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Original Article

Long intergenic non-coding RNA 00152 promotes renal cell carcinoma progression by epigenetically suppressing P16 and negatively regulates miR-205

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Abstract: Long non-coding RNAs (lncRNAs) have been reported to play important roles in the tumorigenesis and development of several human cancers. Long intergenic non-coding RNA 152 (LINC00152) is significantly up-regulated in some solid tumors. However, the role of LINC00152 in the pathogenesis and development of renal cell carcinoma (RCC) remains largely unclear. In the study, we showed that LINC00152 expression was up-regulated in RCC tissues compared with adjacent normal tissues and revealed that LINC00152 expression was positively correlated with lymph node metastasis, higher TNM stage, and poor over survival (OS) time in RCC patients. Furthermore, knockdown of LINC00152 inhibited RCC cell proliferation and S phase cell proportion in vitro. Mechanistically, RNA immunoprecipitation (RIP) and Chromatin immunoprecipitation (ChIP) verified that LINC00152 bound to Enhancer of zeste homolog 2 (EZH2), LSD1 and histone H3 at lysine 27 (H3K27me3) and epigenetically suppressing P16 expression. In addition, LINC00152 expression was negatively correlated with miR-205 in RCC and luciferase reporter assays demonstrated that miR-205 was a target of LINC00152. These findings suggested that LINC00152 may contribute to RCC progression by epigenetically repressing P16 expression and interacted with miR-205. Thus, LINC00152 acted as a novel prognostic marker and a potential therapeutic target for RCC.

Keywords: Renal cell carcinoma, long intergenic non-coding RNA 152, EZH2, P16, miR-205

Introduction

Kidney cancer is one of the ten most frequently occurring cancers in western countries. About 270,000 cases of kidney cancer are diagnosed every year and 116,000 people die from the disease in the world. Renal cell carcinoma (RCC) represents approximately 3% of all malignant tumors and accounts for approximately 90% of all renal malignancies [1]. Despite advances in diagnosis and treatment techniques, the 5-year survival rate in patients with advanced stage RCC is poor (5-10%) due to recurrence or distant metastasis [2, 3]. Thus, it is urgent to investigate some molecular pathways for RCC development and progression, which provide novel methods for the development of treatment that block the RCC progression.

Long non-coding RNAs (lncRNAs) have recently been found to play important roles in numerous cellular functions, some studies have demonstrated that lncRNAs were involved in epigenetic regulation, cell cycle control, nuclear and cytoplasmic trafficking, transcription, translation, splicing, cell differentiation and tumor development [4, 5]. In RCC development and progression, Qiao *et al* found that long non-coding RNA GAS5 functioned as a tumor suppressor in renal cell carcinoma and inhibited the tumor proliferation [6]. MALAT1 could bind to EZH2 and oncogenesis facilitated by MALAT1 was inhibited by EZH2 depletion, thereby blocking epithelial-mesenchymal transition via E-cadherin recovery and β-catenin down-regulation [7]. Another study demonstrated MALAT1 functioned as a competing endogenous RNA to regulate ZEB2 expression by sponging miR-200s and promoted the cell invasion in clear cell kidney carcinoma [8]. Song *et al* also found that long non-coding RNA RCCRT1 promoted cell migration and invasion in RCC [9]. Xiong *et al* revealed that high expression of long non-cod-

ing RNA lncRNA-ATB was correlated with RCC patients metastases and promoted cell migration and invasion in renal cell carcinoma [10]. To sum up, these outstanding evidences suggested that lncRNAs were significantly involved in RCC.

Long intergenic non-coding RNA 152 (LINC00152) was also aberrantly expressed in various types of cancers. For example, Zhao *et al* reported that LINC00152 was involved in cell cycle arrest, apoptosis, epithelial to mesenchymal transition, cell migration and invasion in gastric cancer [11]. In hepatocellular carcinoma study, LINC00152 was showed to act as novel biomarkers in predicting diagnosis of heatocellular carcinoma [12]. Zhou *et al* found that LINC00152 promoted gastric cancer proliferation through the EGFR-dependent pathway [13]. Chen *et al* reported that long intergenic non-coding RNA 00152 promoted tumor cell cycle progression by binding to EZH2 and then repressing p15 and p21 in gastric cancer [14]. However, the role of LINC00152 in the pathogenesis and development of renal cell carcinoma (RCC) remains largely unknown.

In present study, we showed that LINC00152 expression levels were up-regulated in RCC tissues. In vitro, knockdown of LINC00152 inhibited RCC cell proliferation and cell cycle progression in vitro. Furthermore, we verified that LINC00152 interacted with EZH2, LSD1 and H3k27me3 and inhibited P16 expression. In addition, we also demonstrated LINC00152 expression was negatively correlated with miR-205 in RCC and regulated the miR-205 expression. Therefore, our findings suggested that LINC00152 may promote RCC progression by epigenetically repressing P16 expression and interacted with miR-205. LINC00152 might be a novel prognostic marker and a potential therapeutic target for RCC.

Materials and methods

Patient and tissues samples

The 45 cases of RCC tissue samples were obtained from patients who underwent nephrectomies at between March 2009 and January 2012 at the Department of Urology, Hebei Chest Hospital and were histologically diagnosed as RCC. None of the patients had received chemotherapy or radiotherapy before surgery. The histological diagnosis was determined by 2 experienced senior pathologists. Patients were staged according to the 7th AJCC TNM staging system. All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Hebei Chest Hospital (Hebei, China).

