Comprehensive analysis of suppressor of cytokine signaling 2 protein in the malignant transformation of NSCLC

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Abstract. Suppressor of cytokine signaling 2 (SOCS2) plays an essential role in a number of physiological phenomena and functions as a tumor suppressor. Understanding the predictive effects of SOCS2 on non-small cell lung cancer (NSCLC) is urgently needed. The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases were used to assess SOCS2 gene expression levels in NSCLC. The clinical significance of SOCS2 was evaluated through Kaplan-Meier curve analysis and the analysis of related clinical factors. Gene Set Enrichment Analysis (GSEA) was used to identify the biological functions of SOCS2. Subsequently proliferation, wound-healing, colony formation and Transwell assays, and carboplatin drug experiments were used for verification. The results revealed that SOCS2 expression was low in the NSCLC tissues of patients in TCGA and GEO database analyses. Downregulated SOCS2 was associated with poor prognosis, as determined by Kaplan-Meier survival analysis (HR 0.61, 95% CI 0.52-0.73; P<0.001). GSEA showed that SOCS2 was involved in intracellular reactions, including epithelial-mesenchymal transition (EMT). Cell experiments indicated that knockdown of SOCS2 caused the malignant progression of NSCLC cell lines. Furthermore, the drug experiment showed that silencing of SOCS2 promoted the resistance of NSCLC cells to carboplatin. In conclusion, low expression of SOCS2 was associated with poor clinical prognosis by effecting EMT and causing drug resistance in NSCLC cell lines. Furthermore, SOCS2 could act as a predictive indicator for NSCLC.

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Abbreviations: SOCS2, suppressor of cytokine signaling 2; NSCLC, non-small cell lung cancer; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; TCGA, The Cancer Genome Atlas; GEO, Gene Expression Omnibus; GSEA, Gene Set Enrichment Analysis; EMT, epithelial-mesenchymal transition; CCLE, Cancer Cell Line Encyclopedia

Key words: SOCS2, EMT, NSCLC, predictive indicator, prognosis

Introduction

In recent years, lung cancer has become the second most common type of cancer, accounting for 14% of all cancer cases in men and 12% in women, and the leading cause of oncological death (27% for men and 25% for women) globally (1). Lung cancer includes small cell lung cancer (SCLC) and non-SCLC (NSCLC). NSCLC accounts for 85% of lung cancer cases around the world (2). Lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) are the most common histological types of NSCLC (3). There is an urgent need to explore potential molecules or targets that could be used to predict the malignant transformation of NSCLC.

Cytokine and growth factor signals, as the key signals in the process of cell proliferation, bind to cell receptors and produce a cascade reaction to stimulate corresponding changes (4). Suppressor of cytokine signaling (SOCS)1-7 and the cytokine-inducible Src homology 2 (SH2)-domain containing protein are the main members of the SOCS family, and include a central SH2 domain, a variable N-terminal domain and a C-terminal SOCS box (5). SOCS2 is a regulator of numerous cytokines and signaling factors, and abnormal regulation of SOCS2 leads to cancer in most cases (6). Notably, the expression levels of SOCS2 have been reported to be decreased in liver cancer, which affects the invasion and migration of liver cancer, and the prognosis of patients of liver cancer (7). Furthermore, SOCS2 affects prostate cancer by mediating androgen and growth hormone signaling (8). The expression levels of SOCS2 are also decreased in prostate cancer and SOCS2 is associated with its progression (9). SOCS2 can also be used as a predictive indicator for colorectal cancer (10).

Epithelial-mesenchymal transition (EMT) is the process by which epithelial cells lose their cell polarity and ability to adhere to other cells, and gain migratory and invasive properties, becoming mesenchymal stem cells (11). The effects of EMT have been investigated (12,13). EMT serves an important role in tumor metastasis and recurrence, as it breaks down cell-cell and cell-extracellular matrix connections to facilitate cancer cell migration through the extracellular matrix. This allows the tumor to reach new areas. Cancer cells convert early-stage tumors into a dedifferentiated, more malignant state via EMT. Previous studies have demonstrated that EMT is a necessary and key process affecting NSCLC (14-16).

In our previous study, Gene expression data for NSCLC was downloaded from The Cancer Genome Atlas (TCGA) (17) and Gene Expression Omnibus (GEO) (18) databases. The results showed that the expression of SOCS2 was low in NSCLC, and Gene Set Enrichment Analysis (GSEA) demonstrated that SOCS2 was related to metabolism, proliferation, EMT and other physiological behaviors of NSCLC. To the best of our knowledge, there has been little focus on the effects of SOCS2 on NSCLC. The aim of the present study was to investigate SOCS2 as a potential predictive indicator.

Materials and methods

Database resources and gene expression. NSCLC data from the GSE7670 (lung cancer), GSE10072 (LUAD), GSE19188 (NSCLC), GSE19804 (lung cancer), GSE27262 (NSCLC), GSE32863 (LUAD), GSE43458 (LUAD) and GSE75037 (LUAD) datasets were downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/gds/?term=) (19-26). 'limma' and 'impute' (http://www.bioconductor.org/packages/release/data/annotation/html/org.Hs.eg.db.html) packages (R package) were used to explore the expression of SOCS2. The difference in SOCS2 expression was shown using a violin plot, which was drawn using R software (4.0.5). In addition, gene expression data from 1,145 samples were downloaded from TCGA database (https://cancergenome.nih.gov/); 1,037 were tumor samples (LUAD and LUSC) and 108 were paracancerous samples. The difference in expression of SOCS2 between paired normal adjacent tissue and tumor tissue was analysed by the 'BiocManager' (http://www.bioconductor.org/packages/release/data/annotation/html/org.Hs.eg.db.html) packages (R package). Gene expression data and gene mutation data of pan-cancer were downloaded through ucsc xena database (https://xenabrowser.net/). The expression levels of SOCS2 in pan-cancer were analyzed using the 'ggpubr' package (http://www.bioconductor.org/packages/release/data/annotation/html/org.Hs.eg.db.html). Tumor mutation burden (TMB) of SOCS2 in pan-cancer was explored and depicted in a radar chart via 'fmsb' package (http://www.bioconductor.org/packages/release/data/annotation/html/org.Hs.eg.db.html). A protein-protein interaction (PPI) network was generated to show the relationship between SOCS2 and other proteins using the STRING database (http://string-db.org/cgi/input.pl). The relationship between SOCS2 and the clinical prognosis of NSCLC was researched by performing a log-rank test in the KM-plotter database (https://kmplot.com/analysis/) using the following dataset ID: 232539_at (SOCS2). Clinical factors related to SOCS2 were evaluated through UALCAN database (http://ualcan.path.uab.edu/). The expression levels of SOCS2 in different NSCLC cell lines were analyzed using the Cancer Cell Line Encyclopedia website (https://sites.broadinstitute.org/ccle).

