#### **OncoTargets and Therapy**

#### ORIGINAL RESEARCH

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# **RETRACTED ARTICLE: LncRNA MCM3AP-ASI** Promotes Cell Proliferation and Invasion Through Regulating miR-543-3p/SLC39A10/PTEN Axis in **Prostate Cancer**

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Methods: qRT-PCR was conducted measure the expression levels of lncRNA 3-3p in PC MCM3AP-AS1 and miRtissue samples and cell lines. The expression levels of E-cadherin and SL 39A10 oteins were detected by Western blots. CCK-8 trans-wen test were used to evaluate the proliferation, invasion test, cell scratch ies **P** solls, respectively. Annexin V-FITC/PI experiments were and migration abi carried o detei e the status of apoptosis. Bioinformatics analysis and Luciferase were plore the relationship between lncRNA MCM3AP-AS1, miR-543ed to e ass and SI

Res In PCa tissue samples and cell lines, lncRNA MCM3AP-AS1 was up-regulated while he-543-3p was down-regulated. Over-expression of MCM3AP-AS1 could promote the poliferation and invasion of PCa cells. Correlation analysis showed that the pression of MCM3AP-AS1 and miR-543-3p was significantly and inversely correlated. We rther verified that miR-543-3p inhibitor was able to reverse si-MCM3AP-AS1mediated inhibitory effects on the PCa cell proliferation, migration and invasion through regulating the downstream protein axis SLC39A10/PTEN/Akt. Finally, in vivo experiments indicated that knocking down of MCM3AP-AS1 could largely reduce tumor volumes, and decreased the ratio of Ki67-positive cells and the expression of SLC39A10 in tumor samples.

**Conclusion:** LncRNA MCM3AP-AS1 can promote the proliferation, migration and invasion abilities of PCa cells through regulating the miR-543-3p/SLC39A10/PTEN axis, which suggests that lncRNA MCM3AP-AS1 might be a potential target for prostate cancer therapy. Keywords: lncRNA MCM3AP-AS1, miR-543-3p, SLC39A10, PTEN, prostate cancer

## Introduction

Prostate cancer (PCa) is the one of most common heterogeneous tumors occurring among males worldwide.<sup>1</sup> Also, PCa is the second most fatal malignant disease in men, of which the incidence increases with age.<sup>2</sup> Early-stage PCa patients can usually benefit from tumor resection and radiotherapy.<sup>3</sup> However,

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the 5-year survival rate and life quality of other patients at late-stage PCa are markedly affected.<sup>4</sup> Thus, carrying out in-depth research on PCa is of great significance to the health and survival rate. Previous studies have established that the occurrence of PCa is largely related with the dysregulation of multiple molecules, including proteins, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs).<sup>5</sup>

In recent years, extensive studies have investigated the functions of non-coding RNAs.<sup>6</sup> It is reported that non-coding RNAs are a class of RNA molecules that do not encode proteins and can regulate gene expression at different levels.7,8 Among non-coding RNAs, lncRNA transcripts are over 200 nucleotides in length and accounting for 98% of total RNAs.<sup>9</sup> However, biological functions of most lncRNAs are complex, which are involved in various regulatory processes (eg, genomic imprinting, chromatin modification and intranuclear transport).<sup>10</sup> Moreover, it has been discovered that some lncRNAs play important roles in the occurrence and development of PCa, such as PCAT29.<sup>11</sup> GAS5.<sup>12</sup> PCGEM113 and MCM3AP. LncRNA MCM3AP is located on human chromosome 21q22.3 and regulates protein expression in various human diseases. Besid IncRNA MCM3AP-AS1 is an RNA molecule with th reverse sequence of MCM3AP gene.<sup>14</sup> Or study reported that the expression of lncRNA M M3AL AS1 was elevated in glioma microvascular e othelia 00110 and knocking down MCM3AP-AS1\_ould press the cells.<sup>15</sup> viability and migration of oblastoma Furthermore, MCM3AP-AS1 as repried to facilitate PCa development in no and k ck-down of MCM3AP-AS1 was a e to inhibit the proliferation, invasion and migration of Pfr. cells via methylation of DNMT1/DNMT2<sup>16</sup> Sin arly, *j* our preliminary e expression of lncRNA experiments, e found that dysregulated in PCa cells. MCM3AP S1 exploration of the functions of However, de MCM3AP-AS1 the underlying regulatory mechanism are urgently needed.