Cell culture

The four human RCC cell lines (ACHN, Caki-1, Caki-2 and 786-0) and an immortalized primary human proximal tubular cell HK-2 were purchased from the Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). All cells were grown in RPMI supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution (Invitrogen Corporation, Oregon, USA), and incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Vector construction and cell transfection

Two siRNAs targeting LINC00152 and a targeting EZH2 were purchased from RiboBio Co., Ltd. (Guangzhou, China). The ACHN and 786-O cells were transfected by using siRNAs according to the manufacturer's instructions. Their sequences were as follows: siRNA-LINC00152-1, sence: 5'-UGAUCGAAUAUGACAGACACCGAAA-3', anti-sence: 5'-UUUCGGUGUCUGUCAUAUUCGAUCA-3', siRNA-LINC00152-2, sence: 5'-CAGGGAAUCUUUCAGCUGGAUUCCG-3', anti-sence: 5'-CGGAAUGCAGCUGAAAGAUUCCCUG-3'. si-EZH2, sence, 5'-GAGGUUCAGACGAGCUGAUUU-3' anti-sence: 5'-AUCAGCUCGUCUGAACCUCUU-3'. The cDNA encoding LINC00152 was PCR-amplified by the PfuUltra II Fusion HS DNA Polymerase (Stratagene, Agilent Technologies, Santa Clara, CA, USA) and was subcloned into a pcDNA3.1 vector (Invitrogen) to overexpression of LINC00152.

Cell proliferation and assays

The ACHN and Caki-1 cells were seeded in 96-well plates for cell proliferation assay using CCK8 cell proliferation assays (Dojindo, Japan) in accordance with the guidelines. Cell were transfected with the indicated siRNA-NC and siRNA-LINC00152 or pcDNA3.1 and pcDNA3.1-LINC00152 and incubated for, 24, 48, 72 and 96 h. Absorbance was measured at 450 nm using an automatic multi-well spectrophotome-

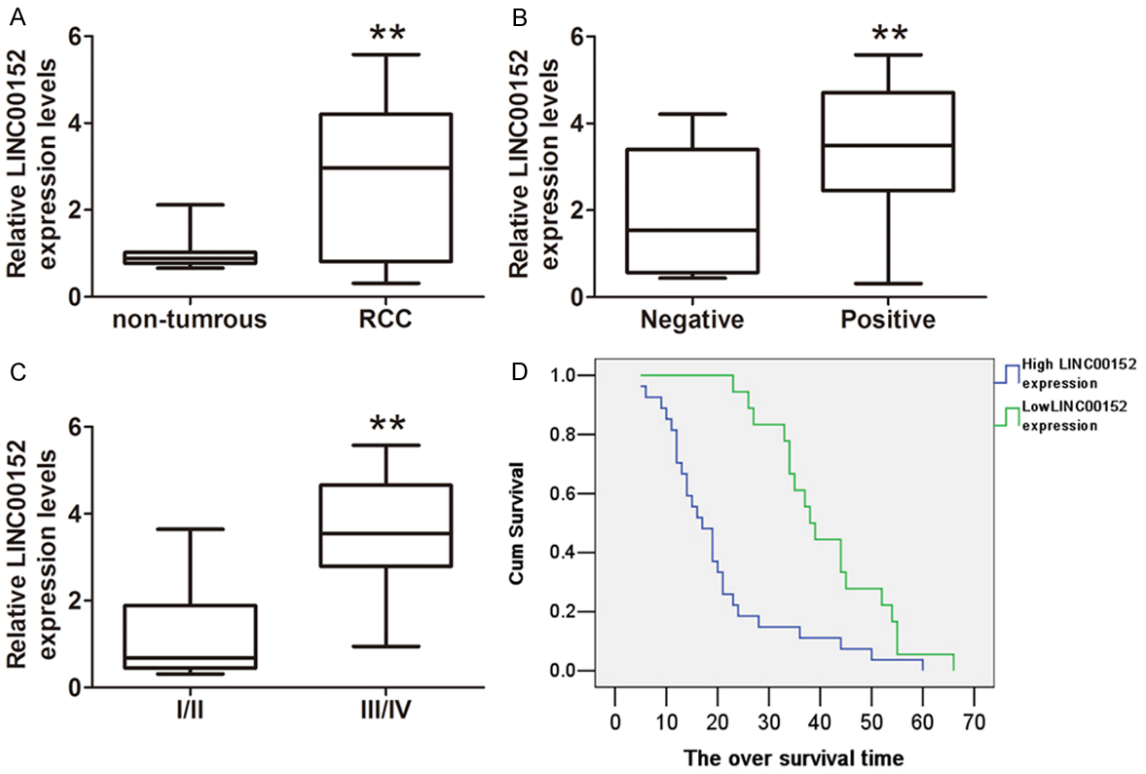

Figure 1. LINC00152 was up-regulated in RCC tissues and was significantly associated with the over survival time. A: Analysis of the LINC00152 expression in 45 cases RCC patient's tissues compared to adjacent non-tumorous samples. The expression level of LINC00152 was measured using qRT-PCR and normalized to GAPDH, **P<0.05. B, C: The association between LINC00152 expression and lymph node metastasis or TNM stage, The expression level of LINC00152 was measured using qRT-PCR and normalized to GAPDH, **P<0.05. D: Kaplan-Meier curves and log-rank test for the overall survival (OS) time in 45 RCC patients, divided according to LINC00152 median expression levels. Higher LINC00152 expression was significantly associated with poor survival time, **P<0.05.

