), compared with those observed in the control group, with an abnormal cell morphology exhibited by the 17-AAG-treated cells (Fig. 1). The proliferation inhibition rate of 17-AAG-treated cells (1.25, 2.5, 5, 10 and 20 mg/l) at 48 h (IC_{50}, 1.71 mg/l) was increased, compared with that observed at 24 h (IC_{50}, 23.24 mg/l; Table II; Fig. 2).

17-AAG induces G_2 stage cell cycle arrest in HCT-116 cells. PI staining detection results revealed that various concentrations (1.25, 2.5 and 5 mg/l) of 17-AAG were able to cause a significant arrest in cell cycle progression of HCT-116 cells at the G_2 stage after 48 h. However, this effect did not appear to occur in a concentration-dependent manner (Fig. 3).

17-AAG promotes HCT-116 cell-apoptosis. The apoptotic rates of the cells in the 17-AAG-treated groups (1.25, 2.5 and 5 mg/l) were markedly increased compared with cells in the control group. Additionally, 17-AAG appeared to increase the apoptosis rate of HCT-116 cells in a dose-dependent manner (Fig. 4).

17-AAG-treatment affects the mRNA and protein expression of STAT3, cyclin D1, cyclin B1 and caspase 3. RT-qPCR and western blot analysis results revealed that the expression levels of STAT3, cyclin D1 and cyclin B1 in the treated groups (1.25 and 2.5 mg/l) were significantly reduced ($P<0.01$), compared with the control group. The expression levels of cyt-*c*, caspase 9 and caspase 3 in the 17-AAG-treated groups were significantly increased ($P<0.01$) compared with the control, in a concentration-dependent manner (Fig. 5).

17-AAG inhibits STAT3 expression. Fluorescence microscopy revealed markedly reduced STAT3 staining in the 17-AAG-treated groups (1.25, 2.5 and 5 mg/l), compared with the control, in a concentration-dependent manner (Fig. 6).

Discussion

The over-expression of hsp90 has been detected in various forms of carcinoma, including breast, prostate, renal, colon, ovarian and hepatocarcinoma, multiple myeloma and leukemia (10-12). As hsp90 is a highly expressed tumor marker (13), hsp90 inhibitors may be an effective anticancer therapy. Geldanamycin, an inhibitor of the hsp90 ATP-binding

Table II. Inhibitory effects of 17-AAG on the proliferation of HCT-116 colon carcinoma cells (mean ± standard deviation; n=6).

	24 h		48 h	
17-AAG, mg/l	Absorbance	Inhibitory rate, %	Absorbance	Inhibitory rate, %
Control	0.450±0.09	0	0.569±0.01	0
1.25	0.399±0.01	26.17[a]	0.472±0.05	30.14[a]
2.5	0.397±0.04	26.61[a]	0.434±0.08	36.82[a]
5	0.392±0.004	27.87[a]	0.366±0.05	48.65[a]
10	0.378±0.019	30.91[a]	0.302±0.04	60.01[a]
20	0.343±0.017	32.45[a]	0.229±0.003	72.73[a]
IC_{50}	60.08		5.24	

[a]P<0.01 vs. control group. IC_{50} = the mean value of 6 repetitions and is defined as the concentration of an inhibitor whereby the response is reduced by half. 17-AAG, 17-allylamino-17-demethoxygeldanamycin; IC, inhibitory concentration.

