

Effect of *Solanum lyratum* Polysaccharide on Malignant Behaviors of Lung Cancer Cells by Regulating the Circ_UHRF1/miR-513b-5p Axis

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ABSTRACT

This study aimed to investigate the effect of *Solanum lyratum* polysaccharide on the malignant behavior of lung cancer cells and its possible mechanism. For this purpose, lung cancer A549 cells were cultured *in vitro* and treated with different doses (0.4, 0.8, 1.2 mg/mL) of *Solanum lyratum* polysaccharide for 24 h. Then cell proliferation was detected by the CCK-8 method and clone formation test. Transwell test was used to detect cell migration and invasion, and flow cytometry was used to detect cell apoptosis. The protein expressions of Bax and Bcl-2 in cells were detected by Western blotting, and the protein expressions of circ_UHRF1 and miR-513b-5p were detected by the qRT-PCR method. Pearson correlation was used to analyze the correlation between circ_UHRF1 and miR-513b-5p expressions in lung cancer tissues. Results showed that compared with the control group, the proliferation inhibition rate and apoptosis rate of A549 cells that intervened with the *Solanum lyratum* polysaccharide and expression of Bax protein in the cells were all increased ($P < 0.05$), but the number of clones, migration and invasion and the protein expression of Bcl-2 were all decreased ($P < 0.05$), and were dose-dependent. The expression of circ_UHRF1 in A549 cells that intervened with the *S. lyratum* polysaccharide was decreased ($P < 0.05$), but the expression of miR-513b-5p was increased ($P < 0.05$). The expression of circ_UHRF1 in lung cancer tissues was higher than that of adjacent tissues ($P < 0.05$), and the expression of miR-513b-5p was lower than that of adjacent tissues ($P < 0.05$). The expressions of circ_UHRF1 and miR-513b-5p in lung cancer tissues were negatively correlated ($r = -0.861$, $P < 0.05$). Circ_UHRF1 could target miR-513b-5p, and the expression of miR-513b-5p in A549 cells knocking down circ_UHRF1 was increased. After knocking down circ_UHRF1, the proliferation inhibition rate and apoptosis rate of A549 cells and protein expression of Bax in the cells were all increased ($P < 0.05$), but the number of clones, migration and invasion and the protein expression of Bcl-2 were all decreased ($P < 0.05$). Up-regulation of circ_UHRF1 reduced the effects of *S. lyratum* polysaccharide on the proliferation, migration, invasion and apoptosis of A549 cells. In general, *S. lyratum* polysaccharide could inhibit the proliferation, migration and invasion of lung cancer A549 cells, and induce cell apoptosis. Its mechanism may be related to the regulation of the circ_UHRF1/miR-513b-5p axis.

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Introduction

Lung cancer is a common malignant tumor of the respiratory system, which is highly malignant and prone to recurrence and metastasis (1). At present, surgery is the main treatment for early lung cancer, while radiotherapy and chemotherapy is the main treatment for late lung cancer. However, the currently used radiotherapy and chemotherapy drugs for lung cancer, such as cisplatin, have great side effects, and lung cancer cells are prone to develop drug resistance to cisplatin, resulting in a poor therapeutic effect (2, 3). Therefore, it is urgent to find effective and low-

toxic drugs for treating lung cancer (4). *Solanum lyratum* polysaccharide is the main active ingredient of Solanaceae, which has many functions such as antiviral, bacteriostasis and immunity regulation. In addition, it also has an anti-tumor effect. Studies have shown that *S. lyratum* polysaccharide can inhibit the proliferation, migration and invasion of osteosarcoma cells by up-regulating Ebp1 (5), promoting the apoptosis of cisplatin-resistant ovarian cancer cells by up-regulating PTEN and down-regulating Surviving (6), and promoting the sensitivity of breast cancer cells to adriamycin by activating Fas and inhibiting

