circITGA7 Acts As miR-370-3p Sponge to Suppress The Proliferation of Prostate Cancer

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Research Article

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Abstract

Objective: Prostate cancer (PCa) refers to one of the most common tumors in male's genitourinary system. Emerging research has confirmed that circRNAs play an important role in the occurrence and development of tumors. However, the correlation between circular RNA circITGA7 and PCa still remains unclear. Here, the role of circITGA7 in PCa was explored and the underlying mechanism was investigated as well.

Methods: The circRNA expression profiles in PCa and the paracancerous tissues were established by high-throughput sequencing. The expression levels of circITGA7 in PCa tissues and cells were detected by qRT-PCR. Cell Counting Kit-8, colony formation, EdU and flow cytometry assays were used to detect the effects of circITGA7 on PCa cell proliferation. To further explore the underlying mechanisms, bioinformatics analysis on downstream target genes was carried out. RNA immunoprecipitation and dual-luciferase reporter assays were used to verify the direct relationship between miR-370-3p and circITGA7 or P21CIP1.

Results: circITGA7 was downregulated in PCa tissues and cells. Gain- or loss-of-function assays showed that circITGA7 inhibited the proliferation of PCa cells in vivo and in vitro. Mechanically, circITGA7 served as a sponge for miR-370-3p and miR-370-3p could target P21CIP1 in PCa cells. The inhibition of cell proliferation induced by circITGA7 could be reversed by transfecting miR-370-3p mimic.

Conclusion: Our data indicated that circITGA7 played an important role in inhibiting tumor proliferation in PCa and might be a potential therapeutic target.

Background

Prostate cancer (PCa) is the second most common cancer in men worldwide, with high morbidity and mortality [1]. Although localized PCa can be cured by surgical resection, many patients were diagnosed with distant metastases and missed the opportunity for surgery. Androgen deprivation therapy (ADT) is the first choice for the treatment of metastatic PCa and can rapidly inhibit tumor progression in the short term. However, almost all PCa patients treated with ADT will develop into hormone resistance prostate cancer (CRPC) and die within 2–4 years [2]. Therefore, it is urgent to better understand the pathogenesis of PCa and find the new therapeutic targets.

Circular RNA is a new type of non-coding RNA without 5' cap and 3' tail. It forms a closed circular structure by back-splicing mechanism and is more stable than linear non-coding RNA [3, 4]. First identified under the electron microscope in 1979, circRNAs were initially thought as the products of precursor mRNA splicing errors [5, 6]. With the advent of high-throughput sequencing, it has been found that circRNAs are widely expressed in eukaryotes [7]. Furthermore, increasing evidence revealed that circRNAs played an important role in the development of malignant tumors, including PCa [8–10].
circITGA7, generated from the exon 4 of integrin subunit alpha 7 (ITGA7) gene by back-splicing, was first reported to inhibit colorectal cancer growth and metastasis by Li et al in 2018 [11]. Subsequently, its regulatory role in thyroid cancer, osteosarcoma and glioma was reported successively [12–14]. However, the expression and biological role of circITGA7 in PCa are still unclear. In this study, we identified the circRNAs differentially expressed in PCa based on high-throughput sequencing, and found that the expression of circITGA7 in PCa tissues was significantly lower than that in paracancerous tissues. We further examined the effects of circITGA7 on the proliferation of prostate cancer cells by CCK-8, EdU, colony formation and flow cytometry, and explored the underlying mechanisms. Our study may provide a potential new target for the diagnosis and treatment of PCa.

**Materials And Methods**

Tissue samples and cell lines

A total of 28 pairs of PCa samples and corresponding adjacent normal prostate tissues were obtained from patients who underwent radical resection of prostate cancer at Wuhan Central Hospital between 2016 and 2019. A summary of clinical data was listed in Supplementary Table 1. Patients who had received chemotherapy, radiation therapy, endocrine therapy, or other anticancer treatments prior to surgery were excluded from our study. The research protocol was approved by the Ethics Committee of the Central Hospital of Wuhan, and informed consent was obtained from each patient before enrollment.

