Screening for the Key lncRNA Targets Associated With Metastasis of Renal Clear Cell Carcinoma

Huibin Liu, MD, Peng Chen, MM, Chunyang Jiang, MD, Jing Han, MM, Bing Zhao, MM, Yujiao Ma, MM, and Mahmut Mardan, MM

Abstract: A large number of long noncoding RNAs (lncRNAs) have been found to be implicated in various tumors. However, the contributions of lncRNAs to tumorigenesis in renal clear cell carcinoma (RCCC) remain largely unknown.

We performed a genome-wide analysis of lncRNA expression in RCCC and matched nontumor (NT) tissues to identify new targets for further study of renal carcinoma.

The genome-wide analysis of lncRNA expression in 3 RCCC and matched NT tissues was conducted using 4 × 180K Agilent lncRNA Chips and 6 lncRNAs were selected and validated by qRT-PCR in 90 RCCC patients. The differentially expressed lncRNAs and mRNAs were recognized through P value and fold-change (FC) filtering. Potential targets associated with RCCC were identified by gene ontology and pathway analyses. Construction of the co-expression network was accomplished using Cytoscape.

A total of 3862 lncRNAs and 2935 mRNAs were deregulated in RCCC tissues, compared with paired NT tissues. PCR results showed that the expressions of these 6 lncRNAs were consistent with the chips. Moreover, the co-expression network analysis portended 641 nodes and 571 connections between 109 lncRNAs and 532 coding genes. Lastly, NONHSAT123350 could be involved in the pathogenesis of RCCC and its expression level was closely related to disease-free survival (DFS) and overall survival (OS) in patients without distant metastasis.

Our results indicated that these abnormal lncRNAs could respond to renal carcinoma progression and NONHSAT123350 may act as a potential target for future treatment of RCCC.

INTRODUCTION

Renal cell carcinoma (RCC) annually accounts for ~3% of adult malignancies, with estimated 150,000 new cases and almost 115,000 deaths.1,2 In kidney tumors, renal clear cell carcinoma (RCCC) is the most prevalent subtype, and basic research has shown that genetic events play a major role in the development of RCCC. Therefore, a better understanding of the global molecular changes that occur during tumorigenesis would help to enhance the effectiveness of current cancer therapies and to find new predictors for chemotherapies.

The sequencing of the human genome led to the astonishing discovery that protein-coding genes compose <3% of human DNA. Yet over 80% of our genome is actively transcribed to a versatile group of RNA transcripts without protein-coding potentials.3,4 Noncoding RNAs (NcRNAs), which were once considered as the “transcriptional noise” in the genome, have been extensively studied and categorized by the size of the RNA transcript into small ncRNAs (<200 bp), such as microRNAs (miRNAs), and long ncRNAs (>200 bp). Although small ncRNAs, in particular miRNAs, have been extensively studied >20 years and many aspects of their biology have been unraveled, still very little is known about the functional role of lncRNAs. Nevertheless, with the application of microarrays and high-throughput RNA-sequencing tools, more and more lncRNAs have recently been found to modulate gene expression at multiple levels, including epigenetic, transcriptional, and post-transcriptional modulation.

Moreover, accumulated evidences demonstrated that lncRNAs could play critical roles in various complex diseases.5,6 Up to now, although lncRNAs in kidney tumors have been reported, only preliminary studies on the roles of lncRNAs in RCCC have been performed,10 and the elaborate molecular mechanism of lncRNAs in carcinogenesis has not yet been well elucidated.

In the present study, we delineated the distinct lncRNA transcription patterns by cis- and/or trans-regulation of protein-coding genes. The lncRNAs’ biological functions were predicted through co-expressed mRNA annotations. We also discovered that lncRNAs may regulate extensive cellular processes and multiple signaling pathways that might be critical for RCCC occurrence and progression. In addition, we have discussed the clinicopathologic and prognostic value of 6 lncRNAs expressions in 90 RCCC samples. These results may provide...
novel insights of gene therapy at the IncRNA level and potential therapeutic targets in cancer patients.

