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MicroRNA-373-3p inhibits prostate cancer progression by targeting AKT1

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Abstract. – OBJECTIVE: This study aims to investigate whether microRNA-373-3p could inhibit the progression of prostate cancer (PCa) by targeting and degrading AKT1.

PATIENTS AND METHODS: Expression levels of microRNA-373-3p and AKT1 in PCa tissues and benign prostate hyperplasia (BPN) tissues were detected by quantitative Real-Time-Polymerase Chain Reaction (qRT-PCR). According to the follow-up data, survival curves and receiver operating characteristic (ROC) curves were drawn to investigate whether microRNA-373-3p could be served as a biomarker for early diagnosis and prognosis of PCa. The effect of microRNA-373-3p on cell proliferation was examined by cell counting kit-8 (CCK-8) assay. Subsequently, we explored the direct binding condition of AKT1 and microRNA-373-3p by dual-luciferase reporter gene assay and Western blot.

RESULTS: QRT-PCR results showed that microRNA-373-3p level was significantly lower in PCa tissues compared with that of BPN tissues, whereas AKT1 expression was significantly increased. By analyzing the survival curve and ROC curve, we found that the overall survival (OS) of PCa patients with higher microRNA-373-3p expression was markedly longer than those with lower expression. Besides, microRNA-373-3p could be used as an early diagnostic marker to distinguish PCa from BPH. Overexpression of microRNA-373-3p in PCa cells (LNCaP and PC3 cells) remarkably inhibited cell proliferation. Dual-luciferase reporter gene assay and Western blot showed that microRNA-373-3p targeted the 3'UTR of AKT1 and inhibited its expression.

CONCLUSIONS: Downregulated microRNA-373-3p promoted the proliferation of prostate cancer cells via targeting AKT1.

Key Words
MicroRNA-373-3p, AKT1, Prostate cancer, Cell proliferation.

Introduction

Prostate cancer (PCa) is the most common malignant tumor of the genitourinary system in males, which ranks the second in the mortality rate of male tumors. As the lifespan of the population increases, the incidence rate of PCa also increases year by year[1]. Men who are over 60 years old are frequently affected by PCa[2,3]. There are no evident (or no specific) clinical manifestations in early PCa. Most of the patients are diagnosed with prostate biopsy when presenting symptoms of urinary tract obstruction due to an elevated level of prostate-specific antigen (PSA). Although PSA screening has been widely used in the diagnosis of PCa, only 6.2% of patients were eventually diagnosed because of the slight elevation on PSA level[4]. Delayed treatment leads to the disease development, the subsequent unsatisfactory treatment effect, and the poor prognosis of PCa. Early diagnosis is crucial for the treatment and prognosis of PCa patients. Therefore, finding reliable, effective and suitable molecular markers for the diagnosis of PCa have become a hotspot in clinical research.

MicroRNAs are a class of non-coding single-stranded RNA molecules with about 22 nucleotides in length. MicroRNAs regulate gene expressions at the post-transcriptional and transcriptional levels. In recent years, accumulating evidence has shown that microRNAs could be used as an ideal biomarker in the diagnosis, prognosis, and treatment of tumors. Dysregulated microRNAs are associated with clinical pathological features of many tumors, including lung cancer, hepatocellular carcinoma, and breast cancer[5-7]. Previous studies have shown that microRNA-141, microRNA-298, microRNA-346, and microRNA-375 are upregulated in serum samples

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of PCa patients. MicroRNA-106a and microRNA-24 were also reported[8,9] to be overexpressed in patients with early stage of Pca. In terms of predictors, microRNA-21 and microRNA-221 showed a significant upward trend in early PCa. MicroRNA-221 has been reported[10,11] as an independent predictor for PCa prognosis. Besides, microRNA-15 and microRNA-16 induced apoptosis through regulating mRNA level of anti-apoptotic gene B lymphocyte 2 (Bcl-2)[12]. Scholars[13] have shown that c-Myc simultaneously activated the transcription of E2F1 and microRNA-17-92 cluster, among which microRNA-17-5p and microRNA-20a inhibited the translation of E2F1.

