European Review for Medical and Pharmacological Sciences 2019; 23: 5640-5647

MiR-101 affects proliferation and apoptosis of cervical cancer cells by inhibition of JAK2

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Abstract. – OBJECTIVE: JAK2 expression and dysfunction play a role in tumor pathogenesis. Bioinformatics analysis revealed a targeted binding site between miR-101 and the 3'-UTR of JAK2 mRNA. This study investigated the role of miR-101 in regulating JAK2 expression and affecting the proliferation and apoptosis of cervical cancer cells.

PATIENTS AND METHODS: The tumo[illegible]tissues and adjacent tissues of patients w[illegible] vical cancer were collected. The expres[illegible] of miR-101 and JAK2 was detected by qRT[illegible]R. The dual luciferase reporter gene assay dated the targeting relationship between m[illegible] 101 and JAK2. The cervical can[illegible] Caski cel[illegible] were cultured *in vitro*, and di[illegible] miR-NC group and miR-101 mimic [illegible]p. [illegible] expression of JAK2 and p-JAK2 [illegible] detecte[illegible] y Western blot, cell apoptosis w[illegible] etecte[illegible] tometry, and cell prolife[illegible] wa[illegible] EdU staining.

RESULTS: Com[illegible] with [illegible]nt tissues, miR-101 expressi[illegible] as significan[illegible] creased, and JAK2 expr[illegible] as increase[illegible] ervical cancer tissue[illegible] her[illegible] a targeted regulatory relationshi[illegible] tween m[illegible] and JAK2. Compared wit[illegible] erEpic cells, [illegible]101 expression in HeLa [illegible] Caski was signi[illegible]tly decreased, and th[illegible] xpress[illegible]n of JAK2 and p-JAK2 was sign[illegible]ntly in[illegible]sed. Transfection of miR-101 mi[illegible] ignifi[illegible]ly red[illegible]ed the expression of JAK2[illegible] K2 in [illegible]ki cells, reduced cell prolifera[illegible]nd inc[illegible]ed cell apoptosis.

[illegible]NCLU[illegible] decrease of miR-101 expr[illegible]n and [illegible]crease of JAK2 expression play[illegible]ole in cer[illegible]al cancer, while the increase of [illegible] expression can inhibit the proliferati[illegible]e the apoptosis of cells by inhibi[illegible] the expression of JAK2.

Words:
[illegible]1, JAK2, Cervical cancer.

Introduction

[illegible]ervical carcinoma is a common female malignant tumor. [illegible]ncidence rate is second only [illegible]east cancer, [illegible]king second in female malignant [illegible]mors[1], p[illegible]g a serious threat to the lives and [illegible]nts[2,3].

The [illegible]ne kinase (JAK)-signal transducer and activator of transcription (STAT) signaling [illegible]s widely expressed in various tissues [illegible]s, and the abnormal activation of JAK-STAT signaling pathway is associated with the occurrence, progression, metastasis and drug resistance of various tumors[4-7]. JAK2, an important regulator of the JAK-STAT3 signaling pathway, is a signaling pathway kinase that plays an important role in activating the JAK-STAT signaling pathway. A number of studies[8-10] have shown that the abnormal expression and function of JAK2 play an important regulatory role in the occurrence and progression of various tumors such as breast cancer, liver cancer and gastric cancer.

MicroRNA is an endogenous non-coding small-molecule single-stranded RNA in eukaryotes with a length of about 22-25 nucleotides, which binds to the 3'-UTR of the target gene mRNA by complementary pairing, resulting in degradation or inhibition of translation. MicroRNAs that regulate the expression of target genes, which account for 1% of human genes, regulate the expression of more than 1/3 human genes[11]. Several studies have found that miR-101 is an important tumor suppressor gene that plays a role as a tumor suppressor gene in the development and progression of various tumors of gastric cancer, bladder cancer and prostate cancer[12-14]. A number

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of studies have shown that the decreased expression of miR-101 is associated with the occurrence and progression of cervical cancer[15-17]. Bioinformatics analysis revealed a targeted binding site between miR-101 and the 3'-UTR of JAK2 mRNA. This study was to detect the expression profiles of miR-101 and JAK2 in tumor tissues and paracancerous tissues of cervical cancer patients, and investigate whether miR-101 regulates JAK2 expression and affects cell proliferation and apoptosis of cervical cancer cells.