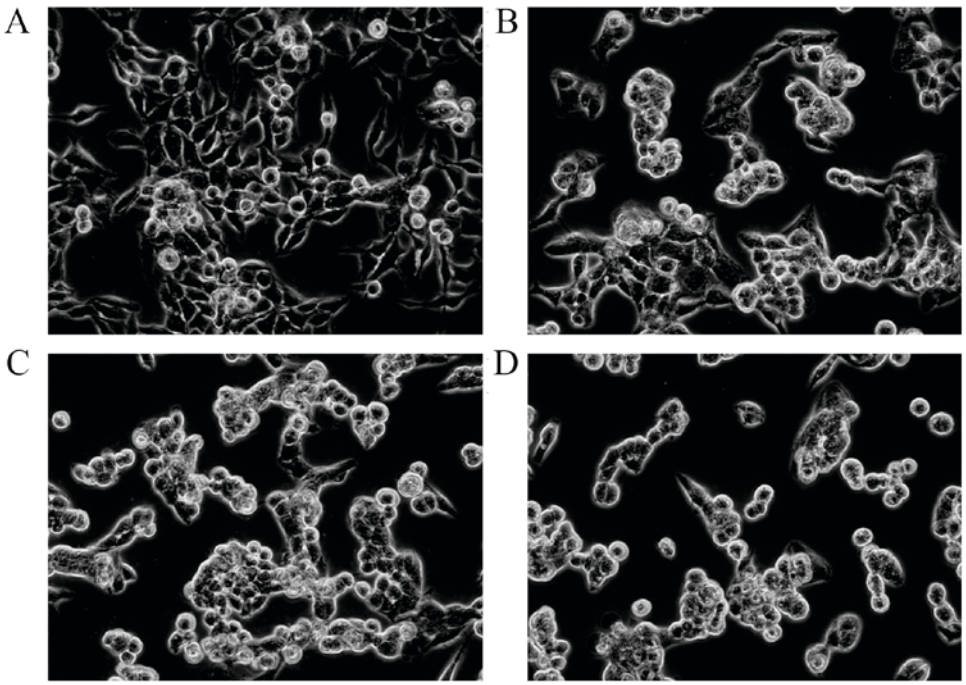

Figure 1. HCT-116 cells following culture for 48 h with various concentrations of 17-AAG; (A) control group; (B) 1.25 mg/l group; (C) 2.5 mg/l group; (D) 5 mg/l group. A decreased number of cells and abnormal cell morphology was observed in the 17-AAG treated groups, compared with the control. 17-AAG, 17-allylamino-17-demethoxygeldanamycin.

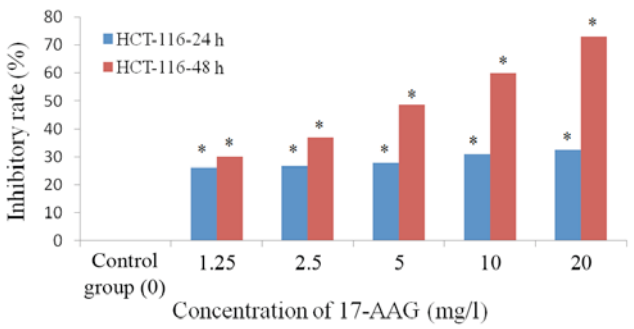

Figure 2. Inhibitory effects of 17-AAG-treatment on HCT-116 cells as assessed by flow cytometry. As the concentration of 17-AAG was increased, the inhibitory effect on the proliferation of HCT-116 cells also increased after 24 and 48 h. *P<0.01 compared with the control group. 17-AAG, 17-allylamino-17-demethoxygeldanamycin.

domain, was the first hsp90 inhibitor to be identified (14). This inhibitor functions by inducing the degradation of hsp90 substrates, which enables the suppression of various signaling pathways, in order to impede carcinoma angiogenesis, histogenesis and cell proliferation as well as induce cell cycle arrest and apoptosis (15). As a number of studies have revealed the high hepatotoxicity of geldanamycin, its derivative 17-AAG was investigated in the present study. 17-AAG has increased water solubility and lower hepatotoxicity (16,17).

Abnormalities in cell cycle control may lead to aberrant proliferation and cancer development (17), suggesting that induction of cell cycle arrest may be a promising therapeutic approach for cancer (18). Numerous studies have reiterated the importance of the cell cycle during carcinogenesis, as well as revealing that hsp90 inhibitors are able to induce arrest

