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LncRNA LINP1 promotes malignant progression of pancreatic cancer by adsorbing microRNA-491-3p

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Abstract. – OBJECTIVE: The purpose of this study was to explore the mechanism by which long noncoding RNA (lncRNA) LINP1 promoted the development of pancreatic cancer (PCa). Meanwhile, the regulatory relationship between lncRNA LINP1 and microRNA-491-3p was further investigated to provide an effective theoretical basis for the treatment of this cancer.

PATIENTS AND METHODS: Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was carried out to examine lncRNA LINP1 and microRNA-491-3p expression in tumor tissue specimens collected from 56 PCa patients, and the interplay between lncRNA LINP1 expression and some clinical indicators, as well as prognosis of patients with PCa was also analyzed. Meanwhile, in vitro, qRT-PCR further verified lncRNA LINP1 level in PCa cell lines. In addition, lncRNA LINP1 knockdown model was constructed using lentivirus in PCa cell lines CFPAC-1 and BxPC-3, and Cell Counting Kit-8 (CCK-8), transwell, and cell wound healing assays were carried out to evaluate the impact of lncRNA LINP1 on the function of PCa cells. Finally, Dual-Luciferase reporting assay and cell reverse experiments were applied to uncover the potential mechanism.

RESULTS: QRT-PCR revealed that lncRNA LINP1 showed a significantly higher expression in pancreatic tumor tissue samples than in adjacent normal ones. Compared with patients with low expression of lncRNA LINP1, patients with highly expressed lncRNA LINP1 showed a higher incidence of distant metastasis, but a lower overall survival rate. In addition, compared to the sh-NC group, the proliferation, invasion, and migration ability of PCa cells decreased remarkably in LINP1 knockdown group. The results of Luciferase reporting assay demonstrated that lncRNA LINP1 could be targeted by microRNA-491-3p through a specific binding site, and qRT-PCR results uncovered a negative correlation between microRNA-491-3p and lncRNA LINP1 expression in PCa tissues. Finally, the recovery experiment revealed a mutual regulation between LINP1 and microRNA-491-3p, which may jointly regulate the malignant progression of PCa.

CONCLUSIONS: LncRNA LINP1 is able to enhance the proliferation and metastasis of PCa cells by modulating microRNA-491-3p, thus affecting the incidence of lymph node or distant metastasis and prognosis of patients with PCa.

Key Words:
LncRNA LINP1, MicroRNA-491-3p, PCa, Malignant progression.

Introduction

Pancreatic cancer (PCa) generally refers to pancreatic exocrine malignancies, most commonly pancreatic ductal adenocarcinoma[1,2]. It develops rapidly and has a high mortality rate, and the five-year survival rate is less than 5%, which is a common malignant tumor threatening human life and health[3]. In China, there are about 300,000 to 400,000 new cases every year, and its incidence is on the rise[4,5]. According to epidemiological statistics, smoking, obesity, diabetes mellitus, and chronic pancreatitis are common risk factors for PCa[6,7]. Currently, there are limited means to diagnose PCa. Clinical screening is mainly conducted by detecting Carbohydrate antigen19-9 (ca19-9) in blood. However, due to the relatively low sensitivity and specificity of ca19-9, its abnormal expression is often found in digestive tract diseases, such as chronic hepatitis and chronic pancreatitis[8,9]. Moreover, patients are

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often in advanced stage when diagnosed of PCa, where nerve infiltration and distant metastasis often occur, thus losing the best treatment opportunity[10]. Although various methods[11,12] including surgery, chemotherapy, and radiotherapy have been applied to treat PCa, their effects are still poor and the mortality rate is still high.

At present, although some progress[13,14] has been made in the research of the occurrence and development of PCa, the molecular mechanism is still not fully clarified. Therefore, to explore the pathogenesis of PCa, there is a need to find a new breakthrough so as to comprehensively understand the pathogenesis of PCa and design corresponding drugs to curb the development of PCa[15,16].

Long noncoding RNA (lncRNA) is a kind of RNA with a transcription sequence greater than 200 bp and cannot encode protein[17]. In the past decade, a large number of lncRNAs have been discovered[17,18] by sequencing and other means, and their functions *in vivo* have yet to be clarified. Most lncRNAs are only expressed at specific physiological stages in corresponding organs, which provides new potential target molecules for clinical diagnosis and treatment of diseases (including tumors)[19,20]. In the past decade, Pardini et al[21] have found that lncRNA is capable of affecting the progression of a variety of diseases, including malignant tumors. Studies[22] on related lncRNAs have revealed that the expression of a series of lncRNAs and related mRNAs are aberrantly expressed in PCa, but only a few lncRNAs have been studied for their specific functions. The results of high-throughput sequencing indicated the high expression of lncRNA LINP1 in a variety of tumors, and the expression level was closely related to the pathological grade, clinical stage, and prognosis of some tumors. However, its expression in PCa has not been reported so far[23,24].

Therefore, we collected tissue samples from PCa patients to further explore whether lncRNA LINP1 modulated the malignant progression of PCa through microRNA-491-3p, which may provide some guidelines for the clinical application of lncRNA LINP1.

