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*Original Article*

# Long non-coding RNA Linc00152 is a positive prognostic factor for and demonstrates malignant biological behavior in clear cell renal cell carcinoma

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**Abstract:** Accumulating evidence demonstrates that lncRNAs play important roles in regulating gene expression and are involved in various pathological processes. In the present study, we screened the lncRNAs profile in clear cell renal cell carcinoma (ccRCC) from The Cancer Genome Atlas (TCGA) database, and got linc00152, a differentially expressed lncRNA that haven't been reported in ccRCC. To further explore its role in ccRCC, the level of Linc00152 was detected in 77 paired ccRCC tissues and renal cancer cell lines by qRT-PCR, and its association with overall survival was assessed by statistical analysis. Linc00152 expression was significantly up-regulated in cancerous tissues and cell lines compared with normal counterparts, and high Linc00152 expression was closely associated with advanced TNM stage. Moreover, Linc00152 was found to be able to serve as an independent predictor of overall survival. Further experiments demonstrated that overexpression of Linc00152 can significantly promote cell proliferation and invasion, inhibit cell cycle arrest in G1 phase and dramatically decrease apoptosis in both 786O and Caki-2 cell lines, whereas the opposite results were observed with attenuated Linc00152 expression. Our data suggest that Linc00152 is a novel molecule involved in ccRCC progression as well as a potential prognostic biomarker and therapeutic target.

**Keywords:** Linc00152, clear cell renal cell carcinoma, long non-coding RNA, renal cell carcinoma

## Introduction

Renal cell carcinoma (RCC) is one of the ten most common cancers, with approximately 202,000 cases and 102,000 deaths worldwide [1, 2]. Despite the wide use of ultrasound and computed tomography, approximately 30% of patients with newly diagnosed disease show evidence of metastases at presentation [3]; the overall median survival of such patients is less than one year. Clear cell renal cell carcinoma (ccRCC) is the most common subtype of renal cancer and accounts for approximately 75% of cases [4]. Therefore, an understanding of the pathophysiological mechanisms contributing to RCC is of paramount importance when developing new diagnosis and treatment strategies and for improving the overall prognosis of RCC patients.

Long noncoding RNAs (lncRNAs), a newly discovered class of noncoding RNAs (ncRNA) greater than 200 nucleotides in length, are being increasingly reported in a variety of cancer types, suggesting an important role in human diseases, especially tumors [5, 6]. Indeed, emerging studies have indicated lncRNA involvement in diverse physiological and pathological processes, such as cell growth, apoptosis, stem cell pluripotency, and development, by acting as transcriptional, posttranscriptional, or epigenetic regulators [7-10]. However, the majority of lncRNAs have not yet been functionally characterized.

Although the dysregulation of lncRNAs has been demonstrated in RCC, including MALAT1 [11, 12], GAS5 [13], H19 [14] and KCNQ1OT1 [15], the profiles of lncRNAs in this malignancy

remain largely unknown. Previous studies verified that lncRNA profiling could be achieved using the database The Cancer Genome Atlas (TCGA) (*http://cancergenome.nih.gov/*), which provides a platform for researchers to search, download, and analyze data sets generated by TCGA. In the present study, the TCGA database was used to search lncRNA gene expression profiles in ccRCC. By applying a comprehensive analysis of lncRNA expression profiles, we identified Linc00152 as a new candidate lncRNA that promotes the development of RCC. Furthermore, its biological role and clinical significance were evaluated.

## Materials and methods

### *Patient samples*

The study was undertaken with the understanding and written consent of each subject, and the methodologies conformed to the standards set by the declaration of Helsinki. All human tissues were collected using protocols approved by the Human Ethics Committee of the Fudan University Shanghai Cancer Center. ccRCC tissues and normal tissues were obtained from 77 patients who had undergone surgical resection of colorectal cancer between 2009 and 2013. No local or systemic treatment had been administered to these patients prior to surgery. All tissue samples were washed with sterile phosphate-buffered saline before being snap frozen in liquid nitrogen and then stored at -80°C until analyzed. The pathological stage and grade were evaluated by an experienced pathologist. The data set from an independent cohort in the TCGA database was utilized for the evaluation of Linc00152 expression as a prognostic factor in ccRCC.

### *Cell lines and culture conditions*

The immortalized normal human proximal tubule epithelial cell line HK-2 and four human RCC cell lines (786O, Caki-2, A498, ACHN, Caki-1) were purchased from Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). The HK-2 cells were cultured in KSFM medium (Gibco) and the 786O cell line in RPMI 1640 medium (HyClone). The remaining cell lines were grown in Dulbecco's modified Eagle's medium (DMEM, HyClone). All media were supplemented with 10% fetal bovine serum (10% FBS, Gibco), 100 U/ml penicillin (Gibco), and 100 mg/ml streptomycin (Gibco), and all cell lines were maintained at 37°C in a humidified atmosphere of 5% $CO_2$.

### *RNA extraction and qRT-PCR analyses*

As described in previous reports [16, 17], total RNA was extracted from tumorous and adjacent normal tissues using Trizol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. RT and qPCR kits were used to evaluate expression of Linc00152 in the tissue samples. The 20 μL RT reactions were performed using a PrimeScript® RT reagent kit (Takara, Dalian, China) and incubated for 30 min at 37°C, for 5 s at 85°C, and then maintained at 4°C. For real-time PCR, 1 μL diluted RT product was mixed with 10 μL of 2 × SYBR® Premix Ex Taq™ (Takara, Dalian, China), 0.6 μL forward and reverse primers (10 μM), and 8.4 μL nuclease-free water in a final volume of 20 μL, according to the manufacturer's instructions. The primers used in this study were 5'-TGAGAATGAAGGCTGAGGTGT-3' (forward) and 5'-GCAGCGACCATCCAGTCATT-3' (reverse). All reactions were carried out using an Eppendorf Mastercycler EP Gradient S (Eppendorf, Germany) under the following conditions: 95°C for 30 s, followed by 40 cycles at 95°C for 5 s and 60°C for 30 s. Real-time PCR was performed in triplicate, and no-template controls were included. Amplification of the appropriate product was confirmed by melting curve analysis following amplification. The relative expression of Linc00152 was calculated using the comparative cycle threshold (CT) (2-ΔΔCT) method with glyceraldehyde-3-phosphate dehydrogenase (ACTIN) as the endogenous control for data normalization. Each sample was analyzed in triplicate.

### *Transfection of RCC cells*

Small interfering RNA (siRNA) and nonspecific control siRNA were synthesized (Biotend, Shanghai, China) and transfected into cells using Lipofectamine 3000 (Invitrogen, USA). To overexpress Linc00152, the full-length coding sequence was amplified and subcloned into the pcDNA 3.1 (+) vector (Invitrogen) according to the manufacturer's instructions. The cell lines 786O and Caki-2 were transfected with a negative control vector or the Linc00152-expressing plasmid according to the manufacturer's protocol, and the cells were harvested after 48 h for qRT-PCR. The siRNA oligonucleotide sequences targeting Linc00152 were siRNA1 (5'-GG-

TCTGGTCGGTTTCCCATTTdTdT-3'), siRNA2 (5'-GGAGAUGAAACAGGAAGCUdTdT-3'), and the sequence of negative control was 5'-UUCUCCGAACGUGUCACGUdTdT-3'.

*Cell proliferation assays*

A total of $2 \times 10^3$ cells per well was seeded in 96-well plates 24 h before the experiment. 7860 and Caki-2 cells were transfected with pcDNA3.1-linc0052 or the negative control, whereas A498 and ACHN cells were transfected with siRNA or scramble siRNA. Proliferation was measured using the CCK-8 kit (Dojindo, Japan) according to the manufacturer's protocol. All experiments were performed in triplicate. Cell proliferation curves were plotted using the absorbance at each time point.

*Colony formation assay*

Cells were digested with trypsin into single-cell suspensions at 48 h after transfection. For the colony formation assay, a sample of 1,000 cells was plated into 6-well plates and incubated in corresponding media with 10% FBS at 37°C. After one week, the cells were fixed and stained with 0.1% crystal violet, and visible colonies were manually counted. Triplicate wells were measured for each treatment group.

