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Laboratory-Prostate cancer

RPS7 promotes cell migration through targeting epithelial-mesenchymal transition in prostate cancer

Yingwu Wen, M.D.[a,1], Zesheng An, Ph.D.[b,1], Baomin Qiao, M.D.[b], Changwen Zhang, M.D.[b,2,*], Zhihong Zhang, M.D.[b,2,*]

[a] *Department of Urology, Kailuan General Hospital, Tangshan, China*
[b] *Department of Urology, The Second Hospital of Tianjin Medical University, Tianjin Institute of Urology, Tianjin, China*

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Abstract

Objectives: Small ribosomal protein subunit 7 (RPS7) is an important structural components of the ribosome involved in protein synthesis, previous studies demonstrated that RPS7 was associated with several malignancies, but the role of RPS7 in prostate cancer (PCa) remains unclear. To decipher such a puzzle, in the current study, we deciphered the role and mechanism of RPS7 during the progression of PCa.

Material and Methods: In this study, the expression of mRNA was performed by quantitative real-time PCR. The protein level was identified by Western blotting. Kaplan-Meier survival analysis was demonstrated the relation between the abnormal expression of RPS7 mRNA and the overall survival. Cell proliferation was assessed by MTT assay and cell counting, meanwhile, cell migration was checked by transwell assay.

Results: RPS7 is higher expressed in PCa ($p < 0.001$), and the overexpression of RPS7 is closely associated with poor outcome of PCa patients after radical prostatectomy ($p < 0.001$). Inhibition the expression of RPS7 with a specific RPS7 siRNA could markedly attenuate prostate tumor growth and migration ($p < 0.05$). Mechanistic data reveals that inhibition of RPS7 could up-regulate the epithelial protein marker, E-cadherin ($p < 0.05$), and down-regulate the mesenchymal protein markers, such as N-cadherin and Snail ($p < 0.001$).

Conclusions: RPS7 is a newly verified tumor promoter in PCa, and promotes cell migration by targeting epithelial-to-mesenchymal transition pathway. Thus, inhibition of RPS7-epithelial to-mesenchymal transition signaling might represent a prospective approach toward limiting prostate tumor progression.

Keywords: RPS7; EMT; Cell migration; Prostate cancer

1. Introduction

Prostate cancer (PCa) has become 1 of the most frequently diagnosed urinary tract malignancy in males all over the world, and its incidence increases yearly [1]. In 2018, the newly estimated cancer cases and deaths of PCa was 164,690 and 29,430 in American, respectively [1]. In recent years, the larger increase of the incidence was found for the PCa, which has become the most important national health problem [2]. Till now, though lots of well-established risk factors have been presented to impact the risk of PCa, including hereditary family factors, ages, and variety of environmental factors, e.g., alcohol consumption, hormones, heavy metals exposure, and body size in recent years [3–5], the etiology, and pathogens for PCa risk are not yet fully understood. As we know, the progression of PCa is a multi-step and multi-pathogen process, once tumors reached to the metastasis stage, no curative therapy is available for the moment [6,7]. Therefore, it is urgently necessary to find new methods and to make improvements for early diagnostics, prevention, and therapeutic strategy of PCa.

*Corresponding authors Tel.: +86 02288329692.
E-mail addresses: zhangchangwen05@hotmail.com (C. Zhang), zhangzhihong8816@hotmail.com (Z. Zhang).
[1]Yingwu Wen and Zesheng An contributed equally to this work and should be considered co-first authors.
[2]Both Changwen Zhang and Zhihong Zhang contributed equally to this work and should be considered as corresponding author.

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Ribosomal small subunit protein 7 (RPS7) is an important component of the 40s small subunit of ribosomes, which is essential in the process of translation and crucial for ribosome assembly [8]. Recently, increasing evidence showed that low-expression of RPS7 was involved in the development of several cancers, e.g., colorectal cancer, ovarian tumor, and hepatoma [9,10]. Among these research, RPS7 regulated tumor progression through deregulating MDM2-P53 signaling, PI3K/AKT, and MAPK signaling [9,10]. However, the potential role and mechanism of RPS7 in PCa needs to be further investigated.