MicroRNAs (miRNAs), a group of non-coding RNAs that are 19–24 nt in length, play regulatory roles in the process of basic metabolism, differentiation, proliferation, survival and cell death.<sup>17</sup> MiR-543-3p functions as a tumor growth inhibitory effector in lung cancer cells and pancreatic cancer cells.<sup>18</sup> Our previous bioinformatics experiments revealed that lncRNA MCM3AP-AS1 might bind with miR-543-3p, and miR-

543 could bind with its downstream regulatory factor SLC39A10, which is a zinc transporter essential for cell survival in PCa.<sup>19</sup> However, the roles that MCM3AP-AS1/miR-543-3p/SLC39A10 axis play in PCa are elusive. Therefore, our study focuses on the interactions among lncRNA MCM3AP-AS1, miR-543-3p and SLC39A10, as well as possible downstream signaling pathways involved in PCa. Our findings may provide a new therapeutic target for PCa.

# Methods

#### **Tissue Samples**

Sixty-four pairs of PCa tissue samples and adjacent normal tissues were collected from the First offiliated Hospital and College of Clinical Medicine of Hence University of Science and Technologic Jacween March 2016 and June 2018. The study was apprived by the Ethics Committees of the First Affiliated Hospital and College of Clinical Medicine of Henan University of Science and Technology, and was called out in accordance with the principles of the Declaration of Helsinki. All patients sign I the written informed consent. Tissue samples were stred at ±0°C before use.

#### Cells and Transfection

Human PCa cell lines (PC-3, DU145, 22RV1, LNCaP) and ormal follicular epithelial cell line (WPMY-1) were provided by ATCC, USA. Cells were incubated in RPMI 1640 with 10% FBS at 37°C. Control siRNA (si-NC), si-MCM3AP-AS1, miR-543-3p mimics, miR-543-3p inhibitor, pcDNA3.1, pcDNA3.1- MCM3AP-AS1, control miRNA, lentivirus-sh-MCM3AP-AS1 (LV-sh-MCM3AP-AS1) and control lentivirus-sh (LV-shRNA-NC) were made by GenePharma, China, and transfected to cells using Lipofectamine 2000 (Invitrogen, USA).

#### **BLAST**

Alignment searches were conducted with NCBI's BLAST suite. Top result with value less than 0.01 was recorded. Through analysis, we selected miR-543-3p, which was highly bound to MCM3AP-AS1, for subsequent experiments.

#### qRT-PCR

The miRNeasy Mini Kit (Qiagen, USA) was used to extract RNAs from tissue samples and cells. The concentration and quality of RNAs were evaluated by

NanoDrop 2000 (Thermo Fisher, USA). First-strand cDNA was synthesized by SuperMix (TransGen, China) following the manufacturer's instructions. RT-qPCR analysis was conducted by SYBR SuperMix (Applied Biosystems, USA). The conditions were: 40 cycles at 55°C for 10 min, 95°C for 30s, 55-59°C for 30 s, and 72°C for 42 s. The fold changes were calculated by  $2^{-\Delta\Delta Ct}$  method. The expression of MCM3AP-AS1 and miR-543-3p was normalized to GAPDH and U6, respectively. Primer sequences were as follows: MCM3AP-AS1, 5'-TGGGATTCAGACGCTAACGC-3' (forward) and 5'-TCCACAGCATCTTTGGCACC-3' (reverse): miR-543-3p, 5'-ATGCCTCGACCACAATCAGA-3' (forward) and 5'- AGGATGCCGGTACTACTCGAT-3' (reverse); GAPDH, 5'-TCGACAGTCAGCCGCATCT TCTTT-3' (forward) and 5'-ACCAAATCCGTTGACTC CGACCTT-3' (reverse); U6. 5'-GCTTCGGCAG CACATATACTAAAAT-3' (forward) and 5'-CGCT TCACGAATTTGCGTGTCAT-3' (reverse).

