MiR-5195-3p Functions as a Tumor Suppressor in Prostate Cancer via Targeting CCNL1

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Abstract

Background

Accumulating evidence indicates miR-5195-3p exerts tumor suppressive role in several tumors. However, there is limited research on the clinical significance and biological function of miR-5195-3p in prostate cancer (PCa).

Methods

Expression levels of miR-5195-3p and Cyclin L1 (CCNL1) were determined using quantitative real-time PCR. The clinical significance of miR-5195-3p in PCa patients was evaluated using Kaplan-Meier survival analysis and Cox regression models. Cell proliferation and cell cycle distribution were measured by CCK-8 assay and flow cytometry, respectively. The association between miR-5195-3p and CCNL1 was analyzed by luciferase reporter assay.

Results

MiR-5195-3p expression levels were significantly downregulated in 69 paired PCa tissues compared with matched adjacent normal tissues. The decreased miR-5195-3p expression was associated with Gleason score and TNM stage, as well as worse survival prognosis. The *in vitro* experiments showed that miR-5195-3p overexpression suppressed the proliferation and cell cycle G1/S transition in PC-3 and DU145 cells. Elevated miR-5195-3p abundance was also demonstrated to impair tumor formation *in vivo* using PC-3 xenografts. Mechanistically, Cyclin L1 (CCNL1) was a direct target of miR-5195-3p in PCa cells, which was inversely correlated with miR-5195-3p in PCa tissues. Importantly, CCNL1 knockdown imitated, while overexpression reversed the effects of miR-5195-3p overexpression on PCa cell proliferation and cell cycle G1/S transition.

Conclusions

Our data suggests that miR-5195-3p functions as a tumor suppressor via downregulating G1/S related CCNL1 expression in PCa.

Background

Prostate cancer (PCa), as the most frequently diagnosed male malignancy, has been the leading cause of tumor-related deaths male tumor with its pathological and clinical heterogeneity in the world [1, 2]. There are more than 220,000 estimated new cases of PCa and over 30,000 men die from this disease per year in the United States [3]. Despite great improvement in outcomes of PCa with early-stage by early prostate-specific antigen (PSA) testing, surgical resection and androgen deprivation therapy [4, 5], the prognosis is still poor for the advanced patients, especially the emergence of castration-resistant PCa [6]. Therefore, elucidation of the molecular mechanisms underlying the initiation and progression of PCa is urgently needed to establish new therapeutic targets for PCa treatment.

MicroRNAs (miRNAs/miRs) consisting of approximately 18-22 nucleotides in length are a group of small, noncoding and single stranded RNAs, which could regulate multiple physiological processes by selectively inhibiting the downstream target mRNAs via binding to their 3'-untranslated region (3'-UTR) [7, 8]. In recent years, growing evidence has suggested that aberrantly expressed miRNAs can act as either oncogenes or tumor suppressors, thereby affecting the pathogenesis of PCa: For example, miR-139 has been associated with improved prognosis and inhibition of growth and migration in PCa cells [9]. Overexpression of miR-589-5p inhibited cell viability, migration and invasion in PCa cells [10]. On the contrary, some oncogenic factors, including miR-410-3p [11], miR-153 [12], and miR-191 [13] have been identified as carcinogenic factors that predict poor prognosis and promote proliferation of PCa cells. Notably, miR-5195-3p, a relatively poorly studied miRNA, has been shown to participate in cell biological processes that regulate the progression of tumors, including non-small cell lung cancer [14], glioma [15], osteosarcoma [16] and bladder cancer [17]. However, there is limited research on the clinical significance and biological function of miR-5195-3p in PCa.

