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MiR-155 regulates the proliferation and apoptosis of pancreatic cancer cells through targeting SOCS3

J. WANG[1], J. GUO[1], H. FAN[2]

[1]Department of Hepatobiliary Surgery, Huangshi Central Hospital of the [illegible] Medical Group, Huangshi, Hubei, China
[2]Department of Ophthalmology Clinic, Huangshi Central Hospital of [illegible] Medical Group, Huangshi, Hubei, China

Abstract. – OBJECTIVE: Reduced expression of suppressors of cytokine signaling 3 (SOCS3) is associated with a variety of tumors. The elevated miR-155 expression is associated with the onset of pancreatic cancer. Bioinformatics analysis revealed a targeted relation between miR-155 and the 3'-UTR of SOCS3. This study investigated whether miR-155 regulates SOCS3 expression and affects the biological effects of pancreatic cancer cells.

PATIENTS AND METHODS: QRT-PCR was used to detect the expression of miR-155 and SCOS3 mRNA in tumor tissues and paracancerous tissues of patients with pancreatic cancer. The dual luciferase reporter gene assay validated the target interaction between miR-155 and SOCS3. Pancreatic cancer cell line SW1990 cells were divided into miR-NC group, miR-155 inhibitor group followed by [illegible] of the expressions of SOCS3, p-JAK2 and p-STAT3, cell apoptosis by flow cytometry, and cell proliferation by EdU staining.

RESULTS: Compared with adjacent tissues, miR-155 expression was increased in tumor tissues of patients with pancreatic cancer, and SOCS3 expression was decreased. There was a targeted regulatory relationship between miR-155 and SOCS3 mRNA. Compared with HPDE6-C7 cells, miR-155 expression in pancreatic cancer SW1990 and Capan-2 cells was increased, and SOCS3 expression was decreased. Transfection of miR-155 inhibitor significantly increased SOCS3 expression in pancreatic cancer [illegible], decreased the expression of p-JAK2 and p-STAT3, increased cell apoptosis, and decreased cell proliferation.

CONCLUSIONS: Increased miR-155 expression and decreased SOCS3 expression are related to the pathogenesis of pancreatic cancer. miR-155 promotes the proliferation and apoptosis of pancreatic cancer cells by inhibition of SOCS3 expression.

Keywords
MiR-155, SOCS3, JAK-STAT, Pancreatic cancer.

Introduction

Pancreatic carcinoma (PC) is a common malignant tumor of the digestive system. It has a very high degree of malignancy, so the treatment effect and prognosis are extremely poor with a high mortality rate.

SOCS3 is a member of the cytokine signal transduction inhibitory protein (SOCS) family, which blocks the activation and transmission of the JAK-STAT signaling pathway by inhibiting activity of JAK kinase[2]. As an important tumor suppressor, the expression or decreased function of SOCS3 plays an important role in the occurrence, progression, and metastasis of various tumors[3-5]. There is research evidence that abnormal expression or dysfunction of SOCS3 is associated with the occurrence, progression, and metastasis of pancreatic cancer[6-8].

MicroRNA (microRNA) is an endogenous non-coding small RNA molecule in eukaryotes that bind to the 3'-untranslated region (3'-UTR) of the target gene mRNA through complementary pairing, leading to degradation of mRNA or inhibition of mRNA translation, thus regulating the expression of target genes and participating in the regulation of biological processes such as cell survival, proliferation, apoptosis, migration. MicroRNA expression and dysfunction have been shown to be associated with non-small cell lung cancer[9], gastric cancer[10], prostate cancer[11], bladder cancer[12], and other tumors. A number of studies[13-16] have shown that the abnormal expression and function of miR-155 are related to the occurrence, progression, metastasis, and prognosis of various tumors such as breast cancer, gastric cancer, lung cancer and intestinal cancer. Several researches[17-19] have shown that abnormal changes in the expression of miR-155 are associated with the

Corresponding Author: Hui Fan, MD; e-mail: songlinzhanw@yeah.net

development, progression, and prognosis of pancreatic cancer. Bioinformatics analysis revealed a targeted binding site between miR-155 and the 3'-UTR of SOCS3 mRNA. This work investigated whether miR-155 plays a role in regulating SOCS3 expression, affecting JAK-STAT pathway activity, pancreatic cancer cell proliferation, and apoptosis.

