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LncRNA LUCAT1 promotes growth, migration, and invasion of oral squamous cell carcinoma by upregulating PCNA

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Abstract. – OBJECTIVE: Recent studies have revealed the important role of long noncoding RNAs (lncRNAs) in the progression of tumorigenesis. This study aims to identify lncRNA LUCAT1 functions in the progression of OSCC (oral squamous cell carcinoma).

PATIENTS AND METHODS: Relative expression of lncRNA LUCAT1 in OSCC cell lines and 50 paired tissue samples was detected by quantitative Real-time polymerase chain reaction (qRT-PCR). Moreover, biological function of LUCAT1 in OSCC was identified by performing transwell assay, wound healing assay and proliferation assay in vitro. The underlying mechanism was explored by qRT-PCR and western blot.

RESULTS: LUCAT1 expression was remarkably upregulated in OSCC tissues when compared with that in adjacent normal samples. Moreover, proliferation, invasion and migration of OSCC cells were inhibited after knockdown of LUCAT1 in vitro. Knockdown of LUCAT1 downregulated PCNA in OSCC cells at mRNA and protein level in vitro. Besides, PCNA expression in OSCC was positively correlated with the expression of LUCAT1.

CONCLUSIONS: Knockdown of LUCAT1 could inhibit migration, invasion and proliferation capacities of OSCC cells through downregulating PCNA, which may offer a new therapeutic intervention for OSCC patients.

Key words:
Long noncoding RNA, LUCAT1, Oral squamous cell carcinoma, PCNA.

Introduction

Oral cancer is the sixth most prevalent cancer in the world, and it ranks the third most common in developing countries[1]. Oral squamous cell carcinoma (OSCC) is the main subtype of oral cancer, contributing to 3% of the newly diagnosed clinical cancer cases[2]. Although the rapid development of diagnosis and treatment methods for OSCC has been achieved[2], the overall 5-year survival rate of OSCC patients still remains lower than 50%. Therefore, it is essential and urgent to find out novel molecular markers to improve the treatment efficacy of OSCC. Long non-coding RNA (lncRNA) is a cluster of non-coding RNA transcripts with over 200 nucleotides (nt) in length and it lacks the ability of encoding proteins. Evidence shows that lncRNAs act as vital regulators in many biological behaviors, including carcinogenesis, apoptosis, proliferation and metastasis in cancer. For example, lncRNA OR3A4 is upregulated in breast cancer, which may

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be a novel prognostic marker and therapeutic target[3]. Activated by H3K27, lncRNA CCAT1 promotes cell proliferation and migration in esophageal squamous cell carcinoma through regulating SPRY4 and HOXB13[4]. LncRNA MALAT1 stimulates the cell migration and invasion in hepatocellular carcinoma by sponging miR-204 to upregulate SIRT1[5]. By suppressing p53 signal pathway, lncRNA ROR facilitates cell proliferation, invasion and chemoresistance in nasopharyngeal carcinoma[6]. Moreover, knockdown of lncRNA CRNDE-h is reported to be associated with poor prognosis of colorectal cancer, which might be used as a novel serum-based biomarker for diagnosis of colorectal cancer[7]. However, the potential influence of lncRNA LUCAT1 on the development of OSCC remains unexplored. In the present study, it is revealed that the expression level of lncRNA LUCAT1 was remarkably upregulated in OSCC samples. Moreover, functional experiments revealed that knockdown of LUCAT1 depressed cell proliferation, invasion and migration capacities of OSCC cells. Furthermore, we discovered that lncRNA LUCAT1 mediated the progression of OSCC by promoting PCNA.

Patients and Methods

Tissue Specimens

Paired OSCC tissues and adjacent non-tu tissues (≥ 5 cm away from the site) we sequentially resected from OS patients during July 2015 and Dec f 2017 he Second Affiliated Hospital musi Heilongjiang Provinc Th Com of Affiliated Stoma gical H l of Jiamusi University in H jiang Pro approved this study pro ll participa provided written inform consc

OSCC s and Cell Tra tion

H OSC l lines Tca8113, TSCCA, CAL-27, 9 an mal cervical epithelium cell line NHO ovided hinese Academy of Sci- (Sha Chi Cells were cultured in Ros- ark Institute-1640 (RPMI-1640; Hy e, Sout gan, UT, USA) containing 10% fet serum (FBS; Invitrogen Life Technolo- urg, MD, USA), and 1% penicillin in % CO_2 incubator at 37°C. Lentivirus expressing t-hairpin RNA (shRNA) directed against LU- were provided by GenePharma (Shanghai, China). cDNA encoding LUCAT1 was amplified and inserted into pcDNA3.1 (Invitrogen, Carlsbad, CA, USA). Next, we transfected the synthesized pcDNA3.1 into OSCC cells with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Transfection efficacy was verified by qRT-PCR.