ter (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

RNA isolation and real-time PCR (RT-PCR)

Total RNA from tissues and cells were extracted using TRIzol reagent (TAKALA, Dalian, China) following the manufacturer's instructions. 1 μg RNA was reverse transcribed into cDNA using the Prime ript RT Master Mix (TAKALA, Dalian, China). Real-time polymerase chain reaction (PCR) was performed using the SYBR Green Real-Time PCR Master Mix (TAKALA, Dalian, China). The real-time PCR assays were performed by using an ABI 7500 Real Time PCR System (Applied Biosystem, Foster City, CA). The primers were designed as follows, for LincRNA 00152, forward primer: 5'-AAAATCACGACTCAGCCCCC-3', reverse primer: 5'-AATGGGAAACCGACCAGACC-3', GAPDH, forward primer: 5'-GGGAGCCAAAAGGGTCAT-3', reverse primer: 5'-GAGTCCTTCCACGATACCAA-3'. p16, forward primer: 5'-CACCGAATAGTTACGGTCGG-3', reverse primer: 5'-GCACGGGTCGGGTGAGAGTG-3'. Relative gene expression level of mRNAs was analyzed using the $2^{-\Delta\Delta CT}$ method, normalized to GAPDH.

Western blot analysis

Cellular protein lysates were isolated in a 10% SDS-polyacrylamide gel and then transferred onto the polyvinylidene fluoride (PVDF) membranes (polyvinylidene difluoride) membrane (Millipore). The membranes were blocked with TBS containing 0.1% Triton X-100 and 5% non-fat milk overnight at 4°C, then were incubated with anti-human antibody P16 (1:1000, Cell Signaling Technology, USA) and β-actin (1:1000, Santa Cruz Biotech, Santa Cruz, CA) at 4°C overnight. After being washed, the membranes were followed by HRP (horseradish peroxidase)-labeled goat-anti rabbit IgG (1:1000, Santa

Table 1. The correlation between LINC00152 expression and clinicopathological factors in 45 cases RCC patients

Factors	Patients number	LINC00152 expression		*p*-value
		Low	High	
Gender				0.619
Female	18	8	10	
Male	27	10	17	
Age				0.626
≤60	23	10	13	
>60	22	8	14	
Lymph node metastasis				0.036**
Negative	19	11	8	
Positive	26	7	19	
Grade				0.264
G1, 2	18	9	9	
G3, 4	27	9	18	
Vein invasion				0.221
No	20	10	10	
Yes	25	8	17	
TNM stage				0.003**
I, II	18	12	6	
III, IV	27	6	21	

**P<0.05.

Cruz Biotechnology, USA) at room temperature for 2 hours. Signal detection was carried out with an ECL system (Amersham Pharmacia, Piscataway, NJ, USA). Bands were analyzed with Image J (National Institutes of Health, MD, USA).

Flow cytometry analysis

RCC cells after transfection at 48 h were harvested then were double stained with stained with PI using the Cycle TEST PLUS DNA Reagent Kit (BD Biosciences) following the protocol and analyzed by FAC Scan. The percentage of the cells in G0/G1, S and G2/M phase was counted and compared.

Dual luciferase reporter assay

Luciferase reporter assay: Human ACHN cells was seeded in the 96-well plate, after 24 h, cell were cotransfected with pmir-GLO-LINC00152-wt or pmir-GLO-LINC00152-wt and miR-205, pmir-GLO-LINC00152-wt, pmir-GLO-LINC00152-wt and miR-205 by using Lipofectamie 3000 (Invitrogen, USA), respectively. After cell transfecting 48 h, firefly and Renilla luciferase activities were measured using the Dual-Luciferase Reporter assay system (Promega) according to the manufacturer's protocol. Firefly luciferase activity was normalized to Renilla luciferase activity.

RNA-binding protein immunoprecipitation (RIP) assay

RIP was performed using a Magna RNA-binding protein immunoprecipitation kit (Millipore, Billerica, MA, USA) according to the manufacturer's instructions. Briefly, ECs were lysed in complete RNA lysis buffer, and then were incubated with RIP buffer containing magnetic beads conjugated with human anti-EZH2, anti-LSD1 and H3k27me3 antibodies (Millipore, Billerica, MA, USA), and negative control normal mouse IgG (Millipore, Billerica, MA, USA). Samples were incubated with Proteinase K and then immunoprecipitated RNA was isolated. Furthermore, purified RNAs extracted and analyzed by qRT-PCR assays.

Chromatin immunoprecipitation (ChIP)

The ChIP experiments were performed using an EZ ChIP™ Chromatin Immunoprecipitation Kit for cell line samples (Millipore, USA) according to the manufacturer's instructions. Briefly, the crosslinked chromatin DNA were sonicated into fragments followed by the fixed with 1% formaldehyde. Then immunoprecipitation using anti-EZH2, anti-LSD1 and H3k27me3 antibodies (Millipore, USA) and normal mouse IgG used as the negative control.

Statistical methods

All data were from at least three independent experiments. Results were given as mean ± SD. Statistical comparison between two groups was carried out using the Student's t-test. Differences among groups were carried out by One-way ANOVA followed by LSD tests. Pearson correlation analysis was used to examine the correlation between the relative expressions of LINC00152 and miR-205. P<0.05 was considered statistically significant.