Human prostate cancer cell lines (DU145, LNCaP, PC3) and normal human prostate epithelial cells (RWPE-1) were acquired from the Institute of Cell Research, Chinese Academy of Sciences (Shanghai, China). Cells were incubated at 37°C and 5% CO₂ in DMEM medium (Gibco, USA) containing 10% FBS (HyClone, USA) and 1% penicillin/streptomycin (Gibco, USA).

RNA-seq analysis

High-throughput sequencing was performed in three pairs of prostate cancer and paracancerous normal tissues, and the samples were processed and sequenced by Kangce Technology Co., Ltd (Wuhan, China). The results were processed with the edgeR software package (version 3.12.1). p-value cutoff of 0.05 and fold-change cutoff of 2 were used to judge the statistical significance of gene expression differences. The RNA-seq data were deposited at the NCBI Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE179321) with accession number GSE179321.

RNA purification and qRT–PCR

Total RNAs from cells and tissues were extracted using TRIzol Reagent (Takara, China) according to the manufacturer’s instructions. RNase R treatment was processed at 37°C with 3 U/mg of RNase R (Epicenter, USA) for 15 min. Complementary DNA was synthesized using the PrimeScript RT Master Mix (Takara, China) or the miScript Reverse Transcription Kit (Qiagen, Germany) from 500 ng of RNA with random or oligo (dT) primer. The quantitative real-time PCR analyze (qRT-PCR) was conducted using
SYBR Premix Ex Taq™ kit (Takara, China) or a miScript SYBR Green PCR Kit (Qiagen, Germany). The values of $2^{-\Delta\Delta CT}$ relative to one of the samples were calculated to analyze relative expression levels of the RNAs. GAPDH or U6 were used as the endogenous control for mRNA and circRNA or miRNA, respectively. All the primers were listed in Supplementary Table 2.

Plasmids construction and transfection

To artificially upregulate or downregulate the expression of circITGA7, the pcD-ciR overexpression vector (circITGA7), empty vector (vector), small interfering RNA targeting circITGA7 (si-circITGA7) and negative control (si-NC) were purchased from Genepharma (Shanghai, China). miR-370-3p mimic and its corresponding negative control (mimic-NC) were purchased from RiboBio (Guangzhou, China). All the vectors were transfected into cells with Lipofectamine 3000 (Thermo Fisher Scientific, USA) following the manufacturer's instructions.

Cell Counting Kit-8 Assay

Transfected cells were plated into 96-well plates at a density of 3000 cells per well and then incubated for 24, 48, 72 or 96 h. Then, 10 µL CCK-8 solution (Sigma-Aldrich, USA) was added in each well, and the cells continued to be cultured for an additional 2 h. The absorbance was measured at 570 nm using a microplate reader.

Colony formation assay

For colony formation assay, PC3 or DU145 cells were seeded into six-well plates with 1000 cells per well and cultured under normal conditions for 2 weeks. The colonies were fixed in methanol and stained with 0.1% crystal violet. Cell colonies with more than 50 cells were counted.

EdU assay.

The EdU assay was carried out using Cell-Light EdU DNA Cell Proliferation Kit (RiboBio, China) according to the manufacturer's protocol. Images were obtained with an Olympus FSX100 microscope (Olympus, Japan). The ratio of EdU-stained cells to Hoechst-stained cells was calculated to evaluate the cell proliferation.

Fluorescence in situ hybridization.

Cy3-labeled circITGA7 were designed and synthesized by RiboBio (RiboBio, China). Cells were firstly fixed in 4% paraformaldehyde and incubated with 0.1% Triton X-100 on ice for 10 min. 2.5 µl circITGA7 probes (20 µM) were hybridized with the cells for 5 h in the dark at 37°C. DAPI was used to stain the nucleus. The images were acquired on Nikon A1Si Laser Scanning Confocal Microscope (Nikon Instruments Inc, Japan).

