

miR-19a-3p targets PMEPA1 and induces prostate cancer cell proliferation, migration and invasion

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Abstract. Prostate cancer (PCa) is one of the most common malignant tumors in men. Studies have observed that microRNA (miR)-19a-3p expression levels are downregulated in several types of cancer, and yet the biological function and its underlying mechanisms in the pathogenesis of PCa remain unclear. In the current study, the expression pattern of miR-19a-3p in PCa tissues and cell lines was detected by reverse transcription-quantitative polymerase chain reaction. The proliferative, migratory and invasive capacity of PCa cells were determined using EdU and Transwell assays following transfection with miR-19a-3p mimics. Additionally, the current study investigated the biological impact and regulation of prostate transmembrane protein androgen induced 1 (PMEPA1) in PCa cells by transfection with PMEPA1 small interfering (si)RNA. It was observed that miR-19a-3p was upregulated in PCa tissue samples and cell lines *in vitro*. Functional analysis also confirmed that miR-19a-3p overexpression promoted the proliferation, migration and invasion of PCa cells. Furthermore, PMEPA1 was identified as a direct target of miR-19a-3p, and siRNA knockdown of PMEPA1 resulted in increased proliferation, migration and invasion of PCa cells, which partially accounts for the effect of miR-19a-3p in tumor metastasis. In conclusion, the findings of the present study suggest that the upregulation of miR-19a-3p expression levels contributes to tumor progression and that one of its underlying mechanisms involves inhibition of PMEPA1 expression.

Introduction

Prostate cancer (PCa) is one of the most frequently diagnosed malignant tumors in men worldwide, and its incidence continues to rise in numerous countries. According to a recent American Cancer Society report, there were 233,000 new

cases and 29,480 PCa-associated mortalities in the United States in 2014 (1). Currently, the early detection of PCa predominantly depends on serum prostate-specific antigen (PSA) testing. Although the routine use of PSA testing increased the detection of PCa, the greatest deficiency of this method is its lack of specificity, as elevated PSA levels may also be detected in men with benign prostatic hyperplasia (BPH) and prostatitis. Although various preventive therapies, such as radical prostatectomy, androgen-deprivation taxane-based chemotherapy, drugs targeting the androgen receptor (enzalutamide) or androgen synthesis (abiraterone acetate), and 5 α -reductase inhibitors, may prevent or manage early disease stages, there is no effective therapeutic target for aggressive PCa; this results in a tumor progression of 40-50% patients with castration resistant prostate cancer (2). Thus, it is necessary to elucidate novel biomarkers that may be used for the development of more sensitive diagnosis and treatment (3).

MicroRNAs (miRNAs/miRs) are small, non-coding RNAs that regulate gene expression at the post-transcriptional and translational levels. The complementarity between miRNAs and target mRNAs causes mRNA cleavage and/or translational suppression, resulting in reduced gene expression levels (4). Multiple miRNAs have been implicated in PCa progression, including the miRNA-17-92 cluster, which has been demonstrated to contribute to tumorigenesis (5-8). In addition, miR-17-5p and miR-17-3p, which are part of the miRNA-17-92 cluster, target TIMP metalloproteinase inhibitor 3 and coordinately function as an oncogene in PCa (9). Furthermore, Yang *et al* (10) confirmed the inhibitory effect of miR-19a-3p in breast cancer progression and metastasis. In serum samples from patients with colorectal adenocarcinoma, the miR-19a-3p level was significantly correlated with the different clinical stages of colorectal adenocarcinoma (11). The high expression of miR-19a-3p was also significantly associated with poor survival of patients with astrocytoma (12). However, there is currently limited knowledge regarding the role of miR-19a-3p in PCa tumorigenesis and metastasis. Thus, the present study investigated the function of miR-19a-3p in PCa. It was observed that miR-19a-3p may promote the progression of PCa progression through the targeting prostate transmembrane protein androgen induced 1 (PMEPA1), which is abundant in the prostate abundant and can negatively regulate the growth of refractory PCa cells.

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Materials and methods

Clinical specimens. A total of 22 PCa samples and 20 BPH tissues were obtained from patients who underwent surgery at Beijing Chao-Yang Hospital, Capital Medical University (Beijing, China) from May 2014 to January 2015. The tissue of each patient was acquired from transrectal biopsy or prostatectomy, and the tumor stage of PCa was assessed according to the modified tumor, node, metastasis system suggested by the UICC International Union Against Cancer (13). All the tissue samples were stored at -80°C following flash freezing in liquid nitrogen. The present study was approved by the ethics committee of Beijing Chao-Yang Hospital, Capital Medical University, and written informed consent was obtained from each patient.

Cell cultures. The BPH cell line, BPH1, and the PCa cell lines, LNCaP and PC3, were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were routinely cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 50 $\mu\text{g}/\text{ml}$ streptomycin, all purchased from Gibco (Thermo Fisher Scientific, Inc., Waltham, MA, USA). All cell lines were cultured in a humidified incubator at 37°C and 5% CO_2 .

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Small RNA was extracted from tissues or cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), in accordance with the manufacturer's instructions. To analyze miR-19a-3p expression levels, an miRcute miRNA First-Strand cDNA Synthesis kit and miRcute miRNA qPCR Detection kit (SYBR Green), purchased from Tiangen Biotech Co., Ltd. (Beijing, China), were used, according to the manufacturer's protocol. All reactions were run on an ABI7500 Sequence Detection System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The PCR conditions included an initial holding period of 2 min at 94°C , followed by a two-step PCR program consisting of 20 sec at 94°C and 34 sec at 60°C for 40 cycles. The forward primer was designed based on the mature sequence of human miR-19a-3p. The forward primer sequence for miR-19a-3p was 5'-CGCATC CCAGTGTGCAAATCT-3' and the forward primer sequence of U6 was 5'-GCAAGGATGACACGCAAATTC-3'. The universal reverse primer was provided in the miRcute miRNA qPCR Detection kit. All samples were normalized against the reference gene (U6 small nuclear RNA) and analyzed using the $2^{-\Delta\Delta\text{C}_q}$ method (14).