*Cell wound-healing and invasion assays*

For the wound-healing assay, cells were seeded into six-well plates and allowed to grow to 90-95% confluence. At 5 h after transfection with pcDNA3.1-linc00152 or siRNA, a single scratch wound was created. The cells were washed three times with PBS to remove cell debris, supplemented with serum-free medium, and monitored. Images were captured by phase-contrast microscopy at 0, 24, 36 and 48 h after wounding.

The cell invasion assay was performed using 24-well insert transwell chambers (8.0 mm, Corning, NY). Cells ($2 \times 10^4$) suspended in 200 μL serum-free medium were added to the upper chamber, and culture medium containing 20% FBS was placed in the bottom chamber. The cells were then incubated for 24 h or 48 h at 37°C; the cells on the upper surface were scraped and washed away, whereas the cells on the lower surface were fixed with 20% methanol and stained with 0.1% crystal violet. The number of invaded cells was counted in five randomly selected fields under a microscope. The experiments were independently repeated in triplicate.

*Cell cycle assays*

Transfected RCC cells were harvested after 48 h of incubation in 6-well plates. The cells were collected and fixed in 70% ethanol (chilled to -20°C) overnight. The cells were then washed with PBS and stained for 30 min with propidium iodide (PI; BD Biosciences) in PBS supplemented with RNase (50 mg/ml) in the dark at room temperature. The assays were performed in triplicate for each sample, and the cell cycle distribution was evaluated using a flow cytometer in accordance with the manufacturer's guidelines (FACS, BD Biosciences).

*Apoptosis assay*

Transfected cells were harvested and double stained with fluorescein isothiocyanate-Annexin V and propidium iodide using a PE Annexin V Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's recommendations. The cells were then analyzed using a flow cytometer (FACScan; BD Biosciences) equipped with Cell Quest software (BD Biosciences). The cells were discriminated into viable cells, dead cells, early apoptotic cells and apoptotic cells, and the relative ratio of early apoptotic cells was compared with control transfection in each experiment.

*Statistical analysis*

Each experiment was repeated 3 times, and the data are presented as the mean with error bars indicating the standard deviation. All statistical analyses were performed using SPSS 18.0 (IBM, SPSS, Chicago, IL), Figures were made by GraphPad Prism 6 (GraphPad Software Inc., San Diego, CA, USA) and R software (R version 3.1.2). The significance of the differences between groups was estimated using the Student t-test, $\chi^2$ test, or Wilcoxon test. OS rates were calculated using the Kaplan-Meier method with the log-rank test for comparisons. Spearman rank order was used to analyze the correlations between clinical parameters and expressions of Linc00152; Univariate analysis and multivariate models were fit using a Cox proportional hazards regression model. A value of $P < 0.05$ indicated a significant difference.

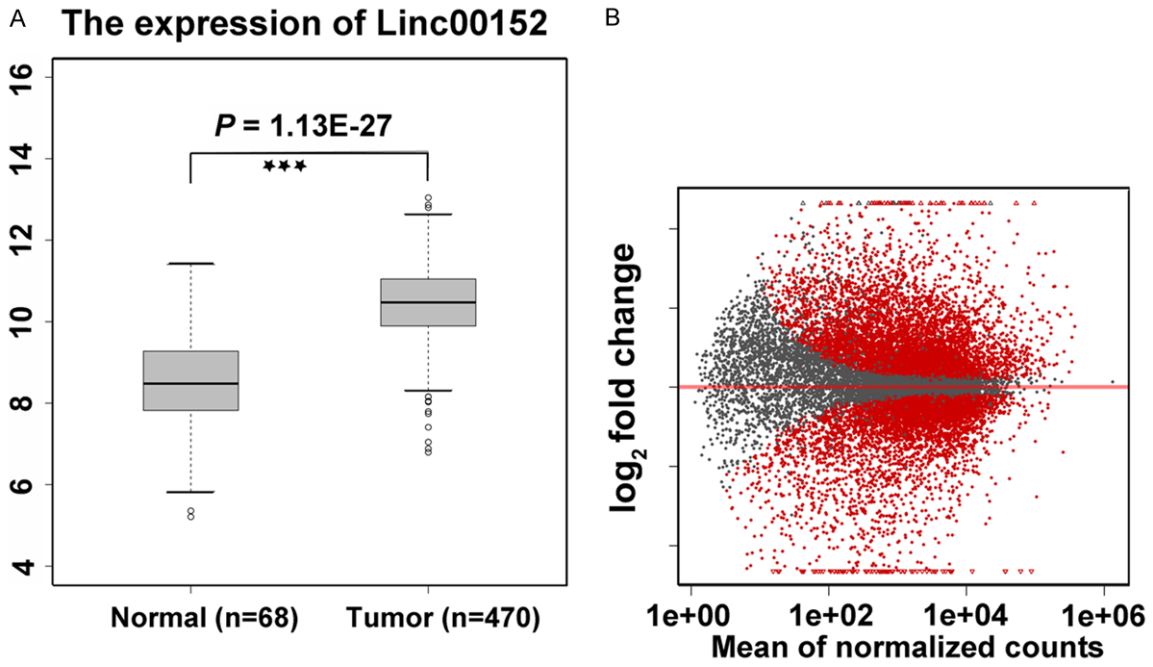



**Figure 1.** Screen of ccRCC-specific LncRNA in the TCGA database. A. Linc00152 was found to be highly over-expressed in ccRCC tissues compared with normal tissues in the TCGA RNA-seq data ($P < 0.001$). B. Differential gene expression analysis was performed based on a negative binomial distribution.

