



Secreted Phosphoprotein 1 Promotes the Development of Small Cell Lung Cancer Cells by Inhibiting Autophagy and Apoptosis

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Abstract

This study aimed to investigate the expression of secreted phosphoprotein 1 (*SPP1*) on lung cancer cells and explore its underlying mechanism on autophagy and apoptosis which effect the development of lung cancer cells. GSE19804 related to lung cancer cells was screened from Gene Expression Omnibus (GEO) database, and we screened the 47 pairs of differential expressed mRNAs in lung cancer cells and adjacent tissues using microarray analysis. The expression of the core gene *SPP1* was detected by qRT-PCR and western-blot. The transfection efficiency of lung cancer cells was detected by qRT-PCR and the expression of transfected group was tested by western-blot. Cell proliferation after transfection was tested by MTT assay and plate cloning experiment. The apoptosis rate of each transfection group was detected by flow cytometry. We use western-blot to test protein expression of autophagy-related proteins Beclin-1, LC3-I, LC3-II and p62 of each transfected group. Through analysis of GSE19804, the heat map showed *SPP1* was the highest expressed in tumor tissues. qRT-PCR and western-blot detected *SPP1* expression in lung cancer tissues was higher than that in normal adjacent tissues and was significantly increased in lung cancer cell lines. After transfection with pcDNA3.1-*SPP1* (p-*SPP1* group), siRNA1-*SPP1* (siRNA1 group) and siRNA2-*SPP1* (siRNA2 group), showed different expression of *SPP1*. Up-regulation of *SPP1* enhanced cell viability and promoted tumor cell proliferation, while knockdown of *SPP1* inhibited tumor cell proliferation. From the results of apoptosis rate, *SPP1* inhibited the tumor cell apoptosis. However, in normal lung cell, *SPP1* had no effect on cell proliferation and apoptosis. And to test autophagy-related proteins, we found that overexpression of *SPP1* inhibited autophagy. High expression of *SPP1* inhibited autophagy and apoptosis to promote the development of small cell lung cancer cells.

Keywords *SPP1* · Small cell lung cancer cells · Autophagy · Apoptosis

Introduction

Among all the cancers, lung cancer remains the highest death rate, accounting for about one-quarter (27%) of all cancer deaths [1]. To date, lung cancer still represents the leading cause of cancer-related deaths worldwide and non-small cell

lung cancer (NSCLC) comprises about 85% of all types of lung cancer [2], with only a small number of patients achieving long-term survival. The specific mechanisms underlying lung cancer development and progression are still unclear. Although cigarette smoking and alcohol consumption are known to directly induce lung cancer, various other environmental risks, such as exposure to second-hand smoke, air pollution, and genetic susceptibility, are also involved in the development of lung cancer [3]. Indeed, multiple genetic and epigenetic changes have been shown to be associated with lung cancer development [4].

Autophagy has been known as a cellular catabolic pathway responsible for the modulation of numerous pathophysiological processes. Autophagy induction in response to stressful stimuli is involved in adaptation of the cells to various stressful conditions, such as shortage of nutrients and oxygen [5], or it can also cause dysregulated cell death [6]. Cancer cells in the tumor tissue are typical example exposed to the stressful

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conditions and growing evidences have suggested the critical role of autophagy in the regulation and/or progression of cancer. The role of autophagy in cancer progression is controversial. For example, cancer cells activate autophagy in response to stressful insults, allowing cells to mitigate damage and promote senescence that limit tumorigenesis, indicating a regulatory role of autophagy in cancer development [7]. However, many other recent studies have indicated autophagy as a cytoprotective adaptive response. Activation of oncogenes and inactivation of tumor suppressor genes in cancer cells enhance autophagy, thereby promoting survival of tumor cells. Furthermore, inhibition of autophagy by pharmacological (e.g. chloroquine or 3-methyladenine) or genetic interference (e.g., knocking down of essential proteins) accelerates cell death in conditions exposed to the cellular stress [8]. In addition, autophagy and apoptosis mutually negatively regulate each other. Autophagic process therefore contributes to the development of cancer [6, 9]. All these reports indicate that autophagy modulates cancer progression in a context-dependent manner.

In this study, the core gene *SPP1* was screened for verifying its effect on the mechanism of lung cancer cells development. It was speculated that *SPP1* might be used as a target of lung cancer cells after verifying the role of *SPP1* in the occurrence and development of lung cancer cells for non-smoking female patients with early lung cancer cells and observing the correlation between *SPP1* and autophagy and apoptosis. The proposition of this speculation based on small cell lung cancer cells was of great significance for patients.

Materials and Methods

Gene Expression Omnibus (GEO) Microarray Data Processing

With “lung cancer cell” as keywords, 17,482 gene expression data were obtained from GEO database (<http://www.ncbi.nlm.nih.gov/geo>), and “expression profiling by array” was selected. “GSE19804” microarray data set was screened, and the platform was GPL570: [HG-U133_Plus_2] Affymetrix Human Genome U133 Plus 2.0 Array. The included samples were 47 cases of stage I to II cancer tissues and paired adjacent tissues respectively. Differentially expressed miRNAs between normal and tumor were screened by SAMR (significance analysis of microarrays) package in R software, under the condition of \log_2FC (fold change) was over 2 and FDR (false discovery rate) less than 0.05.

