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

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Abstract. – OBJECTIVE: MicroRNA-144-3p (miR-144-3p) has been implicated in the tumorigenesis of multiple types of cancer. However, its role in castration-resistant prostate cancer (CRPC) remains largely unknown. This study aimed to explore the biological role of miR-144-3p in the development of CRPC.

MATERIALS AND METHODS: RT-qPCR was performed to measure the expression levels of miR-144-3p in CRPC tissues. CRPC cells were transfected with miR-144-3p or NC. MTT and colony formation assays were used to determine cell growth; flow cytometry was used to measure apoptosis; a luciferase reporter assay was used to predict the target genes regulated by miR-144-3p. Finally, siCEP55 or siNC was transfected into DU145 cells, and the rates of cell proliferation and apoptosis were measured. Protein expression levels were confirmed by Western blot analysis.

RESULTS: MiR-144-3p expression was significantly decreased in both CRPC tissues and cell lines compared with that from androgen-dependent prostate cancer (ADPC) tumors. Overexpression of miR-144-3p in CRPC cells effectively inhibited proliferation and colony formation and promoted apoptosis in these CRPC cells. Additionally, miR-144-3p directly targeted centrosomal protein 55 (CEP55) and suppressed CEP55 expression. Finally, CEP55 silencing remarkably suppressed proliferation and induced apoptosis of CRPC cells, indicating that miR-144-3p affects CRPC cell survival and proliferation by down-regulating CEP55.

CONCLUSIONS: MiR-144-3p serves as a tumor suppressor in CRPC cells by directly targeting CEP55, which appears to be a novel therapeutic target for CRPC.

Key Words:
MiRNA-144-3p, Castration-resistant prostate cancer (CRPC), CEP55, Proliferation, Apoptosis.

Introduction

Prostate cancer (PCa) is the most frequently diagnosed malignant neoplasia and is associated with the highest morbidity and mortality among men in Western countries[1,2]. Approximately 1.7 million new PCa cases will be diagnosed in 2030 as the worldwide population ages[1]. Most PCa-related deaths are a result of metastasis to bones and soft tissues[3]. Currently, androgen deprivation therapy (ADT) is the primary treatment for metastatic disease. Despite initial disease control, almost all patients will develop castration-resistant prostate cancer (CRPC) within 2 to 3 years after treatment[4,5]. CRPC usually results in bone metastasis, and survival of these patients may be prolonged using a variety of therapies, including androgen receptor (AR)-targeted agents, bone-targeted agents, and chemotherapeutic agents[6]. However, the prognosis of patients with CRPC remains poor[7]. MicroRNAs (miRNAs) are a group of small, noncoding, endogenous, single-stranded RNAs 19 to 23 nucleotides in length that play crucial gene-regulatory roles, affecting multiple cellular biological processes, by binding to the 3'-untranslated region (3'-UTR) of mRNA at the transcriptional or post-transcriptional level[8]. Increasing evidence[8-10] has shown that aberrantly expressed miRNAs are implicated in the tumorigenesis and oncogenesis of many types of cancer, including PCa, by acting as either oncogenes or cancer suppressors. MiR-144-3p belongs to the miR-144 family, of which several members are abnormally expressed in numerous cancers, such as lung cancer[11], hepatocellular carcinoma[12], renal cell carcinoma[13], and thyroid cancer[14]. Previous works[15-17] have

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linked miRNA-144-3p to the initiation and development of cancer, as both a potential oncogene or tumor suppressor, depending on the cellular context. Though it has been reported that miR-144-3p is associated with cancer pathogenesis, its role in CRPC has not been explored yet. In our current study, we detected the expression levels of miR-144-3p in cell lines and tissues from patients with either human androgen-dependent prostate cancer (ADPC) or CRPC. We then investigated the biological function of miR-144-3p in CRPC cell proliferation and survival. Furthermore, we identified the potential target of miR-144-3p, and determined the mechanism of miR-144-3p-mediated regulation of CRPC development. Our results suggest that miR-144-3p is a potential therapeutic target for the treatment of CRPC.

Materials and Methods

Tissues Collection

Prostate specimens were obtained from the patients who received treatment at the Traditional Chinese Medicine Hospital of Taian from 2012 to 2016. A total of 20 patients with PCa were diagnosed and included in this study, including 10 with CRPC and 10 with ADPC. Each specimen was histologically identified with prostate specific antigen (PSA) staining, and only specimens with > 60% tumors content were incorporated in our study. All samples were collected with patients' informed consent for tissue donation, and this study was approved by the protocol from Ethics Committee of the Traditional Chinese Medicine Hospital of Taian. A diagnosis of CRPC was defined based on the guidelines published by the European Association of Urology[18].

Cell Culture

Human prostate carcinoma cell lines LNCaP, DU145, and PC3, and normal human prostate epithelial RWPE-1 cells were provided by American Type Culture Collection (ATCC, Manassas, VA, USA). The CRPC (PC3 and DU145) and ADPC (LNCaP) cell lines were routinely maintained in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Gibco BRL, Grand Island, NY, USA). RWPE-1 cells were cultured in keratinocyte serum-free medium (Life Technologies, AG, Carlsbad, CA, USA) containing 5 ng/ml epidermal growth factor, 50 mg/ml bovine pituitary extract, and 5% L-glutamine. All cells were grown in a humidified atmosphere with 5% CO_2 at 37°C.