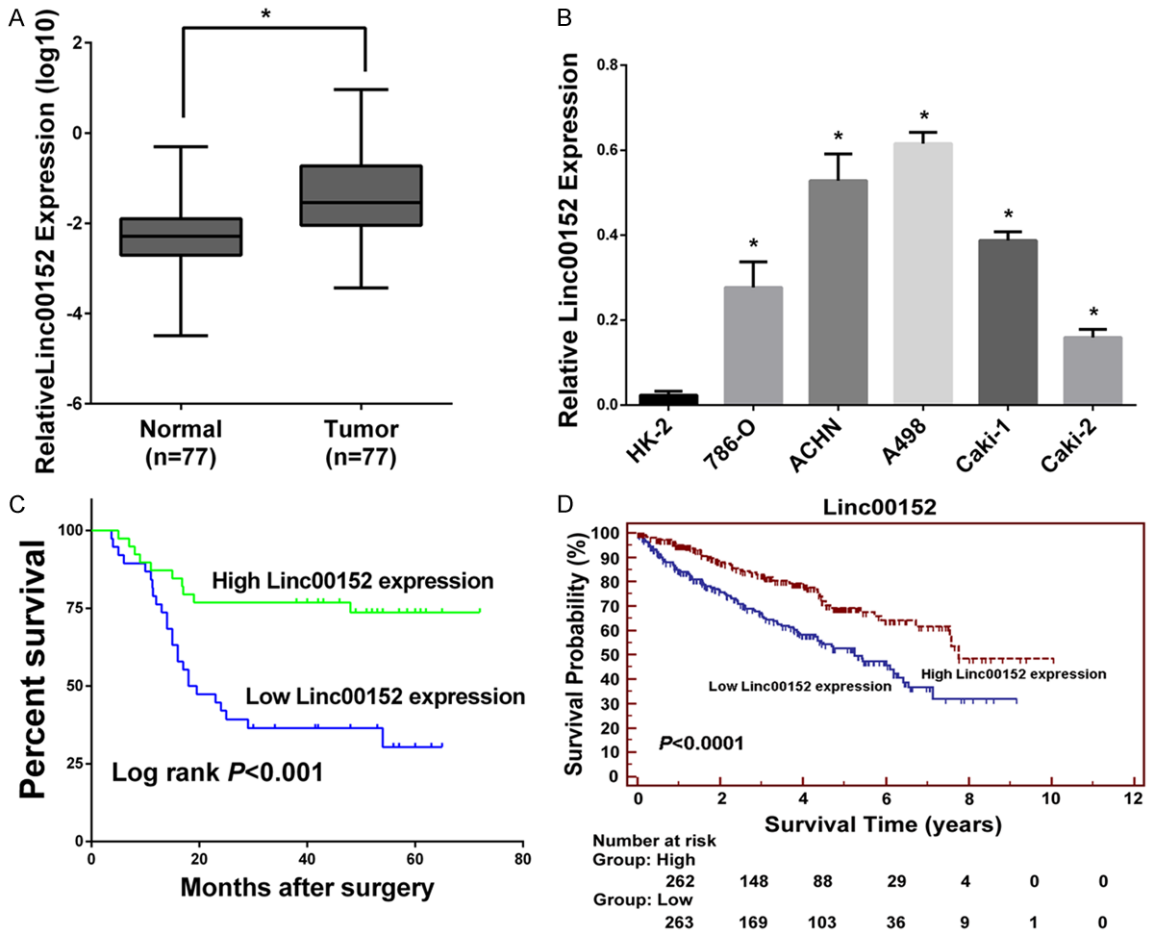


**Figure 2.** The expression of Linc00152 in ccRCC and its association with patient prognosis. A. Linc00152 expression was assessed by qRT-PCR in renal cancer cell lines (786O, Caki-2, A498, ACHN, Caki-1) and compared with the normal human proximal tubule epithelial cell line HK-2. B. Differences in expression levels of Linc00152 between ccRCC tissues and matched non-tumor tissues. The expression of Linc00152 was normalized to ACTIN. Significant differences between samples were analyzed with the paired samples *t*-test. C. Kaplan-Meier curves for overall survival of patients with ccRCC categorized according to Linc00152 expression: significantly poorer overall survival was observed in patients with high Linc00152 expression than those with low Linc00152 expression ($P < 0.001$, log-rank test). $^*P < 0.05$. D. The high Linc00152 expression contributed to a significant poorer survival in another independent cohort available at the TCGA database (n=525, $^*P < 0.0001$, log-rank test).

**Table 1.** The correlation between Linc00152 and clinicopathological parameters

| Variables | n (%) | Expression of Linc00152 | | $\chi^2$ | *P* value |
|---|---|---|---|---|---|
| | | Low | High | | |
| Sex | | | | 0.381 | 0.537 |
| Male | 48 | 23 | 25 | | |
| Female | 29 | 16 | 13 | | |
| Age, y | | | | 1.628 | 0.202 |
| ≤ 60 | 43 | 20 | 23 | | |
| > 60 | 34 | 19 | 15 | | |
| Size | | | | 0.007 | 0.931 |
| ≤ 4 cm | 28 | 14 | 14 | | |
| > 4 cm | 49 | 25 | 24 | | |
| TNM stage | | | | 6.006 | 0.014* |
| I | 22 | 16 | 6 | | |
| II-IV | 55 | 23 | 32 | | |
| Fuhrman grade | | | | 3.163 | 0.075 |
| G1-G2 | 30 | 19 | 11 | | |
| G3-G4 | 47 | 20 | 27 | | |
| Vascular invasion | | | | 3.835 | 0.050 |
| Yes | 15 | 11 | 4 | | |
| No | 62 | 28 | 34 | | |
| Distant metastasis | | | | 0.945 | 0.331 |
| Yes | 20 | 12 | 8 | | |
| No | 57 | 27 | 30 | | |

*: $P < 0.05$.

## Results

### *Screening of ccRCC-specific lncRNAs*

The TCGA database was used to search for differentially expressed lncRNAs between ccRCC tissues and normal tissues. Based on ccRCC RNA-seq data, Linc00152 was found overexpressed in ccRCC tissues. As shown in **Figure 1A**, Linc00152 displayed the highest fold change (Tumor/Normal) in ccRCC. We also performed a differential expression analysis using the R software package ($DEseq_2$). **Figure 1B** shows the relationship between the gene expression data and $log_2$-fold changes.

### *Linc00152 is up-regulated in human ccRCC tissues and is associated with poor prognosis*

To explore the role of Linc00152 in ccRCC progression, we first examined its expression in 77 paired ccRCC tissues and adjacent normal tissues by qRT-PCR; the results were normalized to β-actin. As presented in **Figure 2A**, Linc00152 expression was significantly up-regulated compared with pair-matched noncancerous renal tissues (*P*=0.003). The level of Linc00152 was then detected in five human ccRCC cell lines (786-O, ACHN, A498, Caki-1 and Caki-2) and one normal kidney cell line (HK-2), revealing elevated Linc00152 expression in all five ccRCC cell lines compared with HK-2 cells (**Figure 2B**).

We next analyzed the correlation between Linc00152 expression levels and the clinicopathologic characteristics of the 77ccRCC patients (**Table 1**). Correlation regression analysis showed that high Linc00152 expression was significantly correlated with the TNM stage (*P*=0.014). However, no association was found between the Linc00152 expression level and other parameters such as age and tumor size.

We further examined whether Linc00152 expression correlated with outcome in ccRCC patients. Kaplan-Meier survival analysis and log-rank tests using patient postoperative survival was performed. The Kaplan-Meier survival curve demonstrated that high Linc00152 expression is a significant predictor of overall survival (**Figure 2C**, $P < 0.001$). Consistently, the high Linc00152 expression contributed to a significant poorer survival (n=525, $P < 0.0001$, log-rank test; **Figure 2D**) in another independent cohort available at the TCGA database. Univariate analysis identified that Linc00152 expression, TNM stage and Fuhrman grade are associated with prognosis (**Table 2**), and multivariate analysis confirmed the prognostic value of Linc00152 expression, indicating that the expression level may serve as an

**Table 2.** Univariate and multivariate analyses of clinicopathological factors for overall survival

| Variables | Univariate analysis | | | Multivariate analysis | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *P* value | HR | 95% CI | *P* value |
| Linc00152 expression | 3.616 | 1.734-7.541 | 0.001** | 2.577 | 1.233-5.387 | 0.012* |
| TNM stage (I, II-IV ) | 6.131 | 1.187-20.083 | 0.003** | 3.483 | 1.052-11.534 | 0.041* |
| Fuhrman grade (G1-G2, G3-G4) | 5.826 | 2.259-15.026 | < 0.001** | 4.159 | 1.604-10.787 | 0.003** |
| Sex (male, female) | 0.563 | 0.271-1.169 | 0.123 | | | |
| Age (≤ 60, > 60) | 0.979 | 0.946-1.014 | 0.244 | | | |
| Size (≤ 4 cm, > 4 cm) | 1.189 | 0.594-2.378 | 0.625 | | | |
| Vascular invasion (Yes, No) | 1.507 | 0.706-3.214 | 0.289 | | | |
| Distant metastasis (Yes, No) | 1.470 | 0.723-2.989 | 0.288 | | | |

Abbreviation: HR, hazard ratio. *$P < 0.05$, **$P < 0.01$.

independent prognostic factor for ccRCC patients. All these data suggest that Linc00152 plays a role in ccRCC progression and development.

*Enforced expression of Linc00152 promotes ccRCC cell proliferation and invasion*

To investigate the biological functions of Linc00152 in ccRCC, we first enhanced its expression by transfecting the Linc00152 expression vector pcDNA3.1-linc00152 into 7860 and Caki-2 cells, which showed the highest basic levels of this lncRNA. qPCR assays revealed significantly increased Linc00152 expression in the two cell lines (**Figure 3A**), and cell-counting kit 8 (CCK-8) assays indicated that heightened expression of Linc00152 significantly promoted cell proliferation in both cell lines (**Figure 3B**). Similar effects were observed in the colony formation assay, whereby the numbers of colonies were increased following Linc00152 overexpression compared with control cells (**Figure 3C**).

