Original Article

Stephanthraniline A suppresses proliferation of HCT116 human colon cancer cells through induction of caspase-dependent apoptosis, dysregulation of mitochondrial function, cell cycle arrest and regulation of Akt/p38 signaling pathways

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ABSTRACT — Stephanthraniline A (STA) is a C₂₁ steroidal aglycone isolated from the stem of Stephanotis mucronata (Blanco) Merr. that exerts growth inhibition in human colon cancer cells. However, the intracellular molecular mechanisms whereby this occurs have not been well characterized. In this study, we found that STA significantly inhibits the growth of HCT116 colon cancer cells in a time- and concentration-dependent manner. The inhibitory effect of STA on cell growth was related to the induction of apoptosis. Activated caspase-3, caspase-8 and caspase-9, along with a decreased Bcl-2/Bcl-x ratio and loss of mitochondrial membrane potential (Δψm), were observed in response to STA treatment. Furthermore, treatment of HCT116 cells with STA resulted in G0/G1 phase cell cycle arrest accompanied by decreased mRNA levels of cyclin-dependent kinase 4 (CDK4), p21 and c-myc. Additionally, the inhibition of Akt signaling and activation of p38 signaling were observed after treatment with STA in HCT116 cells. These findings indicate that STA inhibits HCT116 cell growth by promoting apoptosis, the dysregulation of mitochondrial function, and cell cycle arrest.

Key words: C₂₁ steroidal aglycone, Apoptosis, Cell cycle arrest, Colon cancer

INTRODUCTION

Colorectal cancer is one of the most prevalent malignancies and is the fourth leading cause of cancer-related death worldwide. Currently, surgery, radiotherapy and chemotherapy comprise available treatment options for colon cancer (Brenner et al., 2014; Arnold et al., 2017). Among them, chemotherapy is particularly important because it is effective both in patients who have undergone surgery and in those who have been diagnosed at advanced stage with metastasized tumors. However, recent development of novel anticancer drugs failed to effectively prolong the survival time of patients (Afrin et al., 2016), with drug toxicity and resistance remaining the most common challenges to effective treatment. Systemic therapy using classical cytotoxic drugs is poorly tolerated and often ineffective. Thus, there is still an urgent need to identify novel, safe, and effective therapeutic drugs for the treatment of colorectal cancer in clinical practice.

Recently, natural medicine has become a research hotspot in cancer chemotherapy. A variety of components from natural plants have been proven to exhibit both excellent pharmacological activities and low toxicity. C₂₁ steroidal glycosides represent important bioactive components primarily derived from Asclepiadaceae plants. In the past few decades, C₂₁ steroidal glycosides have been reported to possess immunosuppressive, antiepileptic, and antidepressant activities (Li et al., 2015a). Recently, the anticancer activities of many C₂₁ steroidal glycosides have been confirmed both in vitro and in vivo (Liu et al.,...
2010; Peng et al., 2011). *Stephanotis mucronata* (Blanco) Merr. is a plant with abundant C21 steroidal glycosides in the Asclepiadaceae family. Stephananthraniline A (STA) is a C21 steroidal aglycone isolated from the stem of *Stephanotis mucronata* (Blanco) Merr. Compared with some C21 steroidal glycosides, STA loses the sugar chain at the C-3 position and exerts much stronger growth inhibitory effects in cancer cells, especially in colon cancer cells (Zhou et al., 2016). Therefore, STA might represent a potent anticancer compound, necessitating systemic evaluation of its effects and exploration of its anticancer mechanisms. In a previous study, we found that STA induces apoptosis and cell cycle arrest, as well as inhibiting the hedgehog pathway, in HT-29 colon cancer cells (Zhou et al., 2016). However, the precise mechanism for STA-induced apoptosis and the involvement of related upstream pathways are still unclear. In this study, we investigated the anticancer activity of STA in another colon cancer cell line, HCT116, which is more sensitive to STA, to explore the mechanism of STA-induced apoptosis and identify related upstream pathways. The results of this study will be helpful to better understand the anticancer mechanisms of the natural C21 steroidal aglycone STA.

