Association between methylation of tumor suppressor gene SOCS1 and acute myeloid leukemia

XIAO-HUI ZHANG, LIN YANG, XIAO-JUN LIU, YING ZHAN, YU-XIA PAN, XING-ZHE WANG and JIAN-MIN LUO

Department of Hematology, The Second Hospital of Hebei Medical University, Key Laboratory of Hematology, Shijiazhuang, Hebei 050000, P.R. China

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Abstract. Suppressor of cytokine signaling-1 (SOCS1) is a widely recognized tumor suppressor gene. Silencing of SOCS1 expression as a result of promoter methylation is associated with occurrence and development of solid tumors such as liver, cervical and pancreatic cancer. However, the association between SOCS1 gene methylation and acute myeloid leukemia (AML) has not been well explored. In the present study, we examined whether gene expression and methylation status of SOCS1 was altered in AML, and whether this was related to disease occurrence and development. To assess this hypothesis, we analyzed SOCS1 in four groups of AML patients: i) Initial treatment group (IT); ii) relapsed/refractory group (RR); iii) remission group (RE); and iv) normal control group (NC). We also used leukemia cell lines U937 and THP-1 to study the underlying molecular mechanism of SOCS1 in AML, mainly the JAK2/STAT pathway. We used several techniques such as quantitative PCR (qPCR), methylation-specific PCR (MS-PCR), western blotting, flow cytometry and cell transfection techniques to analyze the expression and methylation status of SOCS1. We found that the SOCS1 gene methylation rate in the IT and RR groups was significantly higher than that in the RR and NC groups (48, 80 vs. 0 and 0%, respectively). Furthermore, mRNA and protein expression was significantly lower in the IT and RR groups when compared to the RE and NC groups. We also found that the JAK2/STAT signaling pathway was negatively affected by SOCS1. SOCS1 gene methylation caused gene silencing of SOCS1 which overcame the suppression of the downstream JAK2/STAT signaling pathway by SOCS1, and promoted the growth and proliferation of AML cells.

Correspondence to: Professor Jian-Min Luo, Department of Hematology, The Second Hospital of Hebei Medical University, 215 Heping West Road, Shijiazhuang, Hebei 050000, P.R. China
E-mail: luojm315@163.com

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Introduction

Acute myeloid leukemia (AML) is characterized by malignant proliferation of hematopoietic cells of myeloid lineage. AML has poor prognosis and effective treatments are still lacking despite rigorous research efforts. For AML patients receiving initial treatment, the remission rate can reach as high as 80% after receiving normal chemotherapy, however for some patients, AML will reoccur (1), and the recurrent AML has a poorer prognosis (2). Accurate diagnosis and treatment requires cytogenetics as a prediction tool, and gene overexpression and silencing may provide necessary information.

The suppressor of cytokine signaling-1 (SOCS1) gene, a tumor suppressor gene, belongs to the suppressor of cytokine signaling (SOCS) family. SOCS1 negatively regulates cytokines via the JAK/STAT3 pathway by a negative feedback loop. The SOCS1 gene in humans is located on 16p13.3 and codes for a protein with 221 amino acids. Numerous studies have ascertained that the promoter of SOCS1 is located on the CpG island of the 5'-end this gene, and its abnormal methylation can result in silencing of SOCS1 expression (3). Silencing of SOCS1 expression is associated with carcinogenesis, especially in malignant tumors and proliferative diseases of the hematopoietic system (4).

SOCS1 suppresses the JAK2/STAT signaling pathway to negatively control the expression of cytokines (5) by several mechanisms. SOCS1 combined with phosphorylated tyrosine in the SH2 region blocks activation of JAK2 and transduction of cell signaling (6). Furthermore, the KIR region upstream of the SH2 region can directly act on JAK2 kinase to inhibit binding of the kinase with the substrate, thereby suppressing its activity. In addition, an E3 ubiquitin ligase complex can be formed in the SOCS-Box region at the SOCS1 C-terminal to cause proteasomal degradation of cytokine signal transduction proteins including JAK2 (7). In the present study, we investigated the status of SOCS1 expression in AML patients and the relationship between SOCS1 silencing resulting from methylation and AML occurrence and development.

Materials and methods

Patients. Between February 2015 and October 2017, samples from 110 patients diagnosed with AML and 10 normal controls were obtained from the Department of Hematology at The Second Hospital of Hebei Medical University. Patient clinical characteristics are shown in Table I.

The initial treatment group (IT) included patients who were initially diagnosed, but did not receive any treatment. Patients in the relapsed/refractory group (RR) relapsed after a complete remission or were not yet relieved after treatment of two courses. Patients in the remission group (RE) included those who were fully relieved after treatment. Patients in the normal control group (NC) included those who were healthy or with nutritional anemia. The diagnosis and classification of AML was performed according to French-American-British (FAB) criteria, and included M_0 (2 patients), M_1 (9 patients), M_2 (50 patients), M_4 (36 patients) and M_5 (13 patients).

The research specimens were studied from bone marrow cells which were isolated by lymphocyte separation. This study was approved by the Ethics Committee of Hebei Medical University and written informed consent was obtained from each patient.

Cell lines. Human AML cell lines U937 and THP-1 were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). U937 and THP-1 cells were cultured in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), supplemented with 10% fetal bovine serum (FBS; Clark Bioscience, Claymont, DE, USA) and incubated at 37˚C in a humidified atmosphere of 5% CO_2 in air.