Transfection of miR-19a-3p mimics/inhibitors. miRNA mimics are small, chemically modified double-stranded RNAs that mimic the function of an endogenous miRNA. miRNA inhibitors are single-stranded chemically modified RNAs containing a sequence complementary to the seed region or the complete sequence of the targeted miRNA. They inhibit the incorporation of the endogenous miRNA into the RNA-induced silencing complex, therefore, blocking mRNA cleavage or translational repression. The negative control (NC) used was a scramble miRNA, which is a random sequence of precursor miRNA molecules validated by the manufacturer to not produce any identifiable effects on known miRNA

function (15). In the present study, 4×10^4 PC3 cells were transfected with miR-19a-3p mimics (miR10002869- 1- 5) and inhibitors (miR20000073- 1- 5) in 24-well plates using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. For the miRNA mimic experiments, miR-19a-3p mimics and NC (miR01101-1- 5) (Guangzhou RiboBio Co., Ltd., Guangzhou, China) were used at 50 nM. For the miRNA inhibitors experiments, miR-19a-3p inhibitors and NC (Guangzhou RiboBio Co., Ltd.) were used at 100 nM. In the vehicle group, cells were cultured in medium only. The cells were harvested 24 h after transfection and subjected to various assays.

Small interfering RNA (siRNA) assay. PC3 cells were seeded at a density of 2×10^5 cells per well in 6-well plates in 2 ml of culture medium containing 10% FBS. The cells were transfected with siRNA against PMEPA1 (siG141117152735) and its negative control (siN05815122147) (both purchased from Guangzhou RiboBio Co., Ltd.) using Lipofectamine 2000. The cells were harvested 48 h after transfection and subjected to various assays.

Cell proliferation assay. Cell proliferation was determined by 5-ethynyl-2'-deoxyuridine (EdU) incorporation assay, which was performed using a Cell-Light EdU imaging detecting kit, according to the manufacturer's instructions (Guangzhou RiboBio Co., Ltd.). Incorporation of the EdU thymidine analogue was used to label cells undergoing DNA replication. Images of the EdU positive cells were captured using a light microscope (Leica DM4000B; Leica Microsystems, Wetzlar, Germany) and the cell numbers were counted using Image-Pro Plus version 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA).

Cell invasion and migration assay. Cell invasion was measured using a 24-well Transwell assay (Corning Incorporated, Corning, NY, USA) on a polycarbonate filter pre-coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) at 1:10 dilution. Cells (2×10^4) suspended in 0.2 ml of serum-free medium were added to the upper well of the chamber and 500 μl of medium supplemented with 10% FBS was added to the lower well. Following incubation at 37°C in 5% CO_2 for 24 h, the cells remaining in the upper chamber were removed using a cotton swab. Invaded cells on the bottom of the membranes were fixed with 90% ethanol and stained with 0.1% crystal violet solution (Sigma-Aldrich, St. Louis, MO, USA). Images of the fixed cells were captured with a light microscope (Leica DM4000B; Leica Microsystems) and the cell numbers were counted using Image-Pro Plus version 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA) in five randomly selected fields. Cell migration assays were conducted as described for invasion assays, excluding the use of Matrigel.

miRNA target prediction. Predicted miR-19a-3p target genes were obtained using the miRWalk atlas (zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/), which collates data from the following multiple prediction programs: Targetscan (www.targetscan.org), miRanda (www.microrna.org) and picTar (pictar.mdcberlin.de).

Luciferase activity assay. A luciferase activity assay was performed using a Dual-Luciferase Reporter System developed by Promega Corporation (Madison, WI, USA). In brief, PC3 cells were seeded onto 96-well plates at a density of 2×10^4 cells/well in medium containing 10% FBS and cultured for 24 h. The cells were co-transfected with the luciferase reporter constructs (E1910), corresponding miRNA mimics and a *Renilla* luciferase construct (pmiR- RB- REPORTTM dual luciferase reporter constructs; Promega Corporation) using Lipofectamine 2000. The cells were then lysed using 100 μ l passive lysis buffer (Promega Corporation), (Roche Diagnostics, Basel, Switzerland) and the lysates were centrifuged at $3,000 \times g$ for 10 min for supernatant collection. Supernatant (20 μ l) was mixed with 100 μ l luciferase assay buffer II and the luciferase activities were measured using a Varioskan Flash (Thermo Fisher Scientific, Inc.). For the internal control, 100 μ l Stop & Glo reagent, which diluted 1.0 μ l Dual- Glo Stop & Glo substrate into 0.1 ml of Dual- Glo Stop & Glo buffer, was added to the samples. Luciferase activities between different treatments were compared following normalization to *Renilla* luciferase activities. The miR-19a-3p binding sites included positions 359-366 (5'-AACUUUAAA GACAUAA--UUUGCACA-3') and 611-617 (5'-UAUAAAUGU UGAGAUUUUGCACU-3') of the PMEPA1 mRNA 3'-UTR.

Western blot analysis. Cell lysates (1×10^6 cells) were prepared from cells cultured in 6-well plates by adding 100 μ l lysis buffer containing protease inhibitors to each well. Protein concentrations were measured using a Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Proteins (30 μ g) from each sample were loaded onto 10% SDS-polyacrylamide gels and separated by electrophoresis, then transferred onto polyvinylidene difluoride (PVDF) membrane (GE Healthcare Life Sciences, Chalfont, UK) at 4°C. Following several washes with Tris-buffered saline with Tween 20 (TBS-T; 20 mM pH 7.5 Tris-Cl, 0.15 M NaCl and 0.05% Tween 20), the PVDF membrane was blocked with 10% non-fat milk in TBS-T for 1 h and then incubated with primary goat polyclonal anti-PMEPA1 antibody (dilution, 1:1,000; sc-85829; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at 4°C overnight. The following day, the membrane was washed and incubated with IRDye 800CW donkey anti-goat IgG secondary antibody (dilution, 1:10,000; P/N 926- 32214; LI-COR, Inc., Lincoln, NE, USA) at room temperature for 2 h followed by detection with the Odyssey CLx Infrared Imaging System (LI-COR, Inc.). To verify equal loading of protein, the blots were reprobbed with primary monoclonal antibody against β -actin (ProteinTech Group, Inc., Chicago, IL, USA).