MicroRNA-373-3p is a member of the miRNAs-371-372-373 family, located on chromatin 19q13.42. Differentially expressed microRNA-373-3p is involved in many tumors, such as breast cancer, esophageal cancer, and gliomas. Abnormal expression of microRNA-373-3p in T cell lymphoma affects the proliferation, invasion, and metastasis of tumor cells[14-17]. MicroRNA-373-3p was also reported to induce epithelial-mesenchymal transformation (EMT) by inhibiting the Wnt pathway, thereby promoting tumor metastasis in tongue squamous cell carcinoma[18]. In pancreatic cancer, low expression of microRNA-373-3p in serum was considered to be associated with poor clinical prognosis. MicroRNA-373-3p expression was correlated to tumor grade, staging, and distant metastasis[19]. In addition, microRNA-373-3p inhibited the invasion and metastasis of ovarian cancer *via* targeting Rab22a, thereby acting as a tumor suppressor[20]. Although several studies have revealed the effect of microRNA-373-3p on the occurrence and development of tumors, there was no report on the relationship between microRNA-373-3p and PCa. This study aims to investigate whether microRNA-373-3p was involved in PCa progression.

Patients and Methods

Sample Collection

The serum and tissue samples of 56 PCa patients and 56 BPH subjects were collected at the TaiKang XianLin Drum Tower Hospital The Affiliated Hospital of Nanjing University Medical School from May 2016 to May 2017. Samples were stored in liquid nitrogen. The definite diagnosis of all patients was based on the biopsy results. All subjects included in the study signed the informed consent. Our investigation was approved by the TaiKang XianLin Drum Tower Hospital The Affiliated Hospital of Nanjing University Medical School Research Ethics Committee.

Cell Culture

Normal prostate cell line (WPMY-1) and PCa cell lines (LNCaP, CWR22Rv1, PC3, and DU145) were purchased from Shanghai Cell Research Institute (Shanghai, China). Cells were maintained in Roswell Park Memorial Institute-1640 (RPMI-1640; Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 1% penicillin-streptomycin at 37°C, 5% CO_2.

Transfection

Cells in logarithmic growth phase were seeded in Petri dishes. MicroRNA-373-3p mimics, inhibitor and corresponding negative control were then transfected following the instructions of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Culture medium was changed after 6 hours of transfection. The transfection sequences were as follows: MicroRNA-373-3p mimics: (sense 5'-GAAGUGCUUCGAUUUUGGGGUGU-3', anti-sense 5'-ACCCCAAAAUCGAAGCACUUCUU-3'); MicroRNA-373-3p NC: (sense 5'-UUCUCCGAACGUGUCACGUTT-3', anti-sense 5'-ACGUGACACGUUCGGAGAATT-3').

RNA Extraction

Total RNA from tissues and cells was extracted by TRIzol method (Invitrogen, Carlsbad, CA, USA). Briefly, 1 mL of TRIzol and 250 μL of chloroform were added. After the mixture was shaken for 30 seconds and centrifuged at 4°C, the aqueous phase was aspirated, and an equal volume of pre-cooled isopropanol was added to purify the RNA. After 75% ethanol was used for further purification, the RNA pellet was precipitated, dried and dissolved in 20 μL of DEPC (diethyl pyrocarbonate) water. The RNA concentration was measured using a spectrophotometer (Hitachi, Tokyo, Japan). All RNA samples were placed in a refrigerator at -80°C.

QRT-PCR (Quantitative Real-Time Polymerase Chain Reaction)

A reverse transcription reaction system was prepared on ice, and the reaction was completed according to the instruction of the PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan). The cDNA was then added RNase-free water to a final concentration of 3 ng/μL. Quantitative PCR procedures were performed according to the SYBR Green PCR Kit instructions (TaKaRa, Otsu, Shiga, Japan). The qRT-PCR parameters were

as follows: pre-denaturation at 95°C for 15 min, denaturation at 94°C for 15 s, 55°C for 30 s and extension at 72°C for 30 s, for a total of 40 cycles. The primer sequences were as follows: MicroRNA-373-3p (F: 5'-ATCCAGTGCGTGTCGTG-3', R: 5'-TGCTGAAGTGCTTCGATTTT-3'), U6 (F: 5'-CGCTTCGGCAGCACATATAC-3', R: 5'-TTCACGAATTTGCGTGTCAT-3'), GAPDH (F: 5'-GGAATCCACTGGCGTCTTCA-3', R: 5'-GGTTCACGCCCATCACAAAC-3'), AKT1 (F: 5'-CACAAACGAGGGGAGTACATC-3', R: 5'-GCCATCATTCTTGAGGAGGAAGT-3').

