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Long non-coding RNA LINC00173 serves as sponge for miR-338-3p to promote prostate cancer progression *via* regulating Rab25

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Abstract. – OBJECTIVE: Long non-coding RNA LINC00173 (LINC00173) has been shown to facilitate the progression of a number of malignancies. In this study, we aimed to investigate the function of LINC00173 on prostate cancer (PCa) and discover the potential regulatory mechanism.

PATIENTS AND METHODS: RT-PCR was used to determine the levels of LINC00173, miR-338-3p and Rab25 in PCa patients and cell lines. The clinical significance of LINC00173 was statistically analyzed in 124 PCa patients. CCK-8, colony formation, transwell, scratch wound, Ethynyldeoxyuridine (EdU) assays and flow cytometry assays were used to detect the proliferation, apoptosis, invasion and migration of PCa cells. The mechanism of LINC00173 action was explored through bioinformatics, RNA pull-down assays and Luciferase reporter assays.

RESULTS: We observed that the expression of LINC00173 and Rab25 was distinctly upregulated in both PCa specimens and cell lines, while miR-338-3p expression was significantly down-regulated. High LINC00173 expression was associated with Gleason score, preoperative PSA level and reduced patient survivals. Functional assays revealed that knockdown of LINC00173 suppressed the proliferation, migration and invasion of PCa cells, and promoted apoptosis. Mechanistically, LINC00173 acted as a competitive endogenous RNA in PCa and increased Rab25 expressions via sponging miR-338-3p. Moreover, LINC00173 promoted PCa progression by interacting with miR-338-3p and Rab25.

CONCLUSIONS: Our findings, for the first time, identified a novel PCa-related lncRNA, LINC00173 which might serve as an oncogene in PCa. The discovery of the LINC00173/miR-338-3p/Rab25 pathways provided new thinking for the treatments of PCa.

Key Words:
LncRNA LINC00173, MiR-338-3p, Rab25, Prostate cancer, Biomarker.

Introduction

Prostate cancer (PCa) acts as a representative malignancy specific to men around the world, and ranks 2nd in causing cancer-related death in Western countries[1]. Despite the far lower PCa morbidity in China compared with Western countries, it has presented an evident uptrend in recent years[2]. Based on wide researches, prostate specific antigen (PSA) is still a widely applied molecular marker of PCa, although criticism never stops[3]. Despite the distinct advancement in clinical and experimental oncology for PCa, many patients still exhibit a poor prognosis considering the distant metastasis[4,5]. Notwithstanding, PCa holds extremely proliferation, migration and invasion mechanisms, which have not been determined. On that account, it is necessary to focus on the identification of new biomarkers of PCa and the understanding of its development and progression mechanisms from a new perspective.

As every knows, the number of protein-coding genes occupies only 2% or so of all human genes, while most transcripts come to non-coding RNAs[6]. Long non-coding RNA (lncRNA) refers to a non-coding RNA type longer than 200 nucleotides and, as reported, it participates in the genetic and epigenetic regulation and post-transcriptional regulation[7]. Nowadays, the prevalence of ~10,000 lncRNAs is predicted the ENCODE and GENCODE projects. Nevertheless, a larger number of lncRNAs fails to be determined, let along the corresponding function annotations[8]. In recent years, more and more studies have confirmed the involvement of lncRNAs as the oncogene or the anti-oncogene in the pathogenesis and progression of different diseases such as cancers[9,10]. However, in PCa, only preliminary

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studies are conducted regarding a series of known lncRNAs, like lncRNA PVT1, lncRNA PCAT-1 and lncRNA UCA1[11-13]. It is necessary to explore if other lncRNAs, especially unknown ones, also affect PCa pathogenesis as well as the potential molecular mechanisms.

LncRNA LINC00173 (LINC00173) is a kind of new lncRNA related to tumors, which maps to chromosome 12q24.22. Studies have reported its overexpression in many tumor types like melanoma, colorectal cancer and lung cancer[14-16]. However, whether LINC00173 was dysregulated in PCa and its possible function in the progression of PCa remains unknown. The study is the first one that proved the high expression of LINC00173 as a tumor promotor in PCa patients, suggesting that LINC00173 may be used as a new cancer biomarker and also a treating target for PCa.

Patients and Methods

Patients and Tissue Samples

We acquired human PCa tissues and the adjacent normal tissue samples during surgery from 124 PCa patients aged from 37 to 84 years (57.5±5.3). All tissues were histopathologically confirmed by two experienced pathologists. We froze the obtained specimens and stored them at -80 °C before use. The follow-up data were available and complete for each patient. The study has obtained the approval from the Research Ethics Committee of the Suqian First Hospital, and obtained the written informed consent of all patients.

Cell Culture and Transfection

The normal prostate epithelial cells (RWPE-1) together with PCa cell lines (DU145, PC-3 and LNCap) were provided by the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). We stored all cells in incubator with 5% CO_2 at 37 °C and cultured them in DMEM (Gibco, Hangzhou, Zhejiang, China) supplemented with 10% FBS (Longhu Technology, Kunming, Yunnan, China) and 1% penicillin–streptomycin (Pusi Technology, Chengdu, Sichuan, China).

LncRNA LINC00662 pcDNA3.1 expression vector (5'-CCATCACATTCTTCCATTCCGG-3') and empty vectors (pcDNA3.1-vector; 5′-ATCCCAGCCGTGCAAACTGCACCATT-3') were designed and constructed by Sangon Biotech (Pudong, Shanghai, China). Shanghai Gene Pharma Co., Ltd. (Shanghai, China) took charge of synthesizing miR-338-3p inhibitor and the LINC00173 short interfering RNA (siRNAs). Ribo Co., Ltd. (Hangzhou, Zhejiang, China) took charge of synthesizing miR-338-3p mimic. Cell transfections were performed using Lipofectamine® 2000 reagent (Thermo, Haidian, Beijing, China) at 37°C with 10 nM of vectors, 50 nM of siRNAs and 50 nM of miRNAs. qRT-PCR assisted in confirming the efficiency of infection. The transfection and lentiviral infection methods had been interpreted previously[17].

RNA Extraction and qRT-PCR

TRIzol reagent (Invitrogen, Haidian, Beijing, China) helped to extract the total RNA. A NanoDrop 2000 spectro-photometer (Thermo Fisher Scientific, Kunming, Yunnan, China) was employed for measuring the concentration of RNA. PrimeScriptTM RT reagent Kit with gDNA Eraser (TaKaRa, Hangzhou, Zhejiang, China) assisted in converting 400 ng RNA into cDNA. Prime Script RT reagent Kit together with SYBR Premix ExTaq were applied to the qRT-PCR assay for detecting miRNA, lncRNA and gene expression. GAPDH was taken as the endogenous control. We examined each sample in triplicate and adopted the threshold cycle (Ct) method for the analysis. Table I lists the used primers in qRT-PCR.

