European Review for Medical and Pharmacological Sciences 2018; 22: 8135-8144

Long noncoding RNA-JPX predicts the poor prognosis of ovarian cancer patients and promotes tumor cell proliferation, invasion and migration by the PI3K/Akt/mTOR signaling pathway

J. LI[1], L. FENG[1], C. TIAN[2], Y.-L. TANG[1], Y. TANG[1], F.-Q. HU[1]

[1]Department of Obstetrics and Gynecology, The Second Clinical Medical Institute of North Sichuan Medical College, Nanchong, Sichuan Province, PR China
[2]Department of Burns and Plastic Surgery, The Second Clinical Medical Institute of North Sichuan Medical College, Nanchong, Sichuan Province, PR China

Jun Li and *Lu Feng* contributed equally to this work

Abstract. – OBJECTIVE: To investigate the clinical functions and the detailed mechanism of long noncoding RNA (lncRNA) JPX in human ovarian cancer cell lines.

PATIENTS AND METHODS: The expression of JPX in ovarian cancer tissues and cell lines was detected by Real-time polymerase chain reaction (RT-PCR). The correlation between JPX expression and prognosis was analyzed by follow-up data. The OVCAR-3 cell proliferation, invasion and migration were measured by methyl thiazolyl tetrazolium (MTT) assay, cloning formation assay and scratch assay. The cell apoptosis was detected by Bcl-2, Bax, and Caspase-3 activity. PI3K/mTOR inhibitor treatment and Western blot proved that JPX functions associated with PI3K/Akt/mTOR signaling and test the protein levels of p-PI3K, p-Akt, p-mTOR.

RESULTS: RT-PCR results showed that the expression of JPX was upregulated in ovarian cancer tissues and ovarian cancer cell lines ($p < 0.05$), and it was significantly increased in large tumor tissues and metastatic lymph nodes ($p < 0.05$). The survival rate of high JPX expression patients was much lower than low JPX expression patients ($p < 0.05$), indicating that high expression of JPX predicted poor prognosis in patients with ovarian cancer. MTT assay, colony formation and scratch assay showed the repression of JPX and resulted with significantly decreased in cell proliferation, invasion and migration of OVCAR-3 cells compared with the control ($p < 0.05$). PI3K/mTOR inhibitor treatment showed overexpression of JPX could activate the PI3K/Akt/mTOR signaling pathway. Western blot assay showed that the expressions of p-PI3K, p-Akt, p-mTOR were significantly increased after overexpression of JPX ($p < 0.05$), and after the inhibition of PI3K/Akt/mTOR signaling pathway and overexpression of JPX, the tumor cell proliferation, invasion and migration were significantly repressed, compared with the control ($p < 0.05$).

CONCLUSIONS: JPX could predict the poor prognosis in patients with ovarian cancer, which could promote the tumor cell proliferation, invasion and migration in human ovarian cancer cell lines and inhibited the cell apoptosis through activating PI3K/Akt/mTOR signaling pathway.

Key Words:
LncRNA-JPX, Human ovarian cancer, Proliferation, Invasion, Migration, PI3K/Akt/mTOR signaling.

Introduction

Ovarian cancer is one of the most common malignant cancers that threaten the health and life of women worldwide and it is the top one factor among the death factors of gynecological malignant tumors. It has reached the late stage for more than 70% of patients who show specific symptoms or have been diagnosed because of lack of specific early diagnosis methods, so the prognosis of these patients is extremely poor [1,2]. Therefore, it has always been a difficult problem and hot topic for researchers to find some ways and methods for early diagnosis and reducing the morbidity and the mortality rate. Long non-coding RNAs (lncRNAs) are a class of RNAs longer than 200

Corresponding Author: Feiquan Hu, MD; email: wwwfeiquanhu@gmail.com

nucleotides in length, but cannot be translated into protein. However, these lncRNAs play a quite important role in human tumorigenesis, development and drug resistance since they can regulate gene expressions in transcriptional and post-transcriptional levels[3-5]. Researches[6,7] have shown that many lncRNAs are abnormally expressed in ovarian cancer, playing an important role in the cancer biological processes, such as proliferation, invasion and metastasis. For example, Gao et al[8] found that LncRNA-HOST2 can promote metastasis, invasion and proliferation of epithelial ovarian cancer cells through binding with miRNA let-7 and inhibiting the expression of miRNA let-7. Researchers[9] also found that ARSR could induce epithelial-mesenchymal transition and invasion through increasing the expression of ZEB1 and ZEB2 by competitively binding with the miR-200 family. It has been showed[10] that MALAT1 could increase the cisplatin-resistant treatment by interacting with Notch1 in ovarian cancer. So, further exploring the role of lncRNAs in the pathogenesis of ovarian cancer is needed to provide new ideas for early prevention, clinical diagnosis and treatment of ovarian cancer. Currently, lncRNA-JPX had been reported as an activator of the Xist gene, which could deactivate the transcriptional inhibition of Xist gene by competitively binding with CTCF[11]. After comparing the differences of whole-genome RNA expression between primary and recurrent ovarian cancer, Benoît et al[12] and Huang et al[13] found that Xist gene expression was significantly decreased in recurrent ovarian cancer tissues and the Xist gene expression in ovarian cancer patients was significantly correlated with the length of remission after chemotherapy, which suggested that there was a certain relationship between Xist gene and ovarian cancer drug resistance. However, it is still unclear whether JPX, which is closely related to the activity of Xist gene, plays a role in ovarian cancer. Recent studies[14-16] have found that phospholipid inositol 3 kinase (PI3K), the activation of protein kinase B (Akt) and rapamycin target protein (MTOR), are commonly existed in human ovarian cancer and these proteins co-regulate the proliferation and apoptosis of ovarian cancer cells and participate in the processes of angiogenesis, invasion and metastasis of ovarian cancer. Some other researches have reported that ovarian cancer is closely related to the overexpression and activation of PI3K and Akt in patients with chemotherapy drug resistance[17-19]. The abnormality of PI3K/Akt/mTOR signaling pathway could affect the prognosis of ovarian cancer patients and might provide a new therapeutic target for ovarian cancer. In our previous work, we screened the differential expressions of lncRNAs in ovarian cancer and preliminarily confirmed the high expression of JPX in human ovarian cancer tissues and cancer cell lines by Real-time quantitative PCR. We also found that the inhibition of PI3K/Akt/mTOR signaling was correlated with the expression of JPX by signaling pathway inhibitors in ovarian cancer cell lines. To further study the relationship between JPX and the development and progression of ovarian cancer, we intend to explore the functions of JPX in the development of ovarian cancer through regulating the expression of PI3K/Akt/mTOR signaling pathway.