Cell viability and apoptosis assay. Cell viability was assessed by Cell Counting Kit-8 (CCK-8; Beijing Zoman Biotechnology Co., Ltd., Beijing, China) according to the manufacturer's instructions. U937 and THP-1 cells were plated in 96-well plates at a density of $1\text{x}10^4$ cells/well to investigate the proliferation curves and viability curves effected by 5-azacytidine (5-azaC) and 5-aza-2'-deoxycytidine (5-aza-dC; both from Sigma-Aldrich; Merck, St. Louis, MO, USA) for 48 h. The cell medium was changed every day. The absorbance at a wavelength of 450 nm was assessed using a microplate reader (Molecular Devices Co., Sunnyvale, CA, USA). The assay was performed in triplicate wells and each experiment was repeated three times.

The apoptosis of treated and transfected cells was determined using the FITC Annexin V Apoptosis Detection kit (BD Biosciences, San Jose, CA, USA). The treatment concentrations of 5-azaC and 5-aza-dC in U937 cells were 1, 2, 5 and 10 μmol/l respectively, and those in THP-1 were 10, 20, 50 and 100 μmol/l, respectively. Data were analyzed using BD FACSDiva software v6.1.3 (BD Biosciences, San Jose, CA, USA).

Real-time PCR. Total RNA from bone marrow mononuclear cells and AML cell lines was isolated using RNeasy mini kit (Qiagen Inc., Valencia, CA, USA). cDNA was synthesized from RNA using The RevertAid™ First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.). PCR was performed using the SuperReall PreMix Plus (SYBR-Green) (Tiangen Biotech Co. Ltd., Beijing, China). PCR was carried out by heating at 95˚C for 10 min, followed by 40 cycles at 95˚C for 10 sec, 60˚C for 20 sec, and 72˚C for 15 sec, with a final step for 10 min at 72˚C. β-actin was used as an internal control. The relative gene expression was analyzed by the $2^{-\Delta\Delta Cq}$ method (8) against the NC group which was used as a control. Values were normalized to the expression level of β-actin. The primer sequences are shown in Table II.

Methylation-specific PCR (MSP). Extraction and bisulphite modification of genomic DNA (2 μg) was performed using a DNA extraction kit (Shanghai Generay Biotech Co. Ltd., Shanghai, China) and the EZ DNA Methylation-Gold™ kit (Zymo Research Biotech Co., Irvine, CA, USA) according to the manufacturer's instructions. The cycling conditions were: 10 min at 95˚C, 40 cycles of 30 sec at 95˚C, 40 sec at 58˚C, 45 sec at 72˚C and 72˚C for 10 min. The original cDNA sequence was checked in the gene pool and the primers were designed according to a previously published study (9). PCR products were resolved in 2% agarose gel, stained with ethidium bromide and visualized under UV illumination.

Western blot analysis. Cells were washed three times with PBS and lysed with RIPA buffer (BestBio, Shanghai, China). Total proteins (50 μg) per sample were isolated by 10% SDS-PAGE and then transferred to polyvinylidene fluoride (PVDF) membranes (Millipore Corp, Billerica MA, USA). The membranes were probed with antibodies for SOCS1 (1:1,000; cat. no. 3950), t-JAK2 (1:1,000; cat. no. 4040), p-JAK2 (1:1,000; cat. no. 4406), t-STAT3 (1:1,000; cat. no. 9139), p-STAT3 (1:1,000; cat. no. 52075), t-STAT5 (1:1,000; cat. no. 25656), p-STAT5 (1:1,000; cat. no. 4322) and β-actin (1:2,000; cat no. 3700), and then incubated with anti-rabbit IgG, horseradish peroxidase (HRP)-linked secondary antibody (1:3,000; cat. no. 3700) (all from Cell Signaling Technology, Inc., Danvers, MA, USA) for 1 h for chemiluminescent detection. Specific bindings were visualized with Azure c500 (Azure Biosystems, Dublin, CA, USA).

Cell transfection. Full-length SOCS1 (NM-003745.1) cDNA was synthesized and cloned into pCMV3-C-GFPSpark vector (Sino Biological Inc., Beijing, China). The sequence and orientation of the SOCS1 insert was confirmed by DNA sequencing. pCMV3-SOCS1-GFPSpark was then transfected into U937 and THP-1 cells by Lipofectamine 3000 reagent (1:1 ratio) (Invitrogen; Thermo Fisher Scientific, Inc.). Cells were incubated for 2 days at 37˚C. The empty vector was used as control.

Statistical analysis. Statistical analysis was performed with SPSS software (version 21.0; IBM Corp., Armonk, NY, USA). All data are presented as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with a Fisher's least significant difference and a Chi-squared were used to compare the data. A P-value of <0.05 was considered to indicate a statistically significant difference.

Results

SOCS1 gene mRNA expression and methylation state in AML groups. We first investigated the mRNA expression of SOCS1 in the four groups of AML patients. We found that SOCS1 gene expression was significantly lower in the IT and RR groups when compared to the RE and NC groups (P<0.05) (Fig. 1A).

Table I. Characteristics of patients.

Variables	IT	RR	RE	NC
Male/female (n/n)	22/28	8/2	21/29	5/5
Median age (range)	48.4 (19-67)	45.3 (17-66)	34.8 (22-64)	50 (19-66)

IT, initial treatment group; RR, relapsed/refractory group; RE, remission group; NC, normal control group.

Table II. Primer sequences.