Patients and Methods

Patients

Thirty-six patients with cervical cancer who were treated in our hospital from April 2018 to September 2018 were enrolled. The specimens of cervical cancer tissue confirmed by histopathological examination were collected and the paracancerous tissues which were located at least 2 cm away from the tumor were collected as a control. This study was approved by the Ethics Committee of our hospital and informed co[illegible] were obtained from all patients.

Main Reagents and Materials

Human normal cervical epithelial HcerE[illegible] cells, HEK293T cells were purch[illegible]d from Be[illegible] jing Beina Biological (Beijin[illegible] Human Cervical Cancer HeLa, C[illegible] cells [illegible]re purchased from Guangzhou C[illegible]o Bio ([illegible]zhou, China); RPMI-1640 medi[illegible]pti-M[illegible] serum medium purc[illegible]d [illegible]oco (Grand Island, NY, USA); [illegible]um Feta[illegible]ine Serum (FBS) was purch[illegible]from Shanghai[illegible]aisheng (Shanghai, Ch[illegible]l and Lipo[illegible]00 were purchased fro[illegible]nvitrog[illegible]Carlsbad, CA, USA); qRT-PCR [illegible]erMix was [illegible]ased from Beijing Tra[illegible]n Biotech (Beijin[illegible]hina); miR-101 mimic[illegible]R-NC[illegible]dU Cell Proliferation Assay Kit [illegible]purcha[illegible]from Guangzhou Rui (Guangzhou[illegible]a) [illegible]abbit a[illegible]uman JAK2, p-JAK2 polyclo[illegible]body w[illegible]urchased from Cell Sig-[illegible] Tec[illegible]nvers, MA, USA); rabbit an[illegible]man β-[illegible]olyclonal antibody was purchas[illegible]from Shan[illegible]hai Shenggong Bio (Shanghai, Ch[illegible]jugated secondary antibody was [illegible]hased [illegible]n Jackson ImmunoResearch (West [illegible]ve, PA, USA); pMIR plasmid Purchased from [illegible]sha Youbao Bio (Changsha, China); Du-al-[illegible]erase Reporter Assay System was purchased from Promega (Madison, WI, USA).

Cell Culture

HcerEpic, HeLa, and Caski cells were c[illegible]red in RPMI-1640 medium containing 1[illegible] a 37°C 5% CO_2 cell culture incub[illegible] After t[illegible] cells were over, 0.125% trypsi[illegible]gestion was performed to collect the cells fo[illegible] by a subculture at a ratio of 1:4 to 1:5. Cell[illegible] phase were selected for experim[illegible]

Construction of Lu[illegible]rase Reporter Gene V[illegible]r

The PCR prod[illegible]ct [illegible]-lengt[illegible]UTR fragment of t[illegible]AK2 [illegible]or th[illegible]agment containing th[illegible]tant was c[illegible] the pMIR vector and [illegible]ted as pMI[illegible]K2-WT. A luciferas[illegible]port[illegible]tor containing the 3'-UTR mutant of JAK2 ge[illegible] also constructed and desi[illegible]as pMIR-J[illegible]MUT. pMIR-JAK2-W[illegible] pMIR-JAK2-MU[illegible] was transfected into [illegible]293T cells with miR-101 mimic (or miR-[illegible]) using Lipo[illegible]0 reagent, and cultured for 48 [illegible]llowed by m[illegible]uring the relative luciferase a[illegible] accordi[illegible] the Dual-Luciferase Reporter A[illegible]Kit instructions.

