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MiR-221 inhibits proliferation of pancreatic cancer cells via down regulation of SOCS3

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Abstract. – OBJECTIVE: The over-activation of Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway induced by cytokines are closely correlated with tumorigenesis. Suppressor of cytokine signaling 3 (SOCS3) serves as a negative regulator for JAK-STAT, and its down-regulation is involved in the oncogenesis of pancreatic cancer. We aimed at investigating the effect of miR-221 on the expression and proliferation, cycle and apoptosis of pancreatic cancer cells and determine the related mechanism.

PATIENTS AND METHODS: Dual luciferase reporter gene assay was used to analyze the regulation between miR-221 and SOCS3. The expressions of miR-221, SOCS3, p-JAK and p-STAT3 in normal human pancreatic epithelial cell HPDE6-C7 and pancreatic cancer cell PANC-1 were qua[illegible]d by qPCR and Western blot. Flow cytome[illegible] used to identify cell cycle and proliferation [illegible] *vitro* cultured PANC-1 cells were transfected wi[illegible]R-221 inhibitor or pIRES2-SOCS3. The expressio[illegible]f miR-221, SOCS3, p-JAK and p-STAT3, along [illegible] the cell proliferation or apoptosis, [illegible] compare[illegible].

RESULTS: Bioinformatics a[illegible]owed th[illegible] existence of binding site b[illegible]een [illegible]221 and 3'-UTR of SOCS3 mRNA [illegible]ual lucif[illegible]se gene reporter assay confirm[illegible] targ[illegible] tion between miR-221 and [illegible]3 [illegible] HPDE6-C7 cells, hig[illegible] levels [illegible]-221, p-JAK and p-STAT3 expr[illegible]ion, and l[illegible]xpression of SOCS3, were [illegible] in PANC-1 ce[illegible]g with the increase o[illegible]feration. Tran[illegible]ction of miR-221 inhib[illegible] or pI[illegible]SOCS3 remarkably enhanced SO[illegible]S3 expressio[illegible]ibited the levels of p-JAK an[illegible]-STAT3 express[illegible]d impeded the prolifer[illegible]n of PANC-1 cells.

CO[illegible]LUSIONS: MiR-221 decreases proliferati[illegible]tency [illegible]PANC-1 cells and affects JAK-STA[illegible]g pathw[illegible]via inhibiting SOCS3.

Key Words:
[illegible]221, S[illegible]TAT3, Pancreatic cancer, Proli[illegible]n, Cell cy[illegible]poptosis.

Introduction

[illegible]creatic carcinoma (PC), a commonly occu[illegible]malignant tumor, is characterized by an extreme high malignancy, worse treatment efficiency and prognos[illegible]has b[illegible] mortality and has become top 5 of [illegible]cers[1]. T[illegible]efore, the investigatio[illegible] signa[illegible]hway [illegible]plicated in PC patho[illegible]sis is of cri[illegible]tance for the impro[illegible]f chemothera[illegible]iciency and guidance[illegible] ind[illegible]lized treatment, which benefits patient progn[illegible].

Ja[illegible] kinase (JAK[illegible]nal transducer and a[illegible] of transcription [illegible]STAT) signaling pa[illegible]way are widely expressed in multiple tissues [illegible]d cells of ma[illegible]alians, and participate in the [illegible]ulation of di[illegible]se biological processes such as [illegible] survival, [illegible]oliferation, cycle progression, apo[illegible]on and invasion[2,3]. Suppressors of cyto[illegible] signaling (SOCS) can block pho[illegible]orylation of STAT via suppressing JAK kinase [illegible]us contributing to the inhibitory fun[illegible]ards transducing potency and activity of JAK-STAT[4,5]. SOCS3 is profiled as an important tumor suppressor gene, and its down-regulation is correlated with occurrence and progression of multiple tumors. Various studies[6,7] showed abnormal decrease of SOCS3 expression in PC tissues, and its expression level was correlated with PC progression, tumor growth and distal metastasis. MicroRNA (miR) belongs to the group of endogenous non-coding small molecule single-stranded RNA with 22-25 nucleotide at length in eukaryotes, and can modulate the expression of target gene via complementary binding onto 3'-UTR of its mRNA sequence. Although it takes up 1% of human genes, miR can modulate the expression of more than one third of human genes[8]. Increasing studies showed that miR-221 worked as an oncogene, and its abnormal up-regulation played roles in facilitating occurrence and progression of multiple tumors including breast cancer[9] and gastric carcinoma[10]. Evidence also indicated abnormal rise of miR-221 expression in prostate cancer, and miR-221 was correlated with the development and distal metastasis of PC patients[11]. This work aims to determine the role of miR-221 on the biological process of PC cells and seeks to find related mechanisms.