Cell Transfection

MiR-144-3p mimics and negative controls (NC mimics) were purchased from GenePharma Co., Ltd. (Shanghai, China). CEP55 siRNA (siCEP55) and control siRNA (siNC) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Prior to transfection, DU145 and PC3 cells were cultured in serum-free RPMI-1640 medium (BD Bioscience, San Jose, CA, USA) for 6 h. MiRNAs or siRNAs (50 nM each) were then transiently transfected into cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) per manufacturer's protocol.

3-(4,5)-Dimethylthiazole (-z-y1)-3,5-di- Phenytetrazoliumromide (MTT) Assay

MTT assay was performed following with the protocol described by Mossman[19]. After transfection, DU145 and PC3 cells were seed into 96-well plates (1×10^4 cells/well) in a humidified atmosphere containing 5% CO_2 at 37°C. Wells containing complete medium served as blank controls. 20 µl of MTT reagent (5 mg/ml, Genview, Houston, TX, USA) were added to each well, then maintained at 37°C for another 4 h. The plates were then spun at 1000 rpm for 3 min, and dimethylsulfoxide (DMSO, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was added to each well. Absorbance was detected at 595 nm every 24 h using an automatic multi-well spectrophotometer (Bio-Rad, Hercules, CA, USA). Each experiment was performed in triplicate.

Colony Formation Assay

Transfected cells were maintained in 6-well plates at a density of 1×10^3 cells/well for 10 d. The cells were then stained with 1.0% crystal violet (MedChemExpress, Shanghai, China) and fixed in 4% paraformaldehyde (TCI, Shanghai, China) for 5 min. The number of surviving colonies was counted using an inverted microscope (Olympus, Tokyo, Japan).

Measurement of Apoptosis

After transfection for 24 h, cells were collected, washed, resuspended, and stained with an Annexin V-FITC Apoptosis Detection Kit I (Ab-

cam, Cambridge, MA, USA) per manufacturer's instructions. Apoptotic cells were quantified with a flow cytometer (FACSort, Becton Dickinson, USA).

Target Gene Prediction and Luciferase Report Assays

TargetScan (http://www.targetscan.org/) and Diana lab (http://diana.imis.athena-innovation.gr/) were used to predict target genes for miR-144-3p. Fragments of the CEP55 3′-UTR containing either the predicted binding site of miR-144-3p or mutated binding sites were synthesized and inserted into a luciferase reporter. The reporter was then co-transfected with miR-144-3p mimics or NC mimics into DU145 or PC3 cells using Lipofectamine 2000. After incubation for 48 h, luciferase activities were evaluated using a Dual Luciferase Reporter Assay Kit (Promega, Madison, WI, USA). The relative luciferase activity was normalized against a Renilla control as previously reported[20].

RNA Extraction and Real-Time Quantitative PCR

Total RNAs were extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) per manufacturer's instructions. MiRNAs were reverse transcribed into cDNA using an All-in-One miRNA quantitative reverse transcription-PCR detection kit (GeneCopoeia, Guangzhou, China). Real-time quantitative PCR (RT-qPCR) was performed using the Step One Plus Real-Time PCR System (Applied Biosystems Inc., Foster City, CA, USA) per manufacturer's instructions. Relative gene expression was normalized to that of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (Santa Cruz Biotechnology, Santa Cruz, CA, USA) (for Caspase-3, Bcl-2, Bax, and Survivin) or U6 (for miR-144-3p), as previously reported, using the $2^{-\Delta\Delta Ct}$ method[21]. The sequences of the primers were as follows: miR-144-3p forward, 5'-GGCCCTGGCTGGGATATCAT-3' and reverse, 5'-GGTGCCCGGACTAGTACATC-3'; Caspase-3 forward, 5'-GGGGAGCTTGGAACGCTAAG-3' and reverse, 5'-CCGTACCAGAGCGAGATGAC-3'; Bcl-2 forward, 5'-CTTTGAGTTCGGTGGGGTCA-3' and reverse, 5'-GGGCCGTACAGTTCCACAAA-3'; Bax forward, 5'-CAGAGGCGGGGGATGATTG-3' and reverse, 5'-TGTCCAGCCCATGATGGTTC-3'; Survivin forward, 5'-CGTTCCCCTAACTGTGGCTT-3' and reverse, 5'-ATGCCAAAACACGTGACTCC-3'; GAPDH forward, 5'-CCTACGCAGAAAGCTAGCGA-3' and reverse, 5'-TACGACTGCCGCTTTTTCCT-3'; U6 forward, 5'-CCCTTCGGGGACATCCGATA-3' and reverse, 5'-TTTGTGCGTGTCATCCTTGC-3'.

