SOCS3 protein expression predicts the responses of advanced non-small cell lung cancer patients to platinum-based chemotherapy

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Background: This study sought to assess the relationship between suppressor of cytokine signaling 3 (SOCS3) expression, SOCS3 promoter methylation status, and platinum-based chemotherapy responses in advanced non-small cell lung cancer (NSCLC) patients.

Methods: A total of 400 advanced NSCLC patients with inoperable disease were enrolled in this study. All the patients underwent platinum-based chemotherapy treatment, and the clinical and prognostic outcomes of these patients were analyzed. The SOCS3 protein expression and SOCS3 promoter methylation status of the tumor tissues in these patients were also tested by immunohistochemistry and polymerase chain reaction (PCR), respectively. In addition, we knocked down SOCS3 expression via small-interfering RNA (siRNA) in the lung cancer cell lines and conducted *in vitro* analyses to examine cell viability and apoptosis.

Results: Patients with higher expression levels of SOCS3 were found to have a lower average tumor stage, higher average tumor differentiation, and higher rates of positive chemotherapy responses than those with lower expression levels of SOCS3. SOCS3 promoter methylation was also found to be correlated with chemotherapy responses in these patients. In the prognostic analyses, only SOCS3 expression, but not SOCS3 promoter methylation, was found to be predictive of outcomes in advanced NSCLC patients. We also found that the pro-apoptotic effects of SOCS3 were mediated by the Janus kinase 2 (JAK2)/signal transducer and activator of transcription 3 (STAT3) signaling pathways in the lung cancer cells.

Conclusions: Currently, there is a lack of reliable biomarkers for predicting the responses of NSCLC patients to chemotherapy. Our results may aid in clinical evaluations of NSCLC patients.

Keywords: Suppressor of cytokine signaling 3 (SOCS3); non-small cell lung cancer (NSCLC); promoter methylation; treatment response

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Introduction

Non-small cell lung cancer (NSCLC) is a highly prevalent in lung cancer, and has a low 5-year survival rate (1). Most NSCLC patients are diagnosed at an advanced stage, by which time, it is too late for surgical treatment (2). Platinum-based chemotherapy remains the first-line treatment choice for these patients, but its efficacy is highly variable, even in patients with similar clinical manifestations (2). A study has shown that the 5-year survival rate of advanced NSCLC patients who receive chemotherapy is <5% (3). Given that responsiveness to platinum-based chemotherapy is a key determinant of NSCLC patient prognosis, it is essential to identify new novel biomarkers to predict the treatment responses of patients.

The relationship between inflammation and the development of cancer has been well-established in recent years. Pro-inflammatory interleukins (ILs) and other cytokines drive oncogenesis and affect survival in a range of cancer types, including lung cancer (4). Suppressor of cytokine signaling 3 (SOCS3) is a key inhibitor of inflammatory signal transduction (5). SOCS3 upregulation serves to suppress the IL-6/Janus kinase (JAK)/signal transducer and activator of transcription 3 (STAT3) signaling pathway via a negative feedback mechanism and has been linked to numerous cancer types (6,7). SOCS3 mutations are commonly detected in cancers, have been observed in cases of impaired SOCS3 expression in the A549 NSCLC cell line and are related to enhanced migratory activity (8). In patients with non-Hodgkin lymphoma, SOCS3 upregulation at the messenger RNA level in the peripheral blood has been found to be correlated with a lower tumor stage and poorer treatment responses (9). In light of these findings, SOCS3 has been explored as a potential therapeutic target in a variety of cancers. However, the relationship between SOCS3 and chemotherapy responses in patients with advanced NSCLC is uncertain.

Methylation in the promoter region of the *SOCS3* gene frequently occurs in several types of human malignancy, and SOCS3 methylation leads to low-expression of SOCS3. SOCS3 suppression often occurs in human cancers as a consequence of SOCS3 promoter hypermethylation and histone deacetylation in this region (10-12). The association between SOCS3 promoter methylation and platinum-based chemotherapy responses has not been studied yet. Thus, we sought to analyze SOCS3 expression, SOCS3 promoter methylation, and clinical outcomes in advanced NSCLC patients undergoing platinum-based chemotherapy to determine the prognostic relevance of SOCS3 in a therapeutic context. We present the following article in accordance with the MDAR reporting checklist (available at https://atm.amegroups.com/article/view/10.21037/atm-22-6065/rc).

Highlight box

Key findings

- SOCS3 expression and methylation in the promoter as a platinum chemosensitivity marker for NSCLC.

What is known and what is new?

- SOCS3 has been explored as a potential therapeutic target in a variety of cancers.
- However, the relationship between SOCS3 and chemotherapy responses in patients with advanced NSCLC is uncertain.

What is the implication, and what should change now?

- The expression of SOCS3 and promoter methylation status are closely related to platinum resistance in NSCLC, and can be used as a molecular marker for platinum resistance in NSCLC.