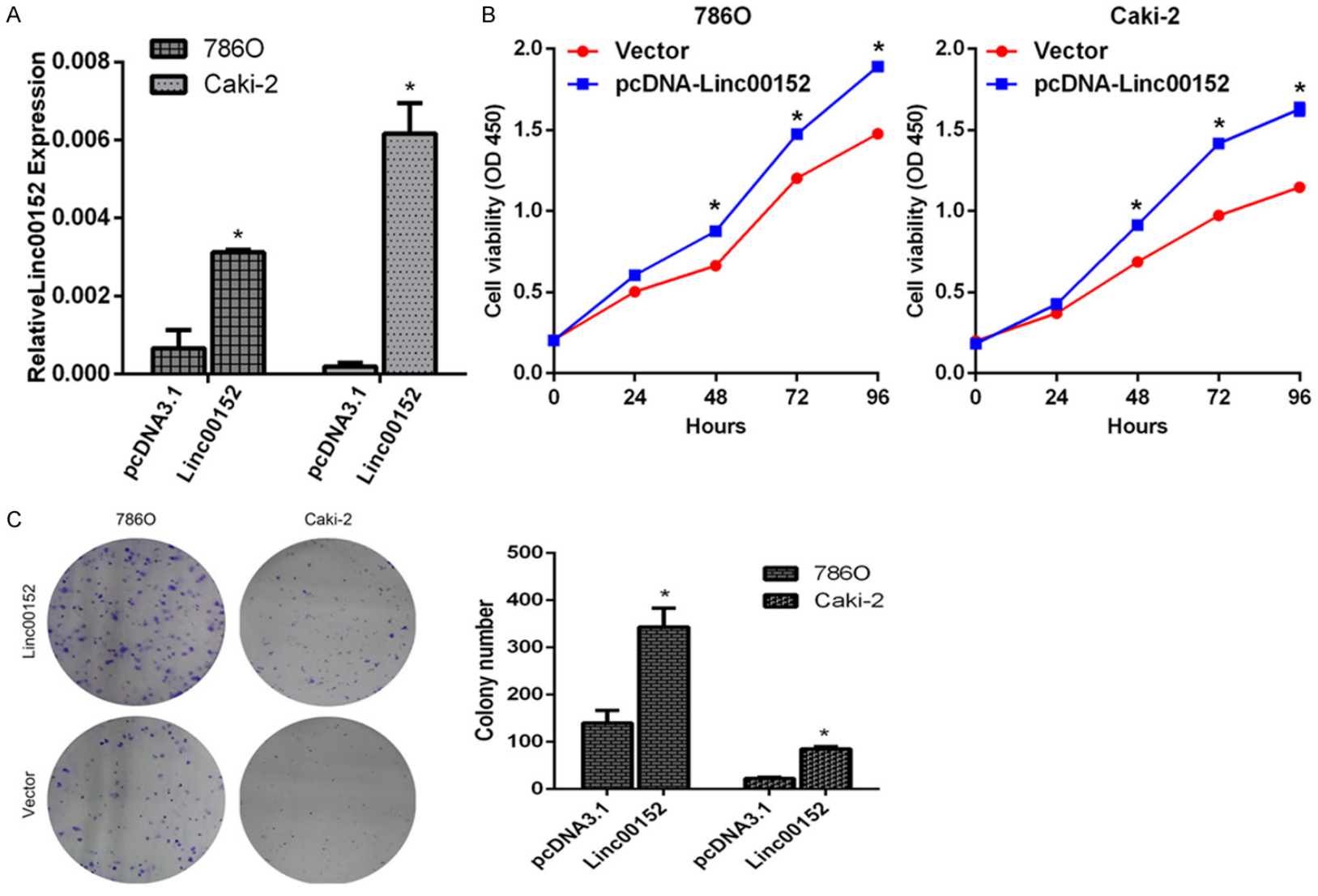
In evaluating the migration efficiency of RCC cells, a wound-healing assay revealed a faster scratch closure rate for cells with increased Linc00152 expression compared with control cells, which suggests enhanced mobility (**Figure 3D**). The transwell assay further confirmed that Linc00152 overexpression significantly increased the migration of 7860 and Caki-2 cells (**Figure 3E**).

To determine whether the proliferative effects of Linc00152 on RCC cells resulted from alteration of the cell cycle or apoptosis, a flow cytometry analysis was performed. The results indicated that Linc00152 overexpression significantly inhibited the arrest of both cell lines in $G_0/G_1$ phase ($P < 0.05$), with an obvious increase in the number of cells in S-phase ($P < 0.05$; **Figure 3F**). In addition, the proportions of apoptotic cells following pCDNA3.1-linc00152 transfection were significantly decreased compared with those in the control groups ($P < 0.05$; **Figure 3G**).

*Attenuated expression of Linc00152 inhibits ccRCC cell proliferation and invasion*

To further verify that Linc00152 expression is positively related to ccRCC progression, we employed two siRNA oligonucleotides to downregulate endogenous expression of this lncRNA in A498 and ACHN cells, which have the lowest levels of Linc00152. qPCR confirmed the efficiency of the siRNAs in the two cell lines (**Figure 4A**). As demonstrated by CCK-8 assays, repression of Linc00152 significantly decreased the proliferation of A498 and ACHN cells (**Figure 4B**), and colony formation in Linc00152-repressed A498 and ACHN cells was significantly reduced compared with the control groups (**Figure 4C**).