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Materials and Methods

Major reagents and materials

Normal human pancreatic epithelial cell line HPDE6-C7 was purchased from ScienCell (Carlsbad, CA, USA). PC cell line PANC-1 was from Gino Biotech (Hangzhou, Zhejiang, China). Roswell Park Memorial Institute-1640 (RPMI)-1640 medium and streptomycin-penicillin were purchased from HyClone (South Logan, UT, USA). Opti-MEM was collected from Gibco (Rockville, MD, USA). Fetal bovine serum (FBS) was acquired from Gemini Bio Products (Shanghai, China). RNA extract reagent Total RNA Kit was obtained from Omega Bio-Tek (Norcross, GA, USA). DNA extraction kit EasyPure Blood Genomic DNA Kit was bought from Quanshijin Biotech (Beijing, China). Fluorescent quantitative PCR reagent PrimerScript RT reagent Kit was purchased from TaKaRa (Kusatsu, Otsu, Shiga, Japan). Annexin V/PI apoptosis kit was from Biolegend (San Diego, CA, USA). Cell proliferation test reagent Click-iT EdU Flow Cytometry Assay Kit was obtained from Molecular Probes (Thermo Fisher Scientific, Waltham, MA, USA). Luciferase activity assay kit Dual-Glo Luci[illegible] Assay System was collected from Promeg[illegible]son, WI, USA). Luciferase reporter plasmi[illegible]UC was from BioVector (Beijing, China). Transf[illegible]n reagent Lip 2000 was bought from Invitrogen ([illegible]lsbad, CA, USA). Mouse anti-human JAK, p-JA[illegible] STAT3 and p-STAT3 antibo[illegible]urchase[illegible] from CST (Danvers, MA, US[illegible] Rabbi[illegible]i-human SOCS3 and β-actin antib[illegible] were ob[illegible]ed from Abcam (Cambridge, MA, [illegible]). H[illegible]roxidase (HRP) conju[illegible]d se[illegible]ntibody was obtained from Sang[illegible]io (Shang[illegible]hina). MiR-221 mimic, miR[illegible]nhibitor and [illegible]C were purchased fro[illegible] (Guangzhou, G[illegible]ngdong, China).

Cell cu[illegible]e

HP[illegible]6-C7 and PANC-1 cells were all incubated [illegible]oswell P[illegible]k Memorial Institute 1640 (RPMI-1[illegible]m cont[illegible]ing 10% fetal bovine serum ([illegible]nd 1% s[illegible]ptomycin-penicillin, and [illegible] incub[illegible] 3[illegible]C chamber with 5% CO_2. [illegible] medi[illegible] changed every three days. Cel[illegible]ere passe[illegible] when reaching 70-80% conflu[illegible]at 1:4 ratio. Those cells at log-growth phase w[illegible]y status were used for experiments.

[illegible]erase reporter gene assay

[illegible]C-1 cells were digested by trypsin and were collected. Genomic DNA was extracted using EasyPure Blood Genomic DNA Kit following the manual instruction. Using DN[illegible]s the template, full-length fragment [illegible] containing mutant sequent of 3'-U[illegible] of SOCS[illegible] gene was amplified. PCR product[illegible]ere extracted from agarose gel, and were l[illegible]into pLUC plasmid, which was used to tra[illegible] DH5α competent cells. Bacteria[illegible]olony w[illegible]tive screening was amplifie[illegible]nd sequenced[illegible] with correct sequen[illegible]ere selected and [illegible]e named as pLUC-S[illegible]wt, [illegible]pLUC-SOCS3-mut. Lipofectamine 2[illegible]used to[illegible]transfect pLUC-S[illegible]3-wt [illegible]UC-S[illegible]S3-mut with miR-22[illegible]mic or miR[illegible]HEK293T cells. After[illegible]cubation, Dua[illegible] Luciferase Assay S[illegible]m w[illegible]ed to measure the relative luciferase activity.

