ABSTRACT
Clear cell renal cell carcinoma (ccRCC) is the most common RCC subtype with high metastasis, poor prognosis and conventional chemotherapy resistance. Prostate cancer associated transcript 1 (PCAT1) is an important lncRNA that was reported to be involved in cell proliferation, migration and invasion of several types of cancer cells. However, its role in ccRCC is still undetermined. This study found that PCAT1 levels were elevated in ccRCC tumors as well as several ccRCC cells, and knockdown of PCAT1 with siRNA (si-PCAT1) alleviated cell proliferation, migration and invasion of Caki-2 and ACHN cells. With bioinformatics analysis, dual-luciferase reporter assay, RNA pull-down assay and Spearman’s correlation analysis, we demonstrated that PCAT1 acted as a sponge for miR-656 and miR-539. Moreover, we found dual competitive interaction of miR-656/539 with PCAT1 and yes-associated protein (YAP), resulting in the identification of PCAT1-miR-656/539-YAP axis in Caki-2 and ACHN cells. With CCK-8 assay and transwell assay, miR-656/539 inhibitor or YAP overexpression could alleviate the effects of si-PCAT1 on the proliferation, migration and invasion of Caki-2 and ACHN cells. Our data indicated that PCAT1 promotes proliferation, migration and invasion of ccRCC cells by upregulating YAP via sponging miR-656 and miR-539. Taken together, this study provided a novel therapeutic target for ccRCC treatment.

INTRODUCTION
Renal cell carcinoma (RCC), one of the ten leading cancer types [1], continues to incur high mortality rates. Clear cell renal cell carcinoma (ccRCC) is one of the majority subtypes accompanied by high metastasis and relapse rate [2]. Several therapeutic protocols have cured early-stage ccRCC; however, their effects on metastatic ccRCC patients are still limited, with a 5-year survival rate of 10% [2]. Thus, more efficient therapeutic protocols should be explored. To date, molecular understanding of the ccRCC cell proliferation, migration and invasion has been limited [3,4].

Long non-coding RNAs (lncRNAs) are RNA transcripts which have been shown to regulate gene expression at both transcriptional and post-transcriptional levels [5,6]. LncRNAs perform a wide variety of functions in biological processes [7] and also have been reported to be involved in cancer processes [8]. LncRNA prostate cancer associated transcript 1 (PCAT-1), an important lncRNA has been shown to contribute to cell proliferation, migration and invasion of several cancer cells, including cervical cancer cells [9], osteosarcoma cells [10], gastric cancer [11], prostate cancer [12,13] and so on. However, its role in ccRCC is still undetermined.

In this study, we characterized the functions of PCAT1 in ccRCC for the first time. We demonstrated that the expression level of PCAT1 was upregulated in both ccRCC tumors and ccRCC cell lines, and further confirmed the correlation between high PCAT1 level and overall survival of ccRCC patients. We also determined that knockdown of PCAT1 inhibited ccRCC cell proliferation, migration and invasion. Furthermore, we wanted to investigate the underlying mechanism behind this inhibition. In previous studies, the involvement of microRNAs (miRNAs) in post-transcriptional...
regulation of lncRNAs has been reported in several kinds of cancers [14–16]. Thus, we sought to investigate whether PCAT1 performed biological functions by lncRNA-miRNA interaction networks. Through a bioinformatics approach, we found PCAT1 directly bound to two target miRNAs (miR-656 and miR-539) and knockdown of PCAT1 upregulated the expression levels of miR-656 and miR-539. We further found the dual competitive interaction of miR-656/539 with PCAT1 and yes-associated protein (YAP), resulting in the identification of PCAT1-miR-656/539-YAP axis in Caki-2 and ACHN cells. YAP has been identified as a crucial oncogene involved in multiple cancers, including ccRCC [17–19], and it was involved in cell proliferation and apoptosis of ccRCC cell lines (786-0 and ACHN). Hence, we concluded that PCAT1 promoted proliferation, migration and invasion of ccRCC cells by upregulating YAP via sponging miR-656 and miR-539.