#### RNA Pull-Down Assay

For miRNA pull-down assay, PC-3 and LNCaP cells were transfected with biotinylated miR-543-3p 543-3p probe) or control probe (Genescript, Nat 'ng, China) and harvested in lysis buffer (20 Tris 7.5, 100 mM KCl, 5 mM MgCl2, 0.5% AP-40 nd 1 A Tota ul Recombinant RNAse inhibitor TaKaP RNAs were pretreated with D asel then heated at 65°C for 5 min, follow, by an inst. ice bath. Afterwards, the RNAs were included with streptavidin-coated magnetic leads (New gland BioLabs, S1420S) at 4°C f 4 h. After incubation, beads were I s buffer and RNAs were washed twice w Trizo (Invit gen, CA, USA). The extracted n of LRNA M3AP-AS1 was detected expressi by RT-CR.

#### Luciferase porter Assays

MCM3AP-AS1 cDNA with miRNA were amplified and cloned into pmirGLO plasmids (Promega, USA) to construct pmirGLO-MCM3AP-AS1. MiR-543-3p mimics or control mi-RNA were co-transfected with pmirGLO-MCM3AP-AS1 or mutant pmirGLO-MCM3AP-AS1 (pmirGLO-MCM3AP-AS1-MUT) into PC-3 or LNCaP cells by Lipofectamine 2000 (Invitrogen). After transfection for 48 h, luciferase activities were detected through Dual-Luciferase Assay.

# RNA Binding Protein Immunoprecipitation Assay

The interaction between miR-543-3p and SLC39A10 using RIP kit (Merck Millipore was assessed Darmstadt, Germany). The cells were washed with precooled PBS and the supernatant was discarded. Cells were then lysed using equal volume of RIPA lysis in ice bath for 5 min and centrifuged at  $2000 \times g$  for at 4°C 10 min to collect the supernatant. Subsequently, the supernatant was incubated with an SLC39A10 antibody for co-precipitation. Specifically magnetic beads of each co-precipitation system were we hed with cold PBS, and resuspended in 100 L RIP Warn Buffer, and incubated with 5 µg \$ <39A10 tibody (1:50) and IgG (1:100) separately text, the bead-attody complex was rinsed and resupported 900 µLRIP Wash Buffer, and incubated w a 100 µL II extract at 4°C overnight. The samples ere hen placed the bead pedestal to collect the complex of ead protein. After the samples were area with proteas K, RNAs were extracted for RT-PCR.

## C Presideration Assay

Cell proliferation rate was detected by Cell Counting Kit-8 (CCL-8; Dojindo, JPN). PC-3 and LNCaP cells were transfected with si-NC, si-MCM3AP-AS1, miR-543-3p inhibitor or si-MCM3AP-AS1 + miR-543-3p inhibitor, harvested and seeded to 96-well plates ( $1 \times 10^3$  cells/well). After 24, 48 72 or 96 h, 10 µL of CCK-8 assay reagent was added to each well, and cells were incubated for 2 h and an enzyme immunoassay analysis (Bio-rad, Hercules, CA, USA) was performed. For cell colony formation assay, cells (500 cells/well) were plated in 6-well plates and incubated in DMEM with 10% of bovine calf serum at 37°C. After two weeks, cells were fixed and stained with 0.1% of crystal violet. Finally, the number of visible colonies was manually counted.

# Wound Healing Assays and Trans-Well Assays

In wound healing assay, cells were seeded in 6-well plate. The culture medium was removed and cells were scraped by a pipette tip. After incubation for 24 h, the wound widths were measured and calculated as percentage. In trans-well assay, chambers were placed with Matrigel. After incubation, cells on the upper chamber were taken off by cotton. The remaining cells were fixed and stained by violet crystalline.

## Western Blots

Cells and tissues were lysed in ice-cold RIPA lysis buffer (Sigma, USA). (Sigma, USA). Cell or tissue lysates were centrifuged and the concentration of proteins was determined by BCA assay. Total proteins were separated by SDS-PAGE and then transferred to PVDF membranes (Millipore, USA). The membranes were blocked by skim milk and incubated with anti-SLC39A10 (1:1000, Abcam) or anti-GADPH (1:1000, Abcam). After primary antibody incubation for overnight, membranes were incubated with secondary goat anti-rabbit IgG (Abcam) at room temperature for 1 h. Signals were detected by ECL.

## Cell Cycle Analysis by Flow Cytometry

Cells were transfected with the indicated siRNA for 72 h, washed with PBS, fixed in 50% ethanol, incubated with RNAase cocktail (Ambion) for 30 min, and stained with propidium iodide (100  $\mu$ g/mL). The stained cells were subjected to flow cytometry.