Cell Transfection and Grouping

[illegible]aski cells cultured *in vitro* were divid[illegible] [illegible]wo transfection groups: miR-NC transfection group and miR-101 mimic transfection group. The general procedure for transfection was to dilute Lipofectamine 2000, miR-NC, miR-101 mimic with Opti-MEM. After incubation for 5 min at room temperature, gently mixing Lip 2000 with miR-NC and miR-101 mimic for 20 min incubation at room temperature followed by addition of the transfectants to the cell culture medium. After 72 hours of culture, cells were collected for analysis.

qRT-PCR Detection of Gene Expression

The RNA was extracted using TRIzol reagent, and the relative expression of the gene was detected by one-step qRT-PCR using TransScript Green One-Step qRT-PCR SuperMix in the 20 μL reaction system including: 1 μg of Template RNA, 0.2 μM of pre-primer, 0.2 μM of post-primer, 10 μL of 2×TransStart Tip Green qPCR SuperMix, 0.4 μL of One-Step RT Enzyme Mix, 0.4 μL of Passive Reference Dye II, and RNase-free water. The qRT-PCR reaction conditions were: 45°C, 5 min; 94°C, 30 s; (94°C, 5 s; 60°C, 30 s) × 40 cycles, and gene expression was detected on a Bio-Rad CFX96 Real-time PCR instrument (Hercules, CA, USA).

Western Blot

The cells were lysed by SDS lysate, and the total protein was extracted. After boiling at 100 °C for 5 min, the protein concentration was determined by BCA method. 40 μg protein was separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) separation gel and 5% concentrated gel, transferred to the polyvinylidene difluoride (PVDF) membrane (300 mA, 100 min), blocked with 5% skim milk powder at room temperature, and incubated with the primary antibody at 4°C overnight (the dilution ratios of JAK2, p-JAK2, and β-actin were 1:2000, 1:800, 1:8000, respectively). After washing times with PBST, the membrane was incubated with the horseradish peroxidase (HRP)-labeled secondary antibody (1:10000 dilution) at room temperature followed by washing the membrane 3 times with PBST, addition of ECL luminescent solution for 1-3 min, exposing and developing.

Flow Cytometry Detection of Apoptosis

The two miR-NC and miR-101 mimic transfected cells were collected by trypsinization and resuspended in 100 μL Annexin V Binding [illegible] fer according to the instructions follo[illegible] addition of 5 μL FITC Annexin V and 10 [illegible]PI. After incubation for 15 min at room tempera[illegible] 400 μL Annexin V Binding Buffer was adde[illegible] stop the reaction and cell apoptosi[illegible]s measure[illegible] using a Beckman Coulter FC[illegible]flow cytometer (Brea, CA, USA).

Flow Cytometry Dete[illegible] of Cell Proliferation

The two miR-N[illegible]d miR-[illegible]imic transfected cells wer[illegible]vested by t[illegible]ization, and resuspend[illegible]complete me[illegible]um containing 10% [illegible]S. Afte[illegible]bation with 10 μM of EdU at [illegible]8C for 2 h, [illegible]lls were further cultured[illegible]48 h. After the[illegible]s were collected by [illegible]zymati[illegible]digestion, cells were washed by [illegible]ifugati[illegible]ixed, and permeabilized, and then [illegible]ac[illegible] soluti[illegible]ntaining Alexa Fluor 488 lab[illegible]added[illegible] the mixture was incu[illegible]at ro[illegible]m[illegible]ure for 30 minutes under da[illegible]d cell p[illegible]ation was measured by flow cyto[illegible]ry.

[illegible]istical [illegible]nalysis

[illegible]tatistical analysis of the data was performed [illegible]SPSS 18.0 software (SPSS Inc., Chicago, IL, [illegible]). The measurement data were expressed as mean ± standard deviation (SD). The comparison between the measurement data of two groups was performed by Studen's *t*-test. The diff[illegible]ce of multiple groups was assessed [illegible] ANOVA with Newman-Keuls mu[illegible] compa[illegible]ison post-hoc analysis. $p < 0.05$[illegible] considered statistically significant.