Patients and Methods

Patients

30 patients with pancreatic cancer who were treated in our hospital from June 2018 to November 2018 were enrolled. The tumor tissues of pancreatic cancer confirmed by pathological examination were collected and the paracancerous tissues located at least 2 cm away from the tumor tissue were collected as control. This research was approved by the Ethic Committee of our hospital and informed consent was obtained from the patients.

Main Reagents and Materials

Human normal pancreatic epithelial cells HPDE6-C7 were purchased from Hunan ... hui organism; pancreatic cancer cells SW19... Capan-1 were purchased from Shanghai Six... organism; Dulbecco's Modifie... Medium (DMEM was purchased fr... ibco ... and Island, NY, USA); Fetal bo... serum ...S) was purchased from Shangh... sai ... nology Company; Li... 20... rchased by Invitrogen (Carlsba... A, US... meScript™ RT reagent Kit, S... Green dye p... ed from TaKaRa (Dalia... miR-155 inh... r, miR-NC, miR-155... mic ... ed and synthesized by Ruibo Bio (Guangzh... China); rabbit anti-human ...TAT3, p-JAK2 ... dy Purchased in the ... CST; rabbit anti-human antibody was purch... from ...ta Cruz Biotechnology (Santa ... CA ...A); rab... anti-human SOCS3, β-acti... , HRP ... jugated secondary antibody w... chas... om Abcam (Cambridge, ...SA); ... smid was purchased from Cha... a, Chin... detection kit was purchased fro... omega (Madison, WI, USA); the EdU-Alex... l proliferation assay kit was purchased from Thermo Fisher Scientific (Waltham, ... USA); the Annexin V-FITC/PI apoptosis a... agent was purchased from Jiangsu Biyuntian ...nce and Technology.

Cell Culture

HPDE6-C7, SW1990, and Capan-1... inoculated in DMEM medium contai... 10% ... and 1% streptomycin, and cultured ... cell culture ... incubator at 37°C with 5% CO_2 u... cells reached a confluence of 90%. After diges... h 0.25% trypsin, the cells were colle... d by c... ation sub-cultured at a ratio of 1:... :5, and cells ... arithmic growth phase w... elected ... experi...

Dual Luciferase ... ...ne Te...

Using the H... 293 ... enome ... template, the ful... gth 3'-... frag... t of the SOCS3 gen... he fragment ... ng the mutant was a... d cloned int... e pMIR vector, tran... med ... H5α competent cells, and the correct plasmids ... equenced and named as ...-SOCS3-WT, ...MIR-SOCS3-MUT. p... SOCS3-WT (or pMIR-SOCS3-MUT) and ...-155 mimic (o... miR-NC) were co-transfected ... HEK293T c... with Lip2000. After 48 h of ...re, the lucif... e activity kit was used to de... relative ...ferase activity.

Cell Transfection

SW1990 cells were cultured *in vitro* and divided ... ansfection groups: miR-NC transfection ...oup ... d miR-155 inhibitors transfection group. The general procedure for transfection was as follows: 10 μL of Lip2000, 50 nmoL miR-NC, and 50 nmoL miR-155 inhibitor were diluted with 100 μL of serum-free Opti-MEM, and incubated for 5 min at room temperature, respectively, and Lip2000 and miR-NC, miR-155 inhibitor, respectively. They were gently mixed, incubated for 20 min at room temperature, and the transfectants were added to the cell culture medium, mixed gently and continued to culture for 72 hours followed by a collection of the cells to measure the relevant parameters.