As cell invasion is an important aspect of cancer progression that involves the migration of tumor cells into contiguous tissues and the dissolution of extracellular matrix proteins, we evaluated the effects of Linc00152 on cell migration and invasion. A wound assay showed that renal cancer cells transfected with two siRNAs have a lower migration capacity than control cells ($P < 0.05$; **Figure 4D**). The results of transwell assays shown in **Figure 4E** demonstrate that transfection of siRNAs targeting Linc00152 impeded the migratory ability of A498 cells ($P < 0.05$), and a corresponding effect on invasiveness was also observed in a parallel invasion assay ($P < 0.05$).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786O
Caki-2
RelativeLinc00152 Expression
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Cell viability (OD 450)
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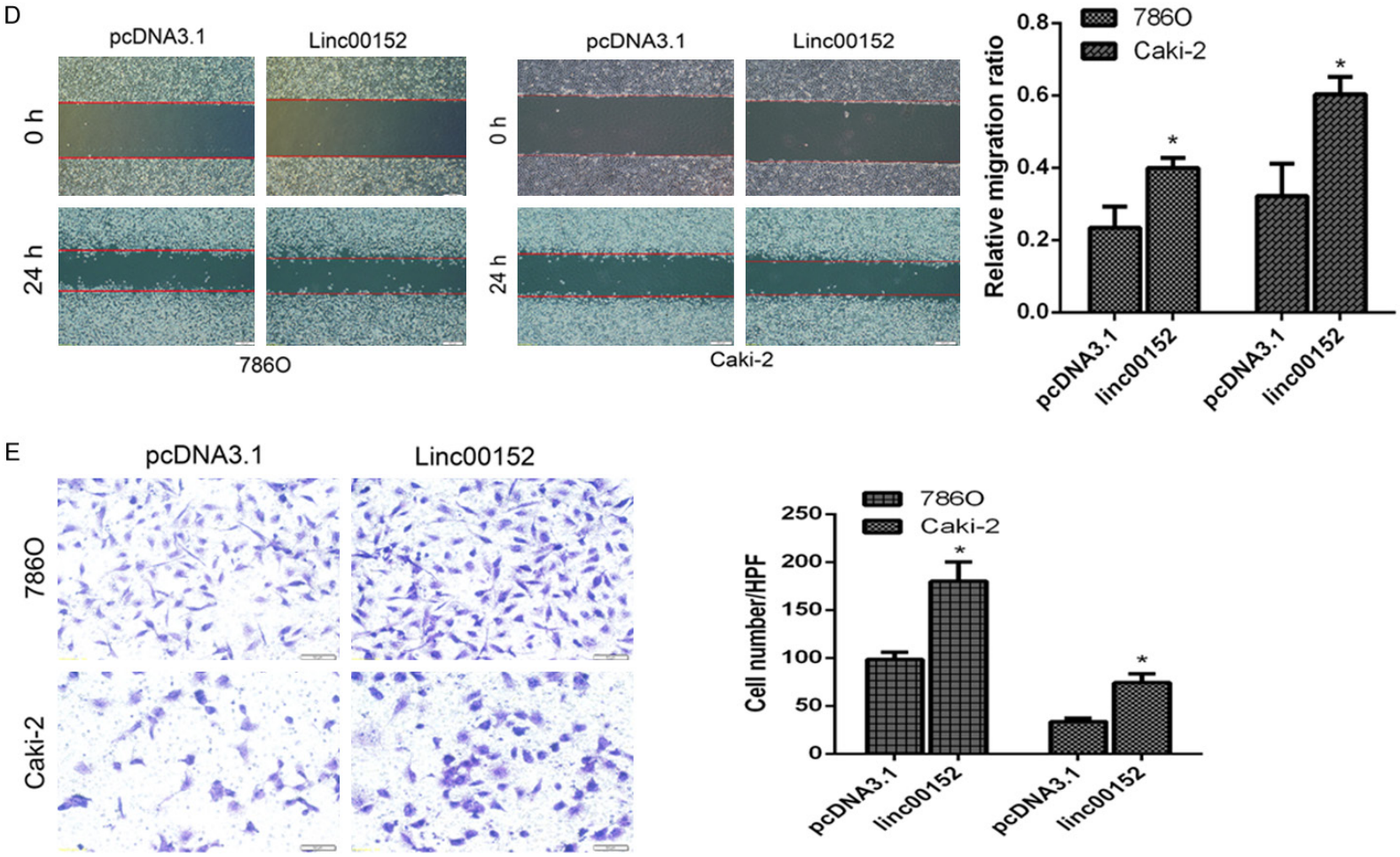
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0.6
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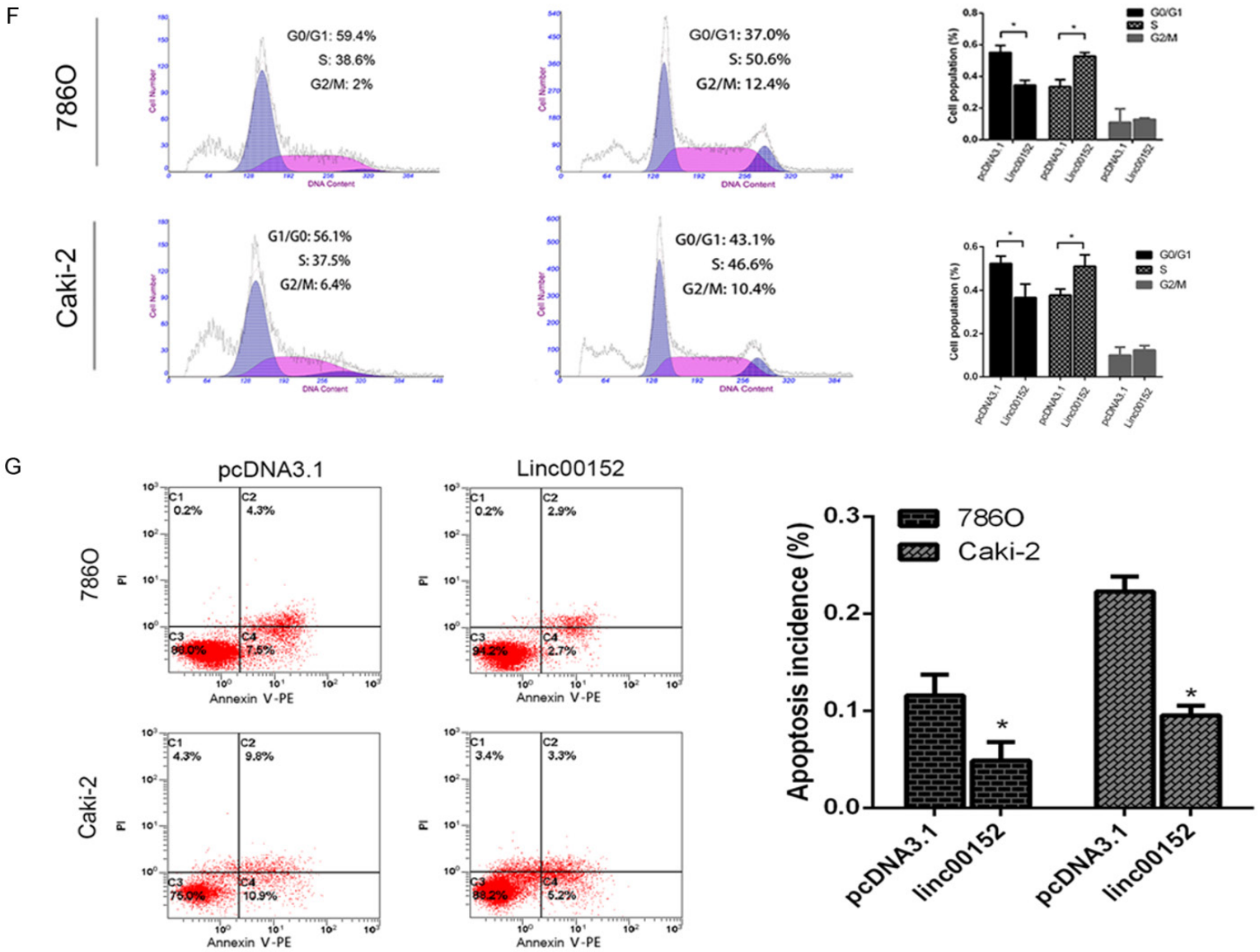
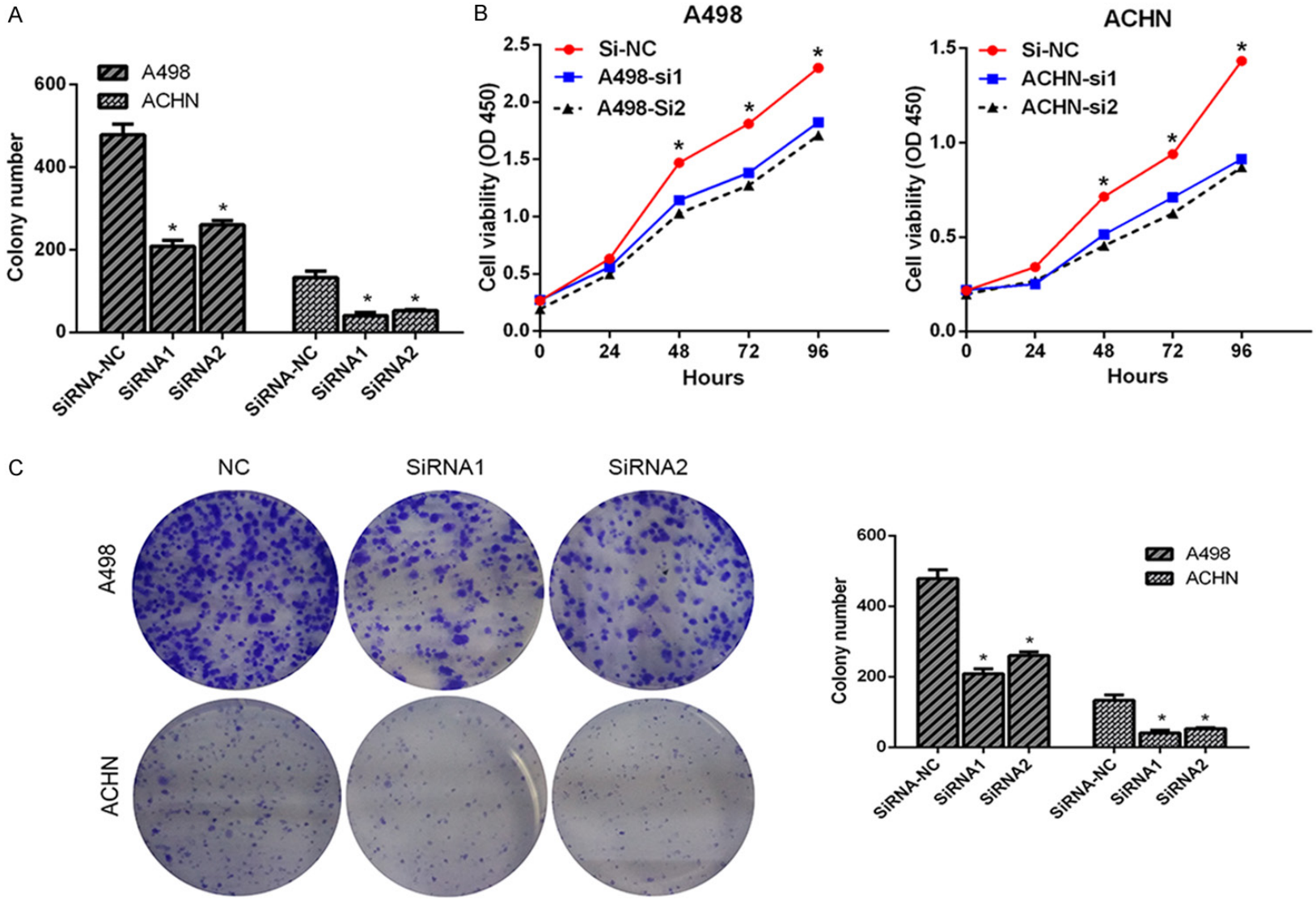
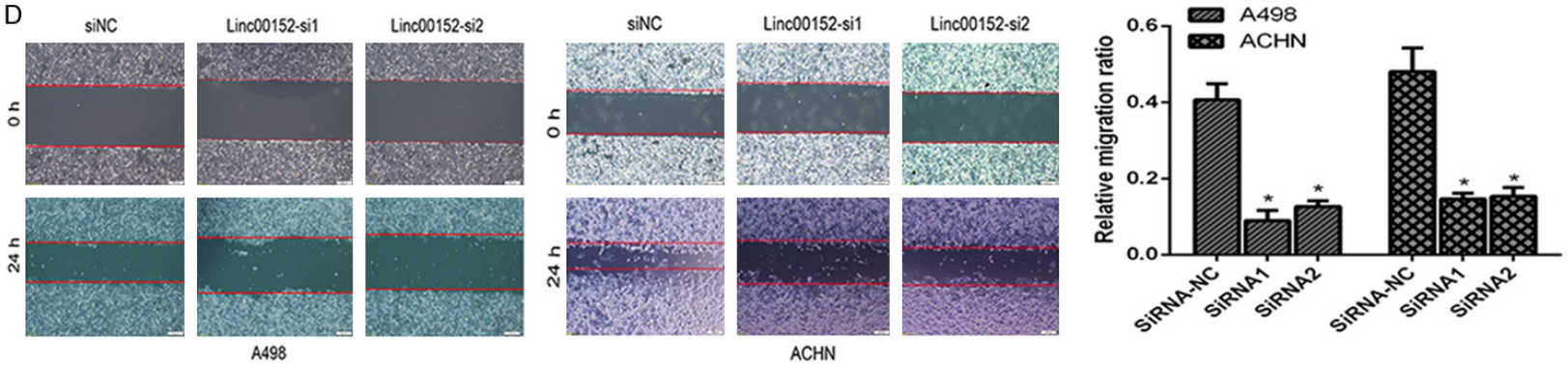



