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Long noncoding RNA LINC00662 functions as miRNA sponge to promote the prostate cancer tumorigenesis through targeting miR-34a

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Abstract. – **OBJECTIVE: Mounting evidence indicates that long noncoding RNAs (lncRNAs) play a critical role in the tumorigenesis. Up-regulation of lncRNA LINC00662 (LINC00662) has previously confirmed in several tumors. However, the study of LINC00662 in prostate cancer (PCa) is limited. Hence, to determine the expression pattern and function of LINC00662 in PCa.**

PATIENTS AND METHODS: LINC00662 expression was first detected in PCa cell lines and tissue samples by qRT-PCR. Based on follow-up data, correlations of LINC00662 expression and clinicopathological features, including overall survival, in PCa patients were evaluated. Cell proliferation, migration, invasion, and apoptosis were detected by CCK-8 assay, colony-forming assay, Wound-healing assay, transwell assay, and flow cytometry, respectively. Additionally, LINC00662-specific miRNA was further confirmed using the dual-luciferase reporter assay and RT-PCR.

RESULTS: LINC00662 was significantly upregulated in PCa tissues and cell lines compared with adjacent normal tissue and a normal prostate epithelial cell line. Higher expression of LINC00662 was positively associated with distant metastasis and shorter overall survival. In addition, multivariate analysis revealed that tissue LINC00662 expression was confirmed to be an independent prognostic factor for PCa. Furthermore, LINC00662 silencing inhibited the proliferation, migration, and invasion of PC-3 and LNCaP cells, and promoted apoptosis in vitro. Bioinformatics methods and luciferase reporter assay revealed the close link within miR-34a and 3'-untranslated region (UTR) of LINC00662 and further confirmed that LINC00662 could function as a sponge of miR-34a in PCa cells. Also, the results of RT-PCR showed that knockdown of LINC00662 suppressed the expression levels of miR-34a.

CONCLUSIONS: The current results further enhanced our understanding of the effects of LINC00662 in PCa and may help to provide a new potential target for PCa treatment.

Key Words
Long noncoding RNA, LINC00662, Prognosis, metastasis, Apoptosis, Prostate cancer, MiR-34a.

Introduction

Prostate cancer (PCa) is one of the most common malignant tumors in men and the third leading cause of cancer death among men in developed countries[1]. The mortality rate has reached the second highest ranking among all male cancers after lung cancer in the USA[2]. Although the incidence of PCa is much lower in Asian than that in Caucasian, patients from China have a rapid increase in incidence[3]. Several therapeutic tools are currently available for PCa, such as radiotherapy, hormonal therapy, and chemotherapy[4]. In addition, surgery is frequently used for young men, but traditional radical prostatectomy can cause distinct urinary adverse effects[5]. On the other hand, the major cause of mortality can be attributed to metastasis[6]. Up to date, the underlying mechanisms of PCa pathogenesis remain poorly understood. Thus, it is indispensable to better understand the tumorigenesis for the development of prognostic markers and novel effective therapies for PCa patients.

Long non-coding RNAs (lncRNAs) are defined as transcribed RNA molecules greater than 200 nt in length cannot be translated into a protein[7]. In the past decades, lncRNAs have been judged as the by-product or 'noise' of gene transcription. However, with the development of bio-

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logical information technology and high-throughput sequencing, more and more lncRNAs were identified to be dysregulated in human tissues, especially in patients with some special diseases[8,9]. Then, emerging biological evidence showed that lncRNAs play a critical role in a wide range of cellular processes, such as X chromosome inactivation, protein synthesis, epigenetic modification, and gene transcription regulation[10-12]. Increasing studies[13-15] provided clear evidence that aberrant lncRNA expression played an oncogenic or tumor-suppressive role in the tumorigenesis of PCa and the dysregulation of lncRNAs may serve as potential prognostic and diagnostic biomarkers for PCa, as well as a potential therapeutic target. Long intergenic non-protein coding RNA 662 (LINC00662), Located at 19q11, firstly was reported to be abnormally expressed in lung squamous cell carcinoma[16]. Then, this up-regulation was found in several other tumors, such as gastric cancer[17] and colorectal cancer[18]. Besides, the tumor-promotive roles of LINC00662 were also confirmed via both gain-of-function and loss-of-function assays. However, the exact role and mechanism of LINC00662 on PCa cell proliferation and metastasis remain unclear.

We first evaluated the expression of LINC00662 in human PCa tissues and cells. Then, we further studied the association between LINC00662 levels and clinical progress of PCa patients. Moreover, loss-of-function assays were performed to explore the potential function of LINC00662 in PCa cells viability proliferation and metastasis. Our results firstly reported that LINC00662 played a tumor-promotive role in PCa and may be useful as a prognostic biomarker and/or a therapeutic avenue for PCa.

Patients and Methods

Patients and Tissue Samples

Prostate cancer tissues and adjacent normal tissue specimens were collected from 105 patients at Xingtai Medical college from Jan 2008 and October 2011. The study protocol was approved by The Ethics Committee of Xingtai Medical College, and the written informed consent was obtained from each patient. None of the patients underwent any anti-tumor therapy before surgery. Tissue samples were snap-frozen in liquid nitrogen after surgical removal and the preserved at -80°C. The clinical information of patients was shown in Table II.