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Bcl-2 gene expression (7). However, it is not known whether the polysaccharide from *S. lyratum* can play an anti-lung cancer role. Circ_UHRF1 is a cyclic RNA (circRNA), which is up-regulated in oral squamous cell carcinoma and liver cancer, and promotes the malignant behavior of tumor cells, thus promoting the development of tumor (8, 9). However, the effect of circ_UHRF1 on the occurrence and development of lung cancer is unknown. On-line software prediction of star target gene shows that circ_UHRF1 may target miR-513b-5p. The expression of miR-513b-5p is down-regulated in lung cancer tissues and cells, and over-expression of miR-513b-5p can significantly inhibit the proliferation, migration and invasion of lung cancer cells. miR-513b-5p can be used as a molecular target for lung cancer treatment (10). In this study, we mainly observed the influence of *S. lyratum* polysaccharide on malignant behavior (proliferation, migration, invasion and apoptosis) of lung cancer cells and whether it can regulate circ_UHRF1/miR-513b-5p axis, so as to provide some experimental basis for its application in the treatment of lung cancer.

Materials and methods

Clinical data

The cancer tissues and adjacent tissues of 110 lung cancer patients, who received surgical treatment in our hospital from May 2017 to May 2020, were collected and stored in liquid nitrogen. There were 62 males and 48 females with an average age of (52.36±7.29) years. Inclusion criteria: First diagnosis by pathological examination. Exclusion criteria: patients with other malignant tumors; Patients with dysfunction of vital organs such as heart and kidney. This study conforms to the principles of the Helsinki Declaration.

Cells and reagents

Cells and reagents that used in this study were A549 cell line, Shanghai Cell Bank, Chinese Academy of Sciences; *Solanum lyratum* thunb, Chinese herbal medicine market in Bozhou, Anhui Province, extracted *S. lyratum* polysaccharide concerning the method of reference (7); RPMI 1640 culture medium, BCA protein detection kit and CCK-8 kit, Beijing Solebao; Fetal bovine serum (FBS), Zhejiang Tianhang Biotechnology Co., Ltd.; Lipofectamine TM 2000 kit, Invitrogen Company of

America; RNA extraction kit, reverse transcription kit and PCR kit, Takara Bio; Sequence, circ_UHRF1 small interfering RNA (si-circ_UHRF1) or overexpression vector (pcDNA-circ_UHRF1), small interfering RNA negative control sequence (si-NC) and empty vector (pcDNA), miR-513b-5p mimetic (mimcs) and simulated control sequence (miR-NC), Shanghai Sango; Bax and Bcl-2 antibodies, China Abcam Company.

Cell culture

The A549 cells were resuscitated and cultured in a CO₂ incubator with RPMI 1640 medium (complete medium) containing 10% FBS. When the cell growth density reached 90%, the cells were digested with 0.25% trypsin and sub-cultured.

Detection of the inhibition rate of cell proliferation by CCK-8 method

200µL logarithmic A549 cells (2.5×10⁴ cells /mL) were inoculated into the 96-well plate and cultured for 4h. Then the culture medium was discarded. After that, the cells were cultured with the culture medium containing 0, 0.4, 0.8 and 1.2 mg/mL (11) of *S. lyratum* polysaccharide, which was marked as the control group, *S. lyratum* polysaccharide L group, *S. lyratum* polysaccharide M group, *S. lyratum* polysaccharide H group. After 24 h of incubation, 10µL CCK-8 was added and incubated for 2 h, and the optical density (OD) was measured by a microplate reader (wavelength 450 nm). Cell proliferation inhibition rate (%)=(OD control group -OD experimental group) /OD control group×100%.

Clone formation experiment

2.5 mL logarithmic A549 cells (2.5×10⁴ cells /mL) were inoculated into the 6-well plate, and after 4h of culture, the culture solution was discarded. The cells were treated in groups according to steps in 1.3.2. The culture solution was changed every 2 days, and cells were cultured for 14 days. After discarding the culture solution, the cells were fixed with paraformaldehyde, stained with crystal violet, washed with PBS, and observed under a microscope. The number of clones was counted.