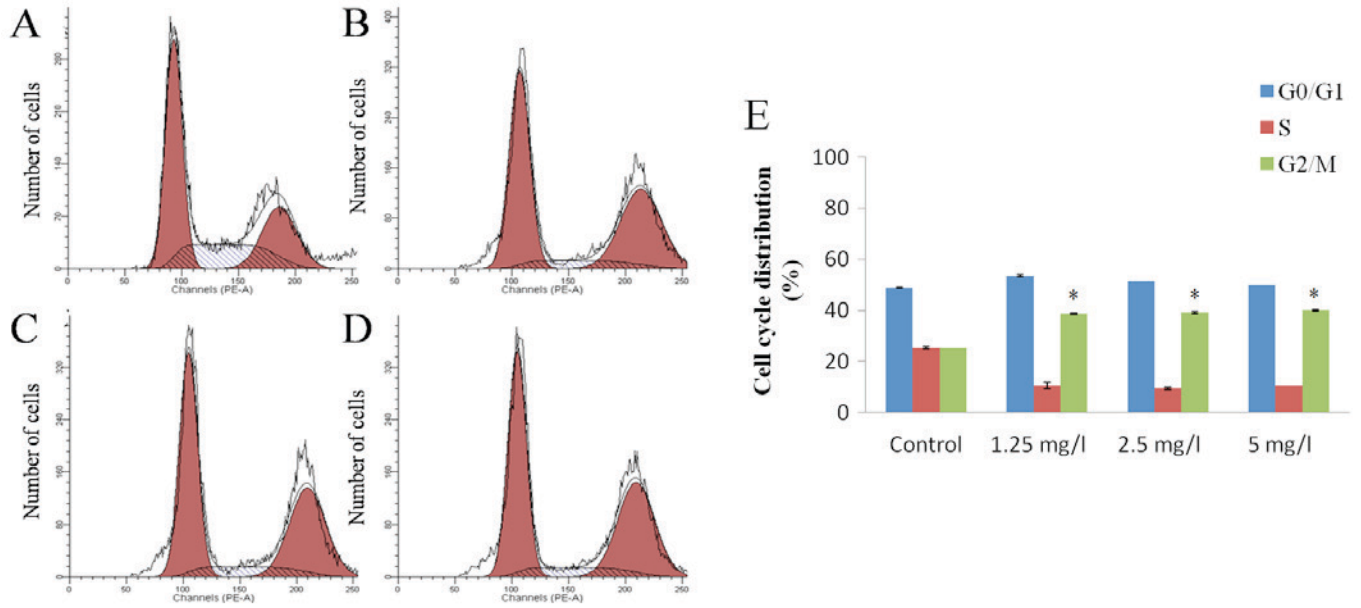

Figure 3. Effect of various concentrations of 17-AAG on the cell cycle of HCT-116 cells. (A) Control; (B) 1.25 mg/l; (C) 2.5 mg/l; (D) 5 mg/l. (E) Bar chart showing the effect of increasing concentration of 17-AAG on cell cycle distribution. The results indicated that, in the 17-AAG treated groups, the S phase fraction was decreased and the G_2/M phase fraction was increased, compared with the control ($P<0.05$), suggesting that 17-AAG induced cell cycle arrest of HCT-116 cells at the G_2/M phase. *$P<0.01$ compared with the control. 17-AAG, 17-allylamino-17-demethoxygeldanamycin; PE-A, phycoerythrin A.

