Safflower polysaccharide induces NSCLC cell apoptosis by inhibition of the Akt pathway

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Abstract. Lung cancer is the leading cause of cancer death in the world. Safflower polysaccharide (SPS) has been used for the improvement of immunomodulatory activities and treatment of cancers. However, studies on the effect of SPS on the progression of lung cancer have rarely been reported. To study the antitumor effect of SPS on human lung cancer and its potential mechanism, non-small cell lung cancer cell lines (NSCLC), A549 and YTMLC-90 were treated with SPS at various concentrations ranging from 0.04 to 2.56 mg/ml and BALB/c nude tumor-bearing mice were injected intraperitoneally with SPS at concentrations ranging from 15 to 135 mg/kg. Results showed that SPS suppressed the proliferation of A549 and YTMLC-90 cells and induced apoptosis by increasing mRNA levels of *bax* and *caspase-3*, and inhibited tumor growth *in vivo*. SPS induced cell cycle arrest in the G2/M phase by decreasing the expression of cdc25B and cyclin B1. Moreover, SPS decreased the expression of Akt, p-Akt and PI3K. In mice, SPS injection enhanced immunomodulatory activities by increasing levels of TNF-α and IL-6 in tumor-bearing mice. Our findings suggest that SPS suppresses tumor growth by enhancing immunomodulatory activities and blocking the PI3K/Akt pathway. This study provides new insight into the anticancer mechanism of SPS.

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Introduction

Lung cancer is the leading cause of cancer death all over the world. It is classified into two main histological groups: non-small cell lung cancer (NSCLC, 85%) and small cell lung cancer (SCLC, 15%) (1). The 5-year survival rate for lung cancer is only 16% (2). Therefore, it is necessary and critical to find a novel approach to increase the survival rate of lung cancer.

The phosphatidylinositol-3kinase (PI3K)/Akt signaling pathway is vital to cell growth and apoptosis (3). Many studies have reported that the PI3K/Akt signaling pathway was aberrantly activated in lung cancer (4-6). Some anticancer-drugs downregulated the expression of Akt and induced G2/M phase arrest. The G2/M checkpoint is regulated by cdc25B and cyclin B1 (7). For example, knocking down Sox2 induced G2/M arrest by decreasing expression levels of cyclin B1 and cdc2 in lung squamous cell carcinomas (8). Genistein induced cell cycle arrest in the G2/M phase by downregulating expression levels of cyclin B1 and cdc25B in H446 NSCLC (9).

Carthamus tinctorius L. (*C. tinctorus*), commonly named safflower, is a herbal plant in the family compositae. Safflower is well known for its function in the promotion of blood flow, removal of blood stasis, promotion of menstruation and alleviation of pain (10). The active components of safflower are quinochalones, flavonoids, alkaloids, and safflower polysaccharide (SPS). In recent years, many pharmacological experiments have demonstrated that safflower has a wide variety of biological activities, including the improvement of acute ischemic stroke (11-13) and the enhancement of antiinflammation (14), antioxidant (15), antitumor (16), and antibacterial activities (17). SPS is one of the most important active components and is used for modulating the immune system and cancer prevention. However, the effect of SPS on lung cancer progression is rarely reported and the underlying mechanisms remain unknown. In this study, we investigated the effect of SPS on the proliferation of A549 and YTMLC-90 cell lines. We also focused on the underlying mechanisms of SPS on the cell cycle and apoptosis in A549 and YTMLC-90

cells. We observed that SPS induced NSCLC cell apoptosis by enhancing immunomodulatory activities and blocking the PI3K/Akt signaling pathway. This study provides new insights into the anticancer mechanism of SPS.

Materials and methods

Chemicals and reagents. RPMI-1640 medium, heat-inactivated fetal bovine serum (FBS), penicillin and streptomycin, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). ELISA kits were from Shanghai Enzme-linked Biotechnology Co., Ltd. (Shanghai, China). BALB/c nude mice were from Vital River Laboratory Animal Technology Co. Ltd., Beijing, China. Antibodies to cyclin B1, Cdc25B, PI3K, Akt and p-Akt were purchased from Abcam (Cambridge, MA, USA). The PrimeScript™ RT-PCR kit and SYBR Premix Ex Taq™ kit were obtained from Takara (Takara, Kusatsu, Japan).

Ethical standard. All procedures performed in studies involving animals were in accordance with the ethical standards of the Authors' institution.

Preparation of SPS. SPS (AR, 90%) was purchased from Xi'an Reain Biotechnology Co. Ltd, Xi'an, China. The SPS was dissolved with DMSO to different concentrations: 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, and 2.56 mg/ml.

Cell culture and treatment. Human lung cancer cell lines, A549 and YTMLC-90 were purchased from the Cell Library Committee on Type Culture Collection of the Chinese Academy of Sciences, Beijing. Cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified, 5% CO_2 atmosphere at 37˚C. The cells were plated at a density of 10^5 cells per well and grown for 24 h.

