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Long noncoding RNA LUCAT1 promotes migration and invasion of prostate cancer cells by inhibiting KISS1 expression

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Abstract. – OBJECTIVE: Recent researches have revealed the role of long noncoding RNAs (lncRNAs) in tumor development. In this study, the potential function of lncRNA LUCAT1 in the progression of prostate cancer was identified.

PATIENTS AND METHODS: Real Time-quantitative Polymerase Chain Reaction (RT-qPCR) was used to detect LUCAT1 expression in both prostate cancer cells and tissue samples. Moreover, the association between LUCAT1 expression level and overall survival of prostate cancer patients was analyzed. In addition, wound healing assay and transwell assay were conducted to evaluate the regulatory effect of LUCAT1 on prostate cancer cells. Furthermore, the underlying mechanism of LUCAT1 in regulating the development of prostate cancer was explored via qRT-PCR and Western blot.

RESULTS: LUCAT1 expression was much higher in prostate cancer samples than controls. Besides, LUCAT1 expression was correlated with the overall survival of prostate cancer patients. Moreover, LUCAT1 overexpression promoted *in vitro* migration and invasion of prostate cancer cells. In addition, the mRNA and protein expression of KISS1 was downregulated after LUCAT1 overexpression. Furthermore, it was found that the expression level of KISS1 was negatively related to the expression level of LUCAT1 in prostate cancer tissues.

CONCLUSIONS: LUCAT1 could enhance migration and invasion of prostate cancer cells by regulating KISS1, which might offer a potential therapeutic target for prostate cancer.

Key Words:
Long noncoding RNA, LUCAT1, Prostate cancer, KISS1.

Introduction

Prostate cancer is one of the most common malignancies in males. It is reported that in 2018, more than 29,000 men has died of prostate cancer in America (https://seer.cancer.gov/statfacts/html/prost.html). Most prostate cancer patients can be treated by surgery and androgen deprivation therapy at early stage although the five-year survival of prostate cancer is 99%, it is still the third-leading cause of cancer-related death in males globally[1-3]. Moreover, the recurrence and recurrence rates of prostate cancer have been significantly increased in developed and developing countries[4,5]. Therefore, it is urgent to find out the underlying mechanism and figure out a new treatment strategy.

As subtypes of non-coding RNAs, long non-coding RNAs (lncRNAs) are longer than 200 nucleotides in length which do not encode proteins. However, evidence has proved that lncRNAs serve crucial roles in the progression of malignant tumors. For example, lncRNA H19 enhanced tumor progression in endometrial carcinoma by negatively regulating miR-612[6]. LncRNA PlncRNA-1 acts as an oncogene in the progression of colorectal cancer cell by regulating the PI3K/Akt signaling pathway[7]. The overexpression of lncRNA CCAT2 was found to promote proliferation and metastasis in intrahepatic cholangiocarcinoma, and indicated a poor prognosis[8]. By activating the Wnt/β-catenin pathway, lncRNA EZR-AS1 functioned as an oncogene in breast cancer[9]. However, the functions and the underlying molecular mechanism of lncRNA LUCAT1 in prostate cancer remain unexplored.

In this work, we showed that the expression of LUCAT1 was remarkably higher in prostate cancer tissues. Moreover, LUCAT1 promoted the migration and invasion of the prostate cancer cell *in vitro*. In addition, our further experiments explored

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the underlying mechanism of how LUCAT1 functioned in the development of prostate cancer.

Patients and Methods

Cell Lines and Clinical Samples

Totally 56 prostate cancer patients were enrolled in this research. They received surgery at the Yantai Yuhuangding Hospital. Before the surgery, written informed consents were gathered. Patients did not have preoperative radiotherapy or chemotherapy. Tissues were collected from the surgery and stored immediately at -80°C. All tissues were independently analyzed by two experienced pathologists. This study was approved by the Research Ethics Committee of Yantai Yuhuangding Hospital.

Cell Culture

The Institute of Biochemistry and Cell Biology, Chinese Academy of Science (Shanghai, China) offered human prostate cancer cell lines (LNCaP, PC3, DU145, and 22Rv1) and normal human prostate epithelial cell lines (P69). Culture medium consisted of 10% fetal bovine serum (FBS; Gibco, Grand, NY, USA), penicillin as well as Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Grand I, NY, USA). Cells were cultured in an incubator containing 5% CO_2 and were set at 37°C.