Cell Counting Kit-8 (CCK-8) Assay

Cells transfected for 24 h were digested, collected and seeded into 96-well plates at a density of 2×10^3 per well. Each group was set up with 6 replicates. After cell adherence for 6 h, 24 h, 48 h, 72 h, and 96 h respectively, cell viability was determined by CCK-8 assay (Dojindo, Kumamoto, Japan). 2 h before the assay, 10 μL of CCK-8 solution was added to each well and the cells were incubated at 37°C for another 1 h. The absorbance of each well at a wavelength of 450 nm was measured with a microplate reader (Bio-Rad, Hercules, CA, USA).

Western Blot

Total protein from cells was extracted by the cell lysate containing the protease inhibitor PMSF (phenylmethylsulfonyl fluoride). The lysate was then collected and centrifuged at 12,000 r/min for 20 min. The supernatant was separated, and the total protein concentration was measured by the BCA (bicinchoninic acid) method (Pierce, Rockford, IL, USA). Totally 50 μg of total protein of each group was loaded on SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) for electrophoresis. After the sample was separated and transferred to PVDF (polyvinylidene difluoride) membrane (Millipore, Billerica, MA, USA), skim milk was used for blockage for 2 h. The specific primary antibodies were added to incubate with the protein bands overnight. The secondary antibody was also added 1 h before imaging.

Dual-Luciferase Reporter Gene Assay

The sequences of wild-type AKT1 (AKT1 WT) and mutant-type AKT1 (AKT1 MUT) were cloned into the psi-CHECK2 luciferase reporter vector, respectively. PCa cells were co-transfected with AKT1 WT or AKT1 MUT, and microRNA-373-3p mimic or control, respectively. Dual-luciferase reporter gene assay was performed to detect the luciferase activity of co-transfected cells.

Statistical Analysis

SPSS 19.0 (Statistical Product and Service Solutions) statistical software (IBM, Armonk, NY, USA) was used for analysis. Measurement data were analyzed by *t*-test. Data were expressed as mean±standard deviation. $p<0.05$ indicated the difference was statistically significant.

Results

MicroRNA-373-3p Was Downregulated and AKT1 Was Upregulated in PCa

We firstly detected expressions of microRNA-373-3p and AKT1 in PCa tissues and BPN tissues by qRT-PCR. The results showed that microRNA-373-3p was downregulated in PCa patients, while AKT1 exhibited a remarkable increase (Figure 1A, 1B). Based on the previous follow-up records, we found that the OS of PCa patients with higher level of microRNA-373-3p was longer than those with lower level (Figure 1C). Meanwhile, ROC curve revealed that microRNA-373-3p could significantly distinguish between PCa patients with BPN patients (AUC = 0.7615, $p<0.001$) (Figure 1D).

Overexpression of MicroRNA-373-3p Reduced Proliferation of PCa Cells

By detecting the microRNA-373-3p expression in normal prostate cells WPMY-1 and PCa cells LNCaP, CWR22Rv1, PC3, and DU145, we found that microRNA-373-3p expression was downregulated in PCa cells than that of normal prostate cells (Figure 2A). PC3 and LNCaP cells were selected for the following experiments. Transfection of microRNA-373-3p mimics in PC3 and LNCaP cells was verified by qRT-PCR (Figure 2B). Cell proliferation of LNCaP and PC3 cells was examined after overexpression of microRNA-373-3p. CCK-8 results showed that overexpression of microRNA-373-3p remarkably inhibited cell proliferation (Figure 2C, 2D).

MicroRNA-373-3p Directly Bound to AKT1 and Reduced Its Expression

By querying bioinformatics software, we predicted that AKT1 was the target gene of microRNA-373-3p (Figure 3A). Meanwhile, we further confirmed the negative correlation between microRNA-373-3p and AKT1 in PCa tissues and BPH tissues (Figure 3B). Dual-lucifer-