Clinical Sample Collection

107 paired human lung cancer tissues and adjacent tissues were all obtained from patients with informed consent during

Sep. 2016 to Dec. 2017. The protocols used in the study had been approved by the Ethic Committee of Qilu Hospital of Shandong University.

Cell Culture and Treatment

Lung cancer cell lines A549, H1975, NCI-H446, SK-MES-1 and human bronchial epithelial cells (BEAS-2B cells) were purchased from the American Type Culture Collection (ATCC, Philadelphia, PA, USA). Lung cancer cells were grown in RPMI 1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA) and 1% penicillin-streptomycin (Invitrogen, Carlsbad, CA, USA). BEAS-2B cells were grown in DMEM (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS, 100 U/mL penicillin and 100 U/mL streptomycin (Invitrogen, Carlsbad, CA, USA). All cells were cultured in a humidified CO₂ incubator at 37 °C.

Cell Transfection

pcDNA3.1 plasmid vectors, pcDNA3.1-*SPP1*, siRNA negative control, siRNA1-*SPP1* and siRNA2-*SPP1* were bought from GenePharma (Shanghai, China). The siRNA sequence of *SPP1* listed in Table 1. NCI-H446 cells were seeded at 10⁵ cells each well into 24-well plates in logarithmic growth phase and grown to 80% confluence. Cells were transfected with corresponding plasmids using the Lipofectamine 2000 (Life Technologies, Gaithersburg, MD, USA) and Opti-MEM serum-free medium (Invitrogen, Carlsbad, CA, USA) on the basis of the manufacturer’s recommendations. qRT-PCR was used for determining the transfection efficiency 48 h post transfection.

qRT-PCR

Initially, RNA was completely extracted using Trizol reagent (Beyotime, Shanghai, China) on the basis of the manufacturer’s guidelines. PrimeScript™ RT reagent kit (Takara, Tokyo, Japan) was used to oppositely transfer RNA into cDNA. qPCR reaction was accomplished using SYBRH Premix Ex Taq™ GC (Takara) and an Applied

Table 1 siRNA sequence of *SPP1*

Gene	Sequence
siRNA1	5'-CCGAGGTGATAGTGTGGTT-3'
siNC-1	5'-CCGAGTGTGATGTGGAGTT-3'
siRNA2	5'-GCTACAGACGAGGACATCA-3'
siNC-2	5'-GCTAGACGAGGACAACACTCA-3'

Biosystems 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). β -actin mRNA was used for *SPP1*'s internal control and the process was done thrice. Samples were standardized to internal controls. And fold changes were obtained through the $2^{-\Delta\Delta CT}$ method.

Western Blot

Briefly, phosphate-buffered saline (PBS) were taken to wash cells and then cell were fostered with lysis buffer (Sangon Biotech, Co., Ltd., Shanghai, China). Protein was parted by SDS-PAGE and shifted to a PVDF membrane. Non-specific binding sites were blocked with TBST which contains 5% (w/v) non-fat dried milk. Next, the membrane was fostered with rabbit anti-*SPP1* (Abcam, 1:200), rabbit anti-Beclin-1 (Abcam, 1:100), rabbit anti-LC3-I (Abcam, 1:200), rabbit anti-LC3-II (Abcam, 1:200), rabbit anti-*p62* (Abcam, 1:200), rabbit anti- β -actin (Abcam, 1:200) at 4 °C all night long. Next, membranes were incubated overnight with a HRP-conjugated goat anti-rabbit IgG (1:10000, Abcam) for 1 h at 37 °C. Signals were observed with ECL chemiluminescence detection kit and semi-quantitated using ImageJ software. Equal protein loading was analyzed by β -actin.

MTT Assay

NCI-H446 cells were plated in 96-well plates. After transfection for 48 h, the culture medium was discarded and 20 μ l (5 mg/ml) 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) solution was added to each well. After culturing for 4 h at 37 °C, 100 μ l dimethylsulfoxide (DMSO) was added to terminate the reaction. The absorbance at 490 nm was measured using a microplate reader.

Annexin V-FITC / PI Double Staining Flow Cytometry

The experiment was carried out according to the Apoptosis Detection Kit (Invitrogen, # BMS500FI-100). 48 h after transfection, cells were digested with trypsin without EDTA, and the cells were centrifuged at 1000 rpm and centrifuged for 5 min. Cells were washed with cold PBS twice, then centrifuged them at 1000 rpm for 5 min. 5 μ l of Annexin V-FITC dye was added into 200 μ l of Binding Buffer. Then they were mixed well, and reacted in room temperature and dark for 15 min. 5 μ l of PI dye was added for reaction. Flow cytometry was performed within 1 h and Cell Quest software was used for analysis.

Statistical Analysis

Data were represented with the mean \pm SD and analyzed by GraphPad Prism 6.0 software (GraphPad Software, La Jolla, CA, USA). Two groups were compared by Student's *t* test. Multiple groups were compared by one-way ANOVA analysis. All experiments were independently performed at least three times. *P* value less than 0.05 was considered statistically significant.