Cell Transfection

After being synthesized, entivi targeting LUCAT1 was clo nto ti-EF1a-EGFP-F2A-Puro vec ia Inc., San Diego, CA, USA). 2 cells ed for packaging LUCAT1 le uses (LU nd the empty vector (cont hich were the used for transfection in pr cells. 48 h later, LUCAT1 expressio these s quantified using Real Time uantitative Po se Chain Reaction (RT-q).

RNA Extra on a Quantitative Real Tim se Ch Reaction (qRT-PCR)

The t RNA ed by using TRIzol reage gen, Ca , CA, USA). The extrac otal A was reverse-transcribed to compl ntary Nucleic Acids (cDNAs) se Tra cription Kit (TaKaRa Biotechn Ltd., Otsu, Shiga, Japan). Thermal cycle was s: 30 sec at 95°C, 5 sec at 95°C and 35 sec at 6 or a total of 40 cycles. Primer sequences used in this study were as f LUCAT1, F: 5'-CGTGAATGATAGTC R: 5'-TGAACGATTTGCCAC AGGA- S1, F: 5'-GACCACGCTCTT GTCTACTG R: 5'-GGCTACGGGCTTC G-3'; G H: F: 5'-CGCTCTCTGCTC TTC R: 5'-ATCCGTTGACT GACC

Western Blot nalysis

Reagent r mmu recipi ion assay (RIPA) (Beyoti ai, Ch was utilized to extract p in f chroninic acid (BCA) pr assay k a, Otsu, Shiga, Japan) w osen for quan ing protein concentrat rget proteins were separated by s n do phate-polyacrylamide gel elec phoresis (S E). Then, they were inc d with antibodi after transferring to the vinylidene difluoride (PVDF) membranes llipore, Bille a, MA, USA). Cell Signaling hnology (CS anvers, MA, USA) provided anti-GA H (glyceraldehyde 3-phosphate d nd rabbit anti-KISS1, as well as goat bit secondary antibody. The chemiluminescent film was applied for assessment of in expression with Image J software.

ound Healing Assay

Cells were inoculated into 6-well plates and cultured in DMEM overnight. After scratching ith a plastic tip, cells were cultured in serum-free MEM. Wound closure was viewed at different time points. Each assay was independently repeated in triplicate.

Matrigel Assay

5×10^4 cells in 200 µL serum-free DMEM were transformed to top chamber of an 8 µm pore size insert (Millipore, Billerica, MA, USA) pre-coated with 50 µg Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). The bottom chamber was supplied with DMEM and FBS. 48 h later, after wiped by cotton swab, cells in the top surface of chambers were immersed with precooling methanol for 10 min and stained in crystal violet for 30 min. Three fields per sample were used to count the invasive cells.

Statistical Analysis

Statistical analysis was conducted through Statistical Product and Service Solutions (SPSS 20.0, Chicago, IL, USA). Data were presented as mean ± SD. Chi-square test and Student's *t*-test were utilized for difference comparison. The Kaplan-Meier

