

Upregulation of LINC00982 inhibits cell proliferation and promotes cell apoptosis by regulating the activity of PI3K/AKT signaling pathway in renal cancer

C. ZHANG, X.-Y. LI, Z.-Z. LUO, T.-W. WU, H. HU

Department of Medical Laboratory, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Abstract. – **OBJECTIVE:** Previous studies have confirmed that lncRNAs are involved in the progression of multiple tumors. However, the role of lncRNAs in renal cancer remains unclear. Our study focused on investigating the expression and function of LINC00982 in renal cancer progression.

PATIENTS AND METHODS: Quantitative Real time-polymerase chain reaction (qRT-PCR) assay was used to detect the expression of LINC00982 in renal cancer tissues and cell lines. LINC00982 expression was upregulated by transfecting with lentivirus. Western blot assay was used to detect the alteration expression levels of the relative protein involved PI3K/AKT signaling pathway.

RESULTS: Downregulated LINC00982 expression was significantly observed in cancer samples when compared with the adjacent specimens, and was related to tumor size and TNM stage. Upregulation of LINC00982 inhibited proliferation and promoted apoptosis of ACHN and A-498 cell lines. LINC00982 could regulate the activity of PI3K/AKT signaling pathway.

CONCLUSIONS: Upregulation of LINC00982 inhibits cell proliferation and promotes cell apoptosis by regulating the activity of PI3K/AKT signaling pathway in renal cancer.

Key Words

LINC00982, Renal cancer, Apoptosis, PI3K/AKT, Proliferation.

Introduction

Globally, renal cancer is one of the most common cancers with high mortality. According to the National Central Cancer Registry of China (NC-CR), the incidence of renal cancer is the second among malignant tumors of the urinary system, which threatens human health¹. The pathological

types of renal cancer are resistant to chemotherapeutics and radiation therapy. Besides, lots of techniques for diagnosing or treating this disease are limited. Thus, new markers for diagnosis and target therapy need to be investigated, and exploring the potential molecular mechanism is urgent. Recent studies demonstrated that long non-coding RNAs (lncRNAs) as novel molecules, influence cancer phenotypes by regulating other cellular macromolecules containing RNA, DNA, and protein. LncRNAs are expressed abnormally in renal cancer and related to tumor development. Ye et al² reported that *in vitro* and *in vivo* lncRNA THOR was overexpressed in renal cancer and could promote cell growth and proliferation. LncRNAs are not only able to regulate renal cancer progression, but also exert as biomarkers for prognosis of the patients. LncRNA HHLA2 was increased in renal cancer tissues, resulting in shorter overall survival and poorer prognosis³. Downregulation of lncRNA TUG1 can repress renal cell proliferation, invasion and induce cell apoptosis⁴. Knockdown of lncRNA H19 can inhibit carcinogenesis of renal cancer⁵. LncRNA PCAT-1 expression can inhibit the proliferation, metastasis and invasion abilities of cervical cancer cells⁶. In lung cancer, decreased LINC00982 expression was related to altered gene expression, damaged pathways and poor survival⁷. In cervical cancer, lncRNA OPA-interacting protein 5 antisense transcript 1 could target SMAD3 to contribute to cancer metastasis *via* sponging miR-143-3p⁸. In hepatocellular cancer, lncRNA-RP11 could inhibit epithelial-mesenchymal transition⁹. In gastric cancer, LINC00982 controlled cell proliferation and was involved in the clinical relevance of the patients¹⁰. Chen et al¹¹ provided new insights into lncRNAs in the development of renal cancer. Xu et al¹² found that LINC00982 was reduced in renal cancer tissues

through a genome-wide comprehensively analyses of lncRNA profiling. However, up to date, the underlying roles of LINC00982 in renal cancer development are not reported. In this study, we found that LINC00982 was low-expressed in renal cancer tissues and cell lines. Aberrant expression of LINC00982 was significantly related to tumor size and poor prognosis in renal cancer. *In vitro*, upregulated LINC00982 inhibited cell proliferation and induce cell apoptosis. Our results suggested that LINC00982 contributed to the progression of renal cancer, and might act as a biomarker and therapeutic target in renal cancer.

Patients and Methods

Patients

96 cases of renal cancer tissues and adjacent tissues were enrolled in The Central Hospital of Wuhan, and were checked with pathological examination. The total samples were stored in a liquid nitrogen tank. This study was approved by the Ethics Committee of the Central Hospital of Wuhan. Signed written informed consents were obtained from all patients before the study.