Genes	Primers (5'-3')	Size (bp)
SOCS-1	F: GACGCCTGCGGATTCTAC	
	R: AGCGGCCGGCCTGAAAG	181
DNMT3A	F: TATTGATGAGCGCACAAGAGAGC	
	R: GGGTGTTCCAGGGTAACATTGAC	111
DNMT3B	F: GGCAAGTTCTCCGAGGTCTCTG	
	R: TGGTACATGGCTTTTCGATAGGA	113
MECP2	F: ACTCCCCAGAATACACCTTGCTT	
	R: TGAGGCCCTGGAGGTCCT	113
MBD2	F: AACCCTGCTGTTTGGCTTAAC	
	R: CGTACTTGCTGTACTCGCTCTTC	101
β-actin	F: GAGCTACGAGCTGCCTGAC	
	R: GGTAGTTTCGTGGATGCCACAG	121
SOCS-1, M-MSP	F: TTCGCGTGTATTTTTAGGTCGGTC	
	R: CGACACAACTCCTACAACGACCG	160
SOCS-1, U-MSP	F: TTATGAGTATTTGTGTGTATTTTTAGGTTGGTT	
	R: CACTAACAACACAACTCCTACAACAACCA	175

F, forward; R, reverse.

Relative expression of SOCS1 mRNA in each group was found to be 0.0306±0.0137 for IT, 0.0164±0.0101 for RR, 1.3346±0.4852 for RE and 1.5983±0.3891 for NC. Fold change differences were compared to NC values. Subsequently, we compared SOCS1 methylation in the four groups. Methylation of SOCS1 was not detected in the RE and NC groups, but it was detected in 24 (48%) IT patients and 8 (80%) RR patients. The frequency of methylation was significantly higher in the IT, RR groups compared with the RE and NC group ($P<0.05$) (Fig. 1B). Thus, SOCS1 gene methylation was negatively correlated with mRNA expression.

Expression of the SOCS1 methylation-related gene. AML patients in the IT group were divided into a SOCS1 methylation group (ME) (24 cases) and a non-methylation group (NM) (26 cases) according to the methylation state of the SOCS1 gene. The NC and RR groups included 10 cases. We found that the mRNA of DNA methyltransferases (DNMTs) such as DNMT1 and DNMT3a in the SOCS1 ME and RR group was higher than that in the NM and NC group ($P<0.05$) (Fig. 1C). There was no change in the mRNA expression of DNMT3b. In addition, gene expression of methylated CpG binding protein MeCP2 was higher in the ME and RR groups than in the NM and NC group ($P<0.05$). MBD2 in the ME and RR groups was significantly higher than that of the NM and NC groups, however, the difference was of no statistical significance ($P>0.05$).

Expression of the SOCS1 protein and downstream pathway proteins. The relative expression of the SOCS1 protein in the AML IT and RR groups was significantly lower than that in the RE and NC groups ($P<0.05$) (Fig. 2A and B). In contrast, p-JAK2, p-STAT3 and p-STAT5 expression was significantly higher in the IT and RR groups ($P<0.05$) in comparison to the RE and NC groups. There was no difference in the expression of t-JAK2, t-STAT3 and t-STAT5 among the four groups.

Cell viability and the apoptosis rate of AML cell lines in response to demethylation drugs. The half maximal inhibitory concentration (IC_{50}) of 5-azaC on U937 and THP-1 cell lines as determined by the cell viability assay was found to be 0.95 and 17.05 μmol/l, respectively, and that of 5-aza-dC was 4.79 and 43.55 μmol/l, respectively (Fig. 3A). With the increase of drug concentration and duration of drug treatment