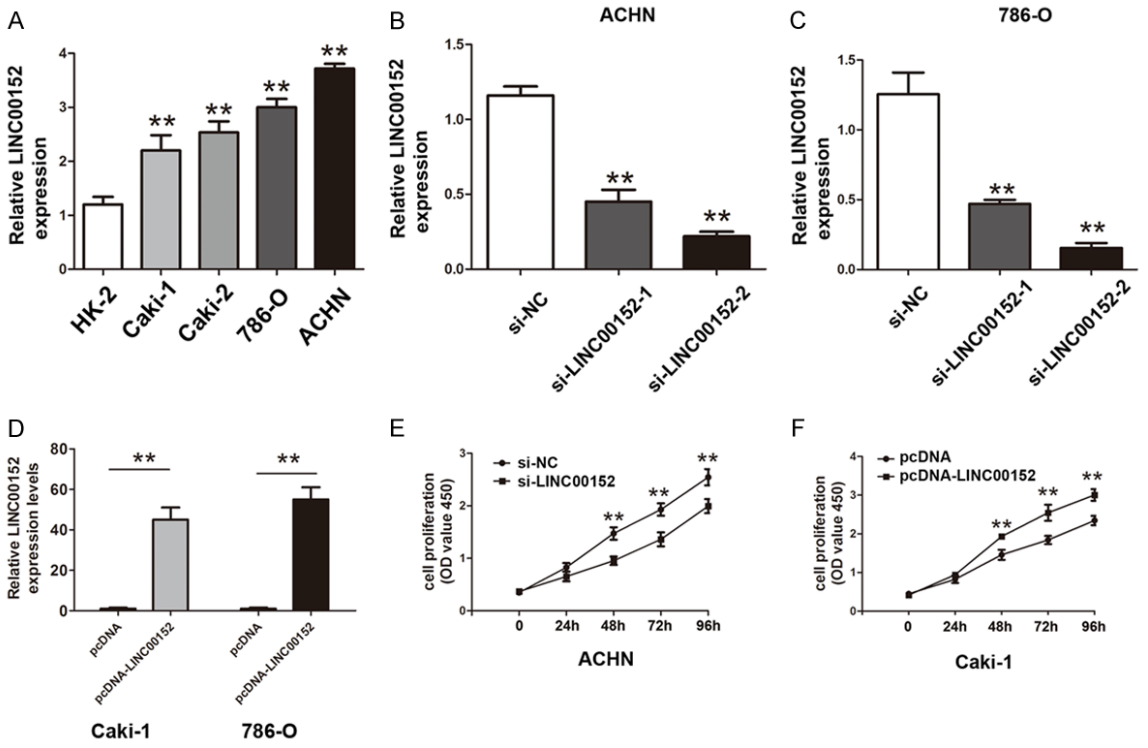

Figure 2. LINC00152 was over-expression in RCC cells and promoted cell proliferation. A: Analysis of LINC00152 expression in RCC cell lines (ACHN, 786-O, Caki-1, and Caki-2) compared to primary human proximal tubular cell HK-2, LINC00152 expression was normalized to GAPDH, **P<0.05. B, C: Analysis of LINC00152 expression after transfecting si-NC, si-LINC00152-1 and si-LINC00152-2 into ACHN and 786-O cells, LINC00152 expression was normalized to GAPDH, **P<0.05. D: Analysis of LINC00152 expression after transfecting pcDNA3.1 or pcDNA3.1-LINC00152 into Caki-1 or 786-O cells, LINC00152 expression was normalized to GAPDH, **P<0.05. E: ACHN cells were seeded in 96-well plates after transfection with si-NC or si-LINC00152, and cell proliferation was assessed at 0, 24 h, 48 h, 72 h and 96 h using a CCK8 assay, **P<0.05. F: Caki-1 cells were seeded in 96-well plates after transfection with pcDNA3.1 or pcDNA3.1-LINC00152, and cell proliferation was assessed at 0, 24 h, 48 h, 72 h and 96 h using a CCK8 assay. The results are represented as the average ± SD based on 3 independent experiments, **P<0.05.

Results

Expression of LINC00152 is significantly up-regulated in RCC samples and cell lines

To examine whether LINC00152 was dysregulated in RCC, we measured LINC00152 expression levels in RCC tissues and cell lines through qRT-PCR analysis. Compared to normal RCC tissues, the expression of LINC00152 was significantly increased in RCC tissue samples (**Figure 1**, P<0.05). Furthermore, we divided the 45 cases RCC patients into two groups: higher LINC00152 expression group (above the mediate LINC00152 expression, n=27) and lower LINC00152 group (below the mediate LINC00152 expression, n=18). Our results showed that higher LINC00152 expression was significantly correlation with lymph node metastasis and higher TNM stage (**Figure 1B**, **1C**; **Table 1**, P<0.05). We also detected the correlation between LINC00152 expression and the over survival (OS) time of RCC patients, the Kaplan-Meier curve and log-Rank test demonstrated that higher LINC00152 was positively correlated with the poor survival time (**Figure 1D**, Log-Rank=13.946, P<0.01). Taken together, these findings indicated that LINC00152 expression in patients with RCC may be a tumor biomarker and predicted the prognosis for patients with RCC.