Flow cytometry analysis of cell cycle
PC3 and DU145 cells transfected with circITGA7 or si-circITGA7 were harvested and stained with propidium iodide buffer (Thermo Fisher Scientific, USA) for 15 min. Cell cycle analysis was performed by FACScalibur flow cytometer (BD Pharmingen, USA). The results were analyzed by the ModFit LT software.

Dual-luciferase reporter assays

Wild-type and mutant sequences of circITGA7 and P21\(^{\text{CIP1}}\) 3′-UTR were cloned into pGL3 promoter vector by RiboBio (RiboBio, China). Then, the luciferase vectors were transfected into cells together with miR-370-3p mimic or mimic-NC, respectively. Luciferase activities were determined by a dual-luciferase reporter assay kit (Promega, USA).

RNA immunoprecipitation

The EZMagna RIP kit (Millipore, USA) was used to evaluate the target relationship between miR-370-3p and circITGA7. Cells were harvested and lysed. The supernatant was mixed with protein A/G beads conjugated with antibodies that recognized Argonaute-2 (Ago2) or Immunoglobulin G (IgG). The immunoprecipitated RNAs were used for identification by qRT-PCR assay.

Western blot assay

Cells were collected after 48 h of transfection and resuspended in RIPA lysis buffer (Beyotime, China). In total, 50 µg of each protein samples were separated by SDS-PAGE, and then transferred onto polyvinylidene difluoride membranes (Millipore, USA). After blocked with 5% blocking buffer, the membranes were incubated with P21\(^{\text{CIP1}}\) antibodies. ECL chromogenic substrate (Beyotime, China) was used to visualize the bands. The antibodies against P21\(^{\text{CIP1}}\) and GAPDH were purchased from Abcam (Abcam, USA).

Tumor xenografts experiments

PC3 cells (3 × 10\(^6\), 200 µl) stably transfected with circITGA7 or control vector were subcutaneously injected into male BALB/c nude mice (4 weeks old). Then, the mice were maintained for three weeks before being sacrificed by cervical dislocation. The tumor volume was recorded every 3 days. The tumor volume was calculated according to the following formula: volume = length \cdot width\(^2\)/2.

**Statistical analysis**

Data were presented as mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 7.0 (La Jolla, USA). All experiments were independently repeated in triplicate. Pearson correlation analysis was used to analyze correlation between the expression of circITGA7 and miR-370-3p. Comparison between tumor and adjacent non-cancerous tissues was analyzed using a paired Student's t-test. Comparison between two independent groups was analyzed via unpaired Student's t-test, whilst comparisons among multiple groups were calculated by one-way ANOVA followed by Tukey's test. P < 0.05 was considered to indicate a statistically significant difference.
Results

circITGA7 is downregulated in PCa tissues and cell lines

To identify the circRNAs differentially expressed in PCa, we performed RNA sequencing in three pairs of prostate cancer and paracancerous tissues, and the results illustrated that 29 circRNAs were significantly upregulated and 87 circRNAs were obviously downregulated in PCa tissues compared with paracancerous tissues (Fig. 1A). The differential expression of circITGA7 in tumors and its ability to participate in tumor development have been reported but its role in PCa remains unclear, so we chose to conduct further studies on circITGA7. The genomic structure indicated that circITGA7 was generated from the exon 4 of the ITGA7 gene and the head-to-tail splicing of circITGA7 was further confirmed by Sanger sequencing (Fig. 1B). Afterwards, we designed convergent primers and divergent primers to amplify linear and circular RNA on the basis of cDNA and genomic DNA (gDNA) from PC3 and DU145 cell lines by PCR. The results gave the information that circITGA7 could only be amplified by divergent primers in cDNA, instead of in gDNA (Fig. 1C). Then, the RNase R assays illustrated that circITGA7 was more resistant to RNase R than linear mRNA (Fig. 1D).

