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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Lappaconitine sulfate induces apoptosis in human colon cancer HT-29 cells and down-regulates PI3K/AKT/GSK3β signaling pathway

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Abstract

Lappaconitine (LA), a diterpenoid alkaloid extracted from the roots of *Aconitum sinomontanum* Nakai, possesses strong central analgesic, local anesthetic, antifebric and anti-inflflammatory effect. Lappaconitine hydrobromide (LH) is used clinically to treat analgesia, but its clinical application is limited because of its poor solubility in water. Studies have suggested that lappaconitine sulfate (LS) is a promising pain reliever, and this particular form not only has readily water soluble, but also exhibits anti-cancer effects. However, the mechanism of LS anti-cancer activity is poorly understood. The aim of this study was intended to investigate the role of LS in apoptosis of human colon cancer HT-29 cells and explore the potential molecular mechanism. Cell proliferation was detected by CCK-8 assay and EdU proliferation assay. Cell morphological change was expressed by Hoechst 33258 staining assay. Expression of apoptosis related proteins were detected by western blot. The effect of LS on cell cycle was detected by flow cytometry. Experimental results showed that LS exhibited anti-proliferative activity and induced apoptosis in HT-29 cells in a dose-dependent manner. LS increased the expression of p53, Bax, cleaved-PARP, cleaved-caspase-3/7/9, and inhibited Bcl-2 expression. LS affected cyclin D1 and p21 expression and induced cell cycle arrest in G0/G1 phase. Additionally, LY294002 signifificantly abrogated the activation of p-PI3K, p-Akt and p-GSK3β. To summarize, these results demonstrated that LS was able to prevent cell proliferation probably via PI3K/Akt/GSK3β signaling pathway.

Keywords LS · Apoptosis · PI3K/AKT/GSK3β signaling pathway · Anti-cancer · HT-29

Abbreviations

LA	Lappaconitine
LS	Lappaconitine sulfate
LH	Lappaconitine hydrobromide
CCK-8	Cell Counting Kit-8
PBS	phosphatebuffered saline
FTIR	fourier transform infrared spectroscopy
OD	absorbance value
EdU	5′-ethynyl-2′-deoxyuridine
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
PVDF	polyvinylidene difluoride

Introduction

Cancer is the world's leading cause of death with uncontrolled proliferation of tumor cells that invade the surrounding tissue and metastasize to other tissues and organs (Zong et al. 2012). Colon cancer is the second most frequently diagnosed cancer in females and the third most frequently diagnosed cancer in males worldwide (Kim et al. 2015). It is predicted that the global colon cancer patients will increase by 60% to more than 2.2 million new cases and 1.1 million deaths up to 2030 (Surachai et al. 2018). Colon cancer remains one of the main challenges due to high mortality rate (Han et al. 2018). Therefore, it is imperative to find new colon cancer drugs.

Many natural compounds are being used as new treatments for cancer because they have the advantage of promoting apoptosis (Wang et al. 2013). *Aconitum sinomontanum* Nakai is a traditional folk medicine mainly distributed in northwestern China (Yü 1979). It has long

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been employed to treat diverse ailments based on its anti-nociceptive, anti-arrhythmic, anti-bacterial and anti-inflammatory activities (Wei et al. 1981; Tang et al. 2016). Lappaconitine (LA) is a non-addictive diterpenoid alkaloid extracted from the root of *A. sinomontanum* with effective central analgesic, local anesthetic, antipyretic, antibacterial and anti-inflammatory activities (Wang et al. 1997; Yang et al. 2015b). And the analgesic effect is 7 times as much as that of aminopyrine and equivalent to the synthetic opioid pethidine (Xu et al. 2011). The hydrobromide salt of lappaconitine (LH) has been widely used in clinic as a non-addictive analgesic drug for treatment of neuropathic pain in China (Wang et al. 2009) and as an antiarrhythmic drug in Uzbekistan (Yunusov 2011). More importantly, it does not possess serious side effects such as respiratory depression, addiction and other upper gastrointestinal symptoms (Yang et al. 2015b). Recently, LA and LH have been reported to have anti-cancer effects. Lin et al. (2005) reported that LH could inhibit the growth of liver cancer of mouse and S180 tumor. Sheng et al. (2013) reported that LA inhibited proliferation and induced apoptosis in lung cancer cells. Wu et al. (2008) reported that LH could induce differentiation and apoptosis of HL-60 cells to a certain extent. However, its hydrophobicity, slow onset time and low bioavailability limited its wider clinical application. Sun et al. (2015) synthesized a series of salt-forming compounds by reaction of LA with corresponding organic or inorganic acid, such as LH, lappaconitine hydrochloride, lappaconitine nitrate, LS, lappaconitine citrate and lappaconitine tartrate. Among them, LS not only exhibited a shortest onset time, longest maintaining time, but also its antinociceptive property was the best. LS has good water solubility and anti-cancer effect (Ma et al. 2017). However, the underlying mechanism of LS inhibited cancer cells growth and induced cell apoptosis have not been precisely elucidated.