Knockdown of LINC00152 inhibits cell proliferation and S phase cell proportion

We further identified the effects of LINC00152 on RCC cell proliferation and cell cycle progression. Firstly, we detected the LINC00152 expre-

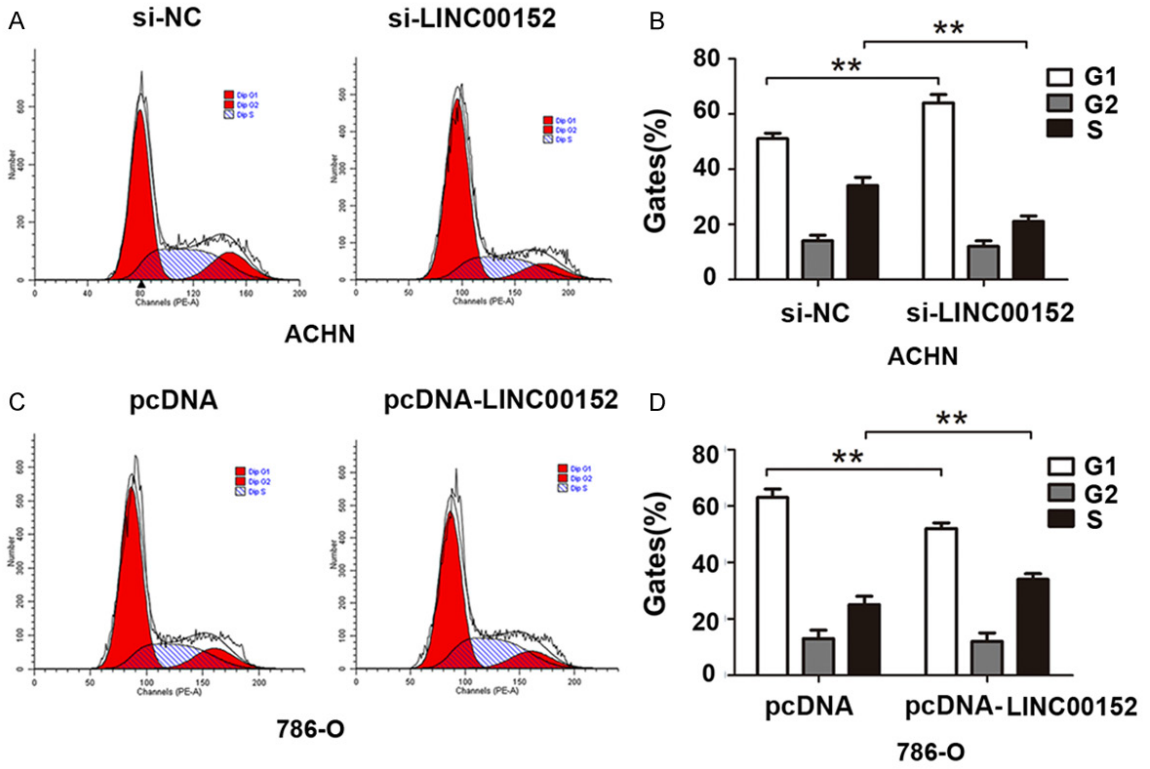

Figure 3. Knockdown of LINC00151 inhibited RCC cell cycle progression. (A, B) Cell-cycle assays and cell cycle analysis in ACHN cells after transfection with si-NC or si-LINC00152 and (C, D) in 786-O cells after transfection with pcDNA3.1 or pcDNA3.1-LINC00152, summarized flow cytometry data are shown. Results are represented as the average ± SD based on 3 independent experiments, **P<0.05.

ssion levels in four RCC cells including ACHN, 786-O, Caki-1 and Caki-2. The results found the LINC00152 was up-regulated in RCC cells compared with HK-2 cells (**Figure 2A**). Furthermore, we performed the gain-of-function and loss-of-function assays in RCC cells. The siRNA-2 was used in knockdown experiments in ACHN and 786-O cells and the pcDNA3.1-LINC00152 plasmid was used to over-expression the LINC00152 in 786-O cells and Caki-1 cells (**Figure 2B-D**). After LINC00152 silencing or over-expression of LINC00152, the results revealed knockdown of LINC00152 inhibited cell proliferation in ACHN cells by using CCK8 cell proliferation assays, compared to the control group, however, over-expression of LINC00152 promoted cell proliferation in Caki-1 cells (**Figure 2E**, **2F**). Moreover, cell cycle assays and cell cycle analysis showed that knockdown of LINC00152 significantly inhibited S-phase cell number in ACHN, but enhancing the S-phase cell number by transfecting pcDNA3.1-LINC00152 into 786-O cells (**Figure 3A-D**). Collectively, these results suggest that LINC00152 acted as an oncogene in RCC and promoted cell proliferation and cell cycle progression.

LINC00152 interacts with EZH2, LSD1 and H3k27me3

LncRNAs have been shown to regulate their target genes by physically connecting their genomic locus with the genomic regions of the target genes. EZH2, a catalytic subunit of PRC2, catalyzed the trimethylation of histone H3 at lysine 27 (H3K27me3) [15]. To investigate the molecular and biological mechanisms of LINC00152 involved in RCC cells, we firstly detected the distribution of LINC00152 in ACHN and 786-O cells. The results showed that LINC00152 was existed in both cytoplasm and nucleus (**Figure 4A**). Next, we performed RIP assays analysis to examine LINC00152 whether bound with EZH2, LSD1 and H3k27me3. As shown the endogenous LINC00152 was enriched in the anti-EZH2, anti-LSD1 and anti-H3k27me3 RIP fraction in ACHN and 786-O

