

Original Article

MicroRNA-144-3p inhibits cell proliferation and induces cell apoptosis in prostate cancer by targeting CEP55

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Abstract: Previous research reported that miR-144-3p functions as tumor suppressor in several tumors, including glioblastoma and hepatocellular carcinoma, but the role of miR-144-3p in prostate cancer (PCa) remains unclear. In this study, we aimed to analyze the role of miR-144-3p in PCa. By RT-qPCR, we found that expression of miR-144-3p was markedly down-regulated in PCa tissues and cell lines compared with that in paired adjacent normal tissues and normal cell lines. Moreover, miR-144-3p overexpression in PC-3 and DU145 cells by transfection with miR-144-3p mimics significantly inhibited cell proliferation and *in vitro* by MTT, colony formation assays and suppressed tumor growth *in vivo* by nude mice model. Flow cytometry analysis further demonstrated that forced expression of miR-144-3p induced cell cycle G1/S phase arrest and apoptosis. Moreover, centrosomal protein of 55 (CEP55) was confirmed as a direct target of miR-144-3p by bioinformatics analysis and luciferase reporter assays. Overexpression of miR-144-3p decreased the CEP5 mRNA and protein levels in PC-3 and DU145 cells. Using Oncomine database analysis, we further found the expression of CEP55 was significantly upregulated in PCa tissues. In addition, knock-down of CEP55 elicited similar effects with miR-144-3p overexpression in PCa cells. Taken together, our results demonstrate that miR-144-3p functions as a tumor suppressor in PCa by downregulating CEP55, supporting the targeting miR-144-3p might be a potentially effective therapeutic approach for PCa.

Keywords: Prostate cancer, miR-144-3p, cell proliferation, CEP55, tumor growth

Introduction

Prostate cancer (PCa) has traditionally been one of most common malignancy [1, 2]. In 2016, the American Cancer Society reported that approximately 180,890 new PCa cases and 26,120 deaths were occurred in America [3]. For all stages combined, the 5-year relative survival rates for PCa patients increased from 83% in 1980s to greater than 95% in 2000, which largely due to widespread prostate-specific antigen (PSA) screening and development of surgical, radio-chemotherapy, primary androgen deprivation therapy, active surveillance and observation treatment strategies [4]. Except for exogenous risk factors such as diet, environmental agents, occupation and other factors have been implicated in the development of PCa, epigenetic and genetic dysregulations are considered as two critical endogenous risk factors involving in carcinogenesis [5].

MicroRNAs (miRNAs) are 20-24-nucleotide-short RNAs that play pivotal roles in almost all

biological processes in mammalian species and other eukaryotes [6]. Accordingly, miRNAs are capable of regulating gene expression at post-transcriptional level, mostly binding to "seedless" pairing sequences in the 3'UTR of target mRNAs, either direct mRNA cleavage or translational depression [7]. A growing literature supports genes alteration mediated by miRNAs are projected to regulate various important processes including cellular growth, apoptosis, metabolic, and development [8]. Indeed, dysregulation of miRNAs signatures are not rare but rather the rule of human cancer [7]. Furthermore, abnormal of miRNAs often result from genetic loss and mutation, epigenetic overexpressing or silencing, and aberrant transcription factor activity in tumors [9]. It was previously reported that miR-144-3p is decreased in glioblastoma [10], bladder cancer [11], laryngeal squamous cell carcinoma [12], etc. Despite miR-144-3p exerts a strong antitumor effect in multiple types of cancer and is largely associated with dysregulation of their target mRNAs,

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Table 1. Clinicopathological features of prostate cancer patients

Clinical characteristics	Number of cases (n = 40)
Age (year-old)	
≤ 60	15
> 60	25
Smoking habits	
Smokers	22
Non-smokers	18
PSA level (ng/mL)	
≤ 4	16
> 4	24
Gleason Score	
7	32
≥ 7	8
Clinical stage	
T1-T2	31
T3-T4	9
Lymph-node metastasis	
Yes	10
No	30

however, the expression and role of the gene in PCa is undefined as yet.

Centrosomal protein of 55 (CEP55), as the name implies, was originally identified as critical regulator of cytokinesis, the end of mitosis that drives cytoplasm roughly equally for each of the two daughter cells [13]. Primary genetic lesion is a failure to successfully complete cytokinesis related to a deficiency of CEP55, contributing to human carcinogenesis and aneuploidy [14]. CEP55 amplification is commonly detected in patients with carcinoma of gastric [15], breast [16], lung [16], and esophagus [17]. In a previous study, CEP55 was found to be a predictive biomarker of head and neck squamous cell carcinoma progression [18].

The present study aims to examine the expression of miR-144-3p and its functional role in PCa progression *in vitro* and *in vivo*. In addition, the cellular effects of miR-144-3p and its interacting targets in PCa cells were investigated. These results may improve understanding of the molecular mechanism of anti-oncogenic miRNAs in the development of PCa and provide opportunities to explore novel strategies to reverse PCa progression.

Materials and methods

Patient samples

Total 40 paired PCa and adjacent non-cancerous prostate tissues were obtained from PCa patients admitted to The Fifth Affiliated Hospital of Sun Yat-sen University (Department of Urology) between 2014 and 2016. All patients had undergone a radical prostatectomy, and hadn't received any radiotherapy, chemotherapy or androgen-deprivation treatment. The summary of clinicopathological characteristics for all patients is presented in **Table 1**. The study protocol was approved by the Institutional Research Review Board at The Fifth Affiliated Hospital of Sun Yat-sen University and signed informed consent was obtained from all study participants.