Materials and methods

Study subjects

Eighty-five (85) patients with ccRCC were selected from Renmin Hospital of Wuhan University between September 1, 2016 and July 31, 2018. We collected tumor tissues as well as the healthy controls from the 85 subjects. All enrolled subjects provided written informed consent, and this research was approved by the ethical committees of Renmin Hospital of Wuhan University.

Cells culture and transfection

The ccRCC cell lines (786-O, Caki-2, 769-P, OS-RC-2, ACHN) and normal human renal epithelial cell line HK2 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and incubated in Dulbecco’s modified Eagles’s medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT, USA) at 37°C in a 5% CO₂ incubator. PCAT1 small interference RNAs (si-PCAT1#1 and si-PCAT1#2) and its corresponding scrambled siRNA control (si-NC) were obtained from Wanleibio Co., Ltd (Shenyang, China). The plasmids were transfected into cells using a Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA).

Animal experiments and xenograft collection

BALB/C nude mice around 4 weeks (n = 12, six per group) were purchased from Charles River laboratories (Beijing, China). A suspension of 786-O cells (5 × 10⁶ cells) was injected subcutaneously into the axilla of the nude mice. After the tumor volume in each nude mouse was greater than 100 mm³, the si-NC (20 nM) or si-PCAT1 pool (10 nM of si-PCAT1#1 and 10 nM of si-PCAT1#2) were infected into the nude mice by Entranster™-in vivo Transfection Reagent according to its protocol (Engreen Biosystem Co, Ltd., Beijing, China). Xenografts were measured weekly and were removed in the fifth week. The animal experiments were approved by the animal Ethics Committee of Renmin Hospital of Wuhan University.

Quantitative real-time PCR (qRT-PCR)

qRT-PCR was conducted to determine the expression levels of PCAT1, miR-656, miR-539 or YAP in the aforementioned tissues or ccRCC cell lines. Total RNA isolation and cDNA synthesis were performed using RNApure total RNA fast isolation kit (BioTeke, Beijing, China) and Super M-MLV reverse transcriptase (BioTeke, Beijing, China), respectively. The PCR reactions were performed using an ABI Prism 7500 (Applied Biosystems, USA). Two pairs of primers (PCAT1 and YAP) were designed by Primer 5.0 and listed in Supplementary Table 1. The relative mRNA expression levels were normalized to β-actin expression and calculated by 2−ΔΔCT method [20]. The levels of miRNAs were examined with specific stem-loop RT-PCR primers (Tiangen, Tianjin, China).

Cell proliferation assay

CCK-8 cell proliferation assay was performed on the PCAT1 silenced Caki-2 and ACHN cells. Furthermore, to determine whether YAP affected the proliferation of PCAT1 knockdown cells, si-PCAT1#1 and miR-656 or miR-539 inhibitor or YAP plasmid were cotransfected into Caki-2 and
ACHN cells. The transfected cells were plated in triplicates in 96-well plates (5 × 10^3 cells per well) and 10 μL of CCK8 solution (Beyotime, Shanghai, China) was added to the cells, followed by incubation at 37°C for 2 h. The absorbance was measured at 450 nm using a SpectraMax 190 microplate reader (Molecular Devices, Sunnyvale, CA).

**Dual-luciferase reporter assay**

The wildtype (WT) or mutant (MUT) PCAT1 and YAP that had the predicted miR-656/539 binding sites were synthesized and integrated into pmirGLO luciferase vector (Promega, Madison, WI, USA). The 150 ng of either PCAT1-WT, PCAT-MUT, YAP-WT or YAP-MUT and either miR-NC, miR-656 or miR-539 reporter vectors were co-transfected into Caki-2 and ACHN by Lipofectamine 2000 (Invitrogen, San Diego, CA, USA). The Firefly and Renilla luciferase activities were determined using a dual luciferase reporter assay system (Promega, Madison, WI, USA). The transfection experiment was performed in triplicate.

**Pull-down assay with biotinylated miRNA**

The Caki-2 and ACHN cells were transfected with 20 nM of biotinylated miR-NC or biotinylated miR-656 or biotinylated miR-539 using Lipofectamine 2000 (Invitrogen, San Diego, CA, USA). RNA pull-down assay was performed using Pierce Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) following the manufacturer’s instructions. The bound RNAs were obtained by TRizol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and the levels were determined by qRT-PCR analysis.