MATERIALS AND METHODS

Patient Cohort Information

The primary tumor samples with paired nontumor (NT) samples were collected from 90 patients at the Affiliated Tumor Hospital of Xinjiang Medical University (Urumqi, China) who underwent nephrectomy or partial nephrectomy for RCCC. The patient donors had pathologically proven primary RCCC and did not receive preoperative radio-chemotherapy. The harvested specimens were snap-frozen and reserved in liquid nitrogen before testing. For these patients, 3 were used for microarray analysis of IncRNAs. All the clinical characteristics of the 90 with RCCC are shown in Table 1.

Ethics Statement

The Ethics Committee of the Affiliated Tumor Hospital of Xinjiang Medical University had approved the protocol of this study and the patients of all participants had been provided written informed consent prior to enrollment.

Coding-Noncoding Gene Profile and Microarray

The Human IncRNA expression microarray (4 × 180K, Agilent) provided global profiling of long transcripts, containing 78,243 IncRNAs and 32,776 mRNAs in human genome, which were selected from the most authoritative databases such as NONCODE, Gencode, Ensembl, and other relevant literatures. In order to improve the confidence of statistical results, every transcript was detected with 2 to 10 specific probes, aiming to exclude highly similar sequences or ncRNAs with the length <200 nt. Both IncRNA and protein-coding gene were represented on a separate array to supply coincident hybridization. RNA labeling and array hybridization were conducted according to the manufacturer’s protocol. In the present study, the genome-wide analysis of IncRNA expression in 3 RCCC and matched NT renal tissues were performed using 4 × 180K Agilent IncRNA Chips. The microarray work was executed by OE Bio-tech, Shanghai, PR China.

Data Analysis for Microarray Result

Microarray image documents (tiff format) were parsed using Feature Extraction software (version 10.7.1.1, Agilent Technologies) to derive source data as pair files for grid alignment and background correction. Expression data were normalized via quantile algorithm and further analyzed using the Agilent GeneSpring Software (version 12.0, Agilent). Differential expression of a IncRNA or mRNA was defined through P value and fold change. The statistical significance of a microarray result was identified through a difference with \( P < 0.05 \), and fold change filtering (FC > 2).

Disease-free survival (DFS) and overall survival (OS) were defined as the time from the date of surgery to the date of regional recurrence or distant metastasis and death or final clinical follow-up, respectively. The correlation between these 6 IncRNAs and clinicopathologic characteristics was assessed using the Pearson test. Actuarial survival rates were plotted against time using the Kaplan–Meier method, and log-rank testing was used to compare the differences between the curves. Because the risk quotient (RQ) value of NONHSAT123350 in most of the 90 RCCC tissues was 0.001 to 0.1, we defined an RQ value of 0.001 to 0.1 as low expression of NONHSAT123350, and an RQ value of 0.1 to 1.0 was considered high expression.

Any value of \( P < 0.05 \) was considered statistically significant.

Gene Ontology (GO) Analysis and Pathway Analysis

The divined target genes in the mRNA expression profile were utilized for the GO term enrichment. They were imported into the Funnet Database (http://www.genome.ad.jp/kegg/), which showed various functional categories of GO terms in the gene profile. In addition, the differentially expressed mRNAs were input to Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.ad.jp/kegg/) to be classified into different annotation groups. The lower the \( P \) value, the more prominent the relevance and a threshold of \( P < 0.05 \) was regarded as obvious discrepancy.

Analysis of IncRNA-Transcript Factor (TF)-mRNA Correlation Network

To categorize IncRNAs that potentially have transregulating functions, the IncRNA target predictions were superimposed onto the IncRNA-TF or IncRNA-TF-mRNA correlation network using Cytoscape (http://www.cytoscape.org). In this network, green circular nodes represent mRNAs, yellow triangular nodes represent transcript factor, whereas red quadrangle nodes represent IncRNAs.

RNA Extraction

Frozen tissues were homogenized by an electric homogenizer, and total RNA was extracted using the TRizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. RNA quantification and purity were assessed using a NanoDrop ND-2000 spectrophotometer (Thermo Scientific) to measure absorbance ratios of A260/A280 and A260/A230, with RNA integrity evaluated by standard denaturing agarose gel electrophoresis. All samples were frozen in liquid nitrogen followed by storing at \(-80^\circ C\) for further analysis.