Cell culture. The LUAD A549 and wild-type LUSC H520 cell lines were purchased from the American Type Culture Collection. Both A549 and H520 cells were cultured in RPMI-1640 medium (Hyclone; Cytiva). 293T cells were cultured in high-glucose DMEM (both from HyClone; Cytiva). The two types of medium were supplemented with 10% fetal bovine serum (FBS; HyClone; Cytiva). The cells were incubated in an atmosphere containing 5% CO_2 at 37˚C.

Silencing SOCS2. For small interfering RNA (siRNA)-induced SOCS1 silencing, H520 and A549 cells were plated at a density of $3x10^5$/60-mm dish. After 24 h of culture, the cell culture medium was replaced with fresh medium. The cells were then transfected with SOCS2 siRNAs or control-siRNA (non-targeting control) purchased from Transheepbio (Shanghai Quanyang Biotechnology Co., Ltd.) using jetPRIME® transfection reagent (Polyplus-transfection SA) at 37˚C. The final concentration of siRNA or control-siRNA was 0.05 μM. The duration of transfection was 24 h and the duration between transfection and subsequent experimentation was 72 h. The siRNA sequences were as follows: Control-siRNA, sense, 5'-UUCUCCGAACGUGUCACGUdTdT-3' and antisense, 5'-ACGUGACACGUUCGGAGAAdTdT-3'; SOCS2, sense, 5'-GCUGAAGCUAAUCUAAUUUdTdT-3' and antisense, 5'-AAAUUAGAUUAGCUUCAGCdTdT-3'.

For short hairpin RNA-SOCS2 (shSOCS2)-induced silencing of SOCS2, a second generation system used. The A549 and H520 cells were cultured for an additional 48 h in 10% FBS-RPMI 1640 medium. 293T cells were transfected with virus packaging plasmids (psPAX2; Addgene, Inc.; cat. no. 12260, 5 μg; pMD2.G, Addgene, Inc.; cat. no. 12259; 2 μg) and pLenti-EF1a-mcherry-P2A-Puro-CMV-MCS-3 Flag (control; 5 μg) or SOCS2 knockout plasmids (5 μg) by transfection reagent (jetPRIME® in vitro DNA and siRNA transfection reagent; Polyplus-transfection® SA). The multiplicity of infection used to infect cells was 5. After the 293T cells were transfected for 48 h at 37˚C, the supernatant was collected and added to A549 and H520 cells for 24 h. The A549 and H520 cells were transfected with plasmid using jetPRIME transfection reagent according to the manufacturer's instructions. The duration of transduction was 3 days. A549 and H520 cells were selected using puromycin (4 ng/μl) for 1 week. The time interval between transduction and subsequent experimentation was 2-3 weeks. The concentration of puromycin used for maintenance was 4 ng/μl.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) in compliance with the manufacturer's standard procedures. RNA samples were reverse transcribed into cDNA using a PrimeScript RT kit (Takara Biotechnology Co., Ltd.) according to the manufacturer's recommendations. The LightCycler 480 Real-time PCR System (Roche Diagnotics) was used to detect the mRNA expression levels. The cDNA was analyzed in RT-qPCR using the SYBR

Green PremixPro Taq HS qPCR Kit (Jiangsu Accuracy Biotechnology Co., Ltd.). The qPCR cycling conditions were as follows: Denaturation at 95˚C for 30 sec (one cycle); PCR at 95˚C for 5 sec, 55˚C for 30 sec and 72˚C for 30 sec (40 cycles); melting at 95˚C for 5 sec, 60˚C for 1 min and 95˚C for 10 min (one cycle); cooling at 50˚C for 30 sec (one cycle). The qPCR primers were designed using Primer3 (primer3.ut.ee/). Each sample was tested three times and 18S ribosomal RNA expression was used to normalize the results. The comparative threshold cycle value method, $2^{-\Delta\Delta Cq}$ (27), was used to evaluate the results. The primer sequences were as follows: 18S ribosomal RNA, forward 5'-AAACGGCTACCACATCCAAG-3' and reverse 5'-CCTCCAATGGATCCTCGTTA-3'; SOCS2, forward 5'-CTAAGGCTGACCAAGACCTGTTGA-3' and reverse 5'-GCCTGGACCTTTGACATAATGGA-3'.

Western blot analysis. A549 and H520 cells were lysed with RIPA lysis buffer (Bio-Rad Laboratories, Inc.). Equal amounts of protein (10 μg protein/lane) were separated by SDS-PAGE (resolving layer, 10%; stacking layer, 5%) and transferred onto PVDF membranes (Pall Life Sciences). The membranes were blocked in 5% bovine serum albumin (BovoStar; Bovogen Biologicals Pty Ltd.) for 1 h at room temperature and then incubated with primary antibodies against SOCS2 (cat. no. ab109245; dilution, 1:1,000; Abcam) and GAPDH (cat. no. ab9485; dilution, 1:1,000; Abcam) overnight at 4˚C. Subsequently, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies [dilution, 1:10,000, cat. nos. ZB-2305 (goat anti-mouse) and ZB-2301 (goat anti-rabbit); OriGene Technologies, Inc.] for 1 h at room temperature after washing. The protein bands were visualized using an enhanced chemiluminescence (ECL) detection reagent (WesternBright™ ECL; cat. no. 180805-33; Advansta, Inc.).