**Nuclear and cytoplasmic RNA extraction**

Nuclear and cytoplasmic RNA of Caki-2 and ACHN cells were isolated using PARIS™ Kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer’s protocol. To determine the location of PCAT1 in nuclear or cytoplasm, qRT-PCR was performed to assess the expression of PCAT1 in nuclear and cytoplasmic RNA of Caki-2 and ACHN cells.

**Western blot**

Proteins were obtained from Caki2 and ACHN cells with RIPA buffer (Beyotime, Beijing, China) and quantified using a BCA Protein Assay Kit (Beyotime, Beijing, China). Equal amounts of protein extracts were loaded to SDS-PAGE gel and then transferred onto PVDF membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% skim milk for 1 h, followed by incubation with YAP antibody (sc-271134, Santa Cruz, CA, USA) at 4°C overnight. The membranes were then probed 45 min at 37°C with HRP-labeled Goat Anti-Mouse IgG(H + L) secondary antibody (A0216, Beyotime, Beijing, China) and the membranes were visualized by a gel imaging system (LIUYI, Beijing, China) with GAPDH (sc-365062, Santa Cruz Biotechnology, CA, USA) as a control. The catalog of the antibodies was listed in Supplementary Table 2.

**Statistical analysis**

All data in this study were analyzed using SPSS 13.0 (SPSS Inc., Armonk, NY). The difference of mean values between two groups was analyzed by Student’s t-test and differences in multiple groups were analyzed by ANOVA with Bonferroni correction. The survival curves were assessed by Kaplan-Meier analysis. Values are expressed as mean ± SD and p value <0.05 was considered statistically significant.

**Results**

**High PCAT1 expression is observed in ccRCC tissues and cell lines**

To evaluate the expression level of PCAT1 in ccRCC, qRT-PCR was performed on 85 pairs of normal and tumor tissues collected from 85 ccRCC patients. Higher PCAT1 expression was observed in ccRCC tissues than that seen in normal tissues (p < 0.001) (Figure 1(a)). The clinical-pathologic features of the 85 patients were shown in Table 1. The subjects were divided into low expression group (n = 43) and high expression group (n = 42) according to the expressing median value in ccRCC tissues. The correlation of PCAT1 expression with clinicopathological data was
assessed by chi-square test and summarized in Table 1. No significant association between gender ($p = 0.157$), age ($p = 0.157$) and PCAT1 expression level was observed; however, significant correlations were observed between PCAT1 expression and tumor size ($p = 0.027$), differentiation ($p = 0.030$), lymph node metastasis ($p = 0.023$), TMN stages ($p = 0.001$).

Table 1. The clinicopathologic features of the 85 patients.

| Clinicopathological characteristics | Total | PCAT1 high expression | PCAT1 low expression | $\chi^2$ | P value |
|------------------------------------|-------|-----------------------|----------------------|---------|---------|
| Gender                             |       |                       |                      |         |         |
| Male                               | 42    | 23                    | 19                   | 0.579   | 0.447   |
| Female                             | 43    | 20                    | 23                   |         |         |
| Age                                |       |                       |                      |         |         |
| $\leq50$                           | 41    | 24                    | 17                   | 2.002   | 0.157   |
| $>50$                              | 44    | 19                    | 25                   |         |         |
| Tumor size                         |       |                       |                      |         |         |
| T1                                 | 22    | 6                     | 16                   | 9.168   | 0.027   |
| T2                                 | 19    | 9                     | 10                   |         |         |
| T3                                 | 18    | 12                    | 6                    |         |         |
| T4                                 | 24    | 16                    | 8                    |         |         |
| Differentiation                    |       |                       |                      |         |         |
| High                               | 28    | 18                    | 10                   | 7.019   | 0.030   |
| Moderate                           | 28    | 16                    | 12                   |         |         |
| Poor                               | 39    | 9                     | 30                   |         |         |
| Lymph node metastasis             |       |                       |                      |         |         |
| Positive                           | 45    | 28                    | 17                   | 5.178   | 0.023   |
| Negative                           | 40    | 15                    | 25                   |         |         |
| TMN stages                         |       |                       |                      |         |         |
| I                                  | 25    | 5                     | 20                   | 17.336  | 0.001   |
| II                                 | 17    | 8                     | 9                    |         |         |
| III                                | 23    | 14                    | 9                    |         |         |
| IV                                 | 20    | 16                    | 4                    |         |         |