Detection cell migration and invasion by Transwell test

Invasion experiment: 2.5 mL logarithmic A549 cells (2.5×10^4 cells/mL) were inoculated into the 6-well plate, and cultured for 4 h. Then the culture solution was discarded, and cells were treated in groups according to steps in 1.3.2. After 24 h of culture, cells were collected, and the cell density of each group was adjusted to 2.5×10^5 cells/mL. The Transwell chamber was placed on a 24-well plate. Matrigel matrix glue was laid on the upper chamber and then was naturally dried. 100 μ L cell suspension of each group was added to the upper chamber and 500 μ L complete culture solution was added to the lower chamber. After culturing for 24 h, the culture solution was discarded. The cells were fixed with paraformaldehyde, dyed with crystal violet, washed with PBS, observed under microscope and counted. Migration experiment: except that Matrigel matrix glue is not laid on the upper chamber of the Transwell chamber, the operation procedure is the same as that of the invasion experiment.

Detection of apoptosis by flow cytometry

2.5 mL logarithmic A549 cells (2.5×10^4 cells/mL) were inoculated into the 6-well plate. After 4h of culture, the culture solution was discarded and treated in groups according to steps in 1.3.2. After 24 h of culture, the cells were collected, and apoptosis was detected by Annexin V-FITC/PI kit and up-flow cytometry.

Western blot was used to detect the expression of Bax and Bcl-2 protein

2.5 mL logarithmic A549 cells (2.5×10^4 cells/mL) were inoculated into the 6-well plate, and after 4h of culture, the culture solution was discarded. The cells were treated in groups according to steps in 1.3.2. After 24 h of culture, the cells were collected. RIPA reagent was used to lyse cells, and centrifugation was carried out at 4°C and 12000r/min for 10 min. The supernatant was taken, and the protein concentration was detected by the BCA method, and then 10% SDS-PAGE electrophoresis was performed. The isolated protein was transferred to PVDF membrane and sealed with 5% skimmed milk powder for 2h. They were incubated overnight with primary antibodies of Bax(1:500), Bcl-2(1:500) and GAPDH(1:1000) in the

refrigerator at 4°C. After washing the membranes, they were incubated for 1h in goat anti-rabbit secondary antibody (1:2000) at 37°C. After adding developer and developing in dark, photos were taken by exposure. The expression of the target protein relative to GAPDH was analyzed by ImageJ software.

Detection of the expressions of circ_UHRF1 and miR-513b-5p by qRT-PCR

Cell inoculation and treatment are the same as 1.3.6. After 24 h of culture, cells are collected; tissue samples were ground under the protection of liquid nitrogen. Total RNA in cells or tissues was extracted by RNA extraction kit and then amplified by PCR after reverse transcription. Amplification procedure: pre-denaturation at 94°C for 3 min; denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 2 min, totaling 35 cycles. The expressions of circ_UHRF1 relative to GAPDH and miR-513b-5p relative to U6 were calculated by $2^{-\Delta\Delta C_t}$.

Double luciferase reporter gene experiment

The circ_UHRF1 nucleotide sequence containing miR-513b-5p binding site was amplified and inserted into pGL3 vector to construct circ_UHRF1 wild type (WT-circ_UHRF1) luciferase reporter gene vector. The mutant circ_UHRF1 (MUT-circ_UHRF1) luciferase reporter gene vector was constructed by inserting the pGL3 vector after mutation of the binding site. This process was completed by Shanghai Sango Bioengineering Co., Ltd. 2.5 mL logarithmic A549 cells (2.5×10^4 cells/mL) were inoculated into the 6-well plate, and the culture solution was discarded after 12H of culture. WT- circ_UHRF1 and miR-NC, WT- circ_UHRF1 and miR-513b-5p mimic, MUT- circ_UHRF1 and miR-NC or MUT-circ_UHRF1 and miR-513b-5p mimics were co-transfected with LipofectamineTM 2000 liposome method. After transfection for 12 h, the culture medium was discarded and the cells were collected. After adding lysis solution for lysis, the cells were centrifuged at 3500 r/min for 10 min. After that, the supernatant was taken, and the luciferase activity was detected with a double luciferase activity detection kit.