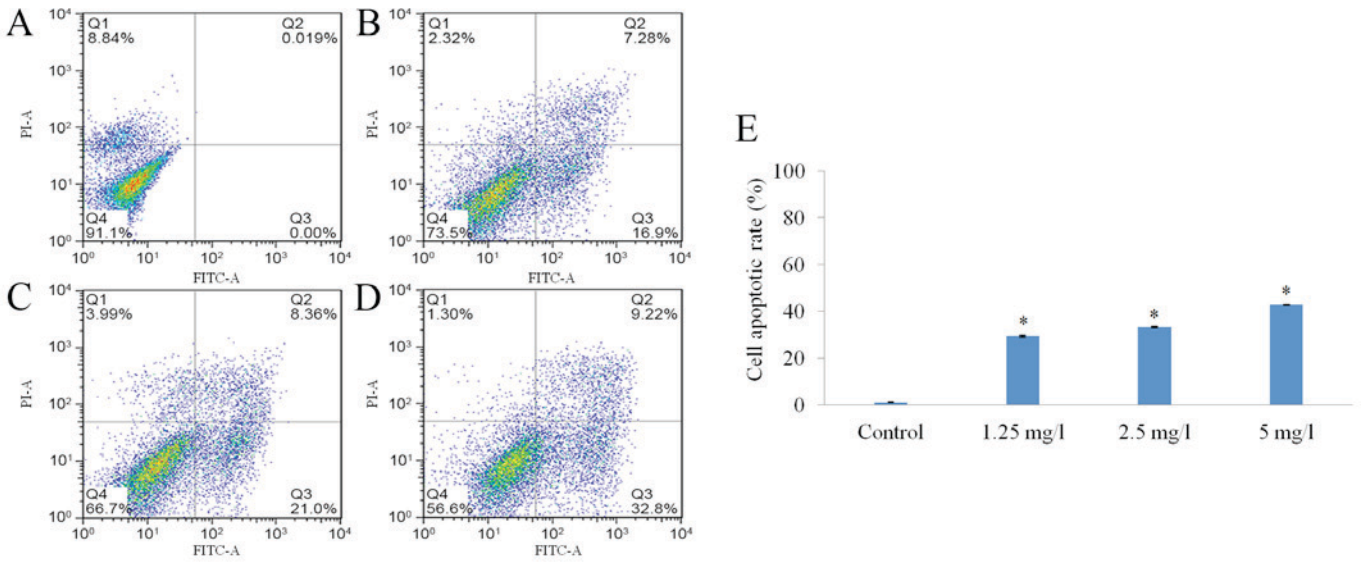

Figure 4. The effect of 17-AAG on the apoptosis of HCT-116 cells. (A) Control: 0 mg/l; (B) 1.25 mg/l; (C) 2.5 mg/l; (D) 5 mg/l. (E) Bar chart showing the effect of increasing concentration of 17-AAG on apoptotic rate. In comparison with the control, the apoptotic rates of the treated groups were elevated as concentration of 17-AAG increased ($P<0.05$). *$P<0.01$ compared with the control. 17-AAG, 17-allylamino-17-demethoxygeldanamycin; Q, quadrant; FITC-A, fluorescein isothiocyanate-Annexin V; PI, propidium iodide.

at different cell cycle stages in various tumor types (19,20). Previous studies have suggested that treatment with 17-AAG is able to arrest SGC-7901 gastric cancer cells and HepG2 liver cancer cell lines at the G_2/M stage (21,22) and bladder cancer cell lines (RT4, RT112 and T24) at the G_1 stage (23). In the present study 17-AAG treatment induced G_2-stage arrest in CT-116 cells in a non-concentration-dependent manner.

In order to control the cell cycle, cyclin-dependent kinases (CDK) are primarily regulated by cyclins and CDK inhibitors (24). High levels of CDK expression are able to trigger anti-apoptotic mechanisms, leading to unrestricted cell proliferation (25). As one of numerous proto-oncogenes, cyclin D1 forms a complex with and activates CDK4, leading to the phosphorylation of retinoblastoma protein and the release of E2 factor (26). This process induces the initiation of S phase-associated gene transcription and the cell cycle transition from G_1 to S phase (26). The present study observed mRNA-level and protein-level changes in cyclin D1, detecting reduced cyclin D1 expression in HCT-116 cells. As cells were arrested at the G_2 phase, it was hypothesized that the effect of 17-AAG on the cell cycle of HCT-116 cells is not dependent on altered cyclin D1 expression levels. The cyclin B1 subunit