Cell Proliferation Assays

For the exploration of LINC00173 expression on the proliferation, we seeded PC3 and DU145 cells with LINC00173 knockdown in the 96-well plates (1×10^3 cells each well) for 0, 24, 48, 72, or 96 h of preincubation in a humidified atmosphere (37°C, 5% CO^2). Then, each well was added with 10 μL of Cell Counting Kit-8 kit (CCK-8) solution

Table I. The primers used in this study for RT-PCR.

Names	Sequences (5′-3′)
LINC00173: F	GCCATCAAGCACTCCAGC
LINC00173: R	ATACCATCCCAATATCACCA
miR-338-3p: F	GCTGGCACCAGCATCAGTGATT
miR-338-3p: R	CCAGTGCAGGGTCCGAGGTA
Rab25: F	AACCAAGCACCAGACCTATGC
Rab25: R	CACTTTTGTTACCCACGAGCA
GAPDH: F	ACAACTTTGGTATCGTGGAAGG
GAPDH: R	GCCATCACGCCACAGTTTC
U6: F	GCGCGTCGTGAAGCGTTC
U6: R	GTGCAGGGTCCGAGGT

(Ningfu Technology, Kunshan, Zhejiang, China). The 96-well plates were shaken overnight in the dark and the absorbance was then determined at 450 nm.

Colony Formation Assays

Cells were transfected with the related factors as described above. Twenty four hours later, the above cells were trypsinized, counted and replated at a density of 600 cells/8 cm dish. Following two weeks of incubation, we employed crystal violet to stain upper layer of agar containing cells, and counted the number of colonies in every dish for estimating the efficiency of plating among various groups.

Cell Migration Assays

The cell migration measurement relied on wound healing assay. Cells received culture in the 6-well plate and seed to 100% confluence. A pipette tip was used to create the wound, followed by another 48 h of culture. The width of wound was photographed with phase-contrast microscope.

Invasion Assays

During the transwell migration assays, we placed transfected PC3 and DU145 cells (3×10^5) in the top chamber which had coated membrane (BD Biosciences, Kunshan, Zhejiang, China). We placed cells in the top chamber in medium free of serum; and filled the lower chamber with 10% FBS. The chemoattractant was 25 ng/ml EGF (Sigma, Pudong, Shanghai, China). Following 24 hours of post-seeding, we used pre-cooled methanol to fix the membrane which then underwent five minutes of staining by using crystal violet (1%) at room temperature. The invasive cells were counted after photographing. Data were expressed as the mean value of cells/field.

Flow Cytometric Analysis of Apoptosis

How LINC00173 knockdown affected cell apoptosis was determined with the FITC-Annexin V Apoptosis Detection Kit (KeyGen Biotech, Xuhui, Shanghai, China). Cells (3×10^5) were first resuspended in 100 μL 1×Binding Buffer, followed by 15 min of incubation by using Annexin V (5 μL) and PI staining solution (5 μL) in the dark at room temperature. Flow cytometry (BD Biosciences, Kunshan, Zhejiang, China) was applied to cell analysis within 1 h. All experiments were performed in triplicate.

Subcellular Fractionation

The RNeasy Midie Kit (Qiagen, Haidian, Beijing, China) was used to detach and harvest cytoplasmic and nuclear fractions. RT-PCR assays were performed for further determination of the cellular localization of LINC00173 with GAPDH as the cytoplasm control and U6 as the nucleus control.

Luciferase Reporter Assays

We seeded PC3 and DU145 cell lines into the 96-well plates to receive the co-transfection by Luciferase (60 ng), pRL-CMV Renilla Luciferase reporter (6 ng) and miR-338-3p mimic or inhibitor. After incubation for 48 hours, a Dual-Luciferase reporter assay (Promega, Madison, WI, USA) was applied to the measurement of the firefly as well as Renilla Luciferase activities.

RNA Pull-Down Assays

A Magnetic RNA Protein Pull-Down Kit (Pierce, Kunming, Yunan, China) was applied to the RNA pull-down assay following the manual. RiboBio (China) assisted in synthesizing biotinylated LINC00173 RNA. Briefly, 60 pmol biotinylated RNA received incubation by using streptavidin-agarose beads (50 μl) following 1 h of prewashing at 4°C. We took 3'untranslated-region of androgen receptor (AR) RNA with UC-rich regions for HuR as the positive control.

Statistical Analysis

SPSS software, version 19.0 (SPSS, Chicago, IL, USA) was applied to the statistical analysis. Two-tailed Student's *t*-tests assisted in assessing the difference between groups. Receiver-operating characteristic (ROC) curves assisted in assessing the performance exhibited by related factors to discriminate PCa specimens from non-tumor tissues. The Pearson χ^2 test was conducted for the analysis of relationships between the LINC00173 expression level and clinicopathological parameters. The Kaplan-Meier curves helped to evaluate how LINC00173 affected the overall survival (OS) and disease-free survival (DFS). The log-rank test was used to validate the significance of the Kaplan-Meier curves. Multivariate analysis was conducted regarding the prognostic factors by virtue of the Cox regression (proportional hazard model). A p value less than 0.05 was considered with statistical significance.

Results

Increased Expression of LINC00173 in Human PCa and its Diagnostic Significance

To explore whether LINC00173 was a PCa related lncRNA, we firstly performed RT-PCR to detect its expression in 124 PCa patients. We observed that the LINC00173 expression in PCa specimens was distinctly higher relative to matched non-tumor specimens (Figure 1A, $p < 0.0$). In addition, PCa tissues with advanced stages and positive metastasis displayed a higher level of LINC00173 than PCa tissues with early stages and negative metastasis (Figure 1B and 1C). The diagnostic assays revealed that high LINC00173 expression had an AUC value of 0.7528 (95% CI: 0.6904 to 0.8151) for PCa (Figure 1D). The sensitivity and specificity of LINC00173 expressions for distinguishing PCa samples from normal samples was 68.34%/81.34%, indicating LINC00173 as an early-diagnosis indicator for PCa patients. Moreover, the LINC00173 expression levels were also clearly upregulated in the three PCa cell lines (Figure 1E). Overall, our findings revealed that LINC00173 exhibited a high expression in PCa patients and could affect PCa progression.