Quantitative RT-PCR

Total RNA was reversely transcribed using a PrimeScript RT Mix (Takara, Dalian, China) in accordance with the manufacturer’s instructions. Following amplified by RT-PCR, melting curve analysis was executed to identify the specific generation of the PCR product with GAPDH used as internal controls. Fold changes for aberrant expression were calculated using the \( 2^{-\Delta\Delta Ct} \) method. Differences in IncRNA expression

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**TABLE 1. Clinical Pathologic Characteristics of Study Cohort**

| Samples | Nonmetastasis Recurrence and Metastasis |
|---------|-----------------------------------------|
|         | \( n = 72 \) | \( n = 18 \) |
| Age     | \( < 60 \)  | 35  | 9  |
|         | \( \geq 60 \) | 37  | 9  |
| Sex     | Male       | 46  | 11 |
|         | Female     | 26  | 7  |
| Tumor stage | 1–2     | 56  | 14 |
|         | 3–4       | 16  | 4  |
| Survival|           | 59  | 10 |
| Death   |           | 13  | 8  |
levels between RCCC samples and NT samples were evaluated by means of Student’s t test, with P < 0.05 viewed to be significantly different. Prime information could be found in Statistical Analysis by means of Student’s t test, with P < 0.05 viewed to be statistically significant. The false discovery rate was reckoned up to rectify the predefined P value (<0.05). The threshold set for up- and downregulated genes was a fold change ≥2.0 and a P value < 0.05. The correlation between lncRNA and clinical and pathological characteristics was analyzed by the Pearson correlation test. P < 0.05 was considered significant.

### RESULTS

**lncRNAs and mRNAs Expression Profiles in RCCC**

To depict abnormally expressed genes in RCCC, we accomplished a global genome expression analysis of lncRNA and mRNA in renal carcinoma and matched nontumor tissues. Among the 78,243 lncRNA transcripts examined, 3862 lncRNAs were differentially expressed (P < 0.05, 2.0-fold change) in RCCC samples relative to their matched counterparts, whereas 2243 were downregulated (Table S1, http://links.lww.com/MD/A622). The miRNA expression profiling data showed 2935 miRNAs to be differentially expressed (P < 0.05, 2.0-fold change) relative to their matched counterparts, with 1619 upregulated, whereas 2243 were downregulated (see Table S1, http://links.lww.com/MD/A622). The miRNA expression profiling data showed 2935 miRNAs to be differentially expressed (P < 0.05, 2.0-fold change) relative to their matched counterparts, with 1619 upregulated, whereas 2243 were downregulated (Table S2, http://links.lww.com/MD/A626). Normalized microarray expression data were used to generate heat map (Figure 1A and B). In order to verify the reliability of our results, differentially expressed lncRNAs acquired by using 4 × 180K Agilent lncRNA Chips were validated by TGCA RCCC RNA-seq database (https://tga-data.ncri.nih.gov/tga/) which included 448 RCCC and 67 matched nontumor (NT) tissues and the same results were obtained. The work was executed by Ruibo Bio-tech, Guangzhou, PR China.

**Real-Time Quantitative PCR Confirmation**

To validate the microarray analysis findings, the expression level of 6 lncRNAs, which may play a role in the regulation of protein-coding genes related to RCCC pathogenesis by lncRNA function prediction, were analyzed using the quantitative real-time polymerase chain reaction (qRT-PCR) (Table S3, http://links.lww.com/MD/A625). For the lncRNAs, the results demonstrated that TCONS_12_00012336, NR_046028.1, and NONHSAT123350 were downregulated, and that NONHSAT024642, NONHSAG019720, and NONHSAG010125 were upregulated in RCCC tissues relative to their matched counterparts, and these results were consistent with the microarray data (Figure 2). By using analysis of Pearson correlation analysis, we found that NONHSAT123350 is closely associated with the metastasis of renal cell carcinoma (r = 0.92). However, there was no significant relationship between the clinicopathologic characteristics (ie age, sex, tumor stage, and metastasis) and the expression levels of the other 5 lncRNAs (r < 0.864) (Table 3).