MTT assay. The MTT assay was used to evaluate cell viability. Cells were seeded in 96-well plates at a density of $5x10^3$ cells per well and grown for 24 h. Cells were then treated with various concentrations of SPS ranging from 0.04 to 2.56 mg/ml for 24, 48, and 72 h. Cells treated with an equivalent volume of DMSO were regarded as the control groups. Each group was treated in triplicate. Following treatment, 10 μl MTT was added to each well and incubated for 4 h. Finally, blue formazan crystals of viable cells were solubilized in 100 μl DMSO. The absorbance was measured at 450 nm using a microplate reader.

Flow cytometric analysis of apoptosis. Apoptosis was assessed in A549 and YTMLC-90 cells using an Annexin V-FITC/propidium iodide (PI) staining assay. Cells were cultured in 6-well plates at a density of $2x10^5$/ml per well overnight. Cells were then treated with various concentrations of SPS ranging from 0.04 to 2.56 mg/ml for 48 h. Control cells were treated with culture medium containing DMSO. Each group was treated in triplicate. After treatment, cells were washed with cold PBS and resuspended in binding buffer (100 mM HEPES, pH 7.4, 100 mM NaCl, 25 mM $CaCl_2$). Cells were stained with Annexin V-FITC/PI at 4˚C for 30 min. Apoptotic cells were analyzed using a fluorescence-activated cell-sorting (FACS) flow cytometer.

Real-time PCR. The expression levels of *bax*, caspase-3 and *cdc25B* were measured using real-time PCR. Cells were treated with various concentrations of SPS ranging from 0.04 to 2.56 mg/ml for 48 h. Following treatment, cells were collected and their total RNA extracted using TRIzol reagent. cDNA was synthesized using the PrimeScript RT-PCR kit (Takara). Real-time PCR was performed using SYBR Premix Ex Taq kit (Takara). Primer sequences were as follows: cdc25B: 5'-TTC ATC AGG GAA CGA GA CCG TG-3', 5'-TTC ACA GAA GTT CGG GTG CTG AG-3'; bax: 5'-GGA GCT GCA GAG GAT GAT TG-3', 5'-CCT CCC AGA AAA ATG CCA TA-3'; caspase-3: 5'-ATG GAG AAC ACT GAA AAC TCA G-3', 5'-GAC CGA GAT GTC ATT CCA GTG-3'; GAPDH: 5'-GAA GGT GAA GGT CGG AGT C-3', 5'-GAA GAT GGT GAT GGG ATT TC-3'. The reaction was repeated 3 times and carried out in an ABI7500 Real-time PCR System (Applied Biosystems, Carlsbad, CA, USA). Templates were initially denatured at 95˚C for 5 min followed by 40 cycles at 95˚C for 5 sec and 60˚C for 34 sec. The relative expression levels of tested genes were calculated using the $2^{-\Delta\Delta CT}$ method.

Flow cytometry analysis of the cell cycle. PI staining followed by flow cytometry was used to assess the effect of SPS on the cell cycle of A549 and YTMLC-90 cells. Cells were treated with different concentrations of SPS for 48 h. Cells were then washed with cold PBS with 75% ethanol for 1 h at 4˚C. The protocol was then followed as previously described (18). Cells were suspended in 1 ml of PBS that contained 1 mg/ml RNase and 50 μg/ml PI, followed by 30 min of shaking at 37˚C in the dark. DNA content was detected using a flow cytometer (BD FACSCalibur System, San Jose, CA, USA).

Western blotting. Protein expression levels of cyclin B1, Cdc25B, PI3K, Akt and p-Akt were measured by western blot according to a previous study (19). After treatment, cells were lysed in RIPA buffer. Protein concentration was determined using the Bradford assay (Bio-Rad, Hercules, CA, USA). Twenty micrograms of protein from each sample were separated by 12% SDS-PAGE and transferred to PVDF membranes. Primary antibodies (rabbit monoclonal anti-human cyclin B1, 1:3000 dilution; rabbit polyclonal anti-human Cdc25B, 1:1000 dilution; rabbit monoclonal anti-human Akt, 1:1000 dilution; rabbit monoclonal anti-human p-Akt, 1:1000 dilution) were then applied and incubated at 4˚C overnight, after which the appropriate HRP-conjugated secondary antibody was added and incubated for 1 h at room temperature. Proteins were detected using the ChemiDoc XRS imaging system and analysis software Quantity One (Bio-Rad).