Patients and Methods

Tissue Specimens

Thirty-two patients with ovarian cancer diagnosed by pathology from January 2010 to August 2017 in our hospital and their cancer tissues and corresponding adjacent tissues were collected by surgical resection. All patients had written informed consent. Age ranged from 35 to 74 years old; 20 cases of tumor size $\leq$ 3 cm, 12 cases of tumor size >3 cm; 17 cases with lymph node metastasis. All tissue samples were frozen in liquid nitrogen until use. Our study was approved by the Faculty of Medicine's Ethics Committee of our hospital.

Survival Curve

In combination with follow-up patients, the duration of follow-up was defined as starting from the date of diagnosis to the date of death or loss of the patient (death = 1; survival =0), and the expression of JPX was divided into high expression group and low expression group bounded by the middle value; then, the survival curve was drawn.

Cell Culture and Cell Transfection

A normal ovarian epithelial cell (ovarian surface epithelium cell, OSE) and two ovarian cancer cell lines (SKOV3, OVCAR3) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). KOV3 and OVCAR3 cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Rockville, MD, USA) with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) at 37°C and 5% CO_2 culture incubator. PcDNA-JPX and its control pcDNA3.1

were synthesized by RiBo Biotech (GuangZhou RiBo Biotech, GuangZhou, China). PI3K/mTOR inhibitor PI-103 was purchased by Calbiochem (Minneapolis, MN, USA). PcDNA-JPX, pcDNA3.1 and PI-103 were transfected by Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to its instructions.

RNA Extraction and Quantitative Real-time PCR

The total RNA of tissue samples and cultured cells were extracted by TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed to generate the first-strand cDNA by PrimeScript™ RT reagent Kit (TaKaRa, Dalian, China). PCR primers were synthesized by Gene Pharma (Shanghai Gene Pharma, Shanghai, China) and the sequence was shown in Table I. mRNA expression was detected with SYBR Premix Ex Taq II (TaKaRa, Dalian, China). The mRNA expressions were normalized to GAPDH and $2^{-\Delta\Delta CT}$ method was used to detect the relative gene expression.

MTT Assay

After transfections for 48 h, cells of control group, pcDNA3.1 group and pcDNA-JPX were digested and cultured in 96-well plate with approximately 1×10^3 cells/well and incubated at 37°C and 5% CO_2. Afterward, 10 μL 5 g/l of MTT (Amresco, Radnor, PA, USA) were added to each well at 1 day, 2 days, 3 days, 4 days, and 5 days, and the resulting solution was incubated for 4 h at 37°C. The absorbance (OD) value of each well was measured at 570 nm. The cell growth curves were plotted using the absorbance value at each time point. Cell viability (P%) was calculated: OD (experimental group)/OD (blank control group) × 100%. This experiment was repeated for three times.

Cloning Formation Assay

After transfections for 48 h, cells of control group, pcDNA3.1 group and pcDNA-JPX were digested and planted in each 60 mm dish with about 1×10^3 cells and the medium was changed every 3 days. After 7 days, cell colony formation was observed under a microscope and the cells were counted.

Table I. Sequences of primers used for quantitative Real-time PCR.

Genes	Primer sequences
JPX	Forward: 5'-GCACCACCAGGCTTCTGTAAC-3'
	Reverse: 5'-GGGCATGTTCATTAATTGGCCAG-3'
GAPDH	Forward: 5'-GGAGTCCACTGGTGTCTTCA-3'
	Forward: 5'-GGGAACTGAGCAATTGGTGG-3'

Scratch Assay

This assay was applied to evaluate the migratory ability of OVCAR-3 ovarian cancer cells. After transfections for 48 h, cells of control group, pcDNA3.1 group and pcDNA-JPX were digested and cultured in 6-well plate at 37°C, 5% CO_2. When cell confluence reached about 90%, a 200 μL sterile tip was used to scratch in each well and then washed by phosphate-buffered saline (PBS) for 3 times. Cell scratch status was observed under the microscope magnified 40 times at 0 h and 24 h, and the healing rate was calculated. Healing rate = (scratch width 0 h - scratch width 24 h) / scratch width 0 h × 100%, the results were calculated by ImageTool (IT).

Transwell Assay

Transwell chambers (BD Biosciences, Franklin Lakes, NJ, USA) were used to evaluate the invasive ability of OVCAR-3 cells. 5×10^4 transfected cells of control group, pcDNA3.1 group and pcDNA-JPX without FBS were put into the top of chamber on the non-coated membrane, and lower chamber filled with 20% fetal bovine serum (FBS) to induce transfected OVCAR-3 cells to invade through the membrane. And the transfected OVCAR-3 cells were put in the upper chamber with the coated membrane for invasion assay. Cells were fixed by methanol for 10 minutes, and then stained with crystal violet solution (Biyuntian, Shanghai, China). Three invasion chambers for experimental group and control group were utilized per condition, and thee random fields were counted per chamber under an inverted microscope.