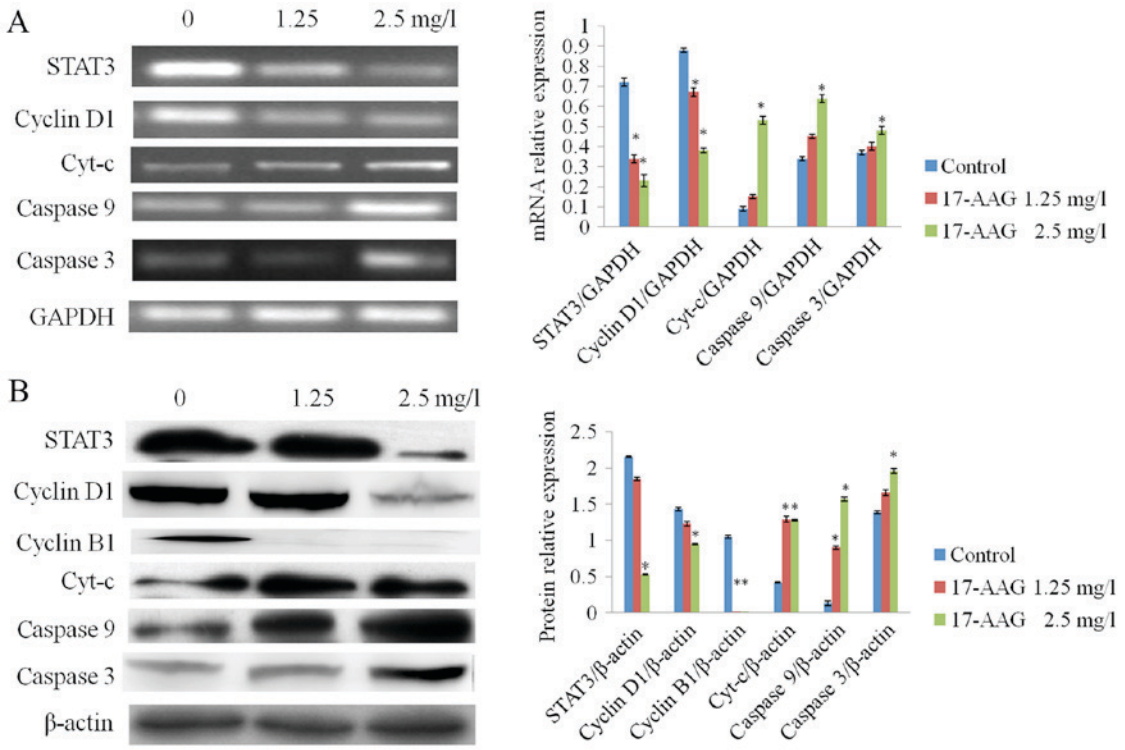

Figure 5. Effect of 17-AAG on the expression levels of various cellular factors. (A) Following treatment with 17-AAG, cyt-*c*, caspase 9 and caspase 3 mRNA expression levels were increased in HCT-116 cells in a concentration-dependent manner compared with the control. By contrast, STAT3 and cyclin D1 mRNA expression levels were decreased in a concentration-dependent manner, compared with the control. (B) Following 17-AAG-treatment, cyt-*c*, caspase 9 and caspase 3 protein expression was significantly increased in a concentration-dependent manner, compared with the control, whereas stat3, cyclin D1 and cyclin B1 expression was significantly decreased in a concentration-dependent manner, compared with the control. *P<0.05 compared with the control. 17-AAG, 17-allylamino-17-demethoxygeldanamycin; mRNA, messenger RNA; cyt-*c*, cytochrome *c*; STAT3, signal transducer and activator of transcription 3.

binds CDK1, and this maturation-promoting factor complex promotes progression from the G_2 to M phase of the cell cycle (27). Following activation, the cyclin B1-CDK1 complex is able to initiate the prophase of mitosis, suggesting that an increase in its activity may lead to alterations in the mitotic behavior of the cell (28). Therefore, the expression levels and activity of cyclin B1 and CDK1 are able to influence cell mitosis. During the present study, HCT-116 cells were arrested at the G_2 phase and cyclin B1 expression levels were significantly decreased. This result is contrary to a previous study, suggesting that the HCT-116 cell cycle is not influenced by 17-AAG treatment (29). However, the present study concluded that 17-AAG is able to arrest HCT-116 cells at the G_2 phase, possibly due to a reduction in the levels of cyclin B1 expression.

Under stress, cells initiate programmed cell death, termed apoptosis, which is important for various physical and pathological processes and is an important regulator of cell growth (30). The hallmarks of apoptosis are changes in cell morphology, including chromatin condensation, nuclear fragmentation and cell shrinkage, as well as biochemical changes, including caspase activation, degradation of DNA and proteins and the rupture of membrane modification factors, which target cells for phagocytosis (31). There are two major apoptosis signaling pathways: The intrinsic mitochondrial apoptosis pathway and the extrinsic death receptor-mediated apoptosis pathway (32). Intracellular toxicity stimuli, including DNA damage and growth factor deficiency, trigger an increase in mitochondrial membrane permeability and the release of intermembrane proteins, particularly cyt-*c*, initiating intrinsic apoptosis (33). Following its release, and aided by ATP or deoxyadenosine triphosphate, cyt-*c* is able to form apoptotic bodies with caspase regulatory factors, and activate caspase 9, and downstream caspase 3 and caspase 7 proteins, to initiate the process of cell apoptosis (34). Abnormal levels of apoptosis disrupt the balance between viable and dead cells to promote tumor development (35); therefore, the regulation of alterations in apoptosis may be a novel anticancer therapy. This present study identified the apoptosis-inducing ability of 17-AAG, but the underlying mechanisms require further investigation. In the present study, an increase in the mRNA and protein expression levels of cyt-*c*, caspase 9 and caspase 3 in HCT-116 cells was detected to varied extents following 17-AAG treatment. These results demonstrated that 17-AAG may induce the apoptosis of HCT-116 cells via the mitochondrial apoptosis signaling pathway in a concentration-dependent manner.