QRT-PCR Detection of Gene Expression

RNA was extracted using TRIzol method and RNA was reversely transcribed into cDNA using PrimeScript™ RT reagent Kit. The resulting cDNA was stored in a refrigerator at –20°C. PCR amplification was carried out under the action of Taq DNA polymerase using cDNA as a template in a total of 10 μL PCR reaction system including 2×SYBR Green Mixture 5.0 μL, 2.5 μm/L forward primer 0.5 μL, 2.5 μm/L reverse primer 0.5 μL, cDNA 1 μL, and ddH_2O. PCR conditions were designed as follows: 95°C for 5 min, 95°C for 15 s, 60°C for 1 min, fluorescence amplification data were stored on a Bio-Rad (Hercules,

CA, USA) CFX96 Real-time PCR instrument for 40 cycles. Primer sequences were: SOCS3-F: 5'-CCTGCGCCTCAAGACCTTC-3', SOCS3-R: 5'-GTCACTGCGCTCCAGTAGAA-3'; β-actin-F: 5'-CATGTACGTTGCTATCCAGGC-3', β-actin-R: 5'- CTCCTTAATGTCACGCACGAT-3'.

Western Blot

The cells were collected, and the protein was extracted from the RIPA lysate. The concentration was determined by bicinchoninic acid (BCA) assay and 40 μg was separated in sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel for 3 h, transferred to a polyvinylidene difluoride (PVDF) membrane by electroporation for 90 min, blocked with 5% skim milk powder, and incubated with the primary antibody at 4°C overnight (the dilution ratios of SOCS3, p-JAK2, p-STAT3, and β-actin were 1:2000, 1:800, 1:800, 1:8000, respectively), and subsequent washing three times with Phosphate-Buffered Saline and Tween (PBST). Next, the membrane was incubated with horseradish peroxidase (HRP)-labeled secondary antibody (1:8000 dilution) for 60 min at room temperature followed by washing 3 times with PBST. Enhanced chemiluminescence (ECL) w... solution was added to blotting membrane fo... at room temperature, and subsequent expos... nd development.

Flow Cytometry Detection of Cell Apoptosis

The above-mentioned m... C a... miR-155 inhibitor transfection gro... W1990... ls were collected by trypsinizati... fter w... with PBS, 300 μL of B... ding ... as added to the cell pellet follow... y addi... 5 μL of Annexin V-FITC fo... min incubati... er dark. After that, 5 μL ... dium iodide (P... staining was added, and 200 μL of Binding Buffer was added followed by an analysis of cell a... flow cytometry.

EdU Staining for Cell Proli... ion

The above two miR-NC and m... inhibitor transfection group SW199... ells we... ected by trypsinization. SW19... ells were re... ed in DMEM complet... dium ... ntaining ... FBS, incubated with ... M Ed... 120 min, and cultured for 48 h. A... zy... c dige... and collection of cel... ell p... tion w... tected by Beckman C... er FC 5... CL f... cytometry accordin... he kit instruc... he prolifer-ation of c... lected by Ed... positive rate.

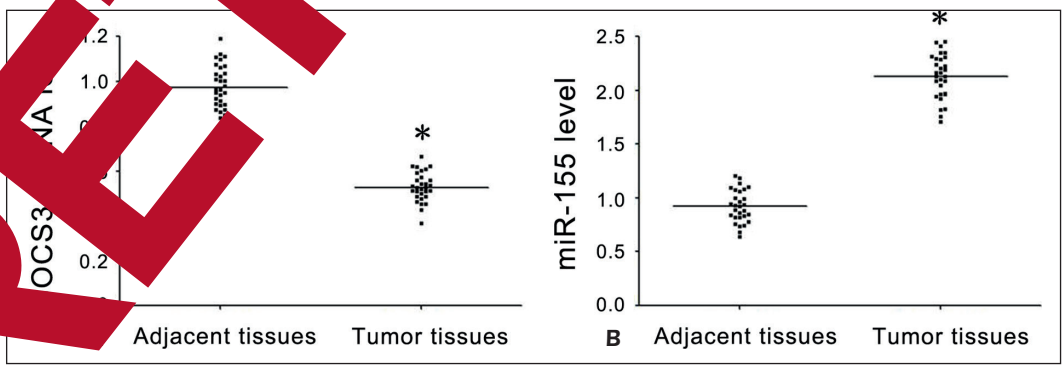
Statistical Analysi...