Cyclin L1 (CCNL1 also termed Ania-6a), localized in the chromosomal 3q25 region, codes for a putative key regulator of pre-mRNA processing and is involved in G1/S transition during the cell cycle [18]. Interestingly, CCNL1 has been implicated in carcinogenesis being potential targets of therapeutic interventions [19]. As reported by Redon et al [20] and Sticht et al [21], CCNL1 was expressed and amplified in human head and neck squamous cell carcinoma. Moreover, CCNL1 has been demonstrated to as a direct target gene of miR-199b-5p and be involved in miR-199b-5p suppressing the cell proliferation, arrested cell cycle progression and promoted cell apoptosis in Ewing's sarcoma [22]. Considering our previous work predicted that CCNL1 as a potential target of miR-5195-3p, we thus speculated that miR-5195-3p might play an important role in PCa tumorigenesis by targeting CCNL1 via affecting cell cycle progression.

Therefore, we first investigated the expression pattern and clinical significance of miR-5195-3p in PCa tissues and conducted a series of in vitro and in vivo functional experiments to observe its effects on cell proliferation and tumor growth. Furthermore, we validated the association between miR-5195-3p and CCNL1 in PCa. This research will enhance our understanding of PCa biology and provide new information for the treated molecular therapy.

Methods

Patients and tissue samples

Total 69 paired of tumor tissues and matched adjacent normal tissues were obtained from patients with PCa who underwent radical prostatectomy at Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Hubei, China). Before the surgery, all patients were confirmed not to receive chemotherapy, radiotherapy or androgen deprivation therapies. All tissue specimens were immediately frozen in liquid nitrogen and kept at -80 °C until further analysis. The basic clinicopathological characteristics of all PCa patients, including age, Gleason score and TNM stage were

summarized in **Table 1**. All enrolled patients underwent five-year follow-up period through the telephone surgery. The written informed consent was signed by all patients and the present study was approved by the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Hubei, China).

Cell culture

Human PCa cell lines (PC-3, 22RV1, DU145 and LNCaP) and one normal prostate epithelial cell line RWPE-1 were purchased from the American Type Culture Collection (Manassas, VA, USA). All cell lines were cultured in RPMI-1640 (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (Gibco) in a humidified incubator containing 5% CO₂ at 37 °C.

Cell transfection

MiR-5195-3p mimics, scrambled miRNA (miR-NC), smaller interfering RNA against CCNL1 (si-CCNL1), negative control (si-NC), the overexpression plasmid of pcDNA3.1-CCNL1 and empty vector pcDNA3.1 were synthesized from Shanghai GenePharma Co., Ltd. For cell transfection, PC-3 and DU145 cells were seeded into six-well plates and transfected with the above oligonucleotides using Lipofectamine 2000 (Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions.

Quantitative real-time PCR

Total RNA was extracted using mirVana miRNA isolation kit (Life Technologies; Thermo Fisher Scientific, Inc.) for miRNA abundance and RNeasy mini kit (Qiagen, Valencia, CA, USA) for mRNA. The synthesis of complementary DNA was performed using miScript II RT kit (Applied Biosystems, CA) for miRNA and superscript VILO cDNA kit (Thermo Fisher Scientific, Inc.) for mRNA according to the manufacturer's instructions. Quantitative real time PCR was performed using miScript SYBR Green PCR kit (Qiagen) for miR-5195-3p or SYBR Green PCR kit (Applied Biosystems, CA) for CCNL1 mRNA levels with the specific primer sequences synthesized by Sangon Biotech (Shanghai). Each experiment was performed in triplicate, and relative abundance was normalized to U6 for miR-5195-3p or GADPH for CCNL1 mRNA by the $2^{-\Delta\Delta CT}$ method.

Cell proliferation assay

Transfected PCa cells in technical triplicates at a density of 3×10^4 cells per well in six-well plates and cultured for 24, 48 and 72 h, respectively. At each time point, cells in each well were incubated with 10 μ l of CCK-8 solution (Sigma-Aldrich) for 2 h. Afterwards, the absorbance at a wavelength of 450 nm was measured in each well using a microplate reader.

Cell cycle analysis

Transfected PCa cells at a density 4×10^5 cells per well were seeded in six-well plates and incubated undisturbed for 48 h. Subsequently, cells were washed with PBS, fixed with cold 70% ethanol overnight,

followed by incubation with 0.1 mg/ml propidium iodide (Sigma-Aldrich) for 30 min in the dark. Next, the cells were analyzed by a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) with FlowJo software (Version 10.0.4; FlowJo LLC).