The effect of SPS injection in vivo. To further analyze the effect of SPS injection *in vivo*, BALB/c nude mice (7 weeks old) were purchased from Vital River Laboratory Animal Technology Co. Ltd. Murine tumor models were induced by subcutaneous injection of A549 cells ($5x10^6$ cells in 0.2 ml of PBS) at one site in the right flank, and tumors allowed to develop for 20 days. When tumors reached ~100 mm^3 in volume, 40 animals were divided randomly into 4 groups (n=10 for each group): low dose (15 mg/kg), middle dose (45 mg/kg) and high dose (135 mg/kg) injection groups, and a control group that was treated with an equal volume of normal saline
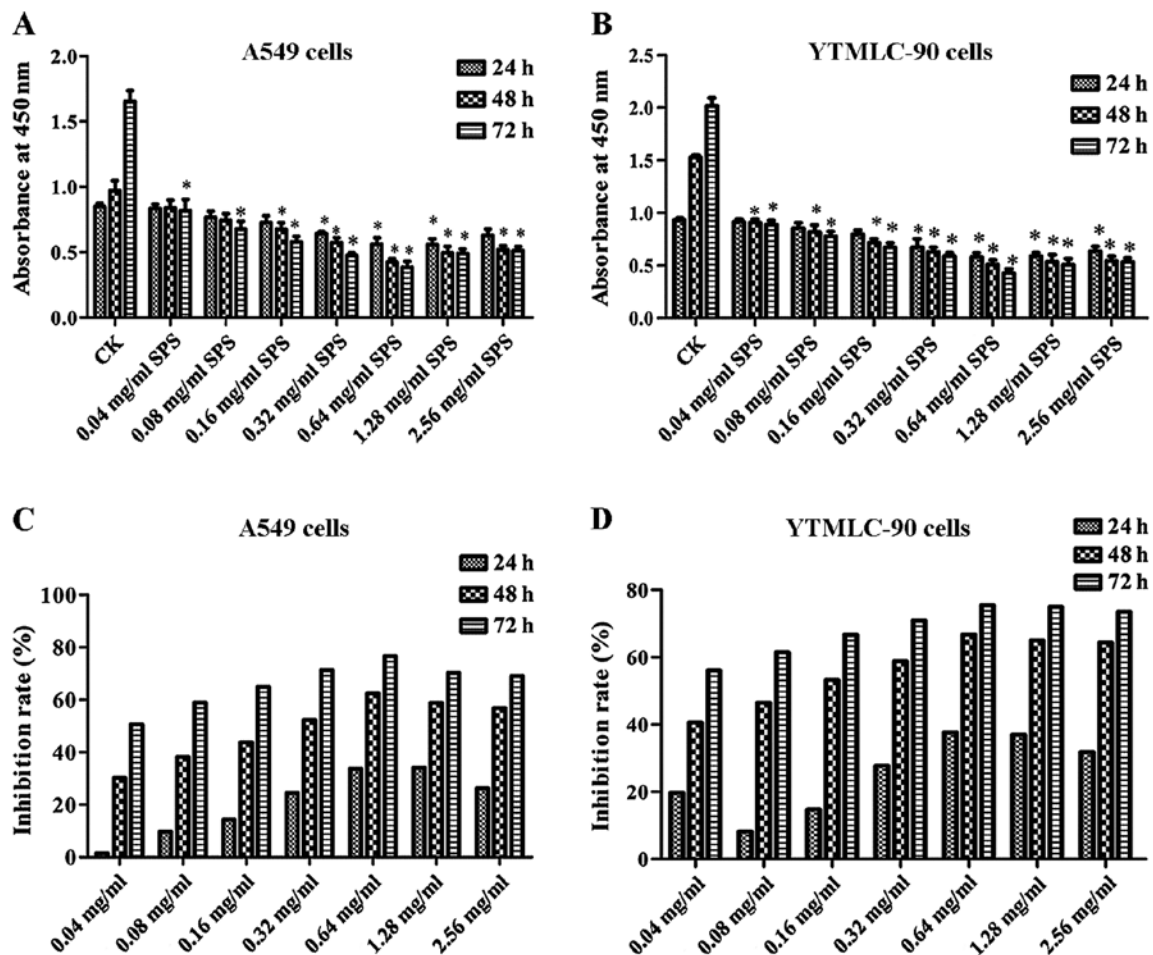
Figure 1. SPS treatment inhibited A549 and YTMLC-90 cell proliferation. Cells were seeded in 96-well plates at a density of 5x10³ cells per well and grown for 24 h. Cells were then treated with various concentrations of SPS ranging from 0.04 to 2.56 mg/ml for 24, 48, and 72 h. Cell viability of A549 (A) and YTMLC-90 (B) cells was measured using the MTT assay. (C) The inhibition rate of A549 cells. (D) The inhibition rate of YTMLC-90 cells. SPS concentrations: 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, and 2.56 mg/ml. *P<0.05 vs. CK group.

(NS). All injections were administered intraperitoneally every day. At 15, 20, 25, and 30 days post-injection, tumor size was measured using calipers and tumor volume was estimated according to a previous study (18). Blood samples were taken from a tail vein to measure the contents of TNF-α and IL-6 using ELISA kits.

Statistical analysis. Data were expressed as mean ± SD and considered significant at P<0.05. Statistical analysis was performed using a Student's unpaired t-test (SPSS release 19.0; SPSS Inc.).

Results

SPS treatment inhibited A549 and YTMLC-90 cell proliferation. To evaluate the effect of SPS on A549 and YTMLC-90 cell proliferation activity *in vitro*, an MTT assay was conducted. Results of the MTT assay are shown in Fig. 1A and B. These results demonstrated that the proliferation of A549 and YTMLC-90 cells was inhibited by different concentrations of SPS. Compared with the CK group, concentrations of 0.04 to 2.56 mg/ml of SPS suppressed the viability of A549 and YTMLC-90 cells, with the exception of 0.04 mg/ml at 24 h (P<0.05). According to Fig. 1C and D, a time-dependent

inhibitory effect of SPS on cell survival was found in A549 and YTMLC-90 cells. At 0.64 mg/ml of SPS, the inhibition rate peaked after 72 h of treatment, being 76.66 and 75.47% in A549 and YTMLC-90 cells, respectively. These results suggested that SPS inhibited A549 and YTMLC-90 cell proliferation.