Protein Extraction and Western Blot

The total protein was extracted from tissue samples and cells by using a RIPA lysis buffer (Biyuntian, Shanghai, China). 50 ug proteins were added to 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then the separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes. After that, the membranes were blocked at room temperature for 1 h. After that, the membranes were incubated with primary antibody (pPI3K/pAkt/pmTOR/Caspase-3) overnight at 4°C and

subsequently incubated with matched secondary antibodies for 1 h. Protein bands were detected by Pierce ECL Western blot substrate (Thermo Fisher Scientific, Waltham, MA, USA) with ECL detection system (Thermo Fisher Scientific, Waltham, MA, USA).

Statistical Analysis

All data were analyzed by SPSS version 21.0 (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA). Data were presented by means± SD. The method of one-way ANOVA was used to analyze these data. Multiple comparisons between groups were performed by S-N-K method. Kaplan-Meier method was used to draw the survival curve. If $p < 0.05$, differences were statistically significant.

Results

JPX Was Upregulated in Ovarian Cancer Tissues and Ovarian Cancer Cell Lines, and It Was Associated With the Prognosis of Patients

32 cases ovarian cancer tissues and para-carcinoma tissues were extracted, and the expressions of JPX were detected by RT-PCR. Expression of JPX was significantly upregulated in ovarian cancer tissues compared with para-carcinoma tissues ($p < 0.05$) (Figure 1A). The JPX expression was also significantly upregulated in ovarian cancer with metastasis compared with patients without metastasis ($p < 0.05$) (Figure 1B). Then, we also detected the level of JPX in tissues from patients with different tumor size. The expression of JPX in cancer tissues larger than 3 cm was significantly higher than that in cancer tissues less than 3 cm ($p < 0.05$) (Figure 1C). Furthermore, we also found that the expression of JPX in SKOV3, OVCAR3 ovarian cancer cell lines was also significantly increased ($p < 0.05$) (Figure 1D). In addition, we divided the ovarian cancer patients into the high JPX expression group and low JPX expression group according to the median expression of JPX and draw a survival curve. Meanwhile, three-year survival rate in high JPX expression group was much lower than that in low JPX expression group ($p < 0.05$) (Figure 1E). These results suggested that JPX expression can predict poor prognosis in patients with ovarian cancer, which was significantly up-regulated in larger tumor tissues and cancer tissues with lymph node metastasis.

Down-Regulation of JPX Inhibited Cell Proliferation, Invasion and Migration in Ovarian Cancer

To exploring the effect of JPX on ovarian cancer cell proliferation, invasion and migration, cell proliferation, invasion and migration were detected in OVCAR3 ovarian cancer cell after transfected with sh-NC or sh-JPX. Sh-JPX was transfected into OVCAR-3 cancer cells and JPX expression was significantly depressed and detected by RT-PCR ($p < 0.05$) (Figure 2A). MTT assay showed that sh-JPX significantly inhibited the proliferation of OVCAR-3 cancer cells at the time of 3 d, 4 d and 5 d, compared with the control ($p < 0.05$) (Figure 2B). Cell cloning assay showed the similar result that sh-JPX significantly inhibited the proliferation of OVCAR-3 cancer cells, compared with the control and sh-NC ($p < 0.05$) (Figure 2C). Transwell assay and scratch assay showed that sh-JPX significantly inhibited the number of invasion and migration distance of OVCAR-3 cancer cells, compared with the control and sh-NC ($p < 0.05$) (Figure 2D, E). Western blot showed that sh-JPX significantly induce the OVCAR-3 cell apoptosis through upregulating the activity of apoptotic factors, including Bax and Caspase-3 ($p < 0.05$) (Figure 2F). These results showed that down-regulation of JPX inhibited cell proliferation, invasion and migration and induced cell apoptosis in ovarian cancer.

Overexpression of JPX Activated PI3K/Akt/mTOR Signaling Pathway in OVCAR-3 Cells

We screened a signaling pathway PI3K/Akt/mTOR associated with JPX-promoting ovarian cancer OVCAR-3 cell proliferation by signaling pathway inhibitors. The OVCAR-3 cells were pretreated with PI3K/mTOR inhibitor PI-103 (10 μM) for 30 min, and pcDNA-JPX was simultaneously transfected along with the control group. The JPX expression was significantly increased after pcDNA-JPX transfection, compared with the control ($p < 0.01$) (Figure 3A). Western blot showed the expressions of p-PI3K, p-Akt and p-mTOR were significantly increased after transfected with pcDNA-JPX, compared to the control ($p < 0.05$) and the expressions of p-PI3K, p-Akt and p-mTOR were significantly decreased after adding PI3K/mTOR inhibitor into pcDNA-JPX group ($p < 0.05$) (Figure 3B). These results indicated that JPX could activate the PI3K/Akt signaling pathway.