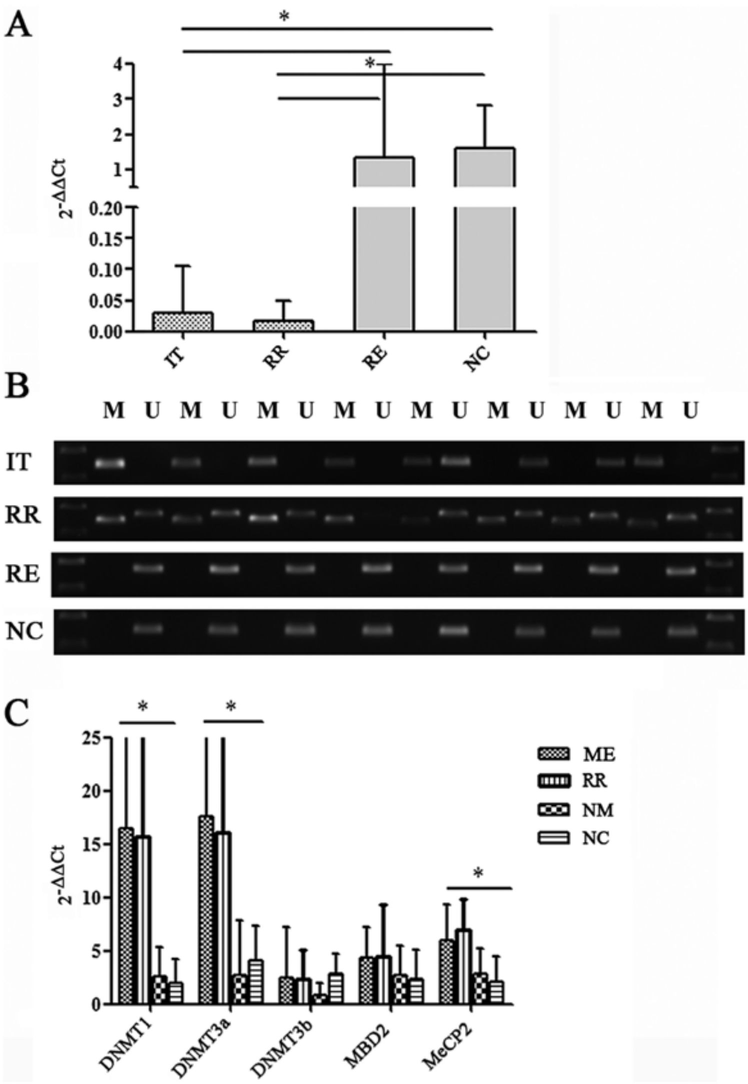

Figure 1. Relative gene expression of SOCS1 and SOCS1 methylation-related genes. (A) qPCR results of SOCS1 in IT, RR, RE and NC groups. Total RNA was extracted from bone marrow mononuclear cells and gene expression was detected with the qPCR method. Fold change difference was compared to a normal control group using the $2^{-\Delta\Delta Cq}$ method. Data represent the mean ± SD from 50 samples in the IT group, 50 samples in the RE group, 10 samples in the RR group and 10 samples in the NC group. *P<0.05. (B) SOCS1 gene methylation state. M indicates the methylation strip, U indicates the non-methylation strip. Eight cases were randomly selected from the IT, RR, RE and NC groups. (C) Relative expression of mRNA of SOCS1 methylation-related genes. Gene expression of DNMT1, DNMT3a and MeCP2 genes. Data is presented as the mean ± SD and is presented as fold change differences between ME, NM, RR and NC. *P<0.05. ME, methylation group; NM, non-methylation group; RR, relapsed/refractory group; RE, remission group; NC, normal control group.

(time <3 days), the viability of U937 and THP-1 cells gradually decreased (Fig. 3B). However, the apoptosis rate of U937 and THP-1 cells gradually increased with the increase of drug concentration (Fig. 3C and D). The results indicated that the viability of the two cell lines was negatively associated with the drug concentration and treatment time, while the apoptosis rate of the two cell lines was positively associated with the drug concentration.

SOCS1 gene expression and methylation state in AML cell lines in response to demethylation drugs. AML cell lines were treated with four concentrations of demethylation drugs. We observed an increase in SOCS1 mRNA expression in response to 5-azaC and 5-aza-dC in a dose dependent manner in both U937 and THP-1 cells (Fig. 4A). Statistical differences existed among the untreated group and the treated groups (P<0.05). Following intervention with demethylation drugs, the non-methylation strip of the SOCS1 gene in the U937 and THP-1 cell lines was light while the methylation strip was darker. As the concentration increased, SOCS1 completely transformed from a methylated state to an unmethylated state (Fig. 4B). As the concentration of the demethylation drugs increased, the mRNA expression of the SOCS1 gene increased, and the relative expression of methylation-related genes DNMT1, DNMT3a, MBD2 and MeCP2 gradually decreased. The expression of DNMT1 and DNMT3a in the U937 cells treated with 5-aza-dC (1 μmol/l) was not significantly different compared with that in the untreated group (P>0.05), while the expression of DNMT1, DNMT3a, MBD2 and MeCP2 in the 5-azaC- and 5-aza-dC-treated U937 and THP-1 cells was significantly different between the untreated group and the drug-treated groups (P<0.05) (Fig. 4C). As the concentration of the demethylation drugs increased, the relative expression of the SOCS1 protein gradually increased, while downstream

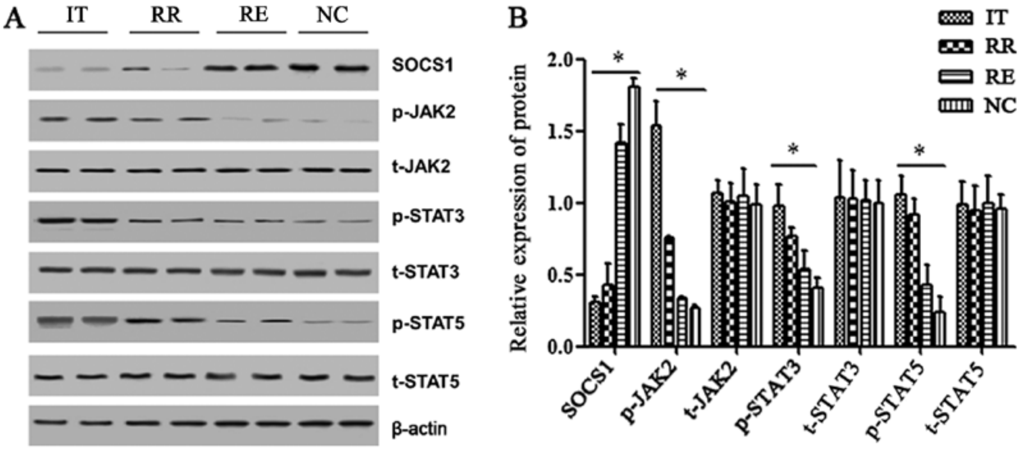

Figure 2. Relative expression of the SOCS1 protein and downstream pathway proteins. (A) Protein expression of SOCS1 and the downstream signaling pathway proteins in each group was determined by western blotting. (B) Relative expression of proteins. Values were normalized against β-actin. The quantification was performed using ImageJ 1.8 software (National Institutes of Health, Bethesda, MD, USA). *P<0.05.