S... al analysis wa... formed using SPSS 1... oftware (SPSS Inc., Chicago, IL, USA). The ... surement data ... ere expressed as mean ± standard deviation (S... The comparison between the ... urement da... the groups was analyzed by S... 's *t*-test... 0.05 was considered statistically

Results

Abnormal Expression of MiR-155 and SOCS3 in Pancreatic Cancer

The results of qRT-PCR showed that the expression of SOCS3 mRNA in tumor tissues of pancreatic cancer patients was significantly lower than that in adjacent tissues (Figure 1A). The results of qRT-PCR showed that the expression of miR-155 was significantly increased in tumor tissues of patients with pancreatic cancer compared with adjacent tissues (Figure 1B).



...1. Abnormal expression of miR-155 and SOCS3 in pancreatic cancer. **A**, QRT-PCR detection of SOCS3 mRNA expressi... creatic cancer tissues; **B**, QRT-PCR detection of miR-155 expression in pancreatic cancer tissues. *represents $p<0.05$ comp... with adjacent tissues.

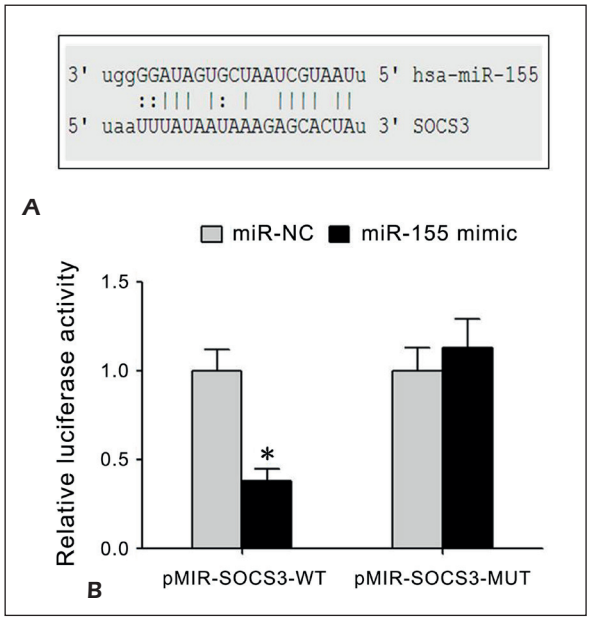


Figure 2. Targeted regulatory relationship between miR-155 and SOCS3 mRNA. A, Schematic diagram of the interaction site between miR-155 and the 3'-UTR of SOCS3 mRNA; B, Dual luciferase gene reporter assay. *represents $p<0.05$ compared to miR-NC.

Targeted Relationship Between miR-155 and SOCS3 mRNA

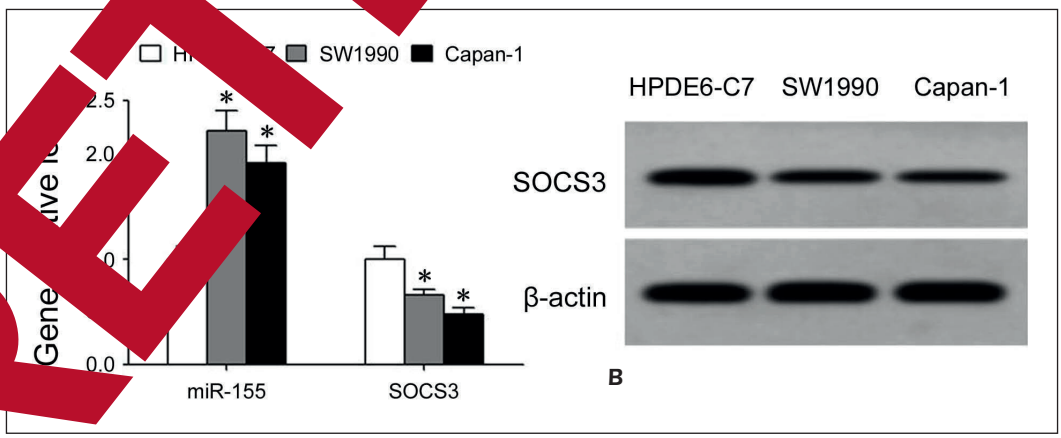
Bioinformatics analysis revealed a comp mentary binding site between miR-155 and the 3 UTR of SOCS3 mRNA (Figur l lucifer- ase gene reporter assays sho that sfection of miR-155 mimic signifi y redu relative luciferase activity in p OCS fected HEK293T cell but or miR-155 mimic did not have significant effect on relative luciferase activity in HEK293T cells with pMIR-SOCS3-MUT (Figure 2 dicat that miR-155 has a targeted reg ry relation- ship with the 3'-UTR region of 3 mRNA.