SPS treatment induced A549 and YTMLC-90 apoptosis. Since a significant decrease in cell viability was found after treatment with SPS, we further tested the effect of SPS treatment on apoptosis. The results of Annexin V-FITC/PI staining showed a dose-dependent effect of SPS on apoptosis in A549 and YTMLC-90 cells (Fig. 2A,B). Compared with CK, apoptosis in A549 and YTMLC-90 cells was significantly increased (P<0.05). Even at 0.64 mg/ml, SPS treatment induced the highest apoptosis rate in both cell lines (P<0.05). When the treatment concentration was >0.64 mg/ml, the level of increase in the apoptosis rate was decreased. To further analyze the mechanism of apoptosis, we also investigated expression levels of *bax* and capase-3. For the two cell lines, expression levels of *bax* and capase-3 were both notably induced after treatment with various concentrations of SPS. The expression level of *bax* peaked at 0.64 mg/ml of SPS treatment, increasing 2.4- and 3.01-fold in A549 and YTMLC-90 cells, respectively, and the transcription level of capase-3 was increased 3.89- and
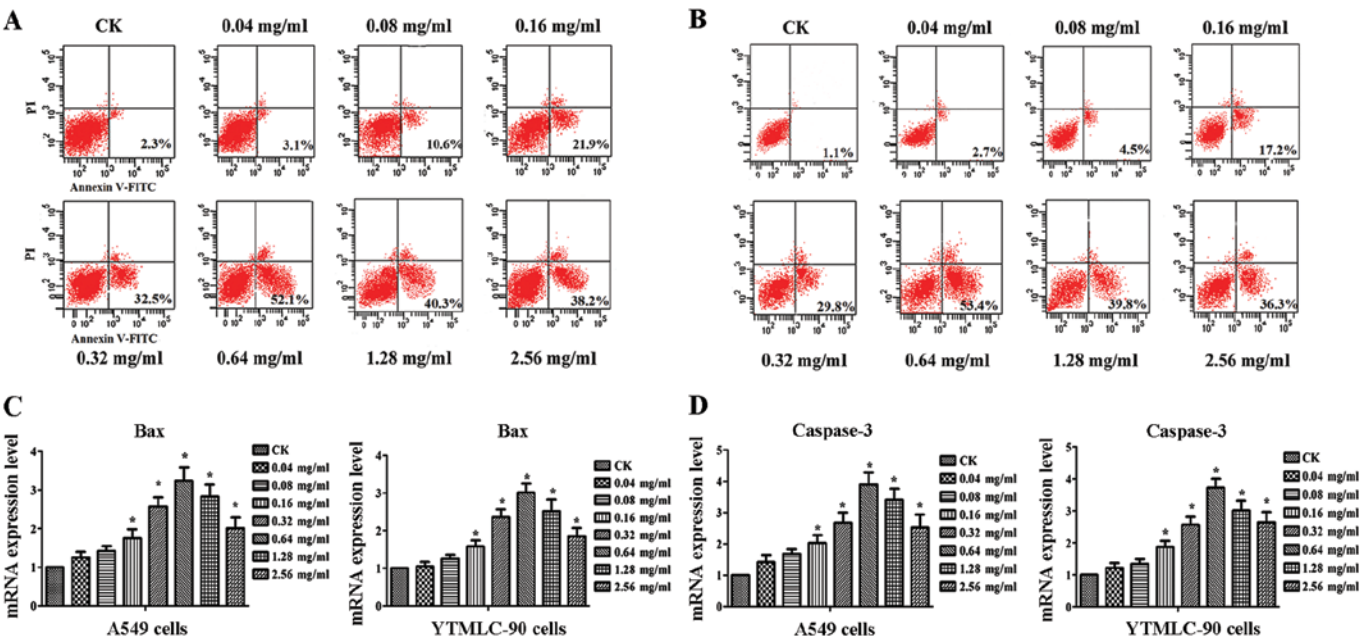
Figure 2. SPS treatment induced apoptosis in A549 and YTMLC-90 cells. Cells were cultured in 6-well plates at a density of 2×10^5/ml per well overnight. Cells were treated with various concentrations of SPS ranging from 0.04-2.56 mg/ml for 48 h. Control cells were treated with culture media containing DMSO. (A and B) Apoptosis in A549 and YTMLC-90 cells after various concentrations of SPS treatment for 48 h and Annexin V-FITC/PI staining assay. (C and D) Transcription levels of *bax* and capase-3 in A549 and YTMLC-90 cells were measured by real-time PCR. SPS concentrations: 0.04, 0.08, 0.16, 0.32, 0.64, 1.28, and 2.56 mg/ml. All data are shown as mean ± SD. *P<0.05 vs. CK group.

Table I. SPS induced A549 cell cycle arrest in G2/M phase.

A549 cells/Groups	Cell cycle distribution (%)		
	G1/G0	S	G2/M
CK	65.7	23.2	11.1
0.04 mg/ml SPS	62.3	24.5	13.2
0.08 mg/ml SPS	59.2	20.9	19.9
0.16 mg/ml SPS	53.2	15.3	31.5
0.32 mg/ml SPS	42.9	12.6	44.5
0.64 mg/ml SPS	29.3	10.4	60.3
1.28 mg/ml SPS	32.6	12.7	54.7
2.56 mg/ml SPS	39.8	16.9	43.3

Table II. SPS induced YTMLC-90 cell cycle arrest in G2/M phase.