Two major mechanisms, death receptor pathways and mitochondrial pathways, for induction of apoptosis have been identified in recent years. Bcl-2 family proteins played a decisive role in regulating the mitochondrial apoptotic pathway. The alteration of the balance between Bcl-2 family proteins causes cytochrome C release, induces caspase-9 and eventually apoptosis (Kuo et al. 2006). Many studies have found that caspases activation is a molecular hallmark of apoptosis (Yan et al. 2017).

Caspase family and Bcl-2 family are not only closely related to apoptosis, but also the downstream effector molecules of PI3K/AKT/GSK3β signaling pathway (He et al. 2018). The PI3K/AKT/GSK3β signaling pathway is very important in the regulation of cell proliferation, apoptosis, survival and metabolism (Zhang et al. 2014). Dysregulation of PI3K/AKT/GSK3β signaling pathway has been found in many cancers such as squamous cell carcinoma, breast cancer, prostate cancer and hepatoblastoma cancer (Sun et al. 2017). The role of PI3K/AKT/GSK3β signaling pathway in LS-induced cell apoptosis and cell cycle arrest of human colon cancer HT-29 cells has never been elucidated.

The main purpose of this research was to investigate the relevant mechanisms of LS on cell apoptosis and cell cycle arrest in HT-29 cells. First, we used CCK-8 assay, EdU proliferation assay and Hoechst 33258 staining assay to detect the effect of LS on proliferation and apoptosis. To further determine the above results, the expression of apoptosis-related proteins in Bcl-2 family and caspase family was examined using the western blot. In addition, detecting the relationship between LS and cell cycle distribution. Finally, the relationship between LS and PI3K/AKT/GSK3β pathway was confirmed. This work will further elucidate the role of LS in proliferation, apoptosis, cell arrest, and better understand the mechanism of LS induces apoptosis in human colon cancer cells.

Materials and methods

Materials

LA and LH were purchased from Gansu Xinlan Pharmaceutical Co., Ltd (Gansu, China), Purity > 97.7%. HT-29 cells were obtained from Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China).

RPMI-1640 medium, fetal bovine serum (FBS), and trypsin were obtained from Gibco (Invitrogen, USA). All antibodies were purchased from Cell Signaling Technology (Boston, MA, USA). Cell Counting Kit-8 (CCK-8) was purchased from EnoGene Cell (Nanjing, China). EdU cell proliferation assay kit was purchased from RiboBio Co., Ltd. (Guangzhou, China). Cell cycle assay kit was provided by Nanjing KeyGEN Biotech (Nanjing, China). BCA protein assay kit was provided by Beijing Solarbio Science & Technology Co., Ltd (Beijing, China). All other chemicals were purchased from Sigma (St. Louis, MO, USA).

Preparation and characterization of LS

LS was prepared as previously reported by Sun et al. (2015). Briefly, 0.584 g of LA was added to 8 mL 0.1 mol/L H_2SO_4 solution, followed by stirring at RT for 6 h. The mixture was then extracted using chloroform three times. The solution was then concentrated under reduced pressure using rotary vacuum evaporation at 60 °C. Finally, LS was prepared and analyzed by fourier transform infrared spectroscopy (FTIR) after compression with potassium bromide. The scanning range was 4000~400 cm^{-1} and the scanning speed was 1 cm^{-1}/s.

Cell culture

HT-29 cells were cultured using RPMI-1640 supplemented with 10% FBS. The cells were cultured in a humidified atmosphere at 37 °C with 5% CO_2.

Cell Counting Kit-8 assay

HT-29 cells were seeded into 96-well plates (3×10^3 cells per well) and incubated at 37 °C for 24 h. Their effects on cell proliferation were quantified using Cell Counting Kit-8 (CCK-8) assay following the manufacturer's direction. As mentioned in the introduction, LH was widely used in China to treat moderate to severe pain, such as cancer and postoperative pain (Xu et al. 2011) and had certain anticancer activity. So, LH was selected as a positive control. The cells were exposed to various concentrations of LS and LH and incubated for 48 h. The cells were then incubated with 10 µL CCK-8 buffer and cultured for 30 min. After incubation for 30 min, the absorbance value (OD) of each well was measured at 450 nm using a Microplate Reader (Thermo Scientific, USA).

EdU proliferation assay

An EdU cell proliferation assay kit was used to measure cell proliferation. HT-29 cells were plated at a density of 5×10^3 cells per well in 24-well plates for 24 h. The cells were exposed to various concentrations of LS and LH for 12 h, then incubated with 5′-ethynyl-2′-deoxyuridine (EdU) for another 90 min. Next, the cells were fixed with 4% paraformaldehyde for 30 min. Finally, cell nuclei were stained for 30 min with 0.5 mL Hoechst 33258 dye. The proportion of nucleated cells incorporating EdU was determined under Olympus CKX41S inverted fluorescence microscope (Japan) at 400× magnifcation (Peng et al. 2009).

Hoechst 33258 staining

HT-29 cells were seeded in 6-well plates containing sterile 5-mm coverslips and incubated with different concentrations of LS and LH for 24 h. Next, 4% paraformaldehyde was added, and cells were incubated for 10 min, washed three times with PBS, and 0.5% Triton X-100 was added for 5 min. Finally, 0.5 mL Hoechst 33258 was added, the cells were stained for 5 min, and then they were observed under fluorescence microscope at ×400 magnifcation (Zeng et al. 2013).