RNA Extraction and qRT-PCR

Total RNA from tissues and c were ex tracted using TRIzol reagent (In en, Carlsbad, CA, USA). The total RNA eversely transcribed to cDNA throu everse cription Kit (TaKaRa Biotec ogy Co., L lian, China). Thermo ng co ditions as follows: 95°C for follo by 40 cycles at 95°C for 5 s, an 6 s. Prim used for qRT-PCR we s foll UCA forward 5′-CCTATCC TCTCTA d reverse 5-ACTTCT AACGTG , glyceraldehyde 3 osph ehydrogenase (GAPDH), forward 5′-CCA CAGATGGGGCAAT-GC and reverse ATGGCATGGACT-G TCATTCA-3′. The ermal cycle was as ws: 95°C fo 0 s, followed by 40 cycles at for 5 s and C for 35 s. $2^{-\Delta\Delta Ct}$ method was u d for calc ng relative expression.

Weste

Total proteins were collected from cells *via* mmunoprecipitation assay (RIPA) buffer e, Shanghai, China) and then quantified by bicinchoninic acid (BCA) protein quantification kit (Pierce, Rockford, IL, USA). The target proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Membranes were incubated with primary antibodies after loading on the polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Then rabbit anti-GAPDH and rabbit anti-PCNA were used for incubation. Pierce electrochemiluminescence (ECL) was utilized for visualizing Western blotting substrate immunoreactive bands (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Cell Proliferation Assay

Cell proliferation of treated cells in 96-well plates was monitored every 24 h by cell counting kit-8 (CCK-8) assay following the instructions (Dojindo Molecular Technologies, Inc., Kumamoto, Japan). Spectrophotometer (Thermo Scientific, Waltham, MA, USA) was utilized to measure the absorbance at 450 nm.

Wound Healing Assay

Cells seeded into 6-well plates were cultured in RPMI-1640 medium overnight. After scratch-

ing with a plastic tip, cells were cultured in serum-free RPMI-1640. Wound closure was detected at the appointed time points. Each assay was independently repeated for three times.

Transwell Assay

Twenty-four hours after transfection, 2×10^5 cells in 100 µL of serum-free RPMI-1640 were applied in the top side of an 8-µm Transwell chamber (Corning, Corning, NY, USA) pre-coated with 50 µg Matrigel (BD Biosciences, Franklin Lakes, NJ, USA). RPMI-1640 containing 20% fetal bovine serum (FBS) was added to the bottom chamber. After 24 h, cells were fixed in methanol for 30 min and stained by hematoxylin for 20 min. An inverted microscope (×20) was utilized for counting migrated and invaded cells in three random fields.

Statistical Analysis

Statistical analysis was conducted by Statistical Product and Service Solutions (SPSS) 18.0 (SPSS Inc., Chicago, IL, USA). Chi-square test and Student *t*-test were performed to analyze categorical data or measurement data, respectively. Kaplan-Meier curve was introduced for s al analysis. Data were presented by mea (Standard Deviation). It was considered st cally significant when $p<0.05$.

Results

Expression Level of L T1 in and Cells of OSCC

First, qRT-PCR conduc for detecting LUCAT1 expres 50-paired C tissues. LUCAT1 was significantly upregulated in tumor tissues than that in adjacent tissues (Figure 1A). When compared with the expression in normal human oral keratinocyte NHOK, LUCAT1 le as significantly higher in OSCC cells (Fig

Knockdown of LUCAT1 Rep d Cell Proliferation In Vitro

According to LUCAT1 e ncer cells, we chose SCC-9 ce or establi LUCAT1 knockdown m . The CAT1 virus (LUCAT1) and mpty or were synthetized, and their ain icacy i C-9 cells was deter d by CR (e 2A). Furthermore, -8 assay s d proliferation of OS was inhibit r LUCAT1 knockdo igu

Kn wn of LUC Repressed Cell M ation and Invasio In Vitro

ound healin ssay revealed that knockdown UCAT1 inhi d OSCC cell migration (Fig- u A). Furthe re, transwell assay showed tha ion SCC cells was inhibited after LUCA down (Figure 3B).