Res[illegible]

A Targeting Rela[illegible]ship [illegible]ween MiR-101 and J[illegible]K2

Bioinformati[illegible]analys[illegible]ealed [illegible]comple-mentary bin[illegible]site betwe[illegible]and the 3'-UTR of [illegible]RNA (Figu[illegible]). The dual luciferas[illegible]e r[illegible]r assay showed that transfection of miR-101 [illegible] significantly reduced relati[illegible]iferase acti[illegible]n pMIR-JAK2-WT tr[illegible]ted HEK293T ce[illegible] compared to miR-[illegible]transfected cells, while transfection miR-NC [illegible]miR-101 mi[illegible]had no significant effect on [illegible]ive luciferas[illegible]ctivity in pKIR-JAK2-MUT t[illegible]ted HE[illegible]3T cells (Figure 1B), indicati[illegible]01 can target 3'-UTR of JAK2 mRNA.

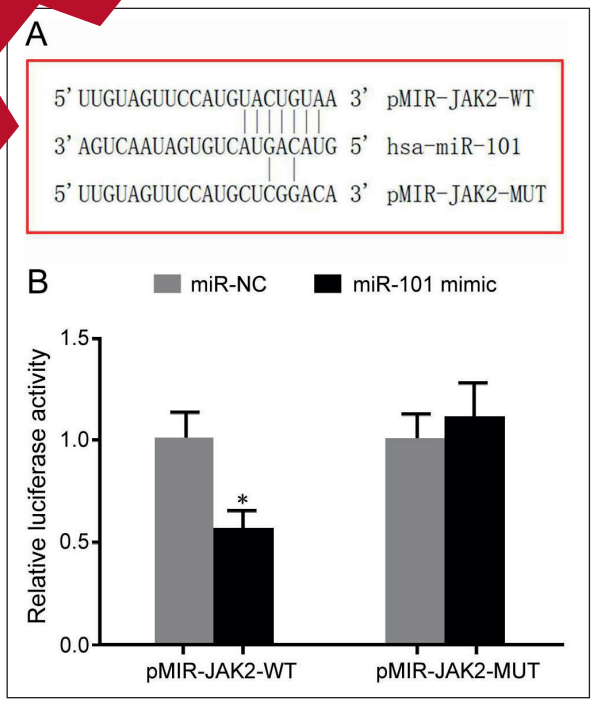


Figure 1. There is a targeting relationship between miR-101 and JAK2 mRNA. ***(A)*** Schematic diagram of the site of action between miR-101 and the 3'-UTR of JAK2 mRNA; ***(B)*** Dual luciferase gene reporter assay. *Represents $p < 0.05$ compared to miR-NC.