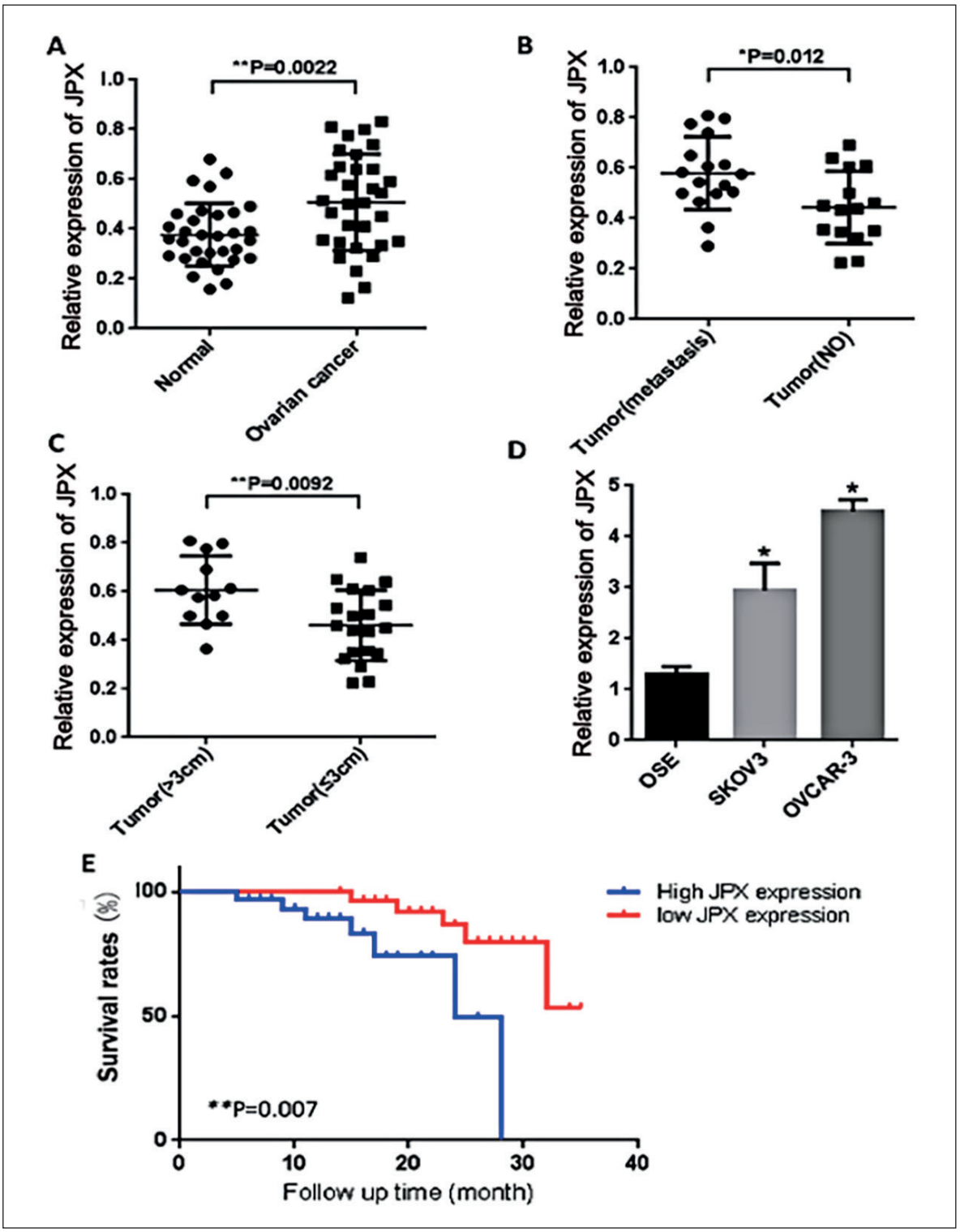

Figure 1. JPX was upregulated in ovarian cancer tissues and ovarian cancer cell lines, and it was associated with the prognosis of patients. **A.** The expression of JPX in ovarian cancer tissues and para-carcinoma tissues was detected by RT-PCR. **B.** The expression of JPX in ovarian cancer patients with lymph node metastasis and without lymph node metastasis was detected by RT-PCR. **C.** The expression of JPX in ovarian cancer tissues larger than 3 cm and less than 3 cm was detected by RT-PCR. **D.** The expression of JPX in SKOV3, OVCAR3 ovarian cancer cell lines was detected by RT-PCR. **E.** Three-year survival rate were analyzed in ovarian cancer patients with high JPX expression and low JPX expression. Data are shown as mean ± SD based on at least three independent experiments, $*p < 0.05$, $**p < 0.01$.