Luciferase reporter assay

According to the predicted putative binding sites for miR-5195-3p with the 3'-UTR of CCNL1 by the online software program TargetScan 7.1 (<http://www.targetscan.org>), we performed luciferase reporter assay to validate the above prediction. In brief, the fragments of CCNL1 3'-UTR containing either putative miR-5195-3p seed sequence or corresponding mutant (MUT) sites using QuickChange Site-Direct Mutagenesis Kit (Stratagene) were subcloned into psiCHECK-2™ vector (Promega, USA) to obtain the reporter plasmids of CCNL1-wild-type (WT) and CCNL1-MUT. Then, PC-3 or DU145 cells were plated in 24-well plates and co-transfected with 1 µg reporter plasmid CCNL1-WT or CCNL1-MUT together with 30 nM miR-5195-3p mimics or miR-NC for 48 h. Relative luciferase activities were determined using a Dual-Luciferase Reporter Assay System (Promega).

Tumor xenograft formation

Equivalent amounts of PC-3 cells (1.8×10^6) stably transfected with miR-5195-3p mimics or miR-NC were subcutaneously injected into the right flank of 4–5-week-old nude mice (Shanghai Laboratory Animal Research Center, Shanghai, China) with five mice in each group. Mice were monitored every five days and the tumor length/width was measured using calipers. Tumor volume was calculated using the modified ellipsoid formula: $\text{Volume} = 1/2 (\text{length} \times \text{width}^2)$. All mice were sacrifice 30 days after injection. Then, the tumor weight was measured and tumor tissues were harvested for analysis of Cyclin L1, CDK4 and Cyclin D1 expression. All animal experiments were performed in accordance with the Huazhong University of Science and Technology Research Institute Animal Care Committee guidelines.

Western blot analysis

Total protein samples were extracted from cell lines or tumor tissues with RIPA lysis buffer (Thermo Fisher Scientific, Inc.) and protein concentration was analyzed using a BCA Protein Assay Kit (Pierce, Rockford, IL, USA) according to the manufacturer's instructions. Then, equal amount of protein sample was subjected to electrophoresis using sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE), which was subsequently transferred onto PVDF membranes. After blocked with 5% nonfat dried milk in TBST for 2 h, the membranes were incubated with primary antibodies against Cyclin L1, CDK4, Cyclin D1 and GAPDH overnight at 4 °C, followed by incubation with horseradish-peroxidase-linked secondary antibodies for 2 h at room temperature. Afterwards, the protein bands were visualized by a chemiluminescence detection kit (ECL, Millipore, USA).

Statistical analysis

The GraphPad Prism 6.0 software (National Institutes of Health, Bethesda, MD, USA) was used to perform all statistical analysis. The association between miR-5195-3p expression and PCa clinicopathologic characteristics was assessed by Chi-square test. The Kaplan-Meier method was performed to generate survival curves. Univariate and multivariate Cox regression models were constructed to estimate the hazard ratios (HRs) of independent factors affecting the overall survival in PCa patients. The Spearman's correlation coefficient was used to analyze the association between miR-5195-3p expression and CCNL1 expression in PCa tissues. All the quantitative data were expressed as mean $\pm$ SD of at least three experimental replicates. The differences among groups were analyzed using either the one-way ANOVA or the Student's t-test. Statistical significance was defined as *p*-value less than 0.05.

Results

MiR-5195-3p was down-regulated in PCa tissues, which correlated with cancer progression

We first performed quantitative real time PCR analysis to determine the expression level of miR-5195-3p in 69 pairs of human PCa and adjacent tissues. As shown in **Figure 1A**, miR-5195-3p expression was significantly lower in PCa tissues than that in adjacent normal tissues. We then sought to explore the correlations between miR-5195-3p expression and clinical characteristics. According to the median value of miR-5195-3p expression, 69 patients were classified into low miR-5195-3p expression (*n* = 35) and high miR-5195-3p expression (*n* = 34). As listed in **Table 1**, miR-5195-3p expression was significantly associated with Gleason score and TNM stage. We further explored whether miR-5195-3p expression was associated with the prognosis of PCa patients. Kaplan-Meier survival analysis revealed that PCa patients with high miR-5195-3p expression afforded longer overall survival than those with low miR-5195-3p expression (**Figure 1B**). Moreover, the results of univariate and multivariate survival analysis demonstrated that miR-5195-3p was an independent prognostic indicator for PCa patients' survival (**Table 2**).