Western Blot Analysis

Tissues and cells were collected and lysed using RIPA buffer (Sigma-Aldrich, St. Louis, MO, USA) containing protease inhibitor (Roche, Basel, Switzerland), and extracted proteins were quantified using a BCA protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA). Western blot analysis was performed per manufacturer's instructions. The following primary antibodies were used: anti-Caspase-3, anti-Bcl-2, anti-Survivin, anti-CEP55, and anti-Bcl-2 (Abcam, Cambridge, MA, USA). Membranes were then incubated with a secondary antibody (1:5000; Abcam, Cambridge, MA, USA) at room temperature for 1 h. GAPDH served as an internal control. Blots were visualized using a Tanon MP imaging system (Tanon Science and Technology Co., Ltd., Shanghai, China).

Statistical Analysis

Quantitative data are expressed as means ± SD (standard deviation) based on three independent experiments. Comparisons between two groups were analyzed using Student's *t*-test. Multiple comparisons among groups were analyzed using one-way analysis of variance followed by Student-Newman-Keuls test for statistical analysis. Statistical differences were determined using the commercially available statistical program SPSS 19 software package (IBM Corp., IBM SPSS Statistics for Windows, Armonk, NY, USA). $p < 0.05$ was regarded as statistically significant.

Results

miR-144-3p Expression is Reduced in CRPC tumors

A significant decrease of miR-144-3p levels was observed in CRPC tumor samples compared with ADPC tumors ($p < 0.001$, Figure 1A), indicating that miR-144-3p may be involved in the development of CRPC. To verify this, we detected the expression levels of miR-144-3p in four different cell lines, including the CRPC cell lines LNCaP, DU145, and PC3, and the ADPC cell line RWPE-1. We found that miR-144-3p expression levels were much higher in normal prostate epithelial cells com-

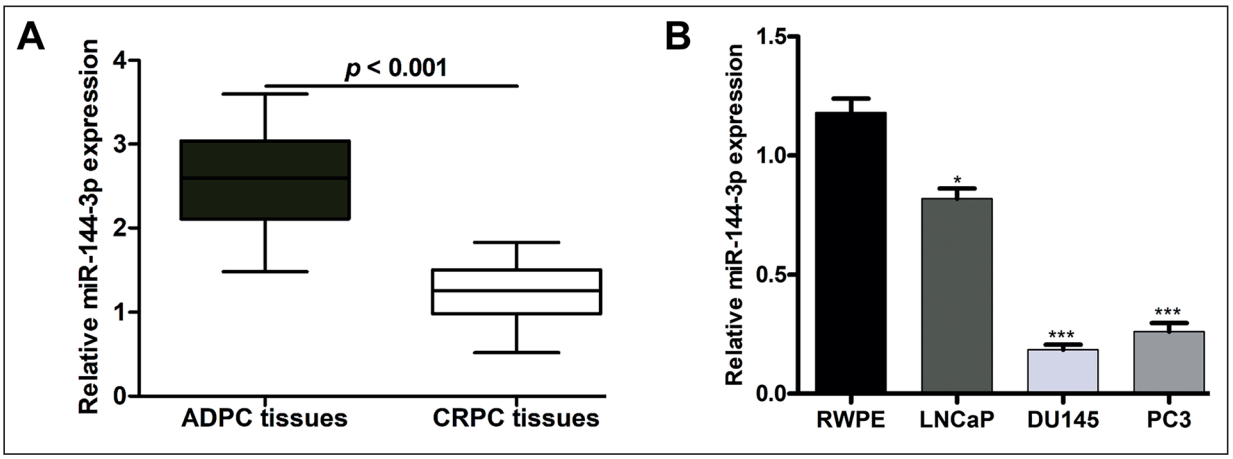

Figure 1. MiR-144-3p is downregulated in CRPC tumors. ***(A)*** RT-qPCR showing relative miR-144-3p expression levels in CRPC and ADPC tumors. ***(B)*** RT-qPCR showing relative miR-144-3p expression levels in four different PCa cell lines (RWPE, LNCaP, DU145, and PC3). *$p < 0.1$, ***$p < 0.001$.

pared with the three prostate carcinoma cell lines ($p < 0.05$, $p < 0.001$, Figure 1B). Furthermore, miR-144-3p expression levels in PC3 and DU145 (PSA-negative cell) were reduced compared with those in LNCaP cells (PSA-positive cell) ($p < 0.001$, Figure 1B). These results suggest that miR-144-3p levels are correlated with the CRPC tumorigenesis. In addition, PC3 and DU145 cells exhibited the lowest expression levels of miR-144-3p among the four PCa cell lines. We, therefore, selected these cell lines for subsequent analysis.

miR-144-3p Overexpression Inhibits CRPC cell growth

To investigate the biological function of miR-144-3p in CRPC, DU145 and PC3 cells were transfected with miR-144-3p or NC mimics. After transfection for 48 h, the efficacy of transfection was determined by RT-qPCR. miR-144-3p expression levels were increased approximately 10-fold in DU145 cells and 9-fold in PC3 cells transfected with miR-144-3p, but not NC, mimics ($p < 0.001$, Figure 2A). Next, we explored the impact of miR-144-3p overexpression on cell proliferation and colony formation. Transfection with miR-144-3p significantly reduced the proliferative capacity of DU145 and PC3 cells ($p < 0.001$, Figure 2B). Similarly, transfection with miR-144-3p significantly reduced colony formation ($p < 0.001$, Figure 2C). These results indicate that miR-144-3p inhibits CRPC cell growth.