Patients and Methods

Patients and Tissue Samples

Human pancreatic ductal adenocarcinoma tissues were obtained from 56 pairs of pancreatic surgical resection specimens in this hospital. All patients had not received drugs and/or radiation before surgery. The excised experimental specimens were immediately immersed in an Eppendorf (EP; Hamburg, Germany) tube containing the preservation solution and stored at -80°C. All tissue samples used in the experiment were clearly diagnosed by two experienced pathologists. Patients and their families had been fully informed that their specimens would be used for scientific research, and all participating patients had signed informed consent. This study was approved by the Ethics Committee of Liaocheng Infectious Disease Hospital. This study was conducted in accordance with the Declaration of Helsinki.

Cell Lines and Reagents

The human PCa cell lines (AsPC-1, PANC-1, MIA PaCa-2, CFPAC-1, BxPC-3) and the normal pancreatic ductal epithelial cell line HPNE were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA). All cell lines were cultured with Dulbecco's Modified Eagle's Medium (DMEM) high glucose medium (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), penicillin (100 U/mL) and streptomycin (100 μg/mL) in a 37°C, 5% CO_2 incubator. When cell density reached to 80%-90%, cells were digested with 1×trypsin+EDTA (ethylenediaminetetraacetic acid; Thermo Fisher Scientific, Waltham, MA, USA).

Transfection

The control group (sh-NC) and the lentivirus group (sh-LINP1) containing the lncRNA LINP1 interference sequence were provided by Shanghai GenePharma Company (Shanghai, China). The cells were seeded in 6-well plates and grown to a cell density of 30-40%, and siRNA transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. After 48 h, the cells were collected for quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) analysis and cell function experiments.

Cell Counting Kit-8 (CCK-8) Test

The cells were collected and plated into 96-well plates at 2000 cells per well. After cultured for 24 h, 48 h, 72 h, and 96 h respectively, CCK-8 reagent (Dojindo Molecular Technologies, Kumamoto, Japan) was added in each well. After

incubation for 2 h, the optical density (OD) value was measured in the microplate reader at 490 nm absorption wavelength.

Transwell Assay

The cells after transfection for 48 hours were digested, centrifuged, and resuspended in serum-free medium to adjust the density to 5 x 10^5 cells/mL. The cells were seeded in the upper chamber while the culture medium was added into the lower chamber. According to the different migration abilities of each cell line, the cells were put back into the incubator and continued to culture for a specific time. The transwell chamber was taken out, washed 3 times with phosphate-buffered saline (PBS), and placed in methanol for 15 min cell fixation. The cells were observed under the microscope (Olympus, Tokyo, Japan), and 10 fields of view were randomly selected for counting and statistical analysis was performed.

Cell Wound Healing

Cell migration ability was tested by wound healing assay. Briefly, CFPAC-1 and BxPC-3 cells were cultured in 6-well plates with serum-free DMEM medium. Then, scratches were generated with sterile pipette tips. 48 h later, scratches in each well were photographed and their area was analyzed with Image J software (NIH, Bethesda, MD, USA).

Quantitative Real Time-Polymerase Chain Reaction (qPCR)

Total RNA was extracted from PCa cell lines and tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and RNA was reversely transcribed into complementary deoxyribose nucleic acid (cDNA) using PrimeScript RT Reagent. Thereafter, qRT-PCR was performed in StepOne Plus Real-time PCR System (Applied Biosystems, Foster City, CA, USA). Data analysis was performed using ABI Step One software (Applied Biosystems, Foster City, CA, USA) and the relative expression levels of mRNA were calculated using the $2^{-\Delta\Delta Ct}$ method. The primers are as follows: lncRNA LINP1: F: 5'-AGCCGGTCCAGTACACCTTT-3', R: 5'-GGAAAGCACCGTCTGTTGTT-3', β-actin: F: 5'-CCTGGCACCCAGCACAAT-3', R: 5'-TGCCGTAGGTGTCCCTTTG-3', microRNA-491-3p: F: 5'-GAGATCAAGGGAACTTGGTTTTTC-3', R: 5'-CAGGGCCCTCTAGGTCACC-3', U6: F: 5'-CCTGGCACCCAGCACAAT-3', R: 5'-TGCCGTAGGTGTCCCTTTG-3'.

Dual-Luciferase Reporting Assay

According to the instructions (Promega, Madison, WI, USA), the PCa cells in the logarithmic growth phase were prepared for Luciferase reporting detection. The relative fluorescence values were measured by a luminometer. The detection principle is that when the selected specific miRNA is complementary to and binds to the target gene sequence in the system, the Luciferase is not expressed, and the relative fluorescence value finally measured is less than that of the sequence.

Statistical Analysis

Statistical analysis was performed using Statistical Product and Service Solutions (SPSS) 22.0 software (IBM Corp., Armonk, NY, USA). The differences between the two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by post-hoc test (Least Significant Difference). Independent experiments were expressed as mean ± standard deviation. $p<0.05$ was considered statistically significant.

Results

LncRNA LINP1 was Highly Expressed in PCa Tissues and Cell Lines

In this study, the lncRNA LINP1 levels in 56 pairs of tumor tissue specimens and paracancerous ones collected from patients with PCa were examined by qRT-PCR. As a result, when compared to the normal tissues, a significant increase in the expression of lncRNA LINP1 was found in the tumor specimens (Figure 1A), suggesting that LINP1 may act as an oncogene in PCa. In addition, qRT-PCR further detected lncRNA LINP1 levels in PCa cell lines, among which, CFPAC-1 and BxPC-3 contained a higher level of lncRNA LINP1 relative to the normal pancreatic ductal epithelial cell line (Figure 1B), which were selected for subsequent experiments. These results showed that lncRNA LINP1 was highly expressed in PCa tissues and cell lines.