Colony formation assay. H520 and A549 were suspended with pancreatin (0.25% Trypsin-EDTA 1X; Gibco; Thermo Fisher Scientific, Inc.) and counted. The cells were then added into 60-mm dishes (1,000 cells/dish) containing 5 ml culture medium and incubated at 37˚C in an atmosphere containing 5% CO_2 and saturated humidity for 7-14 days. Cell culture was terminated when macroscopic cell colonies were visible on the dishes. After discarding the medium, PBS was used to wash the cells, which were then fixed with 4% paraformaldehyde for 30 min at 25˚C and stained with 0.1% crystal violet for 30 mins at 25˚C. Finally, excess dye was gently washed from the cells with running water and the cells were observed under an optical microscope (magnification, x400; Leica Microsystems GmbH). Colony images were obtained by scanning the culture dishes.

Wound-healing assay. Pancreatin (0.25% Trypsin-EDTA 1X; Gibco; Thermo Fisher Scientific, Inc.) was used to lyse the cells. The floating cells were then added into a 6-well plate ($5x10^5$ cells/well). After the 6-well plate was at 100% confluence, the cell layer was scratched using a 100-μl micropipette tip. The cells were then cultured in RPMI-1640 medium containing 1% FBS. Images of the wound widths were obtained under an optical light microscope at 0, 6, 24, 30 and 36 h (Leica Microsystems GmbH).

Cell proliferation assay. A sulforhodamine B (SRB) assay was used to assess cell proliferation. Cells were cultivated in 96-well plates (1,500 cells/well) in an incubator at 37˚C. After the cells adhered to the plate, the 96-well plate was removed from the incubator and cell proliferation was assessed every 12 h. After removing the medium, 60 μl 10% cold trichloroacetic acid (TCA) was added to each well for fixation at room temperature. After all of the samples were fixed, the cold TCA was removed and the plate was washed five times with tap water. After drying overnight at room temperature, 60 μl 0.057% (wt/vol) SRB solution (MilliporeSigma) was added to each well for 1 h at room temperature, after which, 1% acetic acid was used to wash each plate five times. The plates were again dried overnight at room temperature, 150 μl 10 mM Tris base solution was added to each well and the plates were shaken for 20 sec at 2,000 rpm. The cell absorbance was measured at 562 nm using a microplate reader (Thermo Fisher Scientific, Inc.). GraphPad Prism7 (Dotmatics) was used to analyze all results of the cell proliferation assay.

Drug treatment. A549 and H520 cells were cultivated in 96-well plates (3,000 cells/well). Different concentrations (A549: 0, 0.05, 0.1, 0.15 and 0.2 mM; H520: 0, 0.05, 0.1 and 0.2 mM) of carboplatin (MedChemExpress) were added to the plates at 37˚C. The SRB was subsequently performed as aforementioned.

Transwell assay. Matrigel (Clontech; Takara Bio USA) was used to coat the Transwell inserts (Corning, Corning, NY) for the Transwell invasion assay at room temperature for 1 h. The lower chamber of the Transwell insert (pore size, 8.0 μm; 24-well plates) was filled with 1,000 μl RPMI-1640 medium containing 20% FBS (HyClone; Cytiva), and ~$4x10^4$ cells and RPMI-1640 medium without FBS were added to the upper chamber. After the cells were cultured for 24 h, the liquid in the upper chamber was discarded. The chamber was removed and a wet cotton swab was used to remove the cells from the upper chamber. Subsequently, the cells on the lower membrane were fixed for 20 min with 100% methanol, stained with 0.1% crystal violet for 20 min and washed using PBS at room temperature. Images of the Transwell assay were obtained under an optical light microscope (Leica Microsystems GmbH).

GSEA. GSEA was used to explore gene functions. The NSCLC data of 1,145 patients from TCGA database were downloaded and analyzed using GSEA software (http://www.gsea-msigdb.org/gsea/downloads.jsp). GSEA software explores gene function based on gene expression levels in cells. The gene expression of SOCS2 matched the gene function, GSEA provided the relationship between the functions and SOCS2 gene. The present study used the gene set database, c5.all.v6.2.symbols.gmt.

Statistical analysis. GraphPad Prism 7 was used to analyze the data. Data are presented as the mean ± standard deviation of three independent experimental repeats. For experimental data analysis, comparisons between two sets of data were performed using unpaired Student's t-test, whereas one-way ANOVA, followed by Dunnett's multiple comparisons test, was used for the statistical analysis of more than two groups. For gene expression analysis of UALCAN data, comparisons