miR-144-3p Overexpression Promotes Apoptosis in CRPC Cells

Next, we sought to investigate the effect of miR-144-3p on apoptosis in CRPC cells. In DU145 cells, the proportions of apoptotic cells transfected with miR-144-3p or NC mimics were 26.9% and 10.86%, respectively; in PC3 cells, the proportions of apoptotic cells were 40.2% and 18.62%, respectively. Transfection with miR-144-3p resulted in a higher proportion of apoptotic cells compared with control group ($p < 0.05$, Figure 3A). To further identify the pro-apoptotic mechanism of miR-144-3p in CRPC cells, we measured the expression levels of apoptosis-related target genes, including caspase-3, Bcl-2, Bax, and survivin, by RT-qPCR and Western blot analysis. We found that the expression levels of caspase-3 and Bax were enhanced in both DU145 and PC3 cells after transfection with miR-144-3p mimics compared with cells transfected with NC mimics ($p < 0.01$, Figure 3B). In contrast, transfection with miR-144-3p resulted in reduced levels of Bcl-2 and survivin ($p < 0.05$, $p < 0.01$, Figure 3B). We observed a similar effect of miR-144-3p on the protein levels of these apoptosis-related genes in CRPC cells (Figure 3B). These findings suggest that miR-144-3p overexpression promotes CRPC cell apoptosis by regulating the expression levels of apoptosis-related genes.

CEP55 is a Direct Target of miR-144-3p in CRPC cells

To assess the mechanism by which miR-144-3p modulates CRPC cell proliferation, colony

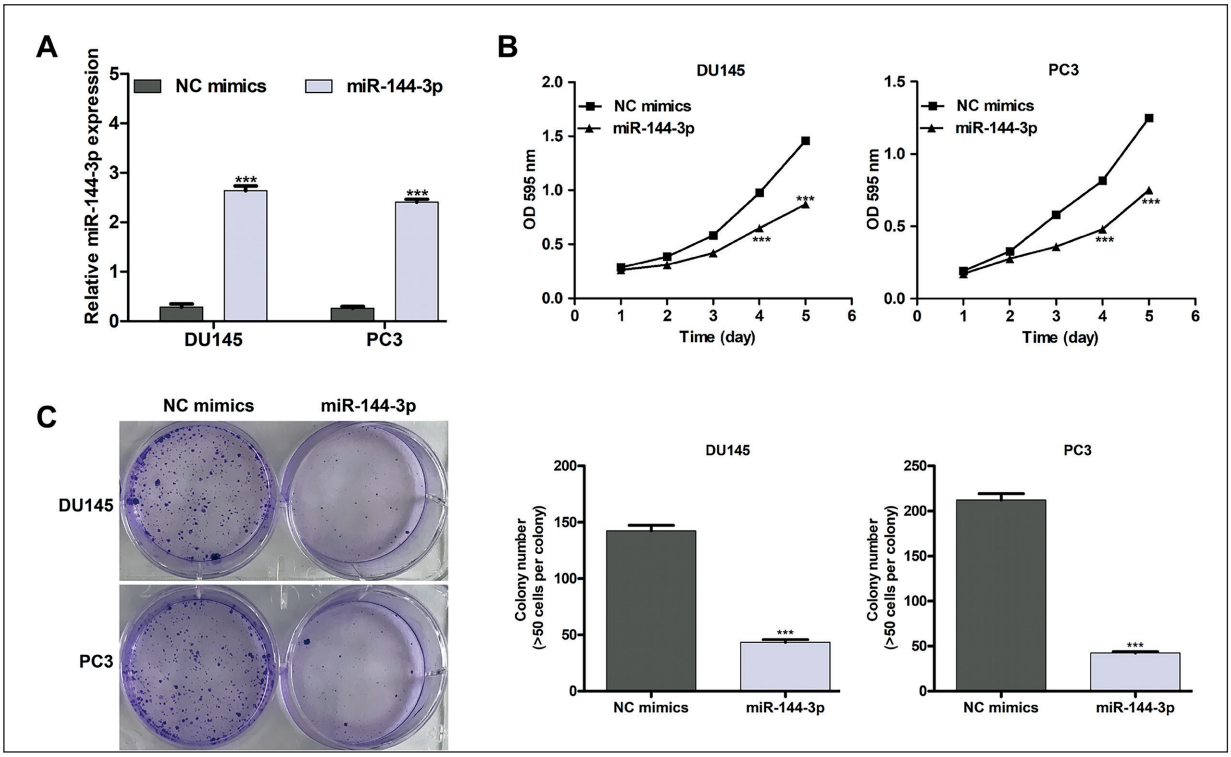

Figure 2. miR-144-3p overexpression inhibits CRPC cells growth. ***(A)*** RT-qPCR showing relative miR-144-3p expression levels in DU145 and PC3 cells transfected with miR-144-3p or NC mimics. ***(B)*** Rates of cell proliferation in DU145 and PC3 cells transfected with miR-144-3p or NC mimics. ***(C)*** Rate of colony formation in DU145 and PC3 cells transfected with miR-144-3p or NC mimics. ***$p < 0.001$.