## Flow Cytometric Analysis of Cell Apoptosis

Apoptotic rate was examined using Annexin V-FIT apoptosis detection kit (Beyotime Biotechnology China) following the manufacturer's instructions. Brindy, the PCa cells were washed twice with PBS, are charves d by Trypsin without EDTA. Afterwards, the centered resuspended in 195  $\mu$ L binding buffer, but L Annexe V-FITC regent and 10  $\mu$ L propidium ion de (to regent, and incubated for 15 min at room temperature in to dark. The cell apoptosis was detected by flow cytometry (BD FACSCalibur cytometre Becom Dickinson, San Jose, CA, USA).

# Lentiving Constructions

Lentiviral vector of MCM3AP-AS1-shRNA was made by Genechem, China, MCM3AP-AS1-siRNA was sub-cloned to pFU-GW-RNAi vector. PC-3 cells were seeded into 6-well plates. After incubation at 37°C with 5% CO2 for 12 h, PC-3 cells were infected with LV-siRNA-NC or LVsiRNA-MCM3AP-AS1.

## In vivo Experiments

In vivo experiments were approved by the Institutional Animal Care and Use Committee of the First Affiliated Hospital and College of Clinical Medicine of Henan University of Science and Technology, and all procedures were in accordance with Council Directive 2010/63 of the European Union. Cells infected with LV-si-MCM3AP-AS1 or LV-si-NC were administered into 6-week-old BALB/c mice. Tumor growth and tumor volume were measured every 3 d.

#### Immunohistochemistry

Tumor tissues were dried, dewaxed and rehydrated. After washing with PBS, tissues were blecked and incubated with Ki67 antibody (Bioss, 1:201 at 4°C overlight. And then, the tissues were treated with K P DAB (Z GB-BIO, China) at 25°C for 20 cm followed by staning with hematoxylin.

## Statistical plysis

The data were expressed as mean  $\pm$  SD. Student's *t*-test (two trans was used for comparisons between two groups. One-way analysis of variance (ANOVA) was used for multi-group comparisons followed by Bonnotoni postone analysis. The correlation of the measured data was analyzed by Pearson correlation analysis of P value less than 0.05 was regarded as statis-acally significant.

## Results

# LncRNA MCM3AP-ASI was Upregulated in Patients with PCa

Tumor tissue samples and adjacent healthy tissue samples from 64 PCa patients were selected for comparison. It was shown that the expression levels of IncRNA MCM3AP-AS1 were markedly increased in PCa patient samples compared to that in healthy tissue samples (Figure 1A). Based on the average expression levels of lncRNA MCM3AP-AS1, all patients were divided into high expression group and low expression group (Figure 1B). Long-term follow-up showed that the long-term survival rate of the high expression group was markedly decreased than that of the low expression group (Figure 1C). In our further analysis, the baseline data suggested that there were great differences between the two groups in Gleason scores for N staging (Table 1). Cox regression analysis also showed similar results (Table 2).



Figure I LncRNA MCM3AP-ASI markedly upregulated in patients with PCa. (A) Lnch A MCM3AP-AS was highly expressed in PCa patient tumor tissues than that in normal tissues. (B) Histogram of PCa with high LncRNA MCM3AP-ASI expression group and low Lnch A MCM3AP-ASI expression group. A total of 37 cases were subgrouped into low LncRNA MCM3AP-ASI expression group, and 27 of the subgrouped into low LncRNA MCM3AP-ASI expression group, and 27 of the subgrouped into low LncRNA MCM3AP-ASI expression group, and 27 of the subgrouped into low LncRNA MCM3AP-ASI expression group and low LncRNA MCM3AP-ASI expression group. A total of 37 cases were subgrouped into low LncRNA MCM3AP-ASI expression group, and 27 of the subgrouped into low LncRNA MCM3AP-ASI expression group and low LncRNA MCM3AP-ASI expression group. A total of 37 cases were subgrouped into low LncRNA MCM3AP-ASI expression group and low LncRNA MCM3AP-ASI expression group. Patients with high lncRNA MCM3AP-ASI expression had poorer survival. \*p < 0.05.

