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MiR-410 affects the proliferation and apoptosis of lung cancer A549 cells through regulation of SOCS3/JAK-STAT signaling pathway

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Abstract. – OBJECTIVE: Janus kinase (JAK) – signal transducer and activator of transcription (STAT) signal pathway participates in regulating cell proliferation, differentiation, and apoptosis, and is correlated with non-small cell lung cancer (NSCLC) onset. Suppressors of cytokine signaling 3 (SOCS3) negatively regulates JAK-STAT pathway. SOCS3 is down-regulated in NSCLC tissues, with an elevation of miR-410 expression. This study thus intends to investigate if miR-410 plays a role in mediating NSCLC onset and underlying mechanism in this regulatory process.

PATIENTS AND METHODS: NSCLC patients were collected for tumor and adjacent tissues, among which, miR-410 and SOCS3 expression were measured. Dual luciferase reporter gene assay was employed to confirm the targeting relationship between miR-410 and SOCS3. Their expression levels were compared between A549 and BEAS-2B cells. Cultured A549 cells were treated with anti-miR-410 and/or SOCS3. Expression levels of SOCS3, p-JAK1/2, p-STAT3, and Bcl-2 were compared along with the apoptotic rate of cells.

RESULTS: Bioinformatics analysis revealed targeted binding site between miR-410 and 3'-UTR of SOCS3 mRNA. Compared to those in tumor tissues, a significant increase of miR-410 and reduction of SOCS3 were found in NSCLC tissue ($p < 0.05$). Dual luciferase reporter gene assay indicated that SOCS3 was targeted regulated by miR-410. Significantly higher miR-410 and lower SOCS3 levels were shown in A549 cells, compared to those in BEAS-2B cells. Transfection of anti-miR-410 and/or SOCS3 in A549 cells, SOCS3 expression and apoptosis were significantly induced, while JAK1, JAK2, and STAT3 phosphorylation were statistically decreased with the reduction of the Bcl-2 level ($p < 0.05$).

CONCLUSIONS: miR-410 level was increased while SOCS3 expression was declined in NSCLS tissues. MiR-410 induces the apoptosis of A549 cells through downregulating JAK/STAT3/SOCS3 signaling pathway, which provides new insights for the therapy of pulmonary carcinoma in clinic.

Key Words:
MiR-410, SOCS3, JAK-STAT3, DDP, Bcl-2, NSCLC.

Introduction

Pulmonary carcinoma is the most leading cause of death among all malignant tumors, and severely threatens public health[1]. Multiple cytokines and receptor-activated Janus kinase (JAK) – signal transducer and activator of transcription (STAT) signal pathway widely participate in cell proliferation/apoptosis, angiogenesis, and other biological processes. Abnormal transduction of JAK-STAT is closely correlated with tumor occurrence, invasion, metastasis, and prognosis[2]. STAT3 belongs to the tumor-facilitating member in STAT family. One of its important mechanisms involves up-regulating Bcl-2 protein expression to promote cell proliferation and tumor occurrence[3]. Suppressors of cytokine signaling (SOCS) can block tyrosine phosphorylation and JAK-STAT pathway transduction via inhibiting JAK kinase activity[4]. As an important member of SOCS family, SOCS3 exerts potent functions to directly inhibit JAK kinase activity and STAT phosphorylation, thus negatively regulating JAK-STAT3 signal pathway[5]. The decreased SOCS3 expression is correlated with occurrence and progression of multiple tumors including breast cancer[6], colorectal carcinoma[7], oral cancer[8], and prostate cancer[9]. Previous studies[10,11] on cancer revealed a significant reduction of

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SOCS3 expression, indicating a possible tumor suppressor role of SOCS3 in lung cancer occurrence.

MicroRNA (miR) is a type of endogenous small molecule non-coding single-stranded RNA with 21-24 nucleotide length. It can regulate target gene expression via degrading mRNA or inhibiting translation, and has become a focus in tumor-related research[12]. Previous studies showed a significantly increased level of miR-410 in pulmonary carcinoma tissues[13], indicating its possible role in the promotion of lung cancer pathogenesis[14].