formation, and apoptosis, we attempted to confirm potential target genes of miR-144-3p. According to the predication by TargetScan and Diana Lab, CEP55 has a conserved miR-144-3p binding site in its 3′-UTR (Figure 4A). To further verify whether CEP55 was a true target for miR-144-3p, the wild-type or mutant 3′-UTR of CEP55 was cloned into a luciferase reporter vector, and luciferase activity was assayed. Our results show that miR-144-3p overexpression remarkably suppressed the luciferase activity of the wild-type-CEP55-3′UTR construct in both DU145 and PC3 cells ($p < 0.01$), whereas no difference was observed using a mutant CEP55-3′UTR (Figure 4B), indicating that miR-144-3p directly targets the 3′-UTR of CEP55. Moreover, CEP55 expression levels decreased significantly in CRPC cells transfected with miR-144-3p compared with those transfected with the negative control (Figure 4C), suggesting that miR-144-3p regulates CEP55 expression. Overall, these results suggested that miR-144-3p regulates CEP55 expression by targeting its 3′-UTR.

CEP55 Silencing Inhibits Cell Proliferation and Induces Apoptosis in CRPC Cells

To better understand the role of CEP55 in CRPC, we performed a meta-analysis using the ONCOMINE database. As shown in Figure 5A, upregulation of CEP55 in pancreatic carcinoma was confirmed in seven previous investigations[22-28], strongly demonstrating the relationship between CEP55 expression and the pathogenesis of CRPC. To identify the role of CEP55 in the development of CRPC, we silenced CEP55 expression in CRPC cells. The rate of proliferation was significantly reduced in DU145 cells transfected with siCEP55 compared with the control ($p < 0.001$, Figure 5B). In addition, CEP55 silencing significantly increased the proportion of apoptotic CRPC cells ($p < 0.001$, Figure 5C), and resulted in increased PARP cleavage and reduced Bcl-2 protein expression levels (Figure 5D). These findings indicate that CEP55 silencing can inhibit cell proliferation and induce apoptosis in CRPC cells. As a whole, our data suggest that miR-144-3p inhibits CRPC tumor growth and induces apoptosis via the downregulation of CEP55 expression.