Upregulation of LINC00173 Confers Poor Prognosis in PCa Patients

For confirming the clinical value possessed by LINC00173 expression in PCa patients, we divided 124 patients into two groups (high: n=61 and low: n=63). The median relative quantity was taken into account for setting the cut-off level for LINC00173. Table II lists the Chi-square test results, indicating the positive relation between high LINC00173 expression and gleason score (p=0.019), preoperative PSA level (p=0.007) and clinical stage (p=0.047). In spite of this, LINC00173 expression level did not remarkably affect other clinical factors ($p > 0.05$). We collected the survival data by five-years follow-up, for further exploring the prognostic value held by LINC00173 in PCa patients. Importantly,

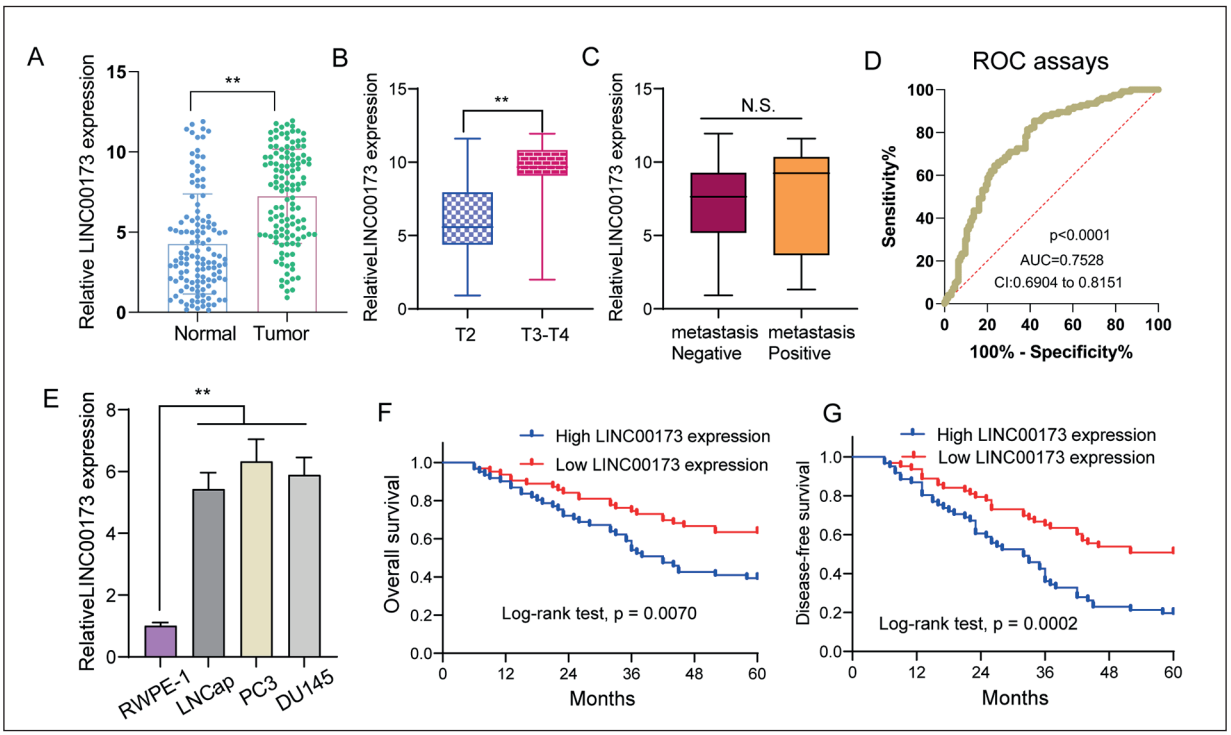

Figure 1. The distinct upregulation of LINC00173 expression and its clinical significance in PCa patients. **A,** The expression of LINC00173 in PCa tissues was significantly higher than that in matched non-tumor specimens. **B,** LINC00173 expression in PCa specimens with different stages. **C,** The PCa samples with positive metastasis exhibited a higher level of LINC00173. **D,** The ROC curve analysis for discriminative ability between PCa specimens and non-tumor controls. **E,** Real-time PCR analysis of LINC00173 expression in RWPE-1 cell lines, including LNCap, PC3 and DU145 cell lines. **F, G,** Kaplan-Meier curves for OS (**F**) and DFS (**G**) time in patients with PCa divided according to LINC00173 expression. $^{**}p < 0.01$, $^{*}p < 0.05$.

Table II. Correlation between LINC00173 expression and different clinicopathological features in patients with PCa.

Characteristics	Group	Total (%)	LINC00173 expression		*p*-value
			High	Low	
Age (years)	< 65	57	30	27	0.480
	≥ 65	67	31	36	
Tumor size (cm)	< 2.5	73	32	41	0.153
	≥ 2.5	51	29	22	
Gleason score	< 7	76	31	45	0.019
	≥ 7	48	30	18	
Preoperative PSA level (ng/mL)	< 10	70	27	43	0.007
	≥ 10	54	34	20	
Clinical stage	T2	76	32	44	0.047
	T3-T4	48	29	19	
Lymph node metastasis	Negative	89	41	48	0.267
	Positive	35	20	15	
Multiple lesions	Negative	78	35	43	0.210
	Negative	46	26	20	

as revealed by the Kaplan-Meier assay result, patients whose LINC00173 expression was high presented a shorter OS (p=0.0070, Figure 1F) and DFS (p=0.0002, Figure 1G) compared with low LINC00173 group. Besides, multivariate assays confirmed that LINC00173 expression was an independent molecular biomarker for the predicting of OS (p<0.05) and DFS (p<0.05) in PCa (Table III). Overall, our findings suggested LINC00173 as a novel cancer biomarker for PCa patients.

LINC00173 Knockdown Hindered PCa Cell Proliferation and Metastasis In Vitro

For exploring how LINC00173 possibly affected PCa cell growth, CCK-8 assay assisted in determining the cell proliferation changes of PC3 and DU145, which suggested that the survival fraction of si-LINC00173 transfected PC3 and DU145 were distinctly decreased compared with the si-LINC00173 control group (Figure 2A and 2B). Similarly, knockdown of LINC00173 prominently depressed colony forming ability of PC3 and DU145 cells to reduce cell proliferation (Figure 2C). Also, as proved by the 5-ethynyl-2'-deoxyuridine (EdU) proliferation assay, LINC00173 depletion caused the cell growth to slow down (Figure 2D). Moreover, flow cytometry analysis indicated the obvious increase in the percentage of early apoptotic cells in si-LINC00173 groups relative to control groups (Figure 2E). To further determine whether LINC00173 was associated with the metastasis potential of PCa, we performed wound-healing assays, finding that knockdown of LINC00173 inhibited cell mobility compared with the control treatments (Figure 3A). Besides, Transwell system helped to find the weakened invasive ability exhibited by PC3 and DU145 cells after si-LINC00173 transfection (Figure 3B). Overall, our data revealed that LINC00173 was involved in cellular progression of PCa cells.

Table III. Multivariate analyses for disease-free survival and overall survival by Cox regression model.