C[illegible]ction of SOCS[illegible] [illegible]er-expression plasmid

[illegible]CDS domai[illegible]agment of SOCS3 gene was [illegible]lified, and t[illegible] target fragment length was d[illegible]ined by a[illegible]rose gel electrophoresis, with 164[illegible]fter enzymatic digestion with XhoI and [illegible]H I, the fragment was ligated into [illegible]RES2 plasmid, which was used to transform [illegible] cell JM109. Positive clones were se[illegible]ected[illegible]y ampicillin resistance. Plasmids were then extracted and sequenced to determine the correct insertion of SOCS3 gene fragment. Those with successful gene insertion were named as pIRES2-SOCS3. Empty plasmid pIRES2-Blank was used as the control group.

Grouping of transfected cells

Cultured PANC-1 cells were divided into 4 groups: miR-NC group, miR-221 inhibitor group, pIRES2-Blank group, and pIRES2-SOCS3 group. All these nucleotide fragments and Lipo 2000 were mixed well in Opti-MEM, and incubated in room temperature for 5 min. Nucleotide fragments were then mixed with Lipo 2000 for gentle mixture, and were incubated for 30 min at room temperature. Original medium of PANC-1 cells was removed, and cells were rinsed twice in phosphate-buffered saline (PBS) to remove the serum. Opti-MEM with serum-free was used, and transfection mixture was added into the culture medium. Cells were incubated for 6 h, and RPMI 1640 medium containing 10% FBS and 1% penicillin-streptomycin was used. After continuous incubation for 72 h, cells were digested by trypsin for collection and for assays of related indexes.

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Quantitative reverse transcription polymerase chain reaction (qRT-PCR) for gene expression

PrimerScript RT reagent Kit was used in qRT-PCR assay to detect relative expression level of genes using RNA extracted by Total RNA Kit.

Reverse transcription system consisted

oligdT Primer (50 µM)	0.5 µL
Random 6 mers (100 µM)	0.5 µL
PrimeScript RT Enzyme Mix	0.5 µL
RNA	1.0 µg
5×PrimeScript Buffer	2 µL
RNase Free dH_2O	Up to 10.0 µL

qPCR system consisted

SYBR Fast qPCR Mix	10.0 µL
Forward Primer (10 µM)	0.8 µL
Reverse Primer (10 µM)	0.8 µL
cDNA	2.0 µL
RNase Free dH_2O	6.4 µL

Reaction parameters were: 95°C pre-denaturing for 10 min, followed by 40 cycles each containing 95°C denaturing for 10 s, 60°C ann[illegible] for 20 s and 72°C elongation for 15 s. P[illegible] performed on Bio-Rad CFX96 Real-time [illegible] detection system. Primer sequences were: miR-[illegible]: 5'-GTTGG TGGGA GCTAC ATTGT CTG[illegible] miR-221P_R: 5'-GTGTC GTGGA CTCGG CAA[illegible] C-3'; U6P_F: 5'-ATTGG AAC[illegible]G AGA[illegible] AG ATT-3'; U6P_R: 5'-GGA[illegible]GCT[illegible]ACGAA TTTG-3'; SOCS3P_F: 5'-C[illegible]C GCC[illegible]AAGAC CTTC-3'; SOCS3P_R: 5'-G[illegible]TG[illegible] TAGAA-3'; β-actinP_F: [illegible]GAA[illegible]AG GCC[illegible]A C-3'; β-actinP_R: 5'-T[illegible]CA CGCA[illegible]TTT CC-3'.