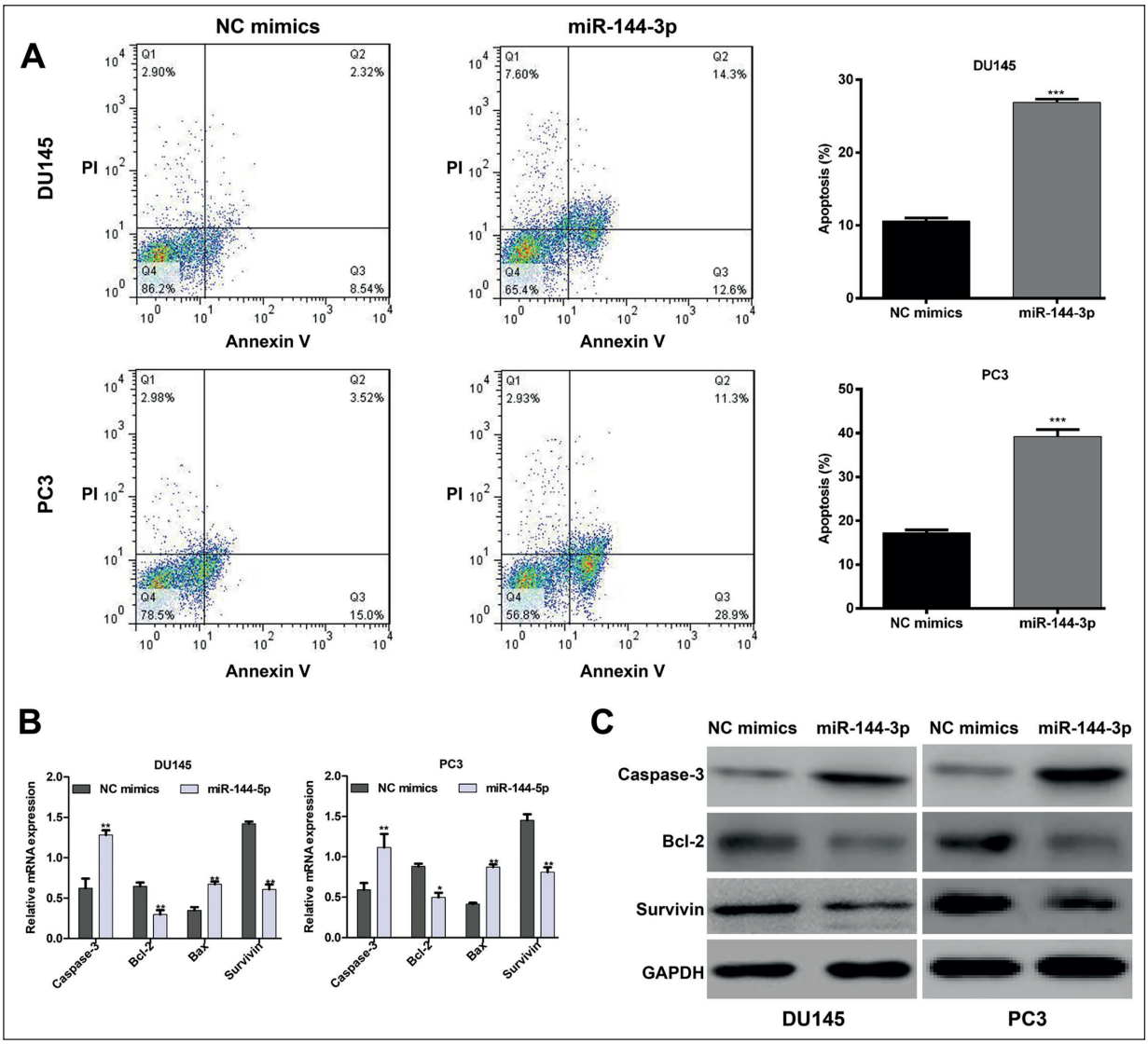

Figure 3. miR-144-3p overexpression promotes CRPC apoptosis. ***(A)*** Flow cytometry analysis indicating the percentage of apoptotic DU145 and PC3 cells after transfection with miR-144-3p or NC mimics. ***(B)*** RT-qPCR showing relative mRNA expression levels of apoptosis-related genes (Caspase-3, Bcl-2, Bax, and Survivin) in DU145 and PC3 cells after transfection with miR-144-3p or NC mimics. ***(C)*** Western blot analysis showing protein expression levels of apoptosis-related genes (Caspase-3, Bcl-2, Bax, and Survivin) in DU145 and PC3 cells after transfection with miR-144-3p or NC mimics. $*p < 0.05$, $**p < 0.01$.

Discussion

MiRNAs play a role in multiple biological processes involved in oncogenesis and tumor development[29]. Generally, miRNAs can regulate the expression of multiple protein-coding genes, and the aberrant expression of tumor-suppressive or oncogenic miRNAs in cancer cells disrupts processes necessary to prevent tumor formation[30]. Therefore, confirming the exact role of these miRNAs in cancer may provide a better understanding of cancer biology, thereby providing a basis to discover novel therapeutics and diagnosis biomarkers[31]. MiR-144 is a family of microRNA precursors found in mammals. MiR-144-3p belongs to this family of miRNAs, and its function has been explored in various neoplasms, including lung cancer[11], hepatocellular carcinoma[12], renal cell carcinoma[13], and thyroid cancer[14]. The abnormal expression of miRNAs usually promotes the occurrence and progression of numerous cancers[32]. Several researches[15,16,33] have reported that miR-144-3p is significantly downregulated in several forms of cancer, and acts