Results

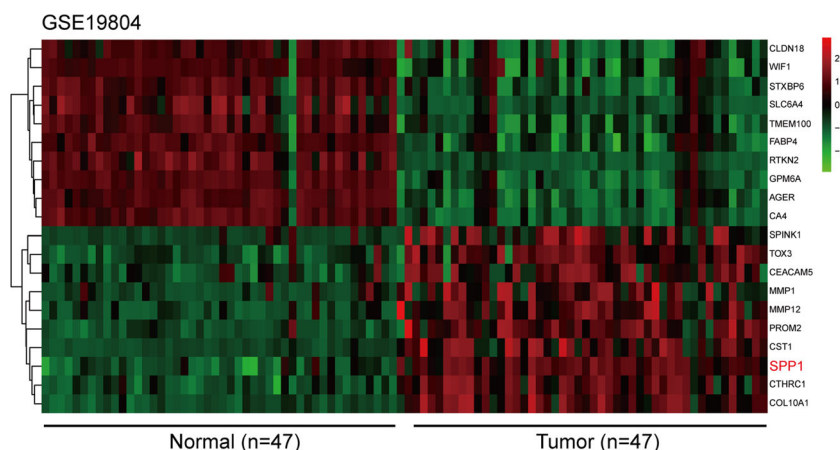
Analysis of GEO19804

We selected 47 cases of stage I to II cancer tissues and paired adjacent tissues as objects of the study from 120 samples obtained from GSE19804 of NCBI. Differentially expressed mRNAs were screened by SAMR (significance analysis of microarrays) package in R software, under the condition of \log_2FC (fold change) was over 2 and FDR (false discovery rate) less than 0.05. In the above conditions, the top 10 mRNA levels were selected and obviously distributed in heat map (Fig. 1). And we found that *SPP1* was the highest expressed mRNA in top 10 mRNA. Based on previous studies, *SPP1* had showed something to do with tumor growth. And the results we got in heat map hinted that *SPP1* were in close contact with tumor growth. So, we chose *SPP1* as our research gene in the following experiments.

SPP1 Was Observably High Expressed in Lung Cancer Cell Lines and Tissues

Total RNA was extracted from lung cancer and adjacent tissues of 107 patients, of whom the clinical characteristics were listed in Table 2. Expression of *SPP1* was evidently increased in 107 lung cancer tissues, compared with adjacent tissues detected by qRT-PCR ($P < 0.01$, Fig. 2a). Besides, protein expression of *SPP1* was remarkably high in 107 lung cancer tissues compared with adjacent tissues detected by qRT-PCR ($P < 0.01$, Fig. 2b) And taking many factors into consideration, we randomly chose 10 samples to do western-blot (Fig. 2c), using β -actin as internal control (Fig. 2c). The correlation between *SPP1* expression and clinic-pathological features in lung cancer was listed in Table 3. There was no significant association between *SPP1* level and age, gender or tumor size, while patients with high expression of *SPP1* are more potential to get an advanced clinical stage ($P < 0.05$). After transfection with *SPP1*, we could see different degree expression of *SPP1* in different cell lines. The expression levels of *SPP1* was remarkably high in lung carcinoma cell lines (A549, H1975, NCI-H446 and SK-MES-1 cells) compared with

Fig. 1 Analysis of GSE19804. There were a total of 120 samples in the GSE19804, the 47 cases of stage I to II cancer tissues and paired adjacent tissues were selected as the research object. Differentially expressed mRNA was screened with the Fold change value greater than 2 and $P < 0.05$ as screening conditions. The cluster heat map showed the top 10 mRNA levels and found that *SPP1* was the highest expressed in cancer tissues



human normal lung epithelial cells (BEAS-2B cell) (Fig. 2d) and found that *SPP1* expression was the highest in NCI-H446 cells in four cancer cells. So, we chose NCI-H446 cells as our experimental subjects in the following experiments to explore the way *SPP1* affect the oncogenesis.

***SPP1* Promotes Small Cell Lung Cancer Cell Proliferation**

NCI-H446 lung cancer cells were transfected with negative control (NC group), pcDNA3.1-*SPP1* (p-*SPP1* group), siRNA1-*SPP1* (siRNA1 group) and siRNA2-*SPP1* (siRNA2 group). Through establishing different grade of *SPP1* expression group, we measured *SPP1*'s impact on lung cancer cell proliferation. The relative expression of *SPP1* was measured by qRT-PCR as well as western blot. The expression of *SPP1* mRNA and protein was obviously down-regulated by siRNA1-*SPP1* and siRNA2-*SPP1* in lung cancer cells, whereas the expression of *SPP1* was significantly facilitated in pcDNA3.1-*SPP1* group ($P < 0.01$, Fig. 3a, b). *SPP1* in pcDNA3.1-*SPP1* group was overexpressed, and was inhibited in siRNA1-*SPP1* and siRNA2-*SPP1* group. MTT assay was performed in order to investigate the effect of *SPP1* on lung cancer cell growth. The result showed that the capacity of cell proliferation with *SPP1* overexpression was obviously higher

than the control group in lung cancer cell line ($P < 0.01$), which suggesting that *SPP1* overexpression could promote cell growth to a certain extent (Fig. 3c). And the low expression of *SPP1* in siRNA1-*SPP1* and siRNA2-*SPP1* group showed that the capacity of cell proliferation was inhibited, compared with NC group. Plate clone assay revealed that the number of cell proliferation was high in p-*SPP1* group, while the number of cell proliferation was decreased in siRNA1 group and siRNA2 group (Fig. 3d, e). Both experiment consistently suggested that *SPP1* overexpression would promoted the cell proliferation in lung cancer cells.