Methods

Patient enrollment and chemotherapy

In total, 400 advanced (stage III/IV) NSCLC patients with inoperable disease were enrolled in this study. All the patients were diagnosed with NSCLC by histology or cytology, and staged according to the 7th edition of the tumor-node-metastases (TNM) system (13). All the patients received the following chemotherapy regimen via intravenous injection: cisplatin (75 mg/m^2) on day 1; carboplatin (6.0 mg/mL/min) on day 1; docetaxel (75 mg/m^2) on day 1; gemcitabine (1,250 mg/m^2) on days 1 and 8; vinorelbine (NVB) (25 mg/m^2) on days 1 and 8; and pemetrexed (PEM) (500 mg/m^2) on day 1. These treatment cycles were repeated every 3 weeks, and every patient accepted 6 courses of treatment (14). All the patients who participated in this study signed an informed consent form, and this study was approved by the Ethics Committee of The Third Affiliated Hospital of Southern Medical University (No. 2016-143). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The clinical trial registration number is ChiCTR1900025905.

Assessment of clinical responses and prognosis

The criteria of the World Health Organization (WHO) were used to classify the responses of the patients to the chemotherapy into the following four categories: complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). CR patients were defined as those in whom the measurable lesions disappeared completely following treatment. PR patients were defined as those who exhibited a reduction of ≥50% in the size of the measurable lesions. SD patients were defined as those who exhibited a decrease of <50% or an increase of <25% in the size of the measurable lesions. PD patients were defined as those in whom new lesions developed or in whom measurable lesions grew by ≥25% in size. In our analyses, the CR+PR patients were grouped together and considered to have responded positively to chemotherapy, while the SD + PD patients were grouped together and were considered to be non-responders (15). For the prognostic analyses, patient overall survival (OS) was measured from the start of chemotherapy to the last follow-up or death due to any cause.

Immunohistochemistry

Tumor tissue samples were collected and fixed via biopsy before the initiation of chemotherapy, embedded in paraffin, and used to prepare 5-μm-thick tissue sections. These sections were then stained with primary anti-SOCS3 (1:200, SIGMA, USA), after which 3,3'-diaminobenzidine (DAB) was used to detect SOCS3 staining. Immunoreactive scores (IRSs) were used to assess SOCS3 expression in a semi-quantitative fashion, and the IRSs were used to classify samples as having either low (an IRS of 0–5) or high (an IRS of 6–12) SOCS3 expression (16).

SOCS3 promoter methylation analysis

Samples of genomic DNA were collected from tumor tissues and were subjected to sodium bisulfite modification with a CpGenome Fast DNA Modification Kit (Chemicon International, USA and Canada). These samples were then subjected to methylation-specific polymerase chain reaction (MSP) amplification using primers specific for methylated and unmethylated versions of the SOCS3 promoter as in previous study (17). The following primers, with their positions relative to the transcriptional start site as indicated, were used: SOCS3-(MF) 5'-GAGGGGTCGTTGTTAGGAAC-3' (nt-1265); SOCS3-(MR) 5'-ACAAAAACCGAAAAAAACGC-3' (nt-1176); SOCS3-(UNF) 5'-GGAGGGGTTGTTGTTAGGAAT-3' (nt-1266); SOCS3-(UNR) 5'-CAAAAACAAAAACCAAAAAAAACA-3' (nt-1175). Using 1.5 U hot-start Taq polymerase (Bioron), the polymerase chain reaction (PCR) reactions were conducted in a 25 μL total volume containing 1× PCR buffer (Bioron, Germany), 1.5 mM of $MgCl_2$, 0.2 mM of dNTP, and 0.4 pmol of each primer. A thermocycler (Bio-Rad, USA) was used for amplification at the following settings: 95 ℃ for 5 min; 40 cycles of 95 ℃ for 30 s, 60 ℃ for 40 s, and 72 ℃ for 40 s; 72 ℃ for 10 min. After amplification 10 μL of each sample was separated via electrophoresis using 6% non-denaturing polyacrylamide gels, after which ethidium bromide staining and ultraviolet light were used to visualize the PCR products (17).

Cell culture and transfection

The human H1299 and H1437 NSCLC lines were cultured at 37 ℃ in a 5%-carbon dioxide incubator using Roswell Park Memorial Institute Medium-1640 containing 10% fetal bovine serum (Sigma-Aldrich, MO, USA) and 1% of an antibiotic-antimycotic solution (10,000 units penicillin, 10 mg of streptomycin, and 25 mg/mL of amphotericin B per mL; Sigma-Aldrich). Three small-interfering RNA (siRNA) constructs specific to SOCS3 and 1 control scrambled construct were designed and synthesized by Ribobio (Canton, China). The sequences of these oligonucleotides were as follows: siSOCS3-1 (targeting sequence: 5'-GGACCAAGAACCTACGCAT-3'), 5'-GGACCAAGAACCUACGCAUdTdT-3' (sense), and 5'-AUGCGUAGGUUCUUGGUCCdTdT-3' (antisense); siSOCS3-2 (targeting sequence: 5'-CCAAGAGAGCTTACTACAT-3'), 5'-CCAAGAGAGCUUACUACAUdTdT-3' (sense), and 5'-AUGUAGUAAGCUCUCUUGGdTdT-3' (antisense); siSOCS3-3 (targeting sequence: 5'-GGAAGACTGTCAACGGTCA-3'), 5'-GGAAGACUGUCAACGGUCAdTdT-3' (sense), and 5'-UGACCGUUGACAGUCUUCCdTdT-3' (antisense). Transfections were conducted with GenMute based on the provided directions, and the cells were harvested after 48 h for the downstream analyses.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assays