Statistical analysis. SPSS version 19.0 (IBM SPSS, Amronk, NY, USA) was used to perform all statistical analyses. Quantitative analysis for immunoblotting was performed using Quantity One software and a Gel Doc 2000 imaging system (version 4.62; Bio-Rad Laboratories, Inc.). For protein quantification, the ratio of PMEPA1 (band density of PMEPA1:band density of β -actin) was expressed as 100% in the NC group. The data from the other groups was expressed as a percentage of that of the NC group. Values are presented as the mean \pm standard error of the mean. Statistical analysis was conducted by one-way analysis of variance followed by

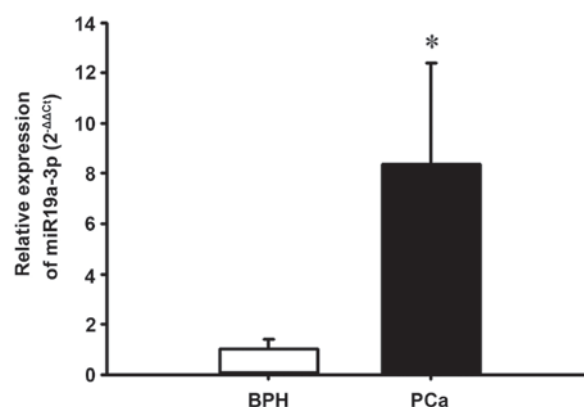


Figure 1. Expression of miR-19a-3p is higher in PCa tissues compared with BPH tissues. The expression levels of miR-19a-3p in tissues was measured by reverse transcription-quantitative polymerase chain reaction. U6 was used as the reference gene. *P<0.05 vs. BPH. Data are presented as the mean \pm standard error of the mean. Each sample was measured in triplicate. miR, microRNA; BPH, benign prostatic hyperplasia; PCa, prostate cancer.

Bonferroni test all pairwise multiple comparison procedures and Student's t test. Spearman's rank correlation was used to perform univariate analysis. P<0.05 was considered to indicate a statistically significant difference.

Results

Upregulation of miR-19a-3p in PCa tissues. Table I presents the clinical and pathological characteristics of the patients in the present study. To determine the expression levels of miR-19a-3p in PCa patients, miR-19a-3p was detected in all the 22 PCa and 20 BPH tissues using RT-qPCR. As demonstrated in Fig. 1, PCa tissues exhibited significantly increased expression of miR-19a-3p compared with that of the BPH tissues (P<0.05). Spearman's rank correlation analysis demonstrated that high expression of miR-19a-3p was not correlated with Gleason grade ($r=-0.3376$, $P=0.1244$) or serum PSA level ($r=-0.0766$, $P=0.735$; data not shown).

Overexpression of miR-19a-3p in the PC3 cell line. The current study further evaluated the expression level of miR-19a-3p in human BPH (BPH1) and PCa (LNCaP and PC3) cell lines. Notably, it was observed that the expression level of miR-19a-3p was increased significantly in human PC3 cells compared with the BPH1 and LNCaP cell lines (P<0.05; Fig. 2A).

Additionally, to investigate the effect of miR-19a-3p on PCa cell growth, the PC3 cells were transiently transfected with miR-19a-3p mimics and corresponding NC. RT-qPCR analysis demonstrated that transfection with the miR-19a-3p mimics caused a significant increase in miR-19a-3p expression levels in PC3 cells compared with cells transfected with NC (P<0.05; Fig. 2B).

miR-19a-3p promotes PC3 cell proliferation. The EdU proliferation assay was conducted in PC3 cells that were transiently transfected with miR-19a-3p mimics. The results in Fig. 3 demonstrate that proliferation was significantly increased in cells transfected with the miR-19a-3p mimics compared with cells transfected with NC (P<0.05).

Table I. Clinicopathological characteristics of patients with PCa or BPH.

Characteristic	PCa	BPH
Patients, n (%)	22 (52.4)	20 (47.6)
Age at diagnosis		
Mean age \pm SD, years	68 \pm 5.8	68 \pm 7.1
\leq 65 years, n (%)	6 (27.3)	7 (35.0)
$>$ 65 years, n (%)	16 (72.7)	13 (65.0)
Mean serum PSA, ng/ml (range)	70.701 (0.002-1000)	5.769 (0.343- 17.420)
Stage, n (%)		
I-II	7 (31.8)	NA
II-IV	15 (68.2)	NA
Primary tumor stage, n (%)		
pT1-pT2	9 (40.9)	NA
pT3-pT4	13 (59.1)	NA
Lymph node metastasis, n (%)		
N0	18 (81.8)	NA
N1-N4	4 (12.8)	NA
Gleason grade, n (%)		
\geq 6	6 (27.3)	NA
7	11 (50.0)	NA
\leq 8	5 (22.7)	NA

PCa, prostate cancer; BPH, benign prostatic hyperplasia; PSA, prostate-specific antigen; SD, standard deviation; NA, not applicable.

