Long Noncoding RNA LINC02747 Promotes the Proliferation of Clear Cell Renal Cell Carcinoma by Inhibiting miR-608 and Activating TFE3

Xiang Ju

soochow university

Yangyang Sun

Soochow University

Feng Zhang

Soochow University

Xiaohui Wei

Soochow University

Zhenguo Wang

Soochow University

Xiaozhou He (✉ Xiaozhouhe123@163.com)

soochow university <https://orcid.org/0000-0002-5437-2948>

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Abstract

Background: With the rapid development of biotechnology, long noncoding RNAs (lncRNAs) have exhibited good application prospects in the treatment of cancer, and they may become new treatment targets for cancer. This study aimed to explore lncRNAs in clear cell renal cell carcinoma (ccRCC).

Methods: Differentially expressed lncRNAs in 54 pairs of ccRCC tissues and para-carcinoma tissues were analyzed in The Cancer Genome Atlas (TCGA), and the most significant lncRNAs were selected and verified in ccRCC tissues.

Results: It was found that lncRNA LINC02747 was highly expressed in ccRCC, which was closely related to high TNM stage and histological grade and poor prognosis of patients. The in vivo and in vitro experiments confirmed that LINC02747 could promote the proliferation of ccRCC cells. We also found that LINC02747 regulated the proliferation of RCC cells by adsorbing miR-608. Subsequent mechanistic research showed that miR-608 is downregulated in ccRCC, and overexpression of miR-608 can inhibit the proliferation of RCC cells. Moreover, it was found that TFE3 is a direct target gene of miR-608. MiR-608 regulated the proliferation ability of RCC cells by inhibiting TFE3. In conclusion, LINC02747 upregulates the expression of TFE3 by adsorbing miR-608, ultimately promoting the proliferation ability of ccRCC cells.

Conclusions: LINC02747 acts as an oncogene in ccRCC and may be developed as a molecular marker for the diagnosis and prognosis of ccRCC. The LINC02747/miR-608/TFE3 pathway may become a new therapeutic target for ccRCC.

Introduction

Renal cell carcinoma (RCC) is the most common malignant tumor originating from the kidney. According to statistics by GLOBOCAN, RCC accounts for approximately 2–3% of all malignant tumors in adults [1]. Recently, the morbidity and mortality rates of RCC have increased significantly, especially in young patients and those with high-grade tumors [2]. According to the latest data from the Cancer Registry of the National Cancer Center of China, the incidence rate of RCC has also increased significantly in China year by year, and there were 45,096 new cases of RCC in 2011 [3]. Clear cell RCC (ccRCC) is the most common and malignant subtype of RCC, accounting for 75–80% of RCC cases [4]. Currently, radical nephrectomy is the major and most effective treatment modality for RCC. However, due to the lack of molecular markers for early diagnosis, approximately 25–30% of patients with RCC are already metastasized when diagnosed, with a median survival time of no more than 2 years [5]. A total of 20–40% of patients with RCC suffer from metastasis after radical nephrectomy [6] and have a poor prognosis. Therefore, it is particularly important to clarify the molecular mechanism of the development of ccRCC and identify molecular markers for early diagnosis and new therapeutic targets.

Approximately 70–90% of the human genome has transcriptional activity, but less than 2% of genes can encode proteins [7]. With the completion of the Human Genome Project and the improvement of deep

sequencing techniques, 95% of transcripts are noncoding RNAs (ncRNAs) [8]. Long ncRNAs (lncRNAs) are longer than 200 nt in length. LncRNAs play an important role in a variety of biological processes [9]. LncRNAs have tissue-specific expression, and they play an important role in cell cycle regulation, cell growth, immune response and cell pluripotency [9]. More importantly, some lncRNAs are involved in multiple tumor-related pathways and play an important role in the occurrence and development of tumors [10]. With the rapid development of biotechnology, lncRNAs have exhibited good application prospects in the treatment of cancer, and they may become new treatment targets for cancer. In this study, differentially expressed lncRNAs in ccRCC were analyzed in The Cancer Genome Atlas (TCGA), and the most significant lncRNAs were selected and verified in ccRCC tissues. It was found that lncRNA LINC02747 was highly expressed in ccRCC tissues, indicating that LINC02747 may play an important role in ccRCC. There is currently no related research on LINC02747 in the literature. Therefore, this study aims to explore the expression of LINC02747 in ccRCC cells and its effect on the proliferation of ccRCC cells, as well as its mechanism of action.