Cell culture

Human PCa cell lines (DU145, PC-3, LNCaP and 22RV1) and normal prostate epithelial cells (RWPE-1) were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). LNCaP, 22RV1 and RWPE-1 cells were cultured in RPMI 1640 medium (Hyclone). PC-3 and DU145 cells were cultured in Ham's/f-12 (Hyclone). All cell lines were supplemented with 10% of fetal bovine serum (FBS, Invitrogen, USA) and maintained in a humidified atmosphere containing 5% CO₂ at 37°C.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted using Trizol reagent (Life Technologies, Carlsbad, CA, USA) following the manufacturer's protocol. For quantification of miR-144d-3p, the TaqMan miRNA Reverse Transcription Kit (Applied Biosystems, USA) was performed reverse transcription and TaqMan miRNA assay (Applied Biosystems, USA) was used to measure the miR-144d-3p expression according to the manufacturers' instructions using U6 small nuclear RNA as an internal control. CEP55 mRNA expression level was investigated by SYBR green PCR assay (Qiagen, USA). The primer sequences were as follows: miR-144-3p forward, 5'-GGGAGATCAGAAGGTG-ATT-3'; reverse, 5'-GTGCAGGGTCCGAGGT-3'. U6 forward, 5'-CTCGCTTCGGCAGCACA-3'; reverse, 5'-AACGCTTCACGAATTTGCGT-3'. CEP55 forward, 5'-AGCAGCAAGAAGAACAACAAGGG-3';

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reverse, 5'-AGTGACTAATGGCTCTGTGATGGC-3'. GAPDH, forward: 5'-GAGTCAACGGATTTGGTCG-TATTG-3'; reverse: 5'-CCTGGAAGATGGTATGG-GATT-3'.

Oligonucleotides and cell transfection

The miR-144-3p mimics (5'-UACAGUAUAGAUG-AUGUACU-3'), small interfering RNA (siRNA) for the CEP55 (siCEP55, 5'-GCAUUGGGUUAC-AUUTT-3') and their corresponding negative control (NC mimics: 5'-UUUGUACUACACAAAA-GUACUG-3' and siNC: 5'-UGACCUCAACUACAU-GGUUTT-3', respectively) were chemically synthesized and purified from GenePharma (Shanghai, China). For cell transfection, PCa cells were seeded in six-well plates and cultured until 60% to 80% confluency. Transfection of miRNA mimics, siRNA, and corresponding NC was performed with a final concentration of 50 nM for RNA mimics, 100 nM for siRNA and their respective NCs using Lipofectamine 2000 (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions.

Cell viability assay

Cell viability was analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cells were seeded at 3,000 cells/well in 6-well plates, with 3 replicates for each group following by the addition of 10 μ l MTT solution (5 mg/ml, Sigma-Aldrich) for 2 hours. After removing the medium, 150 μ l DMSO (Sigma-Aldrich) was added into each well to solubilize the remaining MTT formazan crystals. The optical density (OD) value was measured at 595 nm with a microplate reader (Bio-Rad) and cell viability was determined once per day for consecutive 5 days.

Colony formation assay

Cells were seeded in 6-well plates at a density of 500 cells per well and cultured for 7 days in 5% CO₂ at 37°C. Then cells were fixed with 4% paraformaldehyde for 30 min and stained with 0.5% crystal violet for 30 minutes. The surviving colonies (> 50 cells/colony) in each plate were counted under a light microscope (Olympus Corporation, Tokyo, Japan). The experiments were carried out in triplicate wells.

Flow cytometry analysis

PCa cells (1 \times 10⁵ cells/dish) were seeded on 6-cm dishes and harvested by centrifugation at

1,200 rpm for 5 min. The cells were washed twice with cold PBS and then fixed in 70% ethanol for 30 min at 4°C. The ethanol was discarded after centrifugation at 1,500 rpm for 5 min and cells were re-suspended with PBS. The cell suspension was next filtered through a 50- μ m nylon mesh and centrifuged at 1,200 rpm for 5 min. For cell cycle assay, cells were stained with a propidium iodide (PI, 100 μ g/ml) solution containing 10 μ g/ml of DNase-free RNase A, followed by incubation for 30 min at room temperature and analyzed using flow cytometer (Cell Lab Quanta, Beckman Coulter). For cell apoptosis analysis, the cells were collected and subjected to Annexin V-APC/7-AAD apoptosis detection kit (KeyGEN Biotech, Nanjing, China) following manufacturer's instructions. The experiments were carried out in triplicate wells.

Bioinformatics analysis and luciferase assay

The potential targets of miR-144-3p were predicted by TargetScan (<http://www.targetscan.org/>) and PicTar (<http://pictar.mdcb Berlin.de/>). CEP55 was predicted to be a candidate target gene of miR-144-3p. To confirm this prediction, dual luciferase experiment was performed in PCa cells, respectively. In brief, Luciferase reporter plasmids, psiCHECK-2TM-CEP55-3'-UTR wild-type (WT) and psiCHECK-2TM-CEP55-3'-UTR mutant (MUT) were synthesized and purified by GenePharma Co. Ltd. For cell transfection, PC-3 and DU145 cells were seeded into 24-well plates at a density of 1 \times 10⁵ cells/well and cultured for 24 h. Then co-transfections with 250 ng psiCHECK-2TM-CEP55-3'-UTR WT or psiCHECK-2TM-CEP55-3'-UTR MUT and 50 nM miR-144-3p or NC mimics was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, USA). After 48 h incubation, luciferase activity was determined using Dual Luciferase Reporter Assay Kit (Promega) according to the manufacturer's instructions.

Oncomine data analysis

The differential expression of CEP55 between PCa and their normal counterparts was analyzed using Oncomine database (<https://www.oncomine.org>). Briefly, the Cancer Type was defined as PCa, Data Type was mRNA, and Analysis Type was Cancer versus Normal Analysis. Total three separate datasets, including Arredouani Prostate [19], Grasso Prostate