# Knockdown of IncRNA MCM3AP ASL Markedly Inhibited Turror Proceration and Metastasis

In PCa cell lines (PC-2 DU145, RV1 and LNCaP), the expression level of lncRNA MC 3AP-AS1 were significantly increased empared to that in normal al cells PMY-1 (Figure 2A). human prostate epi. PC-3 and LNC cell, we selected for subsequent experiments be use they had the greatest increase. s were designed for knockdown of Three IncRNA MC (3AP-AS1, and si-MCM3AP-AS1-1 had the highest known efficiency (Figure 2B). In addition, si-MCM3AP-AS1 could inhibit tumor cell proliferation which was detected by CCK-8 assay (Figure 2C) and colony formation assay (Figure 2D), and migration was evaluated by wound healing assay (Figure 2E) and invasion was determined by transwell assay (Figure 2F).

## MiR-543-3p is a Regulatory Target for IncRNA MCM3AP-ASI

Bioinformatics analysis showed that lncRNA MCM3AP-AS1 had a binding site with miR-543-3p (Figure 3A). To validate that miR-543-3p suppressed the expression of MCM3AP-AS1 by directly binding to the 3'-UTR of MCM3AP-AS1, RNA pull down assay and luciferase reporter assay were conducted. Firstly, a biotin-avidin pull-down assay was conducted to evaluate the direct binding of miR-543-3p to MCM3AP-AS1. After transfection with miR-543-3p probe into PC-3 or LNCaP cells for 48 h, streptavidin-coated magnetic beads were used to pull down biotinylated miR-543-3p and the expression of co-precipitated lncRNA MCM3AP-AS1 was measured by RT-qPCR. LncRNA MCM3AP-AS1 could only be precipitated by miR-543-3p probe and was undetectable in the product precipitated by control probe (Figure 3B), indicating that miR-543-3p could

Variables	Low IncRNA MCM3AP-ASI	High IncRNA MCM3AP-ASI	P value
Age (yrs)	57.8±7.6	56.4±8.8	0.814
Tumor TNM stage			0.018
    	17 (53.1%) 10 (31.3%) 5 (15.6%)	7 (21.9%) 16 (50.0%) 9 (28.1%)	
T stage	0.341		
TI T2 T3 T4	9 (28.1%) 19 (59.3%) 3 (9.4%) 1 (3.1%)	7 (21.9%) 18 (56.3%) 5 (15.6%) 2 (6.3%)	
N stage	0.019		
N0 N1 N2	22 (68.8%) 8 (25.0%) 2 (6.3%)	12 (37.5%) 16 (50.0%) 4 (12.5%)	
Gleason score			0.027
2–5 6–8 9–10	15 (46.9%) 13 (40.6%) 4 (12.5%)	10 (31.3%) 17 (53.1%) 5 (15.6%)	

 Table
 I
 Correlation
 Between
 IncRNA
 MCM3AP-ASI

 Expressions and Prostate Cancer Patients

directly interact with lncRNA MCM3AP-A7. IN PC-3 and LNCaP cells.

To evaluate whether miR-543-3p can a ctl olliu IncRNA MCM3AP-AS1, two lucierase reputer plasmids containing MCM3AP-AS c. IA or Mc 3AP-ASI mutant cDNA were constructed and co-transfected with miR-543-3p mimic or miR-NC min. s into PC-3 or LNCaP cells for 48 d. As expected, overexpression of miR-543-3p induced a mately 0% reduction in PC and 60% reduction tivity luciferase repr J. To. inpared to cells the sfected with the control in LNCaP

Table 2 Cox Mult	riate Regression Analysis
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Factors	P value	HR	95% CI
LncRNA MCM3AP-ASI expression	0.023	1.538	1.050–2.274
Age	0.212	1.765	0.603-4.656
TNM stage	0.008	1.652	1.108-3.052
T stage	0.437	1.324	0.587–2.832
N stage	0.042	1.572	0.999–2.384
Gleason Score ≥6	0.032	1.427	0.875–2.547

Abbreviations:	HR,	hazard	ratio;	CI,	confidence	interval	
				- /			

mimic (Figure 3C), whereas miR-543-3p did not affect the mutated reporter activity (Figure 3C)

In order to determine whether miR-543-3p can affect the transcription of *MCM3AP-AS1*, RT-qPCR was used to evaluate the expression levels of lncRNA MCM3AP-AS1 in PCa cells transfected with control mi-RNA, miR-543-3p mimics or miR-543-3p inhibitor. The results showed that miR-543-3p mimics could decrease *MCM3AP-AS1* transcription to lncRNA MCM3AP-AS1, while miR-543-3p inhibitor reversed this effect (Figure 3D). In addition, knockdown of *MCM3AP-AS1* strongly indiced the expression of miR-543-3p in PCa cells (Ligure 3E) It was also found that the expression of *MCA3AP-AS1* and miR-543-3p were inversely correlated Figure F).