### Gene Ontology (GO) and Pathway Analysis

Through GO analysis, we found that the correlated targets of specific lncRNAs were associated with biological process, cellular component, and molecular function (Figure 3 and Table S4, http://links.lww.com/MD/A623). In the classification of GO biological processes, the majority of lncRNA targets were implicated in signal transduction, transmembrane transport, and ion transport. Most of target genes concerned with cellular components were resided in cytoplasm. And target genes associated with ATP binding, calcium ion binding and oxidoreductase activity dominated the top 3 among the GO terms. Pathway analysis was used to identify significant pathways for the differential genes according to the Kyoto Encyclopedia of Genes and Genomes (Figure 4). The maximum-enriched-

**TABLE 2. PrIMERS USED FOR qRT-PCR ANALYSIS OF lncRNA LEVELS**

| Target ID          | Primers                             | Product Length (bp) | Tm (°C) |
|--------------------|-------------------------------------|---------------------|---------|
| TCONS_12_00012336  | Forward: TACGCCTTCTATGACAACTGCCAC  | 86                  | 60      |
|                    | Reverse: ATCAGGCTCACGGTGACCTCAAAG   |                     |         |
| NR_046028.1        | Forward: TCAGTCATCCGGAGTAAAGGTC     | 101                 | 60      |
|                    | Reverse: CTTGAGCCTTCAGGAAAGAGG      |                     |         |
| NONHSAT123350      | Forward: CTTGCTCCGTCCTCAATCTC       | 79                  | 60      |
|                    | Reverse: TGAAAAACTTGCTGAAGACTTC     |                     |         |
| NONHSAG010125      | Forward: TC GGCTGGT CATCTGTGTAAC    | 83                  | 60      |
|                    | Reverse: TACACAGATCGACGGCATGTGG     |                     |         |
| NONHSAG019720      | Forward: CTTCCAGTGCGCTCAAGCCCTC     | 82                  | 60      |
|                    | Reverse: TCTGCAGAAGTGTCACACCTC      |                     |         |
| NONHSAT024642      | Forward: CATACCAAGCAGCAGCCACTC      | 82                  | 60      |
|                    | Reverse: CAATCACAGCCAGGATGACAC      |                     |         |
| GAPDH              | Forward: CATGAGAAGTATGACAACAGCT     | 113                 | 60      |
|                    | Reverse: AGTCCCTCCACAGTACCAAAGT     |                     |         |

lncRNAs = long noncoding RNAs, qRT-PCR = quantitative real-time polymerase chain reaction.
The table lists the 6 primers used for qRT-PCR. The primer for GAPDH was used as control for RNA integrity.
pathway relating to metabolism support the viewpoint that kidney cancer is a metabolic disease.

Construction of the Co-Expression Network

We have built a gene coexpression network of the coding–noncoding genes that comprised aberrantly expressed lncRNAs and neighboring protein-coding genes (distance < 300 kb) with Pearson’s correlation coefficients not < 0.94. This coexpression network consisted of 641 nodes and 571 connections between 109 lncRNAs and 532 coding genes. Within this network, 451 pairs presented as positive and 120 pairs as negative (Table S5, http://links.lww.com/MD/A621). Using the hypergeometric distribution method, we computed $P$ values for enrichment between differentially expressed lncRNAs and transcription factors (TFs) (the recommend $P$ value cutoff is 0.05). The data indicated that most of these potential transregulatory lncRNAs could participate in pathways regulated by 3 TFs: NF-κB1, spi1, and BATF. In the core network of lncRNA-TF pairs, NF-κB1 participates in 222 of the 1092 pairs, spi1 in 439 pairs, and

![Figure 1](image1.png)

**FIGURE 1.** Transcriptomic landscape of kidney cancer. (A) Hierarchical clustering analysis of 3862 aberrant lncRNAs and (B) 2935 aberrant mRNAs. Red and green colors indicate high and low expressions, respectively. In the heatmap, columns represent samples and rows represent each gene. The scale of expression level is shown on the horizontal bar. lncRNAs = long noncoding RNAs.

![Figure 2](image2.png)

**FIGURE 2.** qRT-PCR validation of 6 differentially expressed lncRNAs in 90 RCCC samples. The result showed that expressions of lncRNAs were consistent with the microarray data in neoplastic tissues relative to the paired non-neoplastic tissues. lncRNAs = long noncoding RNAs, qRT-PCR = quantitative real-time polymerase chain reaction, RCCC = renal clear cell carcinoma.
BATF in 431 pairs (Figure 5 and Table S6, http://links.lww.com/MD/A624). As shown in Figure 5, the 3 TFs were potential targets in transcriptional regulation of gene expression. When the top 300 co-expression pairs in P value order were clustered in the lncRNA-TF-mRNA network, NF-κB1 was identified as a transcription regulator acting a central role in signaling pathways (Figure 6).