Cell Lines and Cell Transfection

WPMY-1 cells (normal prostate epithelial cells) and prostate cancer cells (DU145, 22RV1, PC-3, and LNCaP) were purchased from Jennio Biotech Co., Ltd. (Guangzhou, Guangdong, China). The cells were maintained in RPMI-1640 medium (Jihe Biotech, Fengxian, Shanghai, China) with fetal bovine serum (FBS; 10%) and antibiotics (1% penicillin-streptomycin solution). The cells were maintained in an incubator with 5% CO_2 at 37˚C. In addition, the cell transfection was conducted by Lipofectamine 3000 reagent kit (T&L Biological, Haidian, Beijing, China) according to the protocols provided in the kit. We purchased miRNA mimics (miR-34a mimic, NC mimic), small interfering RNAs (siRNA) targeting LINC00662 (siLncRNA1, siLncRNA2) and negative control siRNAs (siControl) from TransSheep Biotechnology Co., Ltd. (Xuhui, Shanghai, China). The pcDNA3.1-LINC00662 vector was purchased from ABACE Biotechnology Co., Ltd. (Tongzhou, Beijing, China).

RNA Purification and Real-Time Quantitative RT-PCR (qRT-PCR)

Total RNA was isolated using Direct-zol reagent (ZYMO Research, Chaoyang, Beijing, China). After the concentration of the purified RNA was detected, the cDNA synthesis was conducted by reverse transcription using a QPCR cDNA Synthesis kit (Agilent, Chaoyang, Beijing, China). Then, the qRT-PCR analysis for LINC00662 detection was performed using a SYBR Green qPCR Mix kit (ZiKe Biotech, Shenzhen, Guangdong, China) on a LightCycler 96 real-time PCR system (Roche, Pudong, Shanghai, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal control for LINC00662 detection. For miR-34a determination, a Genecopoeia All-in-One miRNA qRT-PCR Detection kit (YTHX Biotech, Haidian, Beijing, China) was applied. U6 was used as internal control when detected miR-34a. The primers used are shown in Table I. Relative quantification of LINC00662 and miR-34a expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Table I. Primers sequence for qRT-PCR assay.

Name	Sequences (5′-3′)
LINC00662: F	CACGCTTCTGAAACTGGTGT
LINC00662: R	GCCTGTAGAAGGGAAACGCT
miR-34a: F	GCCCTGGCAGTGTCTTAG
miR-34a: R	CAGTGCGTGTCGTGGAGT
GAPDH: F	AGGTCGGTGTGAACGGATTTG
GAPDH: R	GGGGTCGTTGATGGCAACA

Table II. Association between LINC00662 expression and different features of human PCa.

Clinicopathological features	No. of cases	LINC00662 expression		*p*-value
		High	Low	
Age (years)				0.934
<65	61	31	30	
≥65	44	22	22	
Tumor diameter (cm)				0.274
<2.5	59	27	32	
≥2.5	46	26	20	
Histological grade				0.484
I-II	59	28	31	
III-IV	46	25	21	
Tumor stage				0.186
T2	64	29	35	
T3-T4	41	24	17	
Lymph node metastasis				0.206
Absence	77	36	41	
Presence	28	17	11	
Distant metastasis				0.018
Absence	67	28	39	
Presence	38	25	13	
Capsule invasion				0.628
Absence	50	24	26	
Presence	55	29	26	
Multiple lesions				0.766
Absence	50	26	24	
Presence	55	27	28	

Cell Counting Kit-8 (CCK-8) Assay

CCK-8 assays were utilized to evaluate cell proliferation. LNCaP and PC-3 cells transfected with control or LINC00662 siRNAs were separately planted into 96-well plates (2000 cell/well). After attachment, the cells were incubated with CCK-8 solution (10 µl/well) for about 2 h. Then, at the indicated time (48, 72 and 96 h), the absorbance at a wavelength of 450 nm was detected by a microplate reader (WoYuan Tech, Hongkou, Shanghai, China).

Colony Formation Assay

For the colony formation assay, LNCaP and PC-3 cells transfected with control or LINC00662 siRNAs (500 cells/well) were placed in 6-well plates. Approximately 15 days later, the visible colonies were washed with PBS and stained with crystal violet solution (0.1%). Finally, the colonies were count and photographed using a microscope (WMF-CX40; WuMo, Pudong, Shanghai, China).

TUNEL Assay

The cell proliferation was determined by TdT-mediated dUTP Nick-End Labeling (TUNEL) assay using a One Step TUNEL Apoptosis Assay kit (Beyotime, Haimen, Jiangsu, China). In brief, cells were separately transfected with siLncRNA1, siLncRNA2 or siControl as described above. Afterwards, the cells were collected and placed into 48-well plates. After attachment, TUNEL detecting solution (50 µl/well) was added into each well, and the plates were incubated at 37˚C for 1 h in the dark. After staining with DAPI, the cells were observed and photographed using a WMF-CX40 fluorescence microscope (WuMo, Pudong, Shanghai, China).