Increased MiR-155 and ecrea SOCS3 Expression in creatic Cancer Cells

The qRT-PCR an s show hat compared with normal panc ep al HP -C7 cells, the expre n 155 in creatic cancer SW199 d Capa lls s signifi- cantly incre while the ex of SOCS3 mRNA w ntly decreas (Figure 3A). Western t ana owed that the expression of SOCS3 protein i eatic cancer SW1990 and -1 cells was icantly lower than th HPDE6-C7 cells (Figure 3B).

ibition of 155 Expression ificantly I bits Pancreatic Cell P feration and Promotes Apo

The qRT-PCR analysis showed that the expres- on of miR-155 in SW1990 cells was significantly in miR-155 inhibitor transfection group mpa d with miR-NC transfection group (Fig- ure 4A), while the expression of SOCS3 mRNA was significantly increased (Figure 4B). Western blot analysis showed that compared with miR-NC transfection group, the expression of SOCS3 pro- tein in SW1990 cells was significantly increased in miR-155 inhibitor transfection group, while the expression of p-JAK2 and p-STAT3 protein was



3. Increased expression of miR-155 in pancreatic cancer cells and decreased expression of SOCS3. A, QRT-PCR was us tect the expression of miR-155 and SOCS3 mRNA in cells; B, Western blot was used to detect the expression of SOCS otein in cells. *represents $p<0.05$ compared to HPDE6-C7 cells.