T1 Promoted OSCC Tumorigenesis a A

QRT-PCR results further demonstrated that the mRNA expression of PCNA was downregulated in OSCC cells transfected with LUCAT1 shRNA (Figure 4A). Western blot analysis obtained the same trend at the protein level (Figure 4B). To explore the interaction between LUCAT1 and PCNA, the expression level of PCNA was detected in OSCC tissues. PCNA expression was mark-

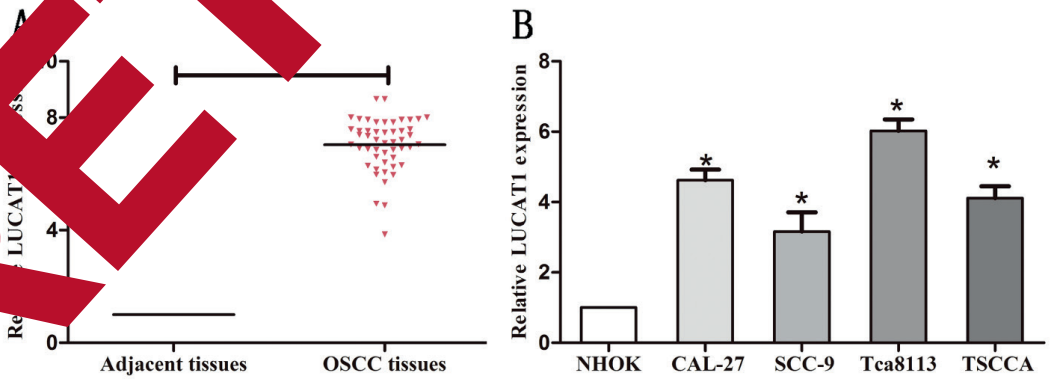

Fig e 1. Expression levels of LUCAT1 were increased in OSCC tissues and cell lines. ***A,*** LUCAT1 expression was significantly increased in the OSCC tissues compared with adjacent tissues. ***B,*** Expression levels of LUCAT1 relative to GAPDH were determined in the human OSCC cell lines and normal human oral keratinocyte (NHOK) by qRT-PCR. Data are presented as the mean ± standard error of the mean. *$p<0.05$.

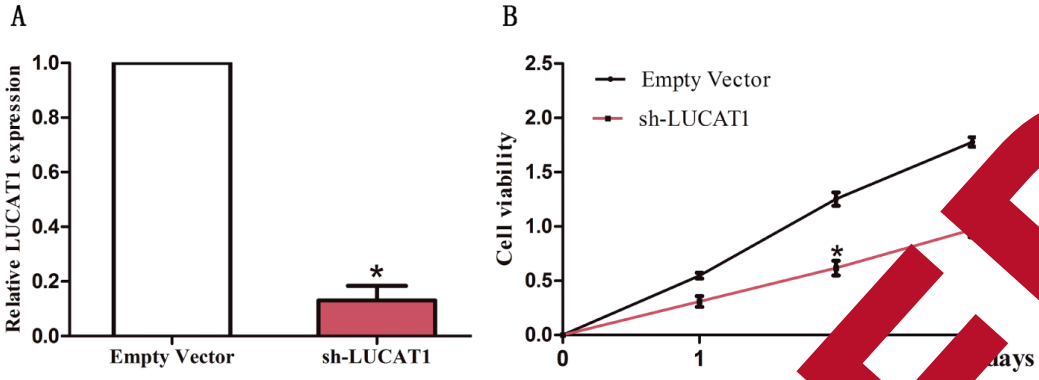

Figure 2. Knockdown of LUCAT1 inhibited OSCC cell proliferation. ***A,*** LUCAT1 ex on in cells tra ed with empty vector or LUCAT1 lentivirus (sh-LUCAT1) was detected by qRT-PCR. GA was used inter ontrol. ***B,*** CCK8 assay showed that knockdown of LUCAT1 significantly repressed cell pro n in SCC-9 The results represent the average of three independent experiments (mean ± standard error). *p<0.05.

edly upregulated in OSCC tissues when compared with that in adjacent tissues (Figure 4C). The results of linear correlation analysis showed that in OSCC tissues, the expression of PCNA was positively correlated to LUCAT1 expre n (Figure 4D).