Methods

Datasets

The expression data on lncRNAs in 54 pairs of ccRCC tissues and para-carcinoma tissues were obtained in TCGA using TANRIC software (https://ibl.mdanderson.org/tanric/_design/basic/main.html), and the expression of lncRNAs and related clinicopathological characteristics in 447 patients with ccRCC were also obtained.

Collection of ccRCC tissue specimens

Cancer and corresponding non-carcinoma tissues were collected from 20 patients undergoing radical nephrectomy at our hospital from June 2018 to December 2019. All patients were diagnosed for the first time and had not undergone any previous treatment. All patients were diagnosed with ccRCC via postoperative pathology, and all the tissue specimens were frozen in liquid nitrogen for later use. The study was approved by the Ethics Committee of the Third Affiliated Hospital of Suzhou University. Before each operation, the patient's consent was obtained, and an informed consent form was signed.

Cell lines and transfection

Cell lines (RPTEC/TERT1, 786-O, ACHN, Caki-1 and Caki-2) were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA). DMEM with 10% FBS was used for the cell culture.

LINC02747 specific siRNA, TFE3 specific siRNA, siRNA negative control, miR-NC, miR-608 mimics, miR-608 inhibitor, control, LINC02747 and TFE3 overexpression plasmid was purchased from BoyaBio Co. Ltd. (Shanghai, China).

Luciferase reporter assay

The binding sites of LINC02747, TFE3 and miR-608 were predicted using a database. LINC02747-WT and TFE3-WT luciferase plasmids containing wild-type binding sites and LINC02747-MUT and TFE3-MUT containing mutated binding sites were constructed. The luciferase plasmids LINC02747-MUT, LINC02747-MUT, TFE3-WT and TFE3-MUT were cotransfected with miR-608 mimic into 786-O and Caki-1 cells, respectively. After 48 h of culture, double luciferase system was used to detect the fluorescence value of each group.

Quantitative Real-time fluorescence RCR (qRT-PCR)

After transfection for 24 h, total RNA was extracted from cells with the manufacturer's instructions. Then, the RNA was reverse transcribed into cDNA using the TaKaRa Reverse Transcription Kit under RNase-free conditions. The primers for the related genes and internal reference were designed and synthesized by Shanghai Ximao Biotechnology Company, and the primer sequences are shown in Table 1. With GAPDH as an internal reference, the experimental results were obtained using the $2^{-\Delta\Delta C_t}$ method.

Western blot analysis

Protein was extracted from 786-O and Caki-1 cells after the transfection for 48 h. SDS-PAGE was performed to separate the protein sample. Then, the samples were transferred to a polyvinylidene fluoride (PVDF) membrane, and followed by incubated with 5% non-fat milk, primary antibodies and secondary antibodies successively. The relative expression of target protein was analyzed with GAPDH as a loading control. Primary antibodies anti- TFE3 (1:1000, Abcam, UK) and anti-GAPDH (1:5000, ProteinTech Group, USA) were used in this research. The experiment was performed three separate times.

CCK-8 proliferation assay

Cells in the logarithmic growth phase were digested and transferred into a 96-well plate (approximately 2000 cells/well). Cells were gently mixed evenly in the wells. After the cells adhered to the wall, they were transfected in each group for 0-96 h. After treatment, the culture solution was aspirated, and 90 μ L of the corresponding complete medium and 10 μ L of CCK-8 reagent were added for incubation in an incubator at 37°C for 1 h. Then, the absorbance value was measured at a wavelength of 450 nm, and the obtained data were statistically analyzed.