LINP1 Expression was Correlated with Pathological Stage, Distance Metastasis and Overall Survival in PCa Patients

According to the qRT-PCR results of LINP1 expression, the above-mentioned tissue samples were divided into LINP1 high and low expression group, and chi-square test was used to analyze the interplay between LINP1 expression

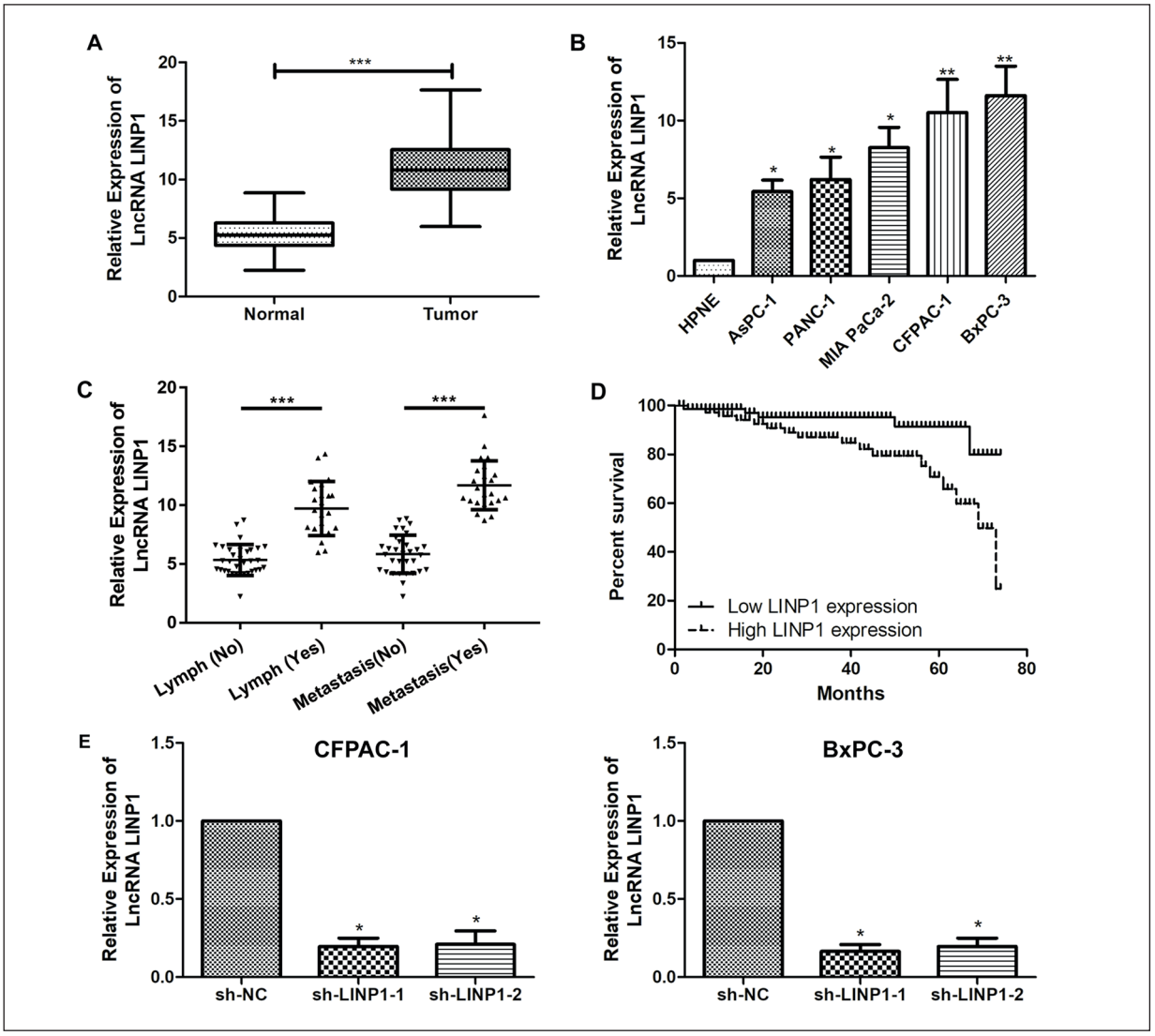

Figure 1. LncRNA LINP1 is highly expressed in PCa tissues and cell lines. **A,** qRT-PCR is used to detect the differential expression of LncRNA LINP1 in tumor tissues and non-tumor tissues of PCa patients. **B,** qRT-PCR is used to detect the expression level of LncRNA LINP1 in PCa cell lines. **C,** qRT-PCR is used to detect the expression level of LncRNA LINP1 in PCa tissues with or without lymph node metastasis. **D,** Kaplan-Meier survival curve of PCa patients based on LncRNA LINP1 expression. Patients with high expression group have significantly worse prognosis than low expression group. **E,** qRT-PCR is used to verify the interference efficiency of the LINP1 knockdown vector after transfection of the CFPAC-1 and BxPC-3 PCa cell lines. Data are mean ± SD, *p<0.05, **p<0.01, ***p<0.001.

and some clinical indicators, including age, sex, pathological stage, and incidence of lymph node metastasis of patients with PCa. As shown in Table I, high expression of LINP1 was positively associated with the incidence of lymph node or distant metastasis, but not with age, gender, and pathological stage (Figure 1C). In addition, Kaplan-Meier survival curve indicated that the higher the expression level of LINP1, the worse the prognosis, suggesting that LINP1 may serve as a predictor for PCa (p<0.05; Figure 1D).