Patients and Methods

Major Reagent and Materials

Lung cancer A549 cell line and normal human pulmonary epithelial cell line BEAS-2B were purchased from Yanyu Biotech (Shanghai, China). Dulbecco's Modified Eagle's Medium (DMEM) medium was purchased from Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS) was purchased from Bioind (Cromwell, CT, USA). Streptomycin-penicillin and Annexin V-Fluorescein isothiocyanate (FITC)/ Propidium Iodide (PI) apoptotic reagent were purchased from Solarbio (Beijing, China). RNA extraction reagent TriPure RNA Isolation Reagent was purchased from Roche (Indianapolis, IN, USA). Lipofectamine 2000 was purchased from Invitrogen (Waltham, MA, USA). QuantiTect SYBR Green RT-PCR Kit was purchased from Qiagen (Venlo, Netherlands). MiR-410 nucleotide fragment and PCR primers were synthesized by Ruibo Bio (Beijing, China). Rabbit anti-human p-JAK1 and p-JAK2 monoclonal antibody were all purchased from Cell Signaling Technology (Danvers, MA, USA). Rabbit anti-human SOCS3 polyclonal antibody was provided by Abcam (Cambridge, MA, USA). Mouse anti-human p-STAT3 monoclonal antibody and Bcl-2 polyclonal antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Goat anti-rabbit and anti-mouse IgG-HRP (H+L) were purchased from Shengxing Biotech (Xi'an, Shan'xi, China). BCA protein quantification kit was purchased from Baitaike Biotech (Beijing, China). Luciferase gene reporter plasmid pLUC Luciferase vector was purchased from Ambion (Waltham, MA, USA). Dual-luciferase Reporter Assay System was purchased from Promega (Madison, WI, USA).

Clinical Information

A total of 38 NSCLC patients who received treatment in China-Japan Union Hospital of Jilin University from December 2015 to August 2016 were recruited for this study. There were 26 males and 12 females in the patient group, with aging between 46 and 71 years (average age = 52.9±12.5 years). All patients received primary surgery in China-Japan Union Hospital of Jilin University. No patient has received any chemo-, radio-, immune therapy or biological therapy. Tumor tissues and adjacent tissues were removed from NSCLC patients during surgery for testing indexes.

This study has been pre-approved by the Ethical Committee of China-Japan Union Hospital of Jilin University. All subjects have signed the informed consent before recruitment in this study.

Cell Culture

A549 and BEAS-2B cells were cultured in DMEM containing 10% FBS and 1% penicillin-streptomycin. After re-suspension, cells were cultured in a chamber with 5% CO_2 at 37°C. Cells were passed at 1:3 ratio after full growth.

Construction of Luciferase Reporter Gene Plasmid and Dual Luciferase Reporter Gene Assay

Full-length fragment of 3'-UTR of SOCS3 gene or polymerase chain reaction (PCR) products containing mutant form were sub-cloned into the pLUC vector. After transformation, the plasmid was sequenced for determining the correct insertion, and were named as pLUC-SOCS3-3'-UTR-wt, pLUC-SOCS3-3'-UTR-mut. Lipofectamine 2000 was used to transfect pLUC-SOCS3-3'-UTR-wt (or pLUC-SOCS3-3'-UTR-mut) and miR-410 mimic (or its negative control sequence miR-NC) into HEK293T cells. After 48 h, dual luciferase activity assay kit (Promega, Madison, WI, USA) was used to test dual luciferase activity following the manual instruction.

Construction of SOCS3 Over-Expression Plasmid

CDS fragment of SOCS3 gene was amplified. The target fragment size was determined by gel electrophoresis. After digestion in Xho I and BamH I enzyme, fragments were ligated into the pIRES2 plasmid to transform JM109 competent cells. Ampicillin resistant plate was used to screen positive colony, which was amplified and extracted recombinant plasmid

containing target fragment. Correct insertion of SOCS3 gene fragment was determined by sequencing and was named as pIRES2-SOCS3. Empty vector pIRES2-Blank was used as the control group.

Transfection of A549 Cells

In vitro cultured A549 cells were divided into five groups: anti-miR-NC group; anti-miR-410 group; pIRES2-Blank group; pIRES2-SOCS3 group; and anti-miR-410 + pIRES2-SOCS3 group. 72 hours after transfection, cells were collected for mRNA, protein expression, and apoptosis assay.

Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR) for Gene Expression

TriPure RNA Isolation Reagent was used to extract total RNA. QuantiTest SYBR Green RT-PCR Kit was used to test gene expression by qRT-PCR. In a 20 μL system, one added 10.0 μL 2X QuantiTest SYBR Green RT-PCR Master Mix, 1.0 μL of forward/reverse primers, 2 μg Template RNA, 0.5 μL QuantiTest RT Mix and ddH_2O. Primer sequences used were: miR-410P_F: 5'-CCGCC AATAT AACAC AGATG GCC-3'; miR-410P_R: 5'-TCAAG TACCC ACAGT GCGGT-3'; U6P_F: 5'-ATTGG AACGA TACAG AGAAG ATT-3'; U6P_R: 5'-GGAAC GCTTC ACGAA TTTG-3'; SOCS3P_F: 5'-CCTGC GCCTC AAGAC CTTC-3'; SOCS3P_R: 5'-GTCAC TGCGC TCCAG TAGAA-3'; Bcl-2P_F: 5'-GGTGG GGTCA TGTGT GTGG-3'; Bcl-2P_R: 5'-CGGTT CAGGT ACTCA GTCAT CC-3'; β-actinP_F: 5'-GAACC CTAAG GCCAA C-3'; β-actinP_R: 5'-TGTCA CGCAC GATTT CC-3'. PCR conditions were: 95°C for 15 min pre-denature, followed by 40 cycles each containing 94°C 15 s denature, 60°C 30 s annealing, and 72°C 30 s elongation. Applied Biosystems 7500 (Waltham, MA, USA) real-time quantitative PCR was used for testing gene expression.

Western Blot

Cells were collected and extracted for proteins using radioimmunoprecipitation assay (RIPA) lysis buffer. After quantification, 40 μg samples were loaded and separated in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were then transferred to polyvinylidene difluoride (PVDF) membrane, which was blocked in 5% defatted milk powder for 60 min room temperature incubation. Primary antibody (SOCS3 at 1:300, p-JAK1 at 1:200, p-JAK2 at 1:200, p-STAT3 at 1:200, Bcl-2 at 1:300, and β-actin at 1:600) was added for 4°C overnight incubation. After phosphate buffer solution Tween-20 (PBST) rinsing, secondary antibody (1:5000 dilution) was added for 60 min incubation. The membrane was rinsed in PBST and quantified for protein expression using enhanced chemiluminescence (ECL) method.

Flow Cytometry for Cell Apoptosis

Cells from all groups were collected and rinsed in PBS by centrifugation. Cells were re-suspended in Binding Buffer, with sequential addition of 5 μL Annexin V-FITC and PI. After 15 min staining, Beckman Coulter Gallios flow cytometry was used to test cell apoptosis (Brea, CA, USA).

Statistical Analysis

SPSS 18.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Continuous data are presented as means ± standard deviation (SD), and were analyzed by using one-way ANOVA, with the Tukey's post-hoc test. A statistical significance was defined when $p < 0.05$.

Results

Abnormal Expression of MiR-410 and SOCS3 in NSCLC Tissues

qRT-PCR results showed that miR-410 expression was significantly elevated in NSCLC tumor tissues compared to that in adjacent tissues ($p < 0.05$) (Figure 1A). Immunofluorescence test showed that SOCS3 level was significantly decreased in NSCLC tissue compared to that in adjacent tissues (Figure 1B). These data indicated that the reduction of SOCS3 expression might be associated with abnormal up-regulation of miR-410, which was involved in NSCLC pathogenesis.

Targeted Regulation Between MiR-410 and SOCS3

Bioinformatics analysis results showed the existence of targeted binding site between miR-410 and 3'-UTR of SOCS3 mRNA (Figure 2B). Transfection of miR-410 mimic significantly depressed relative luciferase activity in HEK293T cells transfected with pLUC-SOCS3-3'-UTR-wt ($p < 0.05$) (Figure 2B), indicating that miR-410 contributed to targeted regulation to SOCS3.

