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Long non-coding RNA CCAT1 promotes the migration and invasion of prostate cancer PC-3 cells

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Abstract. – OBJECTIVE: To investigate the effect of long non-coding RNA CCAT1 on the proliferation, migration, and invasion of prostate cancer PC-3 cells.

PATIENTS AND METHODS: The expression of CCAT1 was detected by Real-time PCR. The effect of CCAT1 down-regulation on the proliferation of PC-3 cells was observed by MTT assay. The regulatory of CCAT1 low-expression on the migration ability of PC-3 cells was investigated by transwell assay. The influence of decreased CCAT1 on the invasion ability of PC-3 cells was detected by Matrigel invasion assay.

RESULTS: Increased CCAT1 was significantly related to lymph node metastasis in prostate cancer. Low-expression of CCAT1 could suppress cell proliferation. Knockdown of CCAT1 inhibited the migration of PC-3 cells. Down-regulation of CCAT1 attenuated the invasion of PC-3 cells.

CONCLUSIONS: CCAT1 promoted the growth and the metastasis of prostate cancer. Our findings might provide a potential target for the diagnosis and treatment of prostate cancer.

Key Words
CCAT1, Prostate cancer, Proliferation, Migration, Invasion.

Introduction

Prostate cancer is one of the most common malignant tumors in men worldwide. It is currently believed that prostate cancer is related to age, androgen, high fat intake and genetic factors, but its pathogenesis is still unclear[1]. Long non-coding RNA (long non-coding RNA, lncRNA) is more than 200 nucleotides in length non-coding RNA, accounting for about 98% of the total RNA. The majority of lncRNA is located in the nucleus or cytoplasm of eukaryotic cells[2]. Many scholars[3-5] found that there is a significant difference in the expression of lncRNA between human tumor tissues and normal tissues. LncRNA is considered to play an oncogene or a tumor suppressor role in various tumors.

The current researches[6-10] have shown that a variety of lncRNA has a close relationship with the development, chemical-resistance, and metastasis of prostate cancer. For example, increased CCAT1 enhanced gallbladder cancer progression through regulating miRNA-218[11]. Additionally, CCAT1 can be activated by c-Myc so as to enhance the development of gastric carcinoma[12]. CCAT1 could enhance chemoresistance in docetaxel-resistant lung adenocarcinoma cells[13]. In epithelial ovarian cancer, CCAT1 could be involved in tumor metastasis, and function as a marker for predicting a poor prognosis[14]. In non-small-cell lung cancer, CCAT1/miR-130a-3p axis could promote cisplatin resistance *via* negative regulation of SOX4[15].

At present, no relevant studies on the role of CCAT1 in prostate cancer have been reported. The purpose of this work was to investigate the expression of CCAT1 in prostate cancer tissues and the effect of CCAT1 on the proliferation, migration, and invasion of prostate cancer PC-3 cells.

Patients and Methods

Materials

Prostate cancer tissues and adjacent tissues were obtained from the patients in our hospital. This study was approved by the Ethics Committee of The First Hospital of Jiaxing. The signed written informed consents were obtained from all participants before the study. Human prostate cancer cells PC-3 were purchased from Shanghai Cell Bank (Shanghai, China); CCAT1 siRNA (interfering RNA) and negative siRNA were synthesized by Shanghai Jima Pharmaceutical Technology Co., Ltd. (Shanghai, China); reverse transcription kit (Thermo Scientific, Waltham, MA, USA); Lipofectamine™2000, TRIzol (Invitrogen, Carlsbad,

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CA, USA); Diluent (Roche, Basel, Switzerland); MTT Kit (Biopharma, Rockville, MD, USA); DMEM/F-12 medium, opti-MEM and fetal bovine serum Matrigel (BD Biosciences, Franklin Lakes, NJ, USA); 6/24/96 well cell culture plates were purchased from Corning Corporation (Corning, NY, USA).