Animal experiments

The stably transfected cell lines in the control group in the logarithmic growth phase and 786-O cell line with LINC02747 knockdown (siRNA) were digested with 0.25% trypsin, centrifuged, mixed with 1 mL of PBS, resuspended and counted. Then, the cells were prepared (approximately 1×10^7 cells per nude mouse) and resuspended in 200 μ L of PBS. The nude mice were randomly divided into two groups: one group was injected with control cells, while the other group was injected with 786-O cells with LINC02747 knockdown. First, the axillary skin of the nude mice was disinfected with 1% iodine, and 200 μ L of cell suspension was withdrawn into a disposable microsyringe and injected into the subcutaneous tissues of

the axillary skin. Liquid leakage was carefully avoided and the grouping was labeled in detail. The nude mice were observed every 3 days to monitor the growth of the tumor and record the volume of the tumor. After 30 days, the nude mice were sacrificed via cervical dislocation, and the transplanted tumors were removed, weighed, imaged and stored for subsequent tests.

Statistical analysis

SPSS 22.0 (IBM, USA) software was used for statistical analysis. The measurement results are expressed as the mean $\pm$ standard deviation (mean $\pm$ SD). A *t*-test was used for the comparison between two groups and one-way ANOVA was used for the comparison between multiple groups. The Kaplan-Meier method was adopted for survival differences.

Results

Identification of differentially expressed lncRNAs in ccRCC

The expression of lncRNAs was detected in a total of 54 pairs of ccRCC tissues and matched para-carcinoma tissues in the TCGA database. With fold change ≥ 4 and $p < 0.01$ as the criteria, it was found that a total of 20 lncRNAs were upregulated and 329 lncRNAs were downregulated in ccRCC tissues (Figure 1A and 1B). The 5 most significantly up-regulated lncRNAs in ccRCC tissues (ENSG00000224490, ENSG00000225174, ENSG00000237471, ENSG00000255774, ENSG00000260877) and the 5 most significantly downregulated lncRNAs (ENSG00000248517, ENSG00000228521, ENSG00000260580, ENSG00000257191, ENSG00000258402) were selected and verified via qRT-PCR in 20 pairs of ccRCC tissues and para-carcinoma tissues. The results showed that the differential expression of ENSG00000255774 (LINC02747) was the most obvious. Compared with that in para-carcinoma tissues, LINC02747 was highly expressed in cancer tissues (Figure 1C). Then, the expression of LINC02747 was detected via qRT-PCR in normal kidney cells (RPTEC/TERT1) and RCC cells (786-O, ACCN, Caki-1, Caki-2). The results revealed that the expression of LINC02747 in RCC cells was significantly higher than that in normal kidney cells. Therefore, LINC02747 was significantly highly expressed in RCC cells and tissues.

The prognostic value of LINC02747

The expression of lncRNAs in TCGA and related clinicopathological characteristics of 447 ccRCC patients were analyzed. The results showed that the expression of LINC02747 in ccRCC patients with tumors > 7 was significantly higher than that in patients with tumors ≤ 7 (Figure 2A). The expression of LINC02747 was significantly higher in patients with Fuhrman grade III-IV ccRCC than Fuhrman grade I-II ccRCC (Figure 2B). The expression of LINC02747 was significantly higher in patients with TNM stage III-IV ccRCC than TNM stage I-II ccRCC (Figure 2C). With the median value as the cut-off value, the overall survival (OS) of patients with high LINC02747 expression was significantly less than that of patients with low LINC02747 expression ($p < 0.001$) (Figure 2D).

Effect of LINC02747 on the proliferation ability of RCC cells

To study the effect of LINC02747 on the proliferation of RCC cells, the expression of LINC02747 in 786-O and Caki-1 RCC cells was upregulated or downregulated by transfection of an overexpression plasmid or siRNA. The expression of LINC02747 after transfection is shown in Figure 3A and 3B. The CCK-8 assay was performed to detect the effect of LINC02747 expression on the proliferation of 786-O cells. It was found that the upregulation of LINC02747 significantly promoted the proliferation of RCC cells (Figure 3C), while the downregulation of LINC02747 inhibited the proliferation of RCC cells (Figure 3D). The same results were obtained in Caki-1 cells (Figure 3E-3F).