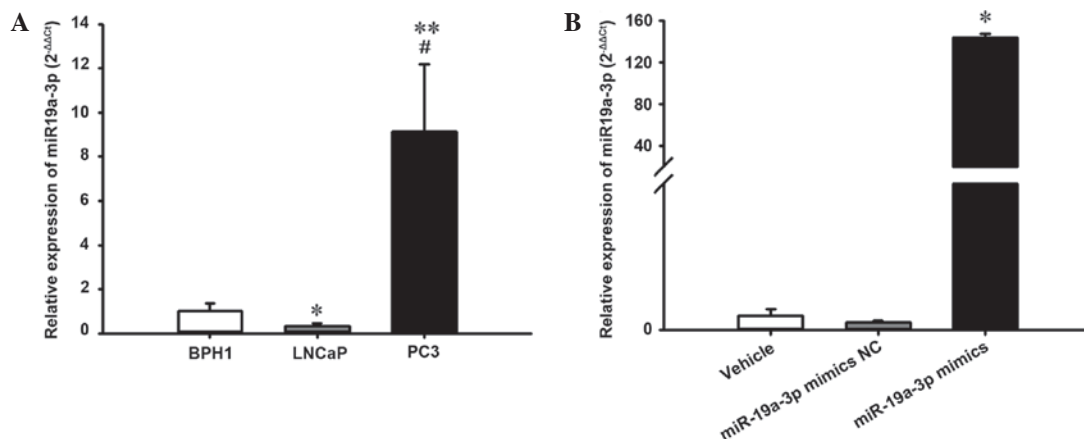


Figure 2. Upregulation of miR-19a-3p. (A) Expression of miR-19a-3p was higher in PC3 cells and reduced in LNCaP cells compared with the BPH1 cell line. * $P<0.05$, # $P<0.05$ vs. BPH1; ** $P<0.05$ vs. LNCaP. (B) Expression of miR-19a-3p was significantly increased in the miR-19a-3p mimic-transfected PC3 cells compared with the NC-transfected PC3 cells. * $P<0.05$ vs. miR-19a-3p mimics NC. The expression of miR-19a-3p in the three cell lines was measured using reverse transcription-quantitative polymerase chain reaction. Data are presented as the mean \pm standard error. Each sample was detected in triplicate. miR, microRNA; NC, negative control.

miR-19a-3p enhances PC3 cell migration and invasion. To evaluate the effect of miR-19a-3p overexpression on the migratory potential of PC3 cells, a Transwell migration assay was performed *in vitro*. The results presented in Fig. 4A and B reveal that miR-19a-3p overexpression increased the migration of PC3 cells compared with cells transfected with NC ($P<0.05$). Similarly, a Transwell invasion assay was performed to investigate the effect of miR-19a-3p on the invasive ability of PC3 cells. Compared with the NC cells, miR-19a-3p overexpression

resulted in an increase in the invasion of PC3 cells ($P<0.05$; Fig. 4C and D).

PMEPA1 is a direct target of miR-19a-3p. To explore the potential function of miR-19a-3p, the current study used bioinformatic analysis to predict putative miR-19a-3p targets. Targetscan, miRanda and picTar systems predicted that PMEPA1 was one of the putative targets of miR-19a-3p. Considering that PMEPA1 is highly expressed in the prostate

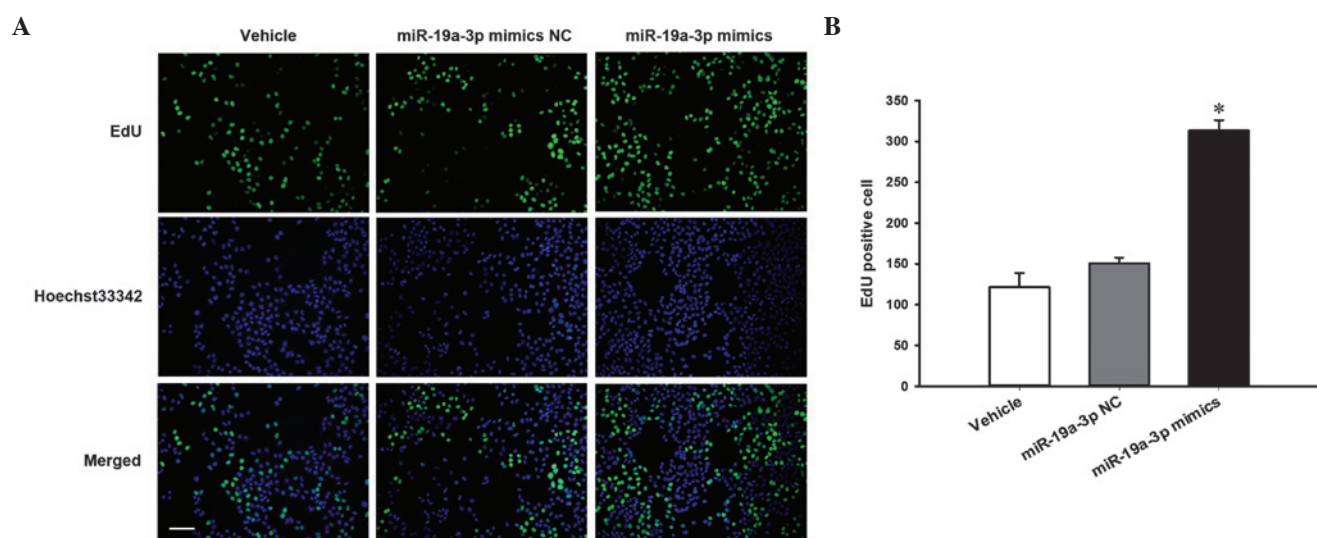


Figure 3. miR-19a-3p increased cell proliferation. (A) Representative EdU proliferation assay images of PC3 cells. Scale bar = 200 μ M. (B) Quantification of EdU-positive cell number indicated that miR-19a-3p mimic-transfected cells displayed increased proliferation compared with the NC group. * $P < 0.05$ vs. miR-19a-3p mimics NC. Data are presented as the mean \pm standard error. $n = 4$ per group. miR, micro RNA; NC, negative control; EdU, 5-ethynyl-2'-deoxyuridine.