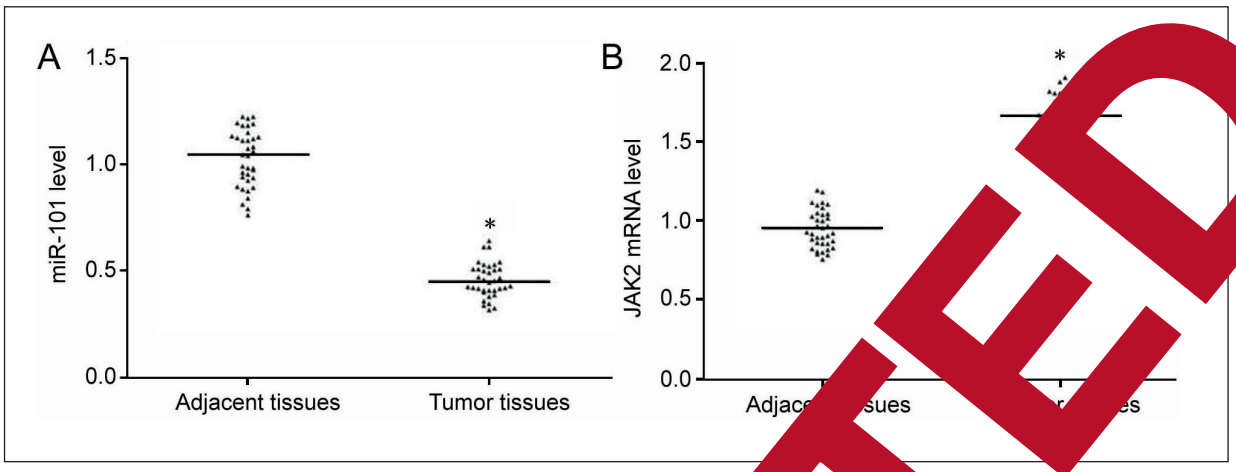


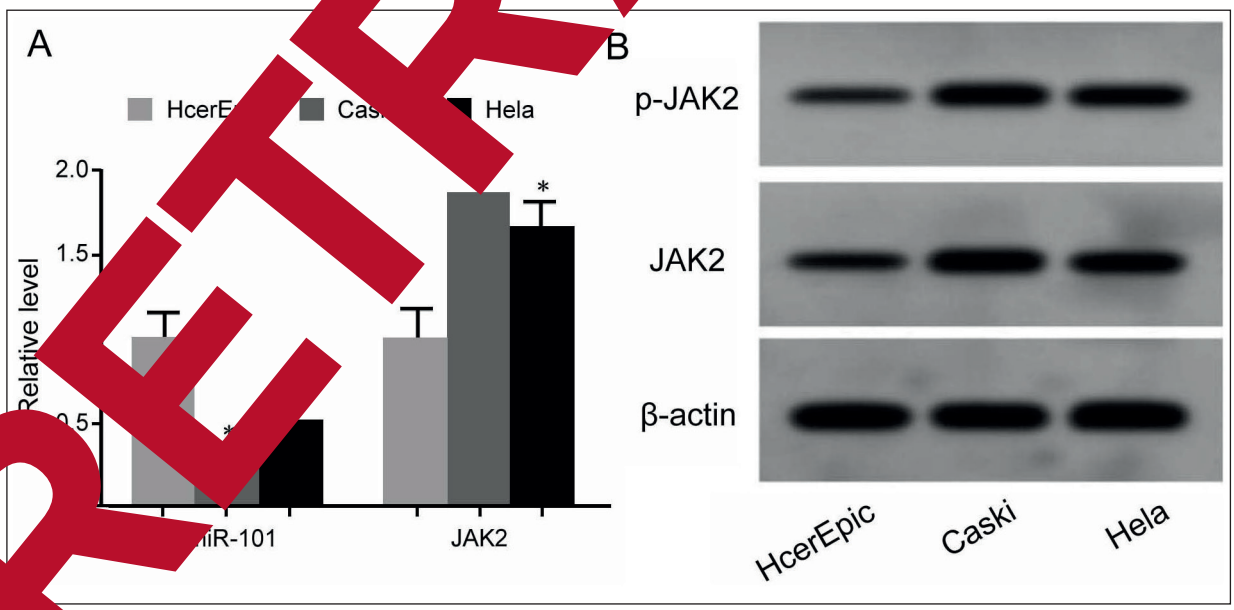
Figure 2. Abnormal expression of miR-101 and JAK2 in cervical cancer. ***(A)*** qRT-PCR ... of miR-101 expression in cervical cancer tissues; ***(B)*** qRT-PCR detection of JAK2 mRNA expres... cervical canc... s. *Represents $p < 0.05$ compared to the two groups.

Abnormal Expression of MiR-101 and JAK2 in Cervical Cancer

The results of qRT-PCR showed that the expression of miR-101 was significantl... creased in tumor tissues of patients w... vical cancer compared with adjacent ... es ($p < 0.05$) (Figure 2A), while the expressi... of JAK2 mRNA was significantly increased (... 0.05) (Figure 2B).