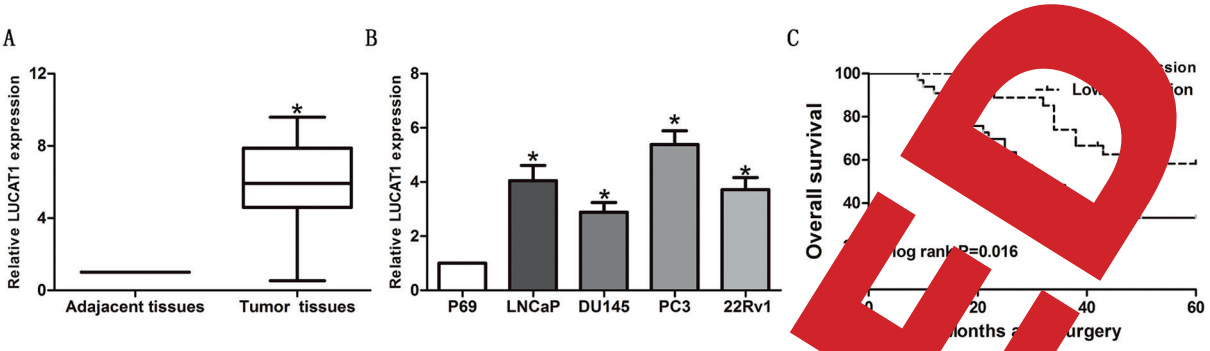


Figure 1. The expression levels of LUCAT1 increased in prostate cancer tissues and cell lines. LUCAT1 expression significantly increased in the prostate cancer tissues compared with adjacent tissues. The expression of LUCAT1 relative to β-actin were determined in the human prostate cancer cell lines and P69 (normal human prostate epithelial cell lines) by qRT-PCR. ***C,*** High level of LUCAT1 was associated with worse overall survival of prostate cancer patients. Data were presented as the mean ± standard error of the mean. *$p<0.05$.

method was performed for evaluating the prognosis. $p<0.05$ was considered statistically significant.

Results

LUCAT1 Expression in Prostate Cancer Tissues and Cells

QRT-PCR was performed to detect the expression of LUCAT1 in 56 tumor tissues and corresponding adjacent normal tissues. The result revealed that LUCAT1 was significantly upregulated in tumor tissue samples (Figure 1A). Identically, LUCAT1 expression in prostate cancer cells was significantly higher than that of HK-2 (human kidney epithelial cell) (Figure 1B).

LUCAT1 Expression was Related with Overall Survival of Prostate Cancer Patients

After the surgery, the Kaplan-Meier method was utilized to analyze the survival time of prostate cancer patients. The prostate cancer patients were divided into two groups, the high-LUCAT1 group and the low-LUCAT1 group, based on their expression level of LUCAT1. The result of Kaplan-Meier analysis showed that prostate cancer patients with low LUCAT1 level had a better overall survival compared to those with high level (Figure 1C).

Overexpression of LUCAT1 Promoted Cell Migration and Invasion of Prostate Cancer Cells

PC3 and DU145 prostate cancer cell lines were chosen in this study. First of all, the transfection efficacy of overexpression lentivirus targeting LUCAT1 was verified (Figure 2A). Moreover, the results of wound healing assay indicated that migrated ability of prostate cancer cells was significantly facilitated after LUCAT1 overexpression (Figure 2B). Furthermore, transwell assay also revealed that the number of

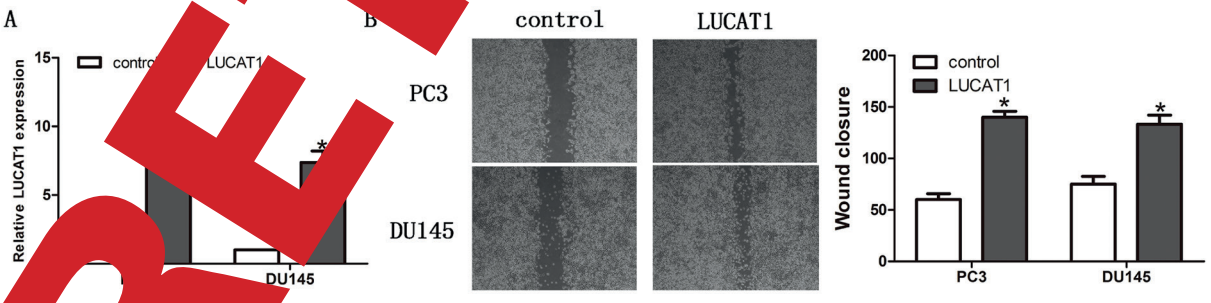


Figure 2. Overexpression of LUCAT1 promoted prostate cancer cell migration. ***A,*** LUCAT1 expression in prostate cancer cells transfected with LUCAT1 lentiviruses (LUCAT1) and the empty vector (control) were detected by qRT-PCR. β-actin was used as an internal control. ***B,*** Wound healing assay showed that the overexpression of LUCAT1 significantly increased cell migration in prostate cancer cells. The results represent the average of three independent experiments (mean ± standard error of the mean). *$p<0.05$.

