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MiRNA-215-5p alleviates the metastasis of prostate cancer by targeting PGK1

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Abstract. – OBJECTIVE: MicroRNAs (miRNAs) are endogenous, non-coding RNAs, which exert crucial functions in regulating biological progressions. Previous studies have demonstrated the anti-tumor effect of miRNA-215-5p. However, its specific role in influencing the progression of prostate cancer (PCa) remains unclear. This study aims to uncover the regulatory effect of miRNA-215-5p on the metastasis and prognosis of PCa.

PATIENTS AND METHODS: MiRNA-215-5p levels in collected PCa tissues (n=52) and paracancerous tissues (n=52) were determined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The relationship between miRNA-215-5p level and pathological indexes as well as overall survival of PCa patients, was analyzed. Regulatory effects of miRNA-215-5p on proliferative and metastatic capacities of LNCaP and DU-145 cells were evaluated through cell counting kit-8 (CCK-8) and transwell assay, respectively. Bioinformatics prediction was performed to search for the target gene of miRNA-215-5p and PGK1 was selected. The biological role of PGK1 in the progression of PCa was finally clarified by a series of rescue experiments.

RESULTS: MiRNA-215-5p was lowly expressed in PCa tissues and cell lines. Low level of miRNA-215-5p predicted poor prognosis in PCa patients. The existence of miRNA-215-5p enhanced viability, migratory and invasive capacities of LNCaP cells, while the overexpression of miRNA-215-5p yielded the opposite trends in DU-145 cells. PGK1 was predicted to be the target of miRNA-215-5p. PGK1 was upregulated in PCa tissues and cell lines and its high level predicted poor prognosis of PCa. Moreover, PGK1 level was negatively correlated to that of miRNA-215-5p in PCa. PGK1 was able to reverse the inhibitory effects of miRNA-215-5p on metastatic potentials of PCa cells.

CONCLUSIONS: Downregulated miRNA-215-5p in PCa is closely related to distant metastasis and poor prognosis of affected patients. MiRNA-215-5p alleviates the malignant progression of PCa by targeting and downregulating PGK1.

Key Words:
MiRNA-215-5p, PGK1, Prostate cancer, Malignancy.

Introduction

Prostate cancer (PCa) is one of the most common malignancies throughout the world, which is the leading cause of cancer-related deaths in the United States and Europe. In recent years, the incidence of PCa in China has rapidly increased every year[1-3]. Detective rate of PCa achieved a peak in the early 1990s after the introduction of prostate-specific antigen (PSA) screening[4,5]. So far, approximately 85% of newly diagnosed PCa cases are limited to early-stage cancer[5]. Although PSA screening greatly improves the diagnostic rate of early-stage PCa, its potential effect on decreasing the mortality of PCa remains controversial[6]. Since PCa is characterized as tumor heterogeneity, developing diagnostic, therapeutic, and prognostic hallmarks is the priority[7,8]. Currently, target therapy based on tumor-related miRNAs presents a promising application[9-11].

MicroRNAs (miRNAs) are small, endogenous non-coding RNAs, exerting negative translation regulation on expressions of protein-coding genes[12,13]. They exert the biological function by degrading or inhibiting translation of mRNAs[14]. It is reported[15] that miRNAs are extensively involved in affecting cellular behaviors and disease progression. Increasing evidence[16,17] has demonstrated the effects of abnormally expressed miRNAs on the occurrence and progression of tumors. These certain miRNAs may be utilized for developing anti-tumor drugs or biological hallmarks[18]. About 30% human genome could be regulated by miRNAs. Most of the human miRNAs (52%) locate on tumor-associated genomic regions or gene

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fragile sites[19,20]. Dysregulation of these certain miRNAs results in tumorigenesis. Bioinformatics has been widely applied to analyze the genome to further uncover the gene expression pattern[21]. By analyzing miRNA profiling microarray of PCa, miRNA-215-5p was selected[22]. Its potential targets and the biological function in PCa were further analyzed[23]. Our findings provide a new idea for the clinical treatment of PCa.