Cell transfection and treatment

2.5 mL logarithmic A549 cells (2.5×10^4 cells/mL) were inoculated into the 6-well plate. After 4 h and 12

h of culture, the culture medium was discarded and RPMI 1640 culture medium without FBS was added. Lipofectamine™ 2000 liposome method was used to transfect si-circ_UHRF1, si-NC, pcDNA-circ_UHRF1 and pcDNA into A549 cells respectively. After transfecting for 12h, the cells were changed into a complete culture medium. The expressions of circ_UHRF1 and miR-513b-5p in cells were detected by qRT-PCR after 24 h of culture, and the method was the same as that in 1.3.7. The above-mentioned transfected A549 cells were all inoculated into the culture plate, and the transfected A549 cells of si-circ_UHRF1 and si-NC were all cultured in the culture medium without *S. lyratum* polysaccharides, which were divided into si-circ_UHRF1 group and si-NC group respectively. PcDNA-circ_UHRF1 and PcDNA-transfected A549 cells were cultured in a culture medium containing 1.2 mg/mL celadone polysaccharide, which were divided into celadone polysaccharide +pcDNA-circ_UHRF1 group and celadone polysaccharide +pcDNA group respectively. According to the above 1.3.2, 1.3.3, 1.3.4, 1.3.5 and 1.3.6, the cell proliferation inhibition rate, the number of clones, the number of migration and invasion, the apoptosis rate and the expressions of Bax and Bcl-2 protein were detected.

Statistical analysis

SPSS.22.0 software was used to analyze the experimental data. The measurement data were expressed in mean standard deviation ($\bar{x} \pm s$). After the normality test and variance homogeneity test, the comparison between the two groups was conducted by independent sample T-test. Univariate analysis of variance was used for comparison among groups, and LSD-t test was used for pairwise comparison among groups. Pearson correlation analysis showed the correlation between circ_UHRF1 and miR-513b-5p expression in lung cancer. $P < 0.05$ showed that the difference was statistically significant.

Results and discussion

Effects of *Solanum lyratum* polysaccharide on proliferation, migration and invasion of A549 cells

Compared with the control group, the inhibition rate of A549 cells was increased ($P < 0.05$), and the number of clones, migration and invasion was

decreased ($P < 0.05$), which was dose-dependent. See Table 1.

Effects of *Solanum lyratum* polysaccharide on apoptosis of A549 cells

Compared with the control group, the apoptosis rate and Bax protein expression in A549 cells were increased ($P < 0.05$), while Bcl-2 protein expression was decreased ($P < 0.05$) in a dose-dependent manner (Figure 1) and (Table 2).

Table 1. Effects of *Solanum lyratum* polysaccharide on proliferation, migration and invasion of A549 cells($\bar{x} \pm s$, n=9)

Group	Inhibition rate%	Number of cells		
		Clone	Migration	Invasion
Control group	0.00±0.00	117.78±6.36	179.22±8.52	142.00±8.07
polysaccharide L	17.23±0.88*	90.78±2.90*	144.89±7.67*	118.22±6.08*
polysaccharideM	34.83±2.44*#	71.33±3.46*#	101.56±5.68*#	87.00±4.24*#
polysaccharideH	52.26±3.09*#&	44.78±2.57*#&	70.78±3.52*#&	52.78±1.87*#&
F	1120.604	507.806	464.913	436.499
P	0.000	0.000	0.000	0.000