...reased Mi...01 and Increased JAK2 E...ssion in ...rvical Cancer Cells

... qRT-PCR showed that the expression of miR-101 was significantly decreased ... the expression of JAK2 mRNA was signifi... reased in HeLa and Caski cells com... ...th normal cervical epithelial HcerEpic cells ($p < 0.05$) (Figure 3A). Western blot analysis showed that the expression of JAK2 and p-JAK2



F... 3. Increased expression of miR-101 in cervical cancer cells and increased expression of JAK2. ***(A)*** qRT-PCR detection of m... 01, JAK2 mRNA expression in cervical cancer cells; ***(B)*** Western blot analysis of JAK2, p-JAK2 protein expression in cervical cancer cells. *Represents $p < 0.05$ compared to HcerEpic cells.

protein in HeLa and Caski cells was significantly higher than those in normal cervical epithelial HcerEpic cells (Figure 3B).

Increased MiR-101 Inhibits JAK2 Expression and Proliferation of Cervical Cancer Cells, Induces Cell Apoptosis

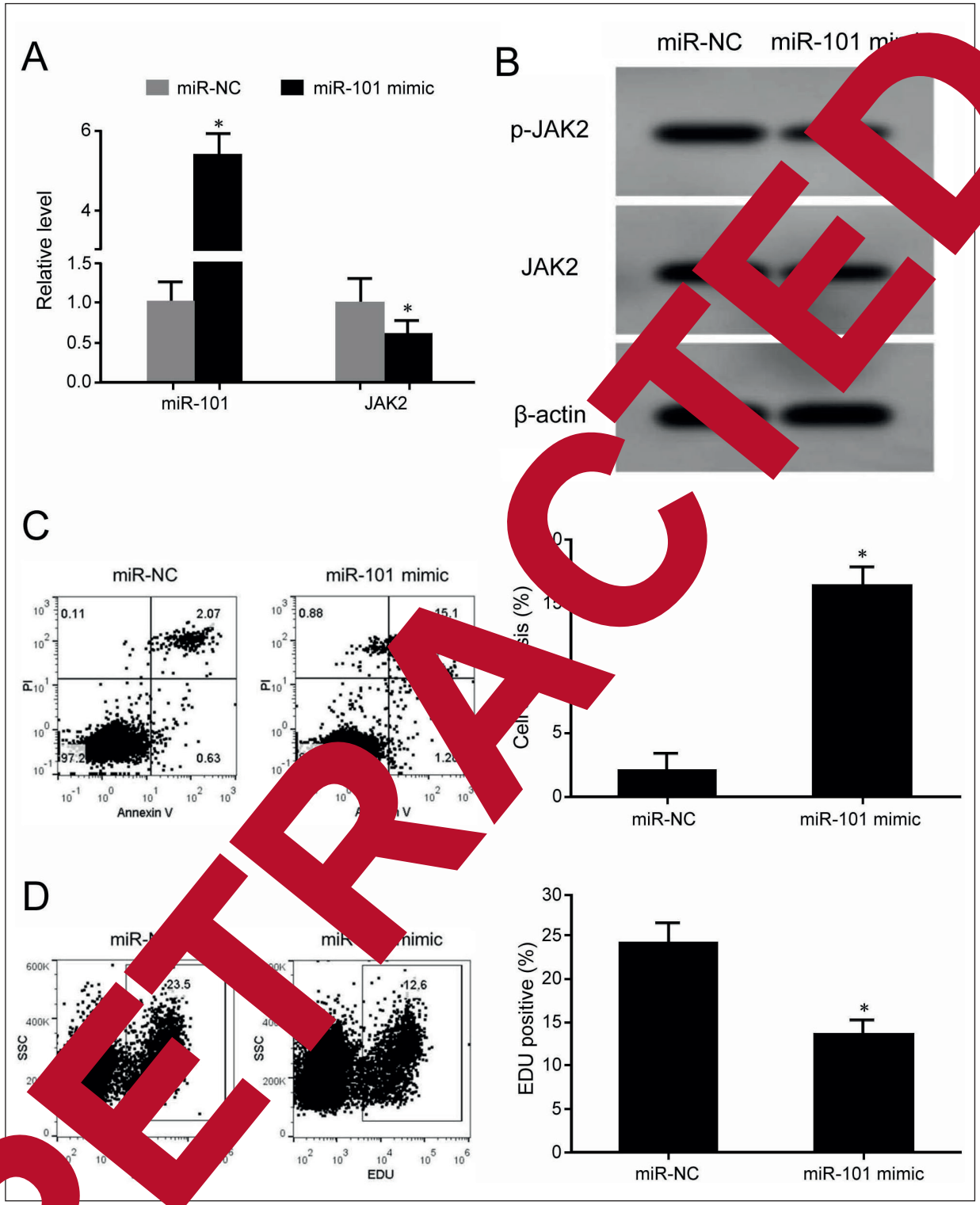
The qRT-PCR assay showed that miR-101 mimic transfection significantly up-regulated the expression of miR-101 in Caski cells compared to the miR-NC group ($p < 0.05$) (Figure 4A), resulting in a significant decrease in JAK2 mRNA expression (Figure 4A). Western blot analysis showed that the expression of JAK2 and p-JAK2 protein in Caski cells was significantly decreased in miR-101 mimic transfection group compared with miR-NC group ($p < 0.05$) (Figure 4B). Flow cytometry analysis showed that transfection of miR-101 mimic significantly increased apoptosis in Caski cells (Figure 4C) and attenuated cell proliferation (Figure 4D).