The sensitivity of the cancer cells to cisplatin was assessed via MTT assays. Briefly, the cells were added to 96-well plates (3,000–4,000 cells per well), after which they were treated for 24 h with 500 μg/mL of cisplatin. Next, 20 μL of MTT (5 mg/mL, Sigma, MO, USA) was added per well, and the plates were incubated for 4 h at 37 ℃. Absorbance was then assessed at 540 nm in each well.

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining

The cells were added to 6-well plates (200,000/well) and were grown for 24 h to 75% confluence, after which an In Situ Cell Death Detection Kit (Roche Applied Science) was used for TUNEL staining in accordance with the instructions provided. Hoechst 33258 (2 μg/mL) was then used to counterstain the cells at room temperature for 30 min, after which aqueous mounting medium was used to cover the cells. Subsequent imaging was performed using a Nikon fluorescence microscope. The rates of cellular apoptosis were calculated as follows: apoptotic rate = total TUNEL positive cell/total cells per field × 100%. All the samples were analyzed in triplicate.

Western blot analysis

Radioimmunoprecipitation assay buffer (Beyotime, Nantong, China) was used to lyse the cells, after which the protein levels in the lysates were measured. Equal amounts of protein from each sample were then separated via 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Millipore, MA, USA). These blots were blocked using 5% non-fat milk before being probed overnight using antibodies specific to SOCS3, phosphorylated JAK2 (p-JAK2), total JAK2 (t-JAK2), phosphorylated STAT3 (p-STAT3), total STAT3 (t-STAT3), caspase-3, or glyceraldehyde-3-phosphate dehydrogenase (all 1:1,000; all from Santa Cruz Biotechnology, CA, USA). An enhanced chemiluminescence detection kit was used to detect the protein bands, and a semi-quantitative densitometric approach was used to quantify the protein expression levels.

Statistical analyses

Associations between SOCS3 expression or promoter methylation and clinical outcomes were compared by chi-squared tests and independent *t*-tests as appropriate. Data from the preclinical experiments assessing the relationship between SOCS3 expression and cisplatin sensitivity were compared using *t*-tests. A P value <0.05 was considered significant, and SAS v9.2 (USA) was used for all the statistical testing.

Results

NSCLC patient characteristics

Of the patients enrolled in this study, 165 were considered to have exhibited good chemotherapeutic outcomes (CR + PR), while the remaining 235 patients were considered to have exhibited poor responses to the treatment (SD + PD). Smoking status differed significantly between the two groups. The "current smokers" comprised a higher proportion of the NSCLC patients with a poor response to chemotherapy (P=0.002). Mean age, sex, TNM stage, and tumor differentiation did not differ significantly between the two groups (all P>0.05; *Table 1*).

SOCS3 expression is associated with NSCLC patient clinical characteristics

We found that SOCS3 expression was primarily restricted to the cytoplasm in the primary NSCLC patient tumor tissue samples (*Figure 1*). Further, 174 patients exhibited high expression levels of SOCS3, while 226 patients exhibited low expression levels of SOCS3. In assessing the relationships between SOCS3 expression and patient clinical characteristics, we found that SOCS3 expression level was significantly associated with tumor stage (P=0.003), tumor differentiation status (P=0.036), and responses to chemotherapy (P=0.001) (*Table 2*). Specifically, patients expressing high levels of SOCS3 had tumors that were more highly differentiated, had a lower tumor stage, and were more likely to exhibit a positive treatment response.

The relationship between SOCS3 promoter methylation and NSCLC patient clinical characteristics

We explored the relationship between SOCS3 promoter methylation and clinical outcomes in NSCLC patients. Methylation status was evaluated via the electrophoresis of MSP products (*Figure 2*). Based on these analyses, 184 and 216 NSCLC patients were determined to have a methylated and unmethylated SOCS3 promoter, respectively. We found

Table 1 The clinical characteristics of NSCLC patients with good and poor responses to chemotherapy

Variables	Good response (n=165)	Poor response (n=235)	P
Age (years), n			0.283
≥55	73	112	
<55	92	123	
Sex, n			0.435
Male	71	98	
Female	94	137	
Smoker, n			0.002
Non-smoker	66	66	
Ever smoker	39	94	
Current smoker	60	75	
TNM stage, n			0.291
IIIB	69	96	
IV	106	129	
Differentiation, n			0.441
Low	61	104	
High	91	144	

NSCLC, non-small cell lung cancer; TNM, tumor-node-metastases.

that SOCS3 methylation status was associated with sex and tumor differentiation status (both P=0.038) (*Table 3*), and chemotherapy response (P<0.001). These data suggest that patients with the unmethylated SOCS3 promoter are more likely to have a positive response to chemotherapy.