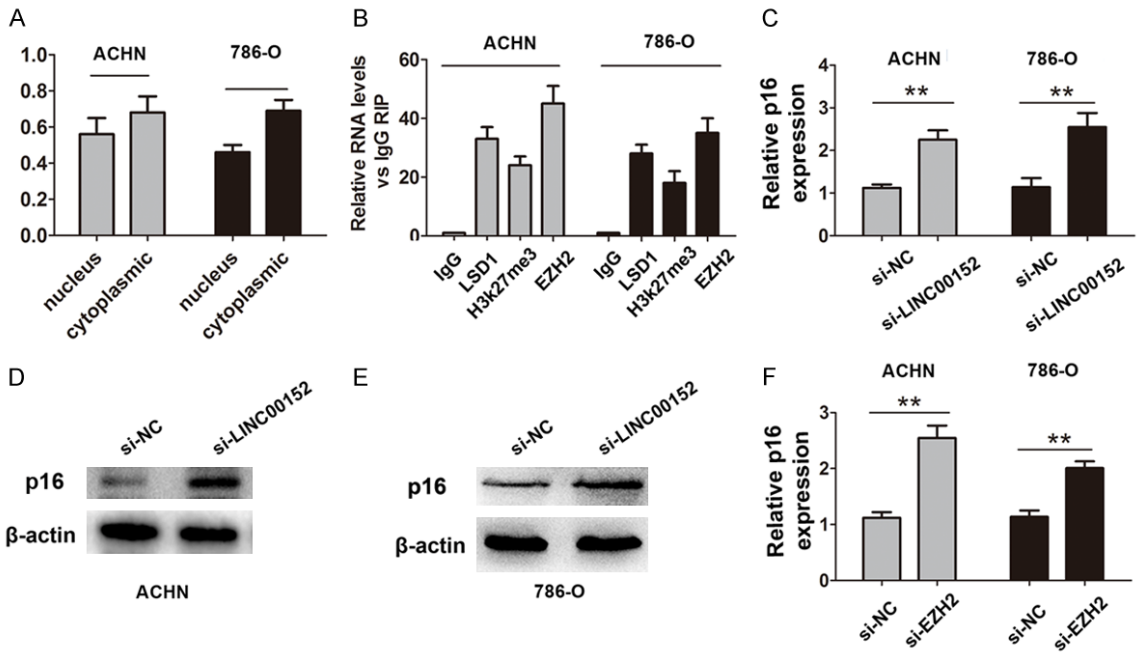

Figure 4. LINC00152 represses P16 by binding with EZH2, LSD1 and H3k27me3 in RCC cells. A: Subcellular localization of LINC00152 was determined using fractionation. After nuclear and cytoplasm separation in ACHN and 786-O cells, RNA was extracted from both fractions and LINC00152 expression was measured by qRT-PCR. GAPDH was used as a cytoplasm marker and U6 was used as a nucleus marker. B: RNA levels in immunoprecipitates with EZH2, LSD1 and H3k27me3 were determined by qRT-PCR. Expression levels of LINC00152 were presented as fold enrichment relative to IgG immunoprecipitate. C: The levels of P16 were detected by qRT-PCR when knockdown of LINC00152 in ACHN and 786-O cells, results are represented as the average ± SD based on 3 independent experiments, **P<0.05. D, E: The P16 protein levels were detected by western blotting analysis in LINC00152 knockdown ACHN and 786-O cells. F: The levels of P16 were detected by qRT-PCR when knockdown of EZH2 in ACHN and 786-O cells, results are represented as the average ± SD based on 3 independent experiments, **P<0.05.

cells (**Figure 4B**). Thus, the above results demonstrated that LINC00152 interacted with EZH2, LSD1 and H3k27me3.

LINC00152 epigenetically represses P16 by interacting with EZH2, LSD1 and H3k27me3

It was reported that EZH2 could bind to certain sets on P16 promoter and inhibited their expression [16]. Furthermore, we focused on whether LINC00152 repressed the expression of P16. After knockdown of LINC00152 in ACHN and 786-O cells, we found the P16 transcriptional levels and protein levels were significantly up-regulated after knockdown of LINC00152 in ACHN and 786-O cells (**Figure 4C**, **4D**). Moreover, we also verified that the protein levels of P16 were also significantly up-regulated after knockdown of EZH2 in ACHN and 786-O cells (**Figure 4F**). In addition, ChIP (Chromatin Immuno-precipitation) assay was applied and the primers to amplify these regions were designed. The results showed that EZH2, LSD1 and H3K27me3 could directly bind to P16 promoter regions in ACHN and 786-O cells (**Figure 5A**). Furthermore, knockdown of LINC00152 resulted in reduced EZH2 and LSD1 binding and H3K27me3 occupany of P16 promoter locus in ACHN and 786-O cells (**Figure 5B**). Functionally, we demonstrated that LINC00152 silencing inhibited the cells proliferation in ACHN cells, but co-transfected siRNA-P16 and si-LINC00152 reversed the effects (**Figure 5C**). Therefore, these data suggested that LINC00152 promoted RCC cell growth partly through epigenetically silencing P16 transcription.