Cell Culture and RNA Transfection

A normal kidney cell line (HK-2) and four renal cancer cell lines (786-O, 769-P, ACHN and A-498) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). 786-O and 769-P were cultured in radioimmunoprecipitation assay Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco BRL, Gaithersburg, MD, USA) with 10% fetal bovine serum (FBS; HyClone Technologies, South Logan, UT, USA). ACHN and A-498 were cultured in Dulbecco's modified Eagle's medium (Gibco Rockville, MD, USA) with 10% fetal bovine serum (FBS). Cells were cultured at 37°C with 5% CO₂. The lentivirus upregulating LINC00982 expression was purchased from GenePharma (Shanghai, China). According to the manufacturer's instructions, cell transfection was performed using Lipofectamine RNAiMAX Reagent (Life Technologies, Carlsbad, CA, USA).

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated from renal tissues and cell lines using the TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) and quantitatively analyzed *via* qRT-PCR assay. All the RNA

was reverse transcribed using the SuperScript First-Strand complementary Deoxyribose Nucleic Acid (cDNA) System kit (Thermo Fisher Scientific, Waltham, MA, USA). DNA was assessed in the 7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control.

Cell Proliferation

A-498 and ACHN cells were seeded in plates (96-well, 100 cells/well). Cell counting kit-8 (CCK-8) assay (Dojindo Molecular Technologies, Kumamoto, Japan) was conducted to assess cell proliferation. The absorbance was detected at 450 nm using a spectrophotometer (xMark; Bio-Rad Laboratories, CA, USA).

TUNEL Staining

An *in situ* cell death detection kit was used for staining cell lines. Cells smears were fixed with 4% paraformaldehyde. Next, cells were permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate. Cells were incubated with TUNEL reaction. Then, cells were stained with 4',6-diamidino-2-phenylindole (DAPI) and visualized. The number of TUNEL-positive nuclei was expressed as a percentage of total nuclei.

Western Blot

Total proteins of cell lines were extracted and quantified. The proteins were subjected to 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking with 5% fat-free milk, the membranes were probed with anti-PI3K, anti-p-AKT, anti-T-AKT, anti-Bcl2 or anti-Bax antibodies (Abcam, Cambridge, MA, USA). The membranes were incubated with secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2 h. The protein levels were analyzed *via* enhanced chemiluminescence kit (Pierce, Rockford, IL, USA) with an imaging system (Bio-Rad, Hercules, CA, USA).

Statistical Analysis

The statistical analyses were executed using Statistical Product and Service Solutions (SPSS) 11. Software (SPSS Inc., Chicago, IL, USA). The *p*-values were analyzed using ANOVA, followed by post-hoc test LSD (Least Significant Difference). *p*-value of < 0.05 was regarded as a statistically significant result.

Table I. LINC00982 level and clinical factors in renal cancer.

Factor		No.	LINC00982 low level	LINC00982 high level	p-value
<i>Age</i>	< 60	59	27	32	0.294
	≥ 60	37	21	16	
<i>Gender</i>	Male	64	31	33	0.665
	Female	32	17	15	
<i>Lymph node metastasis</i>	Yes	13	6	7	0.765
	No	83	42	41	
<i>Tumor size</i>	T1-T2	67	28	39	0.014*
	T3-T4	29	20	9	
<i>TNM stage</i>	I-II	64	27	37	0.030*
	III-IV	32	21	11	

* $p < 0.05$.

Results

Expression of LINC00982 was Reduced in Renal Cancer Tissues

To investigate the role of LINC00982 in renal cancer development, qRT-PCR assay was used to detect the expression of LINC00982 in 96 pairs of renal cancer and the adjacent specimens. LINC00982 expression was significantly downregulated in cancer samples compared with the adjacent specimens (Figure 1A). Then, the analysis of clinical characters suggested that aberrant expression of LINC00982 was related to tumor size and TNM stage (Figure 1B and 1C). However, no significance was observed among age, gender and lymph node metastasis (Table I).

LINC00982 Expression was Upregulated by Transfecting with Lentivirus in ACHN and A-498 Cell Lines

Subsequently, to investigate the potential function of LINC00982 in renal cancer development,

we conducted cell experiments *in vitro*. Firstly, the expression levels of LINC00982 were detected by qRT-PCR analysis in a normal kidney cell line (HK-2) and four renal cancer cell lines (786-O, 769-P, ACHN and A-498). The assay exerted a lower expression of LINC00982 in four renal cancer cell lines (786-O, 769-P, ACHN and A-498) than that in HK-2 cell (Figure 2A), and A-498 and ACHN expressed the lowest LINC00982 among them. Thus, A-498 and ACHN were chosen in this study. LINC00982 expression was then up-regulated by transfecting with lentivirus in ACHN and A-498 cell lines. The transfection effect was detected by qRT-PCR assay (Figure 2B and 2C).