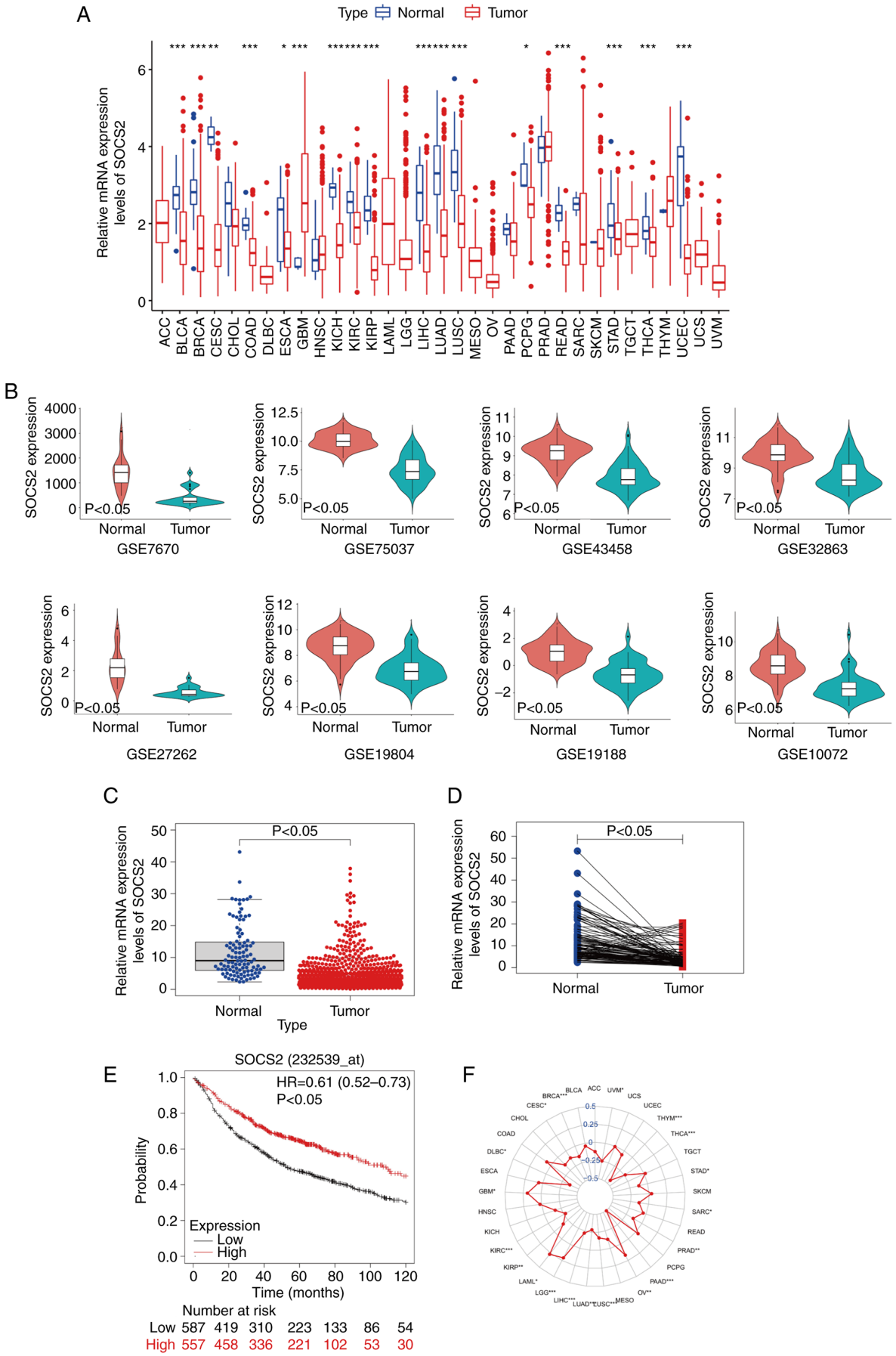

Figure 1. SOCS2 expression is low in NSCLC. (A) Expression levels of SOCS2 in pan-cancer analysis. *P<0.05, **P<0.01, ***P<0.001. (B) Differential expression of SOCS2 between normal and tumor tissue in eight Gene Expression Omnibus datasets. (C and D) Expression levels of SOCS2 were low in NSCLC by TCGA database. (E) Kaplan-Meier curve analysis (P<0.001) of SOCS2 indicated that low expression of SOCS2 mediates poor prognosis. (F) Radar chart of SOCS2 tumor mutational burden in different tumor types. NSCLC, non-small cell lung cancer; SOCS2, suppressor of cytokine signaling 2.