MiR-5195-3p overexpression suppressed PCa cell proliferation and cell cycle G1/S transition *in vitro*

Subsequently, the expression of miR-5195-3p was assessed in several PCa cell lines. Consistently, miR-5195-3p expression levels were found to be significantly decreased in PCa cell lines (PC-3, 22RV1, DU145 and LNCaP) compared with the normal prostate epithelial cell line RWPE-1 (**Figure 2A**). To test the biological function of miR-5195-3p in PCa *in vitro*, PC-3 and DU145 cells were transfected with miR-5195-3p mimics or miR-NC. As shown in **Figure 2B**, miR-5195-3p expression was significantly elevated in both PC-3 and DU145 cells after miR-5195-3p mimics transfection compared with miR-NC transfection. Next, we performed gain-of-function assays in the above constructed miR-5195-3p overexpression cell lines. The results from CCK-8 assay showed that the cell growth curves were remarkably suppressed in PC-3 (**Figure 2C**) and DU145 (**Figure 2D**) cells after miR-5195-3p overexpression, especially at 48 and 72 h, respectively. Considering uncontrolled cell proliferation was correlated with cell cycle progression, we further analyzed the effects of miR-5195-3p overexpression on cell cycle distribution. The results from flow cytometry analysis illustrated that the percentage of cells at G0/G1 phase ($64.71\% \pm 1.04\%$ vs.

52.27% $\pm$ 0.83%, $p < 0.001$) was significantly increased, while cells at S phase (27.68% $\pm$ 1.32% vs. 39.09% $\pm$ 1.17%, $p < 0.001$) and G2/M phase (7.61% $\pm$ 0.28% vs. 8.64% $\pm$ 0.34%, $p < 0.05$) were decreased in miR-5195-3p mimics group compared with miR-NC group in PC-3 cells (**Figure 2E**). Similarly, we observed that miR-5195-3p overexpression caused a significant increase in the proportion of cells at G0/G1 phase and decrease in cells at S phase in DU145 cells (**Figure 2F**). These findings indicated that miR-5195-3p overexpression inhibited the proliferation and induced G1/S arrest in PCa cells.

CCNL1 was a direct target of miR-5195-3p in PCa

To identify the potential effectors of miR-5195-3p in PCa progression, the target genes of miR-5195-3p were searched by performing bioinformatics analysis. We found that CCNL1 3'UTR contains one miR-5195-3p-binding site and then constructed vectors containing the WT or MUT 3'UTR of human CCNL1 fused downstream of the firefly luciferase gene (**Figure 3A**). The results from luciferase reporter assay showed that co-transfection of miR-5195-3p mimics with CCNL1-WT reporters significantly decreased the luciferase activity, while miR-5195-3p-mediated repression of luciferase activity was abolished by the mutations in the putative miR-5195-3p binding site in both PC-3 (**Figure 3B**) and DU145 (**Figure 3C**) cells. What's more, quantitative real time PCR (**Figure 3D**) and western blot analysis (**Figure 3E**) demonstrated that CCNL mRNA and protein expression levels were both significantly suppressed after miR-5195-3p overexpression in PC-3 and DU145 cells. In addition, the quantitative real time PCR results displayed that CCNL1 mRNA expression in PCa tissues was remarkably upregulated compared with that in matched adjacent normal tissues (**Figure 3F**), which was inversely correlated with miR-5195-3p expression (**Figure 3G**, $r = -0.2387$, $p = 0.0483$). Collectively, these results suggest that miR-5195-3p directly targeted CCNL1 in PCa cells to downregulate CCNL1 expression.

