

SUMO4 small interfering RNA attenuates invasion and migration via the JAK2/STAT3 pathway in non-small cell lung cancer cells

JIN-XIAO LIANG¹, WEI GAO², XIAO-WEI ZENG^{1,3}, GUO-PING CHENG⁴,
LEI CAI¹, KAI-YI TAO¹ and XUN YANG¹

¹Department of Thoracic Surgery, Zhejiang Cancer Hospital, Hangzhou, Zhejiang 310022; ²School of Medicine, Zhejiang University City College; ³School of The Second Clinical Medical College, Zhejiang Chinese Medical University City College; ⁴Department of Pathology, Zhejiang Cancer Hospital, Hangzhou, Zhejiang 310000, P.R. China

Received June 24, 2019; Accepted July 9, 2020

DOI: 10.3892/ol.2020.12088

Abstract. Small ubiquitin-like modifier 4 (SUMO4) is the latest member of the sumoylation family, which enhances the stability of protein, regulates the distribution and localization of the protein, and affects the transcription activity of the protein. However, the role of SUMO4 in non-small cell lung cancer (NSCLC) has not yet been reported. The present study first demonstrated that SUMO4 was upregulated in a number of tissues from patients with NSCLC. Immunohistochemistry was performed to demonstrate the expression level of SUMO4 in lung cancer tumor tissues. Following the transfection, The EMT status and signaling pathway activation regulated by SUMO4-siRNA was assessed by western blotting. The Transwell and wound healing assays were performed to investigate the regulatory effect of SUMO4-siRNA on cell migration and invasion. Cell Counting Kit-8 assay was performed to investigate whether SUMO4-siRNA affected the chemosensitivity of the NSCLC cells to cisplatin. Statistical analysis of immunohistochemical results from the tissues showed that the overexpression of SUMO4 was significantly associated with sex, tumor type, history of smoking, T stage and poor prognosis. It was also identified that SUMO4 small interfering RNA attenuated invasion and migration in NSCLC cell lines, as well chemosensitivity to cisplatin via the inhibition of the JAK2/STAT3 pathway. In conclusion, SUMO4 may play an important role in the poor prognosis of patients with NSCLC. The present study indicates that SUMO4 may be a potential therapeutic target for NSCLC.

Introduction

Lung cancer has a high degree of malignancy and ranks first as the cause of cancer-associated mortality in the United States (1). Non-small cell lung cancer (NSCLC) accounts for 85% of all lung cancer cases in the United States (2). Although the diagnosis and treatment methods of NSCLC are continuously improving, metastasis via the lymph nodes and blood system occurs at an early stage, and due to insidious onset and rapid progression, the prognosis of NSCLC remains unsatisfactory. Therefore, an in-depth study on the mechanism of NSCLC metastasis is of great significance for its prevention, and to develop novel molecular targeted therapeutic drugs.

The JAK/STAT signaling pathway was found to have sustained abnormal activation in various malignant tumor cells, such as prostate cancer, sarcomas and lymphomas, regulating tumor proliferation, apoptosis and metastasis (3). As a member of STAT family, STAT3 is closely associated with the prognosis of NSCLC (4). JAK2 activates STAT3 by phosphorylation, allowing it to enter the nucleus and perform its biological function (3). It has been reported that the activation of the JAK2/STAT3 signaling pathway induced metastatic NSCLC (5). Due to the importance of JAK2/STAT3 signaling in tumor development, targeted therapy for this pathway has also become a popular research topic in the study of NSCLC. At present, although several types of JAK2/STAT3-targeted drugs are undergoing clinical trial (and have made progress), their therapeutic effects remain unsatisfactory. Therefore, identifying new targets for JAK2/STAT3 sensitization may enhance the clinical effect of JAK2/STAT3 target-based therapy and improve the prognosis of patients with NSCLC.

Sumoylation is a ubiquitin-like post-translational modification that enhances the stability of protein and regulates their distribution and localization (6). The transcriptional activity of transcription factors can be modulated through sumoylation (7). To date, four members of the small ubiquitin-like modifier (SUMO) family (SUMO1, 2, 3 and 4) have been cloned and identified (7). SUMO4 is a newly discovered member of the sumoylation family, which is mainly expressed in the immune-associated organs and kidneys. It has been confirmed to be closely associated with type I diabetes (8), coronary heart disease (9), psoriasis (10), Behcet's disease (11)

Correspondence to: Dr Jin-Xiao Liang or Dr Xun Yang, Department of Thoracic Surgery, Zhejiang Cancer Hospital, 1 Banshan East Road, Hangzhou, Zhejiang 310022, P.R. China
E-mail: liangjinxiao@163.com
E-mail: zlyy633@163.com

Key words: small ubiquitin-like modifier 4, non-small cell lung cancer, metastasis, prognosis, JAK2/STAT3 pathway

and other diseases. However, research into the potential regulatory effects of SUMO4 on tumorigenesis is somewhat lacking.

A Study has shown that SUMO4 negatively regulates NF- κ B transcriptional activity in a diabetic model (12). Mo *et al.* (11-13) found that SUMO4 decreased oxidative stress by increasing antioxidant enzymatic activity and DNA damage signaling-associated protein activity, thus activating the cellular self-protection mechanisms (13). SUMO4 also directly decreased the DNA-binding activity of the STAT protein, leading to the inhibition of JAK/STAT signaling (14). These findings suggest that SUMO4 may be associated with tumor development and progression. It has been reported that SUMO4 expression was increased in thyroid cancer (15). However, the expression and function of SUMO4 during the tumorigenesis of NSCLC remain unknown. The present study investigated the expression of SUMO4 in NSCLC and identified the mechanisms of SUMO4 in augmenting the proliferation, invasion and migration of NSCLC cells.