Cell cycle analysis

Cell cycle analysis was determined using cell cycle assay kit following the manufacturer's direction (Wang et al. 2013). HT-29 cells were seeded and then incubated with different concentrations of LS and LH for 24 h. After incubation, cells were all collected and then fixed in 75% ice-cold ethanol at –20 °C. After 48 h, the cells were then incubated with RNase A at 0.5 mg/mL and propidium iodide (PI) at 10 µg/mL in the dark at room temperature for 30 min, and then analyzed using a BD FACSCanto flow cytometer (BD Biosciences Pharmingen, USA).

Western blot analysis

HT-29 cells were seeded in 6-well plates and then incubated with different concentrations of LS, LH and LY294002 (25 µM) for 24 h, cells were harvested and washed with ice-cold PBS. The proteins were extracted using RIPA lysis buffer supplemented with protease inhibitors on ice. Subsequently, the protein extracts were quantified using BCA protein assay kit. The protein extracts were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was incubated with primary antibodies (1:500) at 4 °C overnight, and then the membrane was incubated with the secondary antibodies (1:8000) for 2 h at room temperature (Liu et al. 2017). Enhanced chemiluminescence was used to detect the signals, and Image J software was used to quantify the protein levels.

Statistical analysis

Statistical analysis was carried out using SPSS 22.0 software and all data were expressed as the mean ± standard. One-way analysis of variance (ANOVA) was used to determine the difference between groups and $p < 0.05$ was considered as statistically significant.

Results

Chemical analysis of LS by FTIR

The chemical structure of LS was shown in Fig. 1a. LS was confirmed by comparing the FTIR spectra of LA. As shown in Fig. 1b, The FTIR spectrum of LA showed typical peaks at 1686 cm^{-1} (amide C=O), 1594 cm^{-1}, 1527 cm^{-1}, 1450 cm^{-1}, 760 cm^{-1} (benzene ring) and 1267 cm^{-1} (ester or ether C–O–C). The FTIR spectrum of LS also showed these characteristic absorption bands of LA. Additionally, the FTIR spectrum of LS showed two new characteristic absorption bands at 1134 and 585 cm^{-1} of SO_4^{2-}, verifying the formation of LS.

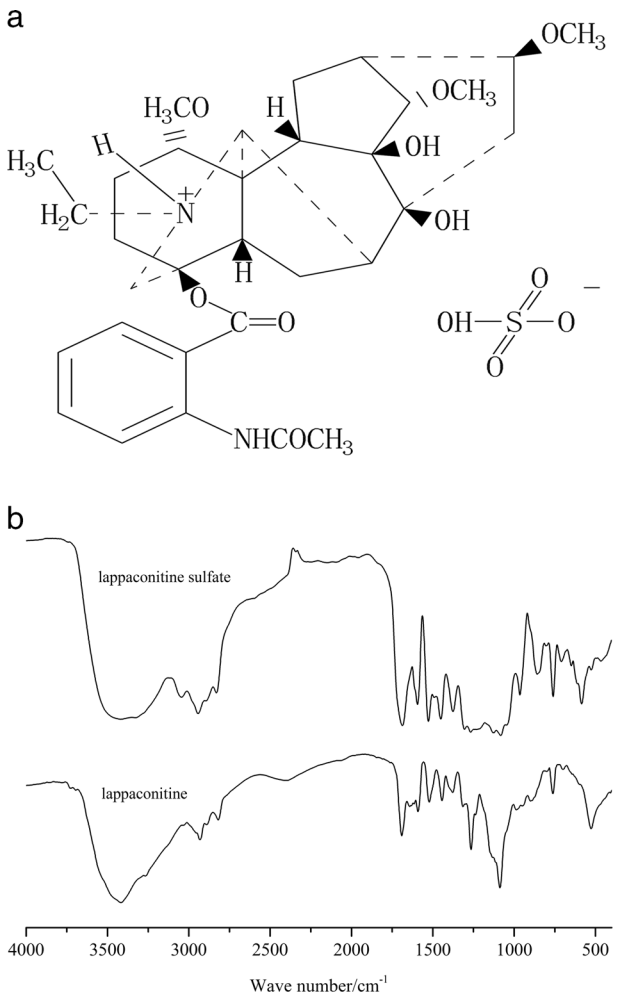

Fig. 1 The chemical structure and FTIR spectra of LS. **a** The chemical structure of LS. **b** The FTIR spectra of LS

LS inhibits HT-29 cell proliferation

The effect of LS in cell proliferation was examined using the CCK-8 assay and EdU incorporation assay. According to Fig. 2a, the CCK-8 assay revealed that LS significantly inhibited the survival of HT-29 cells in a dose-dependent manner ($p < 0.05$). More importantly, the anti-cancer effect of LS was stronger than that of LH, which was the positive control. In addition, the EdU incorporation assay revealed that 400 and 800 μg/mL LS significantly decreased the EdU-positive cell number ($p < 0.05$) (Fig. 2b), demonstrating that LS inhibited the proliferation of HT-29 cells.