Discussion

Evidence demonstrated As participate in diverse biolo behav in the progression of OSCC, ding metastasis and invasi e, lnc A HOTAIR facilitate l inva nd metastasis in OSCC by i ctly recru EZH2 and depressing E- By modula SF1 and suppressing expressio el of miR-184, lncA UCA1 ac erates cell proliferation and latin-resista in oral squamous cell carc a[9]. LncR MALAT1, which may be an imp tic biomarker and therapeutic target C, promotes the progression of OSCC by inducing epithelial-mesenchymal tran- [10]. LncRNA FTH1P3 enhances the devel- of OSCC by modulating the expression of fizzled 5[11]. In addition, lncRNAAC132217.4 promotes cell metastasis in OSCC by regulating IGF2 expression, which is further modulated by KLF8[12]. Lung cancer associated transcript 1 (LUCAT1) was firstly found in the airway epithelium of cigarette smokers, which is a cluster of transcripts located on chromosome 5[13]. Recently, lncRNA LUCAT1 is widely studied for its important role in the progression of tumorigenesis. For instance, through inhibiting

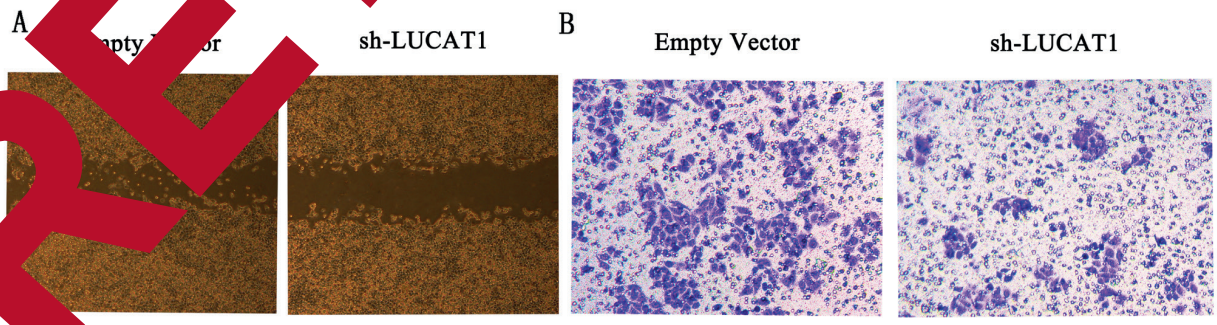

Fig 3. Knockdown of LUCAT1 repressed OSCC cell migration and invasion. ***A,*** Wound healing assay showed that the migrated length of cells in LUCAT1 lentivirus group was significantly decreased compared with empty control group in SCC-9 OSCC cells. ***B,*** Transwell assay showed that knockdown of LUCAT1 significantly repressed cell invasion in SCC-9 OSCC cells. The results represent the average of three independent experiments (mean ± standard error of the mean). *p<0.05.