Patients and Methods

Patients and Tissue Samples

A total of 52 PCa patients undergoing rectal surgery in the Second Affiliated Hospital of Fujian Medical University were enrolled in this experiment. Tumor tissues and matched adjacent normal tissues (5 cm away from tumor edge) were surgically resected from PCa patients and preserved within 5 min *ex vivo*. Pathological indexes and follow-up data of PCa patients were collected for further analyses. Patients and their families have been fully informed. This research was approved by the Ethics Committee of the Second Affiliated Hospital of Fujian Medical Univ

Cell Culture

Prostate epithelial cells (RWPE-1) and P cells (LNCaP, 22RV1, PC3, and 145) wer provided by Cell Bank (Shang). Cells were cultured in Roswell Pa Memo Institute-1640 (RPMI-1640; Hy e, So UT, USA) containing 10% b e serum (FBS; Gibco, Rockville D, US e medium was replaced every days. The passage was conducted at 9 fluence.

Transfection

Transfecti plasmids were p ded by GenePharma anghai China). Cells seeded in the 6-well p were tured until 70% confluence and trans g Lip tamine 3000 (Invitrogen Carl CA, U). At 48 h, cells were ha for ve of transfection efficacy subs ent exp ents.

ll C t-8 (CCK-8)

were seeded in the 96-well plate with 2×1 s per well. At 6, 24, 48 and 72 h, absorban ue at 450 nm of each sample was recorded using the CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan) for plotting the viability curves.

Transwell Assay

Cells were adjusted to a dose of 2.0×10^5/mL. For determining cell invasion, diluted Matrigel was pre-coated on the bottom of e nswell chamber. 200 μL of suspension w pp he upper side chamber (Millipo Billerica, USA) inserted in a 24-well . In the bot side, 700 μL of medium co g 10% was applied. After 48 h incuba ells etrated to the bottom were fixed hol for 15 min, dyed w crysta olet for min, and counted using icros e. The umbers of migratory and inva were nted in 5 randomly sel d fiel sam

Quantit eal Time P merase Chain act RT-PCR)

Total RNA wa acted from cells using TR agent (Invitr , Carlsbad, CA, USA), ed by DNase I treatment, and reversely transbed into c lementary deoxyribose nucleic d (cDNA) u g the Primescript RT Reagent aRa, Ots higa, Japan). The obtained cDNA d to qRT-PCR using SYBR®Premix E q™ (TaKaRa, Otsu, Shiga, Japan). Glyceraldehyde 3-phosphate dehydrogenase H) and U6 were used as internal references. qRT-PCR conditions were as follows: 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s, for a total of 40 cycles. Each sample was performed in triplicate and the relative level was calculated by the $2^{-\Delta\Delta Ct}$ method. Primer 5.0 was used for designing qRT-PCR primers. Primer sequences used in this study were as follows: PGK1, F: 5'-CATGGAGGAACCATTAGTC-3', R: 5'-GCTCCGATGTCCTCCAGAACGGA-3'; microRNA-215-5p, F: 5'-GATGTAGAACAGTCCTGCGACTG-3', R: 5'-GATAAGATGTCATGGAGCTCG-3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Western Blot

Total protein was extracted from cells using radioimmunoprecipitation assay (RIPA) and quantified by bicinchoninic acid (BCA) method (Beyotime, Shanghai, China). The protein sample was loaded for electrophoresis and transferred on a polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Membranes were blocked in 5% skim milk for 2 h and reacted with primary and secondary antibodies. Bands

were exposed by enhanced chemiluminescence (ECL) and analyzed by Image Software (NIH, Bethesda, MD, USA).

Statistical Analysis

GraphPad Prism 6 V6.01 (La Jolla, CA, USA) was used for data analyses. Data were expressed as mean ± standard deviation. Differences between two groups were analyzed by the *t*-test. Kaplan-Meier curves were introduced for survival analysis. Chi-square test was performed to evaluate the correlation between miRNA-215-5p levels with pathological indexes of PCa patients. $p<0.05$ was considered as statistically significant.