Caspase 3/9 Activity Determination

The activity of caspase 3 and caspase 9 in LNCaP and PC-3 cells after their LINC00662 was depletion was determined by a Caspase 3/9 Activity Assay kit (Beyotime, Haimen, Jiangsu, China). Briefly, after LNCaP and PC-3 cells were transfected with siLncRNA1, siLncRNA2 or siControl for 24 h, the cells were harvested and lysis buffer (100 µl) was added into the cells. After incubation for 15 min at 4˚C, the cell lysates were centrifuged (12000 g/min, 4˚C, 15 min) and subsequently the supernatant was collected. Then, Ac-DEVD-pNA (10 µl, 2mM) was added into the supernatant and incubated for 1-2 h at 37˚C. Finally, the absorbance at a wavelength of 405 nm was detected by a microplate reader (WoYuan Tech, Hongkou, Shanghai, China).

Wound-Healing Assay

LNCaP and PC-3 cells were separately transfected with siLncRNA1, siLncRNA2 or siControl as described above. Subsequently, a 12-well plate was applied to culture these cells (1.5×10^6 cells/well). After 24 h, when the cells reached full confluent, a 200 µl pipette tip was employed to scratch across the cells, and a wound was created. At 0 h and 48 h, the wound closure was recorded by a WMF-CX40 microscope (WuMo, Pudong, Shanghai, China).

Transwell Assay

For transwell invasion assays, approximately 1×10^6 cells after siRNAs transfection (suspended in 200 µl serum-free medium) were placed in the upper chamber of a Corning transwell insert (Biodee, Haidian, Beijing, China). In addition, complete medium (15% FBS; 600 µl) was added into the lower chamber. After 48 h, crystal violet solution (0.1%) was applied to stain the invaded cells on the lower surface of the insert membrane. After washing with PBS, the invaded cells were count and photographed by a WMF-CX40 microscope (WuMo, Pudong, Shanghai, China).

RNA Pull-Down Assay

The cells were lysed using cell lysis buffer (Beyotime, Haimen, Jiangsu, China). Then, biotin-labeled probe against LINC00662, which was purchased from Zoonbio Biotechnology Co., Ltd. (Nanjing, Jiangsu, China), was applied to be incubated with the cell lysates. Subsequently, probe was captured by magnetic beads conjugated with M-280 Streptavidin (Thermo Fisher, Pudong, Shanghai, China) and sequentially the precipitants by probe were eluted. Finally, the miR-34a was analyzed by qRT-PCR analysis as described above.

Dual Luciferase Reporter Assay

The wild-type LINC00662 reporter plasmids (LINC00662 wt) and mutant LINC00662 reporter plasmids (LINC00662 mut) were constructed by AtaGenix Biotechnology Co., Ltd. (Wuhan, Hubei, China). Briefly, LINC00662 wt or LINC00662 mut reporter plasmids were co-transfected with indicated miRNA mimics (NC mimics or miR-34a mimics) into LNCaP and PC-3 cells. After culturing for 48 h, we used a Dual-Luciferase Assay kit (Promega, Dongcheng, Beijing, China) to analyze the luciferase activity of these treated cells on a microplate reader (WoYuan Tech, Hongkou, Shanghai, China).

Statistical Analysis

All statistical analysis in this study were conducted by the use of SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). The significance between two groups was calculated using the Student's *t*-test. Comparisons between multiple groups were performed by one-way ANOVA, and comparisons between two groups were performed by the Student-Newman-Keuls (SNK) method. Overall survival curve was calculated using Kaplan-Meier analysis and log-rank test. Univariate and multivariate analysis of the prognostic factors was performed with Cox regression model. A *p*-value of less than 0.05 was considered statistically significant.

Results

The Expression of LINC00662 is Frequently Upregulated in PCa Cell Lines and Tissues

In order to explore whether LINC00662 was abnormally expressed in PCa, the expression levels of LINC00662 in PCa tissues and matched, adjacent, normal tissues were detected by RT-PCR. As shown in Figure 1A, it was found that LINC00662 was significantly upregulated in PCa tissues compared with that in paired adjacent non-tumor prostate tissues ($p<0.01$). We further evaluated the expression of LINC00662 in PCa cell lines. The results showed that the expression levels of LINC00662 in all four PCa cell lines (DU145, 22RV1, PC-3 and LNCaP) were much higher than that in WPMY-1, a human prostate cell line. The C-3 and LNCaP cell lines, which possessed the highest levels of LINC00662 expression among all tested PCa cell lines, was selected for further cells experiments. Overall, we have sufficient evidence to conclude that LINC00662 was highly expressed in PCa patients.

Correlation Between LINC00662 and Clinical Prognosis

To investigate the association between LINC00662 expression level and Clinicopathological features of PCa patients, PCa tissue samples were classified into low expression group (n=52) and high expression group (n=53), according to the median expression level of all PCa samples. Table I showed that high expression of LINC00662 was significantly correlat-