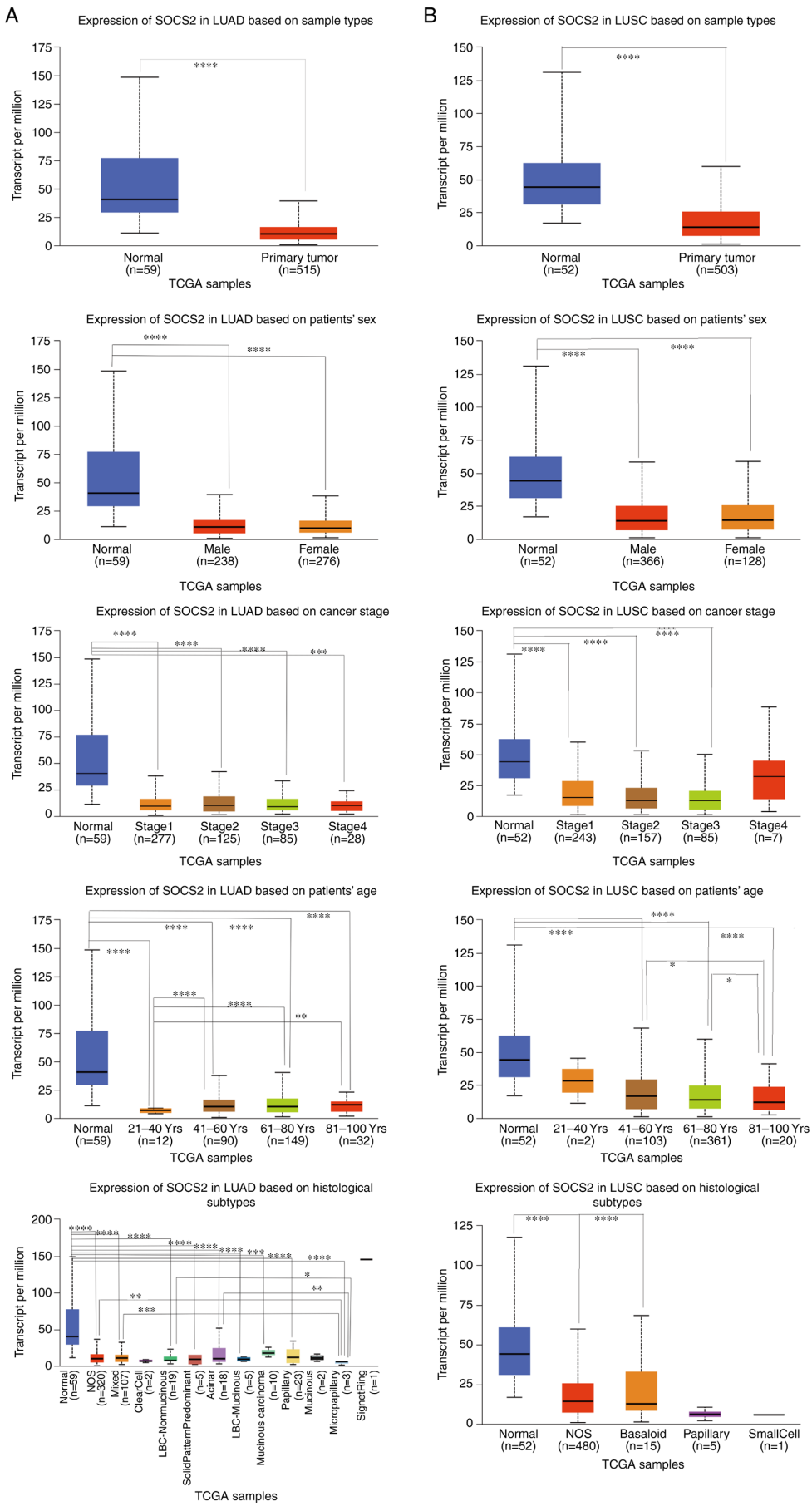

Figure 2. Clinical factors related to SOCS2. (A) Main clinical factors (sample type, age, sex, cancer stage, histological subtype) associated with SOCS2 in LUAD. (B) Main clinical factors (sample type, age, sex, cancer stage, histological subtype) associated with SOCS2 in LUSC. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; SOCS2, suppressor of cytokine signaling 2; TCGA, The Cancer Genome Atlas.

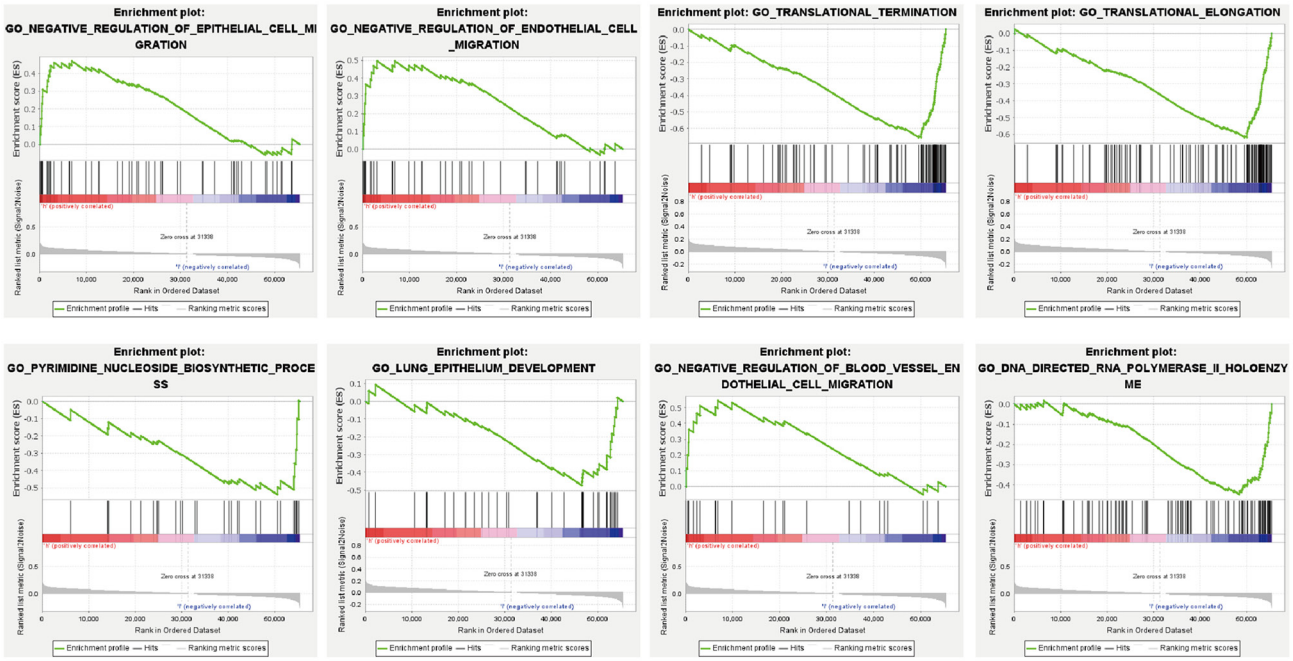

Figure 3. Gene Set Enrichment Analysis of suppressor of cytokine signaling 2.

between two sets of data were performed by unpaired t-test using a PERL script with the Comprehensive Perl Archive Network module 'Statistics::TTest' (http://search.cpan.org/~yunfang/Statistics-TTest-1.1.0/TTest.pm), whereas more than two groups were compared using the Kruskal-Wallis test followed by Dunn's multiple comparisons test (28). All P-values were two-sided and P<0.05 was considered to indicate a statistically significant difference.