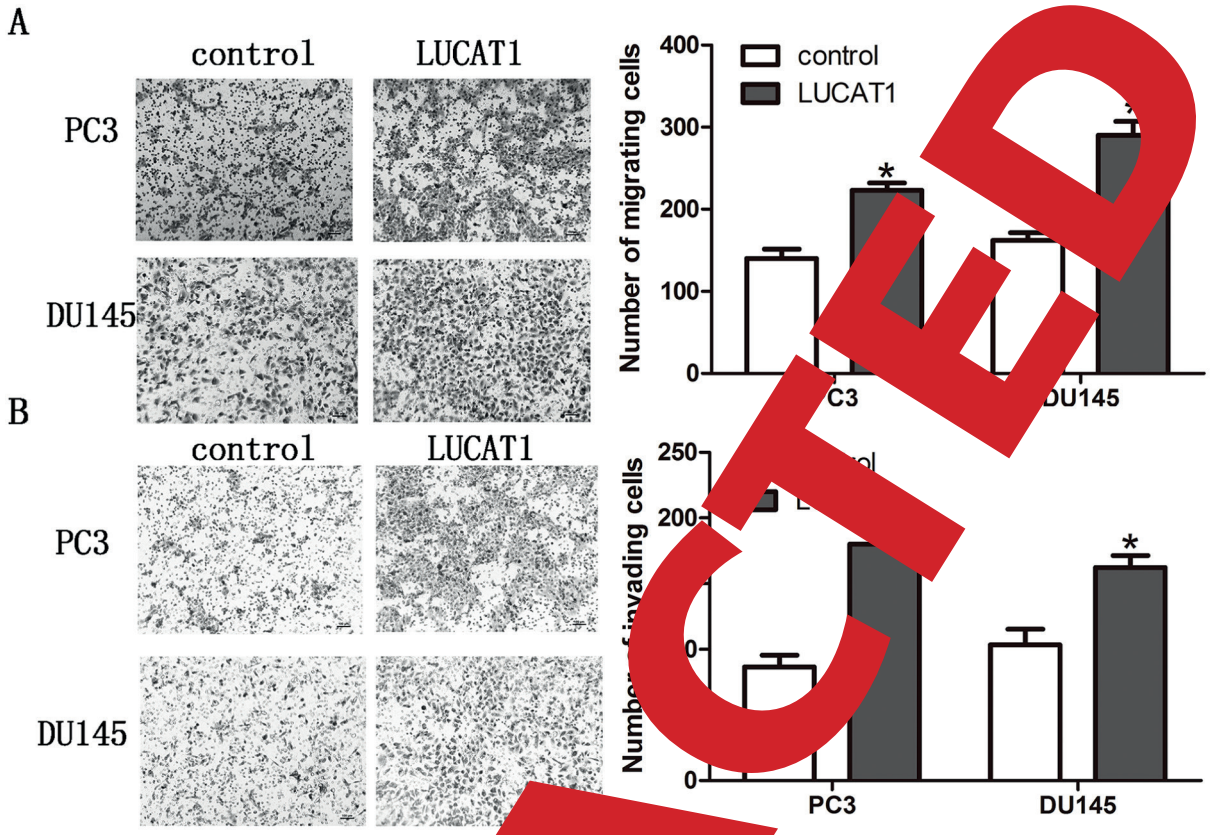


Figure 3. The overexpression of LUCAT1 promote[s] [pros]tate ca[ncer] [mi]gration and cell invasion. ***A,*** The transwell assay showed that number of migrating cells significantly inc[reased] af[ter] [ov]erexpression of LUCAT1 in prostate cancer cells. ***B,*** The transwell assay showed that number of invading cells was [sig]ly increased after the overexpression of LUCAT1 in prostate cancer cells. The results represent the average of three indep[endent] experiments (mean ± standard error of the mean). *$p<0.05$.

migrated cells and invaded c[ells] [sig]ably increased after LUCAT1 o[ver]expr[ession] in prostate cancer cells (Figur[e] and 3B).