Upregulation of LINC00982 Inhibited Proliferation and Promoted Apoptosis of ACHN and A-498 Cell Lines

In order to explore the molecular function of LINC00982 in renal cancer development, this current study sought to evaluate cell proliferation and apoptosis. Thus, CCK-8 and

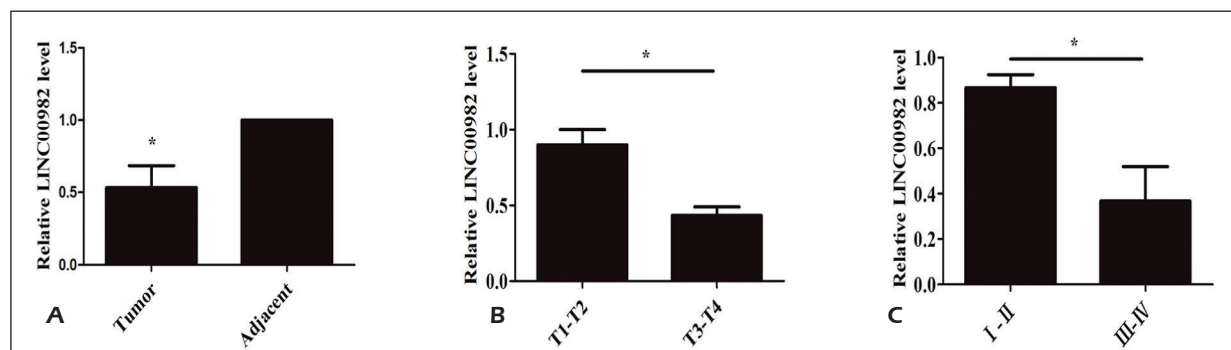


Figure 1. Downregulated LINC00982 expression was significantly observed in cancer samples. **A**, qRT-PCR assay was used to detect the expression of LINC00982 in 96 pairs of renal cancer and the adjacent specimens. **B**, qRT-PCR assay was used to detect the expression of LINC00982 between T1-T2 and T3-T4. **C**, qRT-PCR assay was used to detect the expression of LINC00982 between I-II and III-IV. * $p < 0.05$.

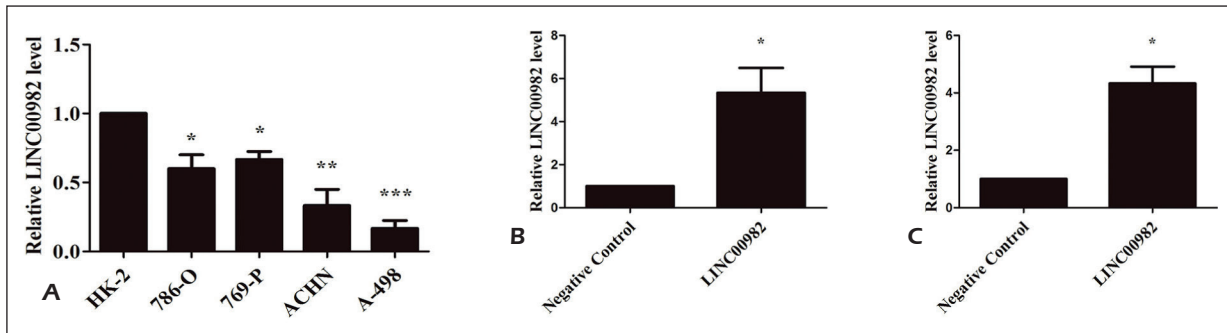


Figure 2. LINC00982 expression was upregulated by transfecting with lentivirus in ACHN and A-498 cell lines. **A**, qRT-PCR assay was used to detect the expression of LINC00982 in a normal kidney cell line (HK-2) and four renal cancer cell lines (786-O, 769-P, ACHN and A-498). **B**, (ACHN) and **C**, (A-498): qRT-PCR assay was used to detect the expression of LINC00982 in cells transfecting with lentivirus. * $p<0.05$. ** $p<0.01$. *** $p<0.001$.