LS induces morphological change in apoptosis of HT-29 cells

To investigate whether LS could induce apoptosis, HT-29 cells were treated with various concentrations of LS (0, 100, 200, 400, and 800 μg/mL) and 800 μg/mL LH. As shown in Fig. 3, Hoechst 33258 staining assay showed that the normal cell nuclei were relatively dispersed with a regular shape and smooth surface. However, as the concentration of LS increased, the cell nuclei became irregular, and the nuclear fragmentation increased (Fig. 3 arrows), which are typical features of apoptosis (Chen et al. 2015). When the concentration of LS reached 800 μg/mL, and the trend of apoptosis was obvious, as compared to the positive control (LH). The result indicated that LS could induce apoptosis of HT-29 cells.

Anti-cancer effect of LS via induction of apoptosis

Apoptosis-associated factors such as Bcl-2, Bax and p53 were detected so as to further explore LS-induced apoptosis in HT-29 cells. As shown in Fig. 4a, compared with the 0 μg/mL control group or the LH positive control group, the experimental data revealed that LS significantly increased the expression of p53 and Bax, decreased the expression of Bcl-2 ($p < 0.05$). In short, LS up-regulated the expression of p53 and Bax along with the down-regulation of Bcl-2.

Bcl-2 family members can regulate cell death induced by caspase (Cheng et al. 1997), As shown in Fig. 4b, when HT-29 cells were treated with LS, the cleaved-caspase-3/7/9 and cleaved-PARP protein levels were significantly up-regulated compared to those in the 0 μg/mL control group cells ($p < 0.05$). Compared with the LH positive control group, LS significantly increased the expression of cleaved-caspase-3/7/9 and cleaved-PARP ($p < 0.05$). Based on these findings, it was concluded that the transmission of LS-induced apoptotic signals involves Bcl-2 apoptotic transcription factor, thus activating the caspase family.

LS participates in the cell cycle

A previous study showed that alkaloids can induce anti-proliferative activity by inducing cell cycle arrest (Ghanemi et al. 2017). Flow cytometry was used to examine the effect of LS on cell cycle distribution in HT-29 cells. The results showed that the distribution of G0/G1 gradually increased after treating with LS, followed by a marked decrease in the distribution of S phase, and a decrease in the G2/M phase ($p < 0.05$). Moreover, there was a significant difference between 800 μg/mL LS and 800 μg/mL LH in S phase ($p < 0.01$) (Fig. 5). Therefore, the knockdown of LS induced cell cycle arrest at the G0/G1 phase, which might lead to the inhibition of proliferation in HT-29 cells. To further dissect the function of LS on cell cycle progression, cell cycle related proteins (cyclin D1 and p21) were detected (Sandor et al. 2000). Compared with the 0 μg/mL control group or the LH positive control group, the result showed that LS significantly increased the expression of p21, decreased the

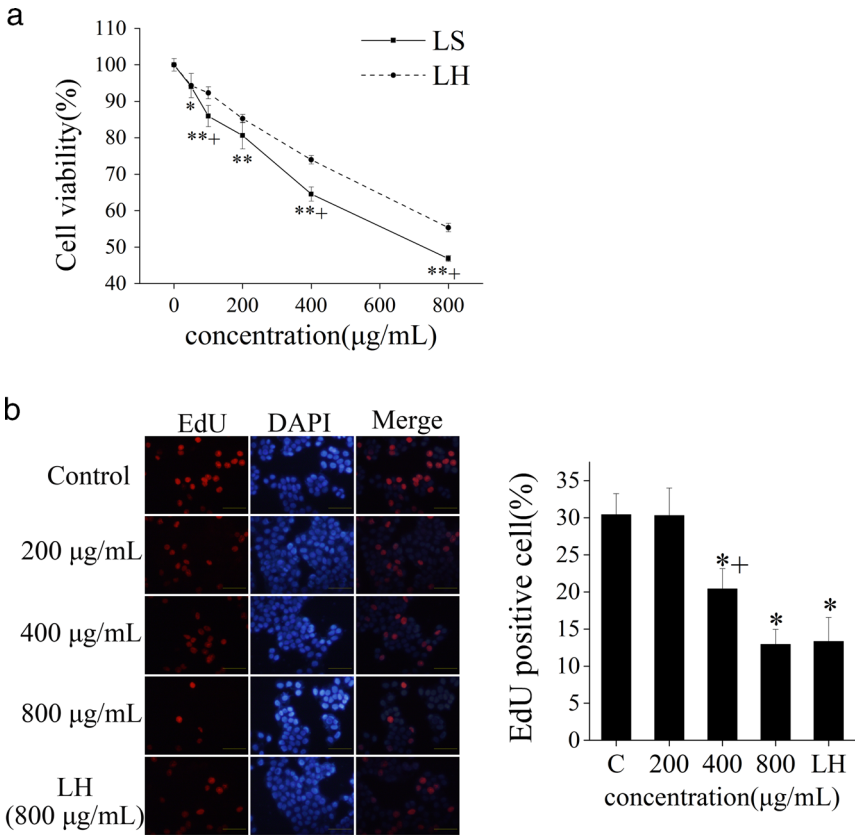

Fig. 2 Effects of LS on cell proliferation in HT-29 cells. **a** CCK-8 assay revealed LS inhibited HT-29 cell proliferation. Data represent the Mean ± SEM of three independent experiments. **b** EdU incorporation assay revealed LS inhibited HT-29 cell proliferation, graph illustrating the EdU score for control and LS-treated cells. $*p < 0.05$; $**p < 0.01$ indicate significant difference with the 0 μg/mL control group. $^{+}p < 0.05$ indicates significant difference with the adjacent concentration