Knockdown of LINP1 Inhibited Cell Proliferation and Metastasis in PCa

To investigate the influence of LINP1 on functions of PCa cells, LINP1 knockdown lentiviral model was constructed in CFPAC-1 and BxPC-3 cell lines, and then, the interference efficiency was verified by qRT-PCR (p<0.05, Figure 1E). Subsequently, CCK-8 test showed a reduced proliferation ability induced by knockdown of LINP1 when compared to the sh-NC group (p<0.05; Figure 2A). In addition, as

Table I. Association of LncRNA LINP1 expression with clinicopathologic characteristics of pancreatic cancer.

Parameters	Number of cases	LINP1 expression		*p*-value
		Low (%)	High (%)	
Age (years)				0.984
< 60	22	13	9	
≥ 60	34	20	14	
Gender				0.656
Male	20	11	9	
Female	36	22	14	
T stage				0.592
T1-T2	34	21	13	
T3-T4	22	12	10	
Lymph node metastasis				0.046
No	33	23	10	
Yes	23	10	13	
Distance metastasis				0.023
No	32	23	9	
Yes	24	10	14	

shown in Figures 2B and 2C, the migration and invasiveness of PCa cells were also significantly attenuated by knockdown of LINP1, confirmed by transwell migration and cell scratch assay. Therefore, it can be concluded that LINP1 was able to remarkably accelerate the migratory ability and invasiveness of PCa cells.

LINP1 was Bound to MicroRNA-491-3p

Bioinformatics revealed that LINP1 may target microRNA-491-3p, therefore, to further validate the targeting of microRNA-491-3p to LINP1, the LINP1 wt or mutant sequence was cloned into the Luciferase reporter plasmid pmirGLO, then, pmirGLO-LINP1-WT, pmirGLO-LINP1-MUT or pmirGLO and microRNA-491-3p were co-transfected into CFPAC-1 and BxPC-3 cell lines. Consequently, Luciferase reporter gene assay detected that LINP1 can be targeted by microRNA-491-3p *via* a specific binding site (Figure 3A). Furthermore, the knockdown of LINP1 significantly elevated the expression level of microRNA-491-3p, measured by qRT-PCR experiment (Figure 3B). These results demonstrated that LINP1 was bound to microRNA-491-3p.

MicroRNA-491-3p was Lowly Expressed in PCa Tissues and Cell Lines

QRT-PCR revealed that the PCa tissue samples contained a higher expression of microRNA-491-3p than adjacent ones (Figure 3C), and the similar result was observed in *in vitro* cell experiments (Figure 3D). In addition, Kaplan-Meier survival curve showed that low expression of microRNA-491-3p was remarkably associated with poor prognosis of PCa, suggesting that microRNA-491-3p may serve as a new biological indicator for predicting the prognosis of PCa ($p<0.05$; Figure 3E). Furthermore, a negative correlation between microRNA-491-3p and LINP1 levels in PCa tissue samples was discovered by qRT-PCR detection (Figure 3F).

LINP1 Modulated MicroRNA-491-3p in PCa

To further verify the interaction between LINP1 and microRNA-491-3p, LINP1 and microRNA-491-3p knockdown vectors were co-transfected into PCa cell lines, and the transfection efficiency was verified by qRT-PCR (Figure 4A). Subsequently, transwell and cell wound healing assays were performed to assess the metastatic characteristics of PCa cells. As a result, the co-transfection remarkably reversed the inhibited PCa cell metastasis induced by knockdown of LINP1 alone (Figure 4B and 4C), suggesting that lncRNA LINP1 regulated the malignant progression of PCa by regulating microRNA-491-3p.

Discussion

As a common malignant tumor threatening human life and health, the etiology and potential mechanism of PCa remain to be explored[1-4]. LncRNA has a variety of key biological functions and important functions in basic life processes,