According to bioin frmatics analysis miR-543-3p might bind to SLC39A. 9 proton (Figure 3G). RIP assay results suggested dat min 43-3p could directly interact with SLC39A. Figure 3H), od af-qPCR results exhibited a significant reduction in the expression of SLC39A10 by knowledge of MCR3AP-AS1 (Figure 3I). The correlation between MCM3AP-AS1 and SLC39A10 was also analyzed and a bisitive correlation with each other was observed (Figure 3J).

## PNA MCM3AP-ASI Regulates the roliferation and Migration of PCa Cells Through Targeting miR-543-3p

Further cellular experiments discovered the inhibitory effects of si-MCM3AP-AS1 on tumor cell proliferation by CCK-8 assay (Figure 4A) and colony formation assay (Figure 4B), migration by cell scratches (Figure 4C), and invasion by Trans-well experiment (Figure 4D). However, those inhibitory effects could be reversed by 543-3p-inhibitor. Interestingly, the same was true for the expression of SLC39A10, and the regulatory signaling pathway involved in this mechanism might be PTEN/Akt (Figure 4E). On the other hand, MCM3AP-AS1 was overexpressed to detect the above-mentioned PCa cell behaviors. The results showed that overexpression of MCM3AP-AS1 significantly promoted PCa cell proliferation (Supplementary Figure A), migration (Supplementary Figure B) and invasion (Supplementary Figure C).

## Si-MCM3AP-AS1 Affects Cell Cycle but Not Cell Apoptosis

To further clarify the function of lncRNA MCM3AP-AS1 /miR-543-3p in PCa cells, si-MCM3AP-AS1 or/and 543-



Figure 2 Knockdown of IncRNA MCM3AP-ASL sig oliferation and metastasis. (A) The expression of MCM3AP-ASI in PC-3, DUI45, intly two types of PCa cell lines with the highest expression levels of MCM3AP-ASI. (B) The expression LNCa 22RVI, LNCaP and WPMY-I cell lines. PC-3 of MCM3AP-ASI-I in PC-3 and LNCaP wi the transfect of control (untransfected cells), si-NC, si-MCM3AP-ASI-I, si-MCM3AP-ASI-2 and si-MCM3AP-ASI -3. si-MCM3AP-ASI-I had the highest own efficiency d then was used to conduct the subsequent experiments. (C) CCK-8 assay was carried out to NC or si-MCAP-ASI. Knocking-down of si-MCM3AP-ASI significantly inhibit the proliferation of both PC-3 on wit detect cell viability under the transfe and LNCaP. (D) Colony formatic assay of PCa us was conducted under the transfection of si-NC or si-MCM3AP-ASI. The number of colonies was largely r si-MCM3AP-AS pmpared to si-NC. (E) Cell scratch tests applied for the evaluation of cell migration ability under si-NC or decreased after the transfection si-MCM3AP-ASI transfection after 24 h of culture. Compared to si-NC, si-MCM3AP-ASIE markedly prohibited cell migration. (F) Trans-well experiments were ander the transfection with si-NC or si-MCM3AP-ASI. The number of invasive cells in si-MCM3AP-ASI transfection group conducted to assess cell asion abili was much smaller than t in sigroup. \*p < 0.05.