MiR-5195-3p suppressed cell proliferation and cell cycle G1/S transition through CCNL1 mediation

To confirm whether CCNL1 was the important downstream mediator involved in miR-5195-3p regulating PCa cell proliferation and cell cycle progression, we performed loss-of-function by transfection with si-CCNL1 or si-NC and rescue experiments by co-transfection with miR-5195-3p mimics and pcDNA3.1-CCNL1 in PC-3 cells. As shown in **Figure 4A**, the protein expression of Cyclin L1 was obviously suppressed by si-CCNL1 transfection, which was recovered by pcDNA3.1-CCNL1 transfection in PC-3 cells. Subsequently, CCK-8 assay depicted that CCNL1 knockdown suppressed, while overexpression promoted the PC-3 cell proliferation (**Figure 4B**). Furthermore, we found that CCNL1 knockdown imitated (**Figure 4C**), while overexpression (**Figure 4D**) reversed the effects of miR-5195-3p overexpression on cell cycle G1/S transition. These results suggested that miR-5195-3p markedly inhibited the proliferation and G1/S transition, at least partially, via targeting CCNL1 in PCa.

MiR-5195-3p overexpression restricted tumor growth *in vivo* with reduced CCNL1 expression

Since miR-5195-3p exerted suppressive role in PCa cell proliferation *in vitro*, we further sought to determine the function of miR-5195-3p in tumorigenesis *in vivo*. In brief, PC-3 cells were transfected with miR-5195-3p mimics or miR-NC, and then injected into nude mice to produce a xenograft model of human

PC-3 tumors. As shown in **Figure 5A**, the tumor size was obviously smaller in miR-5195-3p mimics group compared with miR-NC group in a time course of 30 days. Moreover, the time-dependent analysis illustrated that the tumor volume was significantly suppressed in mice inoculated with miR-5195-3p overexpressed PC-3 cells compared with miR-NC group (**Figure 5B**). Meanwhile, the tumor weight was also remarkably decreased in miR-5195-3p overexpressed mice (**Figure 5C**). We further analyzed the protein content of Cyclin L1, CDK4 and Cyclin D1 in tissues of a subcutaneous xenograft murine model using western blot analysis. As shown in **Figure 5D**, the protein expression levels of Cyclin L1, CDK4 and Cyclin D1 were all significantly suppressed in tumor tissues by the infection with miR-5195-3p mimics compared with miR-NC injection, which certified that the upregulation of miR-5195-3p inhibited the growth of the prostate tumorigenesis by targeting CCNL1.

Discussion

The present study first reported that miR-5195-3p was significantly downregulated in PCa tissues compared with adjacent normal tissues. Lower abundance of miR-5195-3p was associated with Gleason score, TNM stage and worse prognosis in PCa patients, highlighting its potential role as a tumor suppressor miRNA. The in vitro experiments demonstrated that miR-5195-3p overexpression reduced proliferation and induced a G0/G1 cell cycle block in PCa cells (PC-3 and DU145). Consistent with our in vitro data, significant reduction in miR-5195-3p was observed in cancer samples and its reduction was correlated with increased cell proliferation in ovarian cancer [23]. Jiang et al [17] showed that miR-5195-3p suppressed proliferation and invasion of human bladder cancer cells. Wang et al [16] first observed downregulation of miR-5195-3p in osteosarcoma (OS) tissues and further manifested that miR-5195-3p overexpression attenuated OS cell proliferation and induced apoptosis. Additionally, miR-5195-3p plays a suppressive role in cell growth and proliferation in glioma cells [15] and human non-small cell lung cancer cells.