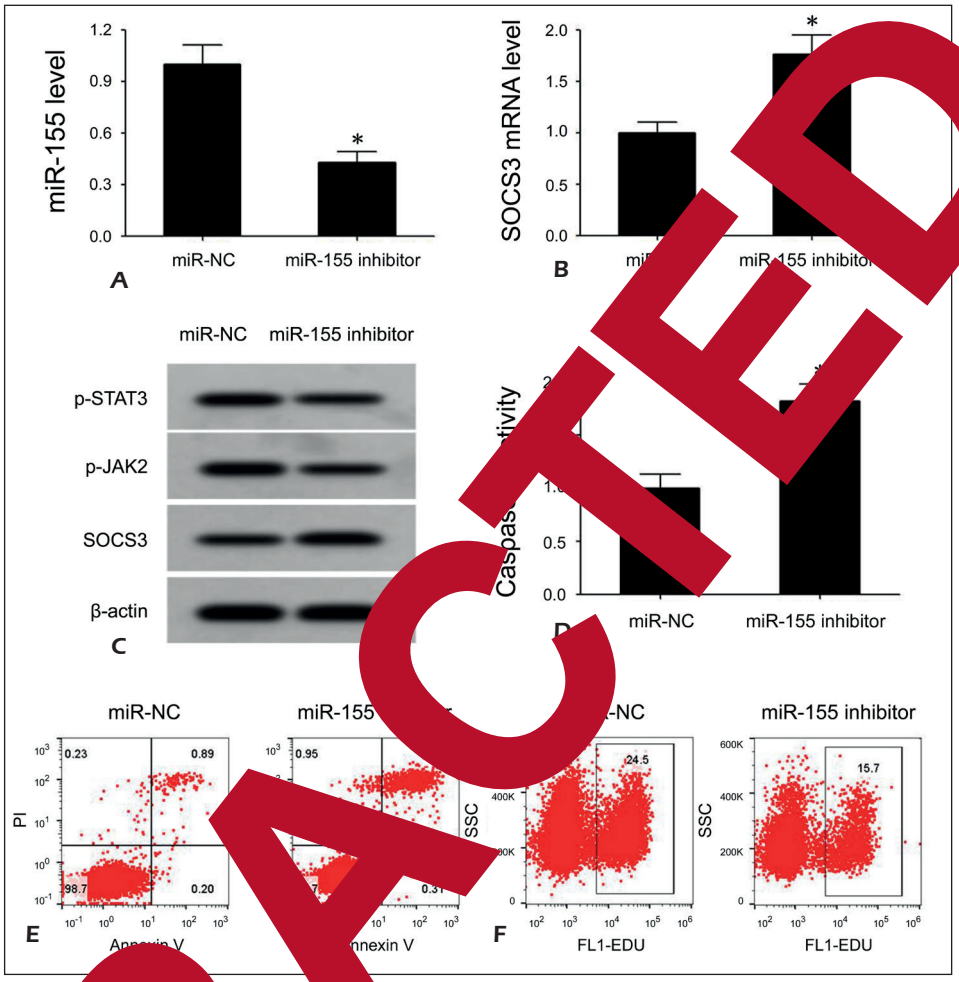


Figure 4. Inhibition of miR-155 expression can significantly inhibit pancreatic cancer cell proliferation and promote apoptosis. **A**, QRT-PCR detection of intracellular miR-155 expression; **B**, QRT-PCR detection of intracellular SOCS3 mRNA expression; **C**, Western blot detection of intracellular protein expression; **D**, Spectrophotometric detection of intracellular caspase-3 enzyme activity;**E**, Flow detection of apoptosis; **F**, EdU staining for cell proliferation. *represents $p<0.05$ compared to miR-NC.

significantly decreased (4C) tometry analysis sho d t -3 activity was significantly i sed in 90 cells of miR-155 inhibito nsfection gr mpared with miR-NC s ure 4D). Flo ytometry analysis s ed t nsfection of miR-155 inhibitor si ificantly inc apoptosis (Figure 4E) and i ited proliferati SW1990 cells (Figure .

Dis ion

tyros e (JAK)-signal transducer and vator o scription (STAT) signaling path is a signal-regulating pathway widely fo tissues and cells of mammals. JAK-STAT pathway is involved in a variety iological processes that regulate cell survival, p ation, migration, and invasion[20,21]. SOCS is a tive feedback regulator in the JAK-STAT signaling pathway and plays an important role in maintaining homeostasis in the cell. SOCS structurally includes an N-terminal kinase inhibitory region (KIR), a central SH2 domain, and a C-terminal SOCS-box structure. As one of the most widely expressed and most functional members of the cytokine signaling inhibitory (SOCS) family, SOCS3 directly inhibits JAK kinase activity and STAT phosphorylation and inhibits JAK-STAT signaling transduction[2]. Researches[17-19] have shown that abnormal changes in the expression of miR-155 are associated with the development, progression, and prognosis of pancreatic cancer. We investigated whether miR-155 plays a role in regulating SOCS3 expression, affecting JAK-STAT pathway activity, and pancreatic cancer cell proliferation and apoptosis.

The results of qRT-PCR showed that compared with the adjacent tissues, the expression of miR-155 was significantly increased in the tumor tissues of patients with pancreatic cancer, while