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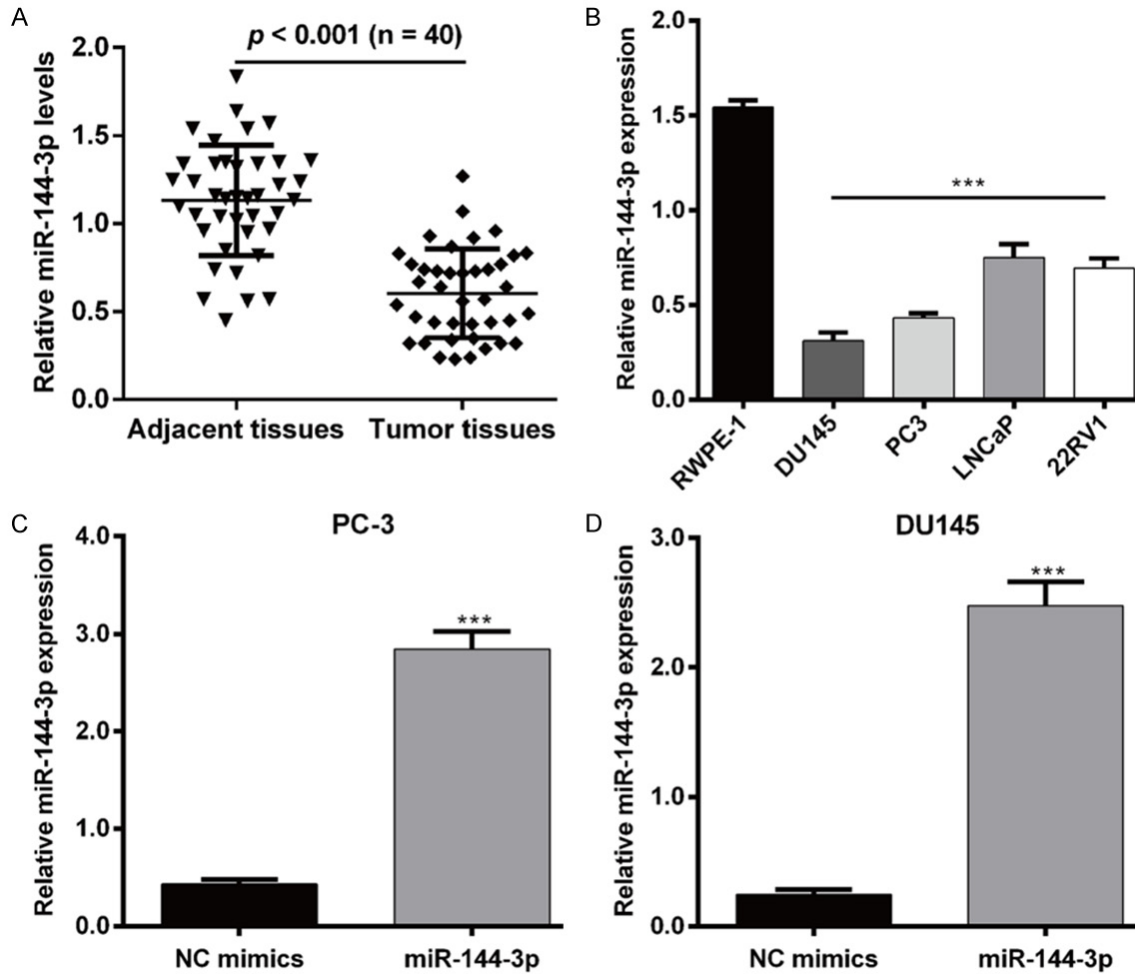


Figure 1. MiR-144-3p expression profiles in PCa tissues, normal prostate tissue, PCa cells and normal prostate epithelial cells. (A) MiR-144-3p expression was downregulated in PCa tissue compared with normal prostate tissue. (B) Lower levels of miR-144-3p expression were detected in four PCa cell lines compared with normal prostate epithelial cells. High levels of miR-144-3p expression were detected in (C) PC-3 and (D) DU145 cells stably transfection with miR-144-3p mimics. *** $P < 0.001$ vs. RWPE-1 cells or NC mimics.

[20] and Tomlins Prostate [21] were screened. Then the log-transformed, median-centered and normalized expression values were extracted, analyzed and graphed as previously described [22].

In vivo tumorigenicity

Two group cells (miR-144-3p-transfected PC-3 cells or NC mimics-transfected PC-3 cells) were suspended in 0.1 ml phosphate-buffered saline (PBS) and injected subcutaneously into the backs of five to six-week-old male BALB/c nude mice, which were purchased from Shanghai SLAC Laboratory Animals Co., Ltd. (Shanghai, China). Correspondingly, six mice were randomized into two groups with three mice in each group. Tumor growth was measured using cali-

pers over the course of 49 days (at least three times every six days). Tumor volume was calculated according to the formula: volume = $0.5 \times \text{length} \times \text{width}^2$. The mice were euthanized with CO_2 and sacrificed on day 49. Tumor weight was measured and compared between two groups. All the animal experiments were strictly performed in accordance with the Guide for the Care and Use of Laboratory Animal, with the approval of the Animal Studies Ethics Committee of Sun Yat-sen University.

Western blot analysis

All protein was extracted using RIPA buffer (Beyotime, Shanghai, China) containing protease inhibitors. The supernatant was collected after the lysates were centrifuged at $12,000 \times$