Subsequently, the expression levels of circITGA7 in 28 cases of PCa tissues and corresponding adjacent normal tissues were detected by qRT-PCR and the results demonstrated that circITGA7 was significantly downregulated in PCa tissues than that in normal tissues (Fig. 1E). However, the expression level of circITGA7 was not remarkably correlated with pathological stage, Gleason score and blood PSA level (Supplementary Table 1). Similarly, the expression of circITGA7 in PCa cells (PC3, LNCaP and DU145) was greatly lower than that in normal prostate epithelial cells (RWPE-1) (Fig. 1F). PC3 and DU145 exhibited relatively lower circITGA7 expression levels and were selected for further study. After that, in order to understand the subcellular localization of circITGA7, we conducted FISH experiment in PC3 cells and the results displayed that circITGA7 was mainly distributed in the cytoplasm (Fig. 1G).

The overexpression of circITGA7 inhibits the proliferation of PCa cells in vivo and in vitro

In order to investigate the functions of circITGA7 in PCa, the circITGA7 overexpression vector was transfected into PC3 and DU145 cells. As shown in Fig. 2A, the expression level of circITGA7 was significantly increased after stable transfection. Afterwards, we performed CCK-8, Colony formation, EdU and flow cytometry analysis. CCK-8 assay revealed that the growth rate of PC3 and DU145 cells was inhibited after the overexpression of circITGA7 (Fig. 2B). Similarly, Colony formation assay and EdU assay exhibited that circITGA7 inhibited the proliferation of PCa cells (Fig. 2C and 2D). Besides, flow cytometry analysis showed that overexpression of circITGA7 induced cell cycle arrest at G0/G1 phase (Fig. 2E). To further explore the effects of circITGA7 on cell proliferation in vivo, PC3 cells stably transfected with circITGA7 were injected into nude mice. As shown in Fig. 2F-2H, compared with the control vector group, the growth rate and tumor weight of the xenograft tumor in circITGA7 group were significantly decreased. Collectively, our results showed that circITGA7 could obviously inhibit PCa cell proliferation in vivo and in vitro.
Knockdown of circITGA7 promotes PC cell proliferation

To further explore the effects of circITGA7 on PCa cell proliferation, we constructed small interfering RNA to knock down circITGA7 expression (Fig. 3A). As shown in Fig. 3B, CCK-8 assay showed that the knockdown of circITGA7 significantly increased the cell growth rate of PCa cells compared with the negative control. Consistently, the following colony formation and EdU assays showed that the proliferation ability of PCa cells was increased after the knockdown of circITGA7 (Fig. 3C and 3D). Moreover, flow cytometry analysis revealed that circITGA7 knockdown could accelerate the cell transition from G1 to S phase (Fig. 3E).

circITGA7 sponges miR-370-3p in PCa Cells

Acting as highly efficient microRNA sponges to regulate protein-coding genes is an important way for circRNAs to exert its function. In order to elucidate the underlying mechanisms of circITGA7 regulating PCa progression, we performed bioinformatics analysis with miRanda, TargetScan and circinteractome, and predicted five microRNAs that might target circITGA7 (Fig. 4A). The analysis of GEO data GSE40026 suggested that the expression level of miR-370-3p in PCa cells was extremely higher than that in normal epithelial cells (Fig. 4B). Moreover, miR-370-3p was reported to promote the proliferation of PCa [15]. Therefore, miR-370-3p was chosen for further study. First, the expression level of miR-370-3p in PCa tissues was examined by qRT-PCR. As shown in Fig. 4C, the expression of miR-370-3p in PCa tissues was obviously increased compared with that in adjacent normal tissues. Then, Pearson correlation analysis illustrated that the expression level of miR-370-3p was negatively correlated with circITGA7 (Fig. 4D). Additionally, the overexpression of circITGA7 remarkably reduced the level of miR-370-3p (Fig. 4E).