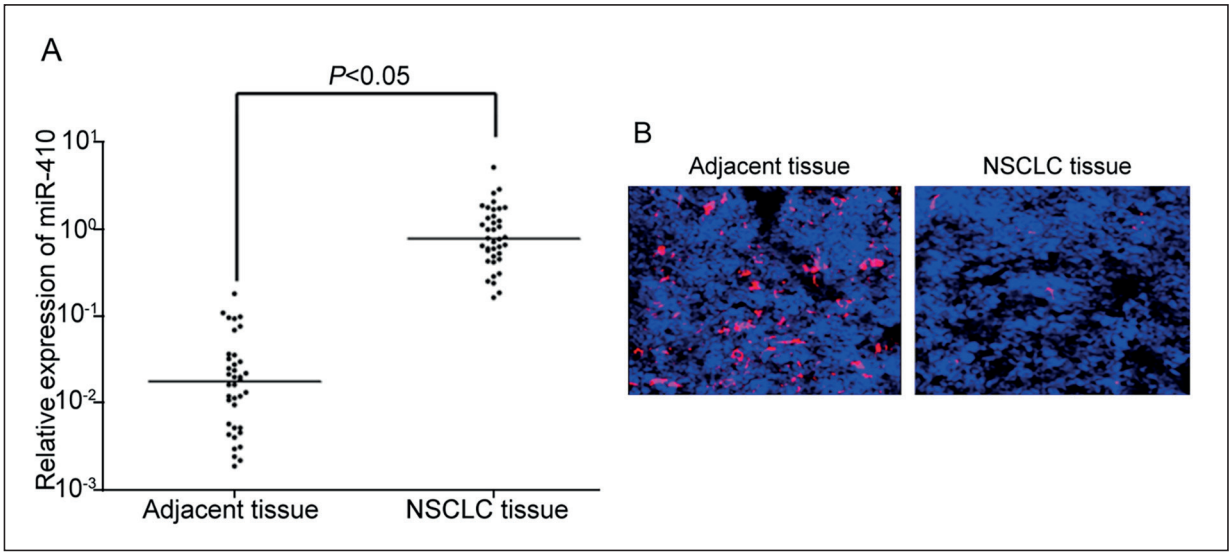

Figure 1. Abnormal expression of miR-410 and SOCS3 in NSCLC tissues. **A,** qRT-PCR for miR-410 expression. **B,**

MiR-410 Inhibition Elevated SOCS3 Expression, Decreased Bcl-2, and Increased A549 Cell Apoptosis

Compared to those in BEAS-2B cells, the levels of miR-410 and bcl-2 were significantly elevated in A549 cells, whilst SOCS3 mRNA expression was statistically decreased ($p < 0.05$) (Figure 3A). Similarly, Western blotting detection showed that SOCS3 protein level was apparently reduced while bcl-2 expression was increased in A549 cells, compared to those in BEAS-2B cells (Figure 3B). We further used A549 cells as the *in vitro* model, on which detailed role of miR-410, SOCS3 in NSCLC pathogenesis mechanism were elucidated. The transfection of anti-miR-410 and/or SOCS3 over-expression plasmid remarkably up-regulated SOCS3 expression in A549 cells (Figure 3C and 3D), and impeded phosphorylation of JAK1, JAK2, and STAT3 (Figure 3D). Our data also indicated that the down-regulation of miR-410 and increase of SOCS3 level decreased expression of anti-apoptotic factor Bcl-2 (Figure 3C and 3D), and increased the apoptosis of cells (Figure 3E).