Variable	Overall survival			Disease-free survival		
	HR	95% CI	*p*	HR	95% CI	*p*
Age	1.452	0.742-2.132	0.234	1.372	0.822-2.341	0.313
Tumor size	1.231	0.423-2.052	0.375	1.349	0.564-1.993	0.231
Gleason score	2.896	1.327-4.672	0.013	3.018	1.426-4.983	0.018
Preoperative PSA level	3.017	1.375-4.883	0.008	3.237	1.427-5.138	0.003
Clinical stage	1.784	0.576-2.138	0.138	1.582	0.633-2.313	0.113
Lymph node metastasis	1.213	0.763-2.381	0.385	1.438	0.832-2.418	0.282
Multiple lesions	1.455	0.673-2.137	0.219	1.389	0.832-2.239	0.176
LINC00173 expression	2.986	1.328-4.789	0.011	3.184	1.427-5.127	0.006

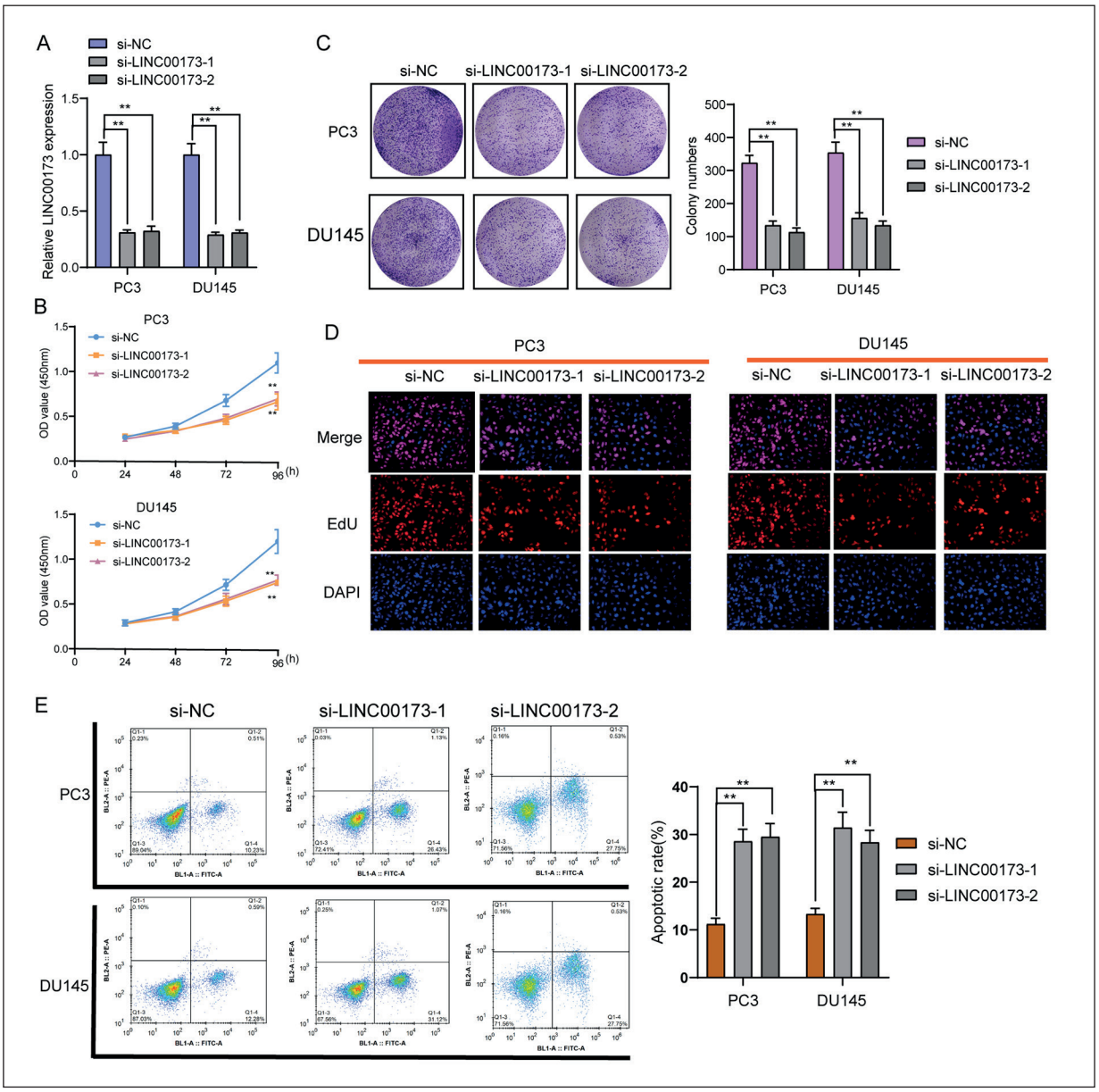

Figure 2. LINC00173 dramatically influenced the proliferation, apoptosis, invasion and migration of PCa cells. **A,** The expression of LINC00173 was analyzed by qRT-PCR in LINC00173-knockout PCa cells (PC3 and DU145 cells). **B-D,** The effect of LINC00173 knockdown on PCa cell proliferation was determined by CCK-8 (**B**), colony formation (magnification: 10×) (**C**) and EdU incorporation assays (magnification: 100×) (**D**). **E,** The apoptosis of PC3 and DU145 cells cells was measured by staining with Annexin V/PI, followed by FACS analysis. $^{**}p < 0.01$, $^{*}p < 0.05$.

LINC00173 Acted as a Sponge for MiR-338-3p

The association of lncRNA with microRNA has been reported in a lot of studies[18]. To further confirm the potential mechanism involved in LINC00173 function in PCa progress, the subcellular location of LINC00173 in PCa cells was confirmed firstly. As revealed by the nuclear-cytoplasmic fractionation, the cytoplasm of PCa cells mainly saw the location of LINC00173 (Figure 4A). As reported, a lot of cytoplasmic lncRNAs can competitively bind microRNAs, thereby acting as the competing endogenous RNAs (ceRNAs)[19]. Therefore, in our study, bioinformatics analysis was used to predict the possible targeting miRNAs. Figure 4B displays the sites where miR-338-3p directly bound to LINC00173. Previous studies have identified the