Materials and methods

NSCLC patient samples. A total of 100 NSCLC 10 adjacent non-cancerous tissues (defined as tissue which is at least 3 cm from cancerous region; samples were collected from 100 patients (71 men and 29 women) during surgery at Zhejiang Cancer Hospital (Zhejiang, China) between January 2009 and March 2011. All patients gave written informed consent to participate in the study and to allow their samples to be biologically analyzed. The age of the patients with NSCLC enrolled in the present study ranged from 39 to 76 years (mean, 61.3 years). The exclusion criteria included patients with other types of cancer and those who had received preoperative chemoradiotherapy. Control samples were obtained from the same patient at a site at least 3 cm from the tumor and were approved as control samples by a pathologist. Tissue sections were fixed with 10% formalin for at least 24 h at room temperature and subsequently embedded in paraffin for immunohistochemistry. The tumors were staged according to the pathological tumor/node/metastasis (pTNM) classification (7th edition) of the International Union against Cancer (16). All procedures for sample collection and processing were ratified by the International Review Board of Zhejiang Cancer Hospital (Hangzhou, China; approval no. IRB-2016-134).

Reagents. Tyrphostin AG490 was purchased from Sigma-Aldrich (Merck KGaA). The Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Molecular Technologies, Inc. The Biotin-Streptavidin HRP Detection kit was purchased from OriGene Technologies, Inc. (cat. no. SP-9000). SUMO4-specific small interfering (si)RNA (forward, 5'-GGA UGGUUCUGUGGUCAGTT-3' and reverse, 5'-CUGCAC CACAGAACCAUCCTT-3') and negative control siRNA (forward, 5'-UUCUCCGAACGUGUCACGUTT-3' and reverse, 5'-ACGUGACACGUUCGGAGAATT-3') were designed and synthesized by Shanghai GenePharma Co., Ltd. Cisplatin was purchased from Jiangsu Hanson Pharmaceutical Co. (<http://www.hansoh.cn>). The SUMO4 antibody was purchased from Abcam (cat. no. ab126606), phosphorylated (p-)JAK2 and p-STAT3 antibodies were from Cell Signaling Technology Inc. (cat. nos. 3771 and 9145), antibodies against

Table I. Summary of SUMO4 expression status in NSCLC and adjacent normal tissues analyzed in the present study.

Group	SUMO4 expression		Total, n
	Negative, n	Positive, n	
Adjacent normal tissue	10	0	10
NSCLC tissue	31	69	100

NSCLC, non-small cell lung cancer; SUMO4, small ubiquitin-like modifier 4.

vimentin, E-cadherin, N-cadherin, JAK2 and STAT3 were purchased from ProteinTech Group, Inc. (cat. nos. 10377-1-AP, 20874-1-AP, 22018-1-AP, 17670-1-AP and 10253-1-AP).

Cell lines. Human NSCLC cell lines A549, NCI-H1650 and SK-MES-1 were purchased from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. All cell lines were cultured in DMEM (Cytvia) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Transgen Biotech Co., Ltd.) in humidified air at 37°C (5% CO₂).

Transfection. Cells were seeded into 6-well plates. At 60-70% confluence (5x10⁵ cells/well), SUMO4 siRNA (100 nM) and scrambled control siRNA (100 nM) were transiently transfected into the cells using Lipofectamine[®] 2000 Reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to manufacturer's instructions. The transfection reagent was removed after 5 h, and the cells were harvested after 48 h.

Immunohistochemical (IHC) staining. IHC staining of tumor tissues was performed on 5- μ m sections. The general procedure for IHC staining was performed as previously described (17). The sections were blocked with blocking serum from the ABC Vectastain kit (cat. no. PK-6100; Vector Laboratories, Inc.) at room temperature for 30 min, followed by incubation with primary antibody against SUMO4 (1:50; cat. no. ab126606; Abcam) overnight at 4°C. Then the sections were incubated with a horseradish peroxidase-conjugated mouse anti-rabbit Ig antibody at room temperature for 1 h, followed by staining with the chromogen diaminobenzidine (Zhongshan, Beijing, People's Republic of China) until a brown color was shown. The slides were counterstained with Mayer's hematoxylin at room temperature for 10 min. Random fields from each slide were viewed under a light microscope (Olympus DP73; Olympus Corporation) at x20 magnification.

Western blotting. Proteins extracted from cells were denatured in RIPA buffer (150 mM NaCl, 0.1% SDS, 25 mM Tris-HCl pH 7.6, 1% sodium deoxycholate and 1% NP-40) with protease inhibitors. The concentrations of protein were detected using a BCA protein assay kit (cat. no. P0010S; Beyotime Institute of Biotechnology). Equal amounts of total protein (50 μ g) were analyzed by SDS-PAGE. Proteins were separated on a 12% polyacrylamide gel and transferred to a nitrocellulose membrane. The membrane was blocked for