SOC3 expression but not promoter methylation predicts the prognosis of NSCLC patients

We next used Kaplan-Meier and log-rank tests to analyze the relationships between clinical variables and patient OS (*Figure 3A*). Compared to patients with high SOCS3 expression, low SOCS3 expression significantly affected OS (26.1±4.2 *vs.* 21.1±2.8 months, P<0.001). However, there was no significant difference between SOCS3 promoter methylation status and patient OS (*Figure 3B*, P=0.125).

Next, Cox proportional hazard models were used to conduct univariate and multivariate analyses to assess the relationship between SOCS3 expression, SOCS3 promoter methylation, pathological findings, and patient prognosis. In the univariate analyses, tumor TNM stage (hazards ratio P=0.015), SOCS3 expression levels (P<0.001), and treatment response (P<0.001) were all significantly related to patient prognosis. In the multivariate analyses, only SOCS3 expression levels (P=0.012) and treatment response were found to predict the OS of NSCLC patients

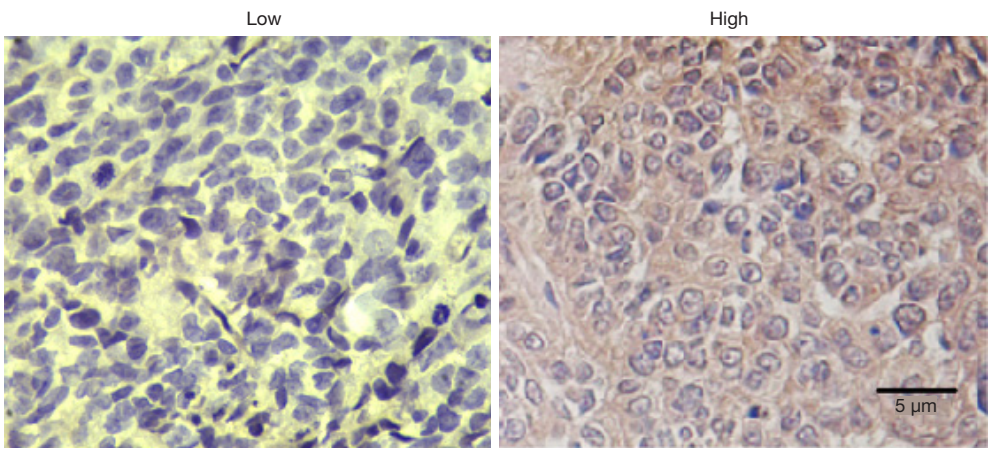

Figure 1 Immunohistochemistry was used to assess SOCS3 expression. Representative images of SOCS3 expression in NSCLC samples, demonstrating that this protein is primarily localized to the cytoplasm. Staining method: IHC. Left: low SOCS3 expression; right: high SOCS3 expression. SOCS3, suppressor of cytokine signaling 3; NSCLC, non-small cell lung cancer; IHC, immunohistochemistry.

(P=0.018; *Table 4*).

Knockdown of SCOS3 enhances lung cancer cell chemosensitivity

There is clear evidence that SOCS3 negatively regulates JAK2/STAT3 signaling in cancer cells (5,6). Thus, we used a siRNA approach to knockdown SOCS3 in lung cancer cells and found that the knockdown of SOCS3 led to the increased expression of caspase-3 and the increased phosphorylation of JAK2 and STAT3. Compared to the control cells, the survival rates of the H1299 and H1437 cells in which SOCS3 had been knocked down were significantly increased (we observed a two-fold increase). Similarly, we determined that SOCS3 knockdown decreased the sensitivity of these NSCLC cells to drug-induced apoptosis, and observed a ~40% reduction in the apoptosis rates of these two cell lines after SOCS3 knockdown compared to that of the control siRNA-transfected cells.

To assess the effects after SOCS3 knockdown of JAK2/STAT3 signaling in the lung cancer cells, we treated these cancer cells with the JAK2/STAT3 inhibitor WP1066 (50 mM) after SOCS3 knockdown. We confirmed that JAK2 and STAT3 phosphorylation were significantly decreased in these cells, as was caspase-3 expression (*Figure 4*). Consistent with these findings, the WP1066 treatment also decreased the survival rates and increased the apoptosis rates of the lung cancer cells, effectively reversing the pro-survival effect of the SOCS3 knockdown.

In the lung cancer cells in which SOCS3 had been knocked down, we found that the WP1066 treatment (50 mM) was able to partially reduce the effects of the SOCS3 knockdown, resulting in reductions in caspase-3 expression and the cell survival rate, and increases in the cellular apoptosis rate (*Figure 5*).