To elucidate whether circITGA7 could directly bind miR-370-3p, we then performed dual luciferase assay and the results showed that miR-370-3p mimic significantly reduced the luciferase activity of circITGA7 compared with miR-NC, while miR-370-3p mimic had no significant effect on luciferase activity of circITGA7 with the mutated target site (Fig. 4F-4H). The next RIP assay exhibited that circITGA7 and miR-370-3p were enriched in RNA complexes in the Ago2 group compared with the IgG group (Fig. 4I and 4J).

P21CIP1 is a target of miR-370-3p in PCa cells

In order to further study the regulation mechanism of miR-370-3p, we predicted the downstream target of P21CIP1 with TargetScan databases. As shown in Fig. 5A, miR-370-3p may target P21CIP1, so luciferase plasmid with WT-P21CIP1 3′ UTR or Mut-P21CIP1 3′ UTR was constructed to further verify the relationship between miR-370-3p and P21CIP1. We observed that miR-370-3p mimic significantly inhibited the luciferase activity in cells that carry a luciferase plasmid containing WT-P21CIP1 3′ UTR, but not the luciferase plasmid containing Mut-P21CIP1 3′ UTR (Fig. 5B). In addition, the mRNA and protein level of P21CIP1 increased significantly after the overexpression of circITGA7, while this effect was reversed by miR-370-3p mimic (Fig. 5C-5E). Collectively, the above results indicated that circITGA7 regulated the expression of P21CIP1 via sponging miR-370-3p.
The overexpression of miR-370-3p eliminates the inhibitory effect of circITGA7 on PCa cell proliferation.

To test whether circITGA7 inhibits the proliferation of PCa cells through sponging miR-370-3p, we conducted a rescue assay. As shown in Fig. 6A, the enforced expression of circITGA7 significantly inhibited PCa cell proliferation, but this inhibition effect was eliminated after transfecting with miR-370-3p mimic. A similar phenomenon was found in subsequent flow cytometry analysis. Cell cycle was arrested in G0/G1 phase after the overexpression of circITGA7, but cells were accelerated into S phase by transfection of circITGA7 and miR-370-3p mimic simultaneously (Fig. 6B). Together, these results indicated that circITGA7 contributed to cell proliferation inhibition by miR-370-3p/P21CIP1 axis.

Discussion

Recently, the role of circRNAs in cancer progression has attracted increasing attention and several circRNAs have been identified as potential biomarkers for cancer [16, 17]. It has been reported that CircSLC19A1 silencing inhibited PCa cell proliferation, migration and invasion by regulating miR-326/MAPK1 axis [18]. Zhang et al proposed that Circ_0057553 affected PCa cell viability, migration, invasion, apoptosis and glycolysis through miR-515-5p/YES1 axis [19]. Shan et al hold the view that circFMN2 promoted the proliferation of PCa cells by facilitating DNA synthesis and inhibiting cell apoptosis [20]. These studies indicated that circRNAs played an important role in the development and progression of PCa.

In this study, we demonstrated that the expression of circITGA7 in PCa tissues and cells was significantly lower than that in normal prostate tissues and cells. However, the expression level of circITGA7 was not significantly correlated with pathological stage, Gleason score and PSA level. Considering the small sample size of our study, this conclusion still needs to be further clarified with a larger sample size. Fang et al reported that circITGA7 promoted the proliferation and metastasis of osteosarcoma cells [13], while Li et al discovered that circITGA7 inhibited the growth and metastasis of colorectal cancer [11]. The two studies with different conclusions revealed the diversity of biological roles of circITGA7 in different tissues. In our present study, the loss- and gain-of-function assays confirmed that circITGA7 inhibited cell proliferation in PCa, but the effects of circITGA7 on cell invasion and migration is still unclear, which will be explored in our future studies.