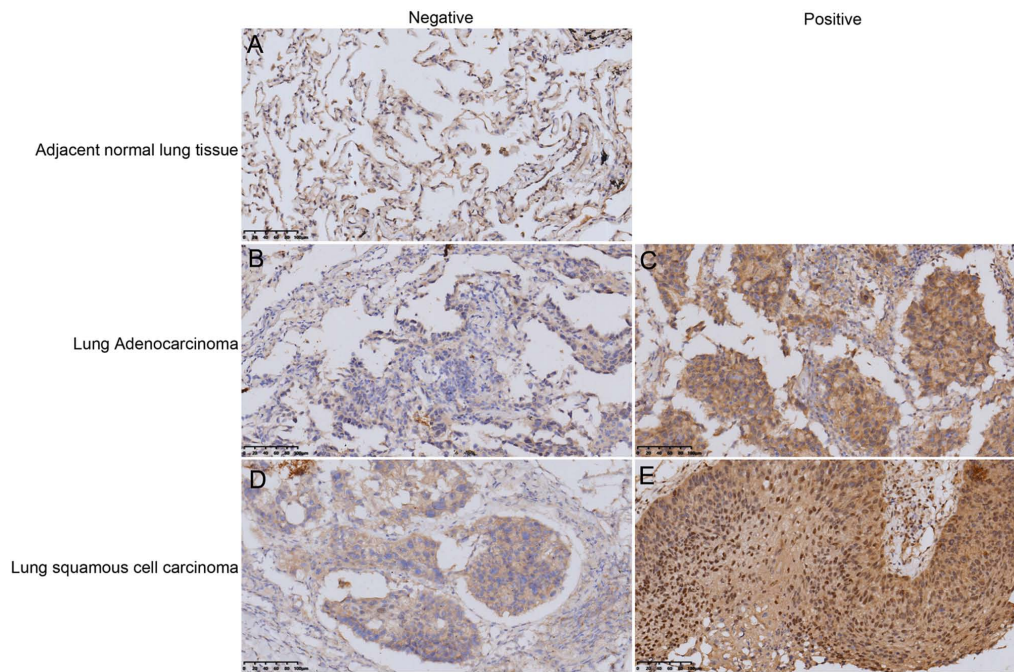


Figure 1. SUMO4 expression in NSCLC tissue. (A) SUMO4 staining in adjacent normal lung tissue. (B) Low SUMO4 staining in lung adenocarcinoma tissue. (C) High SUMO4 staining in lung adenocarcinoma tissue. (D) Low SUMO4 staining in lung squamous cell carcinoma tissue. (E) High SUMO4 staining in lung squamous cell carcinoma tissue. All the images were obtained at x200 magnification. NSCLC, non-small cell lung cancer; SUMO4, small ubiquitin-like modifier 4.

1 h at room temperature using blocking buffer (0.5% fat-free milk). After blocking, the blot was probed with primary antibodies (dilution, 1:1,000) at 4°C overnight. Subsequently, the blot was incubated with HRP-labeled secondary antibodies diluted in PBS buffer (1:2,000; cat. no. SA00001-2; ProteinTech Group, Inc.). ECL reagent was used for visualization. The images were obtained using the Bio-Rad Imaging System. Signal quantification was obtained using Quantity One software (version 4.6.6; Bio-Rad Laboratories, Inc.) and normalized to GAPDH.

Wound-healing assay. A total of 5×10^5 H1650, A549 and sk-mes-1 cells were seeded independently into each well of 6-well plates overnight. A scratch was made on the monolayers with a 200- μ l pipette tip, and washed with PBS to remove the detached cells. Fresh DMEM/F12 medium without serum was added into each well of the 6-well plate. The wounded areas were observed and imaged using a light microscope (magnification x100) at 0 and 24 h. The migration results were quantified using ImageJ software (version 1.52; National Institutes of Health).

Transwell invasion assay. The trypsinized A549, H1650 and sk-mes-1 cells were washed with PBS and resuspended in serum-free DMEM medium. Then, 200 μ l cell suspension (1×10^5 /well) was added to the upper chamber with a 50 μ l solidified Matrigel-coated membrane. The lower chamber was filled with 800 μ l DMEM supplemented with 10% FBS. After 24 h incubation, the chambers were fixed with 100% methanol for 20 min at room temperature, followed by staining with 0.1% crystal violet for 20 min at room temperature. Images were captured with an Olympus fluorescence microscope (magnification x100).

CCK-8 assay. Transfected H1650, A549 and sk-mes-1 cells were collected (100 μ l) at 24 h post-transfection and seeded into 96-well plates at a density of 3×10^3 /well independently. Following incubation for 0, 12, 24, 36 and 48 h at 37°C, 10 μ l CCK-8 reagent was added into each of the wells, and the cells were incubated at 37°C for 2 h. The absorbance was measured at a wavelength of 450 nm using a microplate reader (Bio-Rad Laboratories, Inc.).

Statistical analysis. Statistical analysis was performed using SPSS 23.0 software (IBM Corp.). All experiments were performed in triplicate and data are presented as the mean \pm standard error of the mean. The association between the expression of SUMO4 and clinicopathological parameters was examined using the χ^2 test. The overall survival (OS) and disease-free survival (DFS) curves were produced using the Kaplan-Meier method, the difference in survival was determined using the log-rank test. $P < 0.05$ was considered to indicate a statistically significant difference. Unpaired Student's t-test was performed for western blotting and wound-healing data analysis. One-way ANOVA with Tukey's test was performed for invasion assay and phosphorylation analysis. $P < 0.05$ was considered to indicate a statistically significant difference. Numerical data are presented as the mean \pm standard deviation.

Results

Overexpression of SUMO4 is associated with a poor prognosis in human NSCLC. In the present study, 10 adjacent normal lung tissues and 100 NSCLC tissues were collected for IHC staining, in order to explore the association between SUMO4 expression and NSCLC prognosis. The results revealed that SUMO4 was expressed differently in the cytoplasm of these

Table II. Statistical analysis of the association between the expression of SUMO4 and the clinicopathological parameters of NSCLC.

Clinicopathological parameter	n	SUMO4 expression		P-value
		Negative, n	Positive, n	
Age, years				0.723
<60	33	11	22	
≥60	67	20	47	
Sex				0.017
Male	71	17	54	
Female	29	14	15	
Tumor type				0.013
Adenocarcinoma	46	20	26	
Squamous cell carcinoma	54	11	43	
BMI				0.075
<22	52	12	40	
≥22	48	19	29	
Hypertension				0.108
With	20	3	17	
Without	80	28	52	
Diabetes				1.000
With	7	2	5	
Without	93	29	64	
History of drinking				0.993
Moderate or heavy drinking	42	13	29	
Without or light drinking	58	18	40	
History of smoking				0.002
With	64	13	51	
Without	36	18	18	
Tumor location				0.510
Left lung	37	10	27	
Right lung	63	21	42	
Tumor location				0.327
Upper and middle lung	54	19	35	
Lower lung	46	12	34	
Tumor size, cm				0.262
≤3	34	13	21	
>3	66	18	48	
Tumor differentiation				0.168
Well and moderate	51	19	32	
Poor	49	12	37	
T stage				0.025
T1 and T2	76	28	48	
T3 and T4	24	3	21	
N stage				0.137
N0	47	18	29	
N1, N2 and N3	53	13	40	
M stage				0.526
M0	98	30	68	
M1	2	1	1	
Tumor stage				0.186
Stages I and II	58	21	37	
Stages III and IV	42	10	32	

Table II. Continued.