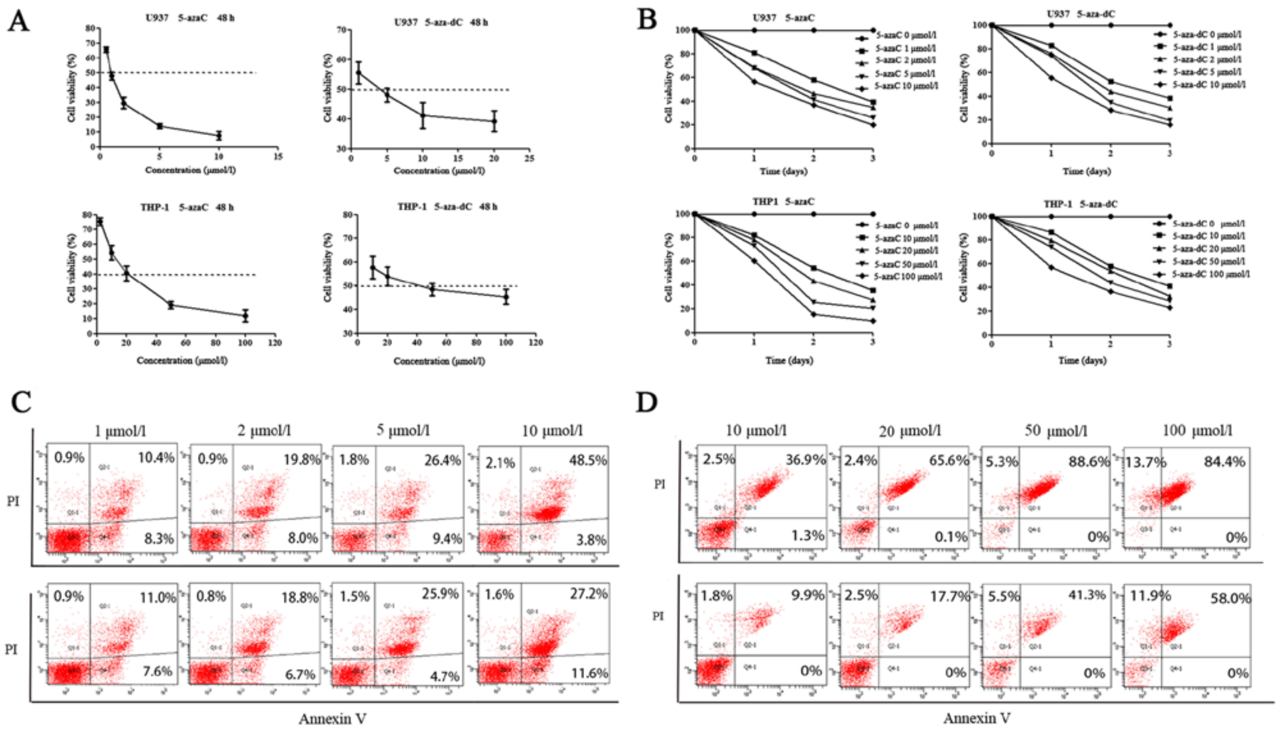

Figure 3. Cell viability and apoptosis of AML cell lines. (A) Cell viability curves of AML cell lines. U937 and THP-1 cells were treated with increasing concentrations of the drugs 5-azaC and 5-aza-dC for 48 h. (B) Cell proliferation curves of AML cell lines. U937 and THP-1 cells were treated with increasing concentrations of the drugs 5-azaC and 5-aza-dC for 3 days. Cell viability was determined by Cell Counting Kit-8 (CCK-8; Beijing Zoman Biotechnology). (C and D) Apoptosis induced by 5-azaC and 5-aza-dC. Flow cytometric analysis of cell apoptosis detected by Annexin V staining in response to increasing doses of 5-azaC and 5-aza-dC treatment for 48 h in (C) U937 cells and (D) THP-1 cells. Cells in lower right quadrant (Annexin V^+, PI^-) were viable apoptotic cells and those in upper right quadrant (Annexin V^+, PI^+) were non-viable apoptotic cells. The total percentage of cells in the two quadrants represents the apoptosis rate of cells. Cells in the upper left quadrant (Annexin V^-, PI^+) were dead cells.

p-JAK2, p-STAT3 and p-STAT5 protein expression gradually decreased, which was negatively associated with the SOCS1 protein. The expression of the p-STAT3 protein between the untreated group and the drug-treated groups was statistically different (P<0.05) except for the 5-azaC low concentration group (1 or 10 μmol/l). Statistical differences in p-JAK2 and p-STAT5 protein expression existed among the untreated group and the treated groups (P<0.05). t-JAK2, t-STAT3 and t-STAT5 protein expression was not markedly altered (Fig. 5).

Protein expression in SOCS1-transfected AML cell lines is altered. SOCS1 was overexpressed in U937 and THP-1 cell lines transfected with the pCMV3-C-GFPSpark vector, and overexpression was confirmed by fluorescence as well as by western blotting (Fig. 6A and D). As time increased (time <3 days), the viability of transfected cells gradually decreased and the apoptosis rate gradually increased (Fig. 6B and C). We found that in cells overexpressing SOCS1, there was a significant decrease in the expression of p-JAK2, p-STAT3 and p-STAT5