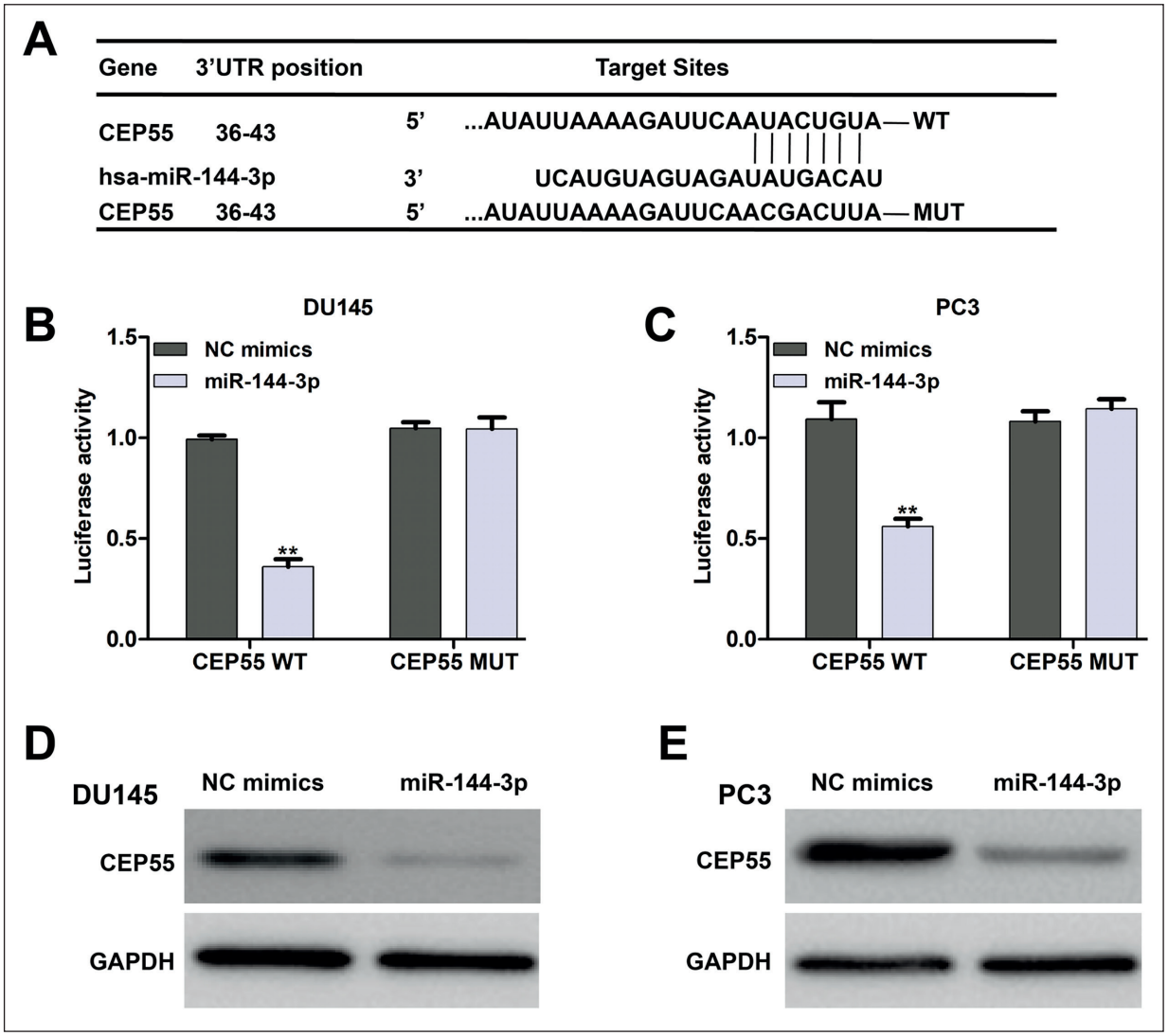

Figure 4. CEP55 is a direct target of miR-144-3p. ***(A)*** Sequence alignment of miR-144-3p and the 3′-UTR of CEP55. ***(B)*** Relative luciferase activities were analyzed in DU145 and PC3 cells co-transfected with the CEP55 3′UTR (wild-type or mutant) reporter plasmid with either miR-144-3p or NC mimics. ***(C)*** Western blot analysis showing the protein expression levels of CEP55 in DU145 and PC3 cells after transfection with miR-144-3p or NC mimics. $^{**}p < 0.01$.

as a tumor suppressor, while other studies have shown that miR-144-3p functions as an oncogene in other cancer types[34,35]. Thus, the biological role of miR-144-3p appears to be tissue specific, though no previous study had investigated the role of miR-144-3p in CRPC. In the present study, we found that miR-144-3p levels are significantly decreased in CRPC tumors and cell lines compared with ADPC tumors, suggesting that miR-144-3p acts as a tumor suppressor in CRPC. To confirm the biological function of miR-144-3p in CRPC, we transfected DU145 and PC3 cells with miR-144-3p or NC mimics to establish an overexpression model for miR-144-3p. Our results showed that miR-144-3p overexpression effectively reduces CRPC cell proliferation and colony formation, confirming the tumor suppressive role of miR-144-3p in CRPC. Because a growing number of studies have shown that miR-144-3p is a key regulator of apoptosis, we explored the effect of miR-144-3p on CRPC apoptosis using flow cytometry. The proportion of apoptotic cells transfected with miR-144-3p was much higher than those transfected with NC mimics, and the levels of apoptosis-related genes, including Caspase-3, Bcl-2, Bax, and Survivin, were also elevated. Caspase-3 is a pro-apoptotic protein that is involved in the late stages of apoptosis[36]. Bcl-