Clinicopathological parameter	n	SUMO4 expression		P-value
		Negative, n	Positive, n	
Recurrence				0.084
With	64	16	48	
Without	36	15	21	

NSCLC, non-small cell lung cancer; SUMO4, small ubiquitin-like modifier 4; BMI, body mass index.

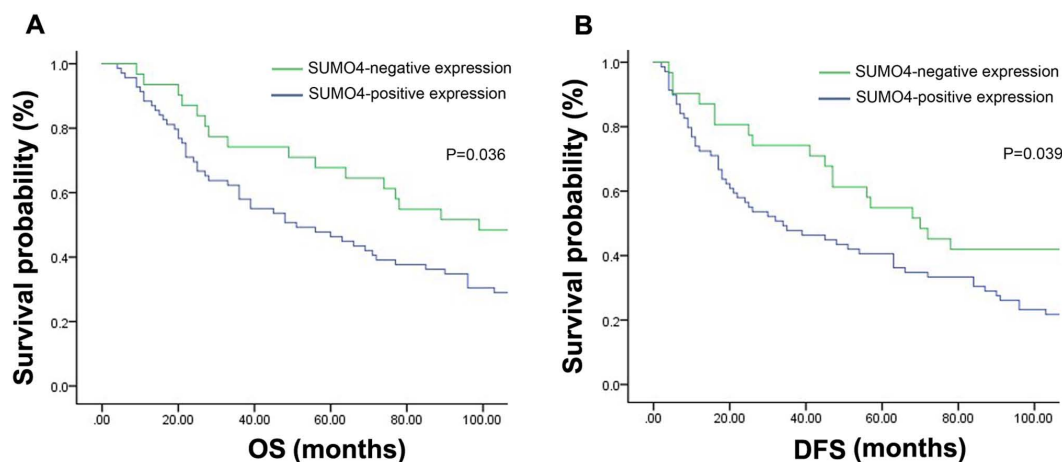


Figure 2. Survival curves of patients with NSCLC with negative or positive expression of SUMO4. SUMO4 expression data were collected from immunohistochemistry experiments in Fig. 1; the prognostic information was collected by the clinical team. (A) OS was based on the expression status of SUMO4. Differences between SUMO4-negative and -positive groups were significant, $P=0.036$. (B) DFS time based on the expression status of SUMO4. Difference between SUMO4-negative and -positive groups were significant, $P=0.039$. NSCLC, non-small cell lung cancer; SUMO4, small ubiquitin-like modifier 4; DFS, disease-free survival; OS, overall survival.

tissues. The tissues were divided into 'positive' and 'negative' groups, according to SUMO4 expression (Fig. 1). The expression of SUMO4 in NSCLC tissues were significantly higher compared with the adjacent normal lung tissue (Fig. 1). Quantitative analysis of the IHC staining results indicated that 69% of NSCLC samples were positive for SUMO4, while all adjacent normal lung tissues were negative via IHC analysis (Table I; $P=1.6862 \times 10^{-5}$).

In order to demonstrate the association between SUMO4 expression and NSCLC prognosis, the expression of SUMO4 was systematically analyzed relative to the clinicopathological characteristics of NSCLC from 100 patients. Based on the IHC staining results of all samples, the following associations were identified: Sex, tumor type, history of smoking and T stage were significantly associated with the expression of SUMO4 ($P<0.05$), however, age, body mass index (BMI), hypertension, diabetes, history of drinking, tumor location, tumor size, tumor differentiation, N stage, tumor stage and recurrence were not associated with the expression of SUMO4 ($P>0.05$) (Table II).

To clarify the associations between SUMO4 and NSCLC prognosis, the OS and DFS curves of these patients were generated using the Kaplan-Meier method. It was identified that positive expression of SUMO4 was significantly associated with short DFS and OS times (Fig. 2), indicating poor prognosis.

SUMO4 siRNA decreases cell migration, invasiveness and epithelial-mesenchymal transition (EMT) in NSCLC cells. To explore whether SUMO4 regulates the migratory and invasive abilities of NSCLC cells, SK-MES-1, NCI-H1650 and A549 cells which expressed SUMO4, were transfected with SUMO4 siRNA and negative control siRNA (Fig. 3A). EMT marker proteins including N-cadherin, E-cadherin and vimentin were examined by western blotting as shown in Fig. 3B. A notable decrease in vimentin and N-cadherin, as well as increase in E-cadherin was observed. Cell migration and invasion were evaluated by wound healing and Transwell assays (Fig. 3C-F). It was found that SUMO4 siRNA significantly inhibited the wound closure and invasion capacities of A549, H1975 and SK-MES-1 cells.

SUMO4 siRNA promotes the sensitivity of NSCLC cells to chemotherapy. To identify whether SUMO4 expression affects NSCLC chemosensitivity, control and SUMO4-siRNA transfected A549, H1975 and SK-MES-1 cells were treated with cisplatin at various concentrations, and the CCK-8 assay was used to assess the inhibition of cell proliferation rate. It was found that the inhibitory effect on the proliferation of cells increased with increasing concentrations of cisplatin. SUMO4 silencing dose-dependently altered inhibition rate compared with the NC group (Fig. 4).