The KM survival curve indicated that the patients with high expression level of PCAT1 had a lower survival percent than that with low expression of PCAT1 ($p < 0.01$) (Figure 1(c)). This result indicated that upregulation of PCAT1 correlated with poor prognosis in ccRCC.

**Knockdown of PCAT1 inhibits ccRCC cell proliferation, migration and invasion**

Among ccRCC cell lines, the expression level of PCAT1 was higher in Caki-2 and ACHN (Figure 1(b)), Caki-2 and ACHNwere selected for further experiments. The si-PCAT1#1 and si-PCAT1#2 plasmids were utilized to inhibit the PCAT1 expression in Caki-2 and ACHN, and the silencing efficiency was confirmed by qRT-PCR. Compared with the expression of PCAT1 in si-NC transfected cells, the lower PCAT1 expression level was observed in si-PCAT1#1 or si-PCAT1#2 transfected Caki-2 and ACHN cells ($p < 0.01$) (Figure 2(a)). No significant difference between si-PCAT1#1 and si-PCAT1#2was detected.

We detected the proliferation, migration and invasion of Caki-2 and ACHN cells which were transfected with si-NC, si-PCAT1#1 or si-PCAT1#2. Our results indicated that si-PCAT1#1 and si-PCAT1#2 notably suppressed the proliferation of Caki-2 and ACHN cells compared to si-NC ($p < 0.01$) (Figure 2(b)). The cell migration ability and migration numbers were also significantly suppressed by si-PCAT1#1 and si-PCAT1#2 in Caki-2 and ACHN (Figure 2(c)). Cell invasion was examined by using transwell invasion assay. The results demonstrated that si-PCAT1#1 and si-PCAT1#2

![Figure 1](image1.png)

**Figure 1.** PCAT1 expression level was significantly elevated in ccRCC tissues and ccRCC cell lines. qRT-PCR revealed that PCAT1 expression level was significantly upregulated in ccRCC tissues compared with adjacent normal tissues (a) and in several ccRCC cell lines (b). *$p < 0.05$, **$p < 0.01$ versus HK2 cells. (c) KM survival curve showed that the patients with high expression level of PCAT1 had a poor prognosis than those with low expression of PCAT1.**
significantly attenuated the invasion ability of Caki-2 and ACHN cells ($p < 0.01$) (Figure 2(d)). The results from either of siRNAs are well consistent (Figure 2).

The role of si-PCAT1 was also investigated in nude mice in vivo. As shown in Figure 2(e), si-PCAT1 pool significantly suppressed the xenograft volume as compared to si-NC group ($p < 0.05$). qRT-PCR result revealed that si-PCAT1 pool markedly suppressed the expression level of PCAT1 in xenograft in vivo.

**PCAT1 acts as a sponge for miR-656 and miR-539**

To determine the subcellular localization of PCAT1 in Caki-2 cells, we tested for the PCAT1 expression level in cytoplasm and nucleus by qRT-PCR. Our results showed that PCAT1 expression level was higher in cytoplasm (Figure 3(a)). Through the miRDB online database, we obtained two top hits for binding with PCAT1: miR-656 and miR-539 sequences which have binding sites with PCAT1 (Figure 3(b)). To provide further evidence on the relationship between PCAT1 and miR-656 or miR-539, the full-length PCAT1 sequence or its mutated sequence was cloned into the pmirGLO dual-luciferase miRNA target expression vector (Promega, Madison, WI, USA). Subsequent luciferase reporter assays showed that miR-656 or miR-539 overexpression significantly repressed the luciferase activity in Caki-2 and ACHN cells when PCAT1 is present, whereas it did not affect the luciferase activity when PCAT1 was mutated (Figure 3(c)).