Discussion

The JAK-STAT signaling pathway is expressed in various tissues and cells, and involved in the regulation of various biological processes such as cell survival, proliferation, cy apoptosis, migration and invasion. d abnorm activation of JAK-STAT sig way are related to the occurrence, ression, metastasis and resistance of seve umors[4-7]. K2, an important regulator of th 2-ST pathway, is a signalin th e that plays an important role i activat the JAK2-STAT signaling ay. In respo ligand binding, the re form a hom ogous or heterodimer a phosp te the JAK2 kinase, and the a ated JAK2 phosphorylate the tyrosine due of STAT, cau STAT to complem AT v he SH2 domain to the tyrosine pho rylatio e of the receptor complex, at whi kina spatially adjacent to STAT osphor s its hydroxytyrosine. hory ET s dissociated from the recep omple s a dimer and is translocated fro e cytopla n to the nucleus, where it acts on A fragments, and regulates gene t cription and expression[18-20]. Several studies[21-24] have shown that the abnormal expression R-101 is related to the occurrence, progression and metastasis of various tumors such as lung cancer, gastric cancer and pancreatic cancer.

This study investigated whether miR-101 plays a role in regulating JAK2 expression and af ting the biological processes of proliferati tosis of cervical cancer cells.

Analysis of the clinical samp n this work showed that compared with t tissues, miR-101 expression in cervical ca atients was significantly decrease ile the io of JAK2 was significan ncreased. Co with normal cervical thelial erEpic c s, miR-101 expression rvica cer H a and Caski cells was si nifi reased e the expression of J was ficantl creased. The results ed that the miR-101 expression ciated with ased expression of J , a abnormalities of miR-101 and JAK2 were ass with the pathogenesis of c cancer. In per, the dual lucifer reporter assay owed that transfection of miR-101 mimic significantly reduced the tive lucifera ctivity of pMIR-JAK2-WT fected HEK T cells compared to miR-NC t tion gr Transfection of miR-NC or miR ad no significant effect on the relative luciferase activity of pMIR-JAK2-MUT transfected HEK293T cells, revealing the target-tory relationship between miR-101 and K2 RNA. In the study of the relationship between miR-101 and cervical cancer, Huang et al[25] found that the expression of miR-101 in cervical cancer HeLa cells was significantly abnormal, and overexpression of miR-101 in HeLa cells inhibits cell proliferation, attenuates cell invasion, and induces apoptosis through inhibiting the expression of COX-2. Jiang et al[15] observed that compared with normal cervical mucosa tissue, miR-101 expression in cervical cancer patients is significantly reduced. In addition to tumor tissue, the expression of miR-101 was decreased in peripheral blood of cervical cancer patients, and was associated with FIGO clinical stage and lymph node metastasis. Compared with those with higher expression of miR-101, the survival rate and prognosis of patients with lower miR-101 expression were lower and worse. Lin et al[26] detected that miR-101 expression was abnormal in tumor tissues of cervical cancer patients compared with the control by using gene expression microarray and RT-PCR. LNA-ISH further showed that the positive rate of miR-101 expression in cervical mucosa was 80%, while the positive rate of miR-11 expression in cervical cancer tissues was only 8%, and the positive rate of target gene COX-2 was significantly increased.