In current study, we gained insights into the function and mechanism of RPS7 on the progression of PCa. We found that RPS7 could largely promoted PCa cell proliferation and migration in vitro. Mechanism research showed that, with the overexpression of RPS7, epithelial-to-mesenchymal transition (EMT) was activated in PCa. Therefore, inhibition of PRS7 induced EMT signaling might appear to be an inspiring strategy for restraining PCa progression.

2. Material and methods

2.1. Cell culture and tissues

The human malignant cell line PC3 cells were obtained from Tianjin Institute of Urology, and cultured in RPMI-1640 (HyClone, Logan, UT) supplemented with 10% fetal bovine serum (HyClone, Logan, UT), and penicillin−streptomycin (100 units/ml) at 37˚C with 5% CO_2.

A total of 142 prostate tumor samples were obtained from the PCa patients undergoing radical prostatectomy at the Second Hospital of Tianjin Medical University from January 2010 to January 2017, and the tissues from benign prostatic hyperplasia (BPH) patients were collected as control from 96 patients at the same time. All patients were pathologically examined. Overall survival was defined as the period between surgical treatment and death or the time of the last follow-up. This study was approved by the Human Ethics Committee at Tianjin Medical University. A written informed consent was obtained from all the patients at the time of admission, with which the blood, tissue, and other samples were authorized for scientific purpose.

2.2. siRNA transfection

A prevalidated siRNA molecules (GeneCopoeia, China) were used to target specific region of RPS7 mRNA. PC3 cells were transfected with siRNA molecules and nontarget siRNAs (the scrambled control) using siPORTTM NeoFXTM Transfection Agent (Ambion Inc.) following the manufacturer‘s instructions. The experiments were performed 48 hours after transfection.

2.3. mRNA extraction and quantitative RT-qPCR

Total mRNA was isolated from cells and extracted using TRizol (Santa Cruz) according to the manufacturer’s instructions, quantitative measurements of gene expression were recorded with DNA Engine Opticon 2 (Bio-Rad, Richmond, CA) equipped with Opticon Monitor 2 software (MJ Research, Waltham, MA). Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was assessed with a kit from Promega according to the manufacturer’s protocol. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. Primer sequences are listed in Table 1.

2.4. Western blot analysis

Cells were harvested into total protein extraction reagent and (Solarbio, China) mixed with 1% Phenylmethanesulfonyl fluoride (PMSF) (Beyotime, China). After measuring the protein concentration, equal amounts of protein (30−50 mg/lane) were subjected to 10% SDS-PAGE and processed for western blot analysis as described previously [11]. Antibodies used in the current study are described in Table 2.

2.5. Cell-proliferation assay

Cell proliferation was determined using Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay (Beyotime, China) and cell counting. Briefly, cells were seeded at

Table 1
Primer lists.

Gene	Sequence (5′ to 3′)	
RPS7	Forward	GTTGGAGATGAACTCGGACCTG
	Reverse	GCCTTCTTGCTGTTGAACTCG
E-cadherin	Forward	GCCGGAGCCCTGCCACCCTG
	Reverse	CTTTCTGTAGGTGGAGTCCC
N-cadherin	Forward	GGAATCCCGCCTATGAGTGG
	Revers	CGTCTAGCCGTCTGATTCCC
Snail	Forward	GCTGCAGGACTCTAATCCAGA
	Reverse	ATCTCCGGAGGTGGGATG
GAPDH	Forward	GGATTTGGTCGTATTGGG
	Revers	GGAAGATGGTGATGGGATT

Table 2
Antibody lists.