The Interaction B[etween] KISS1 and LUCAT1 in Prost[ate] Can[cer]

The expressio[n] level of KI[SS1] in prostate cancer cells w[as] lower in LUCAT1 lentiviruses (LUCAT1) group [was] compared with the KISS1 leve[l] in em[pty] vecto[r] (control) group (Figure 4A). [Wes]tern blot fo[und] out that protein level of KISS[1] [was] dow[nreg]ulated after LUCAT1 ov[er]expressi[on] (Figure 4B). Furthermore, we fo[und] [the] compa[red] with that of adjacent tiss[ues] the KISS1 expression in prostate cancer tissues was significantly lower (Figure 4C). [Pear]son analysis demonstrated that the KISS[1] expression was negatively correlated with the LUCAT1 expression in prostate cancer tissues (Figure 4D).

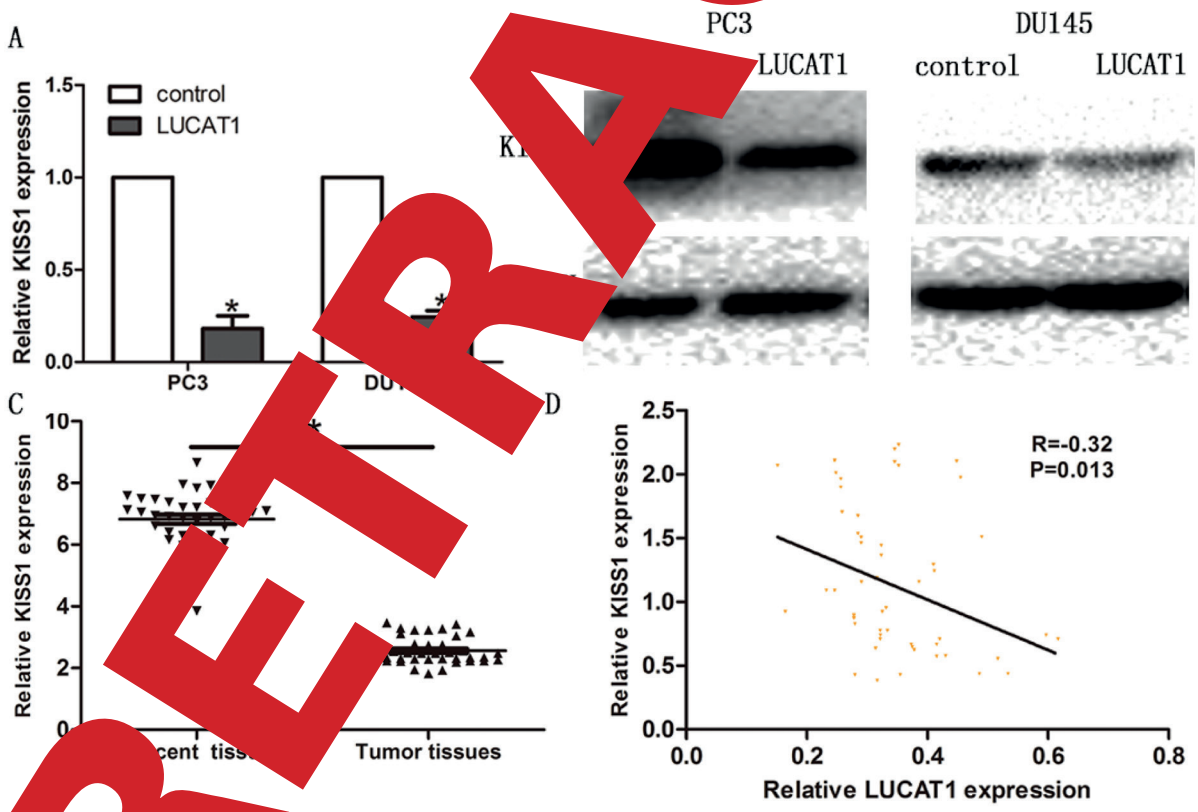
Discussion

Recently, accumulating evidence has indicated that lncRNAs play an important regulatory role in the development of cancers. They are capable of regulating proliferation, apoptosis, migration and invasion of cancer cells. For example, the downregulation of lncRNA LOXL1-AS1 inhibits cell proliferation and cell cycle progression in prostate cancer[10]. In addition, the downregulation of lncRNA PVT1 inhibits the development and migration of prostate cancer by regulating expression and phosphorylation of p38[11]. The repression of lncRNA NEAT1 promotes the development of prostate cancer by disturbing the cell cycle and inhibiting the proliferation of prostate cancer cells[12]. LncRNA PCSEAT functions as an oncogene in prostate cancer by mediating the EZH2 activity, which may offer a potential therapeutic target[13].