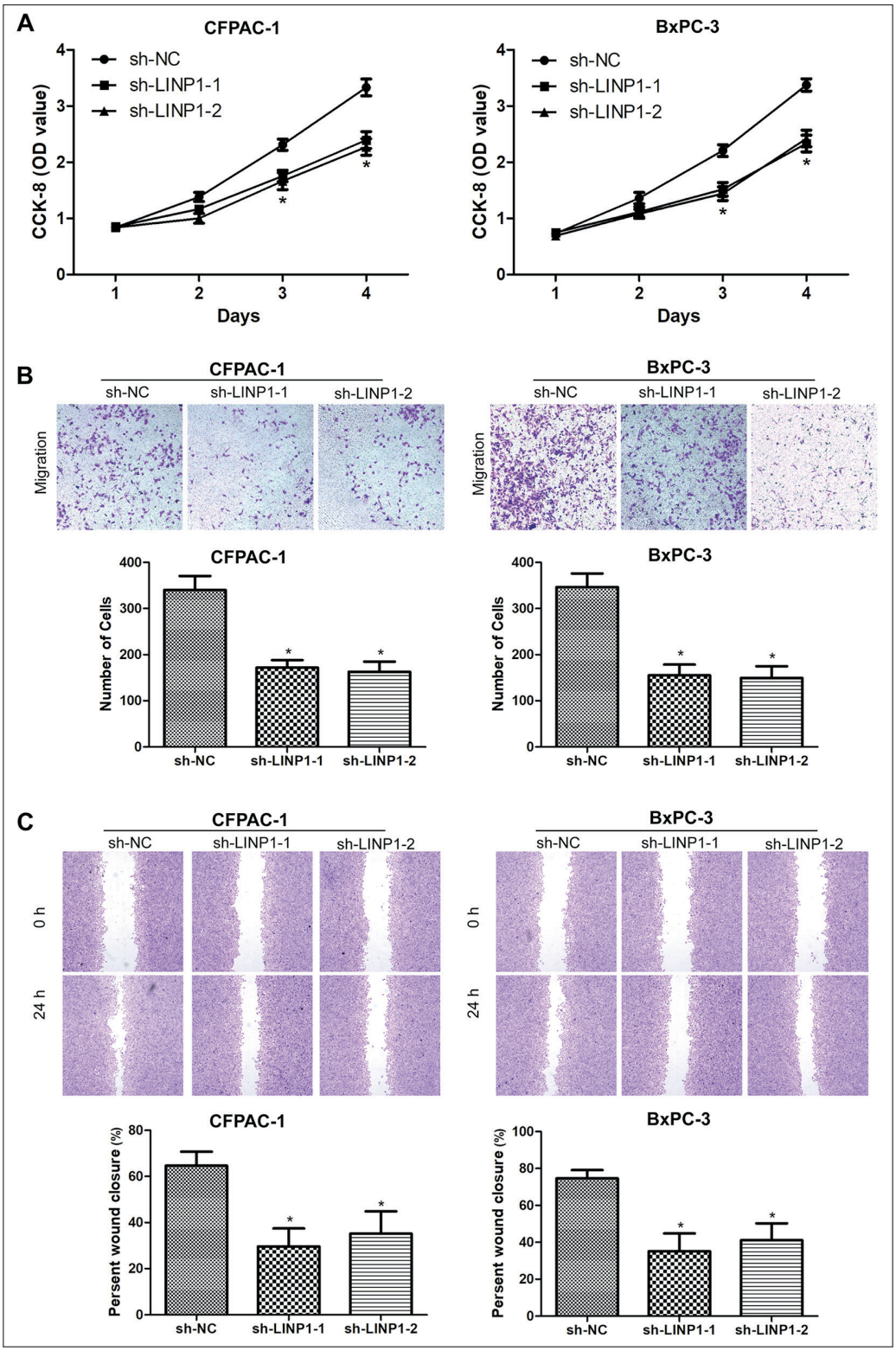

Figure 2. Inhibition of PCa cell proliferation and metastasis following silencing of LncRNA LINP1. **A,** The CCK-8 assay is used to detect the effect of LINP1 knockdown on the proliferation of PCa cells in the CFPAC-1 and BxPC-3 PCa cell lines. **B,** The transwell migration assay is used to detect the effect of LINP1 knockdown on the migration of PCa cells in the CFPAC-1 and BxPC-3 PCa cell lines (Magnification: 40×). **C,** The cell scratch assay examines the effect of LINP1 knockdown on the crawling ability of PCa cells in the CFPAC-1 and BxPC-3 PCa cell lines (magnification: 40×). Data are mean ± SD, *$p<0.05$.