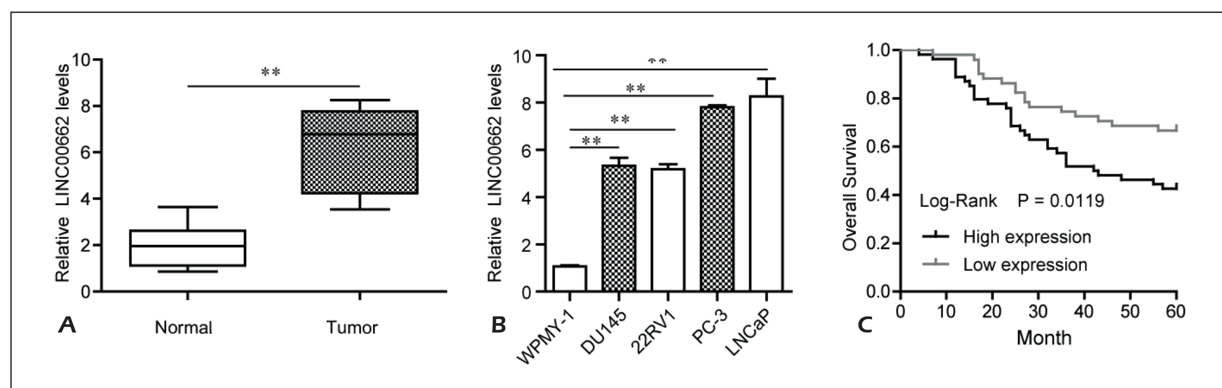

Figure 1. The upregulation of LINC00662 level was associated with favorable prognosis of PCa patients. **A**, Dot plots showing LINC00662 expression levels in PCa tissues compared with adjacent non-tumor tissues. $p<0.01$ *vs.* non-tumor tissues. **B**, The relative expression of LINC00662 was evaluated by qRT-PCR in the PCa cell lines DU145, 22RV1, PC-3 and LNCaP and a human prostate cell line WPMY-1, GAPDH was used as an internal control. **C**, Overall survival curves in patients with PCa according to LINC00662 levels. $^*p<0.05$, $^{**}p<0.01$.

ed with advanced distant metastasis (p=0.018). However, there were no significant correlations of LINC00662 expression with other clinical features. Furthermore, we explored the influence of LINC00662 expression on long-term overall survival. As shown in Figure 1C, Kaplan-Meier survival analysis showed that patients with higher expression of LINC00662 had shorter survival time than those with low expression level of LINC00662 (p=0.0119). In addition, in univariate analysis, we found that the levels of LINC00662 were significantly associated with poor prognosis in PCa patients (HR=3.215, 95% CI: 1.347-4.547, p=0.005). More importantly, further multivariate analysis results showed that LINC00662 expression was also an independent predictor of overall survival (HR=2.984, 95% CI: 1.263-4.198, p=0.011) for PCa patients (Table III).

Repressing the Expression of LINC00662 Suppressed PCa Cell Growth and Accelerated Cell Apoptosis

Due to future uncover the precise roles of LINC00662 in PCa, siRNAs specific targeting LINC00662 (siLncRNA1, siLncRNA2) were applied. Firstly, qRT-PCR analysis was carried out to evaluate the levels of LINC00662 in PCa cells after LINC00662 siRNAs transfection. The data suggested that knockdown of LINC00662 significantly reduced the relative LINC00662 expression in LNCaP and PC-3 cells (Figure 2A). After transfected with LINC00662 siRNAs, the growth of LNCaP and PC-3 cells at indicated time period decreased dramatically when compared with cells transfected with siControl (Figure 2B). Similarly, results from cell colony formation assays demonstrated that depletion of LINC00662 remarkably reduced the colony formation capacity

Table III. Univariate and multivariate analysis of overall survival in PCa patients.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*	HR	95% CI	*p*
Age	0.528	0.556-1.787	0.231	-	-	-
Tumor diameter	0.341	0.672-1.774	0.169	-	-	-
Histological grade	1.132	0.667-2.231	0.326	-	-	-
Tumor stage	0.983	0.644-2.127	0.215	-	-	-
Lymph node metastasis	1.235	0.783-2.334	0.157	-	-	-
Distant metastasis	2.956	1.348-4.271	0.005	2.472	1.184-3.578	0.033
Capsule invasion	0.942	0.457-2.156	0.372	-	-	-
Multiple lesions	1.276	0.784-2.338	0.138	-	-	-
LINC00662 expression	3.215	1.347-4.547	0.005	2.984	1.263-4.198	0.011

of LNCaP and PC-3 cells (Figure 2C). We next performed TUNEL assays to evaluate the effects of LINC00662 on cell apoptosis. As the data presented in Figure 2D, the apoptotic cells were notably increased after the cells were transfected with LINC00662 siRNAs, which suggested that depression of LINC00662 promoted apoptosis of LNCaP and PC-3 cells. We next examined the alternation of critical molecules involved in cell apoptosis in PCa cells after LINC00662 siRNAs transfection. The data confirmed that knockdown of LINC00662 markedly reduced the activity of caspase 3 and caspase 9 in LNCaP and PC-3 cells (Figure 2E). Overall, these results validated that LINC00662 had critically regulatory effects on the cellular growth and apoptosis of PCa cells.