**Figure 3.** Linc00152 promotes the aggressiveness of RCC cells. (A) The relative expression level of Linc00152 in 7860 and Caki-2 cells was significantly increased by transfection of a Linc00152 expression vector. (B) Enhanced expression of Linc00152 in 7860 and Caki-2 cell lines significantly promoted their proliferative capacities, as determined by cell number counting and colony formation assays (C). (D) A wound-healing assay revealed that cells with enhanced Linc00152 expres-

sion exhibited a faster scratch closure rate than control cells. (E) A transwell assay further confirmed that Linc00152 overexpression significantly increased 7860 and Caki-2 cell migration. (F) Enhanced expression of Linc00152 resulted in cell arrest in G1 phase of the cell cycle (G) and a dramatic decrease in apoptosis. $^{*}P < 0.05$.

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Linc00152-si2
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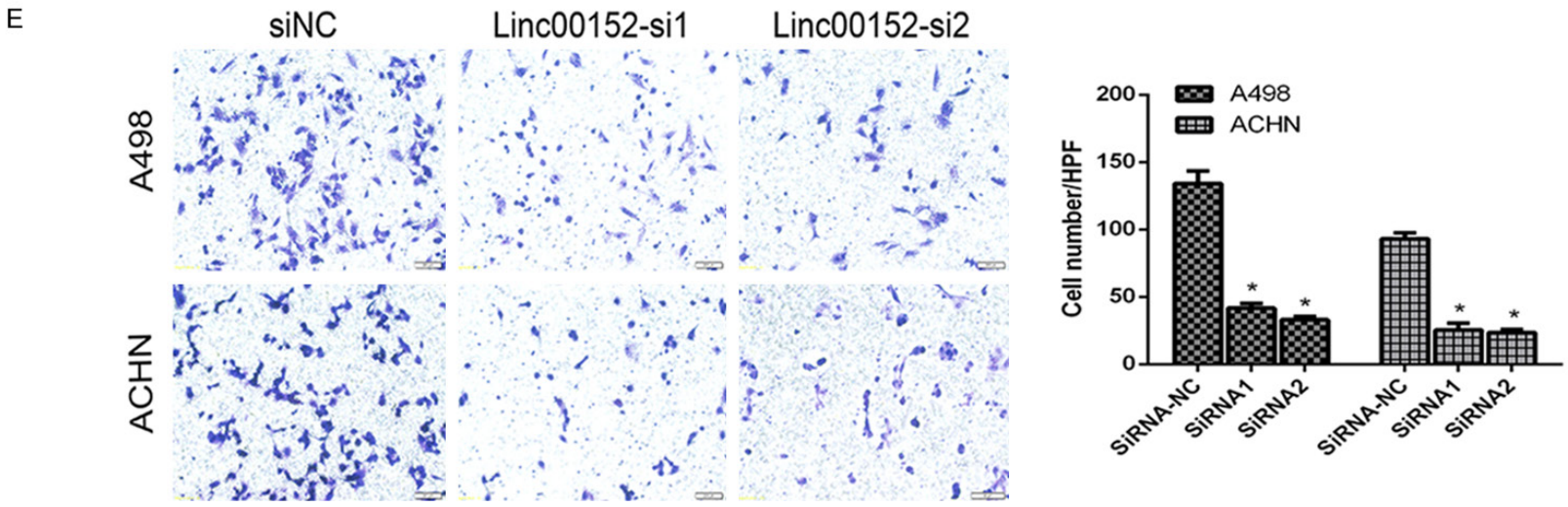
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Linc00152-si1
Linc00152-si2
A498
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Cell number/HPF
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100
50
0
SiRNA-NC
SiRNA1
SiRNA2