Antibodies	Company	Dilution ratio	Secondary species
RPS7	Proteintech	1:1500	Rabbit
E-cadherin	Proteintech	1:1500	Rabbit
N-cadherin	Proteintech	1:1500	Rabbit
Snail	Proteintech	1:1500	Rabbit
GAPDH	Proteintech	1:1500	Mouse

RPS7 = ribosomal protein subunit 7.

concentration of 1.0×10^4 per well in 100 ml culture medium with 1% Fetal calf serum (FCS). Four 96-well plates were prepared and tested at 8 hours following our previous method [12].

2.6. Transwell assay

Matrigel (Thermo) was mixed with RPMI-1640 (Gibco) at a ratio of 1: 4 and place the mixed solution of 50ul in the upper transwell chamber (8 μM pore size, Millipore) at 4˚C for 1 hour. We first transfected cells with RPS7 specific siRNA for 48 hours, 1.0×10^4 cells were then seeded in 100 μl medium onto the upper chambers of transwell chamber under a chemotactic gradient of serum. After cells being cultured at 37˚C overnight, the filters were removed and fixed with 4% paraformaldehyde for 20 minutes, and then treated with 0.5% Triton-100 for 5 minutes. Finally cells were stained with 0.2 μl/ml 4, 6-diamidino-2-phenylindole (Molecular Probes) solution (Sigma). The nucleus of the cells which migrated on the filters were visualized and counted in 5 randomly selected areas under a fluorescent microscope. Three independent experiments were carried out.

2.7. Quantification of western blots and reverse transcriptase–PCR images

The intensities of autoradiograms in western blots and PCR bands in agarose gels were quantified by Image J (NIH, http://rsbweb.nih.gov), and the quantified data of each gene/protein were normalized to those of GAPDH.

2.8. Statistical analysis

The significance of difference between different groups was estimated by 2-tailed Student's t test. The overall survival probability was estimated using Kaplan-Meier