Table 2 The association between SOCS3 expression and the clinical features of NSCLC patients

Variables	Low SOCS3 (n=226)	High SOCS3 (n=174)	P
Age (years), n			0.502
≥55	105	80	
<55	121	94	
Sex, n			0.341
Male	98	71	
Female	128	103	
Smoker, n			0.301
Non-smoker	68	64	
Ever smoker	81	52	
Current smoker	77	58	
TNM stage, n			0.003
IIIB	85	90	
IV	141	84	
Differentiation, n			0.036
Low	131	117	
High	95	57	
Treatment response, n			0.001
Good	66	99	
Poor	160	75	

SOCS3, suppressor of cytokine signaling 3; NSCLC, non-small cell lung cancer; TNM, tumor-node-metastases.

Discussion

In this study, we sought to explore whether SOCS3 could be used as a marker for evaluating the effect of chemotherapy in patients with advanced NSCLC. According to the WHO criteria, we recruited patients with advanced NSCLC and divided them into two groups based on whether they

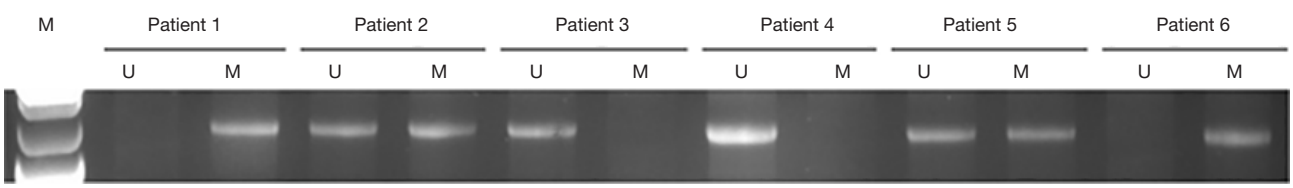

Figure 2 Representative images of SOCS3 promoter methylation status as analyzed by MSP product electrophoresis. Based on these results, patients 1, 2, 5, and 6 were assigned to the methylated group, while patients 3 and 4 were assigned to the unmethylated group. M, methylated; U, unmethylated; SOCS3, suppressor of cytokine signaling 3; MSP, methylation-specific polymerase chain reaction.

Table 3 A comparison between *SOCS3* methylation status and the clinical features of NSCLC patients

Variables	*SOCS3* gene hypermethylation (n=184)	*SOCS3* gene unmethylation (n=216)	P
Age (years), n			0.532
≥55	85	100	
<55	99	116	
Sex, n			0.038
Male	87	82	
Female	97	134	
Smoker, n			0.461
Non-smoker	55	77	
Ever smoker	63	70	
Current smoker	66	69	
TNM stage, n			0.112
IIIB	87	97	
IV	88	128	
Differentiation, n			0.038
Low	79	73	
High	105	143	
Treatment response, n			<0.001
Good	59	125	
Poor	125	111	

SOCS3, suppressor of cytokine signaling 3; NSCLC, non-small cell lung cancer; TNM, tumor-node-metastases.

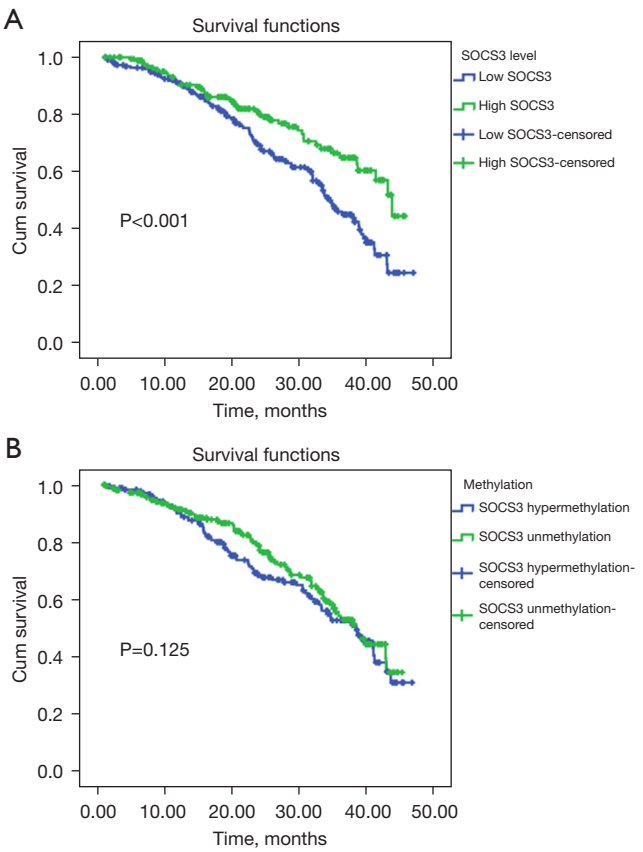

Figure 3 The relationship between SOCS3 expression and promoter methylation status and OS. (A) The relationship between SOCS3 expression and OS; (B) the relationship between SOCS3 promoter methylation and OS. SOCS3, suppressor of cytokine signaling 3; OS, overall survival.

exhibited a good chemotherapy effect (the CR + PR group) or a poor chemotherapy effect (the SD + PD group). By detecting the expression levels of SOCS3 in the tumor tissues of the two groups, we found that the expression levels of SOCS3 in the two groups were significantly correlated with patients' responses to chemotherapy. Specifically, we found that patients with high expression levels of SOCS3 appear to be more likely to show positive responses to chemotherapy.