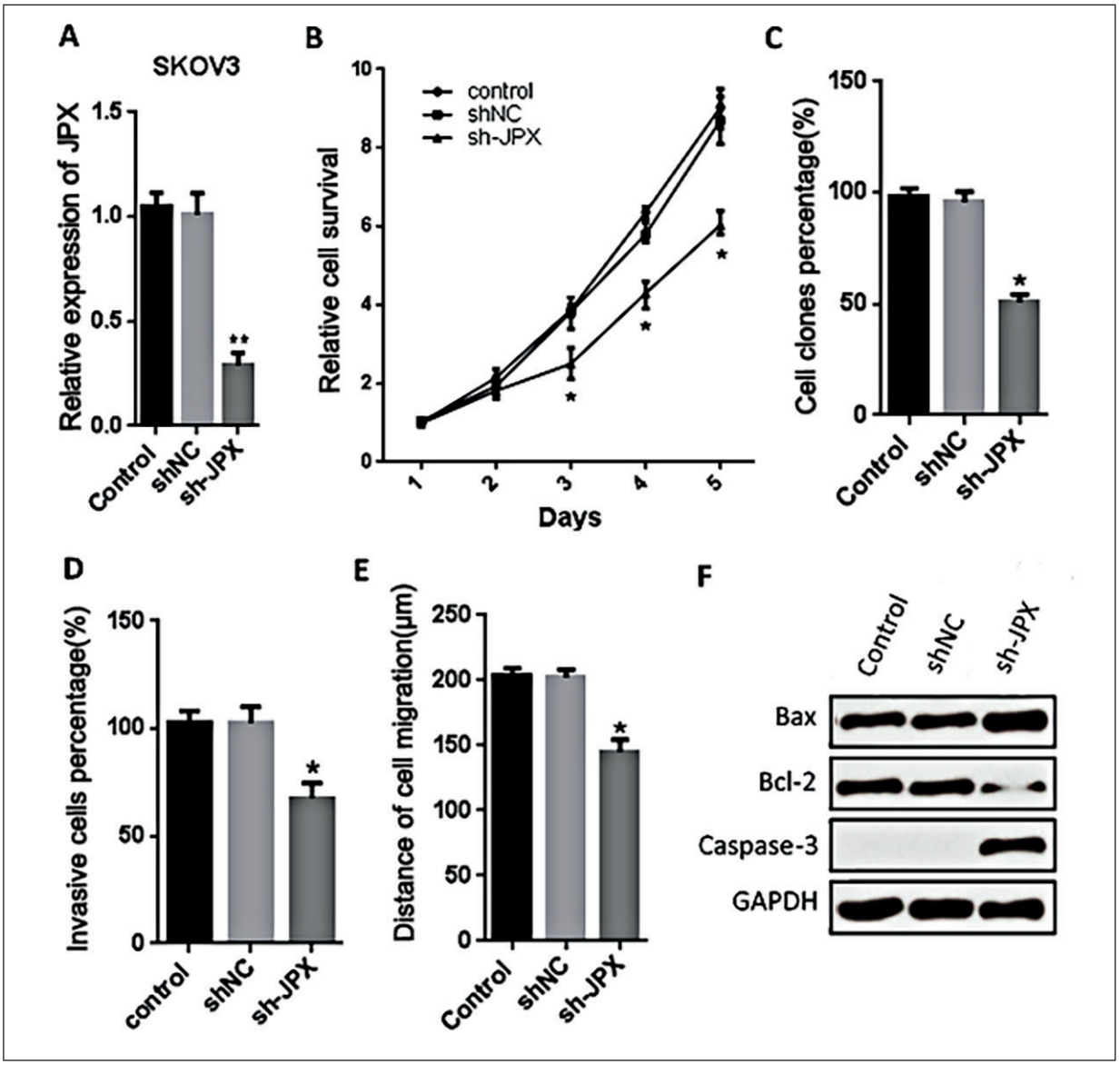

Figure 2. Down-regulation of JPX inhibited cell proliferation, invasion and migration in ovarian cancer**. A.** The expression of JPX was detected by RT-PCR after transfected with control, sh-NC and sh-JPX in OVCAR-3 cells. **B-C.** The cell proliferation rate was detected by MTT assay and cell-cloning assay after transfected with control, sh-NC and sh-JPX in OVCAR-3 cells. **D-E.** The numbers of invasion and migration distance were detected by transwell assay and scratch assay after transfected with control, sh-NC and sh-JPX in OVCAR-3 cells. **F.** Apoptotic factors, including Bax and Caspase-3 were detected by Western blot after transfected with control, sh-NC and sh-JPX in OVCAR-3 cells. Data are shown as mean ± SD based on at least three independent experiments, *$p < 0.05$, **$p < 0.01$.

JPX Promoted Cell Proliferation, Invasion and Migration of Ovarian Cancer Cells Through PI3K/Akt/mTOR Signaling Pathway

The OVCAR-3 cells were pretreated with PI3K/mTOR inhibitor PI-103 (10 μM) for 30 min, and pcDNA-JPX was simultaneously transfected along with the control group. MTT assay showed that the cell number of JPX overexpression group was significantly increased, while the number was significantly decreased after PI-103 in advance ($p < 0.05$) (Figure 4A). Transwell assay and scratch assay showed that overexpression of JPX could significantly promote the number of invasion and migration distance of OVCAR-3 cancer cells ($p < 0.05$) and the number of invasion and migration distance were decreased after the PI3K/mTOR in-

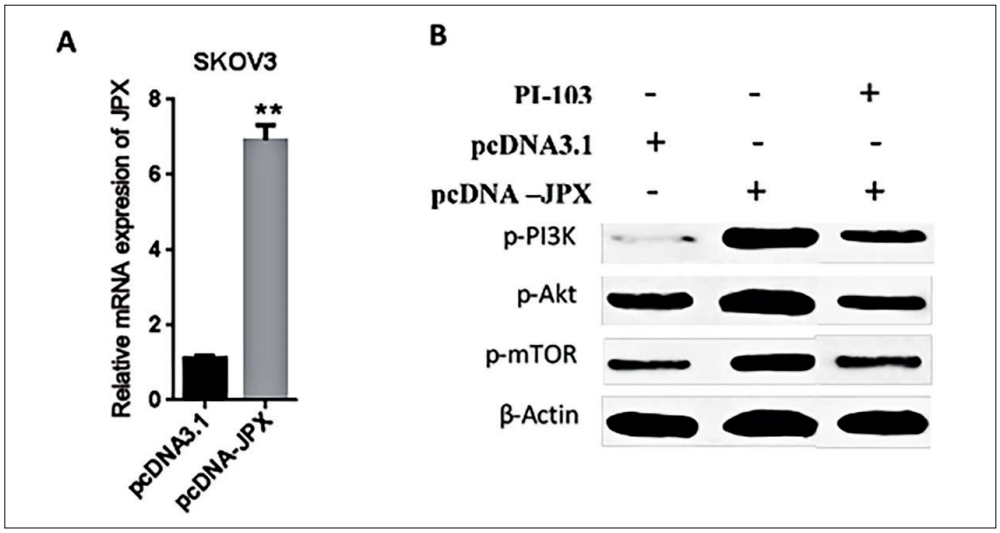

Figure 3. Overexpression of JPX activated PI3K/Akt/mTOR signaling pathway in OVCAR-3 cells. **A.** The mRNA expression of JPX was detected by RT-PCR after transfected with pcDNA-JPX in OVCAR-3 cells. **B.** The expressions of p-PI3K, p-Akt and p-mTOR were detected by Western blot after transfected with PI3K/mTOR inhibitor (PI-103), pcDNA-JPX, PI-103 and pcDNA-JPX. Data are shown as mean ± SD based on at least three independent experiments, $^{**}p < 0.01$.