**MATERIALS AND METHODS**

**Cell culture and reagents**

The human colorectal cancer cell line HCT116 was purchased from the China Academy Sinica Cell Repository (Shanghai, China) and cultured in DMEM supplemented with 10% fetal bovine serum (Gibco, Gaithersburg, MD, USA), 100 U/mL penicillin and 100 μg/mL streptomycin. Cells were incubated with 5% CO2 at 37°C in a humidified atmosphere.

Stephananthraniline A (STA, 12-O-acetyl-20-O-(N-methyl)-anthranilsarcostin, C_{31}H_{43}NO_{8}) was previously isolated from the stems of *S. mucronata* and identified on the basis of chemical and spectroscopic evidence, including two-dimensional NMR spectroscopy and HRESI-MS. STA purity was greater than 98% by HPLC analysis. A stock solution of STA in DMSO was prepared and then diluted as desired in RPMI 1640 medium. The final concentration of DMSO in the assays was less than 0.5% in all experiments and did not exhibit a detectable effect on cell growth.

Flow cytometry analysis of cell cycle arrest and apoptosis

Flow cytometry assays were used for cell cycle and apoptosis assays. Briefly, HCT116 cells were plated in 6-well plates (2 × 10^4 cells/well) and incubated overnight. Cells were then treated with different concentrations of STA (0, 25, 50, 75, or 100 μM) for 24 hr. For cell cycle analysis, cells were washed twice with PBS and then collected with trypsin and fixed in chilled 70% ice-cold ethanol at 4°C overnight. Next, cells were stained with 0.5 mL MTT (5 mg/mL) solution and allowed to adhere for 24 hr. Subsequently, cells were treated with STA (0, 25, 50, 75, or 100 μM) for 24, 48 and 72 hr, respectively. Then, 20 μL MTT (5 mg/mL) solution was added into each well, and cells were incubated at 37°C for another 4 hr. Next, the medium was removed and formed formazan crystals were dissolved in 150 μL DMSO. Finally, absorbance was measured at 490 nm using a multiwell plate reader (Bio-Tek Instruments Inc, WinooSki, VT, USA). Each experiment was performed in triplicate, and the results shown in Fig. 1 are from three independent experiments.

**Cell viability assay**

Cell viability was assessed using the MTT assay. HCT116 cells were seeded in 96-well plates at a final concentration of 5 × 10^3 cells/well in complete medium and allowed to adhere for 24 hr. Subsequently, cells were treated with STA (0, 25, 50, 75, or 100 μM) for 24, 48 and 72 hr, respectively. Then, 20 μL MTT (5 mg/mL) solution was added into each well, and cells were incubated at 37°C for another 4 hr. Next, the medium was removed and formed formazan crystals were dissolved in 150 μL DMSO. Finally, absorbance was measured at 490 nm using a multiwell plate reader (Bio-Tek Instruments Inc, WinooSki, VT, USA). Each experiment was performed in triplicate, and the results shown in Fig. 1 are from three independent experiments.
Evaluation of mitochondrial membrane potential (Δψm)
Mitochondrial membrane potential was assessed by JC-1 staining. HCT116 cells were seeded in 6-well plates (2 × 10^5 cells/well) and incubated overnight. After treatment with STA (0, 25, 50, 75, or 100 μM) for 24, 48 and 72 hr. The values are shown as a percent of the control group, which was normalized to 100. The data are presented as the mean ± S.D. of at least three independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001 compared to control groups.

RNA extraction and quantitative real-time PCR
Total RNA was extracted from HCT116 cells using an RNA extraction kit followed by reverse transcription into cDNA using the iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer’s protocol. Real-time PCR was performed using the SYBR Green PCR master mix (Bio-Rad) on a CFX96 Real-time PCR system (Bio-Rad). Primer designs in real-time PCR are shown in Table 1. The reaction consisted of 40 cycles, with 5 sec denaturation at 95°C, 30 sec annealing at 60°C and 5 sec extension at 65°C. β-actin was used as an endogenous control for each sample. Melting curve data were analyzed to determine PCR specificity. Relative fold expression was analyzed using the 2^−ΔΔCt method.