Knocking down LINC02747 inhibited the proliferation of RCC cells in nude mice

To further explore the effect of LINC02747 on tumor growth in nude mice, 786-O cells with stably downregulated LINC02747 (LINC02747 siRNA) and control cells (Control) were injected into the subcutaneous tissues of nude mice for 30 days. It was found that the growth rate of tumors in the LINC02747 siRNA group was significantly lower than that in the Control group (Figure 4A and 4B). The results showed that knocking down LINC02747 significantly inhibited the growth of RCC cells in nude mice, confirming the effect of LINC02747 on the growth of RCC cells in vivo.

LINC02747 regulated the proliferation of RCC cells by inhibiting miR-608

The nuclear-cytoplasmic fractionation assay demonstrated that LINC02747 was mainly located in the cytoplasm (Figure 4A). Therefore, it was speculated that LINC02747 may regulate the function of RCC cells by inhibiting miRNA. Using the RegRNA2.0 database (<http://regna2.mbc.nctu.edu.tw/>), the miRNAs (miR-187, miR-330, miR-608, miR-593, miR-661) most likely to bind to LINC02747 were identified. Then, LINC02747 was overexpressed in 786-O and Caki-1 cell lines, and the expression changes of the above miRNAs were detected by qRT-PCR. The results showed that miR-608 expression was significantly downregulated (Figure 5B). Then, LINC02747-WT and LINC02747-MUT were cloned into plasmid psiCHECK-2 (Figure 5C), and the constructs were cotransfected with miR-608 mimic into 786-O and Caki-1 cells for the luciferase reporter assay. The results showed that miR-608 overexpression decreased the luciferase activity of the LINC02747-WT vector ($p<0.01$) but did not reduce the luciferase activity of the mutant vector or empty vector (Figure 5D). This result showed that LINC02747 can directly bind to miR-608, and the mutated sites are the binding sites between the two molecules. In addition, miR-608 expression is lower in cancer tissues (Figure 6A), and the expression levels of LINC02747 and miR-608 were negatively correlated in 20 ccRCC tissues (Figure 6B).

Effect of miR-608 on the proliferation ability of RCC cells

To study the effect of miR-608 on the proliferation ability of RCC cells, miR-608 mimic and miR-608 inhibitor were transfected into 786-O and Caki-1 cell lines, and then the expression of miR-608 was detected using qRT-PCR. The expression of miR-608 after transfection is shown in Figure 6C and 6D. In the CCK-8 proliferation assay, it was found that upregulation of miR-608 significantly promoted the proliferation of RCC cells (Figure 6E), while downregulation of miR-608 inhibited the proliferation of RCC cells (Figure 6F). The same results were obtained in Caki-1 cells (Figure 6G-6H).

LINC02747 regulated the proliferation of RCC cells by inhibiting miR-608

To explore whether LINC02747 regulates the proliferation of RCC cells by inhibiting miR-608, 786-O and Caki-1 cells were transfected with Control+miR-NC, LINC02747+miR-NC, Control+miR-608 mimic or LINC02747+miR-608 mimic. The expression levels of LINC02747 and miR-608 in the four groups after transfection are shown in Figure 7A and 7B. The CCK-8 proliferation assay showed that in the cell lines with upregulated miR-608, the upregulation of LINC02747 had no significant impact on the proliferation ability of RCC cells (Figure 7C and 7D). These results indicate that LINC02747 regulates the proliferation ability of RCC cells by inhibiting miR-608.