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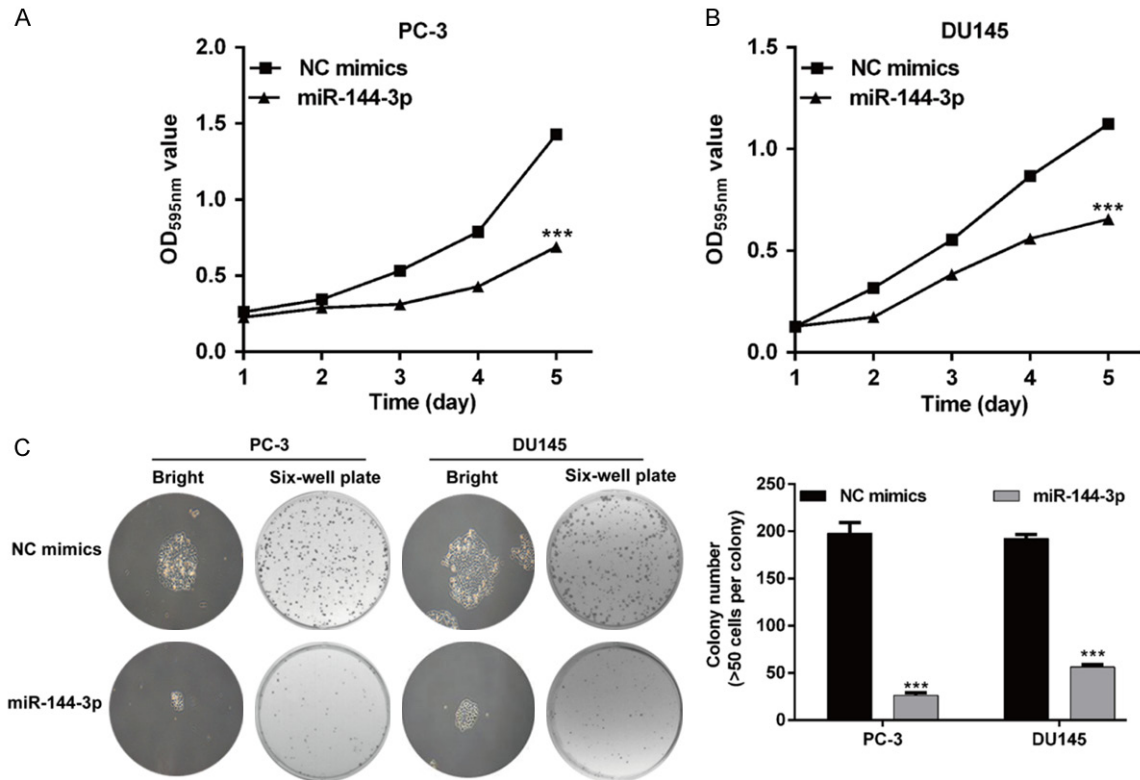


Figure 2. Overexpression of miR-144-3p significantly inhibits cell proliferation in PCa cells *in vitro*. MTT assay was performed to measure cell viability in (A) PC-3 and (B) DU145 cells. Data represent the mean \pm standard deviation of the optical density (OD) value detected at 595 nm from three independent experiments. (C) Colony formation assays indicated fewer colonies in miR-144-3p overexpressing PCa cells. Representative images of colonies were shown in the left panel. Quantification of colonies was presented in the right panel. *** $P < 0.001$ vs. NC mimics.

g for 5 min at 4°C. After protein quantification using a BCA kit (Beyotime, Shanghai, China), the same amount of protein samples were electrophoresed in 10% sodium dodecyl sulfate polyacrylamide gels and transferred onto a polyvinylidene fluoride membrane (Millipore, USA). Then the membrane was blocked with 5% skim milk for 1 h at room temperature and incubated with primary antibodies against CEP55 (1:1000, 23891-1-AP, Proteintech) and GAPDH (1:500000, 10494-1-AP, Proteintech) at 4°C overnight. Subsequently, the membranes were washed with three times and incubated with horseradish peroxidase (HRP) conjugated secondary antibody (1:5000, SC-2054, Santa Cruz Biotechnology Inc.) at 37°C for 2 h. Afterwards, the protein bands were detected and visualized using an enhanced chemiluminescence (Pierce; Thermo Fisher Scientific Inc.).

Statistical analysis

All quantitative experiments were repeated three times. Data in this study were analyzed

with SPSS 17.0 (SPSS Inc., Chicago, IL, USA) and expressed as mean \pm standard deviation (SD). The student t-tests or one-way ANOVA was used to evaluate differences between two groups or more than two groups, respectively. The statistical analysis was considered to be significant when p -value < 0.05 .

Results

MiR-144-3p expression was downregulated in PCa tissues and cell lines

To investigate the expression pattern of miR-144-3p in PCa, we performed qRT-PCR to determine the miR-144-3p expression in 40 pairs of PCa tissues and their adjacent non-cancerous tissues. As shown in **Figure 1A**, PCa tissues expressed significantly lower levels of miR-144-3p compared with adjacent non-cancerous tissues ($P < 0.001$). In addition, expression levels of miR-144-3p were detected in four PCa cell lines (DU145, PC-3, LNCaP and 22RV1), with a normal prostate epithelial

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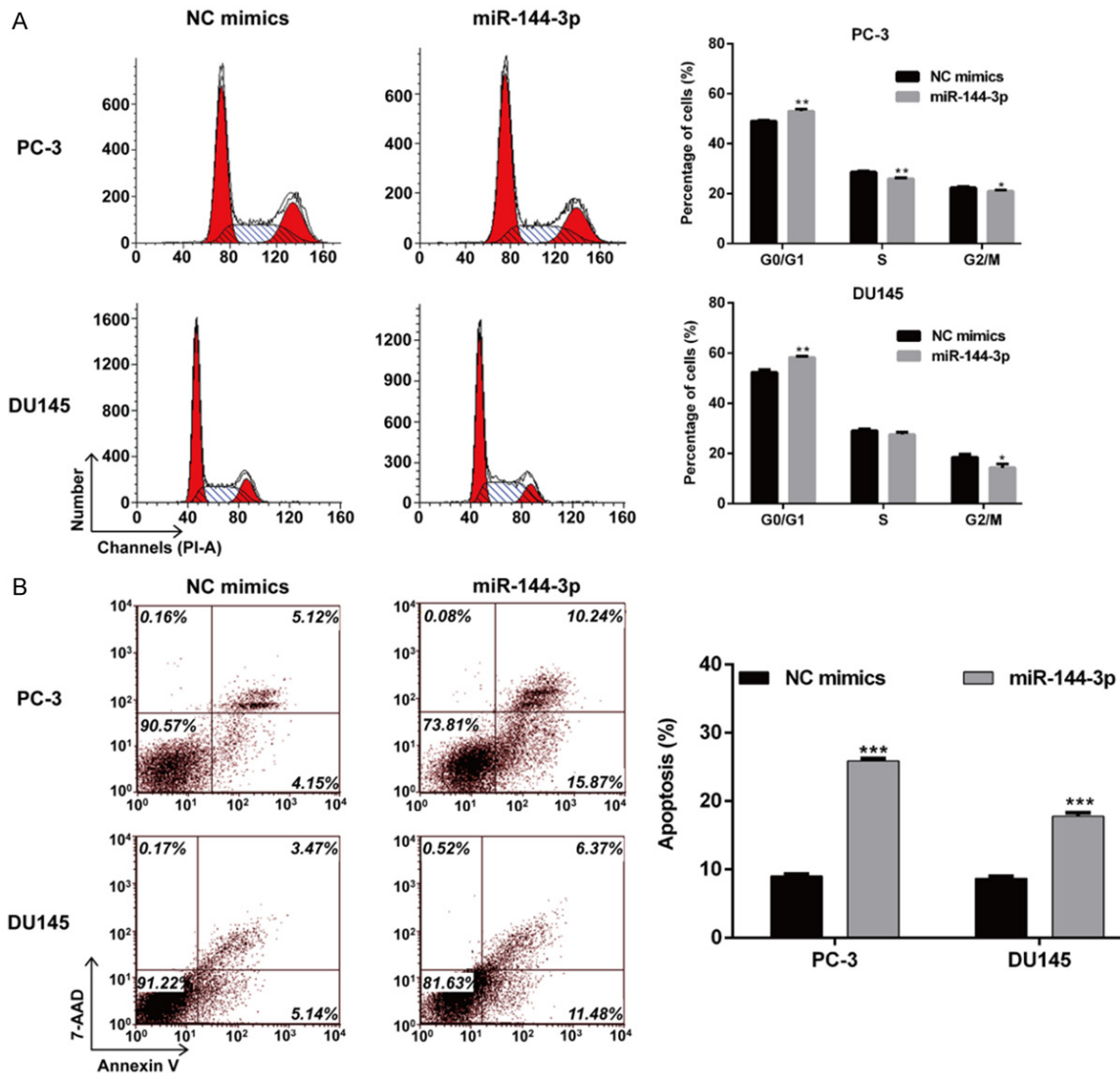