Those circRNAs derived from protein encoding exons and primarily localized in the cytoplasm can function by sponging miRNAs [21]. Jin et al revealed that circLMTK2 acted as a tumor suppressor in PCa via regulating the expression of microRNA-183 [22]. circAMOTL1 was found to serve as a competing endogenous RNA to prompt the expression of AMOTL1 through sponging miR-485-5p in cervical cancer [23]. Given that circITGA7 was derived from exon 4 of ITGA7 gene and was mainly located in the cytoplasm, the next miRNA-targeting analysis illustrated that circITGA7 contained targeting sites for miRNA, suggesting that circITGA7 can act as a miRNA sponge. Through overlapping screening of miRanda, TargetScan and circinteractome, combined with GEO data analysis and literature search, miR-370-3p was selected as the target of circITGA7 for the next step of research. miR-370-3p has been
reported to be involved in a wide range of human diseases, such as acute pneumonia [24], endometriosis [25], hypoxia injury of myocardial cells [26], and especially cancers [27–29]. Through further mechanism research, we revealed that circITGA7 could bind with miR-370-3p and negatively regulate miR-370-3p expression in PCa cells.

P21\textsuperscript{CIP1}, encoded by CDKN1A, is one of the key factors in cell cycle regulation. Several studies have pointed out that P21\textsuperscript{CIP1} is involved in the G1-S phase cell cycle transition [30–32]. In our study, P21\textsuperscript{CIP1} was predicted as a target of miR-370-3p by TargetScan. The overexpression of circITGA7 could upregulate the expression of P21\textsuperscript{CIP1} and induce G0/G1 cell cycle arrest, while this effect was reversed by transfecting of miR-370-3p mimic. Our results preliminarily expounded the mechanism of circITGA7 that regulated the proliferation of PCa cells.

Conclusion

Our study demonstrated that circITGA7 was downregulated in PCa tissues and cell lines for the first time. circITGA7 inhibited the proliferation of PCa cells and induced G0/G1 phase arrest. Mechanistically, functioned as a sponge for miR-370-3p, circITGA7 increased P21\textsuperscript{CIP1} expression. Our data enhanced our understanding of circRNA biology and proposed a novel circRNA/miRNA/mRNA regulatory network for the management of PCa.

Abbreviations

circRNA: circular RNA; miRNA: MicroRNA; ITGA7: integrin subunit alpha 7; CDKN1A: Cyclin-dependent kinase inhibitor 1A; 3\textsuperscript{UTR}: 3\textsuperscript{Un}translational region.

Declarations

Acknowledgements

Not applicable.

Authors’ contributions

Gang Luo and Dong Liu designed the study and wrote the manuscript. Yonglian Guo, Guohao Li, Zhihua Wan and Yuanjie Zhang conducted the experiments. Yonglian Guo and Guohao Li analyzed the data. The final version of the manuscript was approved by all authors.

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Availability of data and materials
The datasets used in the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of the Central Hospital of Wuhan (2021-011) and written informed consent was obtained from each patient before enrollment.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no conflicts of interest.

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Figures
Relative circITGA7 expression levels in PCa. (A) Clustered heat map of the differentially expressed circRNAs in three pairs of human PCa tissues and adjacent normal tissues (GSE179321). (B) Schematic illustration showed the circularization of ITGA7 exon 4 formed circITGA7. The back-splice junction of circITGA7 was identified by Sanger sequencing. (C) PCR assay with divergent or convergent primers indicated the existence of circITGA7 in PCa cells. divergent primers ◀▶, convergent primers ▶◀. (D) The expression of circITGA7 and ITGA7 mRNA in PCa cells treated with RNase R was detected by qRT-PCR. (E) The expression of circITGA7 in 28 pairs of PCa tissues and paracancerous tissues was detected. (F) The relative expression of circITGA7 was detected using qRT-PCR in RWPE-1, PC3, LNCaP, and DU145 cells. (G) RNA-FISH indicated the location of circITGA7 in PC3 cells. Scale bar, 10 μm. Data were presented as mean ± SD of three independent experiments. Statistic significant differences were indicated: *P < 0.05.
Figure 2