Screening and verification of miR-608 target genes

The 5 most likely target genes of miR-608, namely, BRD4, SSBP3, FOXO6, SOD3 and TFE3, were selected as candidate target genes using TargetScan (<http://www.targetscan.org/>) and miRDB (<http://mirdb.org/>). Then, the expression of miR-608 in 786-O and Caki-1 cells was upregulated, and it was found that the mRNA expression of TFE3 changed most significantly, and the difference was statistically significant ($p < 0.01$, Figure 8A). Then, the expression of miR-608 was upregulated in 786-O and Caki-1 cells, and it was found that the expression of TFE3 protein decreased, while the expression of TFE3 increased after downregulating miR-608 expression (Figure 8B). Then, TFE3 wild-type and mutant sequences were cloned into the luciferase reporter plasmid psiCHECK-2 (Figure 8C), and the constructs were cotransfected with miR-608 mimic into 786-O and Caki-1 cells for the luciferase reporter assay. The results showed that overexpression of miR-608 could greatly lower the luciferase activity of the wild-type TFE3 vector ($p < 0.01$) but did not reduce that of the mutant or empty vector (Figure 8D). This result proved that TFE3 is the direct target gene of miR-608, and the mutated sites are the binding sites between the two.

MiR-608 regulated the proliferation of RCC cells by inhibiting TFE3

To verify whether miR-608 can inhibit the proliferation of RCC by inhibiting TFE3, 786-O and Caki-1 cells were transfected with Control+miR-NC, Control+miR-608 mimic, TFE3+miR-NC or TFE3+miR-608 mimics. The mRNA and protein expression levels of TFE3 in the four groups after transfection are shown in Figure 9A and 9B. The CCK-8 assay showed that upregulation of miR-608 expression had no significant impact on the proliferation ability of RCC cells when TFE3 was upregulated (Figure 9C and 9D). These findings show that miR-608 regulates the proliferation of RCC by inhibiting TFE3. In summary, LINC02747 upregulates the expression of TFE3 by adsorbing miR-608, ultimately promoting the proliferation ability of RCC cells.

Discussion

LncRNAs are the most important type of ncRNA in mammals [8], and they played an important role in a variety of biological processes [9]. In recent years, lncRNAs have become a research hotspot in cancer. The roles of lncRNAs in RCC and their biological function in the development of RCC have also attracted increasing attention from scholars. Xiao et al. found that lncRNA FILNC1 deficiency can reduce energy-

induced apoptosis, thus significantly promoting the progression of RCC, whose mechanism is to increase the glucose uptake of tumor cells and increase the production of lactic acid by upregulating c-Myc [11]. Hong et al. reported that lncRNA HOTAIR competitively binds to miR-217, thereby upregulating its target gene HIF-1 α and promoting the expression of downstream AXL genes, ultimately enhancing the proliferation, migration and epithelial-mesenchymal transition of RCC cells and inhibiting the apoptosis of RCC cells [12]. According to another study, lncRNA DLX6-AS1 acts as an oncogene in RCC, and interference with DLX6-AS1 can significantly inhibit the growth of RCC cells. Furthermore, DLX6-AS1 contains binding sites for miR-26a, and it promotes the progression of RCC by competitively binding to miR-26a and upregulating PTEN [13]. Li et al. selected lncRNA MRCCAT1, which is closely related to ccRCC metastasis, through gene chip analysis. Overexpression of MRCCAT1 inhibits the transcription of NPR3, thereby activating the p38-MAPK signaling pathway and promoting the proliferation, migration and invasion of RCC cells [14]. In this study, lncRNAs related to ccRCC were screened in the TCGA database and then verified using qRT-PCR. lncRNA LINC02747, with the most significant difference in expression between cancer tissues and para-carcinoma tissues, was selected for further research. The in vivo and in vitro experiments confirmed that LINC02747 could promote the proliferation of RCC cells.