Cell Culture and Transfection

Prostate cancer PC-3 cells were cultured using Dulbecco's modified Eagle medium (DMEM)/F12 containing 1% double antibodies and 10% fetal bovine serum (FBS) in an incubator with 5% CO_2 at 37°C. At 1 d, PC-3 cells were inoculated into a 6-well plate using the medium without double antibodies. When about 50% cells were fused on the next day, 400 μL transfection medium opti-MEM containing lipofectamine™2000 and small-interfering ribonucleic acid (siRNA) was added into each well, and horizontally mixed evenly, followed by culture in the incubator with 5% CO_2 at 37°C for 4 h. Then, the original medium was discarded, and 2 mL complete medium was added into each well.

qRT-PCR

Messenger RNA (mRNA) was extracted from tumor tissues and PC-3 cells using TRIzol and reversely transcribed into complementary deoxyribonucleic acid (cDNA). Reaction conditions are as follows: 42°C for 30 min, 85°C for 5 min. The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) kit was used for detection. QRT-PCR conditions are as follows: 94°C for 5 min, 94°C for 15 s, 60°C for 1 min, a total of 40 cycles. The temperature of dissolution curve was set at 60-95°C, and 3 repeated wells were set for each sample. Specificity was detected *via* 2% agarose gel electrophoresis.

MTT Assay

After 48 h, cells were digested with trypsin, the complete medium was added, the cell suspension concentration was adjusted and the suspension was paved onto a 96-well plate. 4 rows with 5 repeated wells per row were set for experimental group and control group, respectively. The cell density in each well was adjusted to 2000/well, followed by incubation with 5% CO_2 at 37°C. Methyl thiazolyl tetrazolium (MTT) reagent was added into one row every day in both groups from the next day for 4 consecutive days. After 20 μL MTT reagent was added into each well, cells were incubated for another 4 h. The culture solution in each well was sucked dry, 150 μL dimethylsulfoxide (DMSO) was added into each well, and the 96-well plate was shaken at a low speed on a shaking table for 10 min, followed by detection. DMSO was added into the cell-free wells as zeroing wells, and the optical density (OD) value of each well was measured at 490 nm using an enzyme-linked immunosorbent detector.

Migration Assay

Cells in experimental group and control group after transfection for 48 h were digested with trypsin, and resuspended using serum-free medium, and the cell density was adjusted to 1×10^6 mL. 100 μL cells were added to the upper chamber, while 600 μL complete medium was added to a 24-well plate in the lower chamber. Cells were incubated with 5% CO_2 and 95% saturated humidity at 37°C for 8 h, and the transwell chamber was washed with PBS. The 24-well plate was placed into the chamber, and added with 600 μL 95% methanol into each well for fixation at room temperature for 15 min. After the fixing solution was removed, 600 μL 0.2% crystal violet dye was added to each well of the 24-well plate for staining at room temperature for 20 min. After gentle washing with PBS, the number of cells passing through the filtering membrane was counted under a microscope.

Matrigel Invasion Assay

After Matrigel was thawed at 4°C, it was mixed evenly with serum-free medium on ice and diluted (1:8). The transwell chamber was placed into the 24-well plate, and 200 μL diluted Matrigel was added into the upper chamber, followed by incubation with 5% CO_2 and 95% saturated humidity at 37°C for 1 h to form a Matrigel coating on the surface of chamber. Cells were digested with 0.25% trypsin, centrifuged and added with serum-free medium to be prepared into single-cell suspension. The cell concentration was adjusted into 1 × 106 mL. 100 μL cell suspension was added to the upper transwell chamber, and 600 μL complete medium was added to the 24-well plate in the chamber. Cells in the 24-well plate was incubated with 5% CO_2 and 95% saturated humidity at 37°C for 24 h, fixed with 95% methanol for 20 min, washed with PBS and stained with 0.2% crystal violet for 20 min. After gentle washing with PBS, the number of cells passing through the filtering membrane was counted under the microscope.

Statistical Analysis

SPSS 19.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. All quantitative data were expressed as mean ± standard deviation. The comparison between groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ was regarded as significant difference.

Results

CCAT1 Was Increased in Prostate Cancer Tissues

By using qRT-PCR method, we detected the expression of CCAT1 in prostate cancer tissues and adjacent tissues. The analysis showed that CCAT1 was increased in prostate cancer specimen as compared to the adjacent areas (Figure 1). Furthermore, we found that high expression of CCAT1 was not related to age, PSA and Gleason score, but was significantly involved in lymph node metastasis in the patients with prostate cancer (Table I).