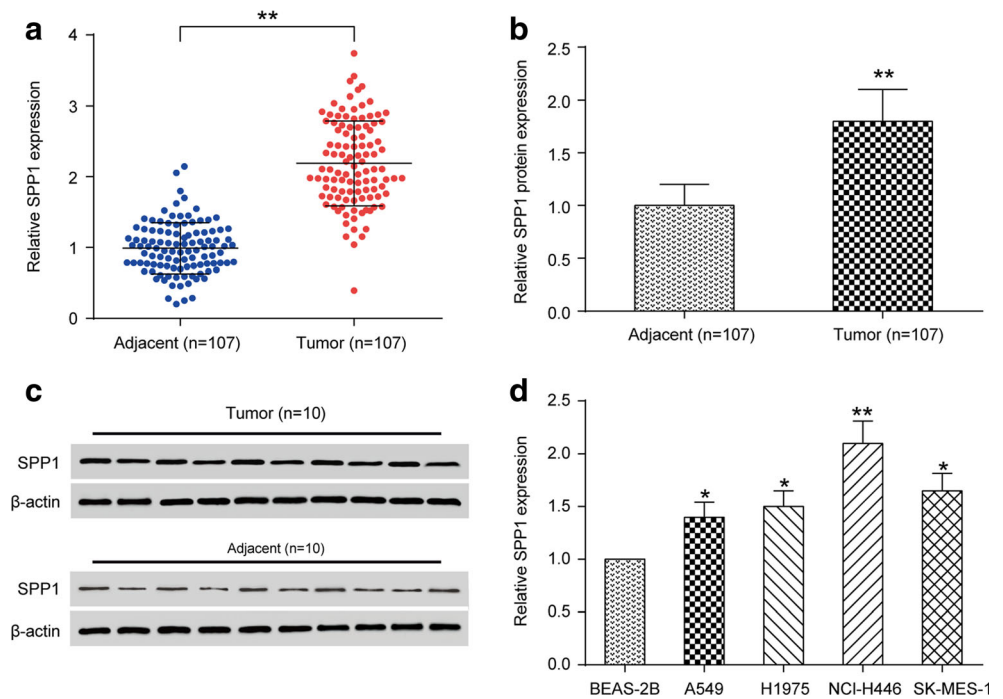
***SPP1* Had no Effect on Bronchial Epithelial Cell Proliferation**

BEAS-2B cells were transfected with negative control (NC group), pcDNA3.1-*SPP1* (p-*SPP1* group), siRNA1-*SPP1* (siRNA1 group) and siRNA2-*SPP1* (siRNA2 group) to measure *SPP1*'s impact on normal cell proliferation. The relative expression of *SPP1* was measured by qRT-PCR and western blot. The expression of *SPP1* mRNA and protein was obviously down-regulated by siRNA1-*SPP1* and siRNA2-*SPP1* in human bronchial epithelial cells, whereas the expression of *SPP1* was significantly facilitated in pcDNA3.1-*SPP1* group ($P < 0.01$, Fig. 4a, b). *SPP1* in pcDNA3.1-*SPP1* group was overexpressed and was inhibited in siRNA1-*SPP1* and siRNA2-*SPP1* group. MTT assay was carried out to explore the effect of *SPP1* on bronchial epithelial cell growth. The result showed that the proliferation capacity of cell with *SPP1* overexpression had no statistic difference compared with the control group ($P < 0.01$, Fig. 4c). And the low expression of *SPP1* in siRNA1-*SPP1* and siRNA2-*SPP1* group showed the similar result compared with NC group. Plate clone assay and flow cytometry revealed that the number of cell proliferation and apoptosis rate among p-*SPP1* group, siRNA1 group and siRNA2 group had no statistic difference (Fig. 4d, e).

Table 2 qRT-PCR primer sequences

Primer	Sequence (5'-3')
SPP1	
Forward primer	TGTTGGTGGAGGATGTCTGC
Reverse primer	TGCCTAGGAGGCAAAGCAA
β-actin	
Forward primer	GGACTTCGAGCAAGAGATGG
Reverse primer	AGCACTGTGTTGGCGTACAG

Fig. 2 *SPP1* was highly expressed in lung cancer tissues. **a** *SPP1* was in a higher expression level in lung cancer tissues by qRT-PCR. $**P < 0.01$, compared with adjacent tissues. **b**、**c** Western blot results revealed that *SPP1* was increased in lung cancer tissues. $**P < 0.01$, compared with adjacent tissues. **d** Expression levels of *SPP1* in lung carcinoma cell lines (A549, H1975, NCI-H446 and SK-MES-1 cells) and human normal lung epithelial cells (BEAS-2B cell). $*P < 0.05$, $**P < 0.01$ compared with BEAS-2B group