Knockdown of LINC00662 Impeded the Metastatic Potentials of PCa Cells

For further investigation, we subsequently verified the effects of LINC00662 on cellular migration and invasion of PCa cells when their LINC00662 was depleted. To achieve that, we next separately undertook wound healing assays and transwell analysis using LNCaP and PC-3 cells. The transwell assays were executed to determine

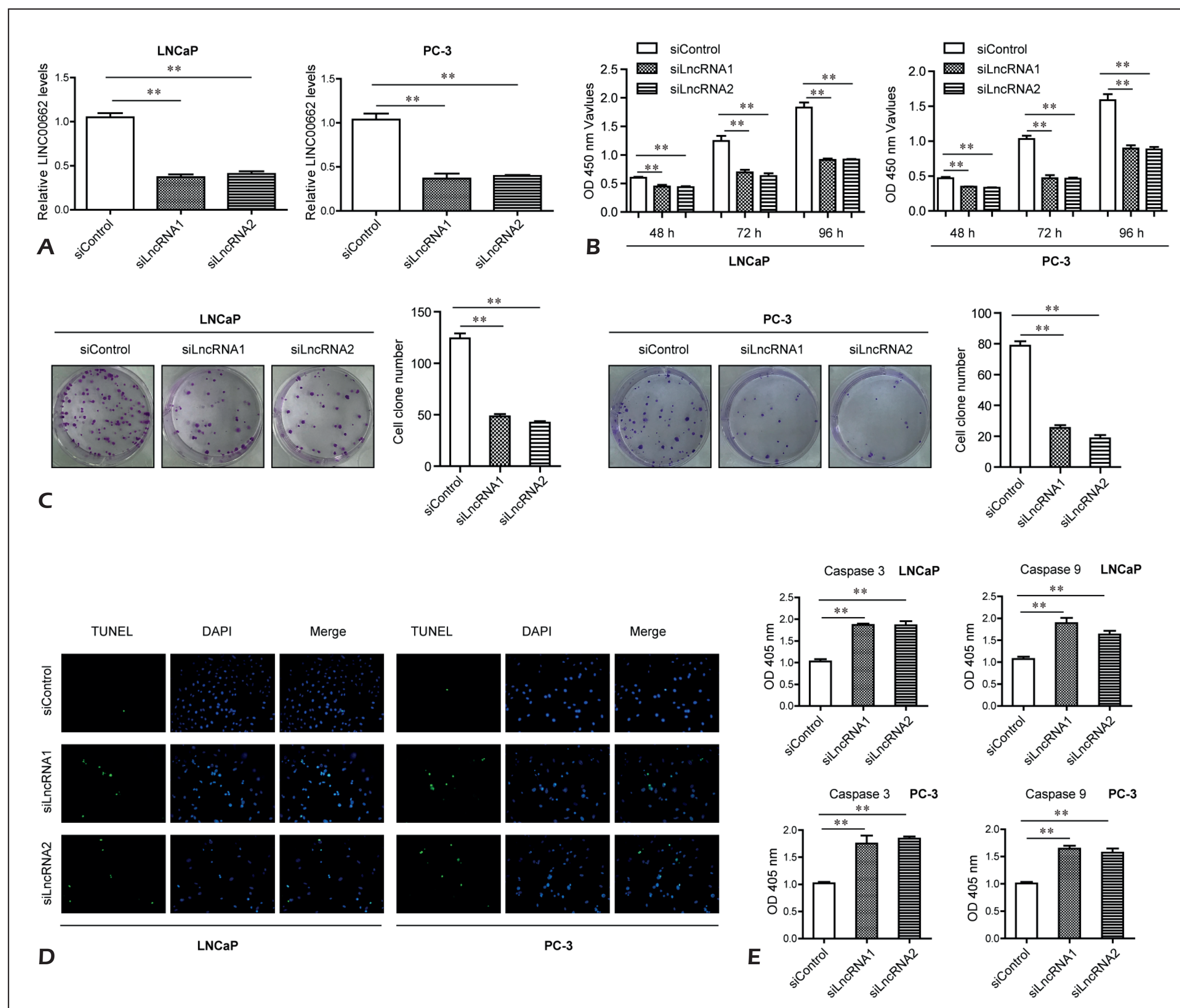

Figure 2. The effects of LINC00662 on the cellular proliferation and apoptosis of LNCaP and PC-3 cells. **A**, The qRT-PCR analysis determined the relative expression of LINC00662 in LNCaP and PC-3 cells. **B**, The proliferation of LNCaP and PC-3 cells transfected with LINC00662 siRNAs or siControl was detected by CCK-8 assays. **C**, Clone formation assays examined the colony formation ability of LNCaP and PC-3 cells. **D**, Cell apoptosis was detected by TUNEL assays. The green fluorescence stained cells were the apoptotic cells. The cellular nuclei were stained by DAPI (blue fluorescence). **E**, The activity of caspase 3/9 was evaluated in LNCaP and PC-3 cells. *p<0.05, **p<0.01.