Results

SOCS2 expression is low in NSCLC samples. The expression of SOCS2 was different in each dataset (P<0.05) as shown below. The expression data of SOCS2 in different tumor types was integrated and analyzed, and the results revealed that the expression levels of SOCS2 were lower in cancer tissues than in normal tissues in most cancer types (Fig. 1A; LUAD, P<0.001 and LUSC, P<0.001). In addition, the GSE7670, GSE10072, GSE19188, GSE19804, GSE27262, GSE32863, GSE43458 and GSE75037 datasets were downloaded from the GEO database. The difference in SOCS2 expression from the eight datasets is shown in violin plots (Fig. 1B). Data from 1,037 NSCLC tissues and 108 normal paracancerous tissues were downloaded from TCGA; the expression information for 56,485 genes was available for these samples. The expression of SOCS2 in normal tissues and tumor tissues was shown in a scatter difference map (Fig. 1C; P<0.001) and a paired difference map (Fig. 1D; P<0.001); the results revealed that the expression of SOCS2 was lower in NSCLC tumor tissues compared with that in normal tissues. The TMB of SOCS2 in different tumors was shown in a radar chart, and the results suggested that gene mutations of SOCS2 in LUAD (P<0.001) and LUSC (P<0.001) may have caused its low expression (Fig. 1F). These results indicated that SOCS2 was highly expressed in normal tissues and lowly expressed in tumor tissues.

Low expression of SOCS2 mediates poor prognosis. A Kaplan-Meier survival analysis showed that the high expression of SOCS2 was a protective factor for patients with NSCLC, whereas low expression of SOCS2 mediated a poor prognosis (Fig. 1E; P<0.001). For both LUAD and LUSC, the clinical factors related to SOCS2 included stage, sample type, TP53 mutation status, nodal metastasis status, histological subtypes, smoking habit, age, sex and ethnicity (Figs. 2 and S1). These findings suggested that SOCS2 has an important effect on the prognosis of patients with NSCLC.

SOCS2 is associated with EMT. GSEA was performed to further understand the effect of SOCS2 on NSCLC. GSEA software provided scores based on the pathways associated with gene function and gene expression level. SOCS2-related functions included 'EMT', 'mesenchymal cell differentiation', 'regulation of endothelial cell differentiation', 'cell proliferation', 'migration' and 'angiogenesis' (Fig. 3). It was thus hypothesized that SOCS2 was related to the progression of NSCLC and mediated EMT.

Silencing of SOCS2 leads to malignant transformation. SOCS2 expression levels were detected in different NSCLC cell lines using data provided by the CCLE (Fig. 4A), and it was revealed that the expression levels of SOCS2 were relatively high in H520 and A549 cells. RT-qPCR and western blot analysis were used to verify the silencing effects of SOCS2 siRNAs and shSOCS2 on the A549 and H520 cell lines. According to the experimental results, SOCS2 siRNA (Figs. 4B and S2) and shSOCS2 (for longer duration experiments; Figs. 5A and S3) were successful at silencing SOCS2 expression in A549 and H520 cells. The two bands of GAPDH may be due to excessive voltage. A549 and H520 cells transduced with stable shSOCS2 plasmids underwent a cell proliferation assay. Knockdown of SOCS2 promoted the