Western blotting analysis
Cells were lysed using RIPA lysis buffer with protease inhibitors on ice, and protein concentrations were determined using BCA protein assay kit. Equal concentrations of denatured proteins were separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore, Bedford, MA, USA), blocked with 5% nonfat dry milk for 1 hr at room temperature and then incubated with specific primary antibodies at a 1:1000 dilution in 5% nonfat dry milk overnight at 4°C. After washing with TBST three times, membranes were incubated with secondary antibody at room temperature for 2 hr. Chemiluminescent signals were detected using an enhanced chemiluminescence detection kit. Images were obtained using a Mini-PROTEAN gel imaging system (Bio-Rad, USA). Optical density was calculated using ImageJ 1.41 software (Bethesda, MD, USA).

Statistical Analysis
Data from three independent experiments are expressed as the mean ± S.D. Values were analyzed by using SPSS 16.0 software for Windows, and statistical significance of difference among the values was evaluated by one-way analysis of variance. The criteria for statistical significance were *p < 0.05; **p < 0.01; ***p < 0.001.

RESULTS
STA inhibits growth of HCT116 cells
To evaluate the growth inhibitory effect of STA on HCT116 cells, cell viability was determined by MTT assay. Cells were treated with different concentrations of STA (0, 25, 50, 75, or 100 μM) for 24 hr, 48 hr and 72 hr.

Table 1. Primers sequences used for qRT-PCR amplification.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
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<tbody>
<tr>
<td>β-Actin</td>
<td>CTGGACCAGGTGAAGGTTACA</td>
<td>AAGGAACCTTCTTGAACAATGCA</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>AAGAGCAGACGGATGAAAAGG</td>
<td>GGGCAAAGAAATGCAAGTGAATG</td>
</tr>
<tr>
<td>Bax</td>
<td>ATGGACGGTGCGGGGAGGCA</td>
<td>GGAGAAATCAACAGAGGCC</td>
</tr>
<tr>
<td>p21</td>
<td>TTACGCAGAAAGAAGGAGT</td>
<td>CGTATGCGGGAAGAGACA</td>
</tr>
<tr>
<td>c-myc</td>
<td>TGAGAGGAGGAGGGGGGAGGG</td>
<td>ATCGATCTTCTCCTCCCTCCC</td>
</tr>
<tr>
<td>CDK-4</td>
<td>CTGCTGGAGCATGAGGAC</td>
<td>GATCTTGATCTGCCGCTG</td>
</tr>
</tbody>
</table>
As shown in Fig. 1, STA inhibited HCT116 cell growth in a time- and dose-dependent manner. After treating for 24 hr, 48 hr, and 72 hr, the growth inhibitory rates of STA were 11.2 ± 9.8%, 38.5 ± 2.3% and 52.3 ± 2.1%, respectively, at 50 μM, and 73.4 ± 1.6%, 91.5 ± 0.9%, and 97.0 ± 0.1%, respectively, at 100 μM.

STA induces cell cycle arrest at G0/G1 phase in HCT116 cells

To explore the mechanisms STA inhibiting HCT116 cell growth, we first examined its effect on cell cycle distribution by flow cytometry. As shown in Fig. 2A and B, increased G0/G1 phase was observed in response to STA treatment. In control groups, the number of HCT116 cells in G0/G1 phase was 28.7 ± 3.0%. After 50 and 100 μM STA, the G0/G1 phase distribution of HCT116 cells was significantly increased to 41.1 ± 3.6% (p < 0.05) and 72.3 ± 2.1% (p < 0.001), respectively.

The cell cycle checkpoint regulators c-myc, p21 and cyclin-dependent kinases CDK-4 play crucial roles in the regulation of cell cycle progression from G0/G1 to S phase (Xiong et al., 1993; El-Deiry 2016). To determine whether cell cycle arrest of HCT116 cells induced by STA was mediated by these regulators and kinases, we measured mRNA expression levels of these genes in HCT116 cells. As shown in Fig. 2C, mRNA expression levels of c-myc and CDK-4 were downregulated, whereas expression of p21 was upregulated in a concentration-dependent manner in response to STA treatment. These results indicate that cell cycle arrest at G0/G1 phase may be associ-
Stephanthraniline A induces apoptosis and cell cycle arrest in HCT116 cells

Fig. 3.  STA induces apoptosis in HCT116 cells. HCT116 cells were treated with STA for 24 hr. (A) Treated cells were stained with Annexin V/FITC. Apoptosis was quantified by flow cytometry. (B) Quantified histograms display the effects of STA on HCT116 apoptosis. (C) and (D) Apoptosis-associated protein levels of pro- and cleaved-caspase 3, 8, 9, and PARP were assessed by western blotting. The values are normalized to controls, which are shown as 1. The data are represented as the mean ± SD of at least three independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001 compared to control groups.