YTMLC-90 cells/Groups	Cell cycle distribution (%)		
	G1/G0	S	G2/M
CK	68.2	27.6	4.2
0.04 mg/ml SPS	65.9	25.7	8.4
0.08 mg/ml SPS	60.3	20.9	18.8
0.16 mg/ml SPS	53.6	15.7	30.7
0.32 mg/ml SPS	42.1	12.3	45.6
0.64 mg/ml SPS	28.9	9.3	61.8
1.28 mg/ml SPS	34.6	14.9	50.5
2.56 mg/ml SPS	40.2	18.3	41.5

3.72-fold, respectively (Fig. 2C and D). This result was consistent with the apoptosis rate. In brief, SPS induced apoptosis in A549 and YTMLC-90 cells.

SPS treatment induced A549 and YTMLC-90 cell cycle arrest in the G2/M phase. To further analyze the effect of SPS treatment on the cell cycle, we examined both mRNA and protein expression levels of cdc25B and cyclin B1 (Fig. 3). The results of real-time PCR showed that after treatment with SPS, the expression levels of cdc25B and cyclin B1 were both decreased with increasing concentration of SPS when compared with the CK group in A549 and YTMLC-90 cells, reaching their lowest levels at 0.64 mg/ml SPS (Fig. 3A and C). Western blot results also confirmed that the activity of cdc25B and cyclin B1 were both decreased, especially after treatment with 0.64 mg/ml of SPS in A549 or TYMLC-90 cells (Fig. 3B and D). As shown in

Table I and Table II, when cells were treated with SPS for 48 h, their DNA contents were significantly increased in the G2/M phase. In the 0.64 mg/ml SPS treatment group, DNA content was markedly increased from 11.1 to 60.3% in A549 cells, and from 4.2 to 61.8% in YTMLC-90 cells. In contrast, the G0/G1 phase population in the SPS treatment group decreased from 65.7 to 29.3% in A549 cells, and from 68.2 to 28.9% in YTMLC-90 cells. These results suggested that SPS induced cell cycle arrest in the G2/M phase.

SPS treatment inhibited the Akt pathway. To investigate whether the inhibitory effect of SPS treatment on NSCLC cell proliferation was mediated by the PI3K/Akt pathway, we analyzed protein expression levels of the PI3K/Akt pathway. In these experiments, cells were treated with SPS (0.04, 0.64,
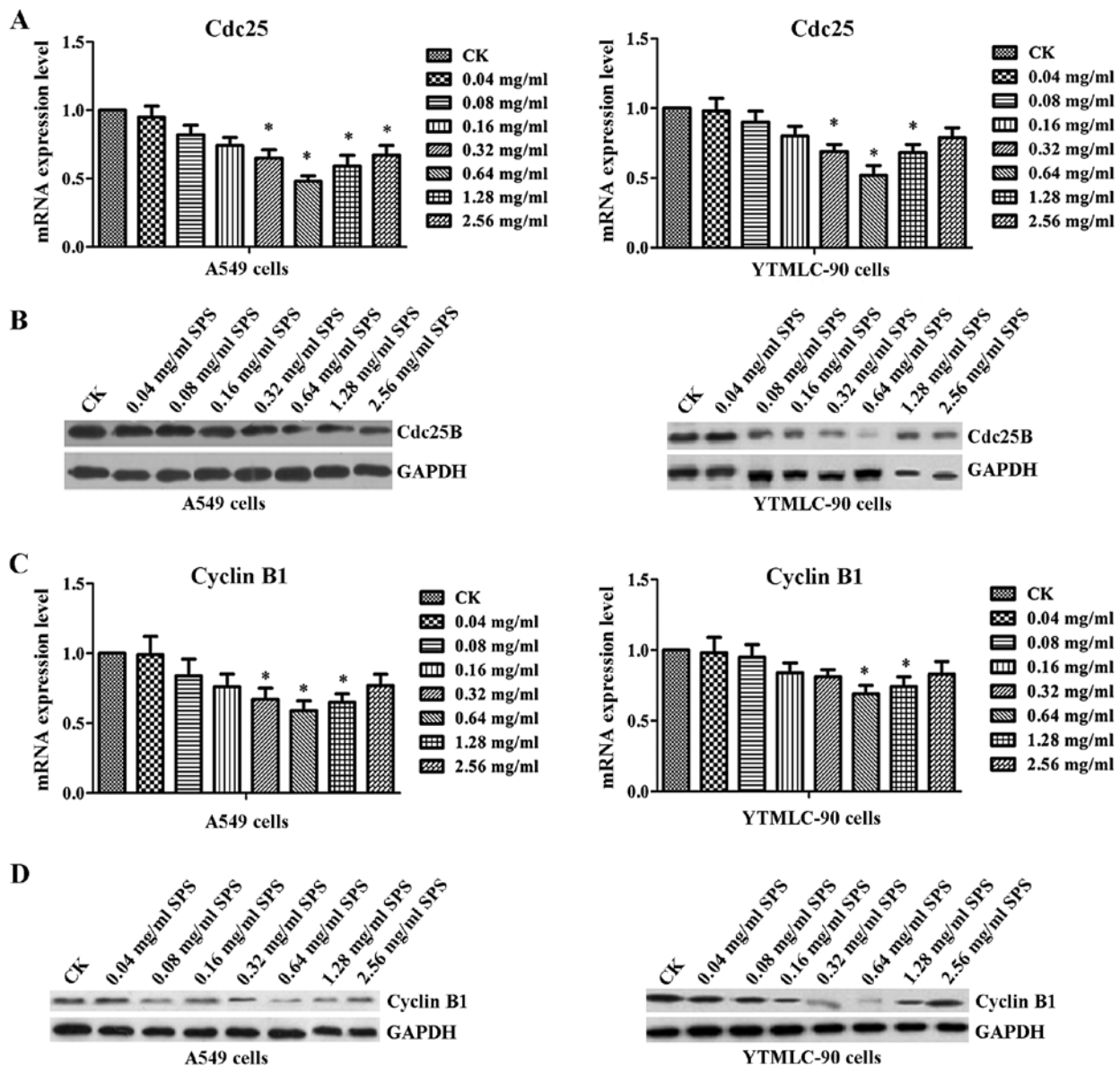
Figure 3. SPS treatment induced A549 and YTMLC-90 cell cycle arrest in the G2/M phase. Cells were then treated with various concentrations of SPS ranging from 0.04 to 2.56 mg/ml for 48 h. Flow cytometry was used to assess the effect of SPS on the cell cycle in A549 and YTMLC-90 cells. (A) The expression level of *cdc25B* in A549 and YTMLC-90 was measured by real-time PCR. (B) The protein expression level of cdc25B in A549 and YTMLC-90 cells was measured by western blotting. (C) The expression level of cyclin B1 in A549 and YTMLC-90 cells was measured by real-time PCR. (D) The protein expression level of cyclin B1 in A549 and YTMLC-90 cells was measured by western blotting. SPS concentrations: 0.04, 0.08, 0.16, 0.32, 0.64 and 2.56 mg/ml. *$P<0.05$ vs. CK group.