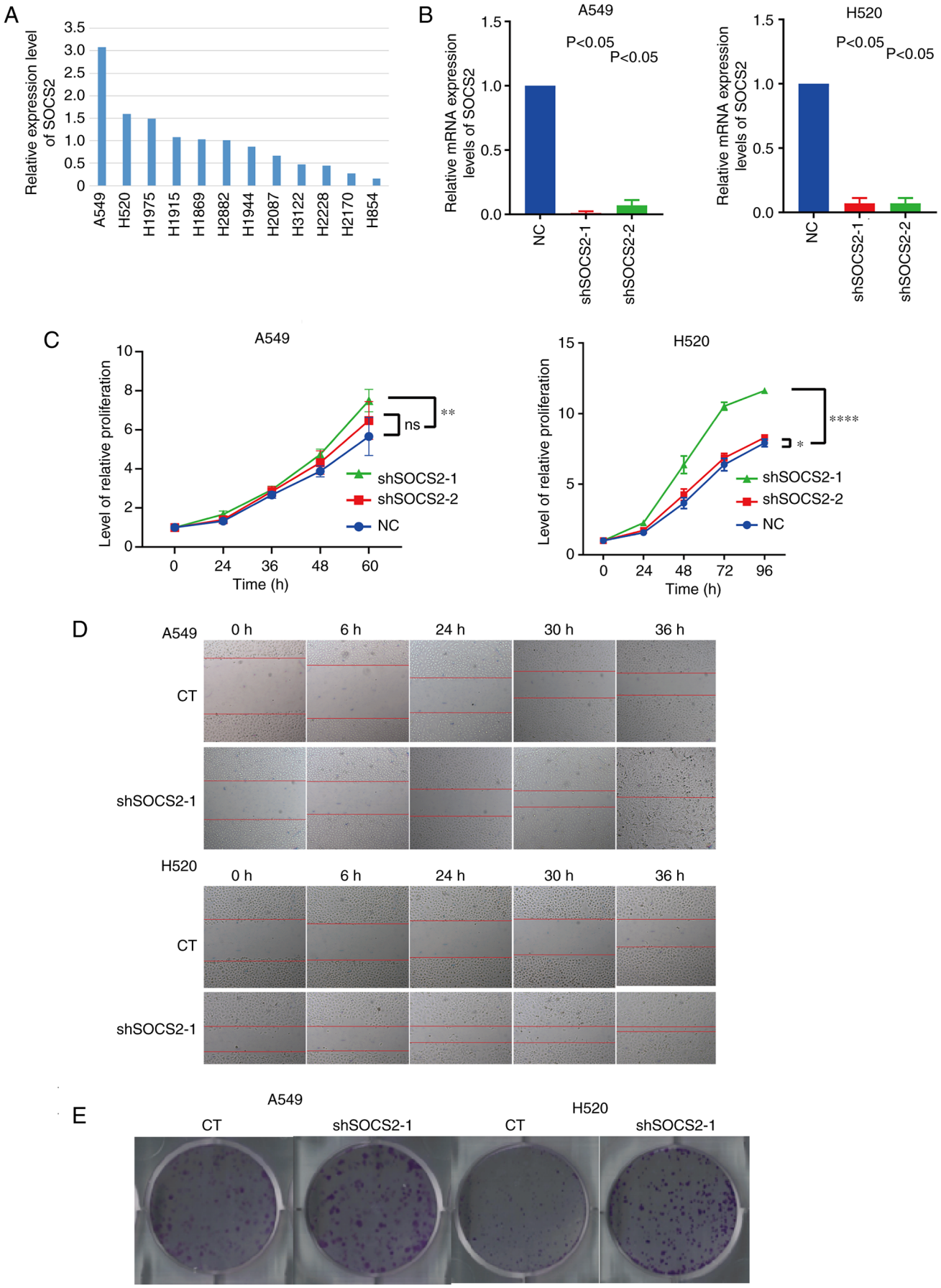

Figure 4. Knockdown of SOCS2 inhibits non-small cell lung cancer cell proliferation and migration. (A) SOCS2 relative expression in different cell lines. (B) Relative expression of SOCS2 after knockdown mediated by shSOCS2. (C) Effect of SOCS2 knockdown on cell proliferation. *P<0.05, **P<0.01, ****P<0.0001. (D) Wound-healing assay and (E) colony formation assay revealed that that knockdown of SOCS2 promoted cell migration. Magnification, x400. CT, control; shSOCS2, short hairpin RNA SOCS2; SOCS2, suppressor of cytokine signaling 2.

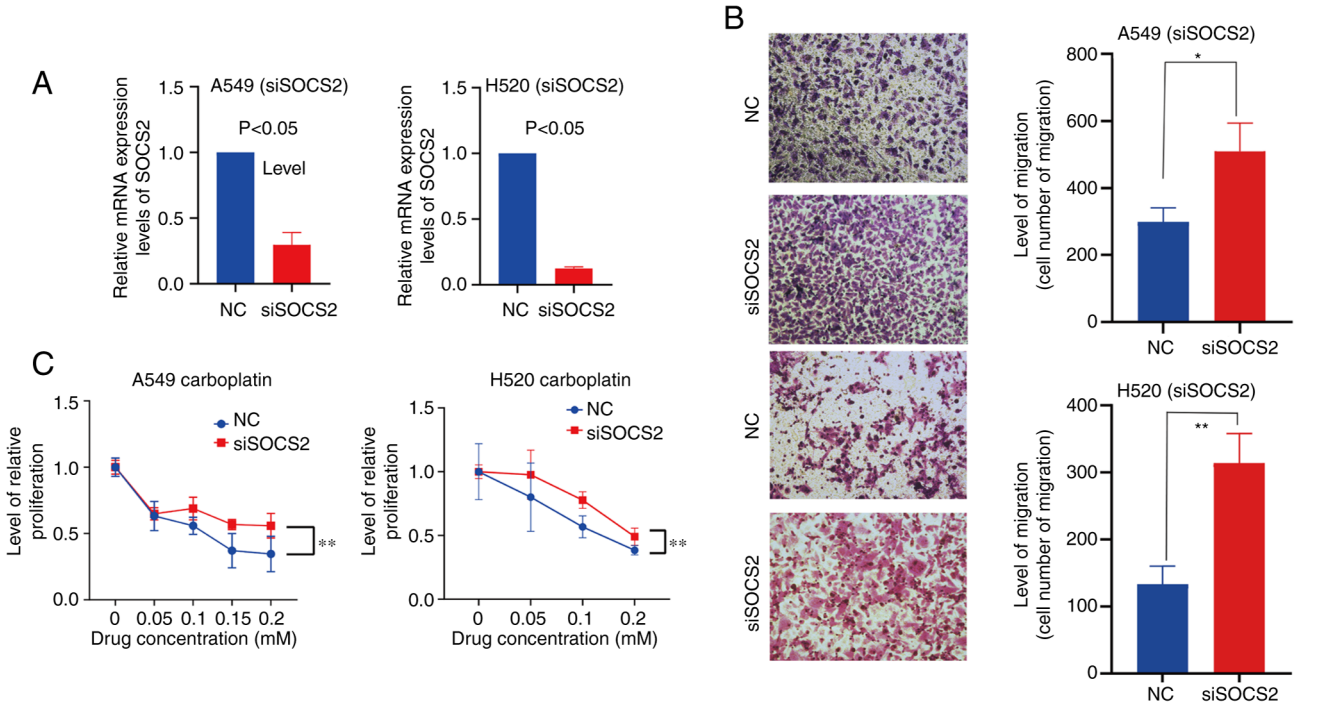

Figure 5. Knockdown of SOCS2 inhibits NSCLC cell migration and promotes drug resistance. (A) Relative expression of SOCS2 after knockdown mediated by siRNA. (B) Transwell assay was used to determine migration in the NC group and siSOCS2 group. Magnification, x400. (C) SOCS2 knockdown promoted carboplatin resistance of NSCLC cell lines. *P<0.05, **P<0.01. NC, negative control; NSCLC, non-small cell lung cancer; si, small interfering; SOCS2, suppressor of cytokine signaling 2.

proliferation of NSCLC cells (Fig. 4C). A549 knocked out with shSOCS2-2 did not have significant proliferation levels, This may be due to the poor state of the cells, which can be considered a normal experimental error. A549 and H520 cells transduced with stable shSOCS2 plasmids were used for the wound-healing assay, and images of cell migration were captured at 0, 6, 24, 30 and 36 h (Fig. 4D). The results showed that the migration ability of A549 and H520 cells was stronger when SOCS2 was knocked down compared with that in the control cell lines. The colony formation assay was performed in A549 and H520 cell lines transduced with stable shSOCS2 plasmids and showed that silencing SOCS2 could accelerate proliferation (Fig. 4E). In addition, A549 and H520 cells transfected with a SOCS2 siRNA underwent a Transwell assay. When SOCS2 was silenced, the invasion ability of cells was increased (Fig. 5B). These findings indicated that silencing SOCS2 could promote the EMT of LUAD (A549) and LUSC (H520) cells. In addition, the SOCS2 gene is likely a tumor suppressor gene and low SOCS2 expression may cause a poor prognosis.