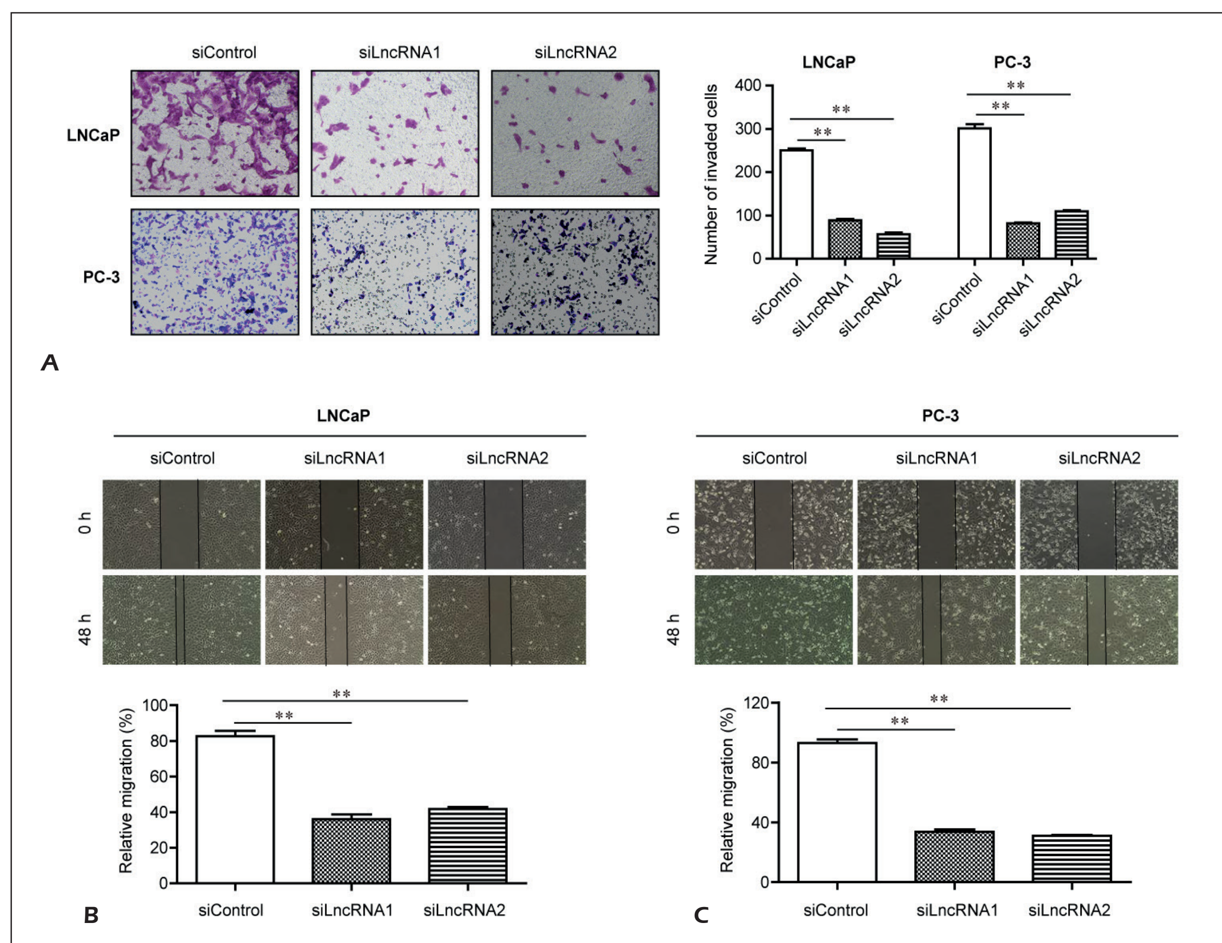

Figure 3. LINC00662 affected the migration and invasion of LNCaP and PC-3 cells. **A**, Transwell assays revealed that the invasion capabilities of LNCaP and PC-3 cells following LINC00662 knockdown. **B**-**C**, Wound healing assays showed significantly decreased migratory abilities in LNCaP and PC-3 cells after transfection of LINC00662 siRNAs. *$p<0.05$, **$p<0.01$.

the invasive capability changes of PCa cells after transfection of LINC00662 siRNAs. Thereafter, the wound healing assays were carried out. The results revealed that the number of invaded cells was remarkably decreased when compared with the cells transfected with siControl (Figure 3A). As the representative figures illustrated in Figure 3C and D, the wound closures in the LINC00662 siRNAs-transfected LNCaP and PC-3 cells were markedly slower than that of the cells transfected with siControl, which indicated that LINC00662 depletion caused significantly inhibitory effects on migratory capability of PCa cells.

LINC00662 Sponged MiR-34a in PCa Cells

We next aimed to discover the detail mechanisms that LINC00662 exerted its functions in PCa. We first performed subcellular fractionation assays and found that the subcellular location of LINC00662 was mainly located in cytoplasm, which implied that LINC00662 might serve as ceRNA to exert its functions (Figure 4A). Considering accumulating evidences had revealed that lncRNA was capable to act as "sponge" to modulate the miRNA expression, we next speculated whether LINC00662 could bind miRNAs to exert its roles in regulating the development and progression of PCa. Therefore, we applied bioinformatics program starBase to predict the potential target miRNAs of LINC00662. As illustrated in Figure 4B, miR-34a, acting as a tumor suppressor in diverse types of cancer, was found to be a potential target of LINC00662. Notably, as revealed by qRT-PCR assays, the expression of miR-34a was decreased in PCa tissues when compared with normal tissues (Figure 4C). Hence, we aimed to validate that miR-34a was the exact target of LINC00662 in PCa cells. Luciferase reporter assays demonstrated that miR-34a mimics notably depressed the activity in LINC00662 wt reporter