Western Blo[illegible]

Sodium do[illegible]cyl su[illegible]DS) lysis buffer was added to l[illegible] cells, and the[illegible] boiled for 5 min. Protein [illegible]centration was m[illegible]ed and 40 µg sampl[illegible] were loaded onto 8-10% separating gel and [illegible] conden[illegible]g gel. Proteins were transferred to p[illegible]li[illegible]e difluo[illegible]de (PVDF) membrane, which w[illegible]cked in[illegible]% defatted milk powder [illegible]0 min a[illegible] t[illegible]erature. Primary antibody w[illegible]nti-SOC[illegible]:1000, anti-p-JAK at 1:1000, anti[illegible]K at 1:200[illegible], anti-p-STAT3 at 1:1000, anti-S[illegible]T at 1:2000, and anti-β-actin at 1:10000. The m[illegible] then used in phosphate-buffered [illegible]ne and Tween (PBST) for three times, followed [illegible]m temperature incubation using horseradish-[illegible]idase (HRP) conjugated secondary antibody (1:10000) for 60 min. The membrane was washed in PBST for three times, and enhanced chemiluminescence (ECL) approach was [illegible] to test protein expression level.

Cell proliferation assay

Transfected cells from all g[illegible] were re-suspended and cultured in 10 µM Ed[illegible]20 min. Cells were inoculated int[illegible]well pla[illegible]48 h continuous culture. Try[illegible] was then use[illegible] gest and collect all ce[illegible] which were fixed fo[illegible]0 min at room tempe[illegible] Cell[illegible]ere washed in wash buffer by centri[illegible] were pe[illegible]abled for 15 min at r[illegible] tempe[illegible] 500 µ[illegible]eaction buffer were a[illegible]d for 30 mi[illegible]ncubation at room te[illegible]re, followed [illegible]one time of wash bu[illegible] cen[illegible]tion. Cells were eventually re-suspended in 50[illegible] wash buffer, and were meas[illegible]d for prolifera[illegible]ing Beckman FC500 M[illegible] cytometry (Bre[illegible], CA, USA).

[illegible]ow cytome[illegible] for cell cycle

Transfected c[illegible] from all groups were digested [illegible]d collect[illegible] After washing in PBS, cells wer[illegible]% ethanol at -20°C for 60 min. Cells we[illegible]ashed twice by centrifugation, and [illegible]re mixed with propidium iodide (PI) staining [illegible]taining RNase A for 30minn dark [illegible]cu[illegible]on at 4°C. Cell cycle was measured by Beckman FC500 MCL flow cytometry apparatus.

Flow cytometry for cell apoptosis

Cells were collected by centrifugation. After washing twice in phosphate-buffered saline (PBS), 100 µL Binding Buffer were added for mixture. 5 µL Annexin V-FITC and 5 µL PI were subsequently added for 10 min dark incubation. 400 µL Binding Buffer were then added to re-suspend cells, which were detected using Beckman FC 500 MCL flow cytometry.

Spectrometry for Caspase-3 activity

Following the manual instruction of Caspase-3 activity assay kit, pNA standard samples were firstly prepared to measure A405 and to plot standard curves. Cells were digested by trypsin and were collected, lysed on ice and centrifuged. The supernatant was saved and transferred to new pre-cold tubes. Assay buffer, test samples, and Ac-DEVD-pNA, were added sequentially into 96-well plate, for 2 h incubation at 37°C. Absorbance value A405 was measured using a microplate reader when founding significant color change, to reflect Caspase-3 activity of test samples.

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Statistical Analysis

SPSS 18.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Measurement data were presented by mean±standard deviation (SD). Student *t*-test was used for comparing measurement data between two groups. The comparison of measurement data among multiple groups was performed using one-way analysis of variance (ANOVA) followed by Least Significance Difference (LSD). A statistical significance was defined when $p<0.05$.

Results

Targeted regulation between miR-221 and SOCS3

Online prediction by microRNA.org showed complementary binding sites between miR-221 and 3'-UTR of SOCS3 mRNA sequence (Figure 1A). Dual luciferase gene reporter assay found that, compared to miR-NC group, miR-221 mimic transfection HEK283T cells gave rise to remarkable decrease in relative luciferase activity. There was no significant change on relative luciferase activity after co-transfection of miR-221 mimic and pLUC-SOCS3-mut into HEK293T cells (Figure 1B), suggesting a targeted regulatory relationship between miR-221 and SOCS3.