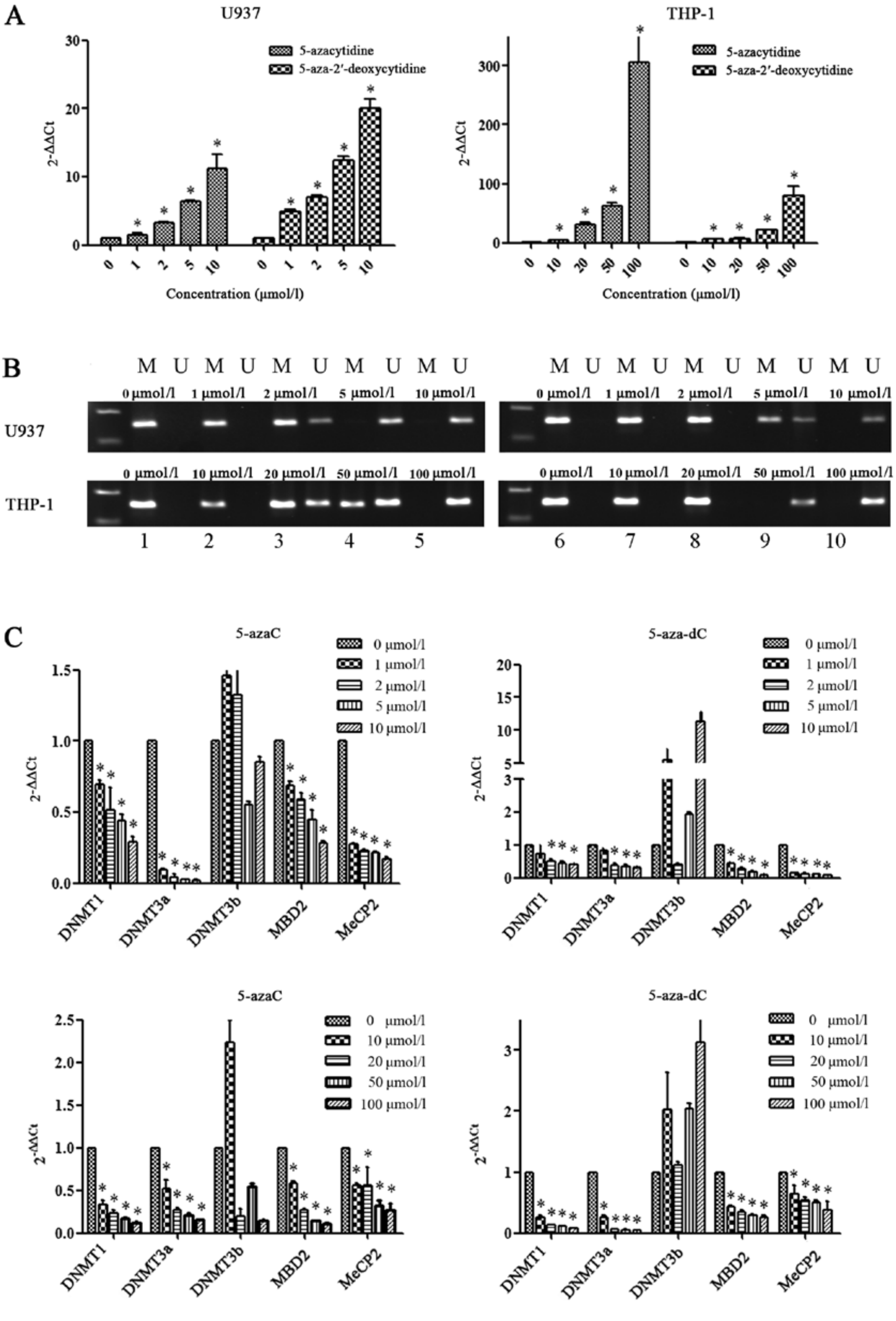

Figure 4. Changes in SOCS1 and SOCS1 methylation-related gene expression in AML cell lines. (A) SOCS1 mRNA expression in response to increasing concentrations of demethylation drugs in U937 and THP-1 cell lines. (B) Changes in SOCS1 methylation state in AML cell lines. M indicates the methylation strip, U indicates the non-methylation strip. Lanes 1 and 6 indicate no-drug intervention. Lanes, 2, 3, 4 and 5 revealed the results from intervention with different concentrations of 5-azaC. Lanes 7, 8, 9 and 10 revealed the results from intervention with different concentrations of 5-aza-dC. (C) Changes in the expression of SOCS1 methylation-related genes in AML cell lines. Relative expression of methylation-related genes DNMT1, DNMT3a, MBD2 and MeCP2 in response to increasing doses of 5-azaC and 5-aza-dC in U937 and in THP-1 cells were examined. The results were represented as the mean ± SD. *P<0.05 vs. the control (untreated group).

proteins (P<0.05) confirming that SOCS1 negatively affects the downstream JAK2/STAT signaling pathway. No significant change in the expression of t-JAK2, t-STAT3 and t-STAT5 proteins was observed.