STA induces apoptosis in HCT116 cells by caspase activation

To further investigate whether the inhibitory effect of STA was associated with induction of apoptosis, we examined apoptotic cell death using flow cytometry. As shown in Fig. 3A and B, treatment with 25-100 μM STA significantly increased apoptotic cells in a dose-dependent manner (p < 0.01). After treatment with 50 and 100 μM STA, apoptotic rates in HCT116 cells were increased by 34.9 ± 3.2% and 67.7 ± 2.0%, respectively,
STA induces apoptosis in a caspase-dependent manner

To evaluate whether STA-induced HCT116 apoptosis was caspase-dependent, we examined the effects of the caspase inhibitor Z-VAD-FMK on preventing STA-induced cell death. After cotreatment with 20 μM Z-VAD-FMK, the percentage of apoptotic cells induced by 75 μM STA was significantly decreased (Fig. 4A and B, \( p < 0.001 \)) and the growth inhibitory effect of STA was weakened (Fig. 4C, \( p < 0.01 \)). Moreover, western blotting analysis revealed that protein expression of cleaved-caspase 3 and PARP was downregulated in response to treatment with caspases inhibitors (Fig. 4D). Taken together, these results suggest that STA induces apoptosis in a caspase-dependent manner in HCT116 cells.

STA regulates mitochondrial membrane potential and expression of Bcl-2 family proteins

The loss of mitochondrial membrane potential (\( \Delta \psi_m \)) is a significant marker for early apoptosis, occurring even prior to caspase activation (Orrenius et al., 2007). Therefore, we examined JC-1 staining after treatment with STA in HCT116 cells. In normal cells with high \( \Delta \psi_m \), JC-1 accumulates in the mitochondrial matrix to form a polymer that produces red fluorescence. In apoptotic cells with low \( \Delta \psi_m \), JC-1 exists as a monomer and produces green fluorescence. As shown in Fig. 5A and 5B, STA induced loss of \( \Delta \psi_m \) in HCT116 cells in a concentration-dependent manner. The percentage of low \( \Delta \psi_m \) was increased from 22.14 ± 2.04% to 57.31 ± 1.51% after treatment with 100 μM STA.

During apoptosis, bcl-2 family proteins are key regulators in mitochondrial structure and function (Schwarz et al., 2007). Anti-apoptotic Bcl-2 and pro-apoptotic Bax are two important members of the bcl-2 family that regulate activation of caspases and play important roles in regulating mitochondrial-related apoptosis by altering cell \( \Delta \psi_m \). The imbalance between Bcl-2 and Bax determines the ultimate fate of cells (Borkan, 2016). To further clarify the mechanism of apoptosis induction in response to STA, we examined the role of bcl-2 family proteins in STA treated HCT116 cells. As shown in Fig. 5C and D, treatment with 25-100 μM STA significantly downregulated mRNA and protein expression levels of Bcl-2, whereas the expression levels of Bax were significantly upregulated in a concentration-dependent manner. These results suggest that STA induces apoptosis of HCT116 cells by inducing mitochondrial dysfunction through deregulation of Bcl-2 family proteins.

STA induces apoptosis through the Akt and p38 MAPK signaling pathways in HCT116 cells

The MAPKs and Akt-mediated signaling pathways have been well defined as involved in tumor suppression and sensitization to apoptosis (Liao and Hung, 2003; Huang et al., 2017). To further determine whether Akt and p38 signaling pathways are related to the mechanisms underlying STA-mediated growth inhibition in HCT116 cells, Akt and p38 protein expression was detected by western blotting. As shown in Fig. 6, STA treatment significantly upregulated expression of phosphorylated p38 in cancer cells. Simultaneously, significant downregulation of phosphorylated Akt was observed in STA-treated HCT116 cells. These results indicate that the p38 and Akt signaling pathways might be involved in STA induced apoptosis in HCT116 cells.