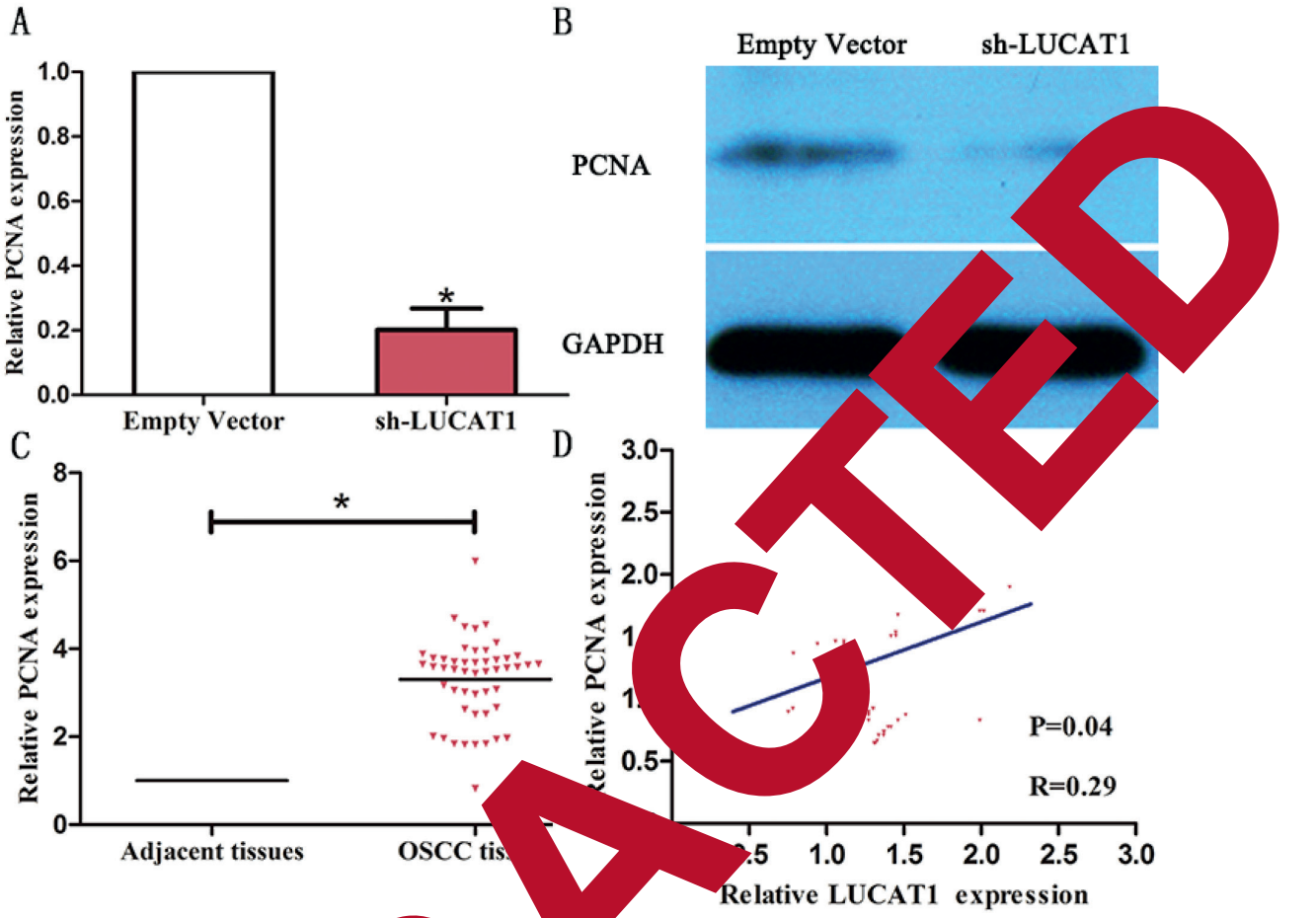

Figure 4. Interaction between PCNA ...CAT1 in C... ***A,*** The RNA expression level of PCNA in LUCAT1 cells was significantly decreased compared wi... ...trol cells ...C-9 cells. ***B,*** Protein expression of PCNA was decreased after knockdown of LUCAT1 in SCC... ...s. ***C,*** ...A was sig... ...ntly upregulated in OSCC tissues compared with adjacent tissues. ***D,*** The linear correlati... ...ween the ...ession level... of PCNA and LUCAT1 in OSCC tissues. The results represent the average of three independe... ...rime... ...nted as the mean ± standard error of the mean. *p<0.05.

the expressions ...1 and p57, ...NA LUCAT1 knockdo... ...es cell pro... ...ation in human non-s... ...ll lung ...cer[14]. Through regulating th... ...bility of D... ...1 and depressing the ex... ...ions of tumor su... ...essors, lncRNA LUC... prom... the tumorigenesis of esophag... ...uam... cell carcinoma formation and tumo... ...asis[15]. ...r knockdown of lncRNA L... ...T1, c... ...iability and invasion are ...sed ... through regulating miR-375 ...pressio... LncRNA LUCAT1 enhances m... ...y of ovarian cancer and promotes ...pro... ...on through regulating miR-612/ ...OXA13 pathway[17]. Moreover, the expression ...ncRNA LUCAT1 is negatively correlated to ... prognosis of clear cell renal cell carcinoma and positively related to malignancy of the tumor[18]. In the present study, LUCAT1 was found to be upregulated in both OSCC tissues and cells. Furthermore, LUCAT1 knockdown inhibited the abilities of cell growth, migration and invasion. These data indicated that LUCAT1 functioned as an oncogene and promoted the tumorigenesis of OSCC. Proliferating cell nuclear antigen (PCNA) is found in cancer cells and proliferating cells. Numerous researches have confirmed that PCNA is directly correlated to the prognosis, tumor stage and tumor differentiation. For instance, the positive expression rate of PCNA was 73% in breast cancer, indicating the important clinical significance in evaluating the prognosis of breast cancer[19]. The expression level of PCNA is significantly higher in colorectal cancer, especially in those with liver metastasis. This may help to evaluate liver metastasis in patients with colorectal cancer[20]. Overexpression of PCNA relieves the repression of miR-363-3p on cell proliferation

and colony formation in lung adenocarcinoma[21]. In addition, upregulation of PCNA is closely related to poor prognosis of patients with osteosarcoma, which may be a potential biomarker[22]. In our study, PCNA was downregulated after LUCAT1 knockdown *in vitro*. PCNA was remarkably upregulated in OSCC samples when compared with that in adjacent tissues. A positive correlation was discovered between PCNA and LUCAT1 expression in OSCC tissues. The above results revealed that LUCAT1 mediated OSCC progression *via* PCNA.

Conclusions

Our research suggests a new biomarker in the development of OSCC. LncRNA LUCAT1 promotes OSCC carcinogenesis *via* regulating PCNA and it may serve as a promising marker for OSCC in the future.

Conflict of Interests

The Authors declare that they have no conflict of

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