lncRNAs bind to miRNAs through complementary sequences, and they competitively downregulate the binding of miRNAs to target mRNAs, thus regulating gene expression, which is one of the main mechanisms of posttranscriptional regulation [15]. In this study, it was found that LINC02747 regulated the proliferation of RCC cells by adsorbing miR-608. Multiple studies have reported that miR-608 play a role as a tumor suppressor gene in a variety of tumors. Wang et al. found that the expression level of miR-608 is significantly reduced in liver cancer tissues, and overexpression of miR-608 can inhibit the proliferation of liver cancer cells through the G1 cell cycle [16]. Yang et al. found that overexpression of miR-608 in colon cancer cell lines can remarkably inhibit the proliferation, cell cycle progression and migration of cancer cells [17]. Wang et al. argued that overexpression of miR-608 in lung cancer cells can suppress the expression of TFAP4, thus promoting the apoptosis of NSCLC cells [18]. Liang et al. found that the relative expression of miR-608 in bladder cancer tissues is low, and overexpression of miR-608 can cause cell cycle arrest and inhibit the proliferation of bladder cancer cell lines. Furthermore, miR-608 affects the downstream AKT/FOXO3a signaling pathway by targeting FLOT1, thus inhibiting the proliferation of bladder cancer [19]. In this study, it was found that miR-608 was expressed at low levels in ccRCC, and overexpression of miR-608 inhibited the proliferation of RCC cells. Moreover, the results showed that TFE3 is a direct target gene of miR-608. MiR-608 regulated the proliferation ability of RCC cells by inhibiting TFE3. Studies have shown that TFE3 is a key pathway regulating cell development, with a wide range of physiological functions. TFE3, a member of the MiTF family, is closely related to the occurrence and development of RCC and is the only driver gene inducing the formation of Xp11.2 [20, 21]. Studies have shown that TFE3 binds to Smad protein to activate the TGF- β signal transduction pathway and also activate the PAI-1 gene [22]. The TFE3 protein possesses potent transcriptional activity, and it can inhibit the p21-mediated pRB pathway, thereby causing uncontrolled cell proliferation and eventually leading to malignant transformation [23]. Recently, it has been reported that the occurrence of RCC is closely related to the inactivation of the tumor suppressor gene FLCN. After inactivation of FLCN, TFE3

protein can be phosphorylated, and its aggregation in the nucleus is reduced, increasing the TFE3 protein transcriptional activity and eventually upregulating the expression of hematopoietic stem cell growth factor-induced neuropeptide (HGFIN), thereby participating in the occurrence of RCC [24]. Fang et al. found that overexpression of TFE3 can promote the proliferation and regulate the cycle of RCC cells mainly through excessive activation of the P13K/AKT/mTOR pathway [25].

In conclusion, it was confirmed by analyzing the relevant data in TCGA and the qRT-PCR results in clinical specimens that lncRNA LINC02747 is highly expressed in ccRCC, which is closely related to the high TNM stage and histological grade and the poor prognosis of patients. LINC02747 upregulates the expression of TFE3 by adsorbing miR-608, ultimately promoting the proliferation ability of RCC cells. The above findings indicate that LINC02747 acts as an oncogene in ccRCC and may be developed as a molecular marker for the diagnosis and prognosis of ccRCC. The LINC02747/miR-608/TFE3 pathway may become a new therapeutic target for ccRCC.

Declarations

Ethics approval and consent to participate

All animal experiments were approved by the ethical review committee from Third Affiliated Hospital of Soochow University. The study protocol was performed in accordance with the guidelines outlined in the Declaration of Helsinki. The Ethics Committee of Third Affiliated Hospital of Soochow University approved the study.

Consent for publication

Not applicable.

Availability of data and material

The lincRNA expression data for ccRCC from TCGA.

Competing interests

The authors declare that they have no competing interests.

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None.

Authors' contributions

J.X and X.Z.H conceived and designed the study and helped to draft the manuscript. Y.Y.S, F.Z. and X.H.W. performed the data collection. Z.G.W performed the statistical analysis. All authors read and critically revised the manuscript for intellectual content and approved the final manuscript.