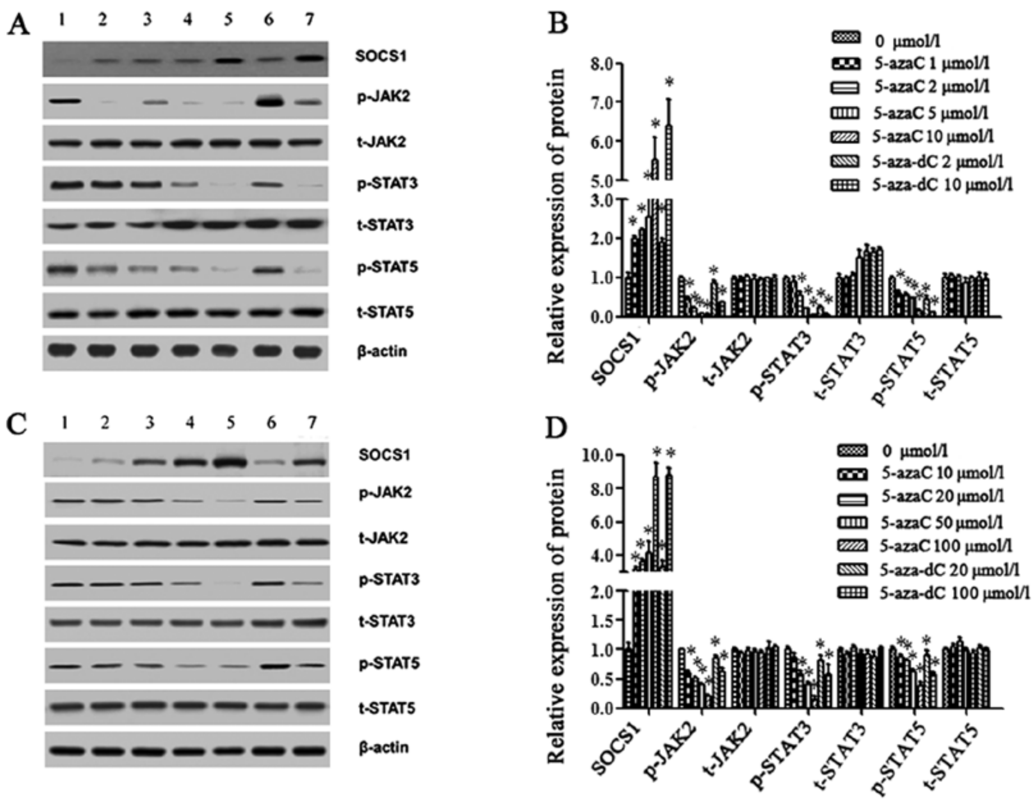

Figure 5. Expression of SOCS1 and downstream pathway proteins in AML cell lines. (A) Protein expression in the U937 cell line analyzed by western blotting. Lane 1 represents the untreated control and lanes 2, 3, 4 and 5 represent cells treated with 1, 2, 5 and 10 μmol/l of 5-azaC respectively. Lanes 6 and 7 represent cells treated with 2 and 10 μmol/l 5-aza-dC respectively. (B) Comparison of relative protein expression in U937 cells. ImageJ software was used for quantification. (C) Relative protein expression in THP-1 cells analyzed by western blotting. Lane 1 represents the untreated control. Lanes 2, 3, 4 and 5 represent cells treated with 10, 20, 50 and 100 μmol/l of 5-azaC respectively. Lanes 6 and 7 represent cells treated with 20 and 100 μmol/l of 5-aza-dC respectively. (D) Comparison of relative expression rates of proteins in THP-1 cells. ImageJ software was used for quantification. *$P<0.05$ vs. the control (untreated group).

Discussion

SOCS1, widely recognized as a tumor suppressor gene, is related to lymphatic metastasis and disease progression of liver cancer (10). Its methylation rate in hepatocellular carcinoma ranges from 39-60%. SOCS1 methylation also exists in other tumors, such as 61% in cervical cancer (11), 45% in esophageal squamous cancers (12) and 40% in hepatoblastomas (13). Recent studies have demonstrated that SOCS1 upregulates the expression of tiny RNAs in multiple myeloma, breast and prostate cancer, further confirming the effect of SOCS1 as a tumor-suppressor gene (14-16). DNA methylation refers to the process of biologically adding a methyl group to cytosine in cytosine-guanine CpG dinucleotides with S-adenosylmethionine (SAM) as a methyl donor under the catalysis of DNA methyltransferases (DNMTs). DNMTs mainly include DNMT1, DNMT3a and DNMT3b. DNA methylation needs to be read by a conserved family of proteins, namely, methyl-CpG binding proteins, which are bound by a methylated DNA-binding domain (MBD) to 5-methylcytosine (5 mC) followed by CpG. Five methylated CpG binding proteins are currently known, MeCP2, MBD1, MBD2, MBD3 and MBD4. Both MeCP2 and MBD2 can bind to methylated DNA and inhibit the transcription of methylated target genes. Our findings revealing that methylated SOCS1 is increased in AML corroborates these studies. In the initial treatment and relapsed/refractory groups, methylated SOCS1 (48 and 80% respectively) decreased the expression of mRNA and protein, while the expression of DNA methyltransferases DNMT1, DNMT3a and CpG binding proteins MBD2 and MeCP2 was increased, indicating their participation in SOCS1 gene methylation. In contrast, in the remission and normal control groups, the SOCS1 gene was found to be in a non-methylated state and its mRNA and protein levels were highly expressed. JAK2/STAT is a major signaling pathway for AML cell growth and proliferation. SOCS1, can directly bind with the JAK2/STAT complex and suppress this signal transduction pathway. Park *et al* (17) reported that SOCS1 silencing increased phosphorylation of STAT and promoted tumor development. In the present study, we found that SOCS1 protein expression in the initial treatment and relapsed/refractory groups was decreased, while the expression of its downstream p-JAK2, p-STAT3 and p-STAT5 proteins was higher than that in the remission and normal control groups. SOCS1 suppressed signal transduction of the JAK2/STAT pathway to exert its biological functions by suppressing p-JAK2, p-STAT3 and p-STAT5 proteins.