and 2.56 mg/ml) for 48 h. As seen in Fig. 4, we found that compared with the CK group, 0.04 mg/ml of SPS treatment had no effect on the expression of Akt, p-Akt, and PI3K, whereas 0.64 mg/ml of SPS treatment markedly decreased the expression of Akt, p-Akt and PI3K; 2.56 mg/ml of SPS treatment also decreased the expression of these four proteins, but the decreased level was lower than in the 0.64 mg/ml group. This result suggested that SPS inhibited NSCLC cell proliferation by decreasing protein expression levels of the PI3K/Akt pathway.

SPS injection inhibited tumor growth and improved immunomodulatory activities in BALB/c nude mice. BALB/c nude mice were used to determine the anti-lung cancer effect of SPS *in vivo*. Mice were injected with different concentrations of SPS (15, 45, and 135 mg/kg). As shown in Fig. 5A,

compared with the control group, SPS injection significantly decreased tumor volume ($P<0.05$). SPS injection of 45 mg/kg and 135 mg/kg dramatically suppressed tumor growth, with the inhibition rate reaching 75 and 65% 25 days after injection (Fig. 5B). This result indicated that SPS inhibited tumor growth in mice. TNF-α and IL-6 levels were measured to evaluate the immunomodulatory activities of SPS injection in mice. According to Fig. 5C and D, SPS significantly increased TNF-α and IL-6 levels when compared with the control group ($P<0.05$). For instance, 30 days after injection of SPS (45 mg/kg) in mice, TNF-α was increased by 33.55%, relative to the control group. The level of IL-6 in the 45 mg/kg SPS injection group was increased by 39.50% relative to the control group. These results indicated that SPS injection increased immunomodulatory activities in mice *in vivo*, suggesting a potential antitumor application in tumor-bearing mice.