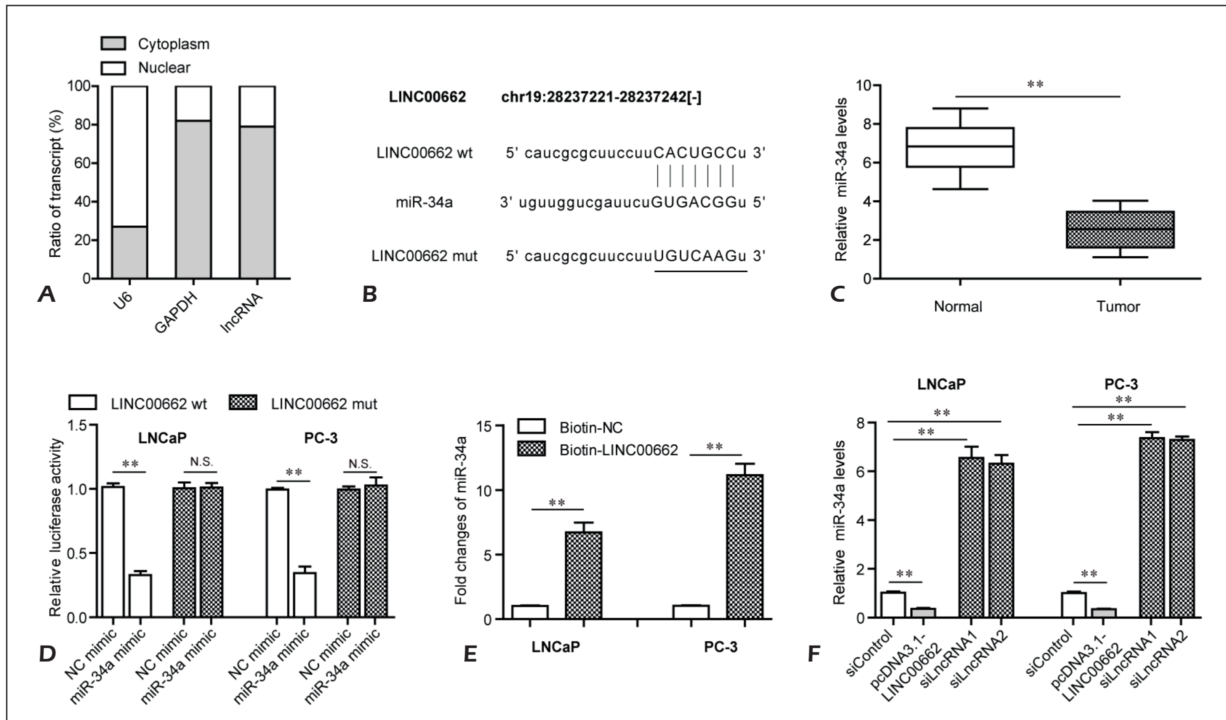

Figure 4. LINC00662 was directly interacted with miR-34a in LNCaP and PC-3 cells. **A**, Subcellular fractionation assays revealed the subcellular location of LINC00662 in LNCaP cells. **B**, The predicted binding site for LINC00662 and miR-34a using "starBase" (http://starbase.sysu.edu.cn/) was shown. **C**, MiR-34a was down-regulated in prostate cancer tissues as determined by qRT-PCR analysis. **D**, Luciferase reporter assays showed that overexpression of miR-34a impeded the activity of LINC00662 wt reporter plasmid-transfected LNCaP and PC-3 cells. **E**, Relative enrichment of miR-34a was evaluated by qRT-PCR after pulldown by biotin-labeled LINC00662 probe. **F**, The qRT-PCR assays evaluated the levels of miR-34a in LNCaP and PC-3 cells when their LINC00662 was overexpression or knocked down. *$p<0.05$, **$p<0.01$.

plasmid-transfected LNCaP and PC-3 cells but not LINC00662 mut reporter plasmid-transfected cells, indicating that LINC00662 directly interacted with miR-34a (Figure 4D). In addition, RNA pulldown assays confirmed that LINC00662 was capable to precipitate miR-34a in LNCaP and PC-3 cells (Figure 4E). Besides, qRT-PCR assays certified that LINC00662 overexpression obviously suppressed miR-34a expression in LNCaP and PC-3 cells, while LINC00662 deficiency dramatically accelerated the expression of miR-34a, suggesting that LINC00662 expression was negatively correlated with miR-34a in PCa cells (Figure 4F). Taken together, these data indicated that LINC00662 sponged miR-34a to repress its expression in PCa cells.

Discussion

PCa is one of the most common and aggressive human malignancies with poor prognosis worldwide. The mechanisms in which the carcinogenesis and progression of PCa remain unclear, which made it hard to screen useful biomarkers for diagnosis and prognosis of PCa[19]. Up to date, many cases of PCa can be diagnosed by the use of serum prostate-specific antigen (PSA) which is a common conventional prognostic factor[20]. However, for some untypical, the specificity of this indicator is limited. Thus, the identification of novel factors would be important for the clinical prognosis of PCa patients. As a very complex disease, the tumorigenesis and progression of PCa were involved in the activities of important tumor-related genes, including oncogenes and tumor suppressors[21-23]. Recently, growing evidence has revealed that lncRNAs can be detected in plasma and tissues as biomarkers for the diagnosis and prognosis of cancers, including PCa[24].