3p-inhibitor were transfected/co-transfected into PC-3 or LNCaP. 1997, we investigated the influence of silencing of lncRNA 1993AP-AS1 or miR-543-3p inhibition on the cell cycle of nove two types of PCa cells according to the cell cycle stage distribution (G1, S, G2/M). As shown in Figure 5A, there was an upregulation of the cell percentage at G1 phase and less S phase-arrested cells were detected, suggesting that suppression of MCM3AP-AS1 arrested cell cycle at G1 phase. Secondly, in order to verify whether lncRNA MCM3AP-AS1 has any effects on PCa cell apoptosis, Annexin V-FITC/PI test and flow cytometry were conducted. The proportions of apoptotic cells in 543–

3p-inhibitor group and si-MCM3AP-AS1+543-3pinhibitor group were not markedly different from that in the control group (Figure 5B), indicating that lncRNA MCM3AP-AS1 did not affect PCa cell apoptosis.

## Knocking Down IncRNA MCM3AP-ASI Can Reduce Tumor Volume of PCa and the Expression of SLC39A10

The LV-sh-Control and LV-sh-MCM3AP-AS1 were constructed, and then intratumorally injected into mice. After 8 weeks, mice were sacrificed, and tumors



Figure 3 MiR-543-3p was a regulatory target for IncRNA MCM3 Bioinfor tics analysis showed that IncRNA MCM3AP-ASI had a binding site with miR-543-3p AST. but not MUT construct. (B) RNA pull-down was conducted to etect the eraction L veen IncRNA MCM3AP-ASI and RNA level of miR-545-3p could be measured by cobe. (C) In luciferase experiments, 543–3p-inhibitor was discovered to markedly reduce RT-qPCR after immunoprecipitation by miR-545-3p probe t not con . (D) RT-qPCR was applied to detect the expression levels of MCM3AP-ASI in PC-3 luciferase activities around WT IncRNA MCM3AP-ASI ut no oct ane Mu and miR-: p inhibitor. Overexpression of miR-543-3p significantly decreased the expression levels of MCM3APand LNCaP with the transfection of miR-543-3p min effect. (E) RT-ASI. However, miR-543-3p inhibitor had the opp R was also conducted to evaluate the expression of miR-543-3p in both PC-3 and LNCaP with the transfection of si-NC and si- MCM3AP-ASI ppi ing MCM3AP-A promoted the expression of MCM3AP-ASI in both cell lines. (F) LncRNA MCM3AP-ASI is negatively correlated with the expression of MR-543-3 tumor samples. (G) MiR-543-3p has a binding site with SLC39A10, which was found by bioinformatics analysis. (H) RNA binding protein immunopreci ation assay was to detect the interaction of miR-543-3p and SLC39A10. IgG and anti- MCM3AP-AS1 were applied for immunoprecipitating SLC39A10 prot and then miR-543-3p ression was detected by RT-qPCR. (I) The expression levels of SLC39A10 were determined by RT-qPCR in n of si-NC nd si-MCM3AP-ASI. Knockdown of MCM3AP-ASI significantly decreased the expression levels of SLC39A10 in both types PC-3 and LNCaP with the transfe on MCM<sup>2</sup> -ASI and SLC39A10 was also analyzed and a positive correlation with each other was observed. p < 0.05; p < 0.001. of cells. (J) The correlation bet

mor samples, it was were remove 5). Fr (n = es of transplanted tumors in LV-sh found that we volu nodel were much lower than that in -MCM3AP-A negative control roup (LV-siRNA-NC) (Figure 6A). Next, those tumor samples were used to test for the expression of lncRNA MCM3AP-AS1 and miR-543-3p, which showed that the expression levels of IncRNA MCM3AP-AS1 were decreased (Figure 6B), and the expression levels of miR-543-3p were promoted in LV-sh-MCM3AP-AS1 group (Figure 6C). Furthermore, LV-sh-MCM3AP-AS1 could induce

decreases in both the expression of Ki67 and SLC39A10 (Figure 6D and E).

#### Discussion

It has been well established that lncRNA MCM3AP-AS1 plays important roles in the regulation of gene expression, as well as the tumorigenesis and tumor development in hepatocellular carcinoma,<sup>20</sup> glioblastoma,<sup>15</sup> and thyroid cancer.<sup>21</sup> LncRNA MCM3AP-AS1 can affect apoptosis, signaling pathways, tumor metastasis and invasion, which has





provided a hay class of clinical early diagnosis and prognostic tool. Our study investigated the regulation roles of lncRNA MCM3AP-AS1 in PCa. Firstly, we found that the expression levels of lncRNA MCM3AP-AS1 significantly increased in PCa patient tissue samples compared to that in healthy tissue samples. Secondly, the long-term survival rate of patients with high expression levels of lncRNA MCM3AP-AS1

markedly decreased than that of patients with low expression levels of MCM3AP-AS1, which might provide new insights into the diagnosis and prognosis of patients with PCa.