Abnormal expression of miR-221 and SOCS3 in PC cells

The result of flow cytometry revealed that the ratio of S phase and G2/M phase in PANC-1 cells significantly increased compared to HPDE6-C7 cells, while G0/G1 phase was significantly lower (Figure 2A). Compared to that in HPDE6-7 cells, positive rate of EdU in PANC-1 cells was significantly elevated, indicating strong proliferation potency (Figure 2B). Moreover, qRT-PCR results showed that compared to that in HPDE6-C7 cells, significantly high miR-221 expression with decrease of SOCS3 mRNA level was found in PANC-1 cells (Figure 2C). Western blot results also showed that, compared to that in HPDE6-C7 cells, the SOCS3 protein expression was remarkably reduced in PANC-1 cells, along with the growing expression of p-JAK and p-STAT3 (Figure 2D).

miR-221 down-regulation effectively inhibited PC cell PANC-1 proliferation and induced cell apoptosis

We determined the effect of miR-221 on the development of PC by using miR-221 inhibitor. Our data demonstrated that, compared to miR-NC transfection group, in PANC-1 cells treated with miR-221 inhibitor, the level of SOCS3 was significantly elevated, while p-JAK and p-STAT3 expressions were remarkably decreased (Figure 3A-B). Flow cytometry showed that the inhibition of miR-221 significantly decreased proliferation potency (Figure 3C), and remarkably enhanced cell apoptosis level (Figure 3D). We also evaluated the role of SOCS3 by over expression of SOCS3 *in vitro*; the results illustrated that the elevation of SOCS3 expression significantly upregulated the levels of p-JAK and p-STAT3 (Figure 3A and 3B), which enhanced proliferation potency (Figure 3C) and suppressed cell apoptosis (Figure 3D).

Discussion

It is estimated that PC incidence has reached 8.3 per 100 000 people worldwide, with mortality rate up to 7.8%[12]. In China, about 91 000 patients were newly diagnosed as PC annually, accompanied with 79 000 cases of death[13]. Generally, PC represents a type of cancers with worse progno-

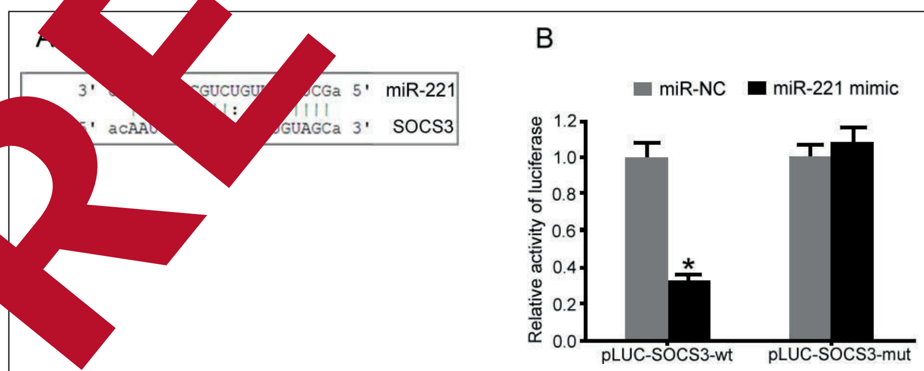

Figure 1. Targeted regulatory relationship between miR-221 and SOCS3. **A**, Schematic diagram for binding sites between miR-221 and SOCS3 mRNA. **B**, Dual luciferase gene reporter assay. *, $p<0.05$ compared to miR-NC.