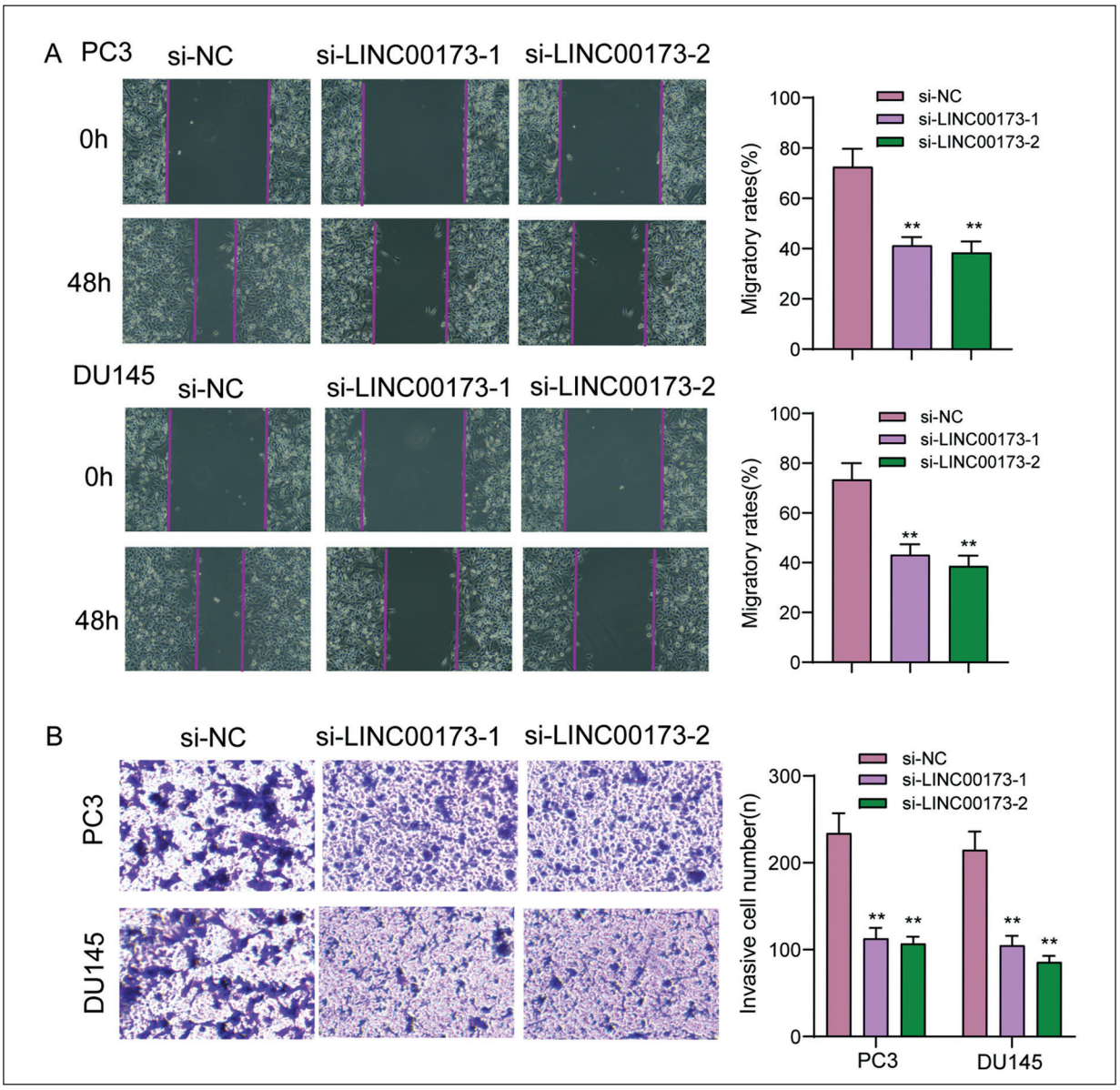

Figure 3. Effects of LINC00173 on PCA cell migration and invasion. **A,** Wound-healing assays were used to investigate the migratory ability of PC3 and DU145 cells (magnification: 10×). **B,** Transwell assays were applied to determine the changes in invasive abilities of PC3 and DU145 cells (magnification: 40×). $^{**}p < 0.01$, $^{*}p < 0.05$.

role of miR-338-3p being a functional miRNA in several tumors as well as a tumor suppressor in PCa. The study also determined miR-338-3p expression in 124 PCa patients, finding that miR-338-3p exhibited a higher level in PCa specimens, especially in PCa tissues with advanced stages (Figure 4C and 4D). The preliminary diagnostic value of miR-338-3p was also detected using ROC assays (Figure 4E). In three PCa cells, miR-338-3p was more highly expressed relative to PWPE-1 cells (Figure 4F). The expressing trend of miR-338-3p in our experiments was consistent with previous studies. Moreover, based on clinical assays, patients whose miR-338-3p expression was low exhibited a shorter OS (Figure 4G, p=0.0082) and DFS (Figure 4H, p=0.0204) than those with high miR-338-3p expression. Furthermore, RNA pull-down assisted in validating the physical relationship of LINC00173 with miR-338-3p in PCa cells *in vitro* assays (Figure 4I). Then, we used a Luciferase reporter assay, finding the reduction in the relative Lucif-