MiR-205 is a direct target of LINC00152

Recent studies have indicated that lncRNAs may act as endogenous sponge RNA to interact with miRNAs and influence the expression of these miRNAs [17]. To investigate the underly-

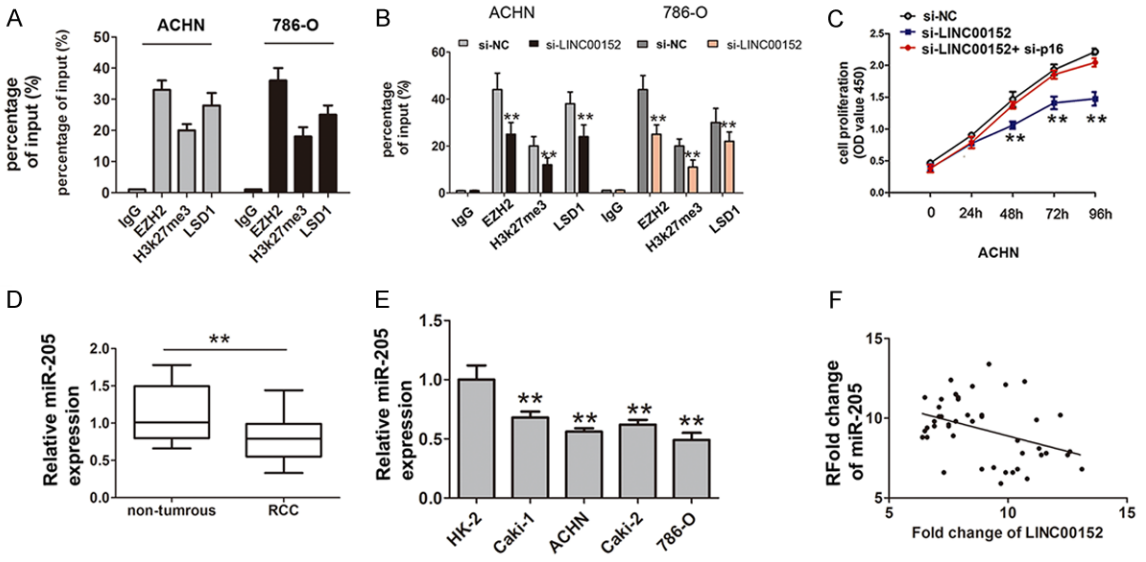

Figure 5. LINC00152 was negatively correlated with miR-205 in RCC. A: Chromatin immunoprecipitation-qPCR analysis of EZH2 and LSD1 occupancy and H3K27me3 binding to the P16 promoter regions in ACHN and 786-O cells, and IgG as a negative control. Results are represented as the average ± SD based on 3 independent experiments, **P<0.05. B: Chromatin immunoprecipitation-qPCR analysis of EZH2 and LSD1 occupancy and H3K27me3 binding to the P16 promoter regions after knockdown of LINC00152 in ACHN and 786-O cells, and IgG as a negative control. Results are represented as the average ± SD based on 3 independent experiments, **P<0.05. C: CCK8 assays was used to determine the cell viability by transfecting si-NC, si-LINC00152 or si-LINC00152 +si-P16 into ACHN cells, results are represented as the average ± SD based on 3 independent experiments, **P<0.05. D, E: MiR-205 was down-regulated in RCC tissues and cells. F: Pearson's correlation was used for correlation analysis between the expression of LINC00152 mRNA and miR-205 mRNA (R=-0.394, P<0.05). Results are represented as the average ± SD based on 3 independent experiments, **P<0.05.

ing mechanism of LINC00152 in RCC progression, we analyzed whether LINC00152 could compete to bind with miRNAs as a miRNA-sponge by online soft miRcode (http://www.mircode.org/). According to the predicted results, miR-205 potentially bind to LINC00152. Previously study had reported that miR-205 functioned as tumor suppressor gene in renal cancer [18]. Our result showed that compared with adjacent normal tissues, miR-205 was down-regulated in RCC tissues and was also significantly lower in RCC cells compared to HK-2 cell (**Figure 5D**, **5E**). Furthermore, we found that LINC00152 was negatively correlated with miR-205 expression levels (r=-0.392, P<0.05). In ACHN and 768-0 cells, we demonstrated that knockdown of LINC00152 significantly increased miR-205 expression (**Figure 6A**), but the results showed that overexpression of LINC00152 significantly decreased miR-205 expression (**Figure 6B**). However, we also showed that LINC00152 had no change by transfecting miR-205 plasmid into ACHN and 768-0 cells (**Figure 6C**). Furthermore, as shown in **Figure 6D**, we predicted the binds site by miR and a (www.mircorna.org), and constructed the luciferase reporter vectors, the results showed that co-transfected miR-205 plasmid reduced the luciferase activities of wild-type (WT) LINC00152 reporter vector, but not a mutant LINC00152 in ACHN cells, which indicated that miR-205 binds to LINC00152 in a sequence specific manner (**Figure 6E**). Taken together, it suggested that LINC00152 down-regulated the RNA levels of miR-205could directly interacted with it in RCC.

Discussion

Long non-coding RNAs (ncRNAs) have been proposed in the last decade and as important regulators of cancer pathways and biomarkers of cancer outcomes [19]. Pang *et al* found LINC00152 in gastric carcinoma was significantly increased and could activate the mechanistic target of rapamycin (mTOR) pathway by binding to the promoter of EpCAM through a cis-regulation [20]. Another study revealed that long non-coding RNA LINC00152 was a positive prognostic factor for and demon-