Figure 3. Overexpression of miR-144-3p induced G1/S phase cell cycle arrest and apoptosis in PCa cells. A: FACS analysis of cell cycle distribution in PC-3 and DU145 cells following miR-144-3p overexpression (left panel). Quantification of the cell percentage in G0/G1 phase, S phase and G2/M phase (right panel); B: PC-3 and DU145 cells stained with Annexin V and 7-AAD analyzed using flow cytometer (left panel). Quantification of the total apoptotic cell percentage (early and late apoptosis) (right panel); Data represent the mean \pm standard deviation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. NC mimics.

RWPE-1 cells as control by qRT-PCR. The results indicated that the expression levels of miR-144-3p were markedly down-regulated in all PCa cell lines compared with that in RWPE-1 cells (Figure 1B, $P < 0.001$). Thus, we speculated that overexpression of miR-144-3p might have a suppressive role in PCa. For the *in vitro* experiments, we stably overexpressed miR-144-3p in PC-3 (Figure 1C, $P < 0.001$) and DU145 (Figure 1D, $P < 0.001$) cells with the lowest miR-144-3p levels by transfection with miR-144-3p mimics, which was confirmed by qRT-PCR.

Overexpression of miR-144-3p inhibits PCa cell proliferation *in vitro*

To investigate whether miR-144-3p plays a role in PCa cell proliferation, the cell viability was first determined in PC-3 and DU145 cells using MTT assay. As depicted in Figure 2A, 2B, overexpression of miR-144-3p significantly induced time-dependent inhibition of cell viability, as described by markedly dropped growth curve of PC-3 and DU145 cells on consecutive five days ($P < 0.001$). Furthermore, the results of colony formation assay showed the number of colo-