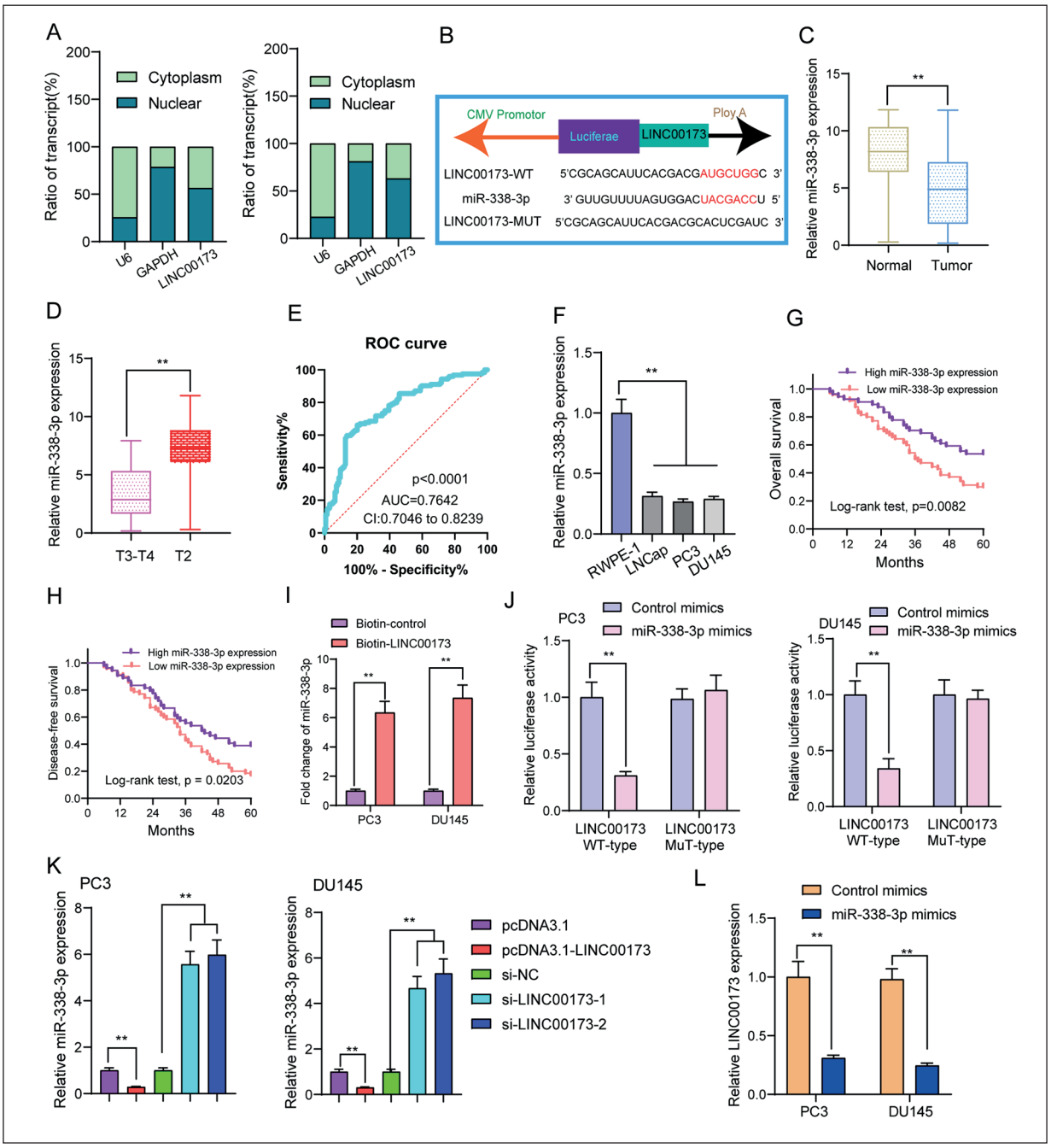

Figure 4. LINC00173 acts as a sponge for miR-338-3p in PCa cells. **A,** Relative LINC00173 expression levels in nuclear and cytosolic fractions of PC3 and DU145 cells. Nuclear controls: U6; Cytosolic controls: GAPDH. **B,** miR-338-3p binding sequence in LINC00173-WT and sequence of LINC00173-MUT. **C,** RT-PCR for the levels of miR-338-3p in 124 PCa patients. **D,** The expression of miR-338-3p in different stages of PCa samples. **E,** The ROC curve analysis for discriminative ability between PCa specimens and non-tumor controls. **F,** Real-time PCR analysis of miR-338-3p expression in RWPE-1 cell lines, including LNCap, PC3 and DU145 cell lines. **G, H,** Kaplan-Meier curves for OS (**G**) and DFS (**H**) time in patients with PCa divided according to miR-338-3p expression. **I,** RNA-pull down assays for the determination of the association between LINC00173 and miR-338-3p. **J,** Luciferase activity in PC3 and DU145 cells co-transfected with miR-338-3p and Luciferase reporters containing LINC00173 wild-type or mutant type (MUT) 3'-UTR. **K,** The dysregulated expression of miR-338-3p in PC3 and DU145 cells after the transfection of pcDNA3.1 or si-LINC00173. **L,** Overexpression of miR-338-3p suppressed the expression of LINC00173 in PC3 and DU145 cells. $^{**}p < 0.01$, $^{*}p < 0.05$.

erase activities after co-transfecting PCa cells with pmirGLO-LINC00173-wt and miR-338-3p, but other groups did not show such reduction (Figure 4J). To demonstrate that LINC00173 can influence miR-338-3p expression, miR-338-3p expression in PCa cells under the transfection of si-LINC00173 or pcDNA3.1 was analyzed, finding that overexpression of LINC00173 suppressed miR-338-3p expression, while miR-338-3p was boosted after LINC00173 downregulation (Figure 4K). Besides, we also observed that miR-338-3p overexpression hindered LINC00173 level in both PC3 and DU145 cells (Figure 4L).

Rab25 Directly Binds to the 3'-UTR of MiR-338-3p

Given that miRNAs were involved in tumor progression via the regulation of mRNA targets, bioinformatics analysis was carried out for detecting miR-338-3p target genes in the study. Unexpectedly, Rab25 acted as one target of miR-338-3p (Figure 5A). We used GEPIA and observed that Rab25 expression was increased in PCa specimens (Figure 5B). As revealed by the pan-cancer assays, the distinct upregulation of Rab25 frequently occurred in many types of tumors, suggesting its extensively regulatory functions in tumor progression (Figure 5C). Also, in our cohort, we observed the distinct upregulation of mRNA Rab25 in both PCa tissues and cell lines (Figure 5D and 5E). Then, we performed Luciferase reporter to confirm whether Rab25 is the direct target gene of miR-338-3p. Figure 5F showed that miR-338-3p mimics transfection suppressed the Luciferase activity exhibited by PC3 and DU145 cells of the