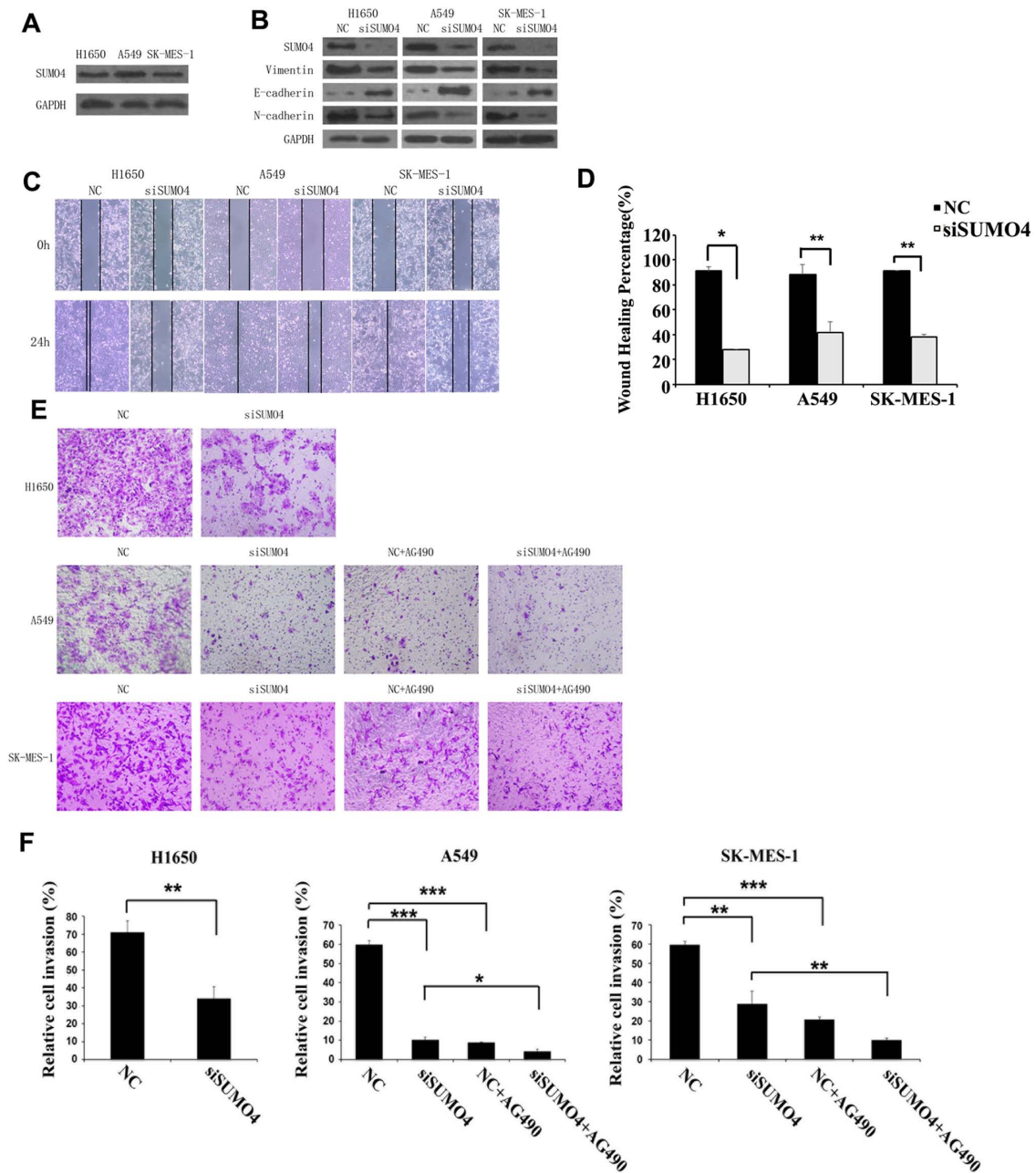


Figure 3. SUMO4-knockdown decreases migration and invasion in NSCLC cells. SUMO4 was downregulated by siRNA in A549, H1650 and SK-MES-1 cells for analysis of its effect on migration and invasion. (A) Western blot analysis shows the expression levels of SUMO4 in the three NSCLC cell lines. (B) Western blot analysis shows the expression levels of SUMO4 and corresponding factors involved in epithelial-mesenchymal transition. (C) Cell migration was detected via the wound healing assay, magnification $\times 100$. (D) Quantitative analysis of (C). (E) Cell invasion was assessed via the Transwell assay. (F) Quantitative analysis of (E) * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. NSCLC, non-small cell lung cancer; SUMO4, small ubiquitin-like modifier 4; siRNA, small interfering RNA; NC, negative control.

SUMO4 siRNA decreases cell invasiveness via the JAK2/STAT3 pathway in NSCLC cells. To explore the underlying molecular mechanism of the augmented invasion by SUMO4 depletion, the JAK2/STAT3 pathway was specifically analyzed in the present study. Using SUMO4 siRNA combined with the application of JAK2 kinase inhibitor AG490, it was found that knocking down SUMO4 slightly decreased JAK2 and STAT3 activity, as reflected by the phosphorylated forms of JAK2 and STAT3 (Fig. 5). In addition, inactivation of JAK2 by a specific inhibitor (AG490) resulted in decreased invasive ability of cells, demonstrated by the transwell invasion assay (Fig. 3D).