Results

MiRNA-215-5p Was Downregulated in PCa

Expression pattern of miRNA-215-5p in PCa was first determined by qRT-PCR. Compared with adjacent normal tissues, miRNA-215-5p was downregulated in PCa tissues (Figure 1A). Similarly, miRNA-215-5p was downregulated in PCa cell lines than that of the prostate epithel line (Figure 1B).

MiRNA-215-5p Expression Was Correlated with Distant Met is and Overall Survival in PC s

Pathological indexes and f v-up d of enrolled PCa patients were c ed. A the median level of miR A-2 patie were assigned to high l and level miRNA-215-5p groups. M A-215-5p was related to distant me but not relat to age, gender, TNM st g, a phatic metastasis of PCa patient Table I). Mo r Kaplan-Meier curves re ed worse prog in PCa patients of l evel g up than that of high-level group (F e 1C).

MiRNA-21 nflu ed Proliferative a stat ities of PCa

iRN 215-5p expression and knockdown m s were constructed in DU-145 and CaP ctively (Figure 2A). Transfec- iRNA-215-5p inhibitor enhanced viability, ers of migratory and invasive LNCaP cells (F 2B, 2C). On the contrary, the viability, migratory, and invasive capacities were attenuated in DU-145 cells overexpressing miRNA-215-5p (Figure 2B, 2C).

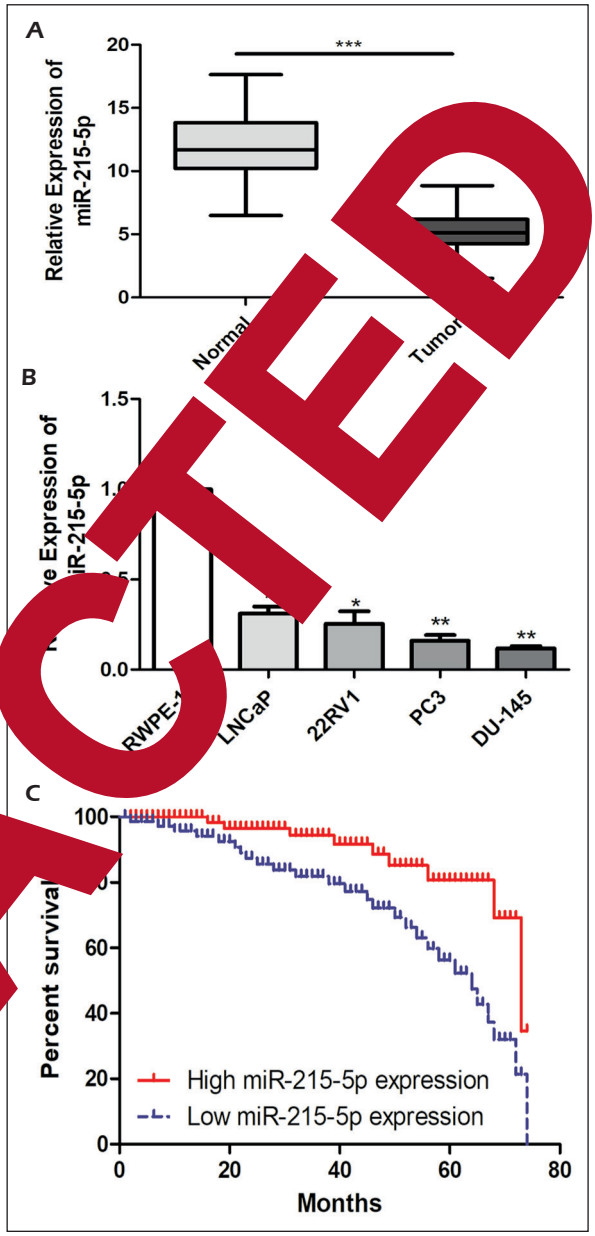

Figure 1. MiR-215-5p was downregulated in PCa. **A,** MiR-215-5p level in adjacent normal tissues and PCa tissues. **B,** MiR-215-5p level in prostate epithelial cells (RWPE-1) and PCa cells (LNCaP, 22RV1, PC3, and DU-145). **C,** Overall survival in PCa patients with high and low expression of miR-215-5p.