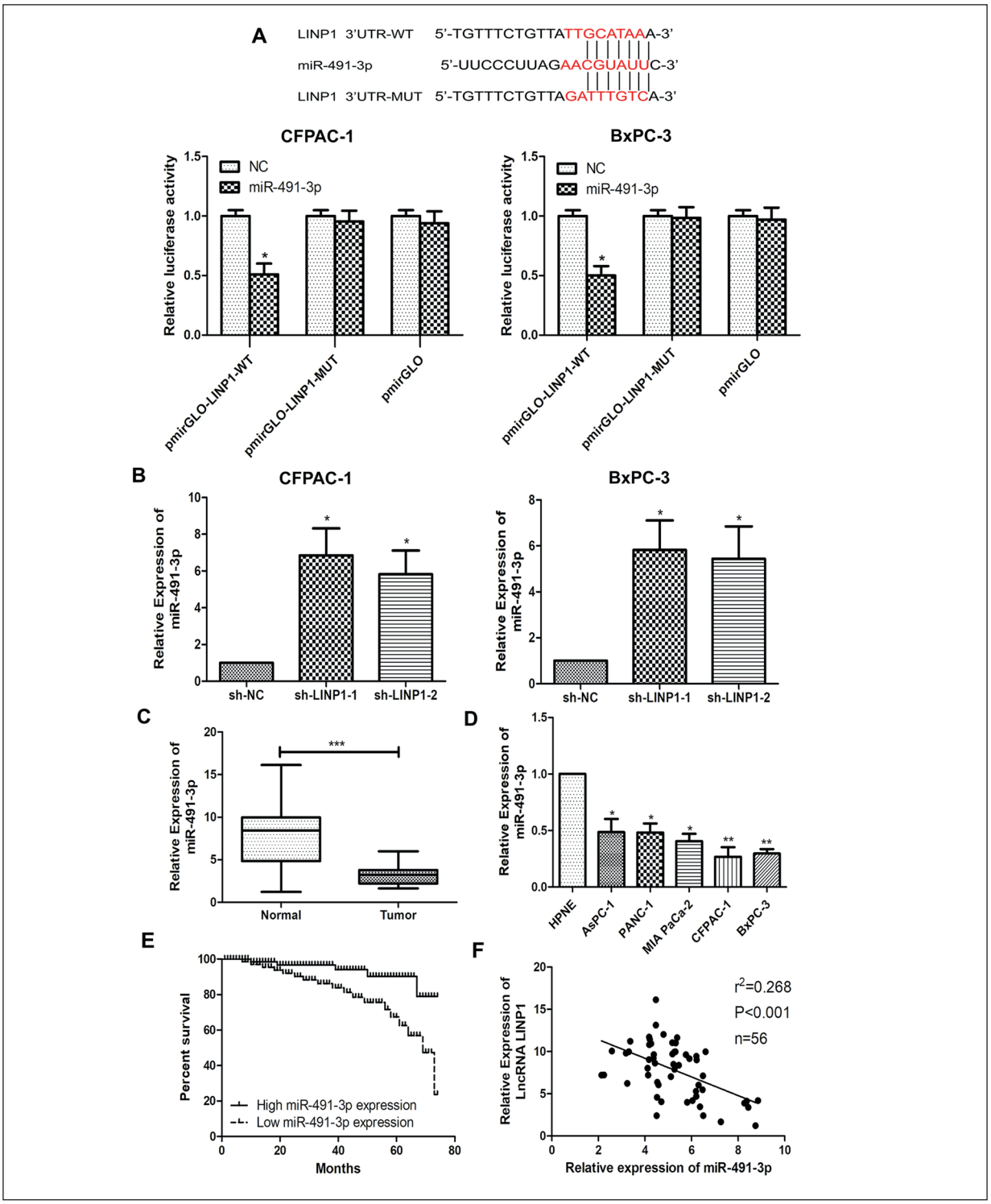

Figure 3. Direct targeting of LncRNA LINP1 to miR-491-3p. **A,** The results of the dual luciferase reporter assay in the CFPAC-1 and BxPC-3 PCa cell lines indicate that LncRNA LINP1 can be targeted by miR-491-3p *via* a specific binding site. **B,** qRT-PCR is used to detect the expression level of miR-491-3p after silencing LncRNA LINP1 in PCa cell lines. **C,** qRT-PCR is used to detect the differential expression of miR-491-3p in PCa tumor tissues and adjacent non-tumor tissues. **D,** qRT-PCR is used to detect the expression level of miR-491-3p in PCa cell lines. **E,** Kaplan Meier survival curve of PCa patients based on miR-491-3p expression, and the prognosis of patients with low expression was significantly worse than that of high expression group. **F,** qRT-PCR shows a significant negative correlation between the expression of LncRNA LINP1 and miR-491-3p in PCa tissues. Data are mean ± SD, *p<0.05, **p<0.01, ***p<0.001.