Subsequently, RNA pull-down assay showed that PCAT1 was pulled down by biotin-labeled miR-656 and miR-539 oligos, but not the biotin-
labeled NC oligos (p < 0.01) (Figure 3(d)). The results demonstrated that PCAT1 directly interacted with miR-656 and miR-539.

Furthermore, we examined the expression levels of miR-656 and miR-539 in PCAT1 knockdown cells. Our results indicated that the expression levels of miR-656 and miR-539 were significantly suppressed by si-PCAT1#1 and si-PCAT1#2 (p < 0.01) (Figure 3(e)). We then compared the levels of miR-656 and miR-539 in ccRCC tumors with that of the normal tissues, and the results showed that the levels of miR-656 and miR-539 in ccRCC tumors were significantly lower than that in normal tissues (p < 0.01) (Figure 3(f)). Upon observing the connection between PCAT1 and miR-656/539, the expression levels of PCAT1 and miR-656/539 had a distinct negative correlation confirmed by Spearman’s correlation analysis (p < 0.0001) (Figure G).

Figure 3. PCAT1 directly binds to miR-656 and miR-539. (a) Subcellular distribution of PCAT1 in Caki2 cells. (b) Bioinformatics analysis shows that two miRNA sequences: miR-656 and miR-539 were the top hits for binding with PCAT1. (c) Dual-luciferase report assay showed that miR-656 and miR-539 reduced the luciferase activity of PCAT1-WT, but not of PCAT1-MUT. (d) RNA pull-down assay was performed to determine the interaction of PCAT1 and miR-656 and miR-539 in Caki2 and ACHN cells, and the expression level of PCAT1 was detected by qRT-PCR. (e) The effects of si-PCAT1#1 and si-PCAT1#2 on the expression levels of miR-656 and miR-539 were measured by qRT-PCR. (f) The expression levels of miR-656 and miR539 in ccRCC tissues and adjacent normal tissues. (g) Spearman’s correlation analysis revealed that the expression levels of PCAT1 and miR-656/539 exhibited a dramatically negative correlation (p < 0.0001).
PCAT1 promotes ccRCC aggressive progression via regulation of miR-656/539-YAP axis

Through the miR and a online database, we found binding sites for miR-656 and miR-539 in YAP 3’UTR sequence, which is known as a ccRCC-related gene [21] (Figure 4(a)). The full-length of YAP 3’UTR sequence or its mutated sequence was cloned into the pmirGLO dual-luciferase miRNA target expression vector (Promega, Madison, WI, USA) and then transfected into Caki-2 and ACHN cells. Compared to miR-NC transfected cells, luciferase activity was significantly suppressed by overexpressed miR-656 or miR-539 when PCAT1 is present, whereas it did not affect the luciferase activity when PCAT1 was mutated (p < 0.01) (Figure 4(b)).

To determine the connection between PCAT1 and YAP gene, si-PCAT1#1 and miR-656 or miR-539 inhibitor were cotransfected into Caki-2 and ACHN cells. We found that PCAT1 knockdown suppressed
the mRNA and protein levels of YAP, while its inhibition was dramatically alleviated by cotransfection of miR-656 or miR-539 inhibitor ($p < 0.01$) (Figure 4(c)). In addition, the higher expression level of YAP was observed in ccRCC tumors compared with that in normal tissues ($p < 0.001$) (Figure 4(d)). Moreover, Spearman’s correlation analysis revealed a positive correlation between the expression levels of PCAT1 and YAP in ccRCC tumors ($p < 0.001$) (Figure E).

To determine whether YAP affected the proliferation, migration and invasion of PCAT1 knockdown cells, we cotransfected si-PCAT1#1 and miR-656 or miR-539 inhibitor or YAP plasmid into Caki-2 and ACHN cells. The results indicated that PCAT1 knockdown suppressed the proliferation, migration and invasion, while its inhibition was dramatically alleviated by miR-656 or miR-539 inhibitor or YAP plasmid ($p < 0.01$) (figure 4(f–h)).