Interaction Between MiRNA-215-5p and PGK1

Potential target genes of miRNA-215-5p were predicted in the miRDB, TargetScan, and StarBase (Figure 3A). At last, the intersection contained 6 potential targets (PGK1, NIPAL1, PRKD3, RPAP2, ZEB2, and RAB2A). Among them, PGK1 was the most differentially expressed one after transfection of miRNA-215-5p

Table I. Association of miR-215-5p expression with clinicopathologic characteristics of prostate cancer.

Parameters	No. of cases	MiR-215-5p expression		
		High (%)	Low (%)	ue
Age (years)				0.8
< 60	20	12	8	
≥ 60	32	20	12	
T stage				0.25
T1-T2	36	24		
T3-T4	16	8		
Lymph node metastasis				16
No	33	22	11	
Yes	19	10	9	
Distance metastasis				0.023
No	31	23		
Yes	21	9	12	

inhibitor in LNCaP cells (Figure 3B). In addition, PGK1 level was markedly downregulated in DU-145 cells overexpressing miRNA-215-5p (Figure 3C). To further uncover the role of PGK1 in PCa, we constructed pcDNA-PGK1 and si-PGK1. MiRNA-215-5p level was found to be remarkably downregulated in LNCaP cells transfected with pcDNA-PGK1, which was regulated in DU-145 cells transfected with si-PGK1 (Figure 3D).

PGK1 Was Upregulated in

In PCa tissues and cell li PGK was remarkably upregulated relati contr 4A, 4B). By detecting ex res of PGK and miRNA-215-5p in a tis a negative correlation between was iden (Figure 4C). Furthermore gnostic po ial of PGK1 was confi d. P tients with a high level of PGK1 ffered wor gnosis relative to those wit level (Figure

MiRNA 5 egula Cell Behaviors of PCa by ting K1

xper re performed to uncover e ro f miR 5-5p/PGK1 axis in influcing t rogression of PCa. In LNCaP cells nsfe RNA-215-5p inhibitor, the up- d PGK1 was reversed after co-transfection PGK1. Moreover, downregulated PGK1 in DU- ells overexpressing miRNA-215-5p was reversed by overexpression of PGK1 (Figure 5A, 5B). Notably, the silence of miRNA-215-5p accelerated migratory and invasive capacities in LN lls, which w versed by co-transfec- of si-PGK1. Similarly, attenuated metastatic pacities of D 145 cells overexpressing miR-215-5p we reversed by overexpression of 1 (Figure).

Discussion

NAs not only influence normal physical activities, but also regulate tumor progression at transcriptional and post-transcriptional levels[10,11]. Whole-genome gene expression analysis and DNA copy number analysis have demonstrated inactivated tumor-suppressor genes (PTEN, ATBF, KLF5, and KLF6) and activated oncogenes (c-MYC, AR and TEMPRESS/ERG) in PCa cells[8,9]. Microarray-based Comparative Genomic Hybridization has identified several chromosomal changes in PCa cell lines and primary tissues[5-7]. In these chromosomal deletion or amplification regions, the presence of cancer-related genes or miRNAs may be existed[10]. Oncogenes or tumor-suppressor genes were used to develop as drug targets for tumor treatment. Nowadays, tumor-related miRNAs have been well concerned, exerting more crucial application in early-stage diagnosis, target therapy, and effective prognosis of tumors[11].