The present study revealed that the expression of STAT3 in the 17-AAG-treated groups was significantly downregulated, indicating that a reduction in STAT3 expression levels may underlie the inhibitory effect of 17-AAG on HCT-116 cells. Members of the STAT family of proteins function in various physical and pathological processes, including cell growth, proliferation and development (36). In particular, STAT3 is important for transcription regulation, post-translational modification and cell functions, including apoptosis and cell death (37). STAT3 is able to translocate into the cell nucleus and bind with specific promoter sequences to regulate transcription. Therefore, STAT3 is able to exert a role in cell proliferation, differentiation and metabolism, processes that are important

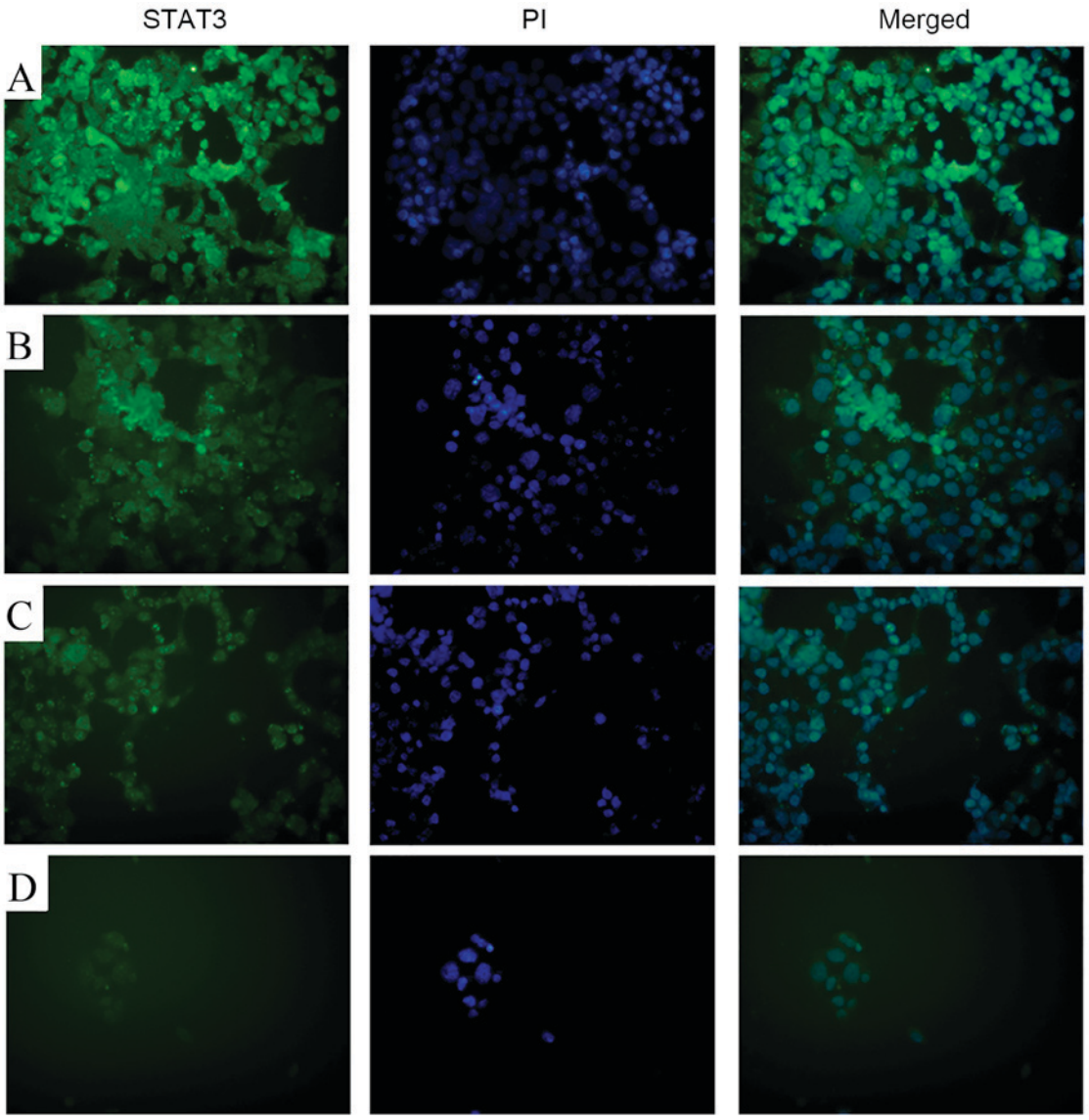

Figure 6. Effect of 17-AAG treatment on STAT3 expression levels in HCT-116 cells as detected by immunofluorescence. (A) Control group; (B) 1.25 mg/l; (C) 2.5 mg/l; (D) 5 mg/l. Following 17-AAG treatment for 48 h, STAT3 expression was markedly decreased. 17-AAG, 17-allylamino-17-demethoxygeldanamycin; STAT3, signal transducer and activator of transcription; PI, propidium iodide.

during the development of inflammation and tumor formation and progression (38). Previous studies have detected STAT3 overexpression in numerous forms of carcinoma, including colorectal cancer, and the loss of control of STAT3 expression in breast, head and neck, prostate, pancreatic, ovarian and brain cancer (39,40). STAT3 is hypothesized to be an important tumor modulatory factor (32), and is able to induce cell proliferation, differentiation and antiapoptotic mechanisms following activation by carcinogenic substances, including B7H3 (41). Leptin is able to elevate hsp90 expression levels via STAT3 regulation, to promote the elevation of human epidermal growth factor receptor 2 expression levels; however, 17-AAG is able to abrogate this effect and inhibit breast cancer cell proliferation (42). This result implicates STAT3 in the anticancer functions of 17-AAG, particularly the inhibition of cell proliferation.