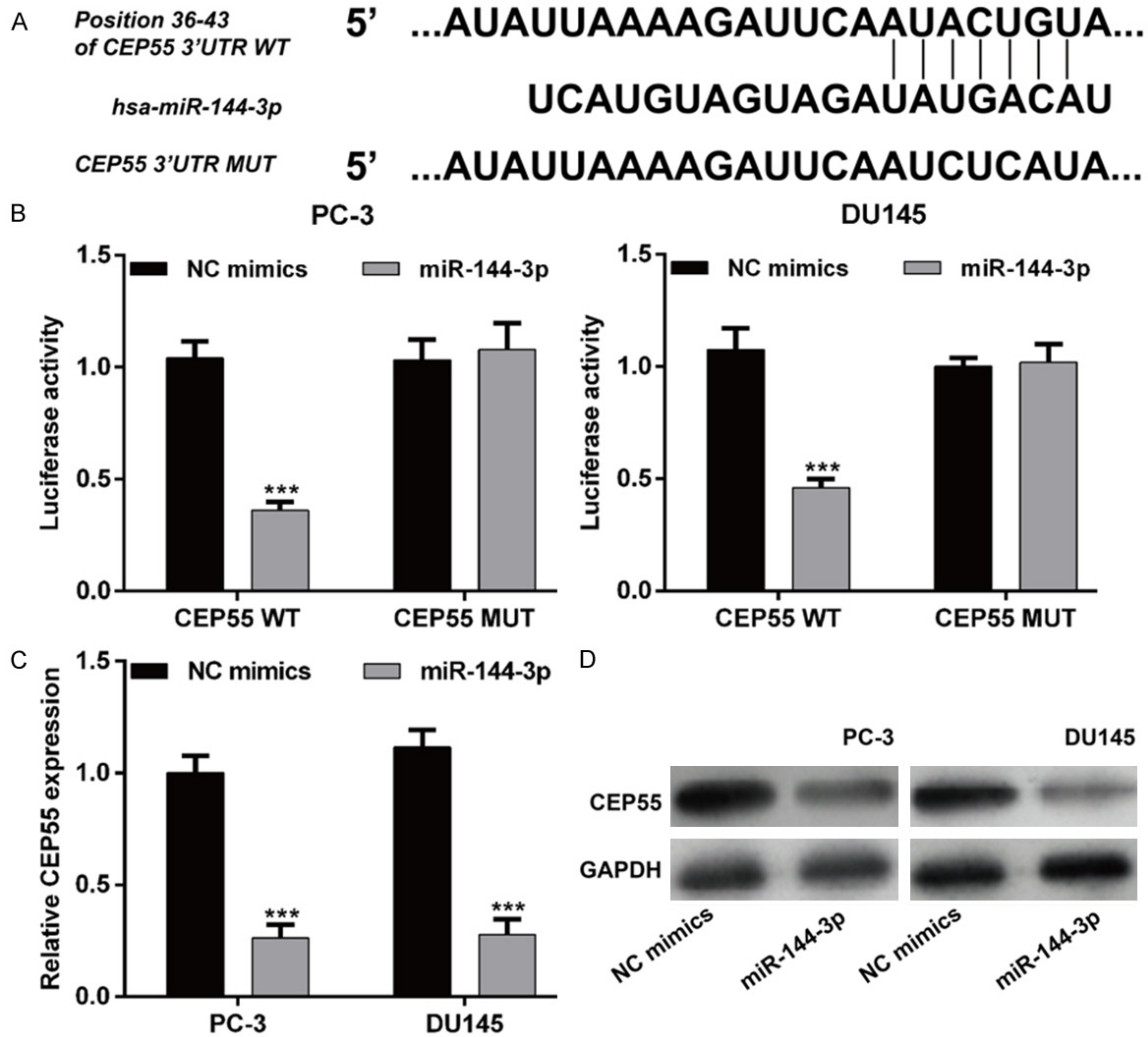


Figure 4. CEP55 was a direct target of miR-144-3p in PCa. (A) The binding sites of wild type and mutant sequences of miR-144-3p were predicted in the 3'UTR-CEP55. (B) PC-3 and DU145 cells were cotransfected with miR-144-3p or NC mimics, and psiCHECK-2TM-CEP55-3'-UTR WT or psiCHECK-2TM-CEP55-3'-UTR MUT. After transfection, luciferase activities were detected using Dual-Luciferase reporter assay system. The mRNA (C) and protein (D) levels of CEP55 in PC-3 and DU145 cells transfected with miR-144-3p or NC mimics were determined by qRT-PCR and western blot analysis, respectively. Data represent the mean \pm standard deviation. *** $P < 0.001$ vs. NC mimics.

nies in miR-144-3p mimics group was remarkably decreased by nearly 86.9% in PC-3 cells and 71.2% in DU145 cells (Figure 2C, $P < 0.001$), indicating that miR-144-3p exerted a suppressive role in PCa cell proliferation.

Overexpression of miR-144-3p induced cell cycle arrest and apoptosis in PCa cells

Now that proliferation inhibition was observed in PCa cells, we used flow cytometry analysis to investigate whether overexpression of miR-144-3p affected cell cycle progression and apoptosis. As shown in Figure 3A, the percent-

age of cells in G0/G1 phase was markedly increased ($49.02\% \pm 0.51\%$ vs. $53.06\% \pm 0.87\%$, $P < 0.01$ in PC-3 cells; $52.31\% \pm 1.12\%$ vs. $58.21\% \pm 0.59\%$, $P < 0.01$ in DU145 cells), S phase ($28.62\% \pm 0.45\%$ vs. $25.95\% \pm 0.45\%$, $P < 0.01$ in PC-3 cells; $29.13\% \pm 0.68\%$ vs. $27.47\% \pm 0.94\%$ in DU145 cells) and G2/M ($22.36\% \pm 0.51\%$, $P < 0.05$ vs. $14.33\% \pm 1.42\%$ in PC-3 cells; $18.47\% \pm 1.23\%$ vs. $14.33\% \pm 1.42\%$, $P < 0.05$ in DU145 cells) phase were accordingly decreased in miR-144-3p group compared with the NC mimics group, suggesting that overexpression of miR-144-3p effectively induced G1/S cell cycle arrest in PCa

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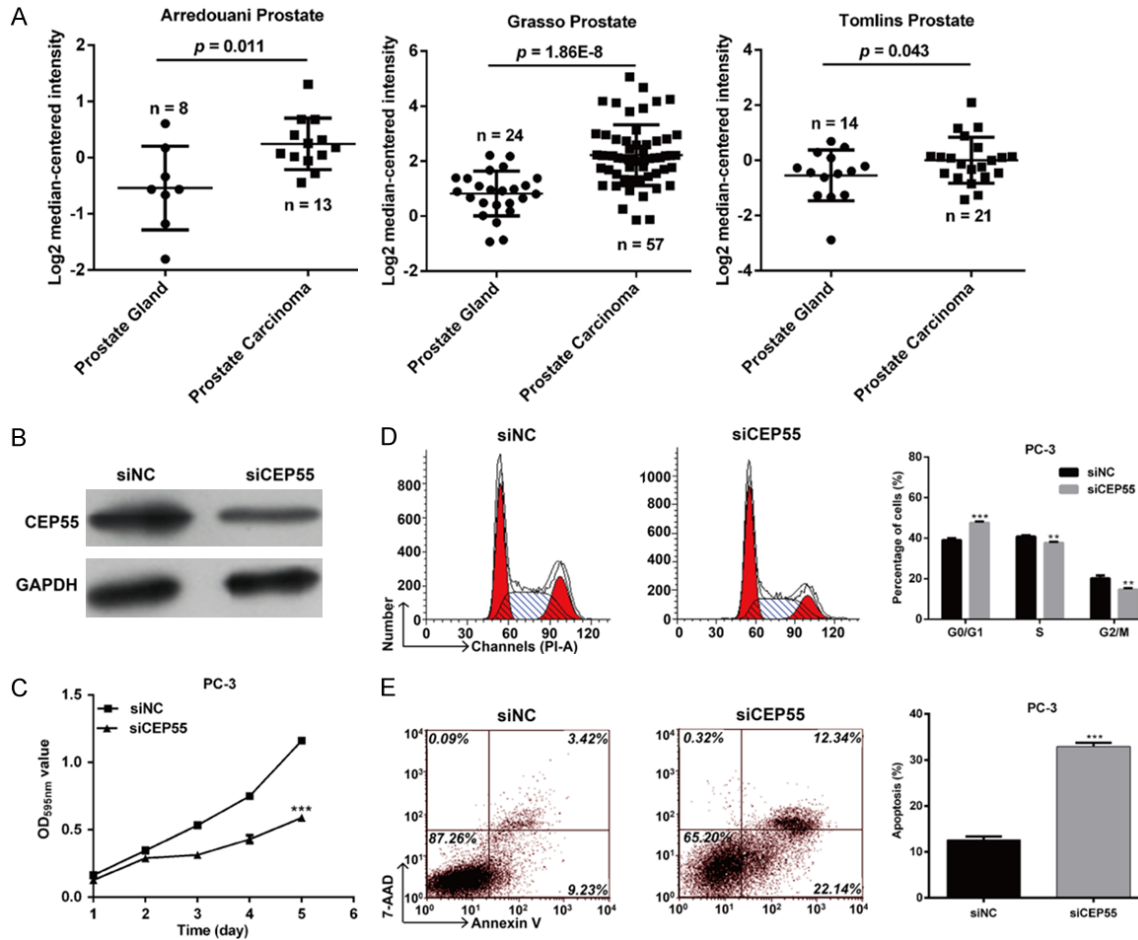