Note: Compared with the control group, * $P < 0.05$; Compared with the *Solanum lyratum* polysaccharide L group, # $p < 0.05$; Compared with *S. lyratum* polysaccharide M group, & $P < 0.05$

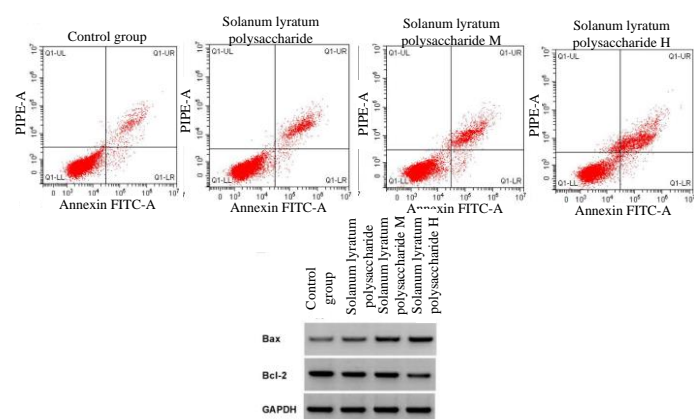


Figure 1. Effects of *Solanum lyratum* polysaccharide on A549 apoptosis

Table 2. Detection of apoptosis of A549 by *Solanum lyratum* polysaccharide ($\bar{x} \pm s$, n=9)

Group	Apoptosis rate/%	Bax	Bcl-2
Control group	7.73±0.45	0.12±0.01	0.80±0.08
polysaccharide L	13.83±0.76*	0.28±0.03*	0.65±0.06*
polysaccharideM	18.25±0.88*#	0.39±0.04*#	0.45±0.04*#
polysaccharideH	23.57±1.76*#&	0.62±0.06*#&	0.29±0.03*#&
F	349.192	256.016	144.072
P	0.000	0.000	0.000

Note: Compared with the control group, * $P < 0.05$; Compared with the *Solanum lyratum* polysaccharide L group, # $p < 0.05$; Compared with *S. lyratum* polysaccharide M group, & $P < 0.05$.

Effects of *Solanum lyratum* polysaccharide on the expression of circ_UHRF1 and miR-513b-5p in A549 cells

Compared with the control group, the expression of circ_UHRF1 in A549 cells was decreased ($P < 0.05$), while the expression of miR-513b-5p was increased ($P < 0.05$) in a dose-dependent manner (Table 3).

Table 3. Effect of *Solanum lyratum* polysaccharide on the expressions of circ_UHRF1 and miR-513b-5p in A549 cells ($\bar{x} \pm s$, $n=9$)

Group	circ_UHRF1	miR-513b-5p
Control group	1.00±0.00	1.00±0.00
polysaccharide L	0.75±0.06*	1.36±0.07*
polysaccharideM	0.46±0.03*#	1.81±0.08*#
polysaccharideH	0.27±0.02*#&	2.46±0.11*#&
F	757.560	609.243
P	0.000	0.000

Note: Compared with the control group, * $P < 0.05$; Compared with the *Solanum lyratum* polysaccharide L group, # $p < 0.05$; Compared with *S. lyratum* polysaccharide M group, & $P < 0.05$.

Expressions of circ_UHRF1 and miR-513b-5p in lung cancer

The expression of circ_UHRF1 in lung cancer was higher than that in adjacent tissues ($P < 0.05$), and the expression of miR-513b-5p was lower than that in adjacent tissues ($P < 0.05$). Pearson correlation analysis showed that circ_UHRF1 was negatively correlated with miR-513b-5p expression in lung cancer tissues ($r = -0.861$, $P < 0.05$). See Figure 2.