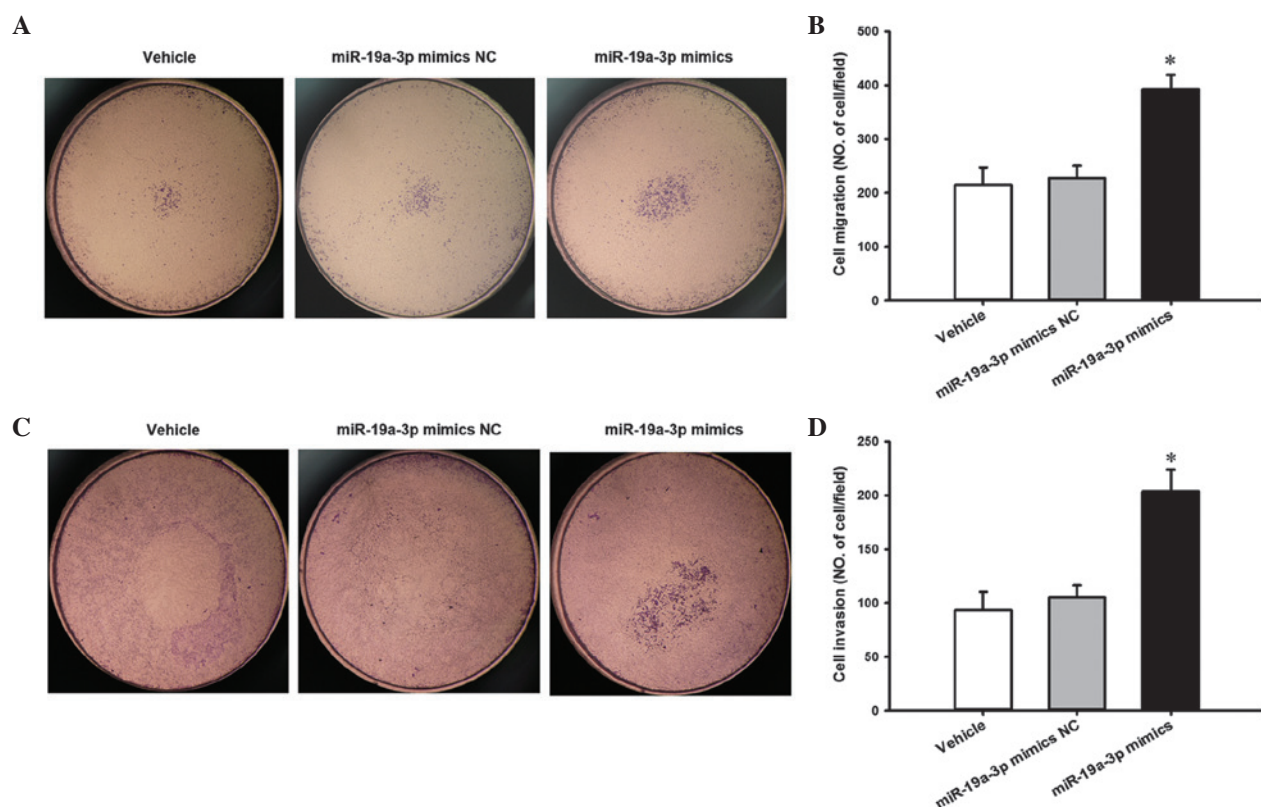


Figure 4. miR-19a-3p promotes cell migration and invasion. (A) Representative images of PC3 cells in a Transwell migration assay (without Matrigel) (magnification $\times 40$). (B) Quantification of cell number indicated that cells transfected with miR-19a-3p mimics exhibited enhanced cell migration compared with the NC group. (C) Representative images of PC3 cells in a Transwell invasion assay with Matrigel (magnification $\times 40$). (D) Quantification of cell number demonstrated that cells transfected with miR-19a-3p mimics were more invasive compared with the NC group. * $P < 0.05$ vs. miR-19a-3p mimics NC. Data are presented as the mean \pm standard error. $n = 4$ per group. miR, microRNA; NC, negative control.

and is involved in androgen receptor (AR)-mediated signaling, which is important during PCa development, the present study investigated the function of PMEPA1 in PCa progression. To verify whether miR-19a-3p directly targets PMEPA1 mRNA in PC3 cells, two wild-type (WT) PMEPA1 luciferase reporter constructs, PMEPA1-WT1 and PMEPA1-WT2, were generated,

containing a miR-19a-3p binding site at nucleotides 359-366 or 611-617 of the PMEPA1 3'-UTR, respectively (Fig. 5A). Two mutant (Mut) constructs were also generated in which the binding sites were mutated (Fig. 5B). As demonstrated in Fig. 5C, compared with PMEPA1-WT1 + NC transfection, luciferase activity was significantly repressed when miR-19a-3p