Located on chromosome 5, lncRNA lung cancer associated transcript 1 (LUCAT1) was firstly found in the airway epithelium of cigarette smokers[14]. Recent researches have revealed the important role of lncRNA LUCAT1 in tumor progression. For instance, the overexpression of lncRNA LUCAT1 facilitates the malignancy of ovarian cancer by regulating the miR-612/HOXA13 pathway[15]. By regulating the stability of DNMT1 and inhibiting the tumor suppressor expressions, lncRNA LUCAT1 promotes the formation and metastasis of esophageal squamous cell carcinoma[16]. In addition, lncRNA LUCAT1 overexpression is remarkably related to malignant stage and poor prognosis of clear cell renal cell carcinoma (ccRCC), which also promotes the proliferation and invasion of ccRCC *via* the AKT/GSK-3β signaling pathway[17].

In this work, we found that LUCAT1 was upregulated both in prostate cancer samples and cells. Besides, the prognosis of prostate cancer was closely correlated to the [illegible]vel of LUCAT1. Furthermore, LU[illegible]T1 over[illegible]sion promoted prostate cancer[illegible] migration [illegible]in-vasion. The above resul[illegible]aled that L[illegible]T1 promoted tumorigenesis [illegible]te can[illegible]and might act as an onco[illegible]ne.

The KISS1 ge[illegible]ncodes KI[illegible]hich is processed quickl[illegible]seru[illegible]nto activ[illegible]peptides called kisspepti[illegible]KPs). [illegible]S1 wa[illegible]first reported in melanoma [illegible]s supp[illegible]ion[18]. Latest studies have repo[illegible]KIS[illegible]exhibited anti-metastat[illegible]nd anti-t[illegible]ies in a variety of cance[illegible]r example, K[illegible]functions as a tumor su[illegible]nd restricts breast cancer brain meta[illegible]s, [illegible]lso sensitizes oncolytic virotherapy[19]. KISS[illegible]own promoted cell proli[illegible]on but inhibite[illegible]ll apoptosis of ccRCC[20]. [illegible]expression level of KISS1 is downregulated [illegible]ing the prog[illegible]ssion of gastric cancer. More [illegible]portantly, the[illegible]w expression of KISS1 indi-[illegible]more ag[illegible]ive histological types or more



F[illegible]. 4. J[illegible]en LUCAT1 and KISS1. ***A,*** QRT-PCR results showed that KISS1 expression was lower in LUC-A[illegible]s (LUCAT1) compared with the empty vector (control). ***B,*** Western blot revealed that KISS1 protein expression decreas[illegible]AT1 lentiviruses (LUCAT1) compared with the empty vector (control). ***C,*** KISS1 was significantly downregulated i[illegible]ancer tissues compared with adjacent tissues. ***D,*** The linear correlation between the expression level of KISS1 and LUC[illegible]n prostate cancer tissues. The results represent the average of three independent experiments. Data were presented as the mean ± standard error of the mean. *$p<0.05$.

advanced tumors[21]. The KISS1 expression is reduced during the malignant transformation of the colonic mucosa, and the upregulation of KISS1 expression is associated with worse prognosis in colorectal cancer[22].

In the present study, the KISS1 expression could be downregulated after overexpression of LUCAT1. Moreover, the KISS1 expression in prostate cancer tissues was negatively related to LUCAT1 expression. All the above results suggested that LUCAT1 might promote tumorigenesis of prostate cancer by targeting KISS1.

Conclusions

We identified that LUCAT1 was remarkably upregulated and negatively related to overall survival of prostate cancer patients. Besides, LUCAT1 could enhance prostate cancer cell migration and invasion by targeting KISS1. These findings suggested that LUCAT1 may contribute to therapy for prostate cancer as a candidate target.

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Conflict of Interest

The Authors declare that they have [illegible]flict

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