Tunnel experiments were conducted, respectively. The analysis of CCK-8 assay showed that LINC00982 expression upregulation significantly suppressed the proliferative ability of ACHN and A-498 cell lines as compared with the control groups (Figure 3A and 3B). In-

versely, the result of Tunnel assay revealed that upregulated LINC00982 expression significantly promoted cell apoptosis (Figure 3C and 3D). Collectively, upregulation of LINC00982 inhibited proliferation and promoted apoptosis of ACHN and A-498 cell lines.

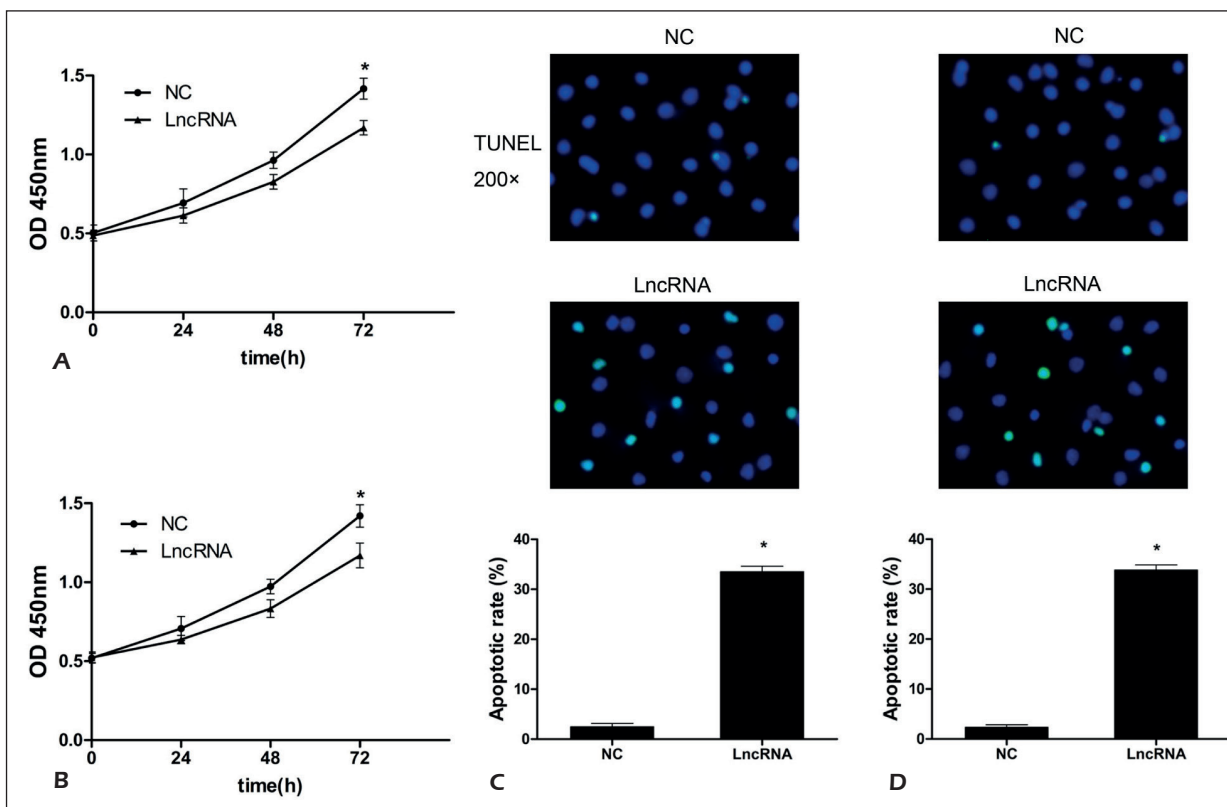


Figure 3. Upregulation of LINC00982 inhibited proliferation and promoted apoptosis of ACHN and A-498 cell lines. **A**, (ACHN) and **B**, (A-498): The analysis of CCK-8 assay showed that upregulated LINC00982 expression significantly suppressed the proliferative ability of cell lines. **C**, (ACHN) and **D**, (A-498): TUNEL assay revealed that upregulated LINC00982 expression significantly promoted cell apoptosis. * $p<0.05$.