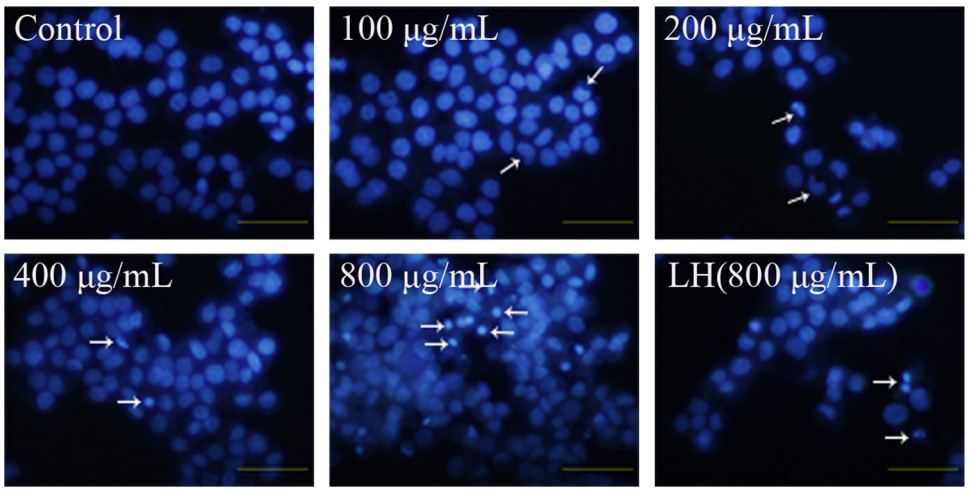

Fig. 3 Hoechst 33258 staining to detect apoptotic morphology in HT-29 cells treated with various concentrations of LS and 800 μg/mL LH for 24 h compared with the 0 μg/mL control group

expression of cyclin D1 ($p < 0.05$). So LS treatment triggered G0/G1 cell cycle arrest in HT-29 cells (Fig. 6).

LS suppresses the PI3K/AKT/GSK3β signaling pathway in HT-29 cells

The disorder of PI3K/AKT/GSK3β signaling pathway has been found in many cancers. The PI3K/AKT/GSK3β signaling pathway was considered and related proteins were detected. As shown in Fig. 7, compared with the 0 μg/mL control group, LS treatment significantly decreased the levels of p-PI3K, p-AKT and p-GSK3β. Compared with the LH positive control group, LS significantly inhibited the expression of p-PI3K, p-AKT and p-GSK3β ($p < 0.05$).

To further determine that LS induced apoptosis in HT29 cells via the PI3K/AKT/GSK3β signaling pathway, HT-29