We also analyzed the relationship between SOCS3 expression levels and patient OS, and found that the prognosis of NSCLC patients was positively correlated to SOCS3 expression levels. We conducted *in vitro* experiments that processed siRNA with SOCS3 knockdown in NSCLC cells, and found that the survival rate of the NSCLC cells knocked down by siRNA was higher than that of the NSCLC cells without SOCS3 knockdown, and the chemosensitivity of the former was also significantly higher. These results indicated that SOCS3 inhibited proliferation while increased NSCLC cells apoptosis. SOCS3 affect the clinical outcome of pediatric acute lymphoblastic leukemia by JAK/STAT pathway. Mechanistically, we found that higher SOCS3 expression levels (without the knockdown of SOCS3 expression) were associated with increased chemosensitivity in lung cancer cells *in vitro* because the JAK2/STAT3 pathway signaling was inhibited by high SOCS3 expression, and the JAK/STAT signaling pathway was involved in the activation of many different cytokine receptors and was ultimately related to cancer cell differentiation, maturation, survival, and proliferation.

Table 4 Identification of the prognostic factors for OS in patients who have undergone TACE treatment

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	P	HR	95% CI	P
Tumor stage	1.56	1.02–4.02	0.015	1.33	0.97–3.98	0.064
SOCS3 expression	2.53	1.33–4.25	<0.001	1.89	1.21–3.48	0.012
Treatment response	2.02	1.15–5.01	<0.001	1.71	1.06–4.32	0.018

OS, overall survival; TACE, transarterial chemoembolization; HR, hazard ratio; CI, confidence interval; SOCS3, suppressor of cytokine signaling 3.