MiRNA-215-5p is a recently discovered miRNA[23]. In this paper, miRNA-215-5p was downregulated in PCa tissues and cell lines. Low level of miRNA-215-5p was correlated with distant metastasis and poor prognosis of PCa patients, suggesting its anti-tumor effect. The strong metastatic ability of PCa cells is the leading cause of

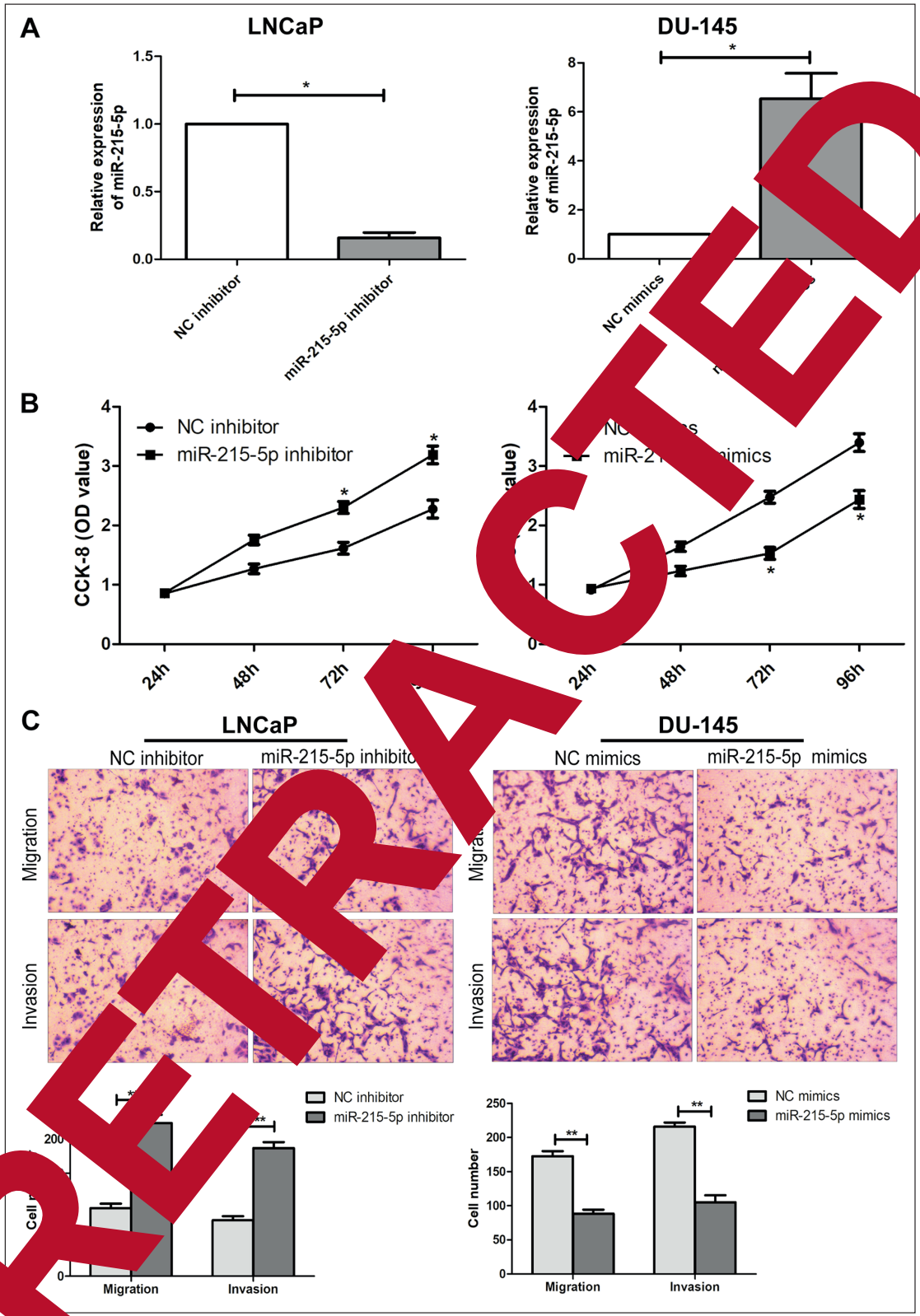

Figure 2. miR-215-5p influences proliferative and metastatic capacities of PCa. **A,** Transfection efficacy of miR-215-5p inhibitor in LNCaP cells, and miR-215-5p mimics in DU-145 cells. **B,** Viability in LNCaP cells with miR-215-5p knockdown and DU-145 cells overexpressing miR-215-5p. **C,** Migration and invasion in LNCaP cells with miR-215-5p knockdown and DU-145 cells overexpressing miR-215-5p (magnification: 200×).