LINC00982 Could Regulate the Activity of PI3K/AKT Signaling Pathway

Many studies¹³ have identified that PI3K/AKT pathway was activated abnormally in different diseases and was implicated in cancer cell proliferation. In this study, to evaluate whether LINC00982 regulated renal cancer cell proliferation by PI3K/AKT signaling pathway, we performed Western blot assays. The alteration expression levels of the relative protein involved PI3K/AKT signaling pathway were detected *in*

vitro. Upregulation of LINC00982 reduced the expression of PI3K and phosphorylated AKT (p-AKT). There was no significant change in the total AKT (T-AKT) expression. Furthermore, the downstream genes of PI3K/AKT signaling pathways including Bcl-2 (anti-apoptosis related factor) and Bax (apoptosis-stimulating protein) were also detected by Western blot assays. As shown in Figure 4A and 4B, upregulated LINC00982 expression resulted in reduced expression of Bcl-2, causing an increased expression of Bax as compared to the control groups.

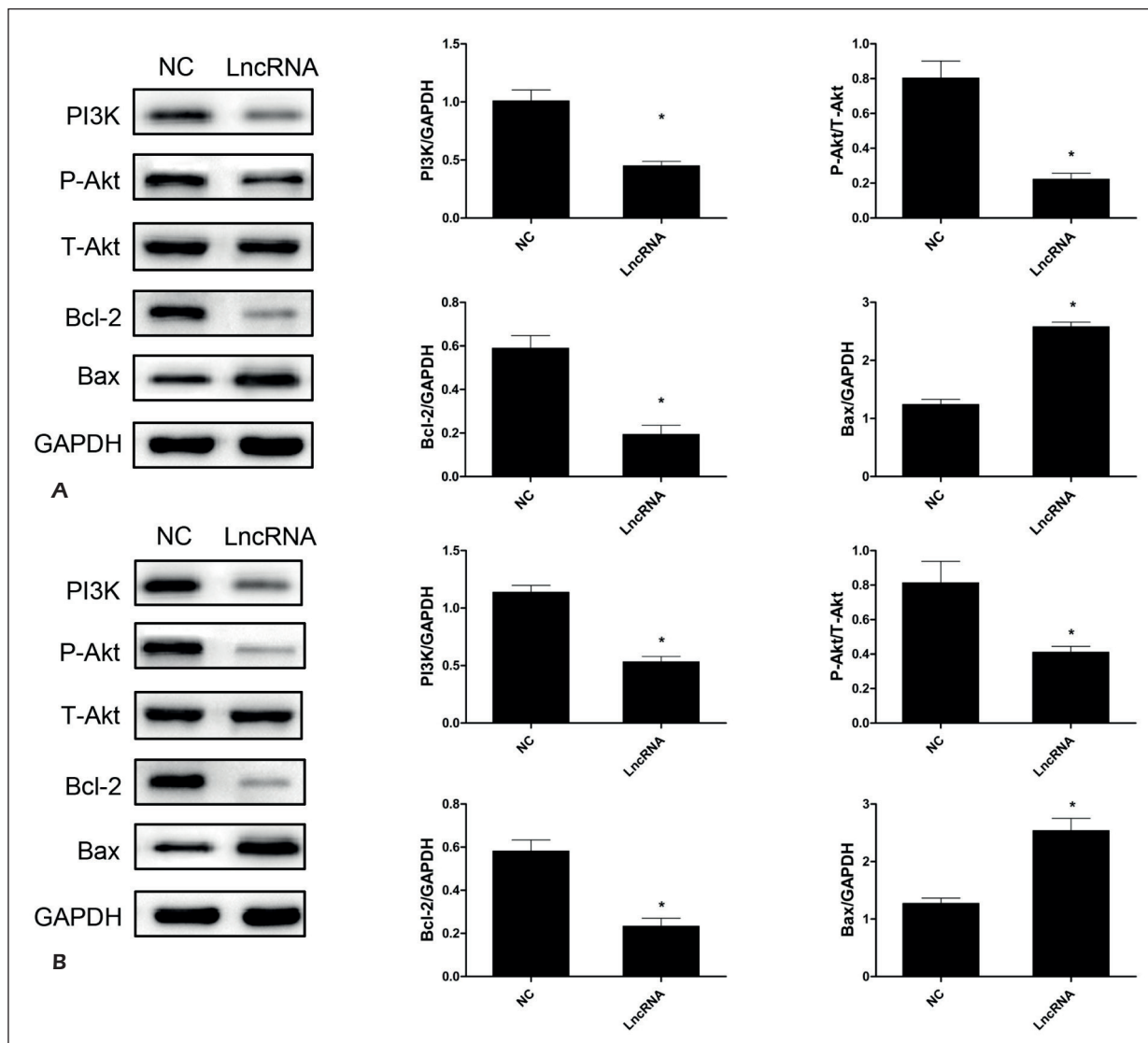


Figure 4. LINC00982 could regulate the activity of PI3K/AKT signaling pathway. **A**, (ACHN) and **B**, (A-498): The Western blot assays were used to detect the altered expression levels of the relative protein involved PI3K/AKT signaling pathway. * $p < 0.05$.