**Relationship Between NONHSAT123350 Expression and RCCC Patient Survival**

Of the 90 patients with RCCC, none was lost to follow-up. The median observation period was 32 months (range, 3 to 60 months), with 53 patients alive and 37 cancer-related deaths at the last clinical follow-up. The median DFS and OS were 27 and 32 months, respectively. The 1-, 3-, and 5-year survival rates for the entire cohort of patients were 86.7%, 60.0%, and 14.4%, respectively. In the Kaplan–Meier analysis, NONHSAT123350 expression was closely associated with DFS and OS. For the whole cohort, median DFS was 27 months longer for patients with high NONHSAT123350 expression (32 months) than for patients with low NONHSAT123350 expression (21 months; \( P = 0.009 \); Figure 7A). Median OS among patients with NONHSAT123350 high expression tumors was also longer than among patients with NONHSAT123350 low expression tumors (38 versus 26 months; \( P = 0.032 \); Figure 7B).

**DISCUSSION**

The recent “noncoding RNA rush” is sparked by 2 new subcategories of molecules: short RNA species represented by miRNAs of 20–24 nt in length and long non-coding RNAs (lncRNAs) of >200 nucleotides in length with lack of protein-coding capability.\(^12\) In the past 20 years, overwhelming evidence has strongly supported the involvement of miRNAs in the pathogenesis of a majority of cancers. However, miRNAs could represent just a particular group of ncRNAs involved in human cancers. Recent findings have showed that lncRNAs are key regulators of gene expression that guide essential processes in tumorigenesis, suggesting that this emerging class of molecules offer a new avenue for diagnosing and treating diseases.\(^13–15\) Xist/Tsix, H19, HOTAIR, and AIR are emblematical examples of lncRNAs whose functions are

| lncRNAs | Age | Sex | Stage | Metastasis |
|---------|-----|-----|-------|------------|
| NONHSAT024642 | 0.52 | 0.54 | 0.30 | 0.81 |
| NONHSAG019720 | 0.67 | 0.33 | 0.47 | 0.81 |
| NONHSAG010125 | 0.15 | 0.24 | 0.36 | 0.10 |
| TCONS_12_00012336 | 0.12 | 0.78 | 0.55 | −0.11 |
| NR_046028.1 | 0.35 | 0.15 | 0.14 | −0.53 |
| NONHSAT123350 | 0.40 | 0.21 | 0.30 | −0.92 |

lncRNAs = long noncoding RNAs.
extensively being investigated. There have been reports of a genome-wide lncRNAs expression patterns in RCCC by microarray, whereas it should be stressed that it may be only the tip of the iceberg in terms of functional characterization of lncRNAs in the renal system.

In the study, we paid close attention to description of the lncRNA expression profile of RCCC, and explanation of differentially expressed genes between RCCC tissues and adjacent normal renal tissues. We examined 3 pairs of cancerous and matched NT specimens by microarray technology and limited lncRNAs were selected and validated by qRT-PCR in ninety pairs of samples. Owing to its large pool of probes including 78,243 lncRNAs and 33,045 mRNAs in the microarray chip, a great deal of lncRNAs can be quantitatively detected. Overall comprehensive analysis of lncRNA expression profile in RCCC was carried out with a view to clarifying the possible part of lncRNAs in the tumorigenesis of RCCC. According to microarray results, 3862 lncRNAs were differentially expressed (P < 0.05, 2.0-fold change) in RCCC samples relative to their matched counterparts, with 1619 upregulated, whereas 2243 were down-regulated, and most of which still required further exploration.