Table I. Association of CCAT1 expression with clinic factors.

Factor	No.	Expression of CCAT1		*p*-value
		High	Low	
NO.	31	16	15	
Age				0.376
<65	14	6	8	
≥65	17	10	7	
PSA (ng/mL)				0.347
<10	18	8	10	
≥10	13	8	5	
Gleason score				0.833
≤6	13	7	6	
≥7	18	9	9	
Lymph node metastasis				0.029
Positive	10	8	2	
Negative	21	8	13	

CCAT1 Expression Was Down-Regulation by Transfecting With siRNA

Firstly, the CCAT1 siRNA and negative siRNA were transfected into the experimental and control groups, respectively. After 48 h, the total RNA was extracted and reverse transcribed into cDNA. The expression of CCAT1 was detected by qPCR in both groups. The results showed that the expression of CCAT1 in the experimental group significantly decreased as compared to the control group (Figure 2). These results suggested that CCAT1 expression was reduced by transfecting with siRNA.

Down-Regulation of CCAT1 Inhibited the Proliferation of PC-3 Cells

Secondly, to investigate the effect of CCAT1 on cell proliferation, the proliferation activity of each group was detected by MTT assay. Compared with the control group, the OD value of the

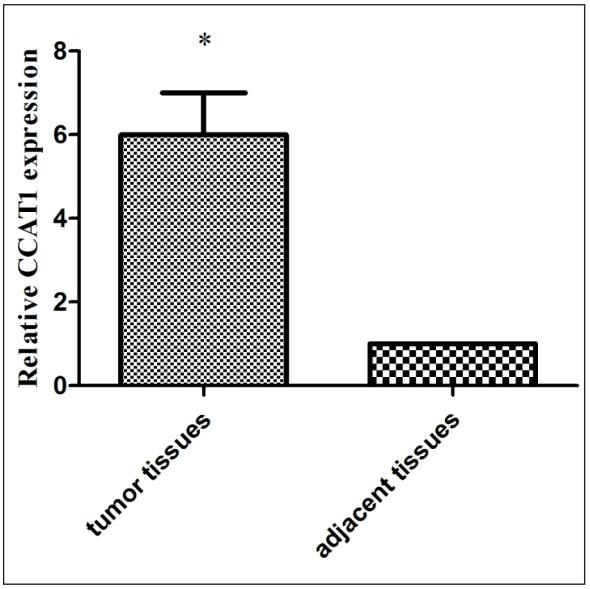

Figure 1. The relative expression of CCAT1 was detected in the prostate cancer tissues and the adjacent tissues via qRT-PCR method. *$p<0.05$.

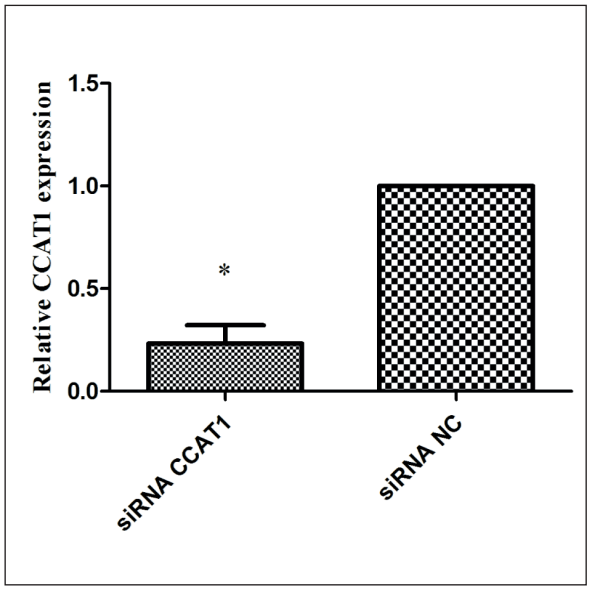

Figure 2. The transfection efficiency was identified by qRT-PCR assay. *$p<0.05$.