SPP1 Promotes Apoptosis and Autophagy

Another way involve to the cancer cell growth was the death of cancer cells. As is known to us all, apoptosis and autophagy are the two maintain ways regulate cell death. The apoptosis rate of p-*SPP1* group was down-regulated, while the apoptosis rate of siRNA1 group and siRNA2 group was up-regulated

detected by flow cytometry (Fig. 5a). Down-regulate of apoptosis rate when *SPP1* was overexpressed could prove that *SPP1* inhibited cancer cell apoptosis, which means decreased the death of cancer cells. When it comes to another way, western blot was used to detect the expression of autophagy-related proteins Beclin-1, LC3-I, LC3-II and p62 in each transfection group to explain the autophagy level.

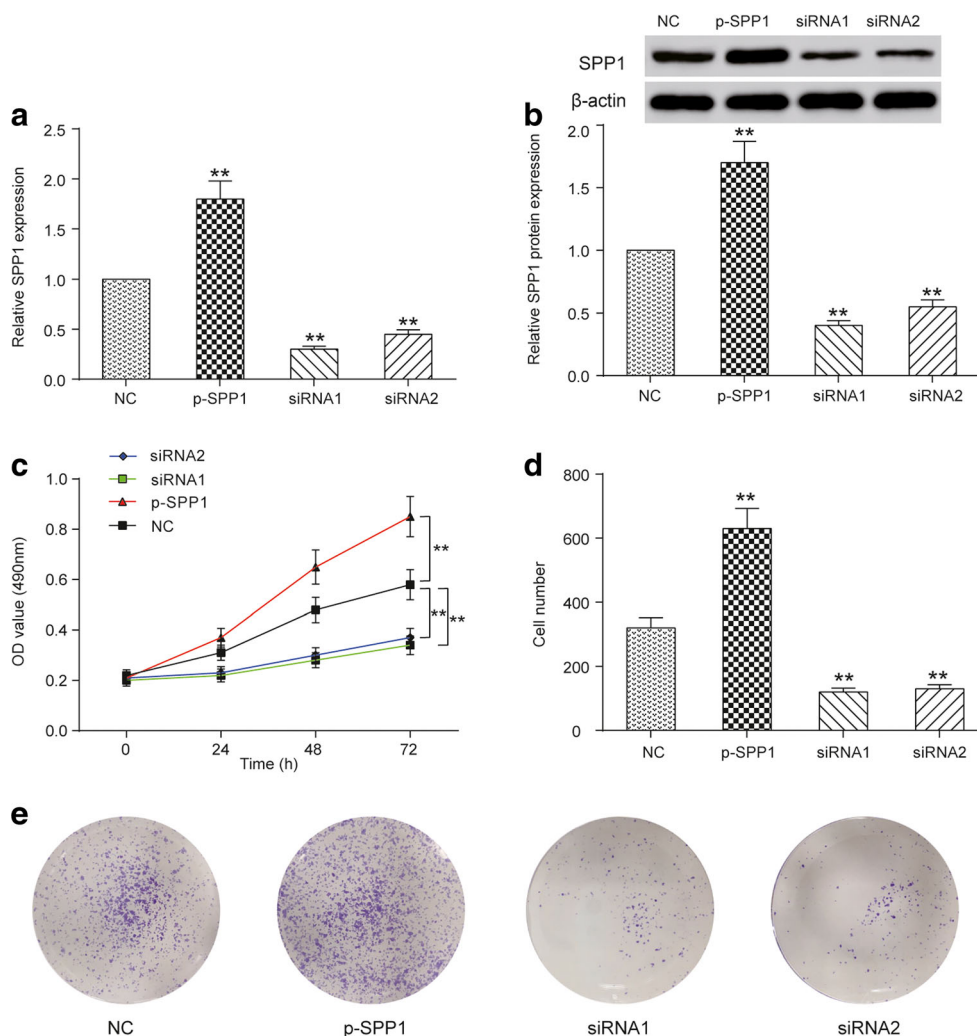
Table 3 The relationship between SPP1 expression and clinicopathological parameters in lung cancer

Clinicopathological parameters	Number (N = 107)	SPP1 expression level		P
		Low expression	High expression	
Age				
≤65	38	18	20	0.5442
>65	69	28	41	
Gender				
Male	32	14	18	0.8306
Female	75	30	45	
Smoking history				
Nonsmoker	35	16	19	0.5350
Smoker	72	28	44	
Tumor size				
≤ 4.5 cm	64	30	34	0.5571
> 4.5 cm	43	23	20	
T stage				
T1 And T2	60	23	37	0.2396
T3 And T4	47	24	23	
Clinical stage				
Early stage (I-II)	48	26	22	0.0494*
Advanced (III-IV)	59	20	39	

* $P < 0.05$ was recognized as a significant difference

Fig. 3 *SPP1* Promotes Lung Cancer Cell Proliferation.