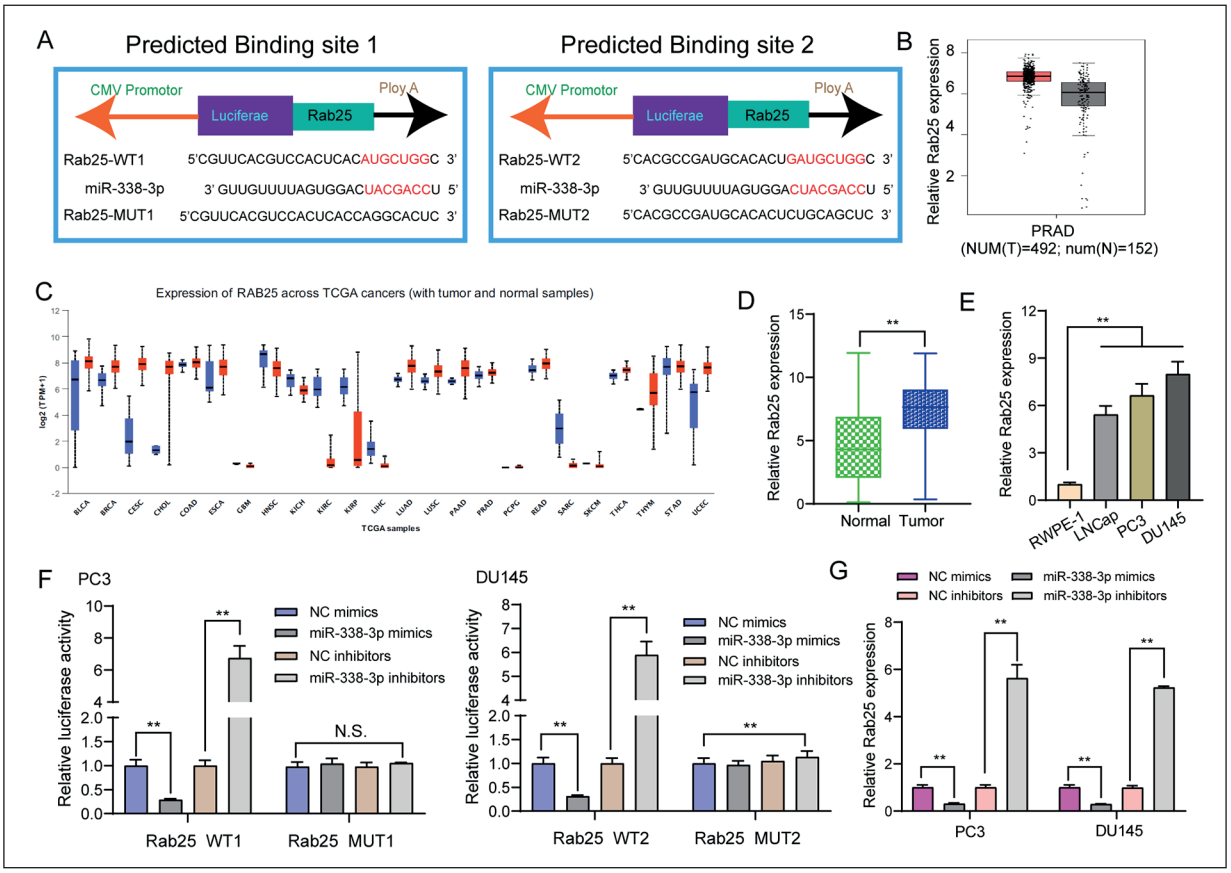

Figure 5. MiR-338-3p negatively regulates Rab25 expression in PCa cells. **A,** Schematic description of the Rab25 3'-UTR with two putative binding sites for miR-338-3p. **B,** The distinct upregulation of Rab25 expression in PCa analyzed by "GEPIA". **C,** The expression of Rab25 across TCGA cancers based on the results of "GEPIA". **D,** RT-PCR determined the levels of Rab25 in 124 PCa patients. **E,** The levels of Rab25 mRNA in three PCa cell lines and RWPE-1 cells. **F,** Luciferase reporter assays in PC3 and DU145 cells. Cells were transfected with 200 ng of wild-type 3'-UTR-reporter or mutant constructs together with miR-338-3p mimics, miR-338-3p inhibitors or negative control (NC). **G,** The expression of Rab25 in PC3 and DU145 cells after miR-338-3p was overexpressed or suppressed. $^{**}p < 0.01$, $^{*}p < 0.05$.

wild-type 3'-UTR of Rab25 while miR-338-3p inhibitor transfection increases the Luciferase activity. Nevertheless, miR-338-3p upregulation or downregulation did not distinctly influence the Luciferase activity in PC3 and DU145 cells of the mutant Rab25 3'-UTR. In addition, the results of RT-PCR also confirmed the suppressing effect of miR-338-3p overexpression and the promoting effect of miR-338-3p knockdown on Rab25 expression (Figure 5G).

LINC00173/MiR-338-3p/Rab25 Axis Affected the Progression of PCa

To explore whether LINC00173 served its biological function through LINC00173/miR-338-3p/Rab25 axis, rescue experiments were designed using miR-338-3p inhibitors. We observed that miR-338-3p knockdown weakened the inhibiting effects imposed by LINC00173 overexpression on the expression of Rab25 (Figure 6A). Moreover, by the of a series of functional