Discussion

Sumoylation has been shown to play important roles in various biological processes such as signal transmission, nuclear transportation, gene expression regulation and cell cycle regulation (18). It also participates in the regulation of mitochondrion division, as well as the maintenance of genome integrity (18). Sumoylation is closely associated with several human diseases, such as cancer, diabetes, Parkinson's disease and Alzheimer's disease (19). For example, sumoylation of β -catenin regulated the proliferation of myeloma (20), CDK6 sumoylation regulated the cell cycle arrest of glioma cells (21),

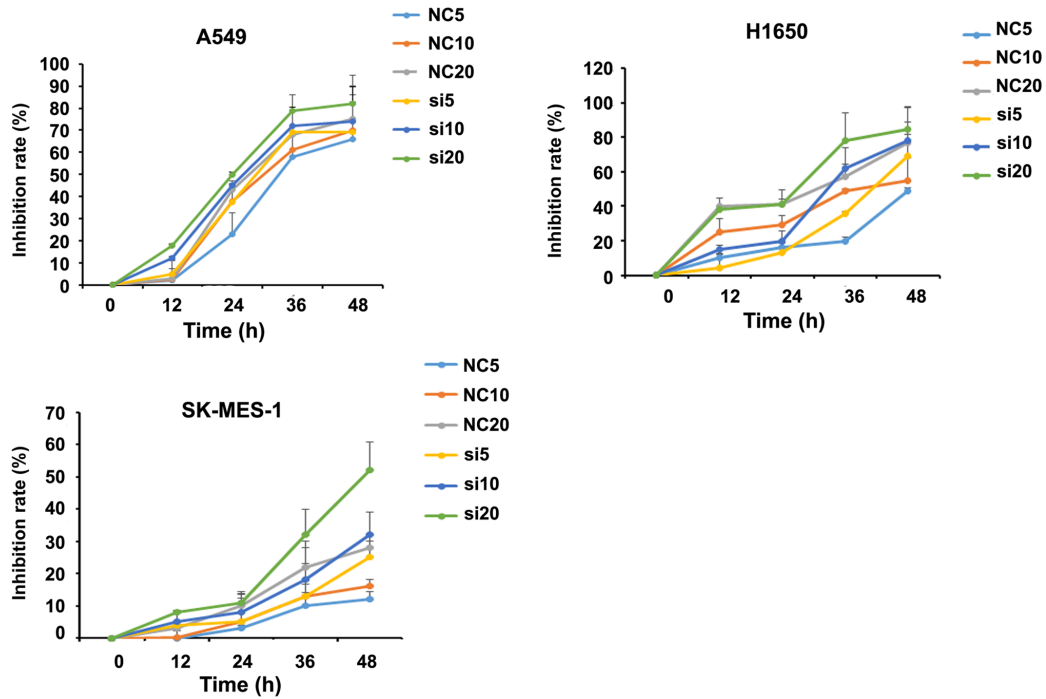


Figure 4. Inhibitory effects of cisplatin on three NSCLC cell lines tested by Cell Counting Kit-8 assay. NSCLC, non-small cell lung cancer; SUMO4, small ubiquitin-like modifier 4; siRNA, small interfering RNA; NC, negative control; NC5, NSCLC cells with negative control siRNA plus 5 μ M cisplatin; NC10, NSCLC cells with negative control siRNA plus 10 μ M cisplatin; NC20, NSCLC cells with negative control siRNA plus 20 μ M cisplatin; si5, NSCLC cells with SUMO4 siRNA plus 5 μ M cisplatin; si10, NSCLC cells with SUMO4 siRNA plus 10 μ M cisplatin; si20, NSCLC cells with SUMO4 siRNA plus 20 μ M cisplatin.

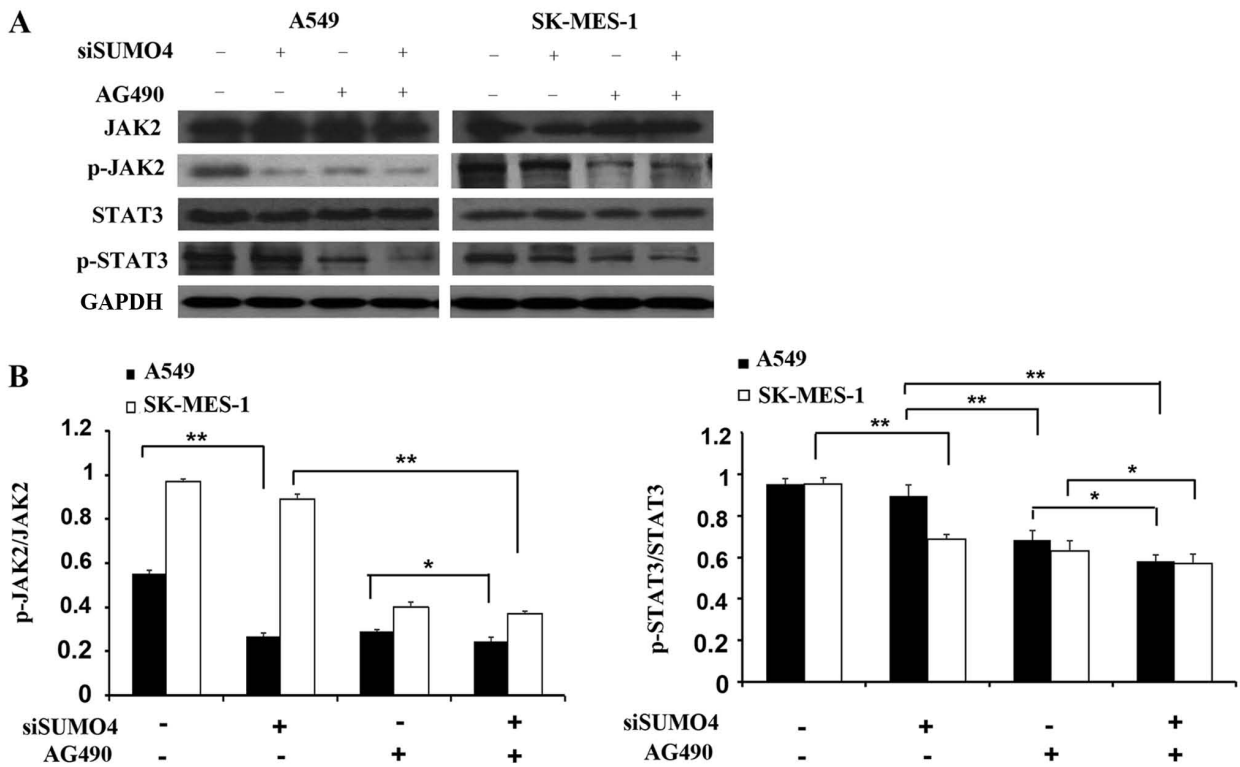


Figure 5. Role of SUMO4 expression in JAK2/STAT3 pathway activation in A549 and SK-MES-1 cells. SUMO4 was downregulated in NSCLC cells in combination with the use of a JAK2 inhibitor (AG490). (A) Equal amounts of total cell lysate were analyzed by western blotting. (B) Comparison of protein levels of p-JAK2 and p-STAT3 to GAPDH ratio. * $P < 0.05$; ** $P < 0.01$. NSCLC, non-small cell lung cancer; SUMO4, small ubiquitin-like modifier 4; p-, phosphorylated.

and sumoylation of Forkhead box protein K2 regulated the sensitivity of breast cancer cells to paclitaxel (22). These

results identified that sumoylation played an important role in the development of tumors.

