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LINC01510 suppresses cell proliferation and invasion by inhibiting Wnt/β-catenin signaling in renal cell carcinoma

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ABSTRACT

The role of long non-coding RNA in Renal cell carcinoma (RCC) tumorigenesis and progression remains largely unknown. Here, we found that LINC01510 functions as a tumor suppressor in RCC tumorigenesis. We screened TCGA database and then found that LINC01510 is significantly down-regulated in malignant RCC tissues, and the lower expression of LINC01510 predicts poor prognosis. Moreover, the down-regulated LINC01510 was further confirmed in our fresh tissues and cell lines. Biological functions assays shown that Ectopic expression of LINC01510 not only inhibits RCC cell proliferation both *in vitro* and *in vivo*, but also impairs cell invasion ability. Moreover, we found overexpression of LINC01510 inhibits the expression of CCND1 and CCNE1, as well as MMPs (MMP2, MMP7 and MMP9), and thus affecting RCC cell cycle and invasion. Meanwhile, Western blot assays revealed that the expression of β-catenin is regulated by LINC01510; overexpression of β-catenin could partly rescue the cell viability and invasion ability caused by ectopic expression of LINC01510. Taken together, we found that LINC01510 regulates cell proliferation and invasion by modulating Wnt/β-catenin signaling in RCC.

1. Introduction

Renal cell carcinoma (RCC) is the most common cancers worldwide, and accounts for 2%–3% of human malignant neoplasm [1]. It is the seventh most common cancer in men and the ninth most common in women [2]. Patients with advanced RCC typically respond poorly to chemotherapy and radiotherapy, which leads to a dramatically decreased 5-year survival rate [3]. Therefore, a better understanding of the potential mechanisms about RCC tumorigenesis and more effective therapeutic approaches are urgent.

As we know, more than 90% of the transcripts from the human genome could not code for proteins. Long non-coding RNAs (LncRNAs) are defined as non-coding transcript with over 200 nucleotides in length [4]. LncRNAs are associated with several important biological progress: X chromatin imprinting, cell differentiation, nuclear and cytoplasmic trafficking, cell cycle control, cancer cells metastasis and drug resistance [5]. More evidences have suggested that the disorders of LncRNAs are closely related to human diseases, including various kinds of cancers. Recently, more and more reports revealed that long non-coding RNA exerts its roles in RCC progression as tumor suppressive or oncogenic factor, such as EGOT [6], DLX6-AS1 [7], CCAT2 [8] and MRCCAT1 [9] etc. Knockdown of lncRNA FTX inhibits proliferation, migration, and invasion in RCC cells [10], and downregulation of LincRNA TUG1 promotes apoptosis of RCC [11].

LINC01510 is located on chromosome 7p31. In current study, we screened TCGA database and found that LINC01510 is dramatically down-regulated in RCC tissues, these findings reminded us that LINC01510 might function as a tumor suppressor in RCC. We explained the clinical significance and function of LINC01510 in RCC by performing functional experiments. With further in-depth investigation, LINC01510 may be of value of a therapeutic target for RCC.

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2. Materials and methods

2.1. Ethic statement

21 fresh tumor tissues and matched adjacent tissues were collected from patients with pathologically and clinically confirmed RCC cancer. All human tissues were obtained with informed consent from patients. Tumor and adjacent tissues were snap frozen in liquid nitrogen immediately after extraction and stored at −80 °C. All experimental protocols were approved by the Shanghai Ruijin Hospital.

2.2. Cell culture

The RCC cell lines (786-O, Caki-2) were purchased from American Type Culture Collection (Manassas, VA, USA). HK-2 and Caki-1 were obtained from the shanghai Institutes. Cells were seeded into P10 dishes containing ATCC recommended medium and in a humidified atmosphere of 5% CO_2 and at 37 °C.

2.3. RNA isolation and quantitative real-time PCR

Total RNA was purified from breast cancer and adjacent tissues or cells using Trizol Reagent (Life Technologies, Caribad, CA, USA) and RNA (1 μg) was reverse transcribed using PrimerScript RT-PCR kit (Takara, China) according to the manufacturer's protocol. Quantitative real time PCR was performed using SYBR green Supermix (Qiagen, Hilden, Germany) in ABI 7500 Fast system. Melting curve analysis was used to monitor the specificity of the PCR product. Primers using in this study were described in Table 1.