Figure 5. CEP55 knockdown resulted similar effects in PCa cells with overexpressing miR-144-3p. A. The mRNA expression of CEP55 in Oncomine datasets including Arredouani Prostate, Grasso Prostate and Tomlins Prostate is upregulated in PCa tissues compared with the normal control tissues. B. Western blot analysis of CEP55 protein expression in PC-3 cells after transfection with siCEP55 or siNC, respectively. C. The cell viability of PC-3 cells after CEP55 knockdown was analyzed by the MTT assay. D. FACS analysis of cell cycle distribution in PC-3 cells following CEP55 knockdown (left panel). Quantification of the cell percentage in G0/G1 phase, S phase and G2/M phase (right panel); E. PC-3 cells stained with Annexin V and 7-AAD analyzed using flow cytometer (left panel). Quantification of the total apoptotic cell percentage (early and late apoptosis) (right panel); Data represent the mean \pm standard deviation. ** $P < 0.01$, *** $P < 0.001$ vs. NC mimics.

cells. In addition, we found overexpression of miR-144-3p induced apoptosis of PC-3 and DU145 cells. Concretely, the total of early and late apoptotic cells (**Figure 3B**) was significantly increased from $9.02\% \pm 0.31\%$ in NC mimics group to $25.87\% \pm 0.35\%$ in miR-144-3p group in PC-3 cells ($P < 0.001$). Similarly, overexpression of miR-144-3p notably elevated the total apoptotic rate from $8.69\% \pm 0.31\%$ to $17.78\% \pm 0.49\%$ in DU145 cells ($P < 0.001$). These results further demonstrated that overexpression of miR-144-3p suppresses the growth of PCa cells might through cell cycle arrest and apoptosis.

CEP55 was a direct target of miR-144-3p in PCa

Bioinformatics analyses were performed using TargetScan and PicTar and CEP55 was predicted as the potential target gene of miR-144-3p (**Figure 4A**). Subsequently, the reporter plasmid psiCHECK-2TM-CEP55-3'-UTR WT or psiCHECK-2TM-CEP55-3'-UTR MUT was co-transfected with miR-144-3p or NC mimics in PC-3 and DU145 cells. Luciferase activity assay showed that miR-144-3p mimics significantly suppressed the luciferase intensity of PC-3 and DU145 cells co-transfected with miR-144-3p in the

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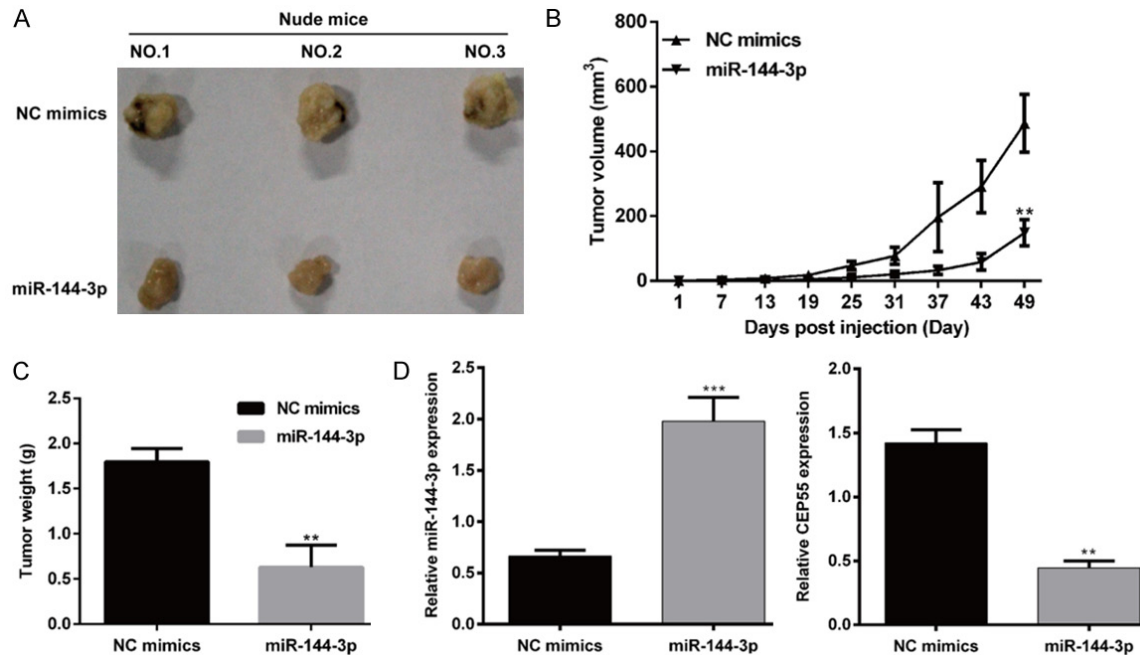


Figure 6. Overexpression of miR-144-3p suppressed tumor growth in nude mice. A. Representative images of the mice carrying tumors are shown after 49 days of inoculation with PC-3 cells expressing miR-144-3p or NC mimics. B. Measurement of tumor volumes at the indicated time points. C. Changes in the tumor weight in mice after miR-144-3p overexpression. D. Expression analysis of miR-144-3p and CEP55 in tumor tissues collected from mice using qRT-PCR assay. Data represent the mean \pm standard deviation. ** $P < 0.01$, *** $P < 0.001$ vs. NC mimics.

wild type but not in mutant type of CEP55, indicating miR-144-3p may directly bind to CEP55 3'UTR (Figure 4B, $P < 0.001$). Moreover, we also assessed the possibility that miR-144-3p serves a functional role in the regulation of endogenous CEP55 expression in PCa cells. The results of qRT-PCR revealed that miR-144-3p overexpression remarkably inhibited CEP55 mRNA expression in PC-3 and DU-145 cells (Figure 4C, $P < 0.001$). The results of the western blot analysis also exhibited a similar inhibitory effect (Figure 4D). These results suggested that miR-144-3p directly targets CEP55 3'UTR to suppress its expression.