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Figures

Figure 1

(A) Heatmap showed differentially expressed miRNAs based on 54 pairs of ccRCC tissues and matched para-carcinoma tissues in the TCGA dataset (Fold Change $\geq$ 4 and P<0.01). (B) Volcano plots were constructed based on TCGA (fold-change >2.0 and P<0.05). The red points represent differentially upregulated genes, and green points represent downregulated genes. (C) Validation of lncRNAs in 20 pairs of cancer tissues and matched para-carcinoma tissues by qRT-PCR. (D) The expression of LINC02747 was detected by qRT-PCR in normal kidney cells (RPTEC/TERT1) and RCC cells (786-O, ACCN, Caki-1 and Caki-2). (**P<0.001, **P<0.01, *P<0.05)

Figure 2

(A) The expression of LINC02747 in different tumor size based on TCGA dataset. (B) The expression of LINC02747 in different Fuhrman grade based on TCGA dataset. (C) The expression of LINC02747 in different TNM stage based on TCGA dataset. (D) The overall survival (OS) of patients with high LINC02747 expression was significantly less than that of patients with low LINC02747 expression. (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$)

Figure 3

Verification efficiency of overexpressed LINC02747 (A) or down-expressed LINC02747 (B) in 786-O and Caki-1 cell lines by qRT-PCR. After altered LINC02747 expression, the proliferation of 786-O (C-D) and Caki-1 (E-F) cells is changed. (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$)

Figure 4

(A) Tumors collected of Control and LINC02747 siRNA for 30 days in vivo. (B) Growth curves of Control and LINC02747 siRNA. (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$)

Figure 5

(A) qRT-PCR analysis of LINC02747 nuclear and cytoplasmic levels in 786-O and Caki-1 cells. U6 was used as a nuclear marker, and GAPDH was used as a cytosolic marker. (B) qRT-PCR detected the expression changes of the candidate miRNAs after overexpressed LINC02747 in 786-O and Caki-1 cells. (C) MiR-608 and LINC02747 binding sequences and LINC02747 mutation sequences. (D) Luciferase reporter assay is used to verify the direct binding between LINC02747 and miR-608. (** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$)

Figure 6

(A) qRT-PCR was used to detect the miR-608 expression in 20 pairs of ccRCC tissues and para-carcinoma tissues. (B) The correlation between LINC02747 and miR-608 in 20 ccRCC tissues. Verification efficiency of overexpressed miR-608 (C) or down-expressed miR-608 (D) in 786-O and Caki-1 cell lines by qRT-PCR. After altered miR-608 expression, the proliferation of 786-O (E-F) and Caki-1 (G-H) cells is changed. (***P<0.001, **P<0.01, *P<0.05)

Figure 7

qRT-PCR analysis of LINC02747 (A) and miR-608 (B) expression in 786-O and Caki-1 transfected with Control+miR-NC, LINC02747+miR-NC, Control+miR-608 mimic, or LINC02747+miR-608 mimic. 786-O (C) and Caki-1 (D) transfected with Control+miR-NC, LINC02747+miR-NC, Control+miR-608 mimic, or LINC02747+miR-608 mimic., and the cell proliferation in each group is detected by CCK-8. (***P<0.001, **P<0.01, *P<0.05)

Figure 8

(A) qRT-PCR detected the expression changes of the candidate mRNAs after overexpressed miR-608 in 786-O and Caki-1 cells. (B) Western blot detected the TFE3 protein in 786-O and Caki-1 transfected with miR-608 mimic or inhibitor. (C) MiR-608 and TFE3 binding sequences and TFE3 mutation sequences. (D) Luciferase reporter assay is used to verify the direct binding between TFE3 and miR-608. (***) $P < 0.001$, ** $P < 0.01$, * $P < 0.05$)

Figure 9

TFE3 mRNA (A) and protein (B) expression in 786-O and Caki-1 transfected with Control+miR-NC, Control+miR-608 mimic, TFE3+miR-NC, or TFE3+miR-608 mimic. 786-O (C) and Caki-1 (D) transfected with Control+miR-NC, Control+miR-608 mimic, TFE3+miR-NC, or TFE3+miR-608 mimic, and the cell proliferation in each group is detected by CCK-8. (** $P < 0.01$, * $P < 0.05$)

Supplementary Files

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- [Supplementarytable1.docx](#)