DISCUSSION

Stephanthraniline A (STA, Fig. 7) is the aglycone form of many active glycosides isolated from the stems of Stephania mucronata (Blanco) Merr. In a previous study, we demonstrated the growth inhibitory effects of STA in human colon cancer cells (Zhou et al., 2016). However, its mechanism is still unclear. Herein, we observed STA-induced growth inhibition in the human colon cell line HCT116 and further explored its anticancer mechanisms. Primary findings of the present study revealed that STA inhibits the growth of HCT116 cells through cell cycle arrest at G0/G1 phase, induction of apoptosis and disruption of \( \Delta \psi_m \). Furthermore, Akt and p38 signaling pathways were also demonstrated to be involved in the multiple anticancer mechanisms of STA.

Apoptosis is an important event targeted by anticancer drugs. Many studies have shown that natural steroid glycosides exert anticancer activities through induc-
Fig. 4. Effects of the pancaspase inhibitor Z-VAD-FMK on STA-induced apoptosis in HCT116 cells. HCT116 cells were pretreated with the pancaspase inhibitor Z-VAD-FMK (20 μM for 2 hr). Then, 75 μM STA was added, and cells were incubated for another 24 hr. (A) Cells were stained with Annexin V/FITC and analyzed by flow cytometry (B) Quantified histograms display the effect of STA and Z-VAD-FMK on apoptosis in HCT116 cells. (C) Cell viability was quantified using MTT assay. (D) Proteins levels of pro- and cleaved-caspase 3 and PARP were assessed by western blotting. The values were normalized to the control group, which is shown as 1. The data are represented as the mean ± S.D. of at least three independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001 compared to control groups.
Fig. 5. STA induces mitochondrial dysfunction and regulates expression of Bcl-2 family proteins in HCT116 cells. HCT116 cells were treated with STA for 24 hr. (A) Cells were stained with JC-1 (10 μg/mL) and analyzed by flow cytometry. Cells with Δψm loss were gated. (B) Quantified histograms display the effect of STA on mitochondrial dysfunction. (C) Quantification of mRNA levels of anti-apoptotic Bcl-2 and pro-apoptotic Bax were analyzed by qRT-PCR. (D) Proteins levels of Bcl-2 and Bax were assessed by western blotting. The values were normalized to the control group, which is shown as 1. The data are represented as the mean ± S.D. of at least three independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001 compared to control groups.
tion of apoptosis in cancer cells (Delebinski et al., 2015). Accordingly, we explored whether the growth inhibitory effect of STA was related to apoptosis. We found that STA treatment for only 24 hr resulted in significant levels of apoptosis in HCT116 cells. The rate of apoptosis at 100 μM STA was much higher than the apoptosis rate induced by STA in HT-29 colon cancer cells that was demonstrated in our previous study (Zhou et al., 2016).

Classic apoptosis is thought to occur through two main pathways: the caspase-9 dependent intrinsic pathway and the caspase-8 dependent extrinsic pathway (Degterev et al., 2003). Caspase-3 is the common downstream molecule between caspase-8 and caspase-9. Our results show that STA significantly downregulated protein levels of pro-caspase 9, pro-caspase 8, and pro-caspase 3, while upregulating expression of cleaved-caspase 9, cleaved-caspase 8 and cleaved-caspase 3. PARP, a substrate of caspase-3, was cleaved in response to STA treatment. Furthermore, the pancaspase inhibitor Z-VAD-FMK reversed PARP and caspase-3 cleavage induced by STA in HCT116 cells. Taken together, these results indicate that STA activates both intrinsic and extrinsic apoptotic pathways and that STA-induced apoptosis is caspase dependent.