**Discussion**

RCC originates from renal epithelium and encompasses more than 10 histological and molecular subtypes. The most common subtype ccRCC has been described as accounting for the highest mortality rate [22]. The previous studies showed that almost all ccRCC patients were diagnosed with metastasis, which is refractory to conventional chemotherapy [22,23]. Thus, it is urgent to explore the novel and effective diagnostic and therapeutic targets for ccRCC.

LncRNAs, which have tens of thousands numbers, perform a wide variety of functions in biological processes such as stem cell renewal, tissue regeneration and tumorigenesis [24,25]. Notably, a number of studies have reported the involvement of lncRNAs in regulation of the growth and metastasis of various malignancies [26–28]. Several lncRNAs have been considered as oncogenes or anti-oncogenes because of their unique expression levels in specific cancer types [29–31], implying their roles in cancer diagnosis and prognosis [32]. To date, upregulation of lncRNAs was found in various cancers [8], for example, lncRNA MRCCAT1 was overexpressed in metastatic ccRCC tissues and its high expression promoted ccRCC cells proliferation, migration, and invasion [33]. LncRNAs also have been reported to function as competing endogenous RNAs (ceRNAs) for miRNAs that can regulate target mRNAs expression at post-transcriptional level [34,35]. A growing body of evidence has shown that the lncRNA-miRNA-mRNA ceRNA network was involved in multiple cellular processes, including cell proliferation, migration and invasion in several cancers [4,36,37]. From the previous research on ccRCC, one lncRNA ZFAS1 has been demonstrated to promote growth and metastasis of ccRCC by targeting miR-10a/SKA1 and it has been identified as a potential biomarker for ccRCC [4]. However, there were no relative researches considering the functions of lncRNA PCAT1 in ccRCC biological and cellular processes. In the present study, we first found that PCAT1 level was significantly upregulated in ccRCC tumors and cells. We identified PCAT1 as an oncogene in ccRCC and further demonstrated that PCAT1 was a prognostic biomarker in ccRCC.

Until now, biological functions of PCAT1 have been previously reported in several cancers, but not in ccRCC. The previous study has revealed that PCAT1 functioned as ceRNA to exert a direct promoting effect on hepatocellular carcinoma (HCC) cells migration and invasion via targeting miR-129-5p/HMGB1 [38]. PCAT1 was identified as a candidate to mediate prostate cancer (PC) risk by bioinformatic analysis [13]. This study provided that PCAT1 was involved in PC development and progression by interacting with androgen late-response genes [13]. PCAT1 also functioned as a potential prognostic biomarker for PC and promoted proliferation, migration, invasion of PC cells through PCAT1-miR-145-5p-FSCN1 ceRNA network [12]. Thus, we inferred that PCAT-1 also performed a series of similar biological functions in ccRCC cells. In this study, we demonstrated that PCAT1 silence with si-RNA alleviated cell proliferation, migration and invasion of Caki-2 and ACHN, indicating that PCAT1 acted as an oncogene in ccRCC.

Yet the molecular mechanism behind this regulation was mostly obscure. In previous studies, several lncRNAs have been proved to possess enhancer-like function to influence the expression of their neighboring genes [39]. The relevant lncRNA PCAT19 was reported to contribute to the upregulation of CEACAM21 expression in
aggressive prostate cancer cells [40]. In this study, through bioinformatics predictions and functional studies, we found for the first time that PCAT1 could act as ceRNA, whose silence elevated miR-656/539 functions and further relieved the expression level of miR-656/539 target gene YAP.

The identified PCAT1-miR656/539-YAP axis was demonstrated to promote the proliferation, migration and invasion of Caki-2 and ACHN cells. From the previous research on ccRCC, the up-regulated expression level of YAP was observed in ccRCC tissues and two ccRCC cell lines 786-O and A498 [41]. The above results confirm that YAP is an oncogene for ccRCC. Therefore, in the present study, we draw a conclusion that PCAT1 promoted cell proliferation, migration and invasion by upregulating YAP via sponging miR-656 and miR-539.

In conclusion, we identified a functional PCAT1-miR656/539-YAP axis in ccRCC cells, which promoted ccRCC cell proliferation, migration and invasion. Our data provided a potential prognostic biomarker and a therapeutic target for ccRCC.