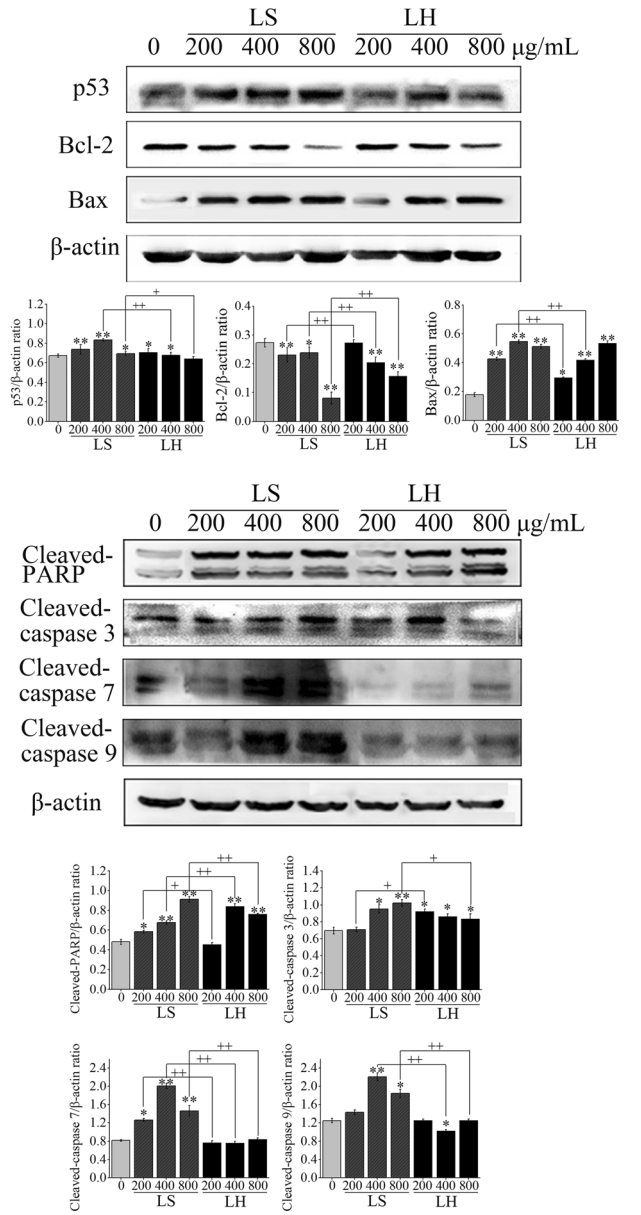

Fig. 4 Effect of LS on cell apoptosis-associated factors in HT-29 cells. **a** Western blot analysis of Bcl-2, Bax and p53 in HT-29 cells after treatment with LS and LH, respectively. **b** Western blot analysis of cleaved-caspase-3/7/9 and cleaved-PARP in HT-29 cells after treatment with LS and LH, respectively. Data are expressed as means ± SD of three independent experiments performed in triplicate. $^{*}p < 0.05$; $^{**}p < 0.01$ indicate significant difference with the 0 μg/mL control group. $^{+}p < 0.05$; $^{++}p < 0.01$ indicate significant difference in LS and LH at a uniform concentration

cells were treated as follows: LS (400 μg/mL), LY294002 (25 μM), LY294002 (25 μM) + LS (400 μg/mL). As shown in Fig. 8, the expression of p-PI3K, p-AKT and p-GSK3β was significantly down-regulated in each treatment group compared to the 0 μg/mL control group ($p < 0.05$). Since LY294002 blocked the PI3K/AKT/GSK3β signaling pathway, the expression of p-PI3K and p-GSK3β was significantly down-regulated compared with LS ($p < 0.01$). After the combined intervention, the LS and LY294002 showed a good synergistic effect, and the expression of p-PI3K, p-AKT and p-GSK3β was more significant than LS alone ($p < 0.05$). The result indicated that LS can suppress PI3K/AKT/GSK3β signaling pathway of HT-29 cells.

Discussion

LA and its derivatives have been shown to exhibit various pharmacological activities, including the inhibition of cancer growth, induction of apoptosis, and cell cycle arrest (Ma et al. 2017). However, the molecular mechanism of LS-induced apoptosis has not yet been elucidated. In this study, HT-29 cells were used to evaluate the anti-cancer activity of LS, and it was shown that LS inhibited cell viability and induced apoptosis in a dose-dependent manner. As the concentration of LS increased, cancer cell viability was significantly decreased, followed by an increase in apoptosis.

Apoptosis is programmed cell death, and in cancer cells, apoptosis does not typically occur. In these cells, a pivotal homeostatic mechanism operates that balances cell division and cell death to maintain the uncontrolled proliferation of cancer cells, and therefore, one of the important properties of anti-cancer drugs is thought to be successful apoptosis induction in cancer cells (Qian et al. 2015). The occurrence of apoptosis is related to characteristic morphological (Yang et al. 2015a). Hoechst 33258 staining assay confirmed that LS inducted cell morphological apoptosis. Bcl-2 family proteins are key regulators of apoptosis, and the role of anti-apoptotic proteins and pro-apoptotic proteins control whether cells undergo apoptosis (Zhao et al. 2016). In the study, pro-apoptotic proteins Bax was up-regulated, and anti-apoptotic protein Bcl-2 was down-regulated, which supported the results of apoptosis. In addition, elevated p53 expression and increased caspase member activity are also thought to be involved in apoptosis regulation (Zuo et al. 2017). On the one hand, western blot showed that expression levels of p53 was increased (Fig. 4a), which also supported the results of LS-induced apoptosis. From the present study, p53 plays a key role in the regulation of apoptosis by inducing activation of the transcription of Bax and repression of the expression of Bcl-2 (Herman-Antosiewicz et al. 2004). On the other hand, the results also showed that LS induced caspase-3, 7, and 9, PARP cleavage and DNA fragmentation in HT-29 cells. Bcl-2 protein activates caspase family. The specific proteolytic breakdown of PARP by caspases is known to occur during the executive phase of apoptosis (Decker et al. 2000), and