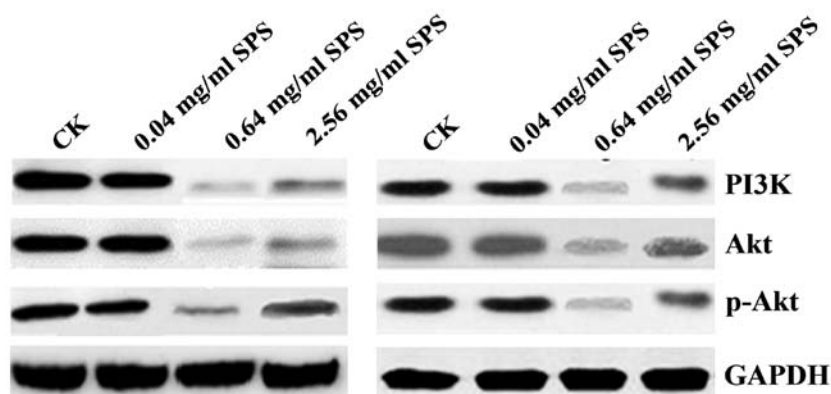
Figure 4. SPS treatment decreased expression levels of PI3K/Akt pathway-related proteins in A549 and YTMLC-90 cells. After A549 and YTMLC-90 cells were treated with SPS (0.04, 0.64, and 2.56 mg/ml) for 48 h, expression levels of PI3K, Akt, and p-Akt were measured by western blotting.
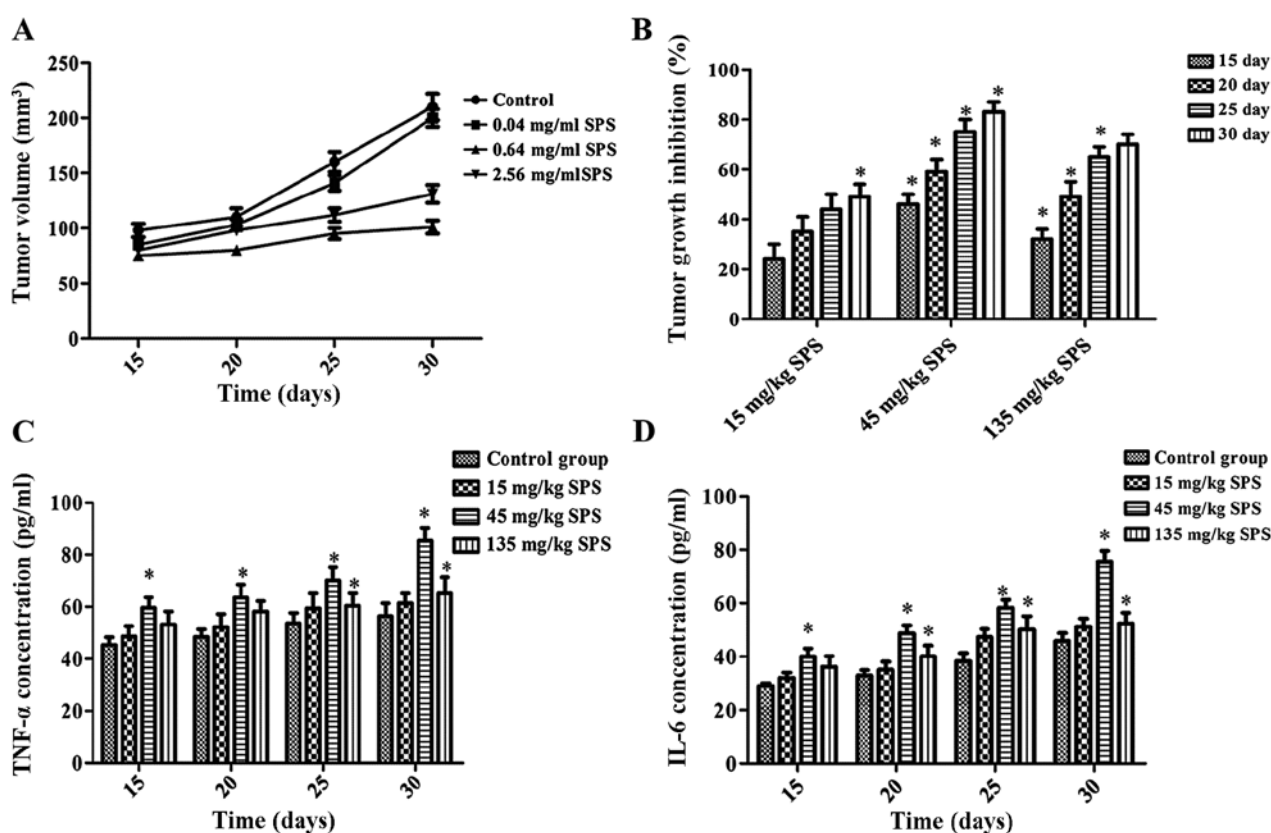

Figure 5. SPS treatment increased immunomodulatory activities in tumor bearing mice. Murine tumor models were induced by subcutaneous injection of A549 cells (5x10^6 cells in 0.2 ml of PBS) at one site in the right flank. When the tumor reached ~100 mm^3 in volume, 40 animals were divided randomly into 4 groups (n=10 for each group): low (15 mg/kg), middle (45 mg/kg) and high (135 mg/kg) dose groups, and a control group that was treated with an equal volume of normal saline (NS). All injections were administered intraperitoneally every day. (A) Tumor volume was measured 15, 20, 25, and 30 days after SPS injection. (B) The inhibition rate of tumor growth. (C) TNF-α concentration (pg/ml) was assayed by ELISA 15, 20, 25, and 30 days after SPS injection. (D) IL-6 concentration (pg/ml) was assayed by ELISA 15, 20, 25, and 30 days after SPS injection. SPS concentrations: 15, 45, and 135 mg/kg. All data are expressed as mean ± SD (n=10). *P<0.05 vs. CK group.