In 2004, Guo *et al* (23) and Bohren *et al* (24) simultaneously discovered the SUMO4 gene on chromosome 6q25, which contains 702 nucleotide residues and encodes 95 amino acids. Guo *et al* (23) also found a close association between SUMO4 and type I diabetes, which may be a novel sensitivity gene for type I diabetes (20). In addition, it was also confirmed that SUMO4 decreased the activity of NF- κ B by binding with I κ B (23). Mo *et al* (13) showed that SUMO4 initiated cell self-protection mechanisms by increasing the activity of antioxidant enzyme and DNA damage signaling protein, thus decreasing oxidative stress. These results indicated that SUMO4 may play an important role in the development of cancer. In previous studies, it was found that SUMO4 expression was significantly increased in thyroid cancer (13). The present findings confirmed that SUMO4 was associated with tumor progression. However, the study of SUMO4 in NSCLC has not been reported, and the mechanism of SUMO4 in tumors is still unclear.

The present study first demonstrated the expression of SUMO4 in NSCLC. The expression of SUMO4 in NSCLC tissues was significantly higher than in the adjacent normal lung tissues. The results showed that SUMO4 may play an important role in the occurrence and development of NSCLC. In the clinicopathological characteristics analysis, the SUMO4 positivity in men, squamous cell carcinoma, patients with a smoking history, T3 and T4 stage was found to be significantly higher compared with women, those with adenocarcinoma and those without a smoking history, and T1 and T2 stage tumors ($P < 0.05$). However, older patients with a lower BMI, hypertension, diabetes, moderate or heavy drinking history, larger tumors, poor differentiation, lymph node metastasis, tumor stage III or IV, and with recurrence were associated with SUMO4 positivity, although not significantly so. These results showed that SUMO4 was closely associated with T stage, but there is no significant difference between N stage and tumor stage, which may be due to the limited number of specimens. Interestingly, it was found that men with squamous cell carcinoma and a history of smoking had high positivity for SUMO4. Since the smoking rate in men is significantly higher compared with women, the association between smoking and squamous cell carcinoma is higher compared with between smoking and adenocarcinoma. Therefore, it is speculated that smoking may increase the positivity for SUMO4 and result in the occurrence and development of NSCLC. In the survival analysis, patients positive for SUMO4 expression were found to have poor prognosis. The results showed that SUMO4 may be a novel prognostic factor for NSCLC.

The mechanism underlying the regulatory effect of SUMO4 in the A549, H1650 and SK-MES-1 cell lines was further explored. The expression of SUMO4 enhanced invasion and migratory abilities, and increased EMT in all three cell lines. These results imply that SUMO4 expression could promote the metastatic capability of NSCLC. It was found that SUMO4 expression decreased cisplatin chemosensitivity. Thus, SUMO4 expression may be involved in chemotherapy resistance in NSCLC.

Wang *et al* (14) found that SUMO4 decreased the DNA binding activity of STAT, thus inhibiting the JAK/STAT signaling pathway. In the present study, the inhibition of

SUMO4 was found to inhibit invasion and migration by downregulating the activation of the JAK2/STAT3 pathway in NSCLC cells. SUMO4 may regulate the stability of molecules which activate JAK2-STAT3 signaling pathways, which lead to tumor progression. However, the molecular mechanism still requires further exploration.

There are limitations of the present study. Firstly, due to the limited number of specimens, some subgroups had insufficient samples. The sample size needs to be increased to obtain more accurate results. Secondly, *in vivo* studies should be performed to explore the effect of SUMO4 on NSCLC metastasis. Furthermore, the mechanisms underlying the effect of SUMO4 on JAK2/STAT3 signal pathway activation requires further exploration, by constructing SUMO4 expression plasmids, in future studies.

In conclusion, SUMO4 was found to be expressed in NSCLC and is significantly associated with sex, tumor type, history of smoking, T stage and poor prognosis in NSCLC. SUMO4 plays a significant role in cell invasion and migration via JAK2/STAT3 pathway activation in NSCLC cell lines, which implies that SUMO4 may be a potential therapeutic target for NSCLC with positive expression of SUMO4.

Acknowledgements

The authors of the present study would like to thank Dr Wei Gao from Zhejiang University City College (Hangzhou, China) and Dr Guoping Cheng from Zhejiang Cancer Hospital (Hangzhou, China) for their technical assistance, and Dr Lei Cai from Zhejiang Cancer Hospital (Hangzhou, China) for providing technical assistance on lung tumor immunohistochemical analyses. The authors would also like to thank Dr Xiaowei Zeng for technical support on the *in vitro* experiments, and Dr Kaiyi Tao, Dr Xun Yang and Dr Jinxiao Liang [all from Zhejiang Cancer Hospital (Hangzhou, China)] for collection, analysis and interpretation of data.