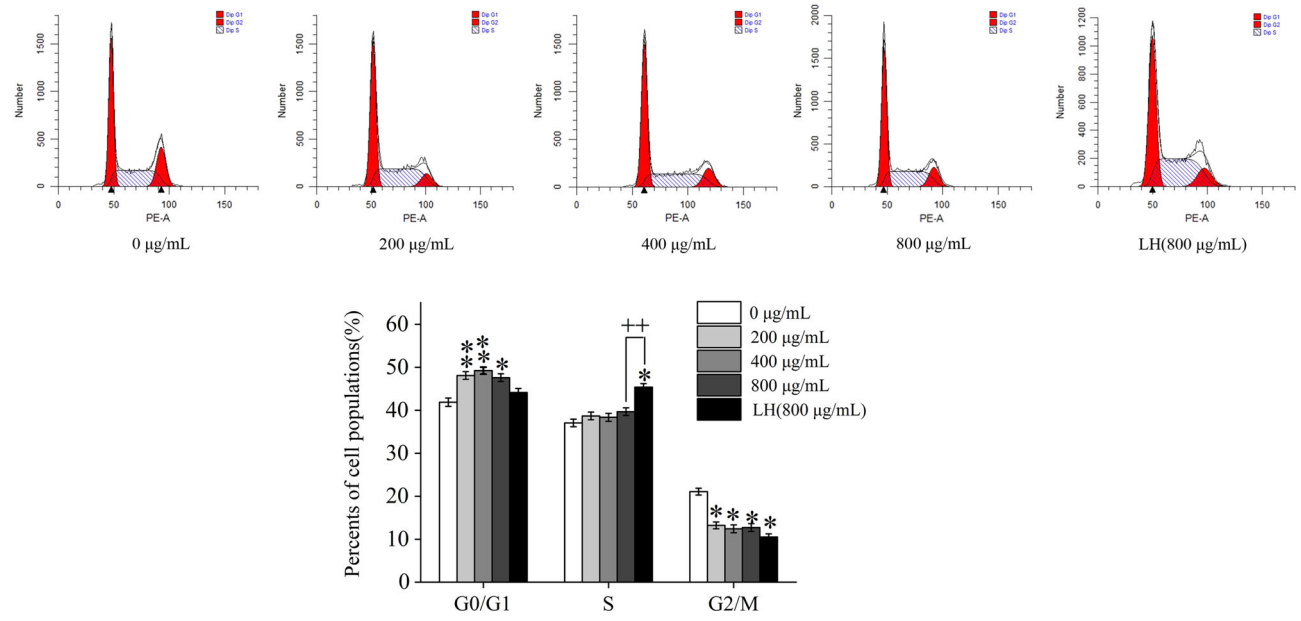

Fig. 5 Effects of LS on cell cycle phase distribution of HT-29 cells. Percentage of cell populations is at the G0/G1, S, and G2/M phases. All values are expressed as mean ± SD of three independent experiments. $*p < 0.05$; $**p < 0.01$ indicate significant difference with the 0 μg/mL control group. $^{+}p < 0.05$; $^{++}p < 0.01$ indicate significant difference in LS and LH at a uniform concentration

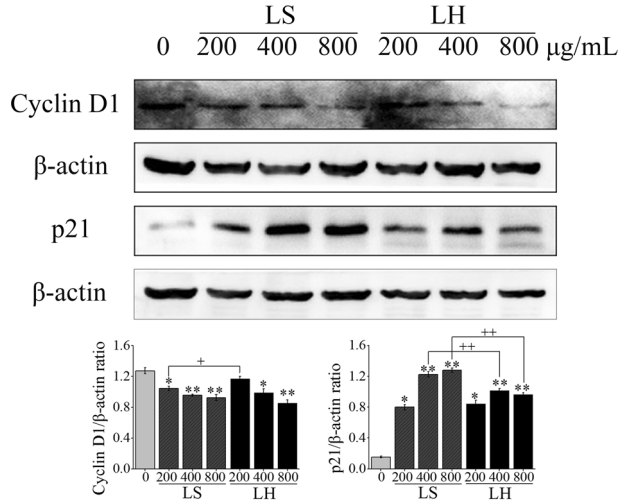

Fig. 6 Western blot analysis of cyclin D1 and p21 in HT-29 cells after treatment with LS and LH, respectively. Data are expressed as means ± SD of three independent experiments performed in triplicate. $*p < 0.05$; $**p < 0.01$ indicate significant difference with the 0 μg/mL control group. $^{+}p < 0.05$; $^{++}p < 0.01$ indicate significant difference in LS and LH at a uniform concentration

breakdown of PARP inhibits DNA repair and activates DNA fragmentation (Livraghi and Garber 2015). These results suggested that cell death by LS was mediated caspase-dependent apoptosis.

Dysfunction cell cycle progression is another feature of cancer and is considered to be an important target for cancer treatment (Wang et al. 2019). Analysis of the cell cycle revealed that LS induced cell cycle arrest in the G0/G1 phase. Magiera et al. (2017) have shown that LCAHA-induced G0/G1 arrest with decreased expression of cyclin D1.Western blot analysis showed that the expression level of cyclin D1 treated by LS also decreased, which was consistent with the above results. The p53 tumor suppressor gene plays an important role in cell cycle arrest, apoptosis and senescence (Gong et al. 2016). p53-mediated cell cycle arrest is caused mainly by p53-dependent transcription of the cyclin-dependent kinases (CDKs) inhibitor p21 (CDKN1A), which inhibited cyclin-CDK complexes and phosphorylation of Rb tumor suppressor gene, thereby preventing cells from entering G1 phase (Guo et al. 2014). It is well known that increased p53 induced p21 expression (Dattaroy et al. 2018), up-regulation of p21 expression can cause the cell cycle arrest at G1 phase (Huang et al. 2018). In summary, LS treatment down-regulated the expression of cyclin D1 and up-regulated the expression of p21, accompany with the increase of p53, which is consistent with the results of previous studies (Huang et al. 2018).