a Negative control (NC group), pcDNA3.1-*SPP1* (p-*SPP1* group), siRNA1-*SPP1* (siRNA1 group) and siRNA2-*SPP1* (siRNA2 group) were transfected into NCI-H446 lung cancer cells respectively. *SPP1* was down-regulated in cell lines after transfected with siRNA1-*SPP1* and siRNA2-*SPP1* and was up-regulated in cell lines after transfected with pcDNA3.1-*SPP1* by qRT-PCR. $**P < 0.01$, compared with NC group. **b** Western blot showed protein expression of *SPP1* was facilitated in p-*SPP1* group and was decreased in siRNA1 group and siRNA2 group. $**P < 0.01$, compared with NC group. **c** MTT assay detected the cell viability of p-*SPP1* group was enhanced while that was inhibited in siRNA1 group and siRNA2 group. $*P < 0.05$, compared with NC group. **d, e** Plate clone assay revealed that the number of cell proliferation was high in p-*SPP1* group, while the number of cell proliferation was decreased in siRNA1 group and siRNA2 group. $**P < 0.01$ compared with NC group.



Upregulation of *SPP1* decreased the protein expression of Beclin-1 and LC3-II/LC3-I, while promoted p62 protein expression. Downregulation of *SPP1* increased Beclin-1 and LC3-II/LC3-I protein expression, but reduced p62 protein expression (Fig. 5b).

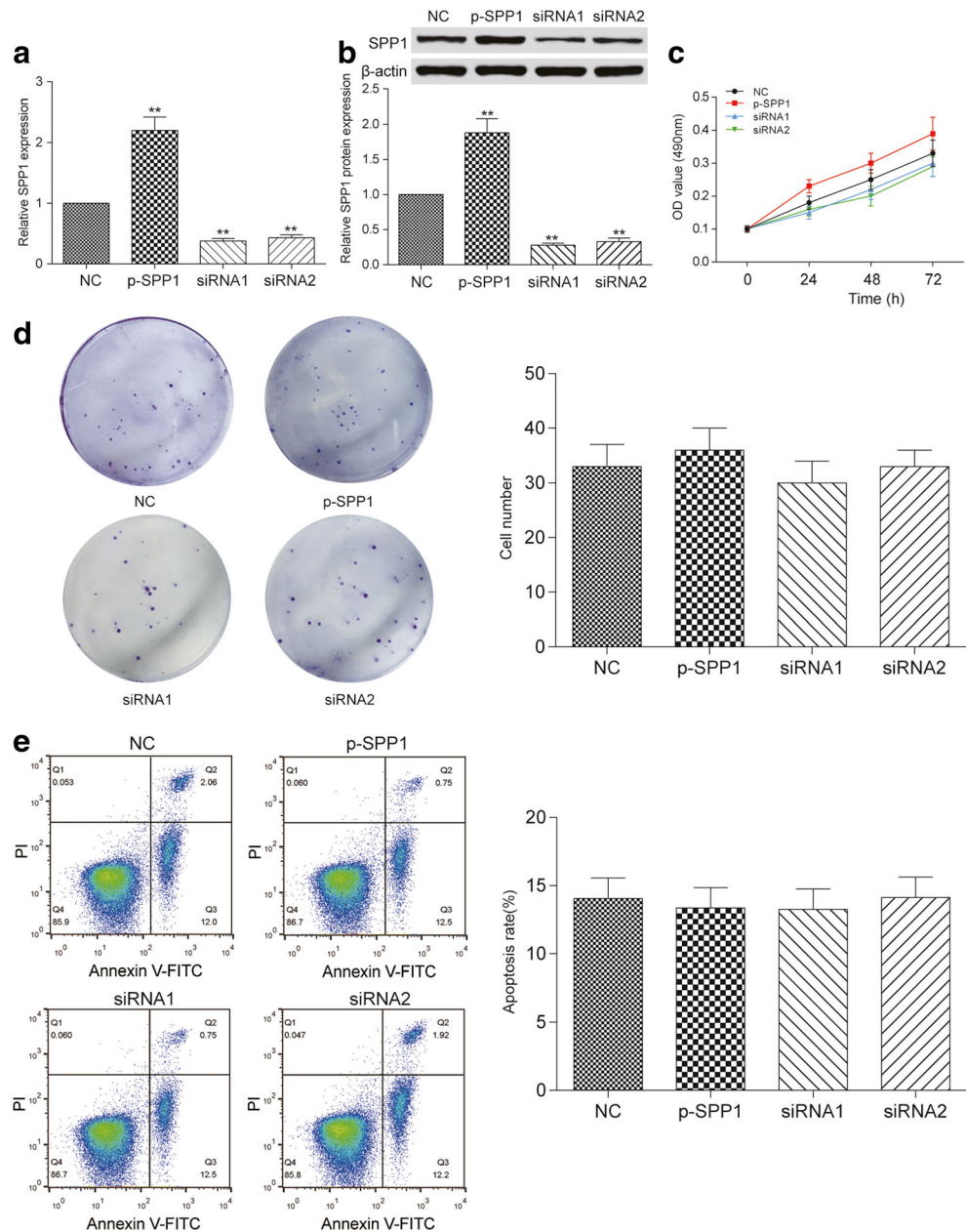
Discussion

In the study, through analyzing the microarray GSE19804, we found the top ten mRNA levels in tumor tissue. *SPP1* was the highest expressed gene of the mRNAs in tumor tissues. For better illustration, we used qRT-PCR to detect the expression levels of *SPP1* and its proteins in 107 lung cancer tissues and adjacent tissues. And 10 cases were randomly selected for western-blot to detect protein expression levels. For a more detailed explanation, we chose four lung cancer cell lines and one normal lung cell line to examine the expression of *SPP1* in lung cancer cell lines. *SPP1* was highly expressed in lung cancer cells detected by qRT-PCR compared with normal lung cells. All of these experiments illustrate the high expression of