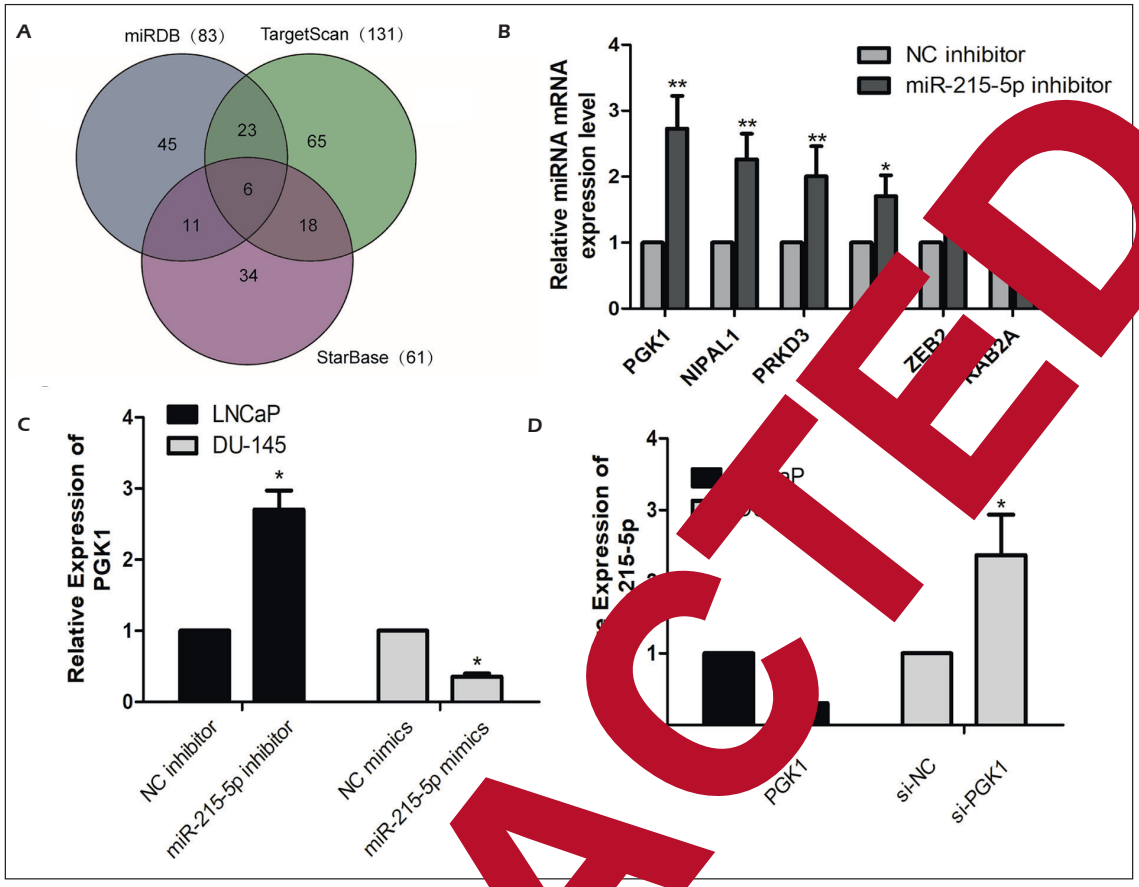

Figure 3. Interaction of miR-215-5p and PGK1. **A,** Poten get genes of miR-215-5p predicted in the miRDB, TargetScan and StarBase. **B,** Expression levels of si ial targets K1, NIPAL1, PRKD3, RPAP2, ZEB2, and RAB2A) of miR-215-5p in LNCaP cells transfected wit r or miR- p inhibitor. **C,** PGK1 level in LNCaP cells with miR-215-5p knockdown and DU-145 cells overe ssing 215-5p. **D** iR-215-5p level in LNCaP cells overexpressing PGK1 and DU-145 cells with PGK1 knockdo

poor prognosis in PC tients. Her results revealed that silenc RNA-215-5p anced viability, migrat and sive capacities of PCa. Converse overexpres f miRNA-215-5p attenuate he proliferative d metastatic capacities.