As mentioned in the introduction, caspase family and Bcl-2 family are downstream effector molecules GSK3β/PI3K / AKT signaling pathway (He et al. 2018). The PI3K/AKT/GSK3β signaling pathway is one of the most frequently activated signal transduction pathways in human cancer. AKT is one of the major downstream effectors of PI3K (Martini et al. 2014). GSK3β is an important effector cell of PI3K/AKT/GSK3β signaling pathway, and some cellular processes such as cell metabolism, cell death, and survival depend on it (Kitagishi et al. 2014). Western blot analysis has confirmed that p-PI3K, p-AKT and p-GSK3β

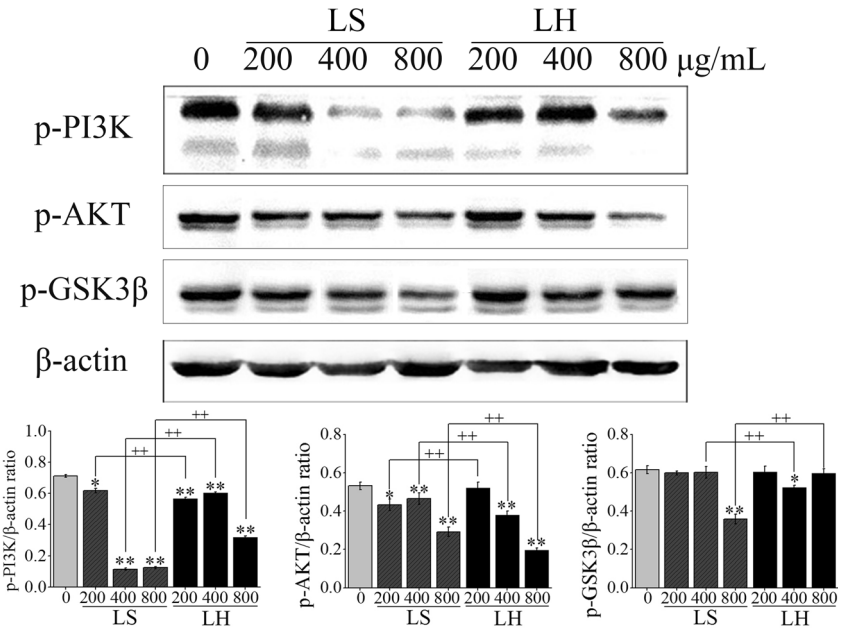

Fig. 7 Western blot analysis of p-AKT, p-GSK3β and p-PI3K in HT-29 cells after treatment with LS and LH, respectively. Data are expressed as means ± SD of three independent experiments performed in triplicate. $^{*}p < 0.05$; $^{**}p < 0.01$ indicate significant difference with the 0 μg/mL control group. $^{+}p < 0.05$; $^{++}p < 0.01$ indicate significant difference in LS and LH at a uniform concentration

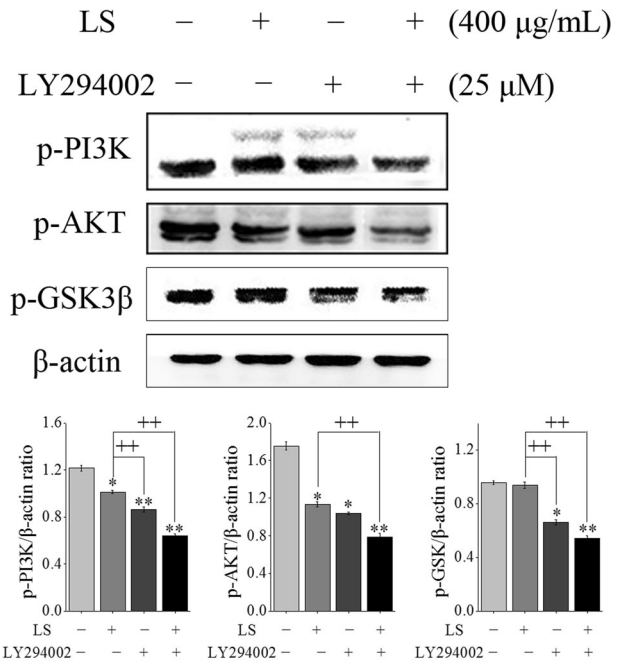

Fig. 8 Western blot analysis of p-AKT, p-GSK3β and p-PI3K in HT-29 cells after treatment with LS and LY294002, respectively. Data are expressed as means ± SD of three independent experiments performed in triplicate. $^{*}p < 0.05$; $^{**}p < 0.01$ indicate significant difference with the 0 μg/mL control group. $^{+}p < 0.05$; $^{++}p < 0.01$ indicate significant difference in LS, LY294002 and LS+LY294002

were dramatically decreased with increasing concentrations of LS, which suggesting that the effect of LS on HT-29 cells may be suppressed the PI3K/AKT/GSK3β signaling pathway. To further demonstrate the above conclusion, the PI3K inhibitor (LY294002) was used in subsequent experiments. The results showed that the activity of p-PI3K, p-AKT and p-GSK3β was significantly decreased by inhibiting the PI3K/AKT/GSK3β signaling pathway, indicating that LS inhibited PI3K/AKT/GSK3β signaling pathway leading to apoptosis of HT-29 cells.

Conclusions

In conclusion, LS induced apoptosis in HT-29 cells, arrested the cell cycle in G0/G1 phase and inhibited PI3K/AKT/GSK3β signaling pathway, indicating that LS had a strong anti-human colon cancer ability. LS might be a potential drug candidate for the treatment of human colon cancer. Further studies in animal models should be conducted to ensure the efficacy of the anti-cancer effect of LS.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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