Dysregulation of LINC00662 expression has been reported in several tumors. However, the expression pattern of LINC00662 in PCa has not been investigated. In this study, we firstly performed RT-PCR to detect the expression in PCa tissues and cell lines and our results revealed that LINC00662 expression was significantly

increased in PCa tissues and cell lines. Interestingly, our results, together with previous findings, indicated that the expression of LINC00662 was up-regulated in PCa, gastric cancer and colorectal cancer[17,18]. Subsequently, the clinical data confirmed the positive association between LINC00662 expression and clinicopathologic features in PCa patients. In other words, high LINC00662 expression was significantly associated with positively distant metastasis, indicating that LINC00662 may contribute to the tumor metastasis of PCa. Moreover, Kaplan-Meier survival analysis was further performed to explore the prognostic value of LINC00662 in PCa patients and the result showed that the overall survival time of patients with higher LINC00662 expression levels was shorter than that of patients with lower LINC00662 expression levels. Further overall survival rates assessed with the Cox proportional hazards regression model, the findings confirmed that the expression of LINC00662 was demonstrated to be an independent factor for predicting the prognosis of PCa patients. Previously, a growing number of lncRNAs have been confirmed to be overexpressed and associated with poor prognosis in PCa. Our findings also provided a new PCa-related lncRNA, LINC00662 which also had potential to be a new candidate as an independent biomarker for PCa patients. However, the sample sizes in this study were small. Further studies on more patients with PCa were required to confirm our findings.

Recently, the functional assays of LINC00662 in several tumors have been performed and the results revealed LINC00662 as a tumor promoter. For instance, Liu et al[17] reported that LINC00662 was significantly upregulated in gastric cancer tissues and cell lines. Knockdown of this lncRNA decreased gastric cancer cell proliferation and increased the chemo-sensitivity of gastric cancer cells via the regulation of the Hippo-YAP1 pathway. In lung cancer, it was reported that LINC00662 was highly expressed in lung cancer tissues compared to normal lung tissues. Functional investigations revealed that overexpression of LINC00662 promoted the migration and invasion of lung cancer cells[25]. Functionally, LINC00662 as a tumor promoter has been confirmed in above two tumors. Given the expression of LINC00662 was also up-regulated in PCa, we thus wondered whether LINC00662 can also display a similar role in PCa progression. In this study, we chose LNCap and PC-3 cells for cells experiments and down-regulated the levels of LINC00662 in above two PCa cell lines. We further used CCK-8 assay and colony formation assay, by which we confirmed the tumor-promotive role of LINC00662 in LNCap and PC-3 cells. Moreover, we next performed TUNEL assays to evaluate the effects of LINC00662 on cell apoptosis, finding that knockdown of LINC00662 significantly promoted cell apoptosis. In addition, we also detected the expression levels of apoptosis-related proteins, Caspase 3 and Caspase 9. The results showed that inhibition of LINC00662 distinctly increased the expression of Caspase 3 and Caspase 9. Metastasis was a critical challenge for the treatment of PCa patients. However, the potential mechanism involved in tumor metastasis remains largely unclear. Emerging evidences have revealed that lncRNAs played a very important role in the progress of metastasis. We also found that knockdown of LINC00662 significantly suppressed the migration and invasion of LNCap and PC-3 cells, which may explain why LINC00662 was associated with the clinical prognosis of PCa patients. Overall, we first showed that LINC00662 acted as a tumor promoter in PCa.

In recent years, the ceRNA hypothesis, proposed by Pandolfi et al[26], which enriches the cross-talk between RNA transcripts, attracted increasing attention on the interaction between lncRNAs and microRNAs since lncRNAs can act as ceRNAs to regulate the expression of miRNAs in various cancers[27-29]. For example, Li et al[30] reported that lncRNA SChLAP1 served as a tumor promoter in PCa and promoted the proliferation and metastasis of tumor cells via targeting miR-198. Zhang et al[31] found that lncRNA PCAT-1 was highly expressed in PCa and its knockdown could invasion and metastasis via targeting miR-129-5p in hepatocellular carcinoma. However, whether LINC00662 could affect the biological behavior of PCa cells through regulating miRNAs is not unknown. In this study, using the bioinformatics tools and luciferase reporter assay, we found that miR-34a shared complementary binding with the 3'-untranslated regions (UTR) of LINC00662. Previous studies[32,33] have reported that miR-34a was abnormally expressed in various tumors and functioned a tumor suppressor which was confirmed by many *in vitro* and *in vivo* assays. In PCa, miR-34a had been well found to act as a tumor suppressor[34]. Then, our results revealed that LINC00662 directly bound to miR-34a in PCa cells. In addition, the results of RT-PCR showed that knockdown of LINC00662 suppressed the expression levels of miR-34a in both LNCap and

PC-3 cells, while up-regulation of LINC00662 had an opposite effect. Thus, our results indicated that LINC00662 may display its tumor-promotive roles by modulating miR-34a. This is the first time to show that LINC00662 negatively regulated miRNA in human PCa.

Conclusions

We determined a novel PCa-related lncRNA, LINC00662 which is an independent prognostic factor for PCa patients and promotes the proliferation and metastasis of PCa cells via regulating miR-34a. Therefore, LINC00662 may be considered a useful tumor biomarker in PCa and, targeting LINC00662 may be a novel therapeutic strategy for patients with PCa.

Conflict of Interests

The authors declare that they have no conflict of interest.

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