SPP1 in lung cancer cells. Therefore, may be *SPP1* overexpression could promote the development of lung cancer cells. The NCI-H446 cell line with the highest *SPP1* expression in four lung cancer cell lines was selected and transfected with negative control (NC), pcDNA3.1-*SPP1* (p-*SPP1*), siRNA1-*SPP1* *SPP1* (siRNA2 group). The expression of *SPP1* was detected by qRT-PCR and western-blot. In the p-*SPP1* group, *SPP1* expression was promoted, and in the siRNA1 group and the siRNA2 group, *SPP1* expression was suppressed. MTT assay found that *SPP1* overexpression could enhance the cell viability of NCI-H446 cells. And the plate clone assay showed that *SPP1* overexpression could increase the number of cell proliferation. The experiments verified that *SPP1* overexpression could promote the development of lung cancer cells.

Lung cancer is one of the most common types of cancer worldwide and is associated with a poor 5-year relative survival rate [10]. The initial treatment of patients hinges on the detection of targetable molecular drivers, now challenges were posed on finding more effective diagnosis and prognosis markers [11]. Accordingly, the role of *SPP1* as a tumor promoter was preferred in the present study. *SPP1* was highly

Fig. 4 *SPP1* had no effect on bronchial epithelial cell proliferation. **a** Negative control (NC group), pcDNA3.1-*SPP1* (p-*SPP1* group), siRNA1-*SPP1* (siRNA1 group) and siRNA2-*SPP1* (siRNA2 group) were transfected into BEAS-2B cell respectively. *SPP1* was down-regulated in cell lines after transfected with siRNA1-*SPP1* and siRNA2-*SPP1* and was up-regulated in cells after transfected with pcDNA3.1-*SPP1* by qRT-PCR. $**P < 0.01$, compared with NC group. **b** Western blot showed protein expression of *SPP1* was facilitated in p-*SPP1* group and was decreased in siRNA1 group and siRNA2 group. $**P < 0.01$, compared with NC group. **c** MTT assay detected the cell viability in p-*SPP1* group, siRNA1 group and siRNA2 group, compared with NC group. **d** Plate clone assay revealed the number of cell proliferation in p-*SPP1* group, siRNA1 group and siRNA2 group. **e** Flow Cytometer revealed the apoptosis rate of cell in p-*SPP1* group, siRNA1 group and siRNA2 group



expressed in lung cancer tissues and cells and promoted cell growth, inhibited cell apoptosis and autophagy at the same time.

Autophagy plays a major role in maintaining cell quality control and homeostasis but under extreme conditions autophagic signaling can be recruited to activate cell death pathways. Thus, autophagy can act either as a tumor suppressor or as a tumor maintenance factor [12, 13]. In the last years, autophagy has gained relevance as a mechanism that, acting in concert with the intrinsic pathway of apoptosis, controls tissue homeostasis and cell survival [14, 15]. Autophagy can be triggered by different causes of environmental stress such as nutritional deficiencies, hypoxia or exposure to high temperatures [16]. This process involves the degradation of cellular components

through lysosomal machinery and is thought to be required for normal turnover of cellular components, especially in response to starvation [14, 15]. The interaction between apoptosis and autophagy is complicated and depends on the cellular context. Autophagy can act both as an alternative death pathway to apoptosis and as stress adaptation mechanism to avoid cell death [17]. In the study, western blot was used to detect the relationship of autophagy-related proteins Beclin-1, LC3-I, LC3-II and p62 and *SPP1*. The first identified mammalian autophagy-related gene, Beclin1, regulates the activity of autophagy particularly in the initiation phase by combining with different ligands [18]. LC3 has been identified to be the most widely used specific marker of autophagy initiation, which is first synthesized as its precursor, then cut up into its cytosolic