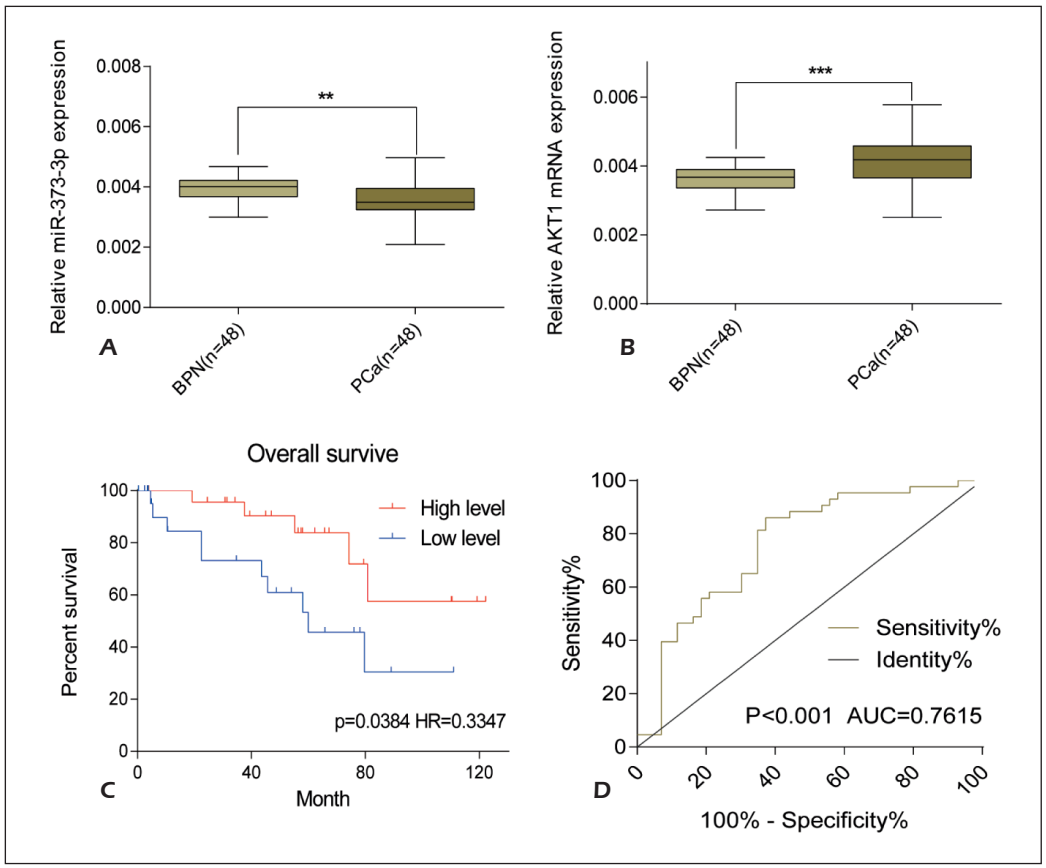

Figure 1. Expression level of microRNA-373-3p and clinical characteristics of prostate cancer patients. **A**, MicroRNA-373-3p was downregulated in 56 prostate cancer tissues compared with that of 56 BPN tissues. **B**, AKT1 was upregulated in 56 prostate cancer tissues compared with that of 56 BPN tissues. **C**, The overall survival of prostate cancer patients with high microRNA-373-3p expression was higher than that of microRNA-373-3p low expression group. **D**, ROC curve was analyzed the potential diagnostic value of microRNA-373-3p in prostate cancer tissues and BPN tissues (AUC=0.7615, $p<0.001$).

ase reporter gene assay found that the binding of microRNA-373-3p mimics to AKT1 WT resulted in a significant decrease of luciferase activity, indicating that microRNA-373-3p could directly bind to AKT1 (Figure 3C, 3D). Western blot results also indicated that overexpression of microRNA-373-3p in PC3 and LNCaP cells remarkably inhibited AKT1 expression (Figure 3E, 3F). The above results demonstrated that microRNA-373-3p exerted its biological function through directly binding to AKT1 and reducing its expression.

Discussion

PCa is the most common malignancy in the male. The incidence of PCa has increased in recent years[21]. The eventual progression to androgen-independent PCa is a major cause of death without effective treatment options[22,23]. MicroRNAs could control expressions of target genes by inhibiting translation or degrading messenger RNA[24]. Over 700 microRNAs have been found to be involved in the evolution of cells, including growth, differentiation, signal transduction, cell development, and tumorigenesis[25-27]. Scholars have observed that microRNA-373-3p was overexpressed in esophageal cancer tissues. MicroRNA-373-3p promoted tumor cell migration and invasion by downregulating matrix metalloproteinase inhibitor TIMP3[15]. In gliomas, microRNA-373-3p was also reported[28] to downregulate expressions of CD44 and TGFBR2, decrease the migration and invasion of glioma cells U251, thereby inhibiting the occurrence of glioma.