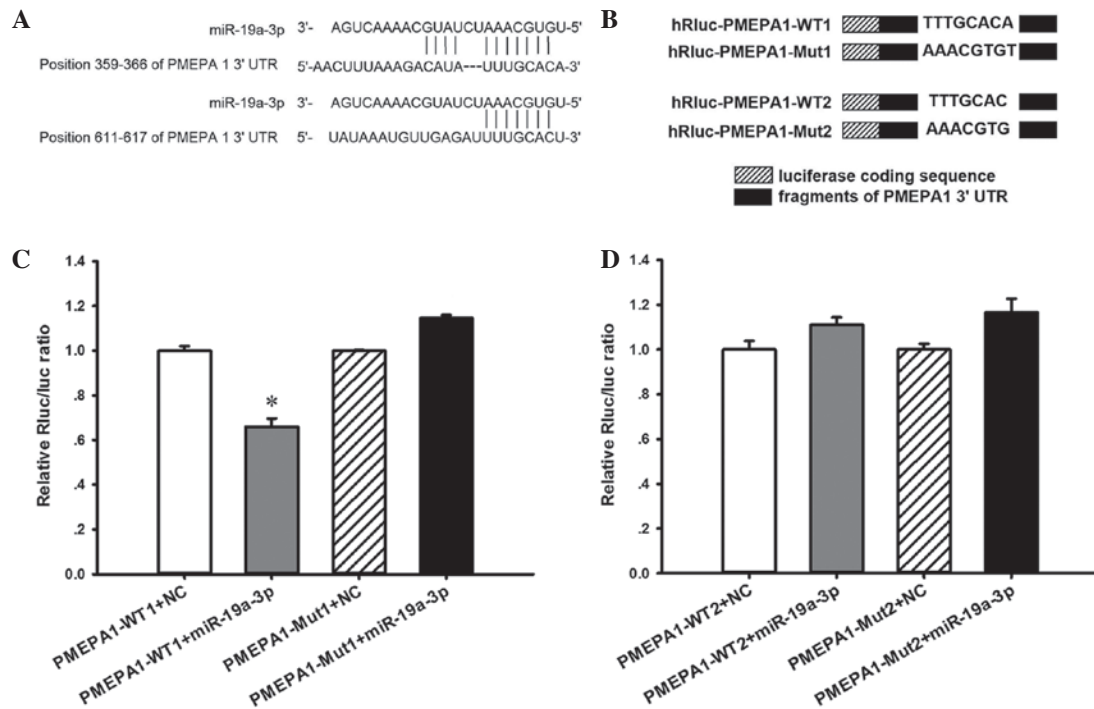


Figure 5. PMEPA1 is a direct target of miR-19a-3p. (A) Bioinformatic sequencing analysis indicated that miR-19a-3p potentially targeted the PMEPA1 3'-UTR at nucleotides 359-366 and 611-617. (B) Two PMEPA1 luciferase reporter constructs, PMEPA1-WT1 and PMEPA1-WT2, were generated, each containing a fragment harboring the miR-19a-3p target sites of the PMEPA1 3'-UTR. Mutations were also generated on the potential target sequence, resulting in two Mut constructs, PMEPA1-Mut1 and PMEPA1-Mut2. PC3 cells were co-transfected with miR-19a-3p mimic and the luciferase reporter constructs. (C and D) Luciferase activity was significantly repressed when miR-19a-3p mimics were co-transfected with PMEPA1-WT1, but not when co-transfected with PMEPA1-WT2. By contrast, co-transfection of miR-19a-3p mimics and PMEPA1-Mut1 abolished the inhibitory effects of miR-19a-3p, while co-transfection with miR-19a-3p mimics and PMEPA1-Mut2 did not. * $P < 0.05$ vs. PMEPA1-WT1 + NC. Data are presented as the mean \pm standard error. $n = 3$ per group. miR, microRNA; PMEPA1, prostate transmembrane protein androgen induced 1; UTR, untranslated region; WT, wild-type; Mut, mutant; Rluc, *Renilla* luciferase; NC, negative control.

mimics were co-transfected with PMEPA1-WT1 ($P < 0.05$). However, no effect was observed upon co-transfection with miR-19a-3p + PMEPA1-WT2 (Fig. 5D). By contrast, co-transfection of miR-19a-3p mimics + PMEPA1-Mut1 abolished the inhibitory effects of miR-19a-3p mimics + PMEPA1-WT1, whereas PMEPA1-Mut2 exhibited no effect.

To investigate whether PMEPA1 was repressed by miR-19a-3p overexpression, cell lysates prepared from cells treated with the miR-19a-3p mimics or inhibitor were analyzed by western blotting. The results indicate that the PMEPA1 expression level was reduced by miR-19a-3p mimics (Fig. 6A and B) and increased by miR-19a-3p inhibitors (Fig. 6C and D) compared with the NC transfection ($P < 0.05$).

Confirmation of PMEPA1 mediation of miR-19a-3p function. To clarify the importance of PMEPA1 in mediating miR-19a-3p functions, PMEPA1 siRNA was transfected into PC3 cells. Silencing of PMEPA1 expression was confirmed by western blot analysis ($P < 0.05$; Fig. 7A and B). Furthermore, the current study examined whether downregulation of PMEPA1 expression levels mimic the effect of miR-19a-3p overexpression. Cell proliferation was increased by PMEPA1 silencing compared with NC transfection ($P = 0.008$; Fig. 7C and D). Additionally, silencing of PMEPA1 increased the migration ($P = 0.003$; Fig. 8A and B) and invasion of PC3 cells compared with NC transfection ($P = 0.026$; Fig. 8C and D), similar to the effect of miR-19a-3p overexpression.

Discussion

The invasion and metastatic potential of PCa is highly significant, thus, various methods have been used to manage PCa; for example, PSA-based screening is increasingly used for PCa detection at the early, curable stage of the disease. However, as the prevalence of non-lethal, non-progressive PCa is high, screening leads to a substantial risk of overdiagnosis and overtreatment (16). Therefore, there is a great need for improved diagnostic and predictive biomarkers to further identify which patients with aggressive disease require treatment and which can be safely observed.

Dysregulation of numerous miRNAs have been demonstrated to influence cellular processes associated with prostate tumorigenesis, including cell proliferation, migration, invasion, apoptosis and the androgen signaling pathway. In PCa cells, the expression levels of the miR-17-92 cluster are upregulated; upregulated miR-17-92 transcripts bind and suppress E2F transcription factors 1-3, facilitating escape from apoptosis (17). Additionally, the expression level of miR-20a was demonstrated to be progressively increased in benign, low-malignant and high-grade malignant prostate tissue, respectively, supporting the oncogenic role of miR-20a in the carcinogenesis of PCa (18). Fuse *et al* (19) demonstrated that the downregulation of miR-145 leads to enhanced cell proliferation, migration and invasion through the direct downregulation of Fascin homolog 1 in the PC3 and