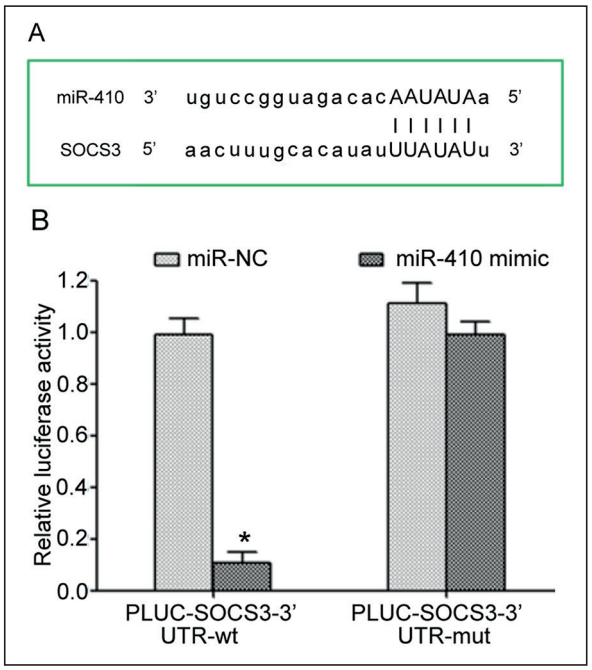

Figure 2. Targeted regulation between miR-410 and SOCS3. **A,** Targeted binding sites between miR-410 and 3'-UTR of SOCS3 mRNA. **B,** Dual luciferase reporter gene assay. *, $p < 0.05$ comparing between miR-410 mimic and miR-NC.

Discussion

After binding with cell surface receptor, cytokines can transduce extracellular signals into the nucleus via signal transduction pathway cascade, in which JAK-STAT plays a critical role in responding extracellular factor, transducing signal cascade response, and regulating cell growth/apoptosis[15]. Cytokines, growth factors dimerize receptor and phosphorylate JAK via the binding with intracellular receptor as ligands, and induce the activation of STAT. Abnormal activation of STAT3 is closely correlated with irregulated cell