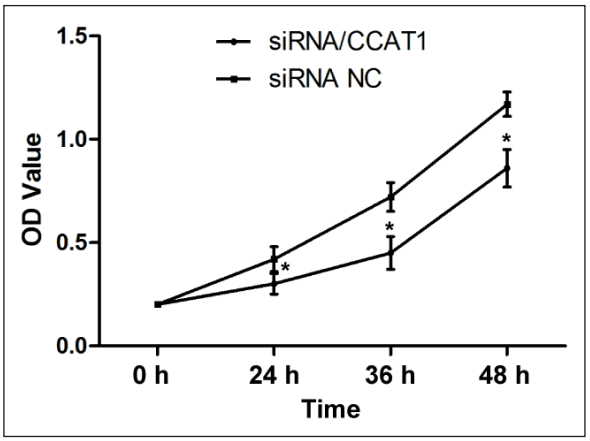

Figure 3. The OD values were examined by MTT assay at 24 h, 36 h, 48 h, 72 h after transfection of siRNA CCAT1 or siRNA NC, respectively. *p<0.05.

experimental group was significantly decreased. The results indicated that the cell proliferation activity was significantly decreased through down-regulation of CCAT1 (Figure 3).

Knockdown CCAT1 Suppressed Prostate Cancer Cell Migration Ability

In addition, to further detect the effect of CCAT1 on cell migration, we conducted a migration assay. The results of migration assay showed that, compared with the control group, the number of PC-3 cells in the experimental group significantly decreased (Figure 4A). These results suggested that knockdown CCAT1 could suppress the migration ability of prostate cancer cell.

Down-Regulation of CCAT1 Also Could Inhibit the Invasive Ability of Prostate Cancer PC-3 Cell

Moreover, the matrixgel invasion assay results showed that the number of PC-3 cells transfected into the lower chamber in the experimental group was significantly reduced (Figure 4B). The results indicated that the down-regulation of CCAT1 also could inhibit the invasive ability of prostate cancer PC-3 cell.

Discussion

Prostate cancer is the second most common tumor in the urinary system, which greatly threaten the health of elderly men[16]. LncRNA, more than 200 nt in length, is a long non-coding RNA that does not has a protein-coding capacity. It is stably present in body fluids such as blood and urine[17,18]. In recent years, lncRNAs have been shown to regulate gene expression at cellular and molecular levels. Previous studies showed that lncRNAs were involved in cell proliferation, apoptosis, cell differentiation, and self-renewal of embryonic stem cells at the cellular level. At the molecular level, lncRNA was related to chromatin remodeling and X chromosome inactivation[19]. At the post-transcriptional level, the target gene can be regulated (inhibited or promoted) by a variety of methods such as alternative splicing, capping, and tailing[20]. In recent years, the researchers showed that lncRNA have a close relationship with a variety of malignant tumors. For example, Prensner et al[21] confirmed that lncRNA SChLAP1 was up-regulated in prostate cancer tissues, and could promote the invasion and metastasis of prostate cancer cells by combating the SWI/SNF chromatin modifier complex. Additionally, Zhu et al[7] clarified that lncRNA H19-miR-675 axis could suppress prostate cancer metastasis by targeting gene TGFBI. Furthermore, Hung et al[22] reported that lncRNA PCGEMl, as a co-activation agent of c-Myc and androgen receptor, could promote the occurrence and development of prostate cancer. Meanwhile, it has been confirmed that lncRNA MEG3 could inhibit cell proliferation, promote apoptosis and eventually inhibit the occurrence and development of prostate cancer[23].

We confirmed that CCAT1 plays an oncogene role in prostate cancer. Decreased expression of CCAT1 could inhibit the proliferation of prostate cancer cells, suggesting that CCAT1 participates in the formation of prostate cancer by promoting proliferation. Through the interference of CCAT1 expression, cell migration and invasion ability weakened, indicating that CCAT1 promotes the metastasis of prostate cancer. Our study further revealed the occurrence, development, and metastasis mechanism of prostate cancer with great significance.

Conclusions

We showed that CCAT1 promotes the metastasis of prostate cancer. Further exploration about the molecular mechanism of CCAT1 in the target genes and signaling pathways will provide new molecular markers and treatment targets for the diagnosis and treatment of prostate cancer.