CEP55 knockdown elicited similar effects with miR-144-3p overexpression in PCa cells

Using the Oncomine database, we examined CEP55 expression in PCa tissues. As seen in Figure 5A, the expression of CEP55 was significantly increased in PCa tissues compared with the corresponding normal prostate tissues in the Arredouani Prostate dataset ($P = 0.011$), Grasso Prostate dataset ($P = 1.86E-8$) and Tomlins Prostate dataset ($P = 0.043$). These observations highlighted a deregulated expres-

sion of CEP55 in PCa. To further explore the functional role of CEP55 in PCa cells, CEP55 was knocked down in PCa cells following transfection of siCEP55 or siNC. The CEP55 expression reduced at 48 h transfection, as detected by western blot analysis (Figure 5B). The results obtained from the MTT assay revealed that the viability of PC-3 cells decreased in a time-dependent manner (Figure 5C, $P < 0.001$). The results of the flow cytometry assay further determined that inhibition of CEP55 induced cell cycle G1/S phase arrest (Figure 5D, $P < 0.01$, $P < 0.001$) and promoted apoptosis (Figure 5E, $P < 0.001$).

Overexpression of miR-144-3p inhibited tumor growth in vivo

The effects of miR-144-3p overexpression on tumor growth were analyzed *in vivo* using nude mouse models. Representative images of the mice carrying tumors after 49 days are shown in Figure 6A. The time-dependent analysis showed that the volume of the tumors was significantly suppressed in mice inoculated with miR-144-3p mimics transfected PC-3 cells compared with NC mimics group (Figure 6B, P

< 0.01). Moreover, the weight of the tumors was also remarkably decreased in miR-144-3p overexpression mice (**Figure 6C**, $P < 0.01$). These results were confirmed by analysis of the mRNA expression of miR-144-3p and CEP55 in tissues of a subcutaneous xenograft murine model. As shown in **Figure 6D**, the expression of miR-144-3p was significantly elevated ($P < 0.001$), but CEP55 expression was remarkably decreased ($P < 0.01$) in tumor tissues by the infection with miR-144-3p (**Figure 6D**).

Discussion

MiR-144-3p, a miRNA frequently down-regulated in diverse human carcinomas, is a potent antitumor molecule controlling cell growth and survival [10-12]. PCa is a highly complex and heterogenous disease, driven by genetic and epigenetic alterations of numerous oncogenes and anti-oncogenes [23]. Despite clinical improvement in diagnosis and management of PCa, little is known about the miR-144-3p in this disease. In the current study, we identified miR-144-3p as inhibitors of PCa proliferation and colony formation and as promoters of apoptosis *in vitro* via directly binding to CEP55. Biological behavior analysis revealed that overexpression of miR-144-3p was responsible for anti-tumorigenesis, also function as a negative regulator of CEP55. Indeed, silencing CEP55 in PCa cells imitated the anti-oncogenic properties of miR-144-3p *in vivo and vitro*.

CEP55 (also known as FLJ10540 or C10orf3) encodes a novel coiled-coil protein, which is mainly localized to centrosome in interphase cells [24]. The recruitment of CEP55 to the midbody is necessary for completion of cell abscission, exerting a controlling influence on the physical separation of two daughter cells in the last stage cytokinesis [25]. Spindle organization and cell cycle progression are controlled by CEP55 in meiotic oocyte [26]. During mitosis, CEP55 can be phosphorylated by a combination of Cyclin-dependent kinase 1, extracellular signal-regulated kinase 2, and PIK1 [27]. Elevation of CEP55 expression levels are observed in a wide range of human cancer and are believed to be implicated in development and progression of tumorigenesis [16]. Tao *et al.* [15] showed that overexpression of CEP55 contributes to the aggressive behavior of human gastric cancer through promoting proliferation, colony formation and oncogenicity,

which associated with increased phosphorylation of AKT and decreased activation of p21 WAF1/Cip1. High CEP55 expression was found to be correlated with advanced tumor stage in oropharyngeal squamous cell carcinoma [28]. A recent report indicates that CEP55 is a predictor of worse survival in epithelial ovarian carcinoma and stage I pulmonary adenocarcinoma following resection [29, 30]. Additionally, CEP55 is a relatively novel negative regulator of apoptosis in human lung cancer and glioma [31, 32].

In the present study, PCa tumor tissues and cell lines exhibited relatively low miR-144-3p expression. CEP55 was a direct target of miR-144-3p and silencing of CEP55 showed a strong G1/S arrest combined with decreased G2/M proportion. We supposed that decreased cell growth associated with overexpression of miR-144-3p was attributable to defects in cell cycle key events such as cytokinesis, spindle organization, and midbody integrity. In a previous study, abnormal in chromosome segregation and abscission resulted in decreased survival in Hela cells with WNK1 knockdown [33]. Here, overexpression of miR-144-3p caused defective abscission, which is a possible mechanism contributing to apoptosis.

In summary, our results revealed that miR-144-3p is pivotal for PCa cell proliferation and tumor growth by regulating G1-S phase transition and apoptosis through directly targeting CEP55. Although the precise molecular mechanisms remain to be investigated, our mechanisms deepen our understanding of targeting miR-144-3p may provide new therapeutic methods to treat PCa.

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Disclosure of conflict of interest

None.

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