Funding

The present study was supported by grants from Medical Health Science and Technology Project of Zhejiang Provincial Health Commission (grant nos. 2017184728 and 2018241087).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

JXL performed *in vitro* experiments and drafted the initial manuscript. WG, GPC and LC collected lung cancer tumor and non-tumor adjacent tissues, analyzed and interpreted the patient data. XWZ performed the *in vitro* experiments. Data analysis was performed by KYT. JXL and XY conceived the study and finalized the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Zhejiang Cancer Hospital and written informed consent was provided by all patients. All procedures involving human participants were performed in accordance with the ethical standards of the institutional and national research committee (Hangzhou, China; approval no. IRB-2016-134).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2018. *CA Cancer J Clin* 68: 7-30, 2018.
2. Molina JR, Yang P, Cassivi SD, Schild SE and Adjei AA: Non-small cell lung cancer: Epidemiology, risk factors, treatment, and survivorship. *Mayo Clin Proc* 83: 584-594, 2008.
3. Pencik J, Pham HT, Schmoellerl J, Javaheri T, Schleuderer M, Culig Z, Merkel O, Moriggl R, Grebien F and Kenner L: JAK-STAT signaling in cancer: From cytokines to non-coding genome. *Cytokine* 87: 26-36, 2016.
4. Yu Y, Zhao Q, Wang Z and Liu XY: Activated STAT3 correlates with prognosis of non-small cell lung cancer and indicates new anticancer strategies. *Cancer Chemother Pharmacol* 75: 917-922, 2015.
5. Song Q, Liu B, Li X, Zhang Q, Cao L, Xu M, Meng Z, Wu X and Xu K: MiR-26a-5p potentiates metastasis of human lung cancer cells by regulating ITGB8- JAK2/STAT3 axis. *Biochem Biophys Res Commun* 501: 494-500, 2018.
6. Wilson VG: Introduction to sumoylation. *Adv Exp Med Biol* 963: 1-12, 2017.
7. Yang Y, He Y, Wang X, Liang Z, He G, Zhang P, Zhu H, Xu N and Liang S: Protein SUMOylation modification and its associations with disease. *Open Biol* 7: 170167, 2017.
8. Zhang J, Chen Z, Zhou Z, Yang P and Wang CY: Sumoylation modulates the susceptibility to type 1 diabetes. *Adv Exp Med Biol* 963: 299-322, 2017.
9. Li H, Yang Z, Pu LM, Li X, Ruan Y, Yang F, Meng S, Yang D, Yao W, Fu H, *et al*: Adiponectin receptor 1 and small ubiquitin-like modifier 4 polymorphisms are associated with risk of coronary artery disease without diabetes. *J Geriatr Cardiol* 13: 776-782, 2016.
10. Alzolibani AA, Settin A, Ahmed AA, Ismail H, Elhefni N and Robae AA: Genetic polymorphisms of NFκB1 -94 del/ins ATTG, NFκB1A 2758 A>G and SUMO rs237025 G>A in psoriasis. *Int J Health Sci (Qassim)* 9: 25-33, 2015.
11. Hou S, Kijlstra A and Yang P: The genetics of behçet's disease in a Chinese population. *Front Med* 6: 354-359, 2012.
12. Chen S, Yang T, Liu F, Guo Y, Yang H, Xu J, Song J, Zhu Z and Liu D: Inflammatory factor-specific sumoylation regulates NF-κB signalling in glomerular cells from diabetic rats. *Inflamm Res* 63: 23-31, 2014.
13. Mo YY, Yu Y, Ee PL and Beck WT: Overexpression of a dominant negative mutant Ubc9 is associated with increased sensitivity to anticancer drugs. *Cancer Res* 64: 2793-2798, 2004.
14. Wang CY and She JX: SUMO4 and its role in type I diabetes pathogenesis. *Diabetes Metab Res Rev* 24: 93-102, 2008.
15. Shen QY, Wu HH, Liang JX, XU JY and Liang Y: Clinical significance of SUMO4 expression in papillary thyroid carcinoma. *Chin J Pathophysiology* 31: 1422-1426, 2015.
16. Sobin LH, Gospodarowicz MK and Wittekind C (eds): TNM Classification of Malignant Tumours, International Union Against Cancer. 7th edition. A John Wiley & Sons, Ltd., Publication, 2009. http://www.inen.sld.pe/portal/documentos/pdf/educacion/13072015_TNM%20Classification.pdf.
17. Gillett CE: Immunohistochemistry. *Methods Mol Med* 120: 191-200, 2006.
18. Wilson VG and Rangasamy D: Incellular targeting of proteins by sumoylation. *Exp Cell Res* 271: 57-65, 2001.
19. Sarge KD and Park-Sarge OK: Sumo and its role in human diseases. *Int Rev Cell Mol Biol* 288: 167-183, 2011.
20. Huang HJ, Zhou LL, Fu WJ, Zhang CY, Jiang H, Du J and Hou J: β-Catenin SUMOylation is involved in the dysregulated proliferation of myeloma cells. *Am J Cancer Res* 5: 309-320, 2014.
21. Bellail AC, Olson JJ and Hao C: SUMO1 modification stabilizes CDK6 protein and drives the cell cycle and glioblastoma progression. *Nat Commun* 23: 4234, 2014.
22. Nestal de Moraes G, Ji Z, Fan LY, Yao S, Zona S, Sharrocks AD and Lam EW: SUMOylation modulates FOXK2-mediated paclitaxel sensitivity in breast cancer cells. *Oncogenesis* 7: 29, 2018.
23. Guo D, Li M, Zhang Y, Yang P, Eckenrode S, Hopkins D, Zheng W, Purohit S, Podolsky RH, Muir A, *et al*: A functional variant of sumo4, a new i kappa b alpha modifier, is associated with type 1 diabetes. *Nat Genet* 36: 837-841, 2004.
24. Bohren KM, Nadkarni V, Song JH, Gabbay KH and Owerbach D: A M55V polymorphism in a novel SUMO gene (SUMO-4) differentially activates heat shock transcription factors and is associated with susceptibility to type I diabetes mellitus. *J Biol Chem* 279: 27233-27238, 2004.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.