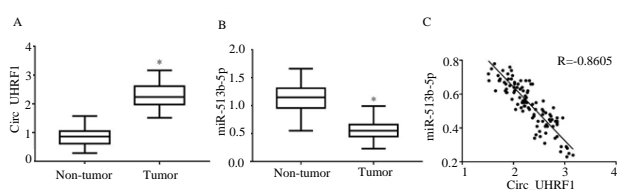


Figure 2. Expressions of circ_UHRF1 and miR-513b-5p in lung cancer and their correlation. Note: A: circ_UHRF1 was highly expressed in lung cancer. B: miR-513b-5p expression was low in lung cancer. C: CIRC_uhrf1 was negatively correlated with miR-513b-5p. Compared with Non-tumor group, * $P < 0.05$.

Circ_UHRF1 targeting miR-513b-5p

Figure 3 for the binding site of circ_UHRF1 and miR-513b-5p nucleotide sequence predicted by Starbase target gene online software. The luciferase activity of A549 cells co-transfected with WT-circ_UHRF1 and miR-513b-5p mimics was

0.39±0.05, which was significantly lower than that of A549 cells co-transfected with WT-circ_UHRF1 and miR-NC ($t = 15.295$, $p < 0.05$). The expression of circ_UHRF1 in A549 cells in the si-circ_UHRF1 group was lower than that in the si-NC group (0.33 ± 0.04 vs 1.00 ± 0.00 , $t = 50.250$, $P < 0.05$), while the expression of miR-513b-5p was higher than that in the si-NC group (2.11 ± 0.10). Therefore, the construction of A549 cells with circ_UHRF1 knockdown was successful, and circ_UHRF1 knocking down promoted the expression of miR-513b-5p in A549 cells.



Figure 3. Complementary sequences of CIRC_uhrf1 and miR-513b-5p

Effects of circ_UHRF1 knockdown on proliferation, migration, invasion and apoptosis of A549 cells

In the si-circ_UHRF1 group, the inhibition rate, apoptosis rate and Bax protein expression in A549 cells were higher than those in the si-NC group ($P < 0.05$), while the clone number, migration number and invasion number and Bcl-2 protein expression were lower than those in the si-NC group ($P < 0.05$) (Figure 4) and (Table 4).

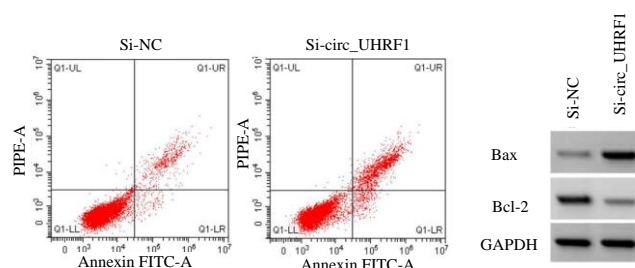


Figure 4. Effects of silencing circ_UHRF1 on apoptosis of A549 cells

Up-regulation of circ_UHRF1 reducing the effect of *Solanum lyratum* polysaccharide on proliferation, migration, invasion and apoptosis of A549 cells

The expression of circ_UHRF1 in A549 cells of *Celastrus sativus* polysaccharide +pcDNA-

circ_UHRF1 group was higher than that of *Celastrus sativus* polysaccharide +pcDNA group. The expression of miR-513b-5p was lower than that of the *S. lyratum* polysaccharide +pcDNA group. The cell inhibition rate, apoptosis rate and Bax protein expression were lower than those of the *S. lyratum*

polysaccharide +pcDNA group (P<0.05), and the number of clones, migration and invasion and Bcl-2 protein expression were higher than those of *S. lyratum* polysaccharide +pcDNA group (P<0.05) (Figure 5) and (Table 5).