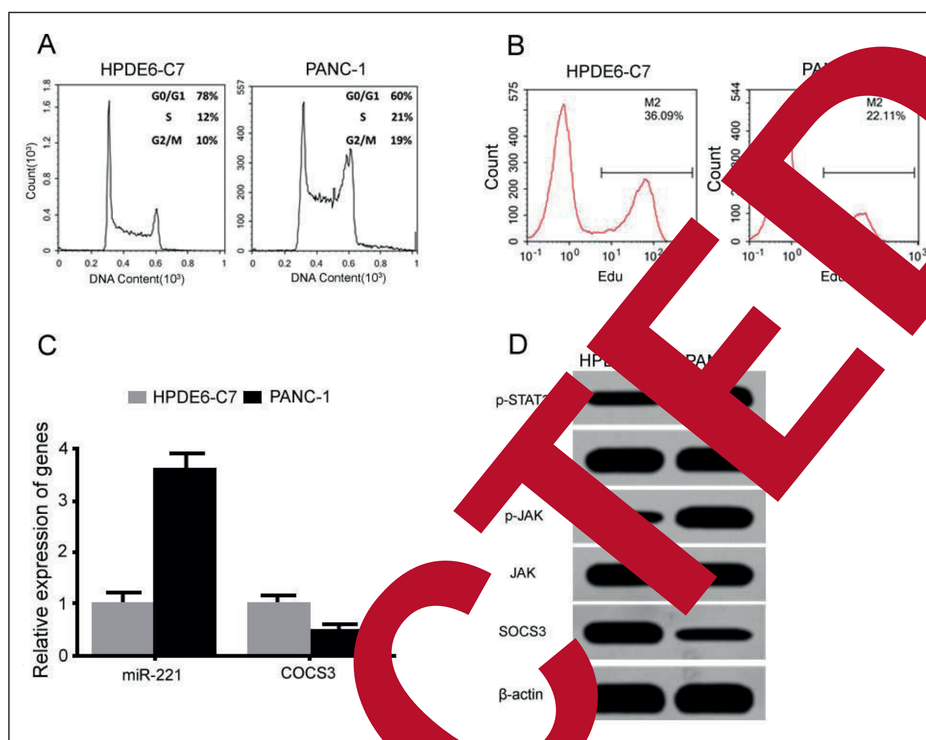

Figure 2. Abnormal expression change of miR-221 and SOCS3 in PC cells. **A**, Flow cytometry for cell cycle; **B**, Flow cytometry for cell proliferation potency; **C**, qRT-PCR for gene expression;D, Western blot for protein expression. *, $p<0.05$ compared to HPDE6-C7 cells.

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sis, an average survival age around 5 [illegible] and a 5-year survival of about 1-5%[14,1[illegible]] [illegible] has been demonstrated that abnormal activat[illegible] of JAK-STAT signaling pathway plays impo[illegible] regulatory roles in facilitating abnormal p[illegible] liferation of cells, accelerati[illegible]ycle pro[illegible] gression, enhancing invasi[illegible] or me[illegible]sis, and decreasing apoptosis[2,3]. [illegible]umulativ[illegible] evidence presented that activated [illegible]T de[illegible] receptor complex, fo[illegible]ed d[illegible] the further transportation from [illegible]toplasm [illegible]leus, where it functions o[illegible]ific DNA se[illegible] to mediate gene tra[illegible] and expressio[illegible][6,17]. The specific proce[illegible] is furt[illegible]sely correlated with tumor pat[illegible]genesis, prog[illegible] and prognosis. Several [illegible]dies illuminated [illegible]ormal change of JA[illegible]TAT signaling pathway activity in vario[illegible]mor ti[illegible]s including prostate cancer[18], colo[illegible]noma[4], [illegible]d lung cancer[19]. This investig[illegible]howed [illegible], compared to normal [illegible]reatic [illegible]PD[illegible]C7, PC cell line PANC-1 s[illegible] p-JA[illegible] p-STAT3 expressions were rem[illegible]ably enha[illegible]ed, with the increase of cell pro[illegible] activity, which suggested the invol[illegible]K-STAT signaling pathway in the [illegible]urrence and development of PC.

[illegible]CS3 can suppress JAK binding with recep[illegible]a its KIR domain, thus suppressing JAK phosphorylation or kinase activity. Alternatively, [illegible]can inhibit JAK phosphorylation on STAT via [illegible]e binding with STAT, thus exerting [illegible] regulatory role on JAK-STAT3 signal pathway activity[20,21]. Recent investigations shed light on the correlation of its down-regulation with the occurrence and progression of multiple tumors including breast cancer[20], colorectal carcinoma[22] and prostate cancer[21]. Particularly, abnormal decrease of SOCS3 expression was validated in PC, and its expression level was related with PC progression, tumor growth and distal metastasis[6,7]. Consistently, our study showed that the over expression of SOCS3 by transfection with pIRES2-SOCS3 decreased p-JAK or p-STAT3 expressions in PANC-1 cells, accompanied with weakened proliferation and potentiated apoptosis of cells, indicating the inhibitory role of SOCS3 in JAK-STAT3 signal pathway as well as PC.