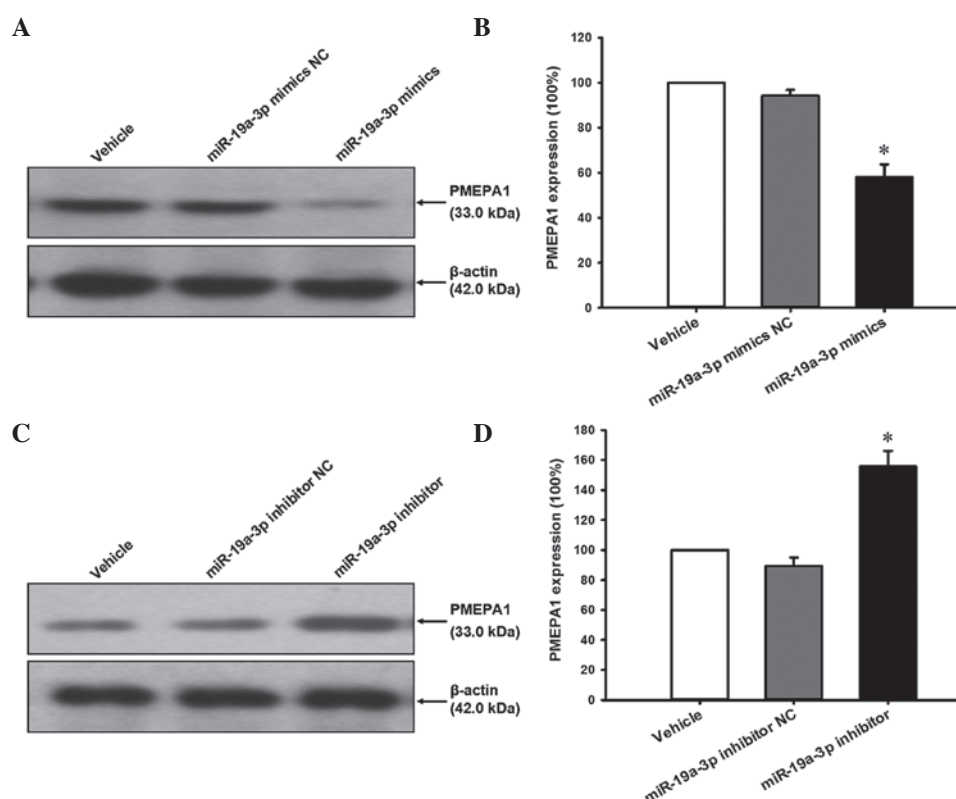


Figure 6. miR-19a-3p represses PMEPA1 expression. Representative blots and quantification of western blot analysis demonstrated the changes in PMEPA1 expression level following (A and B) miR-19a-3p mimics or (C and D) miR-19a-3p inhibitor transfection. * $P < 0.05$ vs. miR-19a-3p mimics/inhibitor NC. Data are presented as the mean \pm standard error. $n = 3$ per group. PMEPA1, prostate transmembrane protein androgen induced 1; miR, microRNA; NC, negative control.

DU145 cell lines. Also, the upregulation or downregulation of several miRNAs are associated with androgen signaling in PCa development. For example, Ribas *et al* (20) observed that transfection of LNCaP cells with a miR-21-expressing retrovirus stimulated androgen-dependent cell growth and rescued cells from androgen-deficient growth arrest (20). Overexpression of miR-221 and miR-222 was also demonstrated to attenuate androgen-induced growth in LNCaP cells and promote androgen-independent growth in LNCaP-derived castration-resistant PCa cells (21). These previous findings provide evidence of the importance of miRNAs in PCa development.

miR-19a-3p has been reported to be dysregulated in various types of cancer. Yang *et al* (10) reported that miR-19a-3p inhibited breast cancer progression and metastasis by inducing macrophage polarization through the downregulation of FOS-related antigen 1 expression levels. Furthermore, exposing MDA-MB-361 breast cancer cells to multi-fractionated radiation inhibited the expression of miR-19a-3p (22). In patients with astrocytoma, high expression of miR-19a-3p was demonstrated to be significantly associated with poor patient survival (12). Additionally, serum miR-19a-3p levels may act as a biomarker for the early diagnosis of colorectal adenocarcinoma (11). Although abnormal miR-19a-3p expression is frequently observed in various types of cancer, the importance of miR-19a-3p in the regulation of PCa remains unclear. Thus, the current study investigated the expression pattern and functions of miR-19a-3p in PCa specimens and cell lines. The results obtained in the current study confirmed the oncogenic effect of miR-19a-3p in PCa.

PMEPA1, also termed TMEPA1, STAG1, ERG1.2 or N4WBP4, is a subcellular protein localized in the cytoplasm. It was originally identified as an androgen-induced protein in LNCaP cells and is highly expressed in prostate epithelial cells. Functional analysis of PMEPA1 has revealed that it is a neural precursor cell expressed developmentally downregulated 4 E3 ubiquitin protein ligase binding protein and is important for AR downregulation through a negative feedback loop (23). Several studies have implicated PMEPA1 in the development of various types of human cancer, including PCa (24), renal cell carcinoma, stomach adenocarcinoma (25), and breast, ovarian (26) and colon cancer (27), due to gene amplification, mRNA expression alterations, and induction by androgens and other growth factors. Although it is known that PMEPA1 is highly expressed in the prostate in comparison with other tissues, the role and mechanism of PMEPA1 in PCa and other types of cancer are not well characterized. Liu *et al* (28) reported that the overexpression of PMEPA1 in PC3 AR-negative cells promotes progression through the cell cycle and increases cell proliferation via suppression of the SMAD 3/4-c-Myc-p21 signaling pathway. Additionally, Hirokawa *et al* (29) demonstrated that a high expression level of PMEPA1 can cause tumors to develop at an increased rate in nude mice injected with a PC3 subclone. However, cell proliferation is inhibited by PMEPA1 overexpression in PCa cell lines, suggesting that PMEPA1 downregulation is correlated with human PCa progression (23). Accordingly, in the present study, it was observed that when PMEPA1 was downregulated by siRNA, the proliferation, migration and invasion potential of PC3 cells were enhanced. These contradictory observations