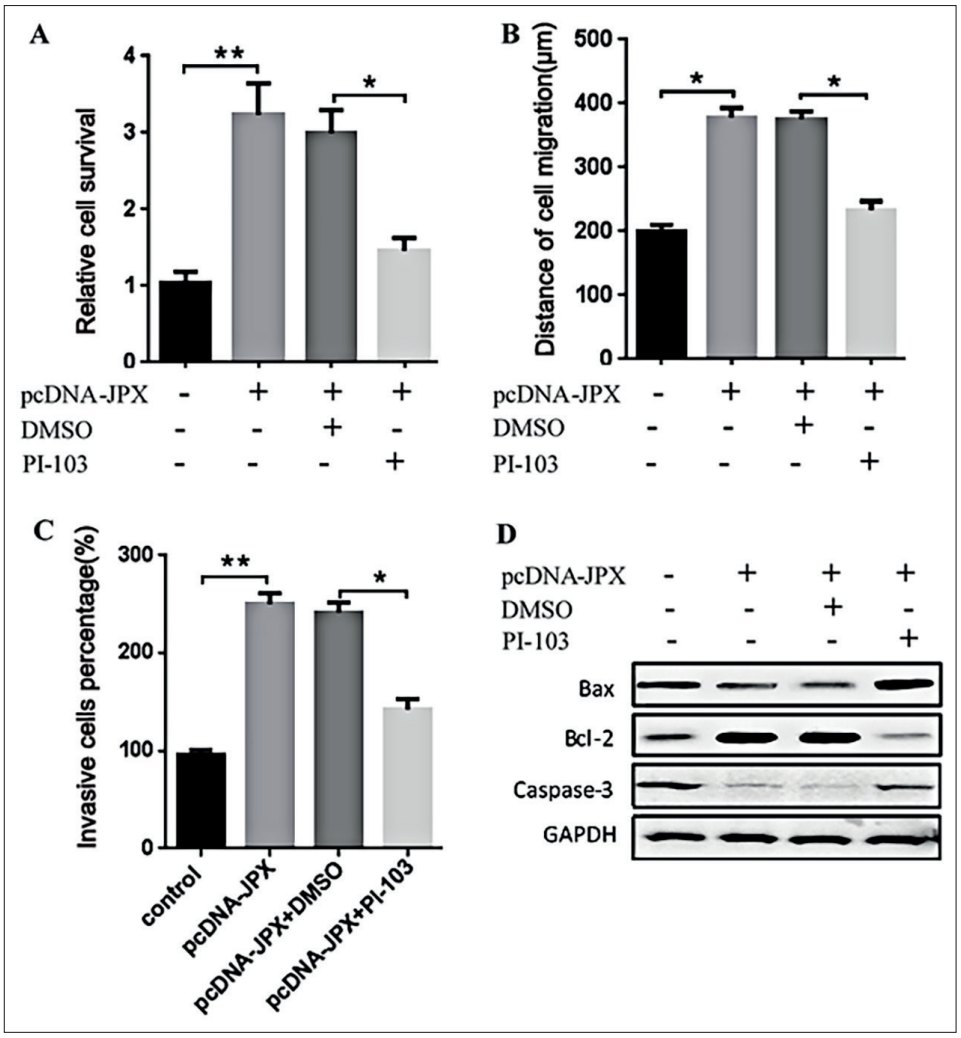

Figure 4. JPX promoted cell proliferation, invasion and migration of ovarian cancer cells through PI3K/Akt/mTOR signaling pathway. **A.** The cell proliferation rate was detected by MTT assay after transfected with control, PI-103, PI-103 and pcDNA-JPX in OVCAR-3 cells. **B-C.** The number of invasion and migration distance were detected by transwell assay and scratch assay after transfected with control, PI-103, PI-103 and pcDNA-JPX in OVCAR-3 cells. **D**. Activities of Caspase-3, Bax and Bcl-2 were detected by Western blot after transfected with control, PI-103, PI-103 and pcDNA-JPX in OVCAR-3 cells. Data are shown as mean ± SD based on at least three independent experiments, $^{*}p < 0.05$, $^{**}p < 0.01$.

hibitor PI-103 treatment ($p < 0.05$) (Figure 4B, C). Furthermore, overexpression of JPX could repress the activity of Caspase-3 and Bax and increase the activity of Bcl-2. However, the activity of Caspase-3 and Bax was significantly increased and Bcl-2 was significantly decreased after PI3K/mTOR inhibitor PI-103 treatment ($p < 0.05$) (Figure 4D). These results suggested that JPX promoted cell proliferation, invasion and migration of ovarian cancer cells through PI3K/Akt/mTOR signaling pathway.