Table 4. Effect of circ_UHRF1 knockdown on proliferation, migration, invasion and apoptosis of A549 cells (x±s, n=9)

Group	Inhibition rate/%	Apoptosis rate/%	Bax	Bcl-2	Number of cells		
					Clone	Migration	Invasion
si-NC	0.00±0.00	7.79±0.60	0.13±0.01	0.78±0.08	116.33±8.71	183.11±8.05	141.33±8.60
si-circ_UHRF1	42.96±2.00*	20.22±1.13*	0.50±0.05*	0.33±0.03*	55.33±2.79*	85.44±3.74*	65.78±2.66*
t	64.440	29.146	21.769	15.801	20.009	33.010	25.178
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: Compared with the si-NC group, *P<0.05

Table 5. Up-regulation of circ_UHRF1 reduces the effects of *Solanum lyratum* polysaccharide on proliferation, migration, invasion and apoptosis of A549 cells (x s, n=9)

Group	circ_UHRF1	miR-513b-5p	Inhibition rate/%	Apoptosis rate/%	Bax	Bcl-2	Number of cells/individual		
							Clone	Migration	Invasion
+pcDNA <i>S. lyratum</i> polysaccharide +pcDNA	1.00±0.00	1.00±0.00	52.69±2.07	23.38±1.68	0.59±0.05	0.32±0.03	43.33±2.31	68.33±4.29	53.67±2.05
+pcDNA-circ_UHRF1 <i>S. lyratum</i> polysaccharide +pcDNA-circ_UHRF1	3.12±0.08*	0.37±0.04*	20.81±1.12*	10.77±0.97*	0.22±0.02*	0.70±0.06*	82.33±2.62*	124.22±5.33*	100.22±6.94*
t	79.500	47.250	40.636	19.501	20.612	16.994	33.496	24.506	19.298
P	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Note: *P<0.05, compared with *Solanum lyratum* polysaccharide +pcDNA group

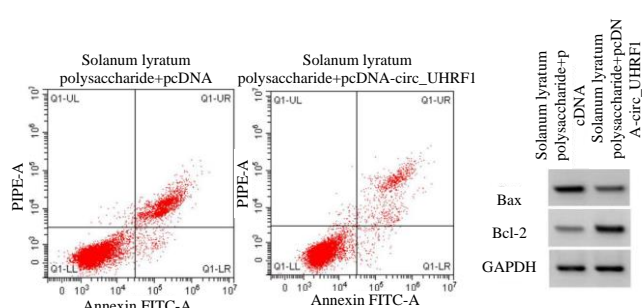


Figure 5. Up-regulation of circ_UHRF1 reducing the effect of *Solanum lyratum* polysaccharide on apoptosis of A549 cells

Natural Chinese herbal medicines or their active ingredients have become the focus of anti-tumor drug research because of their advantages such as fewer side effects, strong efficacy and many targets (12). *Solanum lyratum* Thunb is a kind of Chinese herbal

medicine, which has the functions of clearing away heat and toxic materials, promoting diuresis and expelling wind, and can be used for treating rheumatic arthralgia, malaria, edema and other diseases. *S. lyratum* polysaccharide is the main active ingredient of *S. lyratum*, which has certain anti-tumor activity. It has been reported that *S. lyratum* polysaccharide can inhibit the proliferation of TPC-1 cells of human thyroid papillary carcinoma by regulating Bax/Bcl-2 protein and inhibiting JNK phosphorylation (13), and can inhibit the proliferation of gastric cancer cells and induce apoptosis of gastric cancer cells in a dose-dependent manner (14). The results showed that the inhibition rate and apoptosis rate of lung cancer cells were significantly increased, while the clone number, migration number and invasion number were significantly decreased in a dose-dependent manner,

indicating that the *S. lyratum* polysaccharide inhibited the proliferation, migration and invasion of lung cancer cells and induced the apoptosis of lung cancer cells. This suggested that the *S. lyratum* polysaccharide also has an anti-lung cancer effect. Bax/Bcl-2 is involved in the regulation of tumor cell apoptosis, in which Bax, as a pro-apoptosis gene, promotes tumor cell apoptosis, while Bcl-2 has an inhibitory effect on tumor cell apoptosis (15, 16). The results showed that *S. lyratum* polysaccharide could inhibit the expression of Bcl-2 protein and promote the expression of Bax protein in lung cancer cells in a dose-dependent manner, suggesting that it may induce apoptosis of lung cancer cells by regulating Bax/Bcl-2.