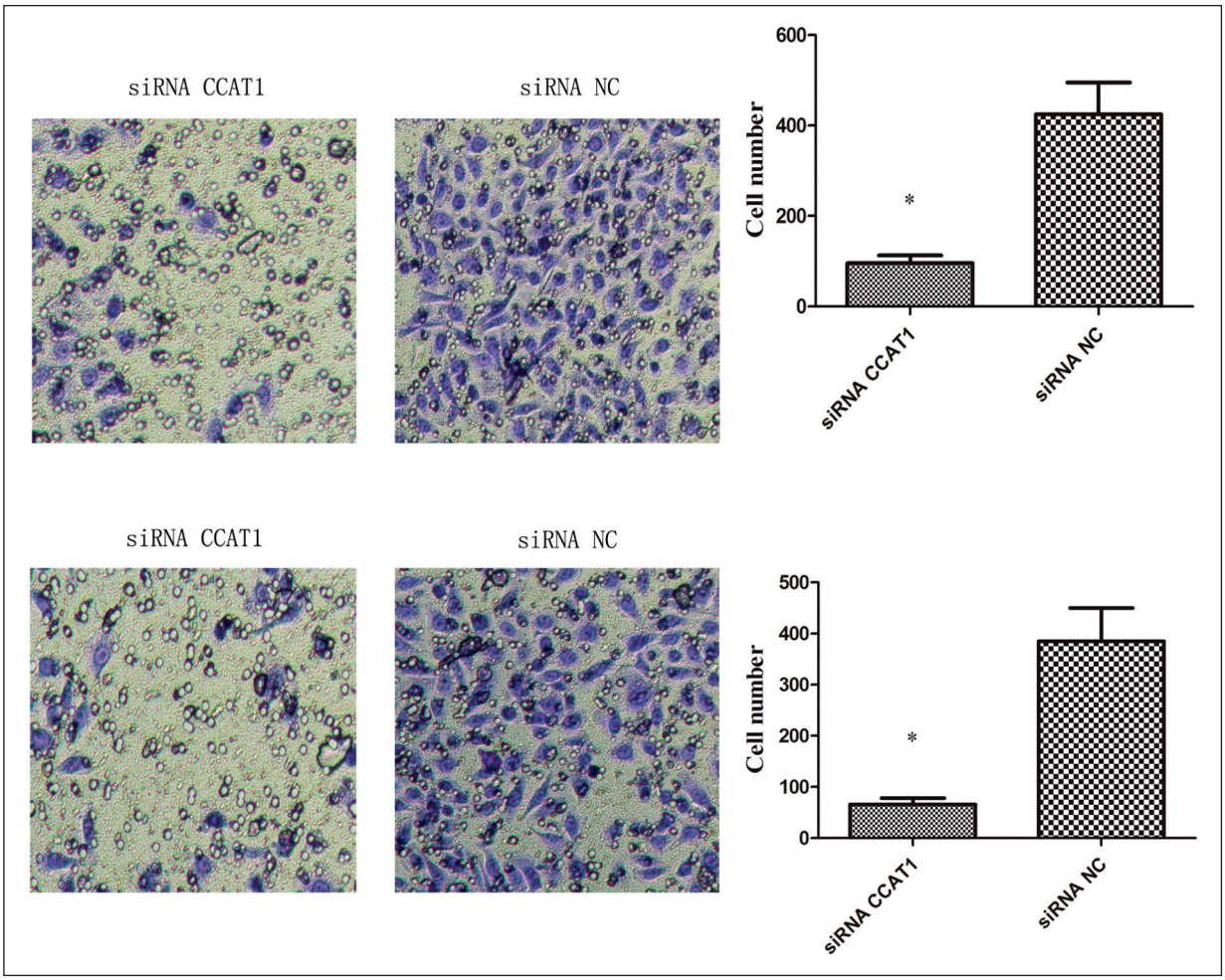

Figure 4. A, Cell migration ability was investigated by migration assay. **B**, Cell invasive ability was evaluated by Matrigel invasion assay. *$p<0.05$.

Conflict of Interest

The authors declared no conflict of interest.

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