Discussion

Safflower is a herb medicine famous for its ability to improve blood circulation and blood stasis and relieve pain. So far, the anticancer effects of safflower have been focused on safflower yellow, which is a kind of flavonoids (11,20). The antitumor effect of SPS is rarely reported, apart from one study indicating that SPS inhibited the proliferation and metastasis of

MCF-7 breast cancer cells (16). In this study, the effect of SPS in human NSCLC was investigated. Results of the MTT assay suggested that SPS suppressed the proliferation of A549 and YTMLC-90 cells and exhibited a dose-dependent effect; in particular, the inhibition rate in the 0.64 mg/ml SPS group reached 76.66 and 75.47% in A549 and YTMLC-90 cells, respectively. Results of the apoptosis assay also showed that SPS induced cell apoptosis and exhibited a dose-dependent

effect. Moreover, expression levels of *bax* and capase-3 were increased after treatment with SPS. In recent years, more and more plant polysaccharides have been used in antitumor studies. For example, cactus polysaccharides induced growth arrest and apoptosis in lung squamous carcinoma cells (21). *Pleurotus nebrodensis* polysaccharide induced apoptosis in A549 cells (22). *Scutellaria Barbata* D. polysaccharides showed anti-tumor growth activity on human lung cancer 95-D (23). In our study, SPS induced apoptosis in NSCLC cells and increased expression levels of *bax* and capase-3.

Cell viability and apoptosis were dose- and time-dependent within the range of 0.04 to 0.64 mg/ml of SPS treatment. When the dose of SPS was higher than 0.64 mg/ml, the rate of increase declined. It is well known that Na^+/K^+-ATPase activity is an important indicator of erythrocyte viability and is downregulated in tumor cells (24). For example, *L. barbarum* polysaccharides prevented the development of cardiovascular disease by increasing the activity of Na^+-K^+-ATPase in heart ischemia reperfusion (IR) in rats (25). Sargassum fusiforme polysaccharides (SFPS) could restore some biochemical functions of erythrocyte membranes in S_{180} mice by increasing Na^+-K^+-ATPase activity (26). SPS may play a similar role to that of *L. barbarum* polysaccharides and SFPS. Na^+-K^+-ATPase activity may be increased when cells are treated with various concentrations of SPS within the range of 0.04 to 0.64 mg/ml, but when the concentration is greater than 0.64 mg/ml, Na^+-K^+-ATPase activity may be decreased. Na^+-K^+-ATPase is very sensitive and regulated in a dose-dependent manner by some drugs, such as ouabain. In this study, SPS within a certain range of concentrations may also have influenced Na^+-K^+-ATPase activity in a dose-dependent manner.

SPS induced A549 and YTMLC-90 cell cycle arrest at the G2/M phase. Cyclin B1 and cdc25B are upregulated in tumor cells and are crucial for the cell cycle. In recent years, many studies have demonstrated that plant polysaccharides induced cell cycle arrest at the G2/M phase by regulating cell cycle-related protein expression. Polysaccharides from Masson pine pollen induced cell cycle arrest at the G2/M phase by downregulating expression levels of CDK1 and Cyclin B (27). Wolfberry *(Lycium barbarum)* polysaccharide induced cell cycle arrest at the G0/G1 phase and the expression of cyclins and CDKs was consistent with changes in cell cycle distribution (28). In this study, we also found that both transcription and protein levels of cdc25B and cyclin B1 were decreased with increasing concentration of SPS treatment. This result indicated that SPS induced cell cycle arrest at the G2/M phase by downregulating expression levels of cdc25B and cyclin B1.

SPS inhibited the Akt pathway. SPS treatment at 0.64 mg/ml markedly decreased the expression of Akt, p-Akt and PI3K. Inhibition of the PI3K/Akt pathway prevented tumor cell proliferation. Such as *Astragalus* polysaccharide could ameliorate doxorubicin-mediated cardiotoxicity via regulation of the PI3k/Akt pathway (29). *Glycyrrhiza* polysaccharide induced apoptosis and inhibited proliferation in human hepatocellular carcinoma cells by blocking the PI3K/Akt pathway (30). Our study also showed that SPS suppressed the PI3K/Akt pathway by decreasing expression levels of PI3K, Akt and p-Akt. To summarize, SPS inhibited NSCLC cell proliferation and induced apoptosis by suppressing the PI3K/Akt pathway.

SPS increased immunomodulatory activities by raising the levels of TNF-α and IL-6. The improvement of immunomodulatory activities in cancer by medicinal plant polysaccharides has been widely investigated. For example, a water-soluble polysaccharide from *Chaenomeles speciosa* increased antitumor and immunomodulatory activities in tumor-bearing mice (31). Polysaccharides from *Cymbopogon citratus* increased immunomodulatory activities by raising levels of TNF-α, IL-2, and IL-6 in transplanted S180 tumors (32). Polysaccharide fractions from safflower petals stimulated the production of IL-1, IL-6 and TNF-α and increased immunomodulatory activity (33). Our results also showed that SPS significantly increased levels of TNF-α and IL-6. We posit that the antitumor activity of SPS may be due to its positive influence on TNF-α and IL-6 expression.

Taken together, our results suggest that, on the one hand, SPS induces cell cycle arrest at the G2/M phase by decreasing expression levels of cyclin B1 and cdc25B. On the other hand, SPS induces A549 and YTMLC-90 apoptosis by decreasing expression levels of capase-3 and *bax*. The underlying mechanism of apoptosis may involve blocking the PI3K/Akt pathway or increasing immunomodulatory activities. Therefore, SPS may have therapeutic implications for the clinical management of lung cancer.

Acknowledgements

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