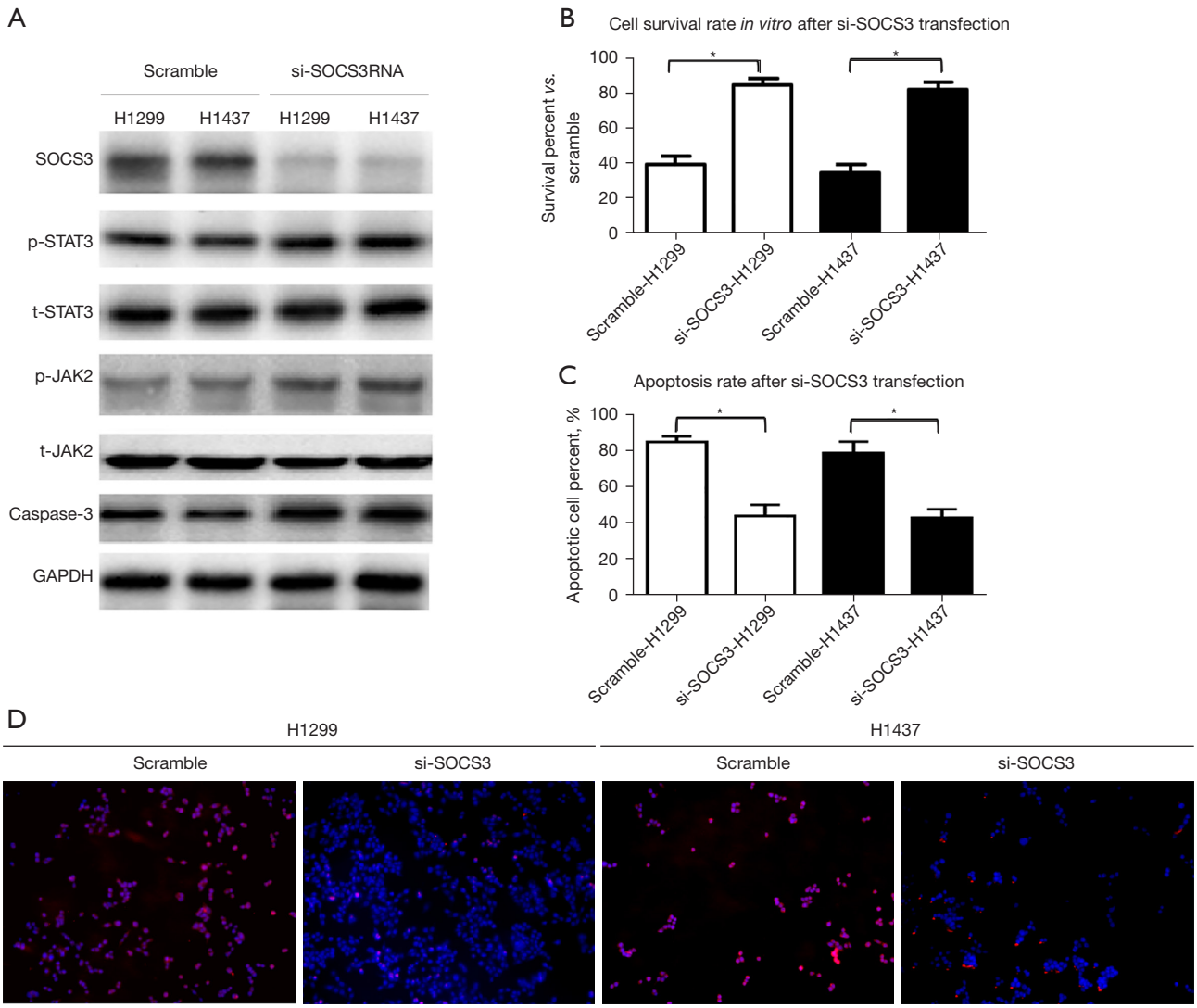

Figure 4 SiRNA-mediated knockdown of SOCS3 (A) enhances JAK2 and STAT3 phosphorylation and the expression of caspase-3 in cultured H1299 and H1437 cells, and is associated with significant increases in the (B) survival rate and (C) drug-induced apoptosis rate of these cells. (D) TUNEL was to assess SOCS3 apoptosis, 200×. *, P<0.05. SOCS3, suppressor of cytokine signaling 3; STAT3, signal transducer and activator of transcription 3; p, phosphorylated; t, total; JAK2, Janus kinase 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; si, small-interfering; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling.