MicroRNAs take a critical part in various malignant tumors[23]. MiR-221 is widely reported to have pro-tumor role, and plays facilitating role in occurrence and progression of various tumors. For example, Li et al[9] showed that miR-221 up-regulation could facilitate proliferation, migration and invasion of breast cancer cells via targeted regulation on PTEN/AKT signaling pathway. Effatpanah et al[10] found significantly higher miR-221 expression in gastric cancer tissues compared to normal gastric mucosa. Li et al[24] reported that compared

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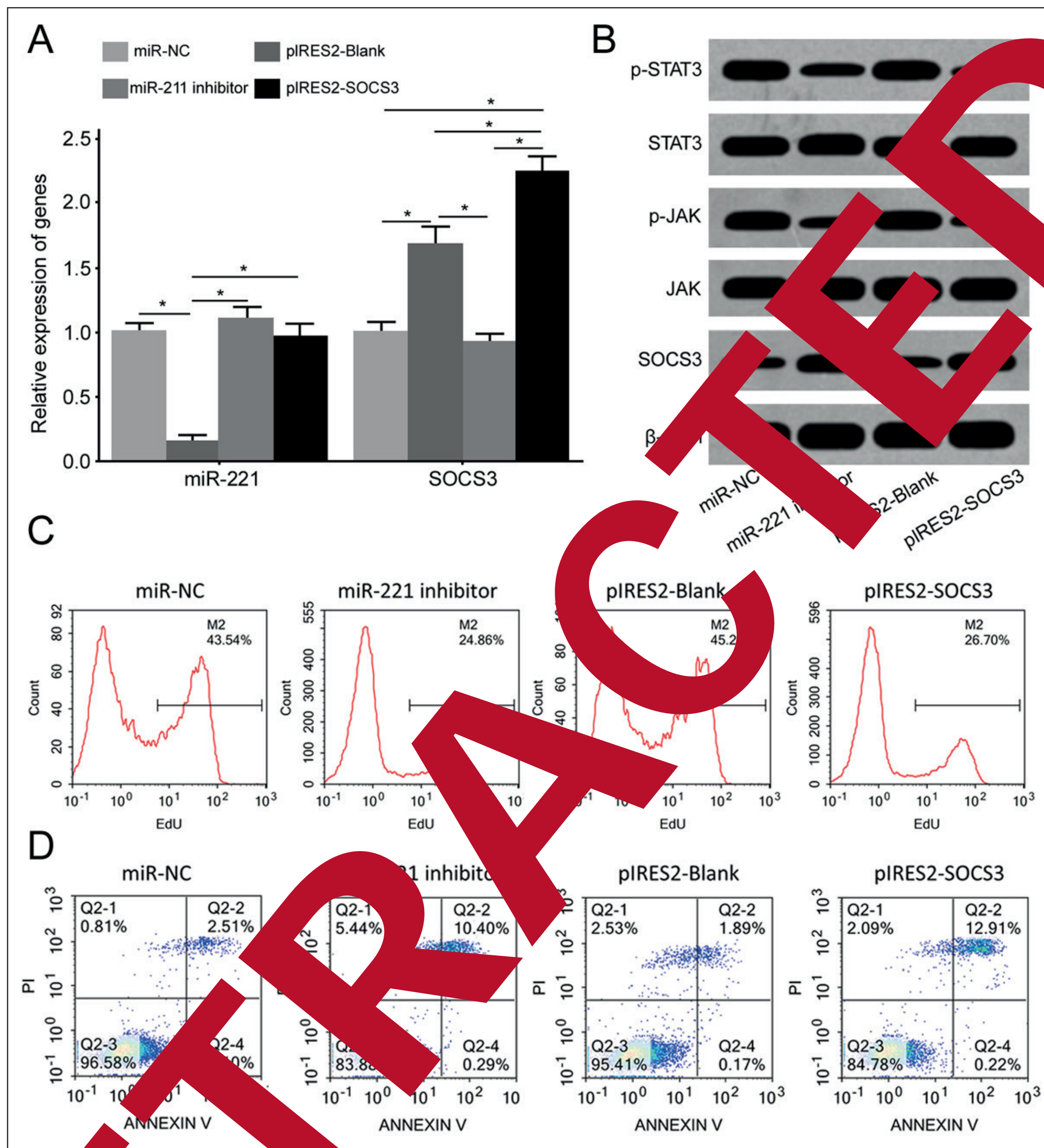

Figure [illegible] [illegible]iR-221 down-regulation [illegible]pressed PC cell proliferation and induced apoptosis. **A**, qRT-PCR for gene expression; **B**, We[illegible] blot for protein expression; **C**, EdU staining for cell proliferation; **D**, Flow cytometry for cell apoptosis. *, $p<0.05$ com[illegible] betwee[illegible]o groups.