Discussion

Recent studies have found that lncRNAs are closely related to the development of ovarian cancer. The abnormal expression of lncRNAs was involved in many biological processes of ovarian cancer cells, such as proliferation, apoptosis, invasion and metastasis. Evidence[20,21] suggested that LncRNAs will be an emerging regulator of cancer biological functions that can serve as a biomarker for potential cancer diagnosis, prognosis, and targeted treatment. However, the functions and mechanisms of many lncRNAs in the development of ovarian cancer remain unclear. In the early stage, we used lncRNA gene chip technology to analyze the gene expressions of 4 ovarian cancer tissues and adjacent tissues and we found that the expression of JPX in cancer tissues was significantly higher than that in adjacent tissues. Expression of JPX was detected by RT-PCR in 32 pairs of ovarian cancer tissues and adjacent tissues and various ovarian cancer cell lines, the level of JPX was significantly increased in ovarian cancer tissues and ovarian cancer cell lines. Furthermore, we also found that the expression of JPX was significantly increased in larger tumor tissues and metastatic ovarian cancer tissues. Survival curves were drawn from the follow-up results of ovarian cancer patients and the prognosis of patients with high expression of JPX was much poorer. To further investigate the role of JPX in ovarian cancer, we used the ovarian cancer cell of OVCAR-3 as a model to investigate the mechanisms of JPK in cell proliferation, invasion and metastasis by MTT assay, cell cloning assay, transwell assay and scratch assays. These results indicated that down-regulation of JPX could depress the capacity of cell proliferation, invasion, metastasis and increase cell apoptosis in OVCAR-3 cells, suggesting that JPX may play a similar role with oncogene in ovarian cancer. To further analyze the role of JPX in the mechanisms of regulating cell proliferation, invasion and metastasis of ovarian cancer, we detected some signaling pathways, including PI3K/Akt/mTOR. We used the signaling pathway inhibitor and the results indicated that PI3K/Akt/mTOR signaling pathway was closely related with JPX expression. In pcDNA-JPX group, the expressions of p-PI3K, p-Akt and p-mTOR were significantly increased after JPX overexpression compared with the control, indicating that JPX activated PI3K/Akt/mTOR signaling pathway. In pcDNA-JPX with PI-103 treatment group, the expressions of p-PI3K, p-Akt and p-mTOR were significantly decreased compared with pcDNA-JPX group. MTT assay, transwell assay and scratch assay further confirmed that JPX overexpression promoted proliferation, invasion and migration of ovarian cancer cells and inhibited apoptosis, while PI3K/mTOR inhibitor PI-103 significantly reversed these processes. These results indicated that the expression of JPX could predict poor prognosis in patients with ovarian cancer and promote tumor cell proliferation, invasion and migration through PI3K/AKT/mTOR signaling pathway. In recent years, more and more studies have shown that PI3K/AKT/mTOR signaling pathway plays an important role in the functions of lncRNAs, which have been reported in epithelial ovarian cancer, gastric cancer, prostate cancer, etc.[22-24]. Xu et al[25] showed that H19 overexpression promotes trophoblast invasion and autophagy through PI3K/AKT/mTOR signaling pathway. Yao et al[26] found ENST00113 was highly expressed in patients with atherosclerosis and promoted proliferation and migration of venous endothelial cells through activating PI3K/AKT/mTOR signaling pathway. In addition, lncRNAs can also interact with miRNAs. Li et al[27] showed that lncRNA-SNHG7 expression was up-regulated in the development of colorectal cancer, which could promote cell proliferation and inhibit apoptosis by binding with miR-34a, and that the PI3K/AKT/mTOR signaling pathway was involved in this process. These aberrantly expressed lncRNAs could regulate cell proliferation and migration by modulating PI3K/AKT/mTOR signaling pathway. In our study, similar findings suggested that JPX was upregulated in ovarian cancer tissues and promoted cell proliferation, invasion, and migration through activating the PI3K/AKT/mTOR signaling pathway.