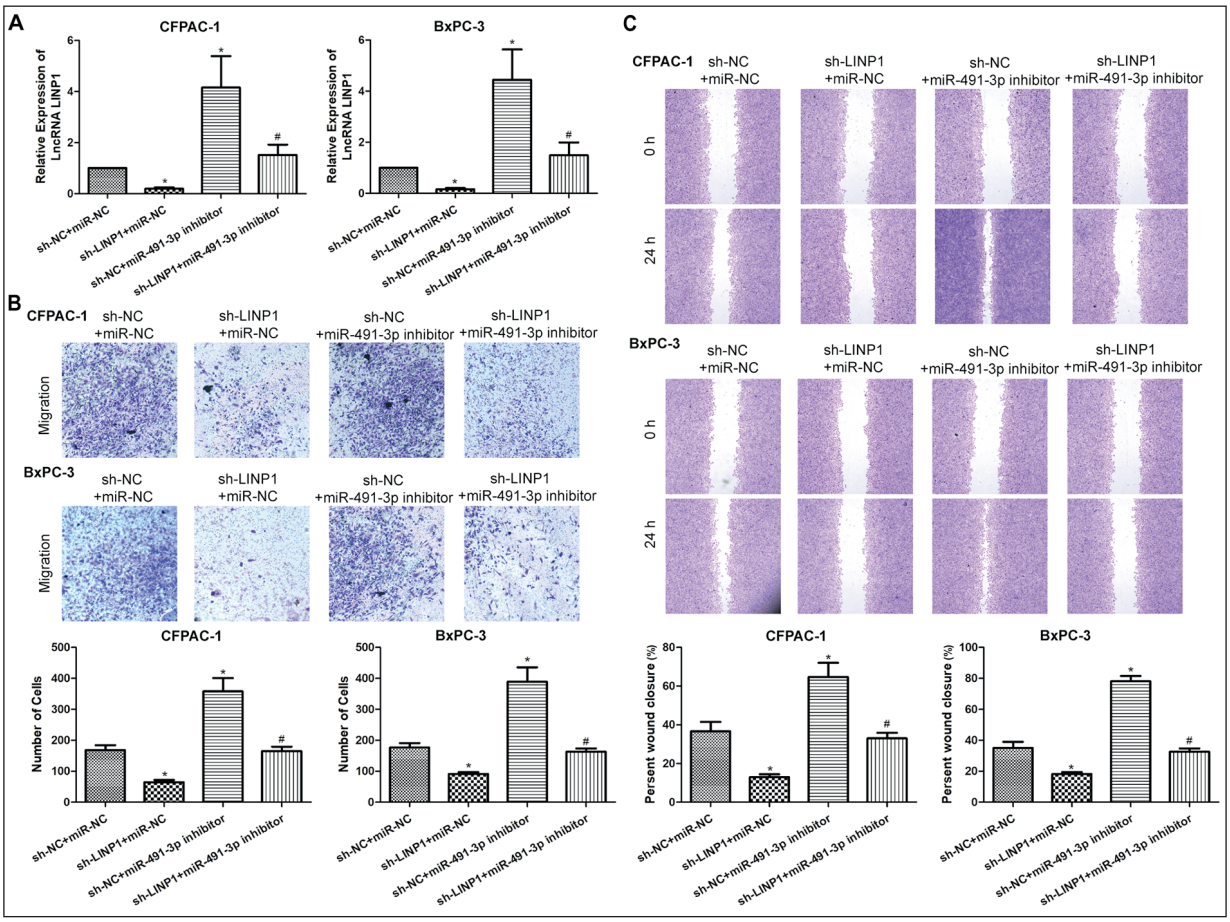

Figure 4. LncRNA LINP1 regulates the malignant progression of miR-491-3p in PCa. **A,** qRT-PCR is used to detect the interference efficiency of LINP1 after co-transfection of LncRNA LINP1 and miR-491-3p knockdown vector in CFPAC-1 and BxPC-3 PCa cell lines. **B,** The transwell migration assay is used to detect the effect of co-transfection of LncRNA LINP1 and miR-491-3p knockdown vectors on PCa cell migration in CFPAC-1 and BxPC-3 PCa cell lines (magnification: 40×). **C,** The cell scratch assay examined the effect of co-transfection of LncRNA LINP1 and miR-491-3p knockdown vectors on the crawling ability of PCa cells in the CFPAC-1 and BxPC-3 PCa cell lines (magnification: 40×). Data are mean ± SD, $*^{\#}p<0.05$.

such as cell growth, proliferation, differentiation, invasion, and metastasis[5,6]. Notably, some key lncRNAs have been shown[7,10] to be aberrantly expressed in malignant processes, such as cancer development and distant metastasis. In addition, it has been reported that lncRNA is involved in the development of PCa at various stages, which has potential clinical value in the diagnosis, treatment, and prognosis of PCa[12-14].

Sequences with coding function were less than 2% of all gene sequences in human body, while other sequences in the genome were considered as "junk DNA" without coding function[14,15]. Most of this DNA is intron DNA, known as noncoding RNA (ncRNA). Based on the natural length of these ncRNA transcripts and the convenience of study, they are generally roughly classified into two categories: small-molecule ncRNA and long-chain ncRNA(lncRNA)[15,16]. At first, little is known about lncRNA, however, some investigations have suggested that lncRNA may play a broad role in transcriptional regulation of genes[17], which plays a very important role in a variety of biological processes, such as maintaining cell growth, differentiation, and proliferation[18,19]. Many researches[20-22] have revealed that lncRNA plays an irreplaceable role in the pathogenesis and progression of a variety of diseases. In order to explore the role of lncRNA in the malignant progression of PCa, the difference in the expression of lncRNA in PCa tissues was detected through high-throughput analysis in the preliminary work, and selected lncRNA LINP1 as the lncRNA related to the malignant progression of PCa for study.

In this study, it was found that the expression of lncRNA LINP1 in the tumor tissues of PCa patients was significantly higher than that in the adjacent tissues, and was positively correlated with lymph node metastasis, distant metastasis, and poor prognosis. Therefore, it is suggested that lncRNA LINP1 may play a role in promoting cancer in PCa. Subsequently, LncRNA LINP1 knockdown model was established in PCa cell lines, and proved that lncRNA LINP1 could promote proliferation and metastasis of PCa cells through CCK-8, transwell migration, and cell recovery experiments, suggesting that LncRNA LINP1 may be a key factor in predicting malignant progression of PCa.

Competitive endogenous RNA (ceRNA) regulatory mechanisms suggest that LncRNAs can competitively bind to common miRNAs with pro-cancer or anti-cancer effects through miRNA response elements, thereby releasing or reducing the inhibitory effect of miRNAs on other target genes, thereby inhibiting or promoting tumor progression[25,26]. In this study, bioinformatics revealed the binding of microRNA-491-3p in lncRNA LINP1 sequence, and the direct binding of lncRNA LINP1 and microRNA-491-3p was verified by qRT-PCR, Dual-Luciferase reporter gene, and other molecular biology experiments. In addition, it was found that silencing microRNA-491-3p can increase the expression level of LncRNA LINP1 and reverse the inhibition of silenced lncRNA LINP1 on the migration ability of PCa cells, which indicated that lncRNA LINP1 regulated the malignant progression of PCa by regulating microRNA-491-3p.