Discussion

This current study revealed that downregulated LINC00982 expression was significantly observed in 96 pairs of renal cancer compared with the adjacent specimens, and aberrant expression of LINC00982 was related to tumor size and TNM stage. CCK-8 and Tunnel experiments showed that upregulation of LINC00982 inhibited proliferation and promoted apoptosis of ACHN and A-498 cell lines. Upregulation of LINC00982 reduced the expression of PI3K and phosphorylated AKT (p-AKT), and resulted in reduced expression of Bcl-2, but caused an increased expression of Bax as relative to the control groups. All the above findings suggested that LINC00982 contributed to the progression of renal cancer, and might act as a biomarker and therapeutic target in renal cancer. The previous studies have confirmed that lncRNAs are involved in the progression of various cancers. In human lung adenocarcinoma, LINC00460 promotes tumor growth *via* targeting miR-302c/FOXA1¹⁴. In breast cancer, MALAT1 is a metastasis-suppressing lncRNA rather than a metastasis promoter¹⁵. In gastric cancer, lncRNA NNT-AS1 enhances cell proliferation and invasion *via* modulating miRNA-363¹⁶. In renal cancer development, hypoxia-regulated lncRNA CRPAT4 enhances cell migration by modulating AVL9 in clear cell renal cancer¹⁷. LncRNA lung cancer associated transcript 1 is able to enhance renal cancer cell proliferation and invasion *via* negatively controlling miR-495-3p¹⁸. LncRNA UCA1 can enhance renal cancer cell proliferation and inhibit cell apoptosis by influencing miR129-SOX4 pathway¹⁹. LncRNA Lucat1 can act as a poor prognostic factor of the patients with renal cancer, and indicate malignant biological behaviors²⁰. Here, we aimed to demonstrate that LINC00982 expression was decreased both in renal cancer tissues and cell lines, and found that LINC00982 might be acted as a tumor suppressor inhibiting the development of renal cancer. Upregulation of LINC00982 inhibited proliferation and promoted apoptosis of ACHN and A-498 cell lines. Lots of researches have illustrated that the regulatory effects of lncRNAs on cancer development partly are owing to the interaction between lncRNAs and many important signal pathways relating to cancer cell biological behaviors. Knockdown of lncRNA SNHG15 can inhibit cell proliferation and EMT *by* controlling

the NF- κ B signaling pathway in renal cancer²¹. Downregulated lncRNA H19 expression can repress melanoma cell migration and invasion *via* inactivating the NF- κ B and PI3K/Akt signaling pathways²². In hepatocellular carcinoma, lncRNA NKILA can promote the anti-cancer effects of baicalein by regulating NF- κ B signaling²³. LncRNA TP73-AS1 is able to enhance cell proliferation and suppress cell apoptosis by inhibiting KISS1 expression and inactivation of PI3K/Akt/mTOR signaling pathway²⁴. LINC003121 can repress thyroid cancer cell proliferation and invasion *via* inhibiting the PI3K/Akt Signaling Pathway²⁵. LncRNA CRNDE can enhance cell proliferation *by* controlling PI3K/Akt / β -catenin signaling in hepatocellular carcinoma²⁶. PI3K/Akt signal pathways contribute to renal cancer cell proliferation. CAV2 can enhance renal cancer cell growth by the EGFR/PI3K/Akt pathway²⁷. The PI3K/Akt pathway has a role in cancer pathogenesis and represents an ideal target for therapeutic intervention in renal cancer²⁸. This study found that upregulation of LINC00982 reduced the expression of PI3K and phosphorylated AKT (p-AKT), and resulted in a reduced expression of Bcl-2, but caused an increased expression of Bax, which indicated that LINC00982 could regulate the activity of PI3K/AKT signaling pathway.

Conclusions

We showed that downregulated LINC00982 expression was significantly observed in renal cancer, and aberrant expression of LINC00982 was related to tumor size and TNM stage. Upregulation of LINC00982 inhibited proliferation and promoted apoptosis of cell lines. LINC00982 could regulate the activity of PI3K/AKT signaling pathway. LINC00982 contributed to the progression of renal cancer, and might act as a biomarker and therapeutic target in renal cancer.

Conflict of Interests

The authors declared no conflict of interest.

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