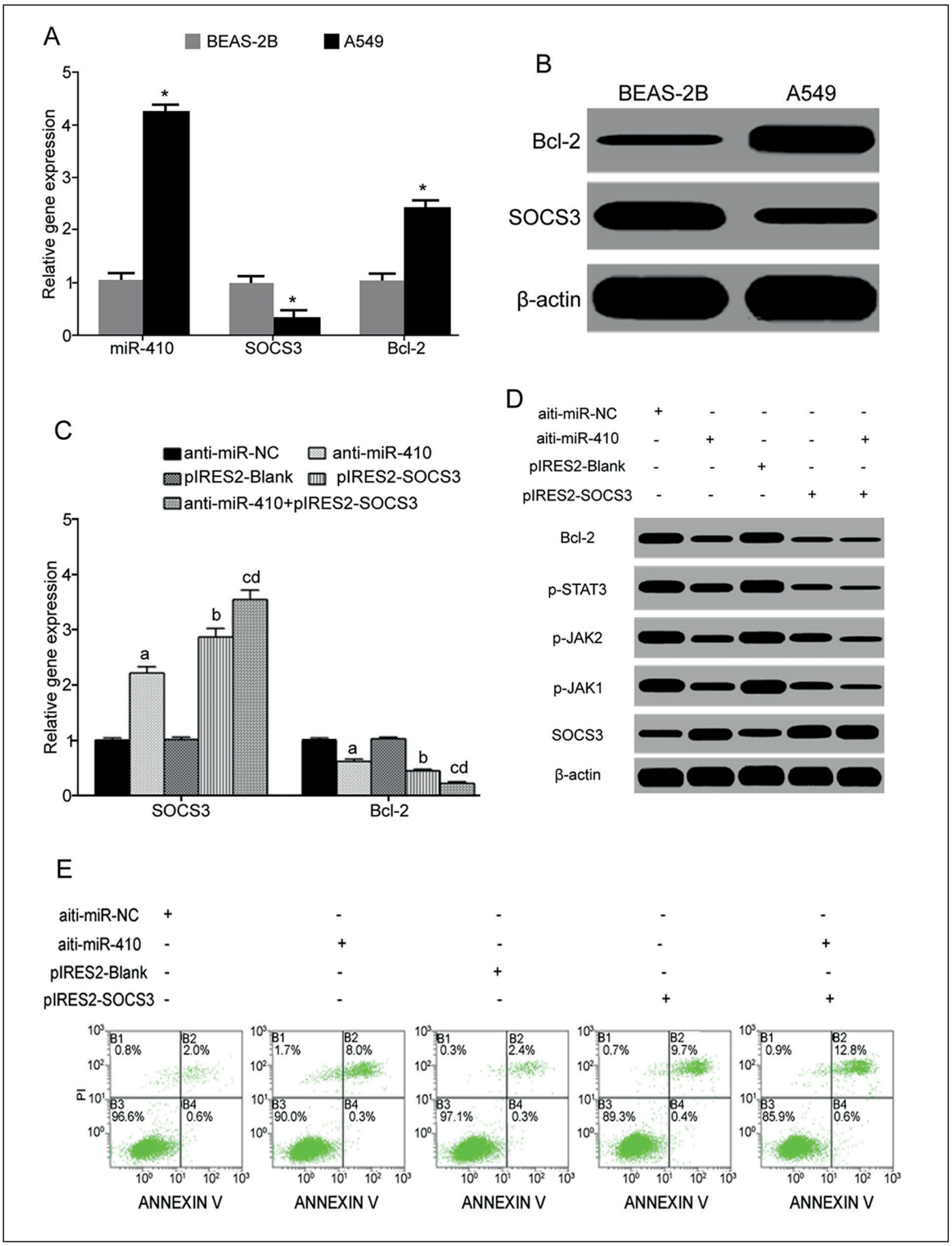

Figure 3. MiR-410 inhibition elevated SOCS3 expression, decreased Bcl-2 and increased A549 cell apoptosis. **A,** qRT-PCR for gene expression in BEAS-2B and A549 cells. **B,** Western blot for protein expression in BEAS-2B and A549 cells. **C,** qRT-PCR for gene expression in A549 cells with transfection. **D,** Western blot for protein expression in A549 cells after transfection. a, $p<0.05$ comparing between anti-miR-410 and anti-miR-NC group; b, $p<0.05$ comparing between pIRES2-SOCS3 and pIRES2-Blank group; c, $p < 0.05$ comparing between anti-miR-410 + pIERS2-SOCS3 and anti-miR-NC group; d, $p < 0.05$ comparing between anti-miR-410 + pIRES2-SOCS3 and pIRES2-Blank group.

proliferation and malignant transformation, and has become one major question in the study of JAK/STAT signal pathway and tumor pathogenesis[16]. SOCS represents the negative feedback regulatory factor in JAK-STAT signal pathway, and exerts a critical role in maintaining intracellular homeostasis. SOCS3 eventually blocks JAK-STAT signal pathway transduction through the inhibition of JAK phosphorylation and JAK kinase activity[17].

This study revealed that miR-410 level was significantly elevated in NSCLS tumor tissues compared to that in adjacent tissues, with a decrease of SCOS3 expression. Li et al[13] also reported higher miR-140 expression in NSCLC tissues compared to adjacent tissues while Zhang et al[14] found abnormally elevated miR-410 expression in NSCLC tissues. Our result agrees with previous finding. Evidence[10,18] revealed abnormally decreased SOCS3 expression in NSCLC tissues. Interestingly, in lung cancer tissues, SOCS3 expression was also decreased[19,20]. In this work NSCLC tissues were detected with lower SOCS3 expression, which was in line with previous studies.