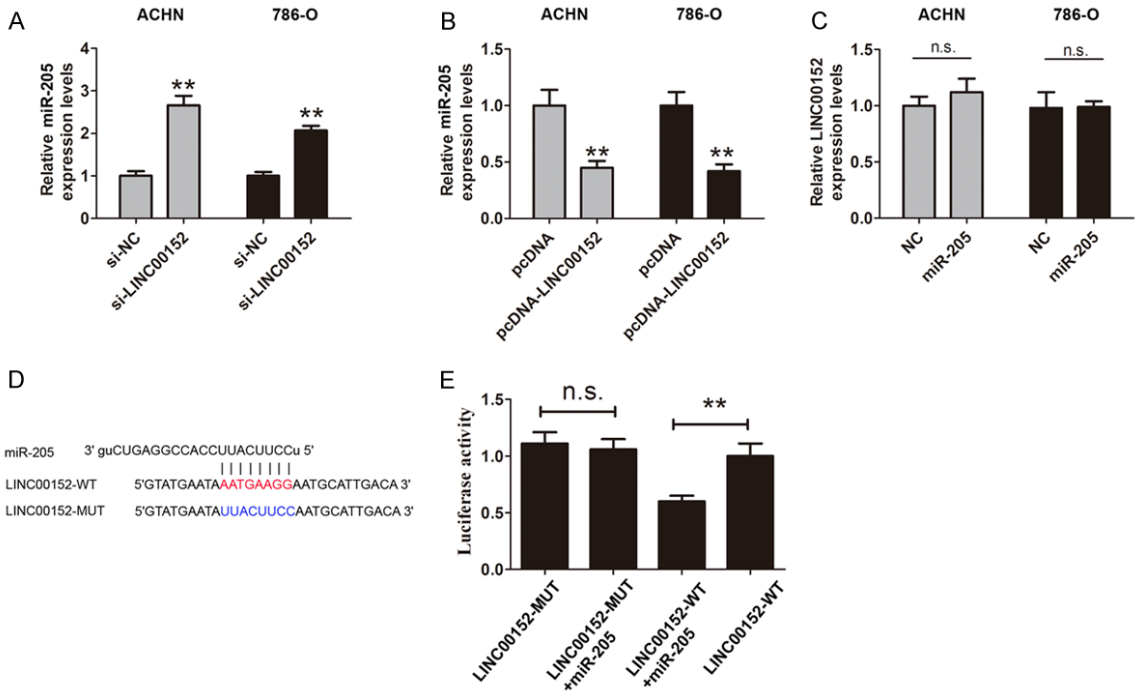

Figure 6. LINC00152 negatively regualted miR-205 expression in RCC cells. (A) The mRNA levels of miR-205 in ACHN or 786-O cells transfected with si-NC or si-LINC00152. (B) The mRNA levels of miR-205 in ACHN or 786-O cells transfected with pcDNA3.1 or pcDNA3.1-LINC00152. (C) The mRNA levels of LINC00152 in ACHN or 786-O cells transfected with miR-NC or miR-205 plasmid. (D) The bind site between LINC00152 and miR-205 was predicted by miRanda (www.microrna.org) and pmir-GLo-LINC00152-WT or pmir-GLo-LINC00152-MUT was constructed for dual luciferase reporter assay (E) luciferase reporter assay was shown by transfected the pmir-GLo-LINC00152-wt, pmir-GLo-LINC00152-wt and miR-205, pmir-GLo-LINC00152-MUT, pmir-GLo-LINC00152-MUT and miR-205 into ACHN cells, respectively. Results are represented as the average ± SD based on 3 independent experiments, **P<0.05.

strates malignant biological behavior in clear cell renal cell carcinoma [21]. In the study, we showed that LINC00152 expression was up-regulated in RCC tissues compared with adjacent normal tissues, and revealed that LINC00152 expression was positively correlated with lymph node metastasis, higher TNM stage, and higher LINC00152 predicted a poor over survival (OS) time in RCC patients. In vitro, knockdown of LINC00152 inhibited RCC cell proliferation and S phase cell number.

We also sought to detect the underlying molecular mechanisms by which LINC00152 regulated downstream effectors in RCC. For example, the well-studied lncRNA HOTAIR acts as a scaffold by connecting PRC2 to the LSD1/CoREST/REST complex, and the HOTAIR/PRC2/LSD1 complex as a whole suppressed gene expression [22]. LINC00152 was reported to regulate downstream effectors via binding to EZH2 and recruited it to the promoter regions of p15 and p21 in gastric cancer [14]. In the study, we found that LINC00152 bound to EZH2 and LSD1, recruited it to the promoter regions of P16 in RCC cells. Furthermore, knockdown of LINC00152 resulted in reduced EZH2 and LSD1 binding and H3K27me3 occupany of P16 promoter locus. Thus, these results suggested that LINC00152 promoted RCC progression partly through epigenetically silencing p16 transcription.

Recently, some reported also revealed that lncRNA shared MREs and acted as decoys to sequester miRNAs to prevent them from binding to targets and hence to modulate many functional mRNA targets through translation [22]. In the current study, the miR-205 expression was negatively association with LINC00152 and we observed that down-regulated LINC00152 led to increased in miR-205 expression, whereas overexpression of LINC00152 inhibited the miR-205 expression. Furthermore, co-transfected miR-205 plasmid reduced the luciferase activities of wild-type (WT)

LINC00152 reporter vector, but not a mutant LINC00152, which indicated that miR-205 binds to LINC00152 in ACHN cells. This result indicated that LINC00152 also functioned as a ceRNA to negatively regulated miR-205 expression in RCC progression.

In summary, our results demonstrated that LINC00152 was up-regulated in RCC and RCC cells. LINC00152 contributed to RCC progression by epigenetically repressing P16 expression and negatively regulated miR-205 expression in RCC. Thus, LINC00152 might act as a novel prognostic marker a potential therapeutic candidate for RCC.

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Disclosure of conflict of interest

None.

Authors' contribution

Yongjun Wang and Jianzhen Liu conceived and designed the study; Hongzhong Bai, Yi Dang and Pei Lv performed the experiment and analyzed the data; Yongjun Wang and Shucai Wu helped with interpretation of the results and wrote the paper.

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