Regul y me ism of miRNAs depends on the e and f ons of their target genes[15,16]. A A de es target mRNA or su its t by base pairing with the mi [17]. The degree of base airing ides the degradation or translation repre NAs, that is, complete base p eads to mRNA degradation. Otherwise, tra n inhibition is achieved[18]. MiRNAs only ac for only 1% of the whole human genome, but they are able to regulate more than 30% protein-encoding genes. Multiple miRNAs could precisely regulate a single target gene[12-15]. Our results showed that PGK1 was the target gene of miRNA-215-5p through prediction in miRDB, TargetScan, and StarBase. PGK1 level was upregulated in PCa and negatively regulated by miRNA-215-5p. Of note, PGK1 could reverse the regulatory effects of miRNA-215-5p on metastatic potentials of PCa cells. Hence, it is concluded that miRNA-215-5p alleviated the malignant progression of PCa *via* negatively regulating PGK1.

Conclusions

We first showed that downregulated miRNA-215-5p is closely related to distant metastasis and poor prognosis of PCa patients. It alleviates the malignant progression of PCa by targeting and downregulating PGK1.

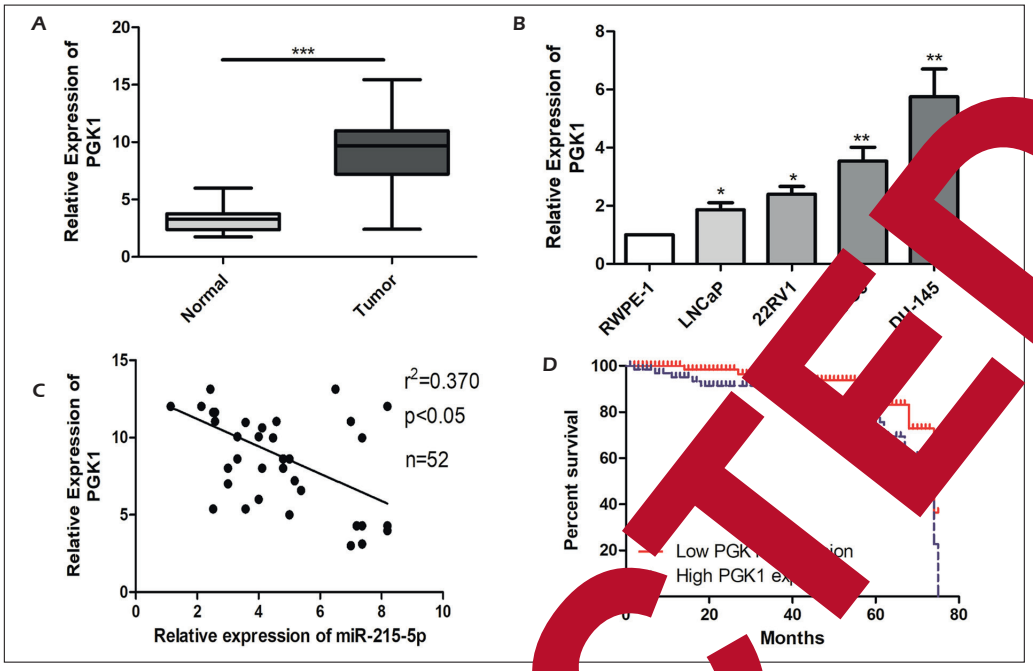

Figure 4. PGK1 was upregulated in PCa. **A,** PGK1 level in adjacent normal tissues and PCa tissues. **B,** PGK1 level in prostate epithelial cells (RWPE-1) and PCa cells (LNCaP, 22RV1, PC3, and DU-145). **C,** A negative correlation between expressions of miR-215-5p and PGK1 in PCa tissues. **D,** Overall survival in PCa patients with high and low expression of PGK1.