AKT, a serine/threonine kinase, also known as PKB, is a member of the most common family of kinases. Three allotypes of AKT with greater than 80% sequence homology have been

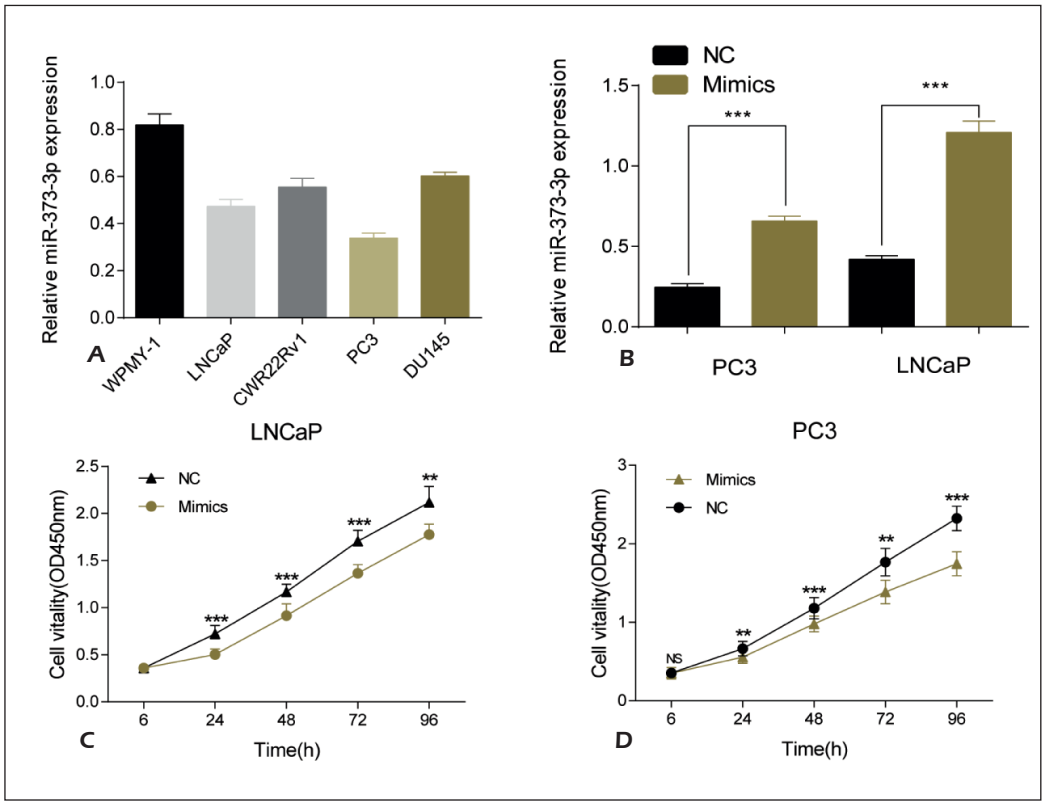

Figure 2. MicroRNA-373-3p inhibited prostate cancer cell proliferation. **A**, Expression of microRNA-373-3p in normal prostate WPMY-1 cells and prostate cancer LNCaP, CWR22Rv1, PC3, and DU145 cells was measured by qRT-PCR. **B**, Transfection efficiency of microRNA-373-3p mimics in PC3 and LNCaP cells. **C**, Upregulation of microRNA-373-3p in LNCaP cells decreased cell viability. **D**, Upregulation of microRNA-373-3p expression in PC3 cells reduced cell viability.

identified in mammals, including AKT1/PKBα, AKT2/PKBβ, and AKT3/PKBγ[29]. AKT was reported[30-33] to play an essential role in regulating cell viability and was closely related to various proliferative diseases. Overexpression of AKT reduced the disease-free survival of patients and promoted the development of PCa, breast cancer, and primary ovarian cancer[34]. However, the role of individual AKT isoforms in different molecular subtypes of PCa has not yet been elucidated. Although studies[35-37] have shown that AKT1 regulated angiogenesis and cell survival, the specific mechanism that AKT1 promotes cell proliferation and migration remained to be studied.

In this work, we found that microRNA-373-3p was downregulated and AKT1 was upregulated in PCa tissues and cells. The survival curves and ROC curves based on follow-up data revealed that longer OS was found in PCa patients with higher microRNA-373-3p expression than those with lower expression. MicroRNA-373-3p was differentially expressed in PCa tissues and BPH tissues. In addition, a negative correlation was observed between microRNA-373-3p and AKT. Overexpression of microRNA-373-3p in LNCaP and PC3 cells inhibited cell proliferation. Dual-luciferase reporter gene assay and Western blot further confirmed the direct regulation between microRNA-373-3p and AKT1.