It has been shown that miR-410 level was abnormally increased in lung cancer cells such as A540, SPC-A1, and H1299, compared to normal human bronchial epithelial cells HBE[13]. Zhang et al[21] also showed lower SOCS3 expression in A549 cells compared to HBE cells. Moreover, lower SOCS3 expression was also found in lung cancer cell lines H460 and A459 compared to normal human embryonic pulmonary cell H1703[18]. We speculated that miR-410 and SOCS3 may be involved in the pathogenesis of lung cancer. Dual luciferase reporter gene assay further showed that transfection of miR-410 mimic significantly suppressed relative luciferase activity, demonstrating a targeted regulation correlation between miR-410 and SOCS3. We then assessed the effect of decreasing level of miR-410 or over-expression of SOCS3 on JAK-STAT signaling pathway and cell apoptosis. Li et al[13] showed that over-expression of miR-410 significantly facilitated proliferation, migration, and invasion of pulmonary cancer cells, probably related with miR-410 targeted inhibition on BRD7 expression and further enhancement of phosphorylation activity of Akt. Zhang et al[14] showed that miR-410 could up-regulate SLC34A2 expression via activating Wnt/β-catenin signal pathway, and could facilitate proliferation, migration, and invasion of lung cancer cells. Over-expression of miR-410 also enhanced survival and viability of pulmonary cancer cells in nude mice. Consistently, our result unrevealed that the reduction of miR-410 induced the cell apoptosis and inhibited the cell proliferation. The decrease of miR-410 also led to the up-regulation of SOCS3. In a similar fashion, previous evidence[21,22] showed that over-expression of SOCS3 significantly weakened motility and migration of A540 cells and decreased proliferation potency of lung cancer cells. Over-expression of SOCS3 significantly increased A540 cell apoptosis, and weakened proliferation/migration potency[10], which was in agreement with our finding. So far, a variety of miRNAs such as miR-520a-3p, miR-92a, miR-378 participate in the regulation of lung cancer[23-25]. Notably, this work verified that the decrease of miR-410 caused A549 cell apoptosis and inactivation of the JAK-STAT pathway by over-expression of SOCS3, indicating the involvement of miR-410 in the regulation of lung cancer. However, the *in vivo* test still requires further evaluation of miR-410 in clinical practice.

Conclusions

We demonstrated that NSCLC patients were detected with a high level of miR-410 and low SOCS3 expression compared to the normal group. MiR-410 can facilitate the activity of JAK-STAT3 signal pathway and cell proliferation via targeted inhibition on SOCS3 expression, which provides leads for the future therapy against lung cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.
2) Hu Y, Hong Y, Xu Y, Liu P, Guo DH, Chen Y. Inhibition of the JAK/STAT pathway with ruxolitinib overcomes cisplatin resistance in non-small-cell lung cancer NSCLC. Apoptosis 2014; 19: 1627-1636.
3) Zhu Z, Li E, Liu Y, Gao Y, Sun H, Ma G, Wang Z, Liu X, Wang Q, Qu X, Liu Y, Yu Y. Inhibition of Jak-STAT3 pathway enhances bufalin-induced apoptosis in colon cancer SW620 cells. World J Surg Oncol 2012; 10: 228.

4) Slattery ML, Lundgreen A, Kadlubar SA, Bondurant KL, Wolff RK. JAK/STAT/SOCS-signaling pathway and colon and rectal cancer. Mol Carcinog 2013; 52: 155-166.

5) Tamiya T, Kashiwagi I, Takahashi R, Yasukawa H, Yoshimura A. Suppressors of cytokine signaling (SOCS) proteins and JAK/STAT pathways: regulation of T-cell inflammation by SOCS1 and SOCS3. Arterioscler Thromb Vasc Biol 2011; 31: 980-985.

6. Barclay JL, Anderson ST, Waters MJ, Curlewis JD. SOCS3 as a tumor suppressor in breast cancer cells, and its regulation by PRL. Int J Cancer 2009; 124: 1756-1766.

7) Igci M, Cakmak EA, Oztuzcu S, Bayram A, Arslan A, Gogebakan B, Igci YZ, Cengiz B, Ozkara E, Camci C, Demiryurek AT. Mutational screening of the SOCS3 gene promoter in metastatic colorectal cancer patients. Genet Test Mol Biomarkers 2012; 16: 1395-1400.

8) Christopher AF, Gupta M, Bansal P. Micronome revealed miR-19a/b as key regulator of SOCS3 during cancer related inflammation of oral squamous cell carcinoma. Gene 2016; 594: 30-40.

9) Pierconti F, Martini M, Pinto F, Cenci T, Capodimonti S, Calarco A, Bassi PF, Larocca LM. Epigenetic silencing of SOCS3 identifies a subset of prostate cancer with an aggressive behavior. Prostate 2011; 71: 318-325.

10) Zhang S, Wang W, Wang E, Qiu X. SOCS3 expression is inversely correlated with Pyk2 in non-small cell lung cancer and exogenous SOCS3 inhibits proliferation and invasion of A549 cells. Pathology 2012; 44: 434-440.

11) Lin YC, Lin CK, Tsai YH, Weng HH, Li YC, You L, Chen JK, Jablons DM, Yang CT. Adenovirus-mediated SOCS3 gene transfer inhibits the growth and enhances the radiosensitivity of human non-small cell lung cancer cells. Oncol Rep 2010; 24: 1605-1612.