the expression of SOCS3 mRNA was significantly decreased, suggesting that increased miR-155 expression may play a role in reducing the expression of SOCS3 and promoting the pathogenesis of pancreatic cancer. In addition, the results of comparative studies showed that compared with normal pancreas HPDE6-C7 cells, the expression of miR-155 in pancreatic cancer SW1990 and Capan-1 cells was significantly increased, while the expression of SOCS3 was significantly decreased. The dual luciferase gene reporter assay showed that transfection of miR-155 mimic significantly reduced relative luciferase activity in pMIR-SOCS3-WT transfected HEK293T cells, but miR-155 mimic did not affect the relative luciferase activity in HEK293T cells transfected with pMIR-SOCS3-MUT, confirming the targeted regulation relationship between miR-155 and SOCS3 mRNA. In the study of the relationship between miR-155 and pancreatic cancer, Ali et al[22] showed that the expression of miR-155 in pancreatic tumor tissues of pancreatic cancer model mice was significantly increased. LaConti et al[23] observed that the expression of miR-155 was significantly el[illegible]ed in tumor tissues of patients with pancrea[illegible] cer compared with normal pancreatic tiss[illegible] ju et al[24] detected that the expression of mi[illegible] was significantly increased in tumor tissues[illegible] patients with pancreatic cancer [illegible]mpared wi[illegible] chronic pancreatitis and nor[illegible]eatic tissue. Liu et al[19] revealed th[illegible]e ex[illegible]ssion of miR-155 was significantly[illegible]eased i[illegible]ncreatic cancer compared wit[illegible]ent t[illegible] the expression of tar[illegible] ge[illegible] was signific[illegible]antly decreased, [illegible] miR-[illegible]motes the pathogenesis of p[illegible]eatic cancer [illegible]h inhibiting BLH1. Pa[illegible]tinou et al[25] [illegible]ted out that the expr[illegible]n o[illegible]155 was abnormally increased i[illegible] pancreatic c[illegible] tissues compared with the [illegible]trol, and the [illegible]e in miR-155 expres[illegible] was associated with tumor stage and prog[illegible]s. Qu[illegible]6 found that the expression of [illegible]155 i[illegible]ipher[illegible]lood of patients with panc[illegible]er was [illegible]rmally elevated. Seux [illegible]27 ind[illegible] that [illegible] expression of the tumor [illegible]ssor g[illegible]NP1 was associated with abn[illegible]ally ele[illegible] expression of miR-155 in pan[illegible]ic cancer tissues, and miR-155 plays a ro[illegible]romotion by inhibiting the ex[illegible]sion of TP53INP1. In this study, the expres[illegible] of miR-155 was significantly increased in p[illegible]tic cancer tumor tissues and pancreatic canc[illegible] cell lines, and elevated miR-155 was a cancer-promoting factor in the pathogenesis of pancreatic cancer, which was consiste[illegible] results of LaConti et al[23], Liu et a[illegible]nd S[illegible] et al[27].

To further investigate the [illegible]nd mechanism of miR-155 in the regulatio[illegible] biological effects of pancreatic c[illegible]er cell[illegible] study transfected miR-155 inhi[illegible] in pancre[illegible] cer cells and observed [illegible]nges i[illegible] the biolo[illegible] effects of pancreati[illegible]ncer [illegible]. The results showed that transfe[illegible] of m[illegible]5 inhi[illegible] significantly down-[illegible]ulat[illegible]xpressi[illegible] of miR-155 in pancre[illegible] cancer c[illegible]nd [illegible]nificantly increased th[illegible]pression of [illegible] while the expressio[illegible]2 and p-STA[illegible] was significantly d[illegible]ased [illegible]educed cell proliferation and increased cell ap[illegible]s. In the study of the rela[illegible] between mi[illegible] and the biological e[illegible] of pancreatic cancer cells, Ali et al[22] found [illegible] the proliferati[illegible]n of pancreatic cancer Rlnk-[illegible]ells can sign[illegible]ntly inhibit cell proliferation [illegible]weaken the [illegible]ty of cell cloning. Lin et al[28] o[illegible]d that t[illegible]xpression of miR-155 is related t[illegible] cancer, and emodin can inhibit the angiogenesis by up-regulating the expression [illegible]f miR-155, thereby exerting an anti-tumor effect [illegible]tic cancer. effect. Wang et al[18] revealed [illegible]at u[illegible]r oxidative stress, K-Ras gene activation can significantly up-regulate the expression of the oncogene miR-155, and miR-155 can inhibit the expression of Foxo3a and reduce the level of SOD and catalase, and the proliferative capacity of pancreatic cancer cells was significantly enhanced. This study combines the targeted regulatory relationship between miR-155 and SOCS3, revealing that miR-155 plays a role in regulating SOCS3 expression, affecting JAK-STAT pathway activity, and pancreatic cancer cell proliferation and apoptosis. However, whether the role of miR-155 targeting SOCS3 in regulating the biological effects of pancreatic cancer cells *in vivo* is unclear, and requires further investigation using animal models in the future.

Conclusions

We showed that the increase of miR-155 expression and the decrease of SOCS3 expression are related to the pathogenesis of pancreatic cancer. miR-155 can inhibit the proliferation and apoptosis of pancreatic cancer cells by targeting the inhibition of SOCS3 expression.

Conflict of Interests

The authors declare no conflict of interest

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