2.4. Western blots

Total protein extracted from patient tissues and cell lines with cell lysis buffer (Beyotime, Shanghai, China) followed the protocol and 30 μg were separated by 10% SDS-PAGE, and transferred onto nitrocellulose membrane (Millipore, Billerica, MA, USA) then the membrane was blocked in TBS buffer containing 5% BSA (Sangon China) for 1 h. The following antibodies were used in this study: β-catenin, β-actin were purchased from Cell signaling technology.

2.5. Construct stable cell lines

To generate over-expressed LINC01510, vectors containing full length of LINC01510 were purchased from GeneCopoeia. Cell line transfected using lipofectamine 2000 (Invitrogen) with those vectors following the manufactures protocols. The supernatant media containing virus was collected by centrifugation to remove cellular contaminant. The resulting viruses were used to infect indicated cells, and then integrated cells were selected by 2 μg/ml puromycin for 2 weeks.

Table 1
Sequence of primers.

Name	Sequence (5′-3′)
LINC01510 primer	F: GCAATGTGATCCTCTAAGCC
	R: GCTGTAACATCAACTCTTCCC
CCND1 primer	F: TCCTCTCCAAAATGCCAGAG
	R: GGCGGATTGGAAATGAACTT
CCNE1 primer	F:GCCAGCCTTGGGACAATAATG
	R: CTTGCACGTTGAGTTTGGGT
MMP2 primer	F:CCCACTGCGGTTTTCTCGAAT
	R: CAAAGGGGTATCCATCGCCAT
MMP7 primer	F:GAGTGAGCTACAGTGGGAACA
	R: CTATGACGCGGGAGTTTAACAT
MMP9 primer	F:AGACCTGGGCAGATTCCAAAC
	R: CGGCAAGTCTTCCGAGTAGT
18sRNA	F: GTAACCCGTTGAACCCCATT
18sRNA	R: CCATCCAATCGGTAGTAGCG

2.6. CCK-8 cell viability assays

Cells were seeded into a 96-well plate at 2 × 103 cells per well with 100 μl cultured medium which containing indicated concentration and cultured at 37 °C, 5% CO2. The cell viability was quantified by addition 10 μl of cell counting kit (CCK-8, Dojindo). After 1.5 h incubation, the plates were monitored by Power Wave XS microplate reader (BIO-TEK) at an absorbance 450 nm.

2.7. Flow cytometry cell cycle assays

The indicated cells (20X 104/well) were seeded in the 6-well plates. Then these cells were collected at 12 h, 24 h and 36 h. Cells were washed twice with 1X PBS, then re-suspended and fixed in 2 ml 70% ethanol at −20 °C. Cells were then stained with PI (BD, USA) and followed as the manufacture's recommended protocol.

2.8. Colony formation assays

Cells were trypsinized and responded in 1.5 ml of 0.35% agarose and poured onto a 1.5 ml of 0.7% agarose bed in 6-well plates. The indicated cells (1000/well) were incubated almost 3 weeks at 37 °C, 5% CO2. Colonies were fixed and stained with 0.5% crystal violet and the number of colonies was counted.

2.9. Transwell cell migration assay

To assess whether LINC01510 involved in the metastatic potential of RCC cells *in vitro*, we performed transwell cell migration assay using transwell chamber (BD Biosciences, USA). In brief, cells were seeded onto the upper chamber at a density of 4×10^4 cells per 200 μl per chamber and maintained in serum-free medium, and lower chambers were filled with 700 μl complete medium. Cells were incubated for 14 h at 37 °C in a 5% CO_2 incubator. Non-invaded cells retaining on the upper surface were removed by scrubbing with a cotton swab. The invaded cells were fixed and then stained with 0.1% crystal violet.

2.10. Subcutaneous tumor formation assay

100×10^4 stable overexpressed LINC01510 786-O cells and control were subcutaneously injected into right flank of 6 BALB/c (nu/nu) mice in each group. Tumor sizes were measured once a week and mice were sacrificed for the analysis of tumor burden after 4 weeks.

2.11. Dual luciferase reporter assays

Indicated cells were seeded in 96-well plates and transfected with TCF/β-catenin reporter plasmid (Promega, USA) and 10 ng Renilla following the recommended protocol for Lipofectamine 2000 transfection system. After 48 h incubation, firefly and Renilla luciferase activities were measured using dual luciferase reporter system (Promega) (Promega, Madison, WI) from the cell lysate. Luciferase activity was measured via GLOMAX 20/20 Luminometer (Promega).

2.12. Statistics analysis

Data are expressed as the mean ± standard deviation. Student's

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