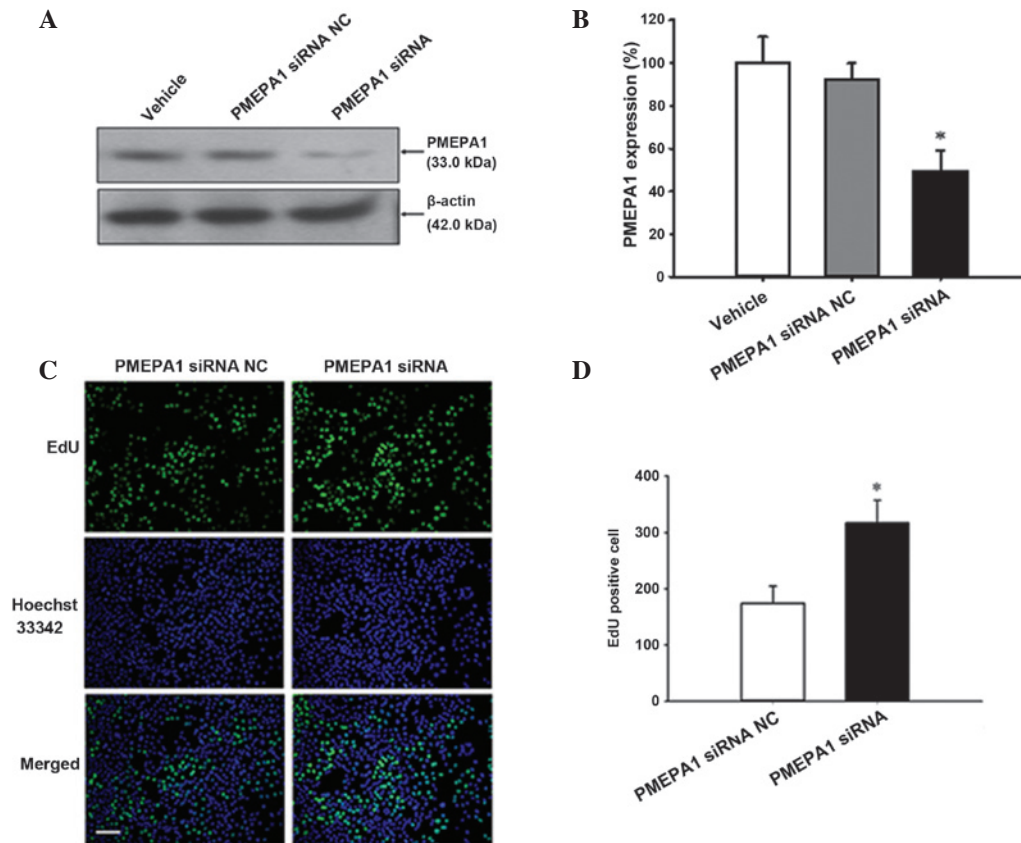


Figure 7. Silencing of PMEPA1 increases PC3 cell proliferation. (A) Representative blots and (B) quantification of western blot analysis demonstrated that PMEPA1 protein expression levels were reduced following transfection with PMEPA1 siRNA compared with the NC group. (C) Representative images of PC3 cells in EdU cell proliferation assay. Scale bar = 200 μ M. (D) Quantification of EdU-positive cell number indicated that PMEPA1 siRNA-transfected cells displayed increased proliferation compared with the NC group. * $P < 0.05$ vs. PMEPA1 siRNA NC. Data are presented as the mean \pm standard error. $n = 3$ per group. PMEPA1, prostate transmembrane protein androgen induced 1; siRNA, small interfering RNA; NC, negative control; EdU, 5-ethynyl-2'-deoxyuridine.

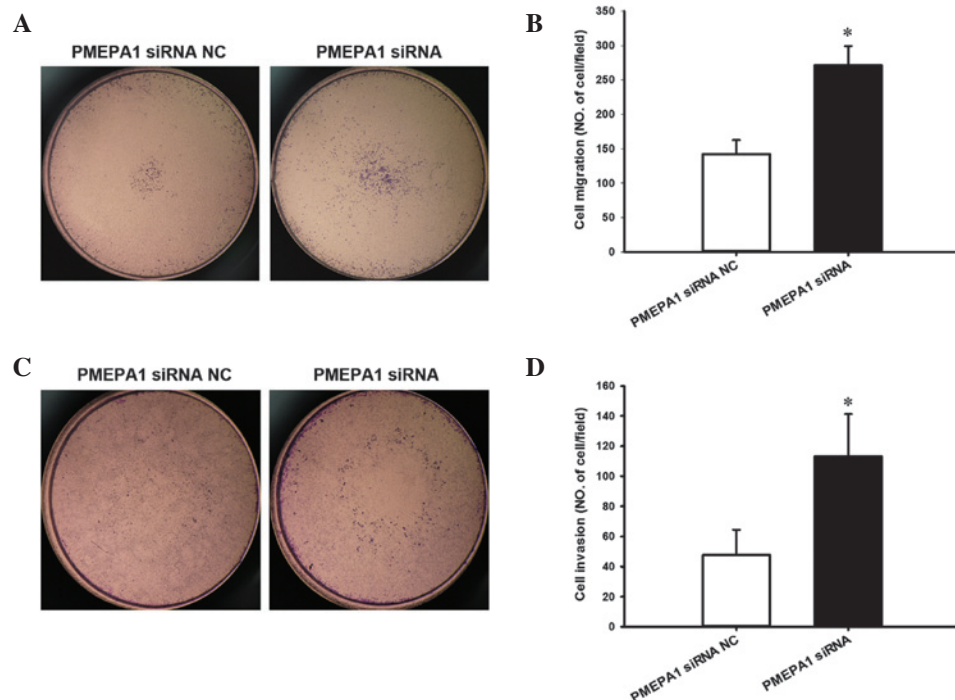


Figure 8. Silencing of PMEPA1 promotes PC3 cell migration and invasion. (A) Representative images of PC3 cell migration during Transwell assay. (B) Quantification of cell number indicated that transfection with PMEPA1 siRNA enhanced cell migration compared with the NC group. (C) Representative images of PC3 cell invasion during Transwell assay (magnification $\times 40$). (D) Quantification of cell number demonstrated that PC3 cell invasion was increased by transfection with PMEPA1 siRNA compared with the NC group. * $P < 0.05$ vs. PMEPA1 siRNA NC. Data are presented as the mean \pm standard error. $n = 3$ per group. PMEPA1, prostate transmembrane protein androgen induced 1; siRNA, small interfering RNA; NC, negative control.

suggest that PMEPA1 may not only be an oncogene in PCa, it may also act as a tumor suppressor, with its function dependent on which proteins it interacts with or the pathways it mediates.

In summary, the results of the present study elucidated the role of miR-19a-3p in PCa progression. The current study demonstrated that the levels of miR-19a-3p were upregulated *in vivo* and *in vitro* in PCa. Functional analysis also revealed that miR-19a-3p overexpression promotes the proliferation, migration and invasion of PCa cells. Additionally, PMEPA1 was identified as a direct target of miR-19a-3p and was demonstrated to partially mediate the effect of miR-19a-3p in tumor metastasis. Considering that PMEPA1 is an androgen-induced protein, further investigation is required to determine the regulatory mechanisms of miR-19a-3p and PMEPA1-associated androgen signaling, and their importance in mediating the development and progression of PCa.

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