Conclusions

We showed that downregulated microRNA-373-3p promoted the proliferation of prostate cancer cells *via* targeting AKT1, which might be served as a potential biomarker for the diagnosis of prostate cancer.

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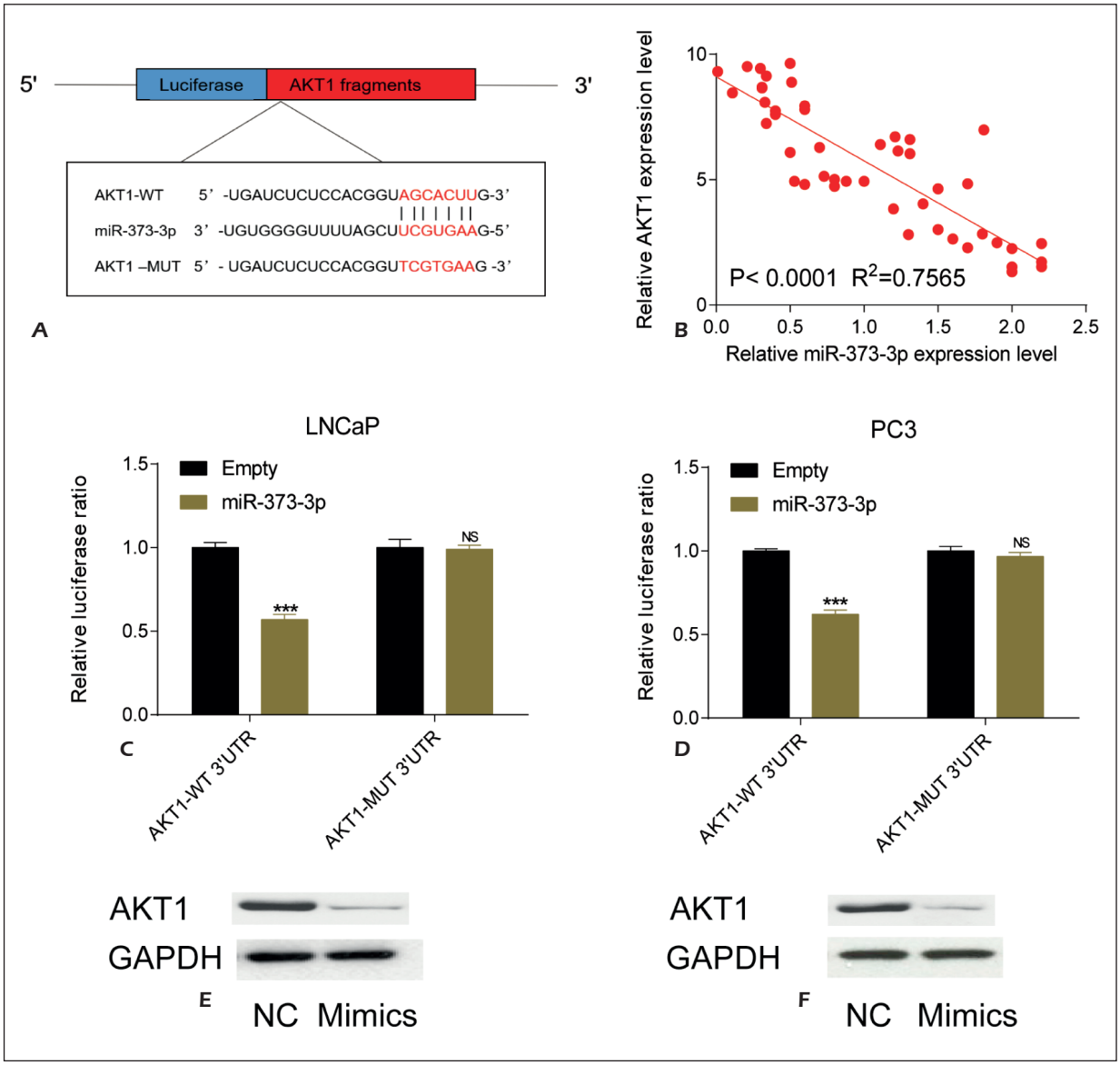

Figure 3. MicroRNA-373-3p bound to AKT1 and inhibited prostate cancer progression. **A**, Construction of AKT1 MUT and AKT1 WT. **B**, Negative correlation between microRNA-373-3p and AKT1. **C**, Luciferase activity was decreased in LNCaP cells co-transfected with AKT1 WT and microRNA-373-3p. **D**, Luciferase activity was reduced in PC3 cells co-transfected with AKT1 WT and microRNA-373-3p. **E-F**, After the upregulation of microRNA-373-3p expression in LNCaP and PC3 cells, AKT1 protein level was significantly reduced.