Furthermore, we analyzed the effects of miR-5195-3p overexpression on tumorigenesis and found that tumor formation in vivo was reduced with elevated miR-5195-3p abundance, further confirming miR-5195-3p functions as a tumor suppressive miRNA in PCa. In molecular level, we confirmed the regulatory of miR-5195-3p on cell cycle G1/S transition regulation, as reflected by decreased expression of CDK4/Cyclin D1 by miR-5195-3p overexpression in tumor tissues. In fact, uncontrol proliferation of tumor cells is closely associated with a deregulation of the cell cycle progression directly driven by a series of heterodimers formed by cyclins and cyclin-dependent kinases (CDKs) [24, 25]. Several lines of evidence indicate that miR-5195-3p are important cell cycle regulators. For instance, miR-5195-3p overexpression significantly downregulated c-MYC and cyclin D1 but upregulated p21 genes in HCT116 cells [26]. MiR-5195-3p sharply suppressed the expression or activation of its downstream promoting cell cycle regulator cyclin D1 in bladder cancer cells [17]. Thus, we speculated that miR-5195-3p exerted its suppressive effects on PCa cell proliferation might via inducing cell cycle G0/G1 arrest through downregulating CDK4/Cyclin D1 expression.

To our best knowledge, several target genes of miR-5195-3p, including HOXB6 in hepatocellular carcinoma [27], MYO6 in lung cancer [14], BIRC2 in glioma [15], EIF4A2 in breast cancer [28], NEDD9 in OS [16] and KLF5 in bladder cancer [17] have been identified and confirmed, which were largely associated with aberrantly tumor cell proliferation. Here, we selected CCNL1 as the potential target gene of miR-5195-3p for its role in G1/S transition [18] and carcinogenesis [19]. Furthermore, we demonstrated that miR-5195-3p downregulated CCNL1 via directly binding its 3'-UTRs. The expression of miR-5195-3p was inversely correlated with the CCNL1 expression level in 69 paired PCa tissues. Similarly, CCNL1 was reported as a direct target gene of miR-199b-5p and be involved in miR-199b-5p suppressing the cell proliferation and arrested cell cycle progression in Ewing's sarcoma [22]. As expected, our data illustrated that CCNL1 knockdown imitated the effects of miR-5195-3p overexpression on PCa cell proliferation and cell cycle G1/S transition, while a converse effect was observed with CCNL1 overexpression.

Conclusion

In summary, this study established the tumor suppressive role of miR-5195-3p in PCa in vitro and in vivo. Most importantly, CCNL1 was demonstrated as the functional regulator by miR-5195-3p, which was associated with cell cycle G1/S transition. Even though the downstream gene regulation by miR-5195-3p/CCNL1 should be further explored, our findings reinforce the importance of miR-5195-3p in regulating PCa aggression.

Abbreviations

PCa, prostate cancer; CCNL1, Cyclin L1; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gels; HRs, hazard ratios

Declarations

Ethics approval and consent to participate

All experimental produces were carried out under the approval of the Ethics Committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Hubei, China).

Consent for publication

Not applicable

Availability of data and materials

The dataset supporting the conclusions of this article is included within the article.

Competing interests

The authors declare that they have no competing interests.

Funding

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Author Contributions

ZX and HZQ performed the experiments and collected the original data. SYQ analyzed the data. WX and GJH participated in the interpretation of data and draft the manuscript. LZ designed the experiments, contributed reagents or materials, authored or reviewed drafts of the paper and approved the final draft. All authors have read and approved the manuscript.

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Tables

Table 1 The relationship between miR-5195-3p expression and clinicopathological characteristics of prostate cancer patients

Characteristics	Cases (n = 69)	miR-5195-3p expression		P-values (chi-square test)
		Low (n = 35)	High (n = 34)	
Age (year)				0.733
< 65	21	10	11	
≥ 65	48	25	23	
Preoperative PSA (ng/mL)				0.368
< 10	43	20	23	
≥ 10	26	15	11	
Gleason score				0.001*
< 7	51	20	31	
≥ 7	18	15	3	
TNM stage				0.006*
I/II	46	18	28	
III/IV	23	17	6	
Metastasis				0.118
No	39	23	16	
Yes	30	12	18	

Note: * $p < 0.05$

Table 2 Univariate and multivariate analysis for overall survival in prostate cancer patients

Characteristics	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Age	0.895 (0.563-1.498)	0.754	—	—
Preoperative PSA (ng/mL)	2.145 (1.284-3.315)	0.415	—	—
Gleason score	1.432 (0.895-2.546)	0.023*	1.365 (0.968-2.584)	0.056
TNM stage	2.312 (1.204-2.978)	0.005*	2.542 (1.432-3.142)	0.024*
Metastasis	3.142 (2.142-4.321)	0.064	—	—
MiR-5195-3p expression	1.759 (1.006-2.153)	0.014*	2.132 (1.354-2.856)	0.007*