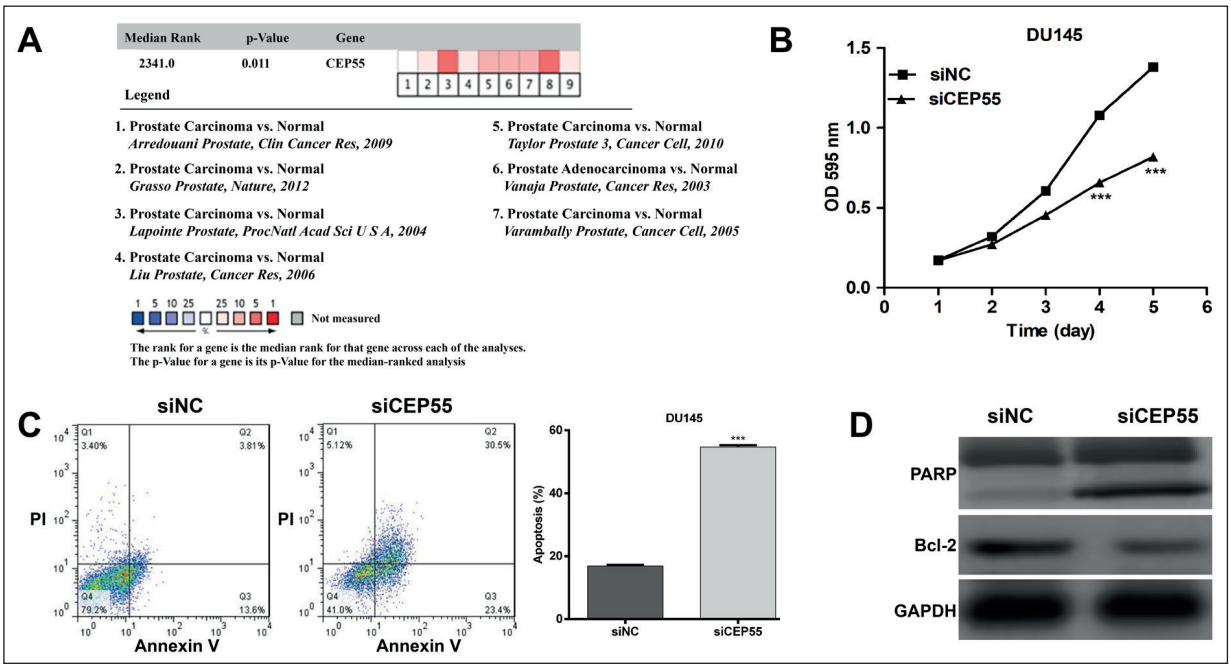

Figure 5. CEP55 silencing inhibits cell proliferation and induces apoptosis in CRPC cells. ***(A)*** Meta-analysis showing the overexpression of CEP55 in seven previous studies. ***(B)*** MTT assay showing rates cell proliferation in DU145 cells transfected with siCEP55 or siNC. ***(C)*** Flow cytometry analysis showing the percentage of apoptotic DU145 cells after transfection with siCEP55 or siNC. ***(D)*** Western blot analysis showing the protein expression levels of PARP and Bcl-2 in DU145 cells transfected with siCEP55 or siNC. ***$p < 0.001$.

2 is a potent suppressor of apoptosis[37], whereas Bax is an effector of the intrinsic apoptotic pathway[38]. Survivin is an anti-apoptotic protein that inhibits the activation of caspase-3. Our findings are supported by several previous studies. For example, Chen et al[11] reported that the administration of miR-144-3p can inhibit proliferation and facilitate apoptosis in lung tumor cells by targeting TIGAR, and Guo et al[39] found that miR-144-3p downregulation promotes the proliferation of bladder cancer cells by regulating Wnt signaling. Overall, our findings provide evidence that miR-144-3p acts as a tumor suppressor in CRPC. MiRNAs can modulate various biological activities by promoting or suppressing target gene expression[40]. Therefore, identifying these targets is crucial in studying the function of miRNAs in cancer. In this study, CEP55 was predicted to be an underlying target gene of miR-144-3p in CRPC via Target Scan and Diana Lab. Our luciferase reporter assay indicated that CEP55 is a direct target of miR-144-3p, a finding that was further supported by Western blot analysis. In these assays, CEP55 expression was inhibited by miR-144-3p overexpression in both DU145 and PC3 cells. CEP55, has been previously reported to participate in the progression of various human cancers, including breast cancer[41], bladder cancer[42], epithelial ovarian carcinoma[43], and gastric specimens[44]. Moreover, knockdown of CEP55 has been found to suppress cell growth in gastric[44] and breast cancer[41], and induce apoptosis in gliomas[45], indicating that CEP55 may serve as an oncogene, and could be a potential target for tumor therapy. However, despite the large amount of studies exploring the role of CEP55 in cancer, the biological function of CEP55 in CRPC has not yet been fully elucidated. To determine the role of CEP55 in CRPC, we silenced the expression of CEP55 in CRPC cells by transfection with siCEP55 or siNC. We found that downregulation of CEP55 significantly inhibits CRPC cell proliferation. Additionally, knockdown of CEP55 increased the percentage of apoptotic DU145 cells, a finding that is consistent with previous reports. Moreover, we found that CEP5 silencing induced the downregulation of Bcl-2, as well as PARP cleavage. Taken together, these findings suggest that miR-144-3p exhibits a tumor-suppressive role in CRPC by regulating CEP55 expression. In summary, our study demonstrates that miR-144-3p is downregulated in CRPC tumors

and cell lines compared with ADPC tumors. Further experimentation revealed that miR-144-3p overexpression inhibits cell proliferation and colony formation, as well as enhances apoptosis, in CRPC cells by downregulating CEP55. These results suggest that miR-144-3p may be a potential therapeutic target in CRPC.

Conclusions

We found that miR-144-3p expression is significantly downregulated in CRPC tumor samples. Overexpression of miR-144-3p in CRPC cells decreases their proliferation capacity, reduces colony formation, and promotes apoptosis, suggesting that miR-144-3p acts as tumor suppressor in CRPC. CEP55 is confirmed as a direct target of miR-144-3p; CEP55 silencing inhibits CRPC cells proliferation and accelerates apoptosis. Therefore, miR-144-3p inhibits tumor growth by downregulating CEP55 expression. Collectively, miR-144-3p could be a potential target for therapeutic approaches to CRPC therapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Authors' Contribution

(1) Bo You analyzed and interpreted the patient data, and he was a major contributor in writing the manuscript. (2) Kaichen Zhang performed the literature research, conceived and designed the study, and revised the manuscript accordingly prior to submission. All authors have read and approved the final manuscript.

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