On the basis of lncRNA function prediction, 6 lncRNAs (TCONS_12_00012336, NR_046028.1, NONHSAT123350, NONHSAT024642, NONHSAG019720, and NONHSAG010125) were selected to validate the consistency. Expressions of these 6 lncRNAs were further evaluated by qPCR in 90 pairs of samples. And real-time PCR results showed the expressions of these 6 lncRNAs were consistent with the chips. NONHSAT123350 is significantly correlated with metastasis of renal cell carcinoma by Pearson correlation analysis. However, there was no significant relationship between the clinicopathological characteristics (ie age, sex, tumor stage, and metastasis) and the expression levels of other 5 lncRNAs (r < 0.864). IncRNA NONHSAT123350 is a 370-bp intragenic lncRNA located on Chromosome 7, whereas this RNA was uncharacterized and the biological functions were still unclear. Although noncoding RNAs in body fluid, especially microRNAs, had been identified as possible biomarkers, it was too early for us to utilize the IncRNA NONHSAT123350 as the possible biomarker to estimate prognosis in RCCC just based on the present data. When analyses focused exclusively on the survival of patients, the NONHSAT123350 expression level was shown to be a significant predictor in surgically resected RCCCs. Median DFS and OS were obviously longer among patients with high NONHSAT123350 expression than among patients with low NONHSAT123350 expression. These findings have not been reported elsewhere, and we cannot make a general statement concerning the influence of NONHSAT123350 expression on the clinical outcome for RCCCs. Nevertheless, we postulate that NONHSAT123350 is maintained at a normal expression level in normal renal epithelial cells, which balances cell behaviors, such as proliferation, differentiation, mitotic cycle, and apoptosis. Collectively, these data show that NONHSAT123350 may be a novel biomarker that is a potentially important predictor of the survival of RCCC patients.

Some studies have demonstrated that lncRNAs are involved in transcriptional regulation and its expression can be deregulated in human cancers. Transregulating lncRNAs dissociate from the site of synthesis, influence genes a long genomic distance away, and tend to converge on and act within large regulatory networks. We predicted functions of transregulatory lncRNAs through the TFs that possibly regulate their expression. In the core network of lncRNA-TF pairs, the lncRNAs can be grouped into 3 categories of pathways.
FIGURE 5. LncRNA-TF core network consisting of the top 100 pairs of lncRNA and TF with the most relevance. The red squares represent lncRNAs, and yellow triangles represent TFs; the edges between them mean that the lncRNAs are potentially regulated by the TFs. lncRNAs = long noncoding RNAs, TF = transcript factor.
regulated by NF-κB1 (p50), BATF, and SPI1. Based on the lncRNA-TF-mRNA analysis (Figure 6), we are fascinated by NF-κB1 and lncRNA NONHSAT123350. NF-κB is a widely expressed molecule with a wide range of biological functions including a role in regulating inflammation, cell differentiation, apoptosis and cell proliferation.25–29 On the basis of our data, NF-κB1 was recruited by >200 lncRNAs in pathway analysis, suggesting that there might be co-ordinated patterns of lncRNAs and transcription factors involved in the development of RCCC.

To understand the functions of lncRNAs further, in the present study we applied pathway analysis to associate these differentially expressed lncRNAs with their target genes and found that most of these potential trans-regulatory lncRNAs could participate in pathways regulated by 3TFs: NF-κB1, spi1, and BATF. The gene category “metabolic pathway” is the maximum-enriched-pathway of these pathways which supported the viewpoint that kidney cancer is a metabolic disease. And as far as we know, this is the first study to describe the possible role of lncRNA NONHSAT123350 in the development and/or progression of this carcinoma, which provides an lncRNA point-of-view on tumor biology and will stimulate new research directions and therapeutic options for considering this lncRNA as novel prognostic markers and therapeutic targets for RCCC.

FIGURE 6. LncRNA-TF-mRNA core network consisting of the top 300 pairs of lncRNA, TF, and mRNA with the most relevance. The red squares represent lncRNAs, yellow triangle represents TF, and green circles represent mRNAs. lncRNAs = long noncoding RNAs, TF = transcript factor.
FIGURE 7. Kaplan–Meier survival analysis of lncRNA NONHSAT123350 expression in RCCC patients: (A) disease-free curves and (B) overall survival for the whole cohort of patients with RCCC. lncRNAs associated long noncoding RNAs and aberrant alternative splicings.

CONCLUSIONS

Emerging studies are starting to link distinct types of mutations in lncRNA genes with a variety of neoplastic diseases. Our results demonstrated that 3862 lncRNAs were differentially expressed between renal carcinoma and non-tumor samples. Further investigations are warranted to clarify the mechanisms lying behind these differences. It is of great interest to filter out particular lncRNAs for the diagnosis, prognosis, and therapeutic evaluation of RCCC. LncRNA NONHSAT123350 is a key molecule closely related to metastasis of RCCC and may provide as a potential target for future treatment of RCCC and offer novel insights into cancer biology.

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