Note: * $p < 0.05$

Figures

Figure 1

MiR-5195-3p was down-regulated in PCa tissues and correlated with overall survival. (A) MiR-5195-3p expression in 69 paired HCC and the matched adjacent normal tissue samples was measured by quantitative real time PCR. (B) The correlation between miR-5195-3p expression and overall survival was analyzed with the Kaplan-Meier method. The p-value was obtained using the log-rank test.

Image not available with this version

Figure 2

Effects of miR-5195-3p overexpression on PCa cell proliferation and cell cycle progression. (A) MiR-5195-3p expression in PCa cell lines (PC-3, 22RV1, DU145 and LNCaP) and one normal prostate epithelial cell line RWPE-1 was measured by quantitative real time PCR. (B) PC-3 and DU145 cells were transfected with the miR-5195-3p mimics or miR-NC. miR-5195-3p expression in PC-3 and DU145 cells was detected by quantitative real time PCR. (C-D) Cell proliferation was tested with CCK-8. MiR-5195-3p overexpression significantly inhibited the proliferation of PC-3 and DU145 cells. (E-F) Cell cycle distribution was determined by PI staining and flow cytometry analysis in PC-3 and DU145 cells. Data are presented as mean $\pm$ standard deviation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with miR-NC group. PI, propidium iodide; NC negative control

Figure 3

CCNL1 was a direct target of miR-5195-3p in PCa. (A) The sequences of the putative miR-5195-3p binding sites in the wild-type and mutant CCNL1 3'UTR. (B-C) Luciferase reporter plasmids carrying the CCNL1 wild-type 3'UTR (CCNL1-WT) or CCNL1 mutant 3'UTR (CCNL1-MUT) were transfected into PC-3 and DU145 cells with miR-5195-3p mimics or miR-NC. MiR-5195-3p up-regulation suppressed luciferase activity of the wild-type but not the mutant 3'UTR of CCNL1. Renilla luciferase activity was used as a

control. (D) The mRNA and (E) protein expression levels of CCNL1 following miR-5195-3p mimics or miR-NC transfection. (F) CCNL1 mRNA expression levels in 69 pairs of human PCa and matched adjacent normal tissues were measured by quantitative real time PCR. Data are presented as mean $\pm$ standard deviation. ** $p < 0.01$ compared with miR-NC group. (G) MiR-5195-3p expression was inversely correlated with CCNL1 miRNA expression in PCa tissues, as demonstrated by the Spearman's correlation coefficient.

Figure 4

MiR-5195-3p suppressed cell proliferation and cell cycle G1/S transition through CCNL1 mediation. PC-3 cells were transfected with si-CCNL1 or si-NC, as well as co-transfection with miR-5195-3p mimics and pcDNA3.1-CCNL1. (A) Western blot was used to determine the Cyclin L1 protein expression levels in the above transfected PC-3 cells. (B) Cell proliferation was tested with CCK-8 assay in the above transfected PC-3 cells. (C-D) Cell cycle distribution was determined by PI staining and flow cytometry analysis in the above transfected PC-3 cells. Data are presented as mean $\pm$ standard deviation. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with si-NC or miR-5195-3p mimics

Figure 5

MiR-5195-3p induced the regression of prostate tumorigenesis in vivo. (A) Image of tumor xenografts in nude mice injected subcutaneously with miR-5195-3p-overexpressing PC-3 cells. (B) Tumor volume was measured every 5 days. (C) Changes in the tumor weight in mice after miR-5195-3p overexpression. Data are presented as mean $\pm$ standard deviation. **p < 0.01, ***p < 0.001 compared with miR-NC. (D) Protein expression levels of Cyclin L1, CDK4 and Cyclin D1 expression were determined by western blot analysis.

Supplementary Files

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- [originalWB.tif](#)