Conclusions

Taken together, lncRNA LINP1 can promote the proliferation and metastasis of PCa cells by regulating microRNA-491-3p. Meanwhile, it exerts effects on the incidence of lymph node metastasis and distant metastasis in PCa patients, and is also remarkably related to poor prognosis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Ilic M, Ilic I. Epidemiology of pancreatic cancer. World J Gastroenterol 2016; 22: 9694-9705.
2) Lo W, Morris MC, Ahmad SA, Patel SH. Screening patients at high risk for pancreatic cancer-Is it time for a paradigm shift? J Surg Oncol 2019; 120: 851-857.
3) Kirkegard J, Mortensen FV, Cronin-Fenton D. Chronic pancreatitis and pancreatic cancer risk: a systematic review and meta-analysis. Am J Gastroenterol 2017; 112: 1366-1372.
4) Lin QJ, Yang F, Jin C, Fu DL. Current status and progress of pancreatic cancer in China. World J Gastroenterol 2015; 21: 7988-8003.
5) Li BQ, Wang L, Li J, Zhou L, Zhang TP, Guo JC, Zhao YP. Surgeons' knowledge regarding the diagnosis and management of pancreatic cancer in China: a cross-sectional study. BMC Health Serv Res 2017; 17: 395.
6) Korc M, Jeon CY, Edderkaoui M, Pandol SJ, Petrov MS. Tobacco and alcohol as risk factors for pancreatic cancer. Best Pract Res Clin Gastroenterol 2017; 31: 529-536.
7) Barone E, Corrado A, Gemignani F, Landi S. Environmental risk factors for pancreatic cancer: an update. Arch Toxicol 2016; 90: 2617-2642.
8) Scara S, Bottoni P, Scatena R. CA 19-9: Biochemical and clinical aspects. Adv Exp Med Biol 2015; 867: 247-260.
9) Luo G, Guo M, Jin K, Liu Z, Liu C, Cheng H, Lu Y, Long J, Liu L, Xu J, Ni Q, Yu X. Optimize CA19-9 in detecting pancreatic cancer by Lewis and Secretor genotyping. Pancreatology 2016; 16: 1057-1062.
10) Hogendorf P, Durczynski A, Strzelczyk J. Metastatic pancreatic cancer. J Invest Surg 2018; 31: 151-152.
11) Mohammed S, Van Buren GN, Fisher WE. Pancreatic cancer: advances in treatment. World J Gastroenterol 2014; 20: 9354-9360.
12) Chiorean EG, Coveler AL. Pancreatic cancer: optimizing treatment options, new, and emerging targeted therapies. Drug Des Devel Ther 2015; 9: 3529-3545.
13) Rouanet M, Lebrin M, Gross F, Bournet B, Cordelier P, Buscail L. Gene therapy for pancreatic cancer: specificity, issues and hopes. Int J Mol Sci 2017; 18. pii: 1231.
14) Makohon-Moore A, Iacobuzio-Donahue CA. Pancreatic cancer biology and genetics from an evolutionary perspective. Nat Rev Cancer 2016; 16: 553-565.
15) Ansari D, Toren W, Zhou Q, Hu D, Andersson R. Proteomic and genomic profiling of pancreatic cancer. Cell Biol Toxicol 2019; 35: 333-343.
16) Hung YH, Hsu MC, Chen LT, Hung WC, Pan MR. Alteration of epigenetic modifiers in pancreatic cancer and its clinical implication. J Clin Med 2019; 8: 903.
17) Bhan A, Soleimani M, Mandal SS. Long noncoding RNA and cancer: a new paradigm. Cancer Res 2017; 77: 3965-3981.
18) Lv Y, Huang S. Role of non-coding RNA in pancreatic cancer. Oncol Lett 2019; 18: 3963-3973.

19) Chi Y, Wang D, Wang J, Yu W, Yang J. Long non-coding RNA in the pathogenesis of cancers. Cells 2019; 8: 1015.

20) Zhu J, Chen S, Yang B, Mao W, Yang X, Cai J. Molecular mechanisms of lncRNAs in regulating cancer cell radiosensitivity. Biosci Rep 2019; 39: BSR20190590.

21) Pardini B, Sabo AA, Birolo G, Calin GA. Noncoding RNAs in extracellular fluids as cancer biomarkers: the new frontier of liquid biopsies. Cancers (Basel) 2019; 11. pii: E1170.

22) Li Y, Yang X, Kang X, Liu S. The regulatory roles of long noncoding RNAs in the biological behavior of pancreatic cancer. Saudi J Gastroenterol 2019; 25: 145-151.

23) Liang Y, Li Y, Song X, Zhang N, Sang Y, Zhang H, Liu Y, Chen B, Zhao W, Wang L, Guo R, Yu Z, Yang Q. Long noncoding RNA LINP1 acts as an oncogene and promotes chemoresistance in breast cancer. Cancer Biol Ther 2018; 19: 120-131.

24) Zhang Y, He Q, Hu Z, Feng Y, Fan L, Tang Z, Yuan J, Shan W, Li C, Hu X, Tanyi JL, Fan Y, Huang Q, Montone K, Dang CV, Zhang L. Long noncoding RNA LINP1 regulates repair of DNA double-strand breaks in triple-negative breast cancer. Nat Struct Mol Biol 2016; 23: 522-530.

25) Zhao J, Li L, Han ZY, Wang ZX, Qin LX. Long noncoding RNAs, emerging and versatile regulators of tumor-induced angiogenesis. Am J Cancer Res 2019; 9: 1367-1381.

26) Abdollahzadeh R, Daraei A, Mansoori Y, Sepahvand M, Amoli MM, Tavakkoly-Bazzaz J. Competing endogenous RNA (ceRNA) cross talk and language in ceRNA regulatory networks: a new look at hallmarks of breast cancer. J Cell Physiol 2019; 234: 10080-10100.