Silencing SOCS2 promotes carboplatin resistance. A549 and H520 cells transfected with a SOCS2 siRNA underwent treatment with different concentrations of carboplatin (A549: 0, 0.05, 0.1, 0.15 and 0.2 mM; H520: 0, 0.05, 0.1, and 0.2 mM). The results revealed that increasing concentrations of carboplatin inhibited A549 and H520 cell proliferation. However, silencing SOCS2 reduced the sensitivity of NSCLC to carboplatin (Fig. 5C); the cell lines in which SOCS2 was silenced exhibited increased proliferation compared with the control groups.

Discussion

Although there are a number of treatments for NSCLC, including surgery (29), chemotherapy (30), radiotherapy (31), targeted medical treatments (32) and immunotherapy, the 5-year survival rate of NSCLC is low (33). Clinical cancer therapy often uses biomarkers to diagnose and predict the prognosis of NSCLC. Molecular oncology has confirmed that ~73.9% of global patients with NSCLC have lung cancer driver gene mutations, including common mutations in EGFR, ALK and ROS1 genes, and rare mutations in the MET gene (34). At present, to the best of our knowledge, no biological markers have been used to screen patients with NSCLC in clinical studies of neoadjuvant immunotherapy. In the future, if markers such as PD-L1 expression or TMB are included in screening criteria, the efficacy of neoadjuvant immunotherapy may be further improved (35-39). Current studies related to biomarkers in NSCLC are lacking, which means that patients with NSCLC may not be diagnosed and treated in a timely manner (40).

The present study aimed to identify predictive indicators of NSCLC. Gene expression data from patients with LUAD and LUSC were downloaded from TCGA and GEO databases, and were analyzed. The results revealed that SOCS2 gene expression was low in NSCLC samples. Previous studies have suggested that SOCS2 can inhibit the metastasis and invasion of cancer, and that for cancer to develop it needs to overcome the interference of tumor suppressor genes, which may include SOCS2. This may explain why SOCS2 is poorly expressed in liver cancer (41), prostate cancer (42) and laryngeal squamous cell carcinoma (43).

We hypothesized that SOCS2 may also have a protective effect on patients with NSCLC, or may act as a predictive indicator for NSCLC. GSEA revealed that SOCS2 may be involved in a number of intracellular physiological reactions, including proliferation, invasion, migration and angiogenesis. Thus, it was inferred that SOCS2 could have an inhibitory effect on the progression of NSCLC, which is similar to the findings of previous studies (44-47). The PPI map of SOCS2 was consistent with that described in previous research and SOCS2 was revealed to be associated with the JAK/STAT signaling pathway, which promotes cancer progression (Fig. S3) (48).

Colony formation, wound-healing, cell proliferation and Transwell assays were performed to further verify our hypothesis. The results revealed that knockdown of SOCS2 resulted in increased invasion, migration and proliferation, which is consistent with the aforementioned hypothesis.

After verifying the effect of SOCS2 on NSCLC in EMT by colony formation assay, wound healing assay, cell proliferation assay and Transwell assay, the present study further assessed its effect on drug resistance. Patients with advanced-stage NSCLC have a median overall survival time of 4-5 months and chemotherapy is still the standard treatment for these patients (49). Carboplatin has been widely used as an anticancer drug, which mainly acts by forming adducts with DNA and inducing apoptosis; however, such platinum drugs can activate some signaling pathways, and cause adverse reactions and drug resistance (50). Therefore, further study on the resistance to platinum drugs would be beneficial to patients with cancer. Since SOCS2 was revealed to be involved in DNA replication, damage repair and some signaling pathways (GSEA analysis), we hypothesized that SOCS2 was related to carboplatin resistance. The results of the drug treatment experiment confirmed that knockdown of SOCS2 could reduced the resistance of NSCLC cells to carboplatin. The results suggested that SOCS2 may inhibit cell proliferation and DNA replication as a tumor suppressor gene, whereas silencing SOCS2 may cause uncontrolled cell proliferation. The drug treatment experiment indirectly confirmed that SOCS2 may act as a tumor suppressor gene that can be used as a predictor for the prognosis of NSCLC, and we hypothesize that the mechanism underlying the effects of SOCS2 may potentially be associated with the control of DNA replication.

The present study has several limitations. First, SOCS2 expression was silenced, but no experiments were performed in cells overexpressing SOCS2, which may affect the conclusions. Second, a LUAD cell line (A549) and a LUSC cell line (H520) were used for *in vitro* experiments only, the lack of *in vivo* experiments mean that the effects of SOCS2 in a living animal were not assessed. Third, there was no in-depth study on the mechanism underlying the effects of SOCS2 on NSCLC proliferation, migration and drug resistance. It was only speculated through drug experiments that SOCS2 was related to DNA replication; nevertheless, such speculation may inspire future research.

In conclusion, low SOCS2 expression was associated with poor clinical prognosis, via its role in EMT, and could cause drug resistance in NSCLC cell lines. SOCS2 may act as a predictive indicator for NSCLC.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the TCGA and GEO repository, [https://cancergenome.nih.gov/; https://www.ncbi.nlm.nih.gov/gds/?term=]. All other datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JD, GM and YZ designed the experiments and performed experiments and analyzed data. WZ, XZ, FB and GW collected and processed the data. YZ wrote and edited the article. All authors contributed to data analysis, drafting and revising of the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work. JD and GM confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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