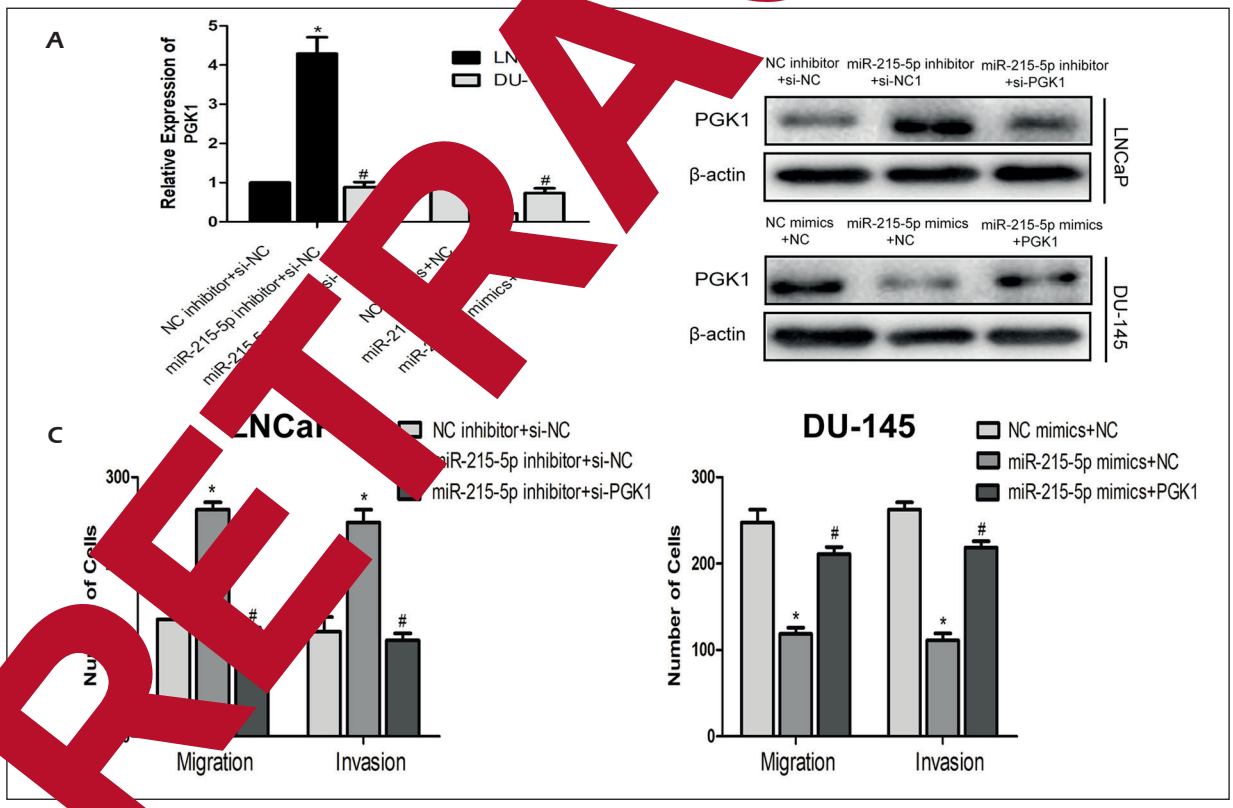

Figure 5. miR-215-5p regulated PCa cell behaviors by targeting PGK1. LNCaP cells were transfected with NC inhibitor + si-NC, miR-215-5p inhibitor + si-NC or miR-215-5p inhibitor + si-PGK1. DU-145 cells were transfected with NC mimics + NC, miR-215-5p mimics + NC or miR-215-5p mimics + pcDNA-PGK1. **A,** mRNA level of PGK1; **B,** Protein level of PGK1; **C,** Migration and invasion abilities.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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