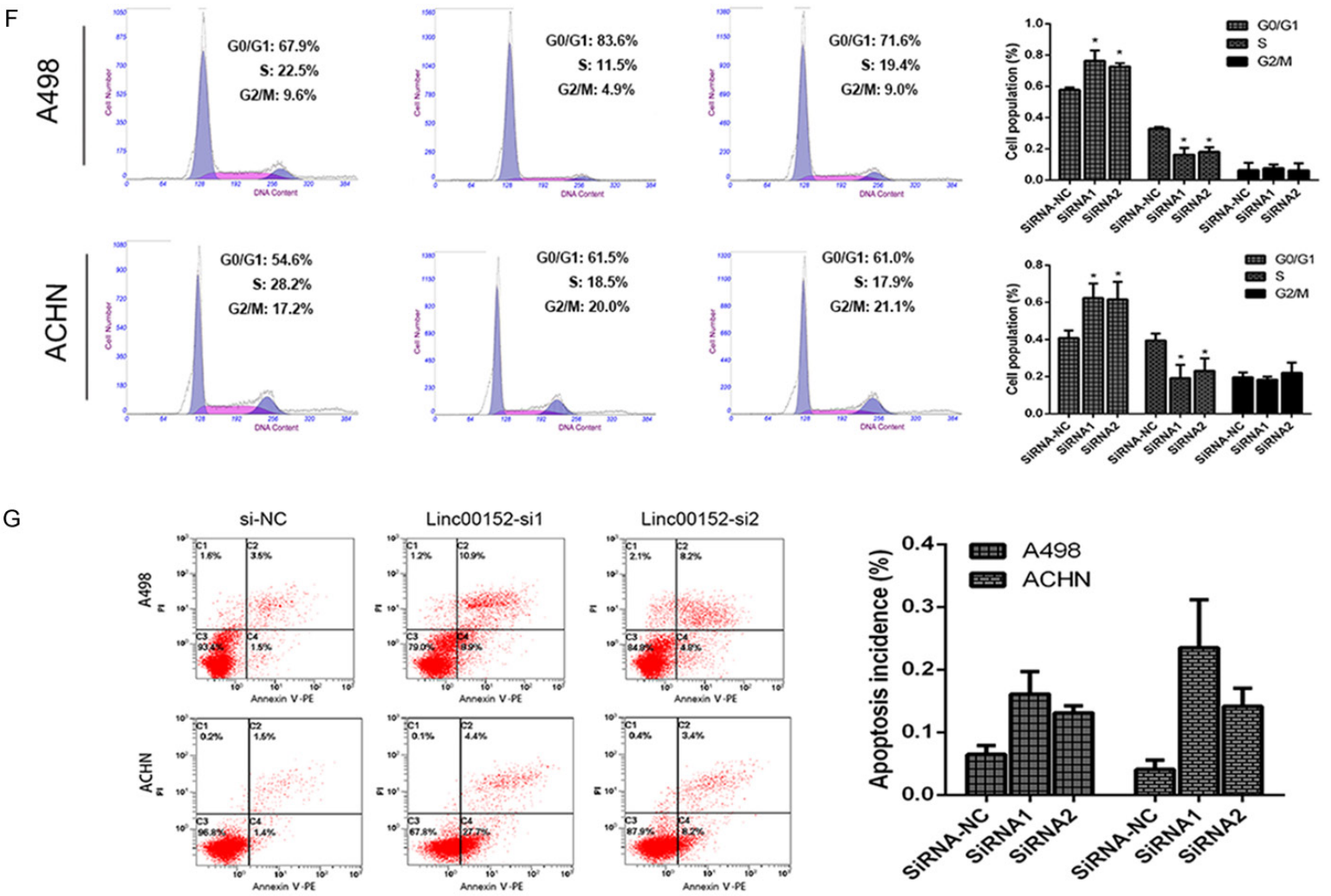


**Figure 4.** Knockdown of Linc00152 inhibits RCC cell proliferation and invasion. A. Knockdown efficiency in A498 and ACHN cells was determined by qRT-PCR. B. Repression of Linc00152 decreases proliferation in A498 and ACHN cells. The proliferative capacity was determined by the CCK-8 assay. C. Colony-forming assays were performed to determine the proliferation of A498 and ACHN cells. The colonies were counted and captured. D. A wound-healing assay revealed that cells with attenuated Linc00152 expression showed a slower scratch closure rate than control cells. E. Transwell assays were performed to investigate the invasive ability of A498 and ACHN cells. The numbers inside the bars represent the relative ratio of invaded cells (normalized to the control). F. The cell cycle was arrested in G1 phase in A498 and ACHN cells with Linc00152 silencing. G. Annexin V/PE staining and flow cytometry analysis was used to assess apoptosis in A498 and ACHN cells, with a significant increase in apoptosis was observed. $^{*}P < 0.05$.

Next, to further assess whether the effect on RCC cell proliferation occurred by altering cell cycle progression, we examined cell cycling in A498 and ACHN cells by flow cytometry. The data revealed that after transfected with two siRNAs, the progression of A498 and ACHN cells was significantly stalled at $G_0$-$G_1$ phase ($P < 0.05$; **Figure 4F**). In addition, we performed flow cytometric analysis of si-linc00152-treated A498 and ACHN cells to determine whether apoptosis was a contributing factor to cell growth inhibition. The data showed a significant increase in apoptotic cells in the si-linc00152-treated A498 and ACHN cells compared with the siNC-treated groups (**Figure 4G**).

## Discussion

At present, with the aid of high-throughput techniques, a variety of systematic cancer genomics projects, such as TCGA (*http://cancergenome.nih.gov/*), are being used to investigate different molecular pathways and genomic, transcriptomic, proteomic, and epigenomic alterations in each specific type of cancer. These investigations not only focus on the role of protein-coding genes but also include long non-coding transcripts, which have been shown to be involved in the regulation of a diverse array of biological processes [5, 6, 18-20]. For example, Hirata *et al.* [11] demonstrated that MALAT1 is markedly increased in RCC tissues and cell lines and that its overexpression promotes aggressive RCC through Ezh2 and interaction with miR-205. In addition, Guo *et al.* [21] identified three single-nucleotide polymorphisms (SNPs) in ZNRD1-AS1 and reported that these SNPs influence the susceptibility of cervical cancer by influencing ZNRD1 expression. By comparing tumor to peritumoral tissues using microarrays, Yang *et al.* [22] identified a small number of lncRNAs that are aberrantly expressed in hepatitis B virus-related HCC and found that hypoxia-induced histone deacetylase 3 represses lncRNA-LET by reducing histone acetylation-mediated modulation of the lncRNA-LET promoter region. In the present study, we analyzed ccRCC datasets based on the TCGA platform and identified a long non-coding RNA: Linc00152 and confirmed that the expression of Linc00152 was significantly higher in ccRCC tissues and cell lines. Clinically, increased expression of Linc00152 is an independent predictor of OS in ccRCC patients in our corhort. And the reliability of prognostication based on Linc00152 was further confirmed in a large-scale sample from the TCGA database, indicated that Linc00152 may potentially a reliable prognosis predictor of ccRCC.

Previous studies have demonstrated that Linc00152 functions as an oncogene in gastric cancer and cell lines [23, 24]; indeed, knockdown of Linc00152 inhibits the proliferation of gastric cancer cells [25]. These findings are consistent with our present report of increased Linc00152 expression in ccRCC, indicating that Linc00152 may be a common oncogenic lncRNA. Although a variety of lncRNAs are aberrantly expressed in several tumors, the mechanisms underlying such dysregulation are poorly understood. According to National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/gene/112597), Linc00152 is an intergenic lncRNA located on 2p11.2; BANP and HSD17B14 genes may interact with Linc00152 to promote tumor progression. In addition, to determine whether Linc00152 functions as a competing endogenous RNA (ceRNA) by competitively binding to common microRNAs (miRNAs), we performed a search for possible binding sites using the online software programs *starBase v2.0* (http://starbase.sysu.edu.cn/mirLncRNA.php) and *miRcode 11* (http://mircode.org) and found that Linc00152 contains complementary sites for the seed region of multiple ccRCC related miRNAs such as miR-138/138ab [26, 27] and miR-376c-3p suggests that Linc00152 may also function as a "sponge" [28] in ccRCC. Moreover, together with recent literature, we assume that Linc00152 may be involved in the cell cycle via binding to specific protein, similar to ZFAS1 [29], MINCR [30] and GAS5 [31]. Nonetheless, the precise mechanism requires further study.

The role of Linc00152 in cellular proliferation was also investigated in our study. We identified the function of Linc00152 in RCC cells by employing gain-of-function and loss-of-function approaches: overexpression of Linc00152 in lower-expressing A498 and ACHN cells could notably promote proliferation and migration and also inhibit G1 phase arrest and apoptosis, whereas inhibition of Linc00152 produced the opposite results. Thus, Linc00152 has an oncogenic function in ccRCC and represents a potential target for RCC treatment.

However, it is worth noting some unavoidable limitations in this study. First, whether the promotion of Linc00152 on the migration and

invasion of ccRCC cells was machaniclly based on it induce the epithelial-mesenchymal transition (EMT) or other intracellular course is remains unknown. Second, although we demonstrated that Linc00152 is overexpressed in ccRCC, the mechanisms underlying this dysregulation deserve further investigation.

## Conclusions

In summary, although our understanding of RCC pathogenesis has improved via the identification of activating mutations in and the amplification of oncogenes [32, 33], such as the VHL gene mutation [34], the mechanism of RCC progression, including the role of cell proliferation, apoptosis resistance, invasion, metastasis, and angiogenesis, has not been fully elucidated. Our study provides key evidence to support the hypothesis that overexpression of the oncogenic lncRNA, in addition to deregulation of protein-coding genes, is clinically and functionally relevant in the progression of human RCC. Cumulatively, these findings indicate that Linc00152 is a novel oncogene in ccRCC. Exploration of the precise role of Linc00152 in the pathogenesis of RCC will increase our understanding of the biological basis of cancer progression and may lead to the development of a novel diagnostic marker and therapeutic strategy for ccRCC.

## Disclosure of conflict of interest

None.