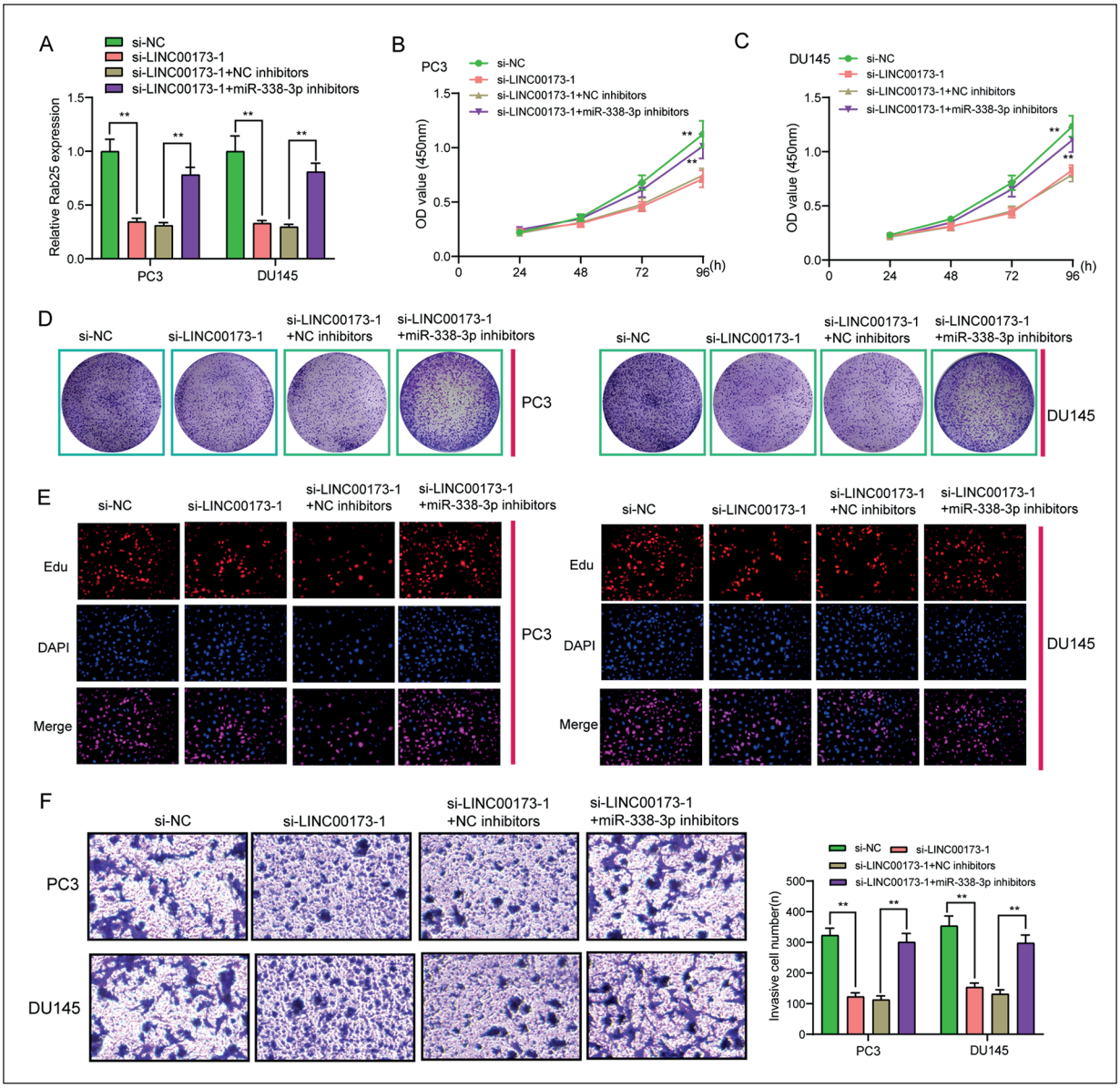

Figure 6. LINC00173 promotes the progression of PCa cells via miR-338-3p/Rab25 axis. **A,** The expression of Rab25 mRNA was qualified in PC3 and DU145 cells transfected with si-LINC00173 and/or miR-338-3p inhibitors according to RT-PCR. The cell viability (**B, C**), colon formation (magnification: 10×) (**D**), cell proliferation (Magnification: 100×) (**E**) and invasive ability (magnification: 40×) (**F**) of PC3 and DU145 cells were assessed. $^{**}p < 0.01$, $^{*}p < 0.05$.

assays, we observed that miR-338-3p inhibitors could rescue the proliferation and invasion-suppressing effects of LINC00173 knockdown in PC3 and DU145 cells (Figure 6B-6F). In summary, these data demonstrated that LINC00173 might serve as a ceRNA to assist miR-338-3p in regulating to Rab25 expression, which led to the positive metastasis and cell cycle out of control of PCa cells.

Discussion

PCa acts as a representative solid cancer for males in developed countries[20]. Irrespective of the prevalence, its molecular mechanisms regarding the occurrence and development are still not well known. Up to date, many PCa patients with advanced stages exhibited a poor clinical outcome, which required novel sensitive biomarkers for the early screening and prediction of long-term survival before clinical treatments[21,22]. Recently, growing studies have paid attention to the potential of lncRNAs used as novel cancer biomarkers due to their frequent dysregulation in various types of tumors and important function in the progression of tumors[23,24]. Our present study identified a novel PCa-associated lncRNA, LINC00173, which was indicated to be overexpressed in PCa tissues, especially in tumor specimens with metastasis and advanced stages. ROC assays revealed the possible diagnostic value of LINC00173 for PCa patients. Clinical assays indicated the relationship between high LINC00173 expression and Gleason score, preoperative PSA level as well as a shorter OS and DFS. The independently prognostic value of increased LINC00173 expression was also confirmed using multivariate analyses. Overall, our findings indicated LINC00173 as a novel biomarker for the diagnosis and prognosis of PCa patients.

It has been investigated a lot the expression pattern and potential functions of LINC00173 in several types of tumors. For instance, Zeng et al[14] reported that LINC00173 was highly expressed in lung cancer, and its overexpression promoted chemoresistance, proliferation and metastasis *via* sponging miRNA-218 to increase Etk expressions. In breast cancer, LINC00173 was shown to be overexpressed in breast cancer, and its silencing suppressed breast cancer cells regarding proliferation, colony formation, and migration via modulating miRNA-490-3p, whereas its forced expression displayed opposite effects[25]. In addition, the overexpressed levels of LINC00173 and its oncogenic roles were also reported in gastric cancer, cervical cancer and melanoma[16,26,27]. Interestingly, previous evidences studies indicated that LINC00173 served as a tumor promoter. Thus, we wondered whether LINC00173 had a similar function in PCa progression. Based on the loss-of-function assays, we confirmed that LINC00173 knockdown hindered the proliferation, migration and invasion of PC3 and DU145 cells, but facilitated the apoptosis, suggesting LINC00173 as an oncogenic lncRNA in this tumor. Thus, our findings, together with previous results, indicated that high LINC00173 expression might be positively involved in tumor progression.

Initially, researches showed that lncRNAs could participate in a ceRNA mechanisms as the miRNA sponge, thus competitively regulating miRNA targets[28]. As suggested by more and more studies, dysregulated lncRNAs are capable of absorbing miRNAs for modulating the expression exhibited by tumor promotors and anti-oncogenes in carcinogenesis and tumor developments[29,30]. As reported, a series of cytoplasmic lncRNAs serve as a kind of competing ceRNA via binding microRNAs in a competitive manner. The study found the expression of LINC00173 in the nucleus and cytoplasm, and the cytoplasm saw more obvious LINC00173 expression, which made a candidate miRNA sponge. StarBase v3.0 software assisted in predicting that miR-338-3p was more likely to bind to LINC00173. In addition, we observed a distinct increase of miR-338-3p expression in PCa specimens and cell lines, which could lead to poor OS and DFS of PCa patients. Previous studies have found miR-338-3p as a tumor promotor in many tumors types, including PCa, which was consistent with our findings[31-33]. Then, we performed the Luciferase assays and RNA-pull down assays, confirming that LINC00173 served as a sponge for miR-338-3p in PCa cells.

Rab25 constitutes the Rab11 subfamily with 23 kDa and shows an exclusive expression in the epithelial cells[34]. Rab25 has been frequently reported to be overexpressed in many tumors[35,36]. In PCa, Rab25 overexpression facilitated PCa cells regarding the proliferation and the metastasis[37]. There are three main mechanisms that govern the expression of Rab25, (1) epigenetic regulation, (2) miRNAs and (3) copy number change. In this study, we searched TargetScan database and miRDB database, finding that Rab25 may be

a potential targeting gene of miR-338-3p. In the proposed cohort, Rab25 expression was distinctly upregulated in PCa specimens, which was consistent with previous findings. Then, as indicated by Luciferase reporter assay together with RT-PCR assay, miR-338-3p may directly target Rab25 to suppress its expression. Thus, based on our results, miR-338-3p could target Rab25, thereby suppressing PCa progression. To more deeply explore the association among LINC00173, miR-338-3p and Rab25, we performed rescue experiments, finding that knockdown of miR-338-3p might increase Rab25 expression suppressed by the LINC00173 down-regulation in PC3 and DU145 cells. Furthermore, miR-338-3p knockdown could reverse the negative effect imposed by LINC00173 silencing on PCa cells in terms of the proliferation, the colony formation and the invasion. Overall, our findings indicated that LINC00173 may exert its biological effects via modulating miR-338-3p/Rab25 axis.

Conclusions

To sum up, the finding of the study identified a novel lncRNA LINC00173, which exhibited an upregulated expression in PCa and can lead to weak PCa prognosis. LINC00173 may promote the progression of PCa via promoting Rab25 expression by sponging miR-338-3p. Our data imply that LINC00173 would be a potential biomarker and therapeutic target for PCa.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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