DNA methylation is a reversible change. In the present study, we performed demethylation on AML cell lines U937 and THP-1 with drugs 5-azaC and 5-aza-dC. We found that following treatment, the SOCS1 gene changed from a methylated state to a non-methylated state and this was accompanied by increased mRNA and protein expression in a drug concentration-dependent manner. In contrast, the

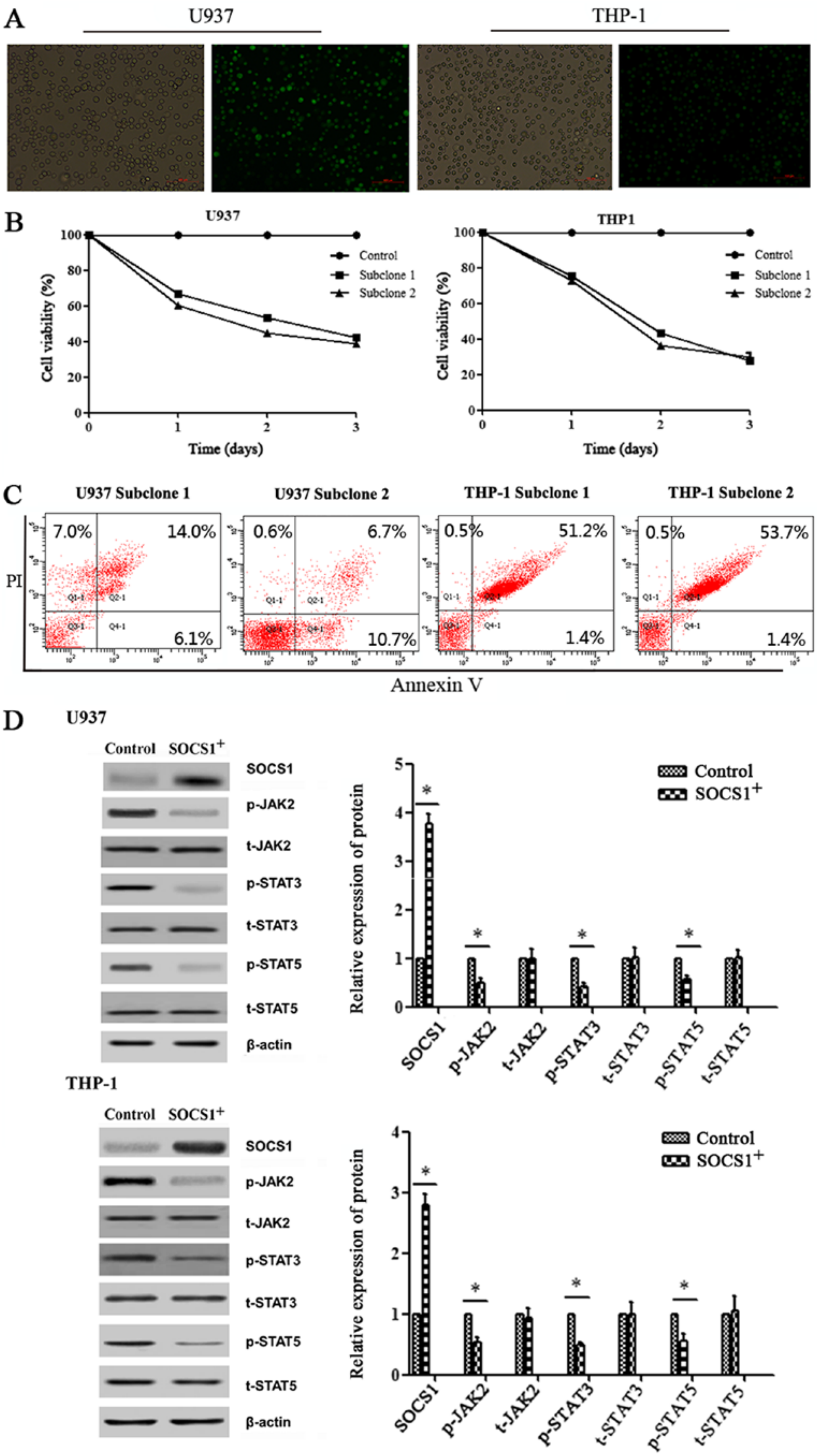

Figure 6. Cells overexpressing SOCS1 reveal inhibition of the JAK2/STAT pathway. (A) U937 and THP-1 cells transfected with the SOCS1-GFP vector. Cells were imaged with LSM 510 (ZEISS AG, Oberkochen, Germany). (B) Proliferation curves of transfected cells. Cell viability was determined by the CCK-8 assay. (C) The apoptosis rate of transfected cells. Flow cytometric analysis of cell apoptosis detected by Annexin V staining. This experiment was performed twice. (D) Expression of downstream pathway proteins in cells with the empty vector and after transfection of the SOCS1 gene in U937 and THP-1 cell lines. Subclone 1 and 2 are each from SOCS1-transfected AML cell lines. The control represents the empty vector, SOCS1+ represents cells transfected with SOCS1. *P<0.05.

expression of downstream p-JAK2, p-STAT3 and p-STAT5 proteins and the tumor cell viability rate was decreased, while the apoptosis rate was increased. Furthermore, the expression of the p-JAK2, p-STAT3 and p-STAT5 proteins was downregulated in cells transfected with the SOCS1 protein. This further ascertained that SOCS1 negatively regulates the downstream JAK2/STAT signaling pathway. In addition, in transfected cells, we also observed that the cell viability rate was decreased and the apoptosis rate was increased.

The relationship between SOCS1 methylation and AML should be further explored in gene methylation sequencing and with siRNA, which is the study aim in our future study.

Thus, this study revealed that SOCS1 may be used a therapeutic target and interventions that may induce expression of SOCS1 may be used for anticancer therapy (18-20). Whether SOCS1 can suppress other types of tumors should be verified by studies on different types of tumors. The demethylated SOCS1 gene may possibly become a new target for future tumor therapy and provide a new hope for tumor therapy and prognosis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

XZ and JL conceived and designed the study; XZ, LY, XL and YZ collected, analysed and interpreted the data; YP and XW designed the experimental techniques and XZ drafted the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Hebei Medical University and written informed consent was obtained from each patient.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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