LncRNA and miRNA are two kinds of non-coding RNA. LncRNA can regulate the expression of miRNA target genes by competitive adsorption of miRNA, participate in physiological or pathological processes such as cell proliferation, apoptosis and inflammation, and play an important role in the occurrence and development of various diseases including tumors (17-19). Studies have shown that a variety of lncRNA are involved in regulating the development of lung cancer. For example, Shen *et al.* (20) showed that lncRNA MCM3AP-AS1 was over-expressed in lung cancer tissues and cells, which promoted the malignant behavior of lung cancer cells by targeting the miR-195-5p/E2F3 axis, and further promoted the development of lung cancer. The research of Feng *et al.* (21) shows that the expression of lncRNA PGM5P4-AS1 in lung cancer cells is down-regulated, and LZTS3 up-regulation inhibits the proliferation, migration and invasion of lung cancer cells in vitro and tumor growth in vivo by competitively adsorbing miR-1275. lncRNA PGM5P4-AS1 may be a potential therapeutic target for lung cancer. This indicates that different lncRNA has different effects on the occurrence and development of lung cancer. The results of this study show that circ_UHRF1 is highly expressed in lung cancer tissues. Knocking down circ_UHRF1 can obviously inhibit the proliferation, migration and invasion of lung cancer cells, and knocking down circ_UHRF1 can promote the apoptosis of lung cancer cells, which indicates that circ_UHRF1 is a potential molecular target for lung cancer treatment. In addition, this study showed that *S. lyratum* polysaccharide could significantly inhibit

the expression of circ_UHRF1 in lung cancer cells while up-regulating the expression of circ_UHRF1 reduced the effect of *S. lyratum* polysaccharide on malignant behavior of lung cancer cells, which suggested that *S. lyratum* polysaccharide might regulate the malignant behavior of lung cancer cells by targeted inhibition of circ_UHRF1.

To further explore the molecular mechanism of *S. lyratum* polysaccharide regulating malignant behavior of lung cancer cells by targeting circ_UHRF1, this study confirmed that circ_UHRF1 targets and negatively regulates miR-513b-5p, which is consistent with the negative correlation between circ_UHRF1 and miR-513b-5p expression in lung cancer tissues. The research showed that the expression of miR-513b-5p in liver cancer was decreased, and up-regulation of miR-513b-5p could inhibit the malignant behavior of liver cancer cells in vitro and tumor growth in vivo by targeting PIK3R3. miR-513b-5p could be used as a molecular target for liver cancer treatment (22); the expression of miR-513b-5p was down-regulated in pancreatic cancer (23) and osteosarcoma (24, 25), and it inhibited the occurrence and development of this tumor as a tumor suppressor gene. The results of this study showed that *S. lyratum* polysaccharide could promote the expression of miR-513b-5p in lung cancer cells in a dose-dependent manner, while over-expression of circ_UHRF1 decreased the promoting effect of *S. lyratum* polysaccharide on the expression of miR-513b-5p in lung cancer cells, which further suggested that *S. lyratum* polysaccharide might inhibit the malignant behavior of lung cancer cells by targeting circ_UHRF1 to up-regulate the expression of miR-513b-5p in cells.

To sum up, a certain dose of *S. lyratum* polysaccharide can inhibit the proliferation, migration and invasion of lung cancer cells and induce apoptosis, and its mechanism may be related to the regulation of circ_UHRF1/miR-513b-5p axis. The *S. lyratum* polysaccharide has potential value in treating lung cancer, but its anti-lung cancer effect needs to be verified in vivo by animal experiments.

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None.

Interest conflict

None.

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