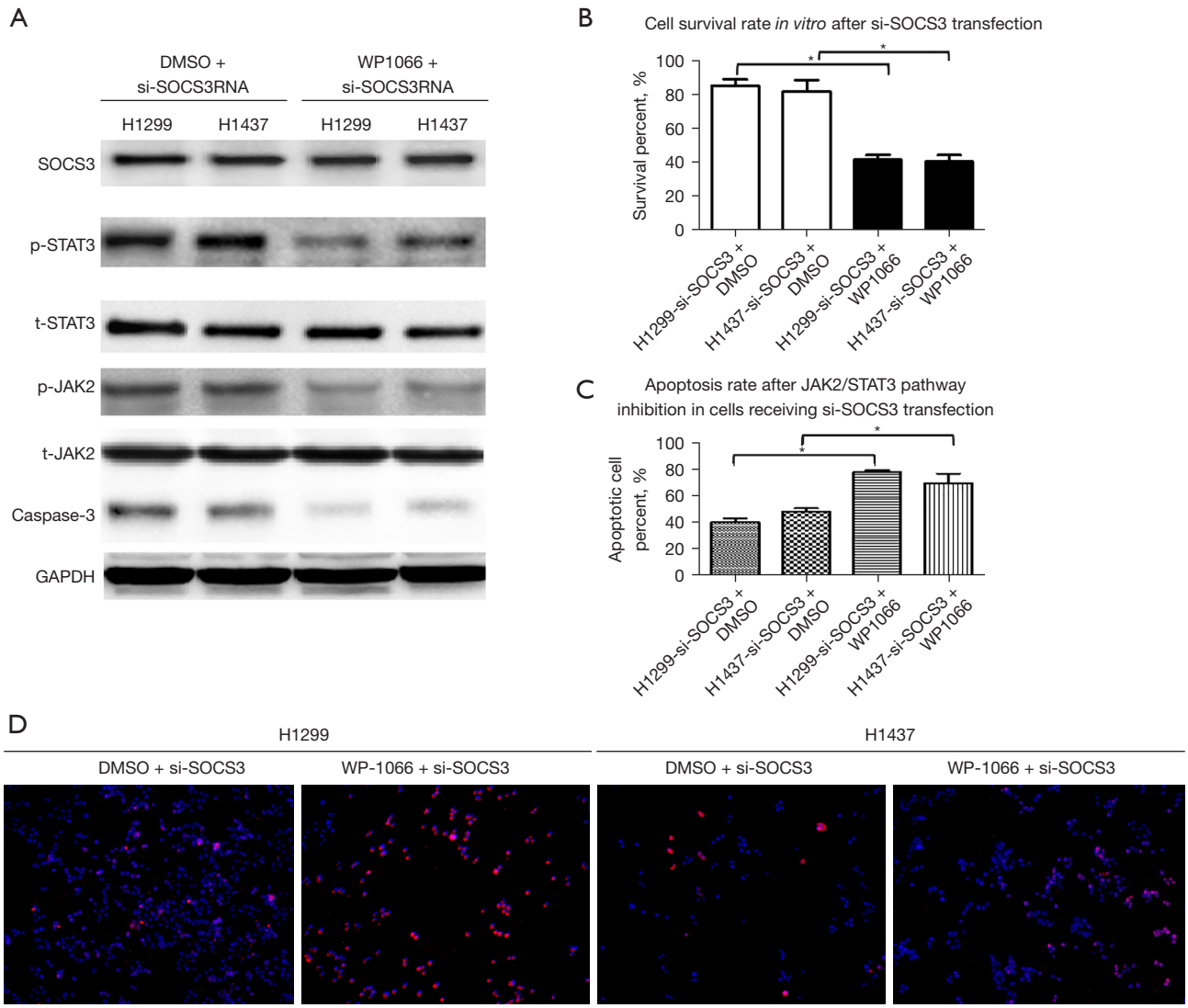

Figure 5 JAK2/STAT3 signal pathway mediates SOCS3 to regulate the death of NSCLC cells. (A) The inhibition of JAK2/STAT3 phosphorylation processed with WP1066 (50 mM) dramatically reduced caspase-3 expression in si-SOCS3-transfected lung cancer cells compared to the control cells. WP1066 treatment impaired the pro-survival effects of SOCS3 knockdown in these lung cancer cell lines as evidenced by (B) reductions in the cell survival rate and (C) increases in the cell apoptosis rate. (D) TUNEL was to assess SOCS3 apoptosis, 200×. *, P<0.05. SOCS3, suppressor of cytokine signaling 3; STAT3, signal transducer and activator of transcription 3; p, phosphorylated; t, total; JAK2, Janus kinase 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; DMSO, dimethyl sulfoxide; si, small-interfering; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; NSCLC, non-small cell lung cancer.

Previous research on SOCS3 protein expression in lung cancer cells reported that SOCS3 is decreased in the A549 lung cancer cell line (7). Another recent study found that the adenoviral overexpression of SOCS3 inhibited the growth of human NSCLC cells while increasing the sensitivity of these cancer cells to radiotherapy (12). SOCS3 upregulation is associated with the impaired proliferation and invasion of lung cancer cells (17). In our study, we found that the expression level of SOCS3 was closely related to the effect of platinum-based chemotherapy and prognosis in advanced NSCLC patients. However, the relationship between SOCS3 expression and the development of NSCLC has not yet been fully elucidated. The present study confirmed that a high expression of SOCS3 may inhibit JAK2/STAT3

pathway signaling, and conversely, may promote the correlation between SOCS3 expression levels and platinum-based chemotherapy in advanced NSCLC patients. Thus, SOCS3 may be a predictive indicator for the treatment of NSCLC patients.

Promoter methylation is a key mechanism involved in the transcription of SOCS3 and other SOCS family genes (18). Thus, SOCS3 promoter methylation status has become an increasingly popular area of research in the field of cancer. In previous study, epigenetic regulation or the silencing of gene expression has been reported to be closely related to many human pathological conditions. In particular, DNA methylation is a relatively conserved regulatory mechanism that is frequently dysregulated in cancer cells and inhibiting the hypermethylation of oncogene promoters and the demethylation of oncogene promoters can rapidly drive cancer progression (19). A cytosine poly guanine (CpG) island methylation phenotype has been observed in some cancers, which is manifested by the simultaneous hypermethylation of multiple loci (20,21). The hypermethylation of the SOCS3 promoter in prostate cancer tissue is associated with decreased enzalutamide efficacy (22). In patients with glioblastoma multiforme, SOCS3 promoter hypermethylation is a favorable prognostic indicator (23). SOCS3 methylation status has also been shown to predict postoperative survival outcomes in patients with hepatocellular carcinoma (24). However, to date, no research has been conducted on the relationship between SOCS3 promoter methylation and responses to platinum-based chemotherapy in NSCLC. Thus, to explore whether the level of SOCS3 methylation is related to the chemotherapy responses of NSCLC patients, this study used PCR technology for the first time to analyze the tumor tissue of two groups of patients.

We analyzed the SOCS3 methylation levels in the tumor tissues of the two groups, and found that the SOCS3 methylation status was correlated with chemotherapy responses, such that patients with unmethylated SOCS3 promoters were more likely to respond positively to chemotherapy. By analyzing the relationship between SOCS3 promoter methylation and OS, we found that SOCS3 methylation status was associated with chemotherapy responses in our NSCLC patient cohort; however, it was not found to be a significant predictor of overall patient outcomes. Thus, SOCS3 methylation may not be a reliable indicator for predicting chemotherapy responses and prognosis in NSCLC patients.

This study had some limitations. First, our sample was small, and mainly comprised Chinese patients with advanced NSCLC. Further study with larger populations needs to be conducted. Second, we only assessed the effect of SOCS3 on chemosensitivity *in vitro* through the JAK2/STAT3 pathway, but some other study has found that SOCS3 inhibits the cell cycle by inhibiting the transcriptional activity of the E2F transcription factor 1 (E2F1)/dodeca-satellite-binding protein 1 (DP-1) transcription factor (25); thus, more studies need to be conducted to elucidate the role of SOCS3 in this respect.

In conclusion, we used the tumor tissues of NSCLC patients to explore the relationship between SOCS3 protein expression levels, SOCS3 promoter methylation, and responses to platinum-based chemotherapy, and found that SOCS3 protein expression levels, but not SOCS3 promoter methylation levels, can predict chemotherapy responses and the prognosis of NSCLC patients.

Conclusions

SOCS3 protein expression and promoter methylation can be reliable biomarkers for predicting the responses of NSCLC patients to chemotherapy.

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Footnote

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aspects of the work, including ensuring that any questions related to the accuracy or integrity of any part of the work have been appropriately investigated and resolved. All the patients who participated in this study signed an informed consent form, and this study was approved by the Ethics Committee of The Third Affiliated Hospital of Southern Medical University (No. 2016-143). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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