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## References

[1] Siegel R, Ma J, Zou Z and Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.
[2] Wang C, Yu C, Yang F and Yang G. Diagnostic accuracy of contrast-enhanced ultrasound for renal cell carcinoma: a meta-analysis. Tumour Biol 2014; 35: 6343-6350.
[3] Gupta K, Miller JD, Li JZ, Russell MW and Charbonneau C. Epidemiologic and socioeconomic burden of metastatic renal cell carcinoma (mRCC): a literature review. Cancer Treat Rev 2008; 34: 193-205.
[4] Russo P. Renal cell carcinoma: presentation, staging, and surgical treatment. Semin Oncol 2000; 27: 160-176.
[5] Esteller M. Non-coding RNAs in human disease. Nat Rev Genet 2011; 12: 861-874.
[6] Prensner JR and Chinnaiyan AM. The emergence of lncRNAs in cancer biology. Cancer Discov 2011; 1: 391-407.
[7] Gibb EA, Brown CJ and Lam WL. The functional role of long non-coding RNA in human carcinomas. Mol Cancer 2011; 10: 38.
[8] Ponting CP, Oliver PL and Reik W. Evolution and functions of long noncoding RNAs. Cell 2009; 136: 629-641.
[9] Li X, Wu Z, Fu X and Han W. Long noncoding RNAs: insights from biological features and functions to diseases. Med Res Rev 2013; 33: 517-553.
[10] Rinn JL and Chang HY. Genome regulation by long noncoding RNAs. Annu Rev Biochem 2012; 81: 145-166.
[11] Hirata H, Hinoda Y, Shahryari V, Deng GR, Nakajima K, Tabatabai ZL, Ishii N and Dahiya R. Long Noncoding RNA MALAT1 Promotes Aggressive Renal Cell Carcinoma through Ezh2 and Interacts with miR-205. Cancer Res 2015; 75: 1322-1331.
[12] Kuiper RP, Schepens M, Thijssen J, van Asseldonk M, van den Berg E, Bridge J, Schuuring E, Schoenmakers EF and van Kessel AG. Upregulation of the transcription factor TFEB in t(6;11)(p21;q13)-positive renal cell carcinomas due to promoter substitution. Hum Mol Genet 2003; 12: 1661-1669.
[13] Qiao HP, Gao WS, Huo JX and Yang ZS. Long non-coding RNA GAS5 functions as a tumor suppressor in renal cell carcinoma. Asian Pac J Cancer Prev 2013; 14: 1077-1082.
[14] Frevel MA, Sowerby SJ, Petersen GB and Reeve AE. Methylation sequencing analysis refines the region of H19 epimutation in Wilms tumor. J Biol Chem 1999; 274: 29331-29340.
[15] Chiesa N, De Crescenzo A, Mishra K, Perone L, Carella M, Palumbo O, Mussa A, Sparago A, Cerrato F, Russo S, Lapi E, Cubellis MV, Kanduri C, Cirillo Silengo M, Riccio A and Ferrero GB. The KCNQ1OT1 imprinting control region and non-coding RNA: new properties derived from the study of Beckwith-Wiedemann syndrome and Silver-Russell syndrome cases. Hum Mol Genet 2012; 21: 10-25.
[16] Dong L, Qi P, Xu MD, Ni SJ, Huang D, Xu QH, Weng WW, Tan C, Sheng WQ, Zhou XY and Du X. Circulating CUDR, LSINCT-5 and PTENP1 long noncoding RNAs in sera distinguish patients with gastric cancer from healthy controls. Int J Cancer 2015; 137: 1128-35.

[17] Qi P, Xu MD, Ni SJ, Shen XH, Wei P, Huang D, Tan C, Sheng WQ, Zhou XY and Du X. Down-regulation of ncRAN, a long non-coding RNA, contributes to colorectal cancer cell migration and invasion and predicts poor overall survival for colorectal cancer patients. Mol Carcinog 2015; 54: 742-750.
[18] Chen LL and Carmichael GG. Decoding the function of nuclear long non-coding RNAs. Curr Opin Cell Biol 2010; 22: 357-364.
[19] Fatica A and Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet 2014; 15: 7-21.
[20] Lalevee S and Feil R. Long noncoding RNAs in human disease: emerging mechanisms and therapeutic strategies. Epigenomics 2015; 7: 877-9.
[21] Guo L, Wen J, Han J, Jiang J, Xie S, Feng X, Wei B, Dai J, Zhang K, Qi J, Ma H, Shi J, Ren J, Zhang Y, Dai M, Hu Z and Li N. Expression quantitative trait loci in long non-coding RNA ZNRD1-AS1 influence cervical cancer development. Am J Cancer Res 2015; 5: 2301-2307.
[22] Yang F, Huo XS, Yuan SX, Zhang L, Zhou WP, Wang F and Sun SH. Repression of the long noncoding RNA-LET by histone deacetylase 3 contributes to hypoxia-mediated metastasis. Mol Cell 2013; 49: 1083-1096.
[23] Pang Q, Ge J, Shao Y, Sun W, Song H, Xia T, Xiao B and Guo J. Increased expression of long intergenic non-coding RNA LINC00152 in gastric cancer and its clinical significance. Tumour Biol 2014; 35: 5441-5447.
[24] Cao WJ, Wu HL, He BS, Zhang YS and Zhang ZY. Analysis of long non-coding RNA expression profiles in gastric cancer. World J Gastroenterol 2013; 19: 3658-3664.
[25] Zhao J, Liu Y, Zhang W, Zhou Z, Wu J, Cui P, Zhang Y and Huang G. Long non-coding RNA Linc00152 is involved in cell cycle arrest, apoptosis, epithelial to mesenchymal transition, cell migration and invasion in gastric cancer. Cell Cycle 2015; 14: 3112-23.
[26] Liang J, Zhang Y, Jiang G, Liu Z, Xiang W, Chen X, Chen Z and Zhao J. MiR-138 induces renal carcinoma cell senescence by targeting EZH2 and is downregulated in human clear cell renal cell carcinoma. Oncol Res 2013; 21: 83-91.
[27] Song T, Zhang X, Wang C, Wu Y, Cai W, Gao J and Hong B. MiR-138 suppresses expression of hypoxia-inducible factor 1alpha (HIF-1alpha) in clear cell renal cell carcinoma 786-0 cells. Asian Pac J Cancer Prev 2011; 12: 1307-1311.
[28] Lu M, Tian H, Cao YX, He X, Chen L, Song X, Ping P, Huang H and Sun F. Downregulation of miR-320a/383-sponge-like long non-coding RNA NLC1-C (narcolepsy candidate-region 1 genes) is associated with male infertility and promotes testicular embryonal carcinoma cell proliferation. Cell Death Dis 2015; 6: e1960.
[29] Thorenoor N, Faltejskova-Vychytilova P, Hombach S, Mlcochova J, Kretz M, Svoboda M and Slaby O. Long non-coding RNA ZFAS1 interacts with CDK1 and is involved in p53-dependent cell cycle control and apoptosis in colorectal cancer. Oncotarget 2016; 7: 622-37.
[30] Doose G, Haake A, Bernhart SH, Lopez C, Duggimpudi S, Wojciech F, Bergmann AK, Borkhardt A, Burkhardt B, Claviez A, Dimitrova L, Haas S, Hoell JI, Hummel M, Karsch D, Klapper W, Kleo K, Kretzmer H, Kreuz M, Kuppers R, Lawerenz C, Lenze D, Loeffler M, Mantovani-Loffler L, Moller P, Ott G, Richter J, Rohde M, Rosenstiel P, Rosenwald A, Schilhabel M, Schneider M, Scholz I, Stilgenbauer S, Stunnenberg HG, Szczepanowski M, Trumper L, Weniger MA; ICGC MMML-Seq Consortium, Hoffmann S, Siebert R and Iaccarino I. MINCR is a MYC-induced lncRNA able to modulate MYC's transcriptional network in Burkitt lymphoma cells. Proc Natl Acad Sci U S A 2015; 112: E5261-5270.
[31] Liu Y, Zhao J, Zhang W, Gan J, Hu C, Huang G and Zhang Y. lncRNA GAS5 enhances G1 cell cycle arrest via binding to YBX1 to regulate p21 expression in stomach cancer. Sci Rep 2015; 5: 10159.
[32] Fenner A. Kidney cancer: creating a molecular atlas of clear cell renal cell carcinoma genetics. Nat Rev Urol 2013; 10: 489.
[33] Fenner A. Genetics: a molecular atlas of clear cell renal cell carcinoma. Nat Rev Clin Oncol 2013; 10: 485.
[34] Ma X, Yang K, Lindblad P, Egevad L and Hemminki K. VHL gene alterations in renal cell carcinoma patients: novel hotspot or founder mutations and linkage disequilibrium. Oncogene 2001; 20: 5393-5400.