Conclusions

We demonstrated that JPX could predict the poor prognosis of ovarian cancer patients and promote cell proliferation, invasion and migration human ovarian cancer OVCAR-3 cells by activating the PI3K/Akt/mTOR signaling pathway and inhibits apoptosis. It may be a new biomarker and therapeutic target for clinical application and treatment.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Schauer IG, Sood AK, Mok S, Liu J. Cancer-associated fibroblasts and their putative role in potentiating the initiation and development of epithelial ovarian cancer. Neoplasia 2011; 13: 393-405.
2) Aust S, Pils D. Epithelial ovarian cancer - more data, more questions? Wien Med Wochenschr 2014; 164: 479-486.
3) Heery R, Finn SP, Cuffe S, Gray SG. Long non-coding RNAs: key regulators of epithelial-mesenchymal transition, tumour drug resistance and cancer stem cells. Cancers (Basel) 2017; 9: 38.
4) Lv Z, Xu Q, Yuan Y. A systematic review and meta-analysis of the association between long non-coding RNA polymorphisms and cancer risk. Mutat Res 2017; 771: 1-14.
5) Morris KV, Mattick JS. The rise of regulatory RNA. Nat Rev Genet 2014; 15: 423-437.
6) Huang K, Geng J, Wang J. Long non-coding RNA RP11-552M11.4 promotes cells proliferation, migration and invasion by targeting BRCA2 in ovarian cancer. Cancer Sci 2018; 109: 1428-1446.
7) Zhan L, Li J, Wei B. Long non-coding RNAs in ovarian cancer. J Exp Clin Cancer Res 2018; 37: 120.
8) Gao Y, Meng H, Liu S, Hu J, Zhang Y, Jiao T, Liu Y, Ou J, Wang D, Yao L, Liu S, Hui N. LncRNA-HOST2 regulates cell biological behaviors in epithelial ovarian cancer through a mechanism involving microRNA let-7b. Hum Mol Genet 2015; 24: 841-852.
9) Shu C, Yan D, Mo Y, Gu J, Shah N, He J. Long non-coding RNA lncARSR promotes epithelial ovarian cancer cell proliferation and invasion by association with HuR and miR-200 family. Am J Cancer Res 2018; 8: 981-992.
10) Bai L, Wang A, Zhang Y, Xu X, Zhang X. Knockdown of MALAT1 enhances chemosensitivity of ovarian cancer cells to cisplatin through inhibiting the Notch1 signaling pathway. Exp Cell Res 2018; 366: 161-171.
11) Sun S, Del Rosario BC, Szanto A, Ogawa Y, Jeon Y, Lee JT. Jpx RNA activates Xist by evicting CTCF. Cell 2013; 153: 1537-1551.
12) Benoît MH, Hudson TJ, Maire G, Squire JA, Arcand SL, Provencher D, Mes-Masson AM, Tonin PN. Global analysis of chromosome X gene expression in primary cultures of normal ovarian surface epithelial cells and epithelial ovarian cancer cell lines. Int J Oncol 2007; 30: 5-17.
13) Huang KC, Rao PH, Lau CC, Heard E, Ng SK, Brown C, Mok SC, Berkowitz RS, Ng SW. Relationship of XIST expression and responses of ovarian cancer to chemotherapy. Mol Cancer Ther 2002; 1: 769-776.
14) Zhang H, Liu T, Zhang Z, Payne SH, Zhang B, McDermott JE, Zhou JY, Petyuk VA, Chen L, Ray D, Sun S, Yang F, Chen L, Wang J, Shah P, Cha SW, Aiyetan P, Woo S, Tian Y, Gritsenko MA, Clauss TR, Choi C, Monroe ME, Thomas S, Nie S, Wu C, Moore RJ, Yu KH, Tabb DL, Fenyö D, Bafna V, Wang Y, Rodriguez H, Boja ES, Hiltke T, Rivers RC, Sokoll L, Zhu H, Shih IM, Cope L, Pandey A, Zhang B, Snyder MP, Levine DA, Smith RD, Chan DW, Rodland KD; CPTAC Investigators. Integrated proteogenomic characterization of human high-grade serous ovarian cancer. Cell 2016; 166: 755-765.
15) Cai J, Xu L, Tang H, Yang Q, Yi X, Fang Y, Zhu Y, Wang Z. The role of the PTEN/PI3K/Akt pathway on prognosis in epithelial ovarian cancer: a meta-analysis. Oncologist 2014; 19: 528-535.
16) Chen J, Bai M, Ning C, Xie B, Zhang J, Liao H, Xiong J, Tao X, Yan D, Xi X, Chen X, Yu Y, Bast RC, Zhang Z, Feng Y, Zheng W. Gankyrin facilitates follicle-stimulating hormone-driven ovarian cancer cell proliferation through the PI3K/AKT/HIF-1alpha/cyclin D1 pathway. Oncogene 2016; 35: 2506-2517.
17) Gasparri ML, Bardhi E, Ruscito I, Papadia A, Farooqi AA, Marchetti C, Bogani G, Ceccacci I, Mueller MD, Benedetti Panici P. PI3K/AKT/mTOR pathway in ovarian cancer treatment: are we on the right track? Geburtshilfe Frauenheilkd 2017; 77: 1095-1103.
18) Li H, Zeng J, Shen K. PI3K/AKT/mTOR signaling pathway as a therapeutic target for ovarian cancer. Arch Gynecol Obstet 2014; 290: 1067-1078.
19) Mabuchi S, Kuroda H, Takahashi R, Sasano T. The PI3K/AKT/mTOR pathway as a therapeutic target in ovarian cancer. Gynecol Oncol 2015; 137: 173-179.
20) Malek E, Jagannathan S, Driscoll JJ. Correlation of long non-coding RNA expression with metastasis, drug resistance and clinical outcome in cancer. Oncotarget 2014; 5: 8027-8038.
21) Lin L, Gu ZT, Chen WH, Cao KJ. Increased expression of the long non-coding RNA ANRIL promotes lung cancer cell metastasis and correlates with poor prognosis. Diagn Pathol 2015; 10: 14.
22) Jin Y, Feng SJ, Qiu S, Shao N, Zheng JH. LncRNA MALAT1 promotes proliferation and metastasis in

epithelial ovarian cancer via the PI3K-AKT pathway. Eur Rev Med Pharmacol Sci 2017; 21: 3176-3184.

23) Li C, Liang G, Yang S, Sui J, Yao W, Shen X, Zhang Y, Peng H, Hong W, Xu S, Wu W, Ye Y, Zhang Z, Zhang W, Yin L, Pu Y. Dysregulated lncRNA-UCA1 contributes to the progression of gastric cancer through regulation of the PI3K-Akt-mTOR signaling pathway. Oncotarget 2017; 8: 93476-93491.

24) Xue D, Zhou C, Lu H, Xu R, Xu X, He X. LncRNA GAS5 inhibits proliferation and progression of prostate cancer by targeting miR-103 through AKT/mTOR signaling pathway. Tumour Biol 2016; 37: 16187-16197.

25) Xu J, Xia Y, Zhang H, Guo H, Feng K, Zhang C. Overexpression of long non-coding RNA H19 promotes invasion and autophagy via the PI3K/AKT/mTOR pathways in trophoblast cells. Biomed Pharmacother 2018; 101: 691-697.

26) Yao X, Yan C, Zhang L, Li Y, Wan Q. LncRNA ENST00113 promotes proliferation, survival, and migration by activating PI3K/Akt/mTOR signaling pathway in atherosclerosis. Medicine (Baltimore) 2018; 97: e0473.

27) Li Y, Zeng C, Hu J, Pan Y, Shan Y, Liu B, Jia L. Long non-coding RNA-SNHG7 acts as a target of miR-34a to increase GALNT7 level and regulate PI3K/Akt/mTOR pathway in colorectal cancer progression. J Hematol Oncol 2018; 11: 89.