Conflict of Interest:

The authors declare that they have no conflict of interest.

References

1) Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.

2) Rebillard A, Lefeuvre-Orfila L, Gueritat J, Cillard J. Prostate cancer and physical activity: adaptive response to oxidative stress. Free Radic Biol Med 2013; 60: 115-124.

3) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.

4) Peyromaure M, Debre B, Mao K, Zhang G, Wang Y, Sun Z, Xu D, Jiang J, Sun Y. Management of prostate cancer in China: a multicenter report of 6 institutions. J Urol 2005; 174: 1794-1797.

5) Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M, Fabbri M, Campiglio M, Menard S, Palazzo JP, Rosenberg A, Musiani P, Volinia S, Nenci I, Calin GA, Querzoli P, Negrini M, Croce CM. MicroRNA gene expression deregulation in human breast cancer. Cancer Res 2005; 65: 7065-7070.

6) Murakami Y, Yasuda T, Saigo K, Urashima T, Toyoda H, Okanoue T, Shimotohno K. Comprehensive analysis of microRNA expression patterns in hepatocellular carcinoma and non-tumorous tissues. Oncogene 2006; 25: 2537-2545.

7) Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, Stephens RM, Okamoto A, Yokota J, Tanaka T, Calin GA, Liu CG, Croce CM, Harris CC. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell 2006; 9: 189-198.

8) Selth LA, Townley SL, Bert AG, Stricker PD, Sutherland PD, Horvath LG, Goodall GJ, Butler LM, Tilley WD. Circulating microRNAs predict biochemical recurrence in prostate cancer patients. Br J Cancer 2013; 109: 641-650.

9) Moltzahn F, Olshen AB, Baehner L, Peek A, Fong L, Stoppler H, Simko J, Hilton JF, Carroll P, Blelloch R. Microfluidic-based multiplex qRT-PCR identifies diagnostic and prognostic microRNA signatures in the sera of prostate cancer patients. Cancer Res 2011; 71: 550-560.

10) Sita-Lumsden A, Dart DA, Waxman J, Bevan CL. Circulating microRNAs as potential new biomarkers for prostate cancer. Br J Cancer 2013; 108: 1925-1930.

11) Kneitz B, Krebs M, Kalogirou C, Schubert M, Joniau S, van Poppel H, Lerut E, Kneitz S, Scholz CJ, Strobel P, Gessler M, Riedmiller H, Spahn M. Survival in patients with high-risk prostate cancer is predicted by miR-221, which regulates proliferation, apoptosis, and invasion of prostate cancer cells by inhibiting IRF2 and SOCS3. Cancer Res 2014; 74: 2591-2603.

12) Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. MiR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A 2005; 102: 13944-13949.

13) O'Donnell KA, Wentzel EA, Zeller KI, Dang CV, Mendell JT. C-Myc-regulated microRNAs modulate E2F1 expression. Nature 2005; 435: 839-843.

14) Qu YH, Liu HT, Zheng LJ, Qin Z, Li HX. [Effects of microRNA-373 on the proliferation and invasiveness of breast carcinoma and its mechanisms]. Zhonghua Yi Xue Za Zhi 2017; 97: 603-607.

15) Liu W, Li M, Chen X, Zhang D, Wei L, Zhang Z, Wang S, Meng L, Zhu S, Li B. MicroRNA-373 promotes migration and invasion in human esophageal squamous cell carcinoma by inhibiting TIMP3 expression. Am J Cancer Res 2016; 6: 1-14. eCollection 2016. Erratum in: Am J Cancer Res 2016; 6: 1458-1459.

16) Jing SY, Jing SQ, Liu LL, Xu LF, Zhang F, Gao JL. Down-expression of miR-373 predicts poor prognosis of glioma and could be a potential therapeutic target. Eur Rev Med Pharmacol Sci 2017; 21: 2421-2425.

17) Tian YY, Jia CM, Li Y, Wang Y, Jiang L, Liu AC. Restoration of microRNA-373 suppresses growth of human T-cell lymphoma cells by repressing CCND1. Eur Rev Med Pharmacol Sci 2016; 20: 4435-4444.