[illegible]rmal p[illegible]ati[illegible]uctal epithelium, PC cells s[illegible] remar[illegible]levated miR-221 expression. The[illegible]so found that compared to tumor adjacent tiss[illegible] PC tissues showed abnormally elevated m[illegible]ssion, and those up-regulated serum [illegible]R-221 were correlated with patient TNM stage [illegible]istal metastasis, plus relatively higher value for [illegible]rential diagnosis of distal metastatic PC (AUC=0.689). Xu et al[25] demonstrated that compared to non-infiltrative PC cell line Bxpc-3, infiltrative PC cell line SW-1990, PANC-1 and Miapaca-2 showed remarkably elevated miR-221 expression. Similarly, we observed that compared to normal pancreatic epithelium, miR-221 expression was abnormally elevated in PC cells showed, indicating its potential oncogene role in PC pathogenesis, which was in line with previous finding that compared to normal pancreatic tissues. PC tumor

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tissues also showed remarkably elevated miR-221 expression, which was inversely correlated with patient survival[26].

Of note, our data on dual luciferase gene reporter assay confirmed the targeted regulatory relationship between miR-221 and SOCS3. The expression of SOCS3 and G0/G1 phase arresting were significantly decreased. We proposed that miR-221 up-regulation might play a role in suppressing SOCS3 expression, enhancing JAK-STAT3 pathway activity, and facilitating PC cell proliferation and cycle progression. Further study elucidated that the suppression of miR-221 remarkably up-regulated SOCS3 expression in PANC-1 cells, which also impeded the cell proliferation. Sarkar et al[26] found that after using drugs such as G2535, BR-DIM or CDF to suppress miR-221 expression, the expression of tumor suppressor genes including PTEN, p27 (kip1), p57 (kip2) and PUMA was significantly elevated in PC cells such as MiaPaCa-2 or PANC1, whilst cell proliferation or migration potency was compromised. Tanaka et al[27] found abnormally elevated miR-221 expression in PC cells. After metformin drug treatment, the expression of target gene p27Kip1 was remarkably elevated, accompanied with [illegible] G1 phase arresting and weakened prolife[illegible] PC cells, which was in agreement with ou[illegible]dy. Furthermore, Lesina et al[28] found that knockd[illegible] of SOCS3 expression remarkably up-regulated ST[illegible] transcriptional activity, thus facilitating progress[illegible] of PC. Patel[29] showed that SOC[illegible]egulatio[illegible] was correlated with malignan[illegible]ologic[illegible]roperties of PC cells. When SOCS3 e[illegible]ession wa[illegible]nhanced, activity of JAK kinase and [illegible]3 we[illegible] weakened, and growth[illegible] mig[illegible]tency of PC cells was significan[illegible]estricted. [illegible]vestigation observed the corr[illegible] between STA[illegible]ynregulation and malig[illegible]ical behaviors [illegible]PC cells, and SOCS3 u[illegible]-regulat[illegible]gnificantly weakened PC malign[illegible] features. Ho[illegible]whether such modulatory [illegible]ationship existed b[illegible]n miR-221 and SOCS[illegible] PC patients *in vivo* still remains unclear, and [illegible]her inve[illegible]ations are thus required to elucidate [illegible] profiles [illegible]miR-221 and SOCS3 in PC tissu[illegible]

[illegible]onclusions

[illegible] that miR-221 inhibits SOCS3 [illegible]ression. Down-regulation of miR-221 enhan[illegible]OCS3 expression, suppresses activity of JA[illegible]AT3 pathway, remarkably weakening proliferation potency of PC cell line PANC-1.

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Conflict of Interest:

The Authors declare that they ha[illegible] no co[illegible]nterests.

Ref[illegible]nces

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