The enforced expression of circITGA7 inhibits cell proliferation in vitro and in vivo. (A) The expression levels of circITGA7 in PCa cells stably transfected with circITGA7 overexpression vector or negative control vector were detected by qRT-PCR. (B) CCK-8 assay was used to detect the growth rate of transfected PCa cells. (C and D) The cell proliferation was measured by the colony formation and EdU assays. Scale bar, 100 μm. (E) The cell cycle was analyzed by flow cytometry analysis. (F-H) Xenograft tumors derived from circITGA7 group showed a smaller volume and lower weight than those derived from empty vector group. Data were presented as mean ± SD of three independent experiments. Statistic significant differences were indicated: *P < 0.05.
Figure 3

The knockdown of circITGA7 promotes the proliferation of PCa cells. (A) qRT-PCR assay was used to measure the efficiency of small interfering RNAs. (B) CCK-8 assay confirmed that the cell growth rate was increased after the knockdown of circITGA7. (C and D) Colony formation and EdU assays were used to detect cell proliferation after the knockdown of circITGA7. Scale bar, 100 μm. (E) Flow cytometry analysis revealed circITGA7 knockdown promoted the cell transition from G1 phase to S phase. Data were presented as mean ± SD of three independent experiments. Statistic significant differences were indicated: *P < 0.05.
circITGA7 sponges miR-370-3p in PCa cells. (A) Bioinformatics analysis with miRanda, TargetScan and CircInteractome. (B) The GEO dataset analysis showed the expression of miR-370-3p in PCa cells and normal prostate epithelial cells. (C) The expression level of miR-370-3p in 28 pairs of PCa and paracancerous tissues was detected. (D) Evaluating the correlation of circITGA7 and miR-370-3p expression in PCa tissues by Pearson' s correlation coefficient. (E) miR-370-3p was downregulated after the overexpression of circITGA7. (F) qRT-PCR was used to detect the relative expression of miR-370-3p in cells transfected with miR-370-3p mimic or negative control. (G) Schematic representation of the binding sites of miR-370-3p with circITGA7. (H) Relative luciferase activity was determined by dual-luciferase reporter assay in PCa cells co-transfected with miR-370-3p mimic and WT-circITGA7 or Mut-circITGA7. (I and J) Interaction between miR-370-3p and circITGA7 was further confirmed by RIP assay. Data were
presented as mean ± SD of three independent experiments. Statistic significant differences were indicated: *P < 0.05.

Figure 5

circITGA7 upregulates the expression level of P21CIP1 via miR-370-3p. (A) The binding sites of miR-370-3p in 3' UTR of P21CIP1 were predicted by bioinformatics analysis. (B) Luciferase reporter assays were performed following the co-transfection of the cells with WT-P21CIP1 or Mut-P21CIP1 and miR-370-3p mimic or NC mimic. The mRNA (C) and protein levels (D and E) of P21CIP1 were examined after the co-transfection of circITGA7 overexpression plasmid and miR-370-3p mimic. Data were presented as mean ± SD of three independent experiments. Statistic significant differences were indicated: *P < 0.05.
Figure 6

Transfection of miR-370-3p mimic reverses the inhibitory effect of circITGA7 on cell proliferation. (A) The cell growth rate was detected by CCK-8 assay after the co-transfection with circITGA7 overexpression plasmid and miR-370-3p mimic. (B) Cell cycle distributions were presented by flow cytometry analysis. Data were presented as mean ± SD of three independent experiments. Statistic significant differences were indicated: *P < 0.05.

Supplementary Files

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