Disclosure statement

The authors report no conflict of interest.

References

[1] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2013. CA Cancer J Clin. 2013;63(1):11–30.
[2] Motzer RJ, Molina AM. Targeting renal cell carcinoma. J Clin Oncol. 2009;27(20):3274–3276.
[3] Wang K, Sun Y, Tao W, et al. Androgen receptor (AR) promotes clear cell renal cell carcinoma (ccRCC) migration and invasion via altering the circH1AT1/miR-195-5p/29a-3p/29c-3p/CDC42 signals. Cancer Lett. 2017;394:1–12.
[4] Dong D, Mu Z, Wei N, et al. Long non-coding RNA ZFAS1 promotes proliferation and metastasis of clear cell renal cell carcinoma via targeting miR-10a/SKA1 pathway. Biomed Pharmacother. 2019;111:917–925.
[5] Beermann J, Piccoli MT, Viereck J, et al. Non-coding RNAs in development and disease: background, mechanisms, and therapeutic approaches. Physiol Rev. 2016;96(4):1297–1325.
[6] Adams BD, Parsons C, Walker L, et al. Targeting non-coding RNAs in disease. J Clin Invest. 2017;127(3):761–771.
[7] Yang L, Lin C, Jin C, et al. lncRNA-dependent mechanisms of androgen-receptor-regulated gene activation programs. Nature. 2013;500(7464):598–602.
[8] Huar.te M. The emerging role of lncRNAs in cancer. Nat Med. 2015;21(11):1253–1261.
[9] Ma TT, Zhou LQ, Xia JH, et al. LncRNA PCAT-1 regulates the proliferation, metastasis and invasion of cervical cancer cells. Eur Rev Med Pharmacol Sci. 2018;22(7):1907–1913.
[10] Huang J, Deng G, Liu T, et al. Long noncoding RNA PCAT-1 acts as an oncogene in osteosarcoma by reducing p21 levels. Biochem Biophys Res Commun. 2018;495(4):2622–2629.
[11] Bi M, Yu H, Huang B, et al. Long non-coding RNA PCAT-1 over-expression promotes proliferation and metastasis in gastric cancer cells through regulating CDKN1A. Gene. 2017;626:337–343.
[12] Xu W, Chang J, Du X, et al. Long non-coding RNA PCAT-1 contributes to tumorigenesis by regulating FSCN1 via miR-145-5p in prostate cancer. Biomed Pharmacother. 2017;95:1112–1118.
[13] Guo H, Ahmed M, Zhang F, et al. Modulation of long noncoding RNAs by risk SNPs underlying genetic predispositions to prostate cancer. Nat Genet. 2016;48(10):1142–1150.
[14] Ge Y, Yan X, Jin Y, et al. MiRNA-192 [corrected] and miRNA-204 directly suppress lncRNA HOTTIP and interrupt GLS1-mediated glutaminolysis in hepatocellular carcinoma. PLoS Genet. 2015;11(12):e1005726.
[15] Wang X, Ren Y, Yang X, et al. miR-190a inhibits epithelial-mesenchymal transition of hepatoma cells via targeting the long non-coding RNA treRNA. FEBS Lett. 2015;589(24 Pt B):4079–4087.
[16] Wang X, Li M, Wang Z, et al. Silencing of long non-coding RNA MALAT1 by miR-101 and miR-217 inhibits proliferation, migration, and invasion of esophageal squamous cell carcinoma cells. J Biol Chem. 2015;290(7):3925–3935.
[17] Zender L, Spector MS, Xue W, et al. Identification and validation of oncogenes in liver cancer using an integrative oncogenomic approach. Cell. 2006;125(7):1253–1267.
[18] Zhang X, George J, Deb S, et al. The hippo pathway transcriptional co-activator, YAP, is an ovarian cancer oncogene. Oncogene. 2011;30(25):2810–2822.
[19] Cao JJ, Zhao XM, Wang DL, et al. YAP is overexpressed in clear cell renal cell carcinoma and its knockdown reduces cell proliferation and induces cell cycle arrest and apoptosis. Oncol Rep. 2014;32(4):1594–1600.