Cyclin D1 is an established downstream target of STAT3, and cyclin D1 expression is decreased upon STAT3 suppression (43). The present study revealed that STAT3 expression in HCT-116 cells was decreased in the 17-AAG-treated groups using RT-qPCR, western blotting and immunofluorescence data. In addition, the current study detected significantly reduced cyclin D1 mRNA and protein expression levels in 17-AAG-treated cells. Therefore, it was hypothesized that 17-AAG may downregulate the expression of cyclin D1 and cyclin B1 via STAT3, in order to induce G_2 phase arrest in HCT-116 cells.

Previous studies have suggested that, upon STAT3 inhibition, the expression levels of caspase3 may be increased (44,45). In addition, Duan *et al* (46) revealed that thymic stromal lymphopoietin may downregulate caspase 3 expression through the activation of the STAT3 signaling pathway, thereby suppressing the apoptosis of decidual γδ T cells (17), indicating that caspase 3 may be a target of STAT3. Previous studies reported that some novel medicines, including geraniin are able to induce the intrinsic apoptosis signaling pathway via the STAT3 pathway (47-49), catalyzing the caspase cascade response. Therefore, it has been suggested that STAT3 may translocate into mitochondria and regulate apoptosis (50).

In conclusion, the present study revealed the downregulation of STAT3 expression following 17-AAG-treatment in HCT-116 cells, hypothesizing that 17-AAG may regulate mitochondrial apoptosis via the STAT3 signaling pathway, leading to apoptosis induction. However, further studies are required to elucidate the role of STAT3 in the 17-AAG-induced apoptosis of HCT-116 cells.

Acknowledgements

The present study was supported by the Hebei Province Education Department (grant no. ZD20140003) and University Emphasis Subject of Hebei Province and Immunology Emphasis Subject of Chengde Medical University

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