12) Ge C, Wu S, Wang W, Liu Z, Zhang J, Wang Z, Li R, Zhang Z, Li Z, Dong S, Wang Y, Xue Y, Yang J, Tan Q, Wang Z, Song X. miR-942 promotes cancer stem cell-like traits in esophageal squamous cell carcinoma through activation of Wnt/beta-catenin signalling pathway. Oncotarget 2015; 6: 10964-10977.

13) Li D, Yang Y, Zhu G, Liu X, Zhao M, Li X, Yang Q. MicroRNA-410 promotes cell proliferation by targeting BRD7 in non-small cell lung cancer. FEBS Lett 2015; 589: 2218-2223.

14) Zhang X, Ke X, Pu Q, Yuan Y, Yang W, Luo X, Jiang Q, Hu X, Gong Y, Tang K, Su X, Liu L, Zhu W, Wei Y. MicroRNA-410 acts as oncogene in NSCLC through downregulating SLC34A2 via activating Wnt/beta-catenin pathway. Oncotarget 2016; 7: 14569-14585.

15) Pencik J, Pham HT, Schmoellerl J, Javaheri T, Schleder-er M, Culig Z, Merkel O, Moriggl R, Grebien F, Kenner L. JAK-STAT signaling in cancer: from cytokines to non-coding genome. Cytokine 2016; 87: 26-36.

16) Gao B, Shen X, Kunos G, Meng Q, Goldberg ID, Rosen EM, Fan S, Constitutive activation of JAK-STAT3 signaling by BRCA1 in human prostate cancer cells. FEBS Lett 2001; 488: 179-184.

17) Collins AS, McCoy CE, Lloyd AT, O'Farrelly C, Stevenson NJ. miR-19a: an effective regulator of SOCS3 and enhancer of JAK-STAT signalling. PLoS One 2013; 8: e69090.

18) He B, You L, Uematsu K, Zang K, Xu Z, Lee AY, Costello JF, McCormick F, Jablons DM. SOCS-3 is frequently silenced by hypermethylation and suppresses cell growth in human lung cancer. Proc Natl Acad Sci U S A 2003; 100: 14133-14138.

19) Hofmann HS, Bartling B, Simm A, Murray R, Aziz N, Hansen G, Silber RE, Burdach S. Identification and classification of differentially expressed genes in non-small cell lung cancer by expression profiling on a global human 59.620-element oligonucleotide array. Oncol Rep 2006; 16: 587-595.

20) Wikman H, Kettunen E, Seppanen JK, Karjalainen A, Hollmen J, Anttila S, Knuutila S. Identification of differentially expressed genes in pulmonary adenocarcinoma by using cDNA array. Oncogene 2002; 21: 5804-5813.

21) Zhang S, Guo D, Jiang L, Zhang Q, Qiu X, Wang E. SOCS3 inhibiting migration of A549 cells correlates with PYK2 signaling in vitro. BMC Cancer 2008; 8: 150.

22) Wan J, Che Y, Kang N, Wu W. SOCS3 blocks HIF-1alpha expression to inhibit proliferation and angiogenesis of human small cell lung cancer by downregulating activation of Akt, but not STAT3. Mol Med Rep 2015; 12: 83-92.

23) Lv X, Li CY, Han P, Xu XY. MicroRNA-520a-3p inhibits cell growth and metastasis of non-small cell lung cancer through PI3K/AKT/mTOR signaling pathway. Eur Rev Med Pharmacol Sci 2018; 22: 2321-2327.

24) Zhu Q, Zang Q, Jiang ZM. Enhanced expression of non coding miR 92a expression is implicated in the development of lung cancer. Eur Rev Med Pharmacol Sci 2018; 22: 1028-1034.

25) Ji KX, Cui F, Qu D, Sun RY, Sun P, Chen FY, Wang SL, Sun HS. MiR-378 promotes the cell proliferation of non-small cell lung cancer by inhibiting FOXG1. Eur Rev Med Pharmacol Sci 2018; 22: 1011-1019.