[20] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402–408.
[21] Hu X, Chen J, Fu Q. Downregulation of YAP in clear cell renal cell carcinoma contributes to poor prognosis and progressive features. Ann Clin Lab Sci. 2017;47(1):36–39.
[22] Hsieh JJ, Purdue MP, Signoretti S, et al. Renal cell carcinoma. Nat Rev Dis Primers. 2017;3:17009.
[23] Gong J, Maia MC, Dizman N, et al. Metastasis in renal cell carcinoma: biology and implications for therapy. Asian J Urol. 2016;3(4):286–292.
[24] Lin C, Yang L. Long noncoding RNA in cancer: wiring signaling circuitry. Trends Cell Biol. 2018;28(4):287–301.
[25] Wapinski O, Chang HY. Long noncoding RNAs and human disease. Trends Cell Biol. 2011;21(6):354–361.
[26] Schmitt AM, Chang HY. Long noncoding RNAs in cancer pathways. Cancer Cell. 2016;29(4):452–463.
[27] Qi P, Du X. The long non-coding RNAs, a new cancer diagnostic and therapeutic gold mine. Mod Pathol. 2013;26(2):155–165.
[28] Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. Cell. 2013;152(6):1298–1307.
[29] Iyer MK, Niknafs YS, Malik R, et al. The landscape of long noncoding RNAs in the human transcriptome. Nat Genet. 2015;47(3):199–208.
[30] Serviss JT, Johansson P, Grandâ®r D. An emerging role for long non-coding RNAs in cancer metastasis. Front Genet. 2014;5:234.
[31] Martens-Uzunova ES, Bottcher R, Croce CM, et al. Long noncoding RNA in prostate, bladder, and kidney cancer. Eur Urol. 2014;65(6):1140–1151.
[32] Malek E, Jagannathan S, Driscoll JJ. Correlation of long non-coding RNA expression with metastasis, drug resistance and clinical outcome in cancer. Oncotarget. 2014;5(18):8027–8038.
[33] Li JK, Chen C, Liu JY, et al. Long noncoding RNA MRCCAT1 promotes metastasis of clear cell renal cell carcinoma via inhibiting NPR3 and activating p38-MAPK signaling. Mol Cancer. 2017;16(1):111.
[34] Salmena L, Poliseno L, Tay Y, et al. A ceRNA hypothesis: the rosetta stone of a hidden RNA language? Cell. 2011;146(3):353–358.
[35] Jalali S, Bhartiya D, Lalwani MK, et al. Systematic transcriptome wide analysis of lncRNA-miRNA interactions. PLoS One. 2013;8(2):e53823.
[36] Jiang H, Liang M, Jiang Y, et al. The lncRNA TDRG1 promotes cell proliferation, migration and invasion by targeting miR-326 to regulate MAPK1 expression in cervical cancer. Cancer Cell Int. 2019;19:152.
[37] Ren Y, Shang J, Li J, et al. The long noncoding RNA PCAT-1 links the microRNA miR-215 to oncogene CRKL-mediated signaling in hepatocellular carcinoma. J Biol Chem. 2017;292(43):17939–17949.
[38] Zhang D, Cao J, Zhong Q, et al. Long noncoding RNA PCAT-1 promotes invasion and metastasis via the miR-129-5p-HMGBl signaling pathway in hepatocellular carcinoma. Biomed Pharmacother. 2017;95:1187–1193.
[39] Orom UA, Derrien T, Beringer M, et al. Long noncoding RNAs with enhancer-like function in human cells. Cell. 2010;143(1):46–58.
[40] Gao P, Xia JH, Sipeky C, et al. Biology and clinical implications of the 19q13 aggressive prostate cancer susceptibility locus. Cell. 2018;174(3):576–589e518.
[41] He C, Chen ZY, Li Y, et al. miR-10b suppresses cell invasion and metastasis through targeting HOXA3 regulated by FAK/YAP signaling pathway in clear-cell renal cell carcinoma. BMC Nephrol. 2019;20(1):127.