F sed miR-101 inhibits JAK2 expression and proliferation of cervical cancer cells, induces apoptosis. ***(A)*** qRT-PCR detection of miR-203, JAK2 mRNA expression; ***(B)*** Western blot analysis of JAK2, p-JAK2 protein ession; ***(C)*** flow detection of apoptosis; ***(D)*** flow detection of cell proliferation. * represents $p < 0.05$ compared to -NC group.

Liu et al[27] demonstrated that miR-101 expression was significantly decreased in cervical cancer tissues compared with normal cervical tissues. In this study, miR-101 expression was significantly decreased in cervical cancer tissues and tumor cells, indicating that miR-101 plays a role as a tumor suppressor gene in the pathogenesis of cervical cancer, which was consistent with Jiang et al[15] and Lin et al[26].

Further studies showed that transfection of miR-101 mimic significantly reduced the expression of JAK2 and p-JAK2 in cervical cancer Caski cells, which significantly attenuated cell proliferation and increased cell apoptosis. In the study of the relationship between miR-101 and the biological effects of cervical cancer cells, Liang et al[16] showed that there is a targeted regulatory relationship between miR-101 and Fos gene in cervical cancer HeLa cells, overexpression miR-101 can block the G1/S phase of the cell cycle by inhibition of Fos expression. Lin et al[28] observed that miR-101 plays a role as a tumor suppressor gene in cervical cancer. Overexpression of miR-101 in cervical cancer SiHa cells can inhibit cell proliferation, r[illegible]e cell invasion and increase cell apoptosi[illegible] hibition of COX-2 gene expression. Liu [illegible][27] showed that overexpression of miR-101 i[illegible] vical cancer SiHa cells significantly inhibi[illegible] cell proliferation and cell cycle [illegible]t, and pr[illegible] moted apoptosis. Shen et al[illegible] that the expression of miR-101 was [illegible]eased [illegible]relation to cervical cancer. The [illegible]ession [illegible]R-101 could inhibit the prolifer[illegible]f c[illegible] cells and decrease [illegible]yth [illegible]and tumorigenicity in anim[illegible]by inhib[illegible]of CXCL6 expression. This [illegible]y combines [illegible]rgeting regulation bet[illegible]124 and JAK[illegible], revealing that the [illegible]crease[illegible]ression of miR-101 and the in[illegible]sed expressi[illegible]JAK2 play a role in cervi[illegible]ancer, while the [illegible]ession of miR-101 i[illegible]reased. It has a negative anti-cancer effe[illegible] the m[illegible]nant biological characteristics of c[illegible]r cells. However, whether miR-101 reg[illegible]he bio[illegible]cal effects of JAK2 on [illegible]al ca[illegible]ells *in vivo* remains unclear and req[illegible]s furth[illegible]stigations.

Conclusions

[illegible] showed that the decrease of miR-101 expr[illegible] and the increase of JAK2 expression play a role in cervical cancer, while the increase of miR-101 expression can inhibit the proliferation of cells and promote the apoptosis of [illegible] by inhibiting the expression of JAK2.

Conflict of Interest

The Authors declare that they h[illegible] conflict[illegible]sts.

R[illegible]nce

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