The expression of lncRNA MCM3AP-AS1 was elevated in lung cancer cells, and accelerated angiogenesis and progression in lung cancer.<sup>22</sup> In our study, we firstly carried out comparison experiments in PCa cell lines to



Figure 5 LncRNA MCM3AP-ASI and miR-543-3p did not affect apoptosis. (A) The percentee of apoptotic of twas evaluated by flow cytometry after the transfection with si-NC or si-MCM3AP-ASI or/and 543–3p-inhibitor for 24 h. There was no any significate in alterations of poptotic cells percentage among those groups. (B) The roles of silencing of lncRNA MCM3AP-ASI or/and miR-543-3p inhibition in the cell cycle of Percentage LncRP or swere assessed by flow cytometry according to the cell cycle stage distribution (GI, S, G2/M). Si-MCM3AP-ASI could induce cell to be aposted on GI photo S43–3p-inhibitor force more cells to roll into S phase. \*p < 0.05 compared to si-NC in GI phase; p < 0.05 compared to si-NC in S phase.

investigate the expression levels of lncRNA 3AP-AS1 in vitro. It was obvious that the expression ion lev ls of IncRNA MCM3AP-AS1 significantly inc ased cell lines than that in normal hum 1 prosta epithelial cells. Our study is the first RNA port that MCM3AP-AS1 was dysregulated in Ca tissues and cells, and its knockdow could signific suppress tlv tumor metastasis.

Previous studies indicated that lncRNA vith R-211.<sup>15</sup> miR-340-MCM3AP-AS1 bina 5p.<sup>20</sup> 1R-194  $5p^{22}$  and interactions between lncRNA M3 nd these miRNAs exert important bio vical functions in different tumor v, we report that miR-543-3p is types. In this st a regulatory target of lncRNA MCM3AP-AS1 in PCa. Specifically, lncRNA MCM3AP-AS1 can bind with miR-543-3p and there is a negative correlation between them.

Overexpression of lncRNA MCM3AP-AS1 has been shown to promote proliferation, migration and invasion by

sponging miR-138-5p in pancreatic cancer.<sup>23</sup> Similarly, our sults demonstrated inhibitory effects of si-MCM3AP-AS1 on the proliferation, invasion, migration and the expression of SLC39A10 in PCa cells, and these effects could be reversed by 543–3p-inhibitor. In order to further confirm the role of IncRNA MCM3AP-AS1 in PCa cell behaviours, MCM3AP-AS1 was overexpressed and PCa cell proliferation, migration and invasion abilities were detected. The results indicate that MCM3AP-AS have promotive effects on PCa cell above behaviors, which has confirmed our hypothesis. We also found the potential down-stream signaling pathway involved in the MCM3AP-AS1/miR-54-3p-mediated tumor metabolism might be PTEN/Akt. Moreover, our in vivo experiments demonstrated that tumor proliferation and metastasis abilities in mice with the injection of lncRNA MCM3AP-AS1 became much weaker. Additionally, a reduction in the expression levels of MCM3AP-AS1 and an elevation in the expression levels of miR-543-3p were revealed in tumor samples, which suggested that MCM3AP-AS1 and miR-543-3p might be key regulators in the development of PCa.



d day pression of Ki67 and SLC39A10 in vivo. (A) The volume of mice tumors infected s in tea Lv-sh-MCM3AP-AS1 largely shrank compared to that infected with Lv-sh-MCM3AP-AS1 in tumor samples. (C) The expression of miR-543-3p was elevated in ed out to indicate the ratio of Ki67-positive cells in tumor slice. The number of Ki67polots were used to show the expression of SLC39A10 in PCa tumor, which indicates a samples infected with Lv-sh-MCM3AP-AS1. \*p < 0.05.

# Conclusion

MCM3AP-AS1 promoted PCa cell provieration, migration and invasion by recutating pCR-543-3p/SLC39A10/PTEN axis, suggesting that it much be a potential and important target for PCC prapy.

## Fund. g

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## Disclosure

The authors report no conflicts of interest in this work.

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