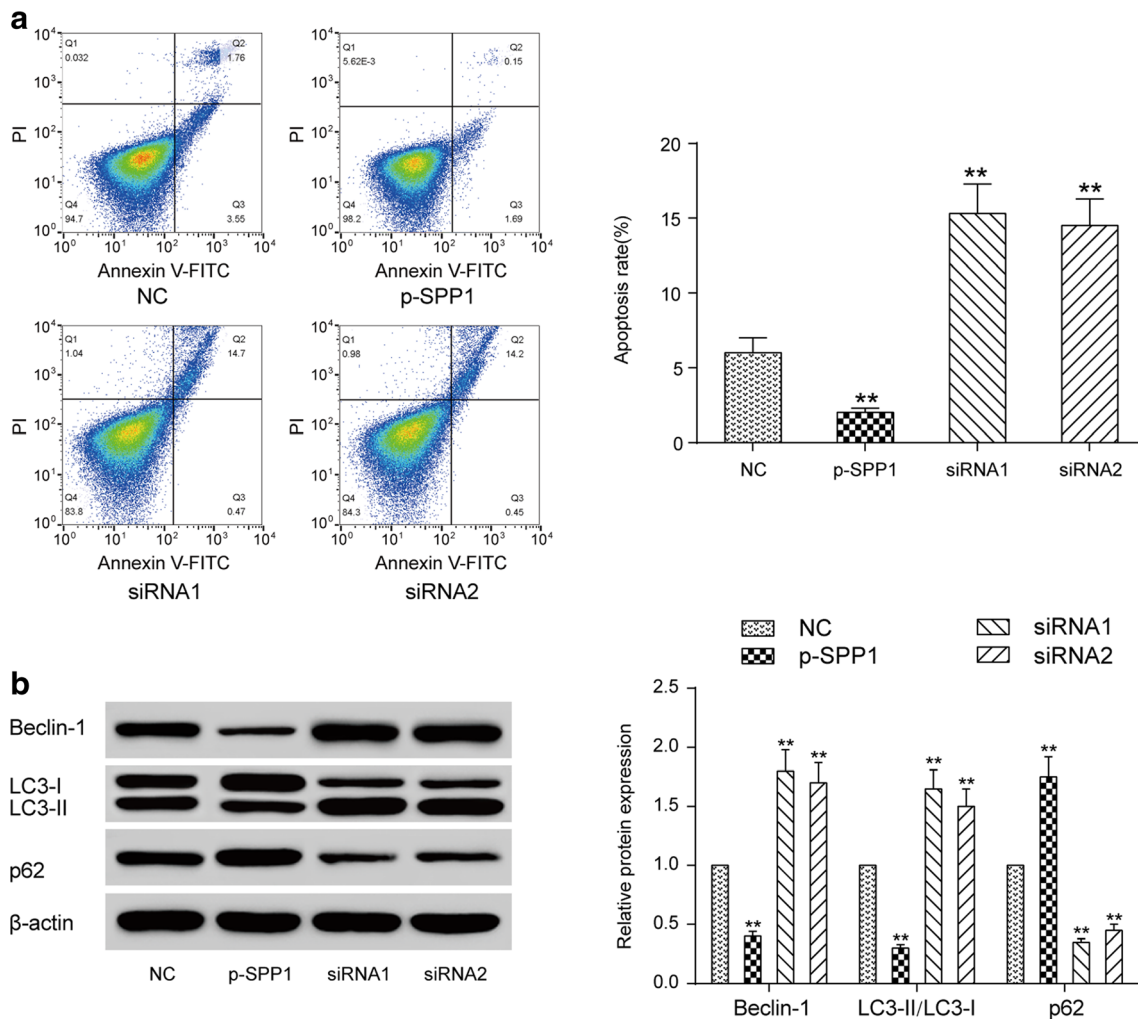


Fig. 5 *SPP1* promoted autophagy and apoptosis in lung cancer cells. **a** The apoptosis rate of p-*SPP1* group was down-regulated, while the apoptosis rate of siRNA1 group and siRNA2 group was up-regulated detected by flow cytometry, ** $P < 0.01$ compared with NC group. **b**

Western blot was used to detect the expression of autophagy-related proteins Beclin-1, LC3-I, LC3-II and p62 in each transfection group. ** $P < 0.01$ compared with NC group

form, LC3-I, which can be processed into LC3-II [19]. Autophagosome is essential for the elongation stage of the phagophore membrane and LC3-II specifically binds to it. To test the amount of autophagosome can be measured by the expression of LC3-II or LC3-II/LC3-I [20]. So we investigate expression of LC3-II/LC3-I. In addition, P62 is negatively correlated with autophagy activity, reflecting the degradative capability of autophagy and the intensity of autophagic flux [21]. Observable changes of autophagy-related proteins showed that upregulation of *SPP1* decreased the protein expression of Beclin-1 and LC3-II/LC3-I, while promoted p62 protein expression. These markers of autophagy told us *SPP1* inhibited the autophagy. The more complex transduction pathways associated with autophagy under the influence of *SPP1* and whether autophagy-related proteins can regulate lung cancer cells or not need further study.

Nevertheless, limitations in this report are to be taken into consideration. For example, we identified *SPP1* could promote the development of lung cancer cells by inhibiting autophagy and apoptosis, but upstream mechanism of *SPP1* was not explored. In addition, the relationship between *SPP1* and survival time of patient with lung cancer needs investigation by future experiments.

Conclusion

In conclusion, we detected *SPP1* expression was highly expressed in lung cancer tissues as well as cell lines. High expression of *SPP1* was capable to significantly promote the development of lung cancer cells via inhibiting the autophagy and apoptosis.

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Compliance with Ethical Standards

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

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of Qilu Hospital of Shandong University.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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