Mitochondrial dysfunction is one of the hallmarks of intrinsic pathway-induced apoptosis. Upon apoptotic stimuli, Δψm becomes reduced, and mitochondrial permeability increases, resulting in the release of cytochrome c and activation of caspase-9 (Haga et al., 2003). Cancer cells can acquire resistance to apoptosis by overexpressing anti-apoptotic proteins, such as Bcl-2, or by downregulating pro-apoptotic proteins, such as Bax. Increasing the Bax/Bcl-2 ratio results in disruption of the integrity of the mitochondrial membrane, forming the mitochondrial permeability transition pore that help release cytochrome c and initiate the intrinsic apoptotic pathway (Schwarz et al., 2007). In our experiment, loss of Δψm decreased expression of Bcl-2 and increased expression of Bax in HCT116 cells in response to STA treatment. These results

Fig. 6. Effects of STA on Akt and p38 MAPK signaling pathways in HCT116 cells. HCT116 cells were treated with STA for 24 hr. Protein levels of p38, p-p38, Akt and p-Akt were assessed by western blotting. The values were normalized to the control group, which is shown as 1. The data are represented as the mean ± S.D. of at least three independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001 compared to control groups.

Fig. 7. The chemical structure of stephanthraniline A
suggest that STA induces apoptosis in HCT116 cells through induction of mitochondrial dysfunction.

Loss of cell cycle control and unlimited proliferation are essential attributes in human cancer. Therefore, disruption of the cell cycle by therapeutic agents can lead to tumor growth arrest and inhibit cell proliferation, contributing to cancer therapy (Malumbres and Barbacid, 2009; Roskoski, 2019). In this study, we found that the number of HCT116 cells at G0/G1 phase was significantly increased in a concentration-dependent manner in response to STA treatment. These results indicate that cell cycle arrest at G0/G1 phase could be one mechanism of STA against cancer cells. Cell cycle regulation is affected by activation of a group of related enzymes known as cyclin-dependent kinases (CDK) whose activity depends on regulatory subunits called cyclins. CDK4 (CDK4/cyclin D) regulates the G1-to-S-phase transition (Asgar et al., 2015). Our results demonstrated that STA treatment downregulated mRNA expression levels of CDK4 in HCT116 cells, resulting in G0/G1 arrest in response to STA. p21 is a universal CDK inhibitor that exists in mammalian cyclin-CDK protein complexes (Xiong et al., 1993; Abbas and Dutta, 2009). In a previous study, we demonstrated that STA upregulates gene expression of p21 by PCR array analysis in T lymphocytes (Chen et al., 2012). In this study, we also observed an effect on p21 in response to STA treatment in HCT116 cells. Results illustrated that STA treatment enhances mRNA expression of p21, suggesting that CDK inhibition and cell cycle arrest induced by STA might be mediated by the CDK inhibitor p21. In addition, c-myc is an important transcription factor implicated in cell cycle progression, proliferation and survival. c-myc and YAP-TEAD integrate mitogenic and mechanical cues at the transcriptional level to control cell proliferation and cell cycle entry (Croci et al., 2017). Consistently, our results demonstrated that gene expression of c-myc was significantly downregulated. These results suggest that cell cycle arrest at G0/G1 could be one mechanism of STA against cancer cells.

Akt kinase suppresses apoptosis by maintaining mitochondrial integrity and mitigating cell response to the release of cytochrome c into the cytoplasm (Franke et al., 2008). Inhibition of the Akt pathway can promote pro-apoptotic Bax and Bad, subsequently inducing mitochondrial dysfunction through decreased mitochondrial potential and reactive oxygen species (ROS) production and leading to apoptosis. Therefore, the inhibition of Akt is an important target for anticancer drugs (Li et al., 2015b; Han et al., 2018). p38 is one of the MAPK subgroups that transduces a diverse spectrum of extracellular and intracellular stimuli into alterations in gene expression and cell function (Fan and Chambers, 2001). Recently, many anticancer drugs were shown to induce cancer cell apoptosis through activation of p38 signaling (Hui et al., 2014; Li et al., 2015b; Kim et al., 2018). In our study, activation of p38 and inhibition of Akt were also observed after treatment with STA in HCT116 cells. These data suggest that STA inhibits cells growth via interrelated mechanisms and multiple signaling pathways.

In conclusion, the present study demonstrated that STA induces caspase-dependent apoptosis and cell cycle arrest in HCT116 cells. Furthermore, Akt and p38 signaling pathways are involved in STA-induced apoptosis in HCT116 cells. Our study highlights STA as a potential anticancer drug in colon cancer therapy.

ACKNOWLEDGMENTS

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Conflict of interest---- The authors declare that there is no conflict of interest.

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