18) Weng J, Zhang H, Wang C, Liang J, Chen G, Li W, Tang H, Hou J. MiR-373-3p targets DKK1 to promote EMT-Induced metastasis via the Wnt/beta-Catenin pathway in tongue squamous cell carcinoma. Biomed Res Int 2017; 2017: 6010926.

19) Hua Y, Chen H, Wang L, Wang F, Wang P, Ning Z, Li Y, Liu L, Chen Z, Meng Z. Low serum miR-373 predicts poor prognosis in patients with pancreatic cancer. Cancer Biomark 2017; 20: 95-100.

20) Zhang Z, Cheng J, Wu Y, Qiu J, Sun Y, Tong X. LncRNA HOTAIR controls the expression of Rab22a by sponging miR-373 in ovarian cancer. Mol Med Rep 2016; 14: 2465-2472.

21) Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007; 57: 43-66.

22) Pienta KJ, Bradley D. Mechanisms underlying the development of androgen-independent prostate cancer. Clin Cancer Res 2006; 12: 1665-1671.

23) Damber JE, Aus G. Prostate cancer. Lancet 2008; 371: 1710-1721.

24) Farh KK, Grimson A, Jan C, Lewis BP, Johnston WK, Lim LP, Burge CB, Bartel DP. The widespread impact of mammalian MicroRNAs on mRNA repression and evolution. Science 2005; 310: 1817-1821.

25) Ambs S, Prueitt RL, Yi M, Hudson RS, Howe TM, Petrocca F, Wallace TA, Liu CG, Volinia S, Calin GA, Yfantis HG, Stephens RM, Croce CM. Genomic profiling of microRNA and messenger RNA reveals deregulated microRNA expression in prostate cancer. Cancer Res 2008; 68: 6162-6170.

26) Galardi S, Mercatelli N, Giorda E, Massalini S, Frajese GV, Ciafre SA, Farace MG. MiR-221 and miR-222 expression affects the proliferation potential of human prostate carcinoma cell lines by targeting p27Kip1. J Biol Chem 2007; 282: 23716-23724.

27) Sun T, Wang Q, Balk S, Brown M, Lee GS, Kantoff P. The role of microRNA-221 and microRNA-222 in androgen-independent prostate cancer cell lines. Cancer Res 2009; 69: 3356-3363.

28) Wei F, Wang Q, Su Q, Huang H, Luan J, Xu X, Wang J. MiR-373 inhibits glioma cell u251 migration and invasion by down-regulating CD44 and TGFBR2. Cell Mol Neurobiol 2016; 36: 1389-1397.

29) Yang ZZ, Tschopp O, Baudry A, Dummler B, Hynx D, Hemmings BA. Physiological functions of protein kinase B/Akt. Biochem Soc Trans 2004; 32: 350-354.

30) Gonzalez-Angulo AM, Blumenschein GJ. Defining biomarkers to predict sensitivity to PI3K/Akt/mTOR pathway inhibitors in breast cancer. Cancer Treat Rev 2013; 39: 313-320.

31) Matsuoka T, Yashiro M. The role of PI3K/Akt/mTOR signaling in gastric carcinoma. Cancers (Basel) 2014; 6: 1441-1463.

32) Cheung M, Testa JR. Diverse mechanisms of AKT pathway activation in human malignancy. Curr Cancer Drug Targets 2013; 13: 234-244.

33) Fransecky L, Mochmann LH, Baldus CD. Outlook on PI3K/AKT/mTOR inhibition in acute leukemia. Mol Cell Ther 2015; 3: 2.

34) Grottke A, Ewald F, Lange T, Norz D, Herzberger C, Bach J, Grabinski N, Graser L, Hoppner F, Nashan B, Schumacher U, Jucker M. Downregulation of AKT3 increases migration and metastasis in triple negative breast cancer cells by upregulating S100A4. PLoS One 2016; 11: e146370.

35) Cho H, Thorvaldsen JL, Chu Q, Feng F, Birnbaum MJ. Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J Biol Chem 2001; 276: 38349-38352.

36) Lee MY, Luciano AK, Ackah E, Rodriguez-Vita J, Bancroft TA, Eichmann A, Simons M, Kyriakides TR, Morales-Ruiz M, Sessa WC. Endothelial Akt1 mediates angiogenesis by phosphorylating multiple angiogenic substrates. Proc Natl Acad Sci U S A 2014; 111: 12865-12870.

37) Xu N, Lao Y, Zhang Y, Gillespie DA. Akt: a double-edged sword in cell proliferation and genome stability. J Oncol 2012; 2012: 951724.