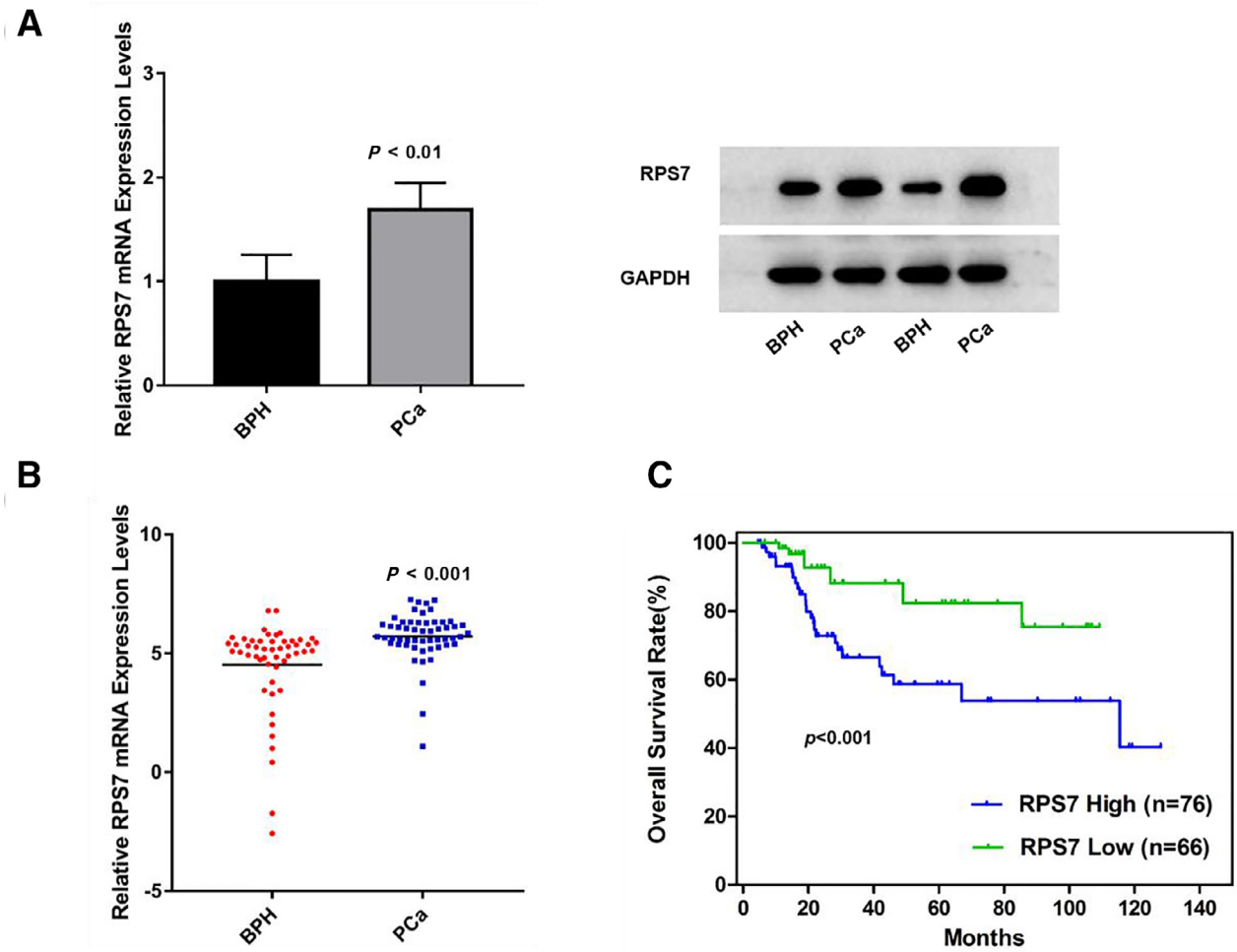

Fig. 1. High expression of RPS7 in PCa. (A). Left: The mRNA expression of RPS7 in PCa ($n = 142$) and BPH tissues ($n = 96$) by RT-qPCR analyses. Right: Western blot analyses of the protein levels of RPS7 in PCa and BPH tissues. (B). The mRNA expression of RPS7 in PCa and BPH tissues by analyzing the data from a PCa cohort database. (C). Overall survival in PCa patients related to the abnormal expression of RPS7. BPH = benign prostatic hyperplasia; PCa = prostate cancer; RPS7 = ribosomal small subunit protein 7; RT-qPCR = real-time polymerase chain reaction.

methods (SPSS 19.0, Inc.). The data are represented as the mean ± SE. P value < 0.05 was considered significant.

3. Results

3.1. RPS7 is overexpressed in PCa

To investigate the potential role of RPS7 in the tumorigenesis and progression of PCa, we first assessed the expression of RPS7 in PCa tissues. As shown in Fig. 1A, co mpared with the benign tissues, a tendency toward increased expression of RPS7 was found in PCa tissues as revealed by RT-qPCR analysis ($P < 0.01$), which was confirmed by the results of a PCa cohort database (Fig. 1B, https://www.oncomine.org. Singh Prostate Statistics). Consist with the results at mRNA level, the protein level of RPS7 was also increased as demonstrated by western blot analysis (Fig. 1A), suggesting a potential role of RPS7 in tumorigenesis and progression of PCa. To further validate our hypothesis, we characterized the association between abnormal expression of RPS7 and overall survival in PCa patients. We established an appropriate cutoff of RPS7 abnormal expression following previous studies [13], above which was considered as overexpressed and below as low expressed, and the mean level was used. The results indicated that high expression of RPS7 was closely correlated with poor outcome of PCa patients after radical prostatectomy ($P < 0.001$, Fig. 1C). All these results suggested that overexpression of RPS7 was significantly involved in the progression of PCa, and RPS7 might be served as an important biomarker to predict clinical outcome of PCa patients after radical prostatectomy.

3.2. Inhibition of RPS7 attenuated PCa cell invasion

To investigate the biological behavior of RPS7 in PCa cells, we examined the level of RPS7 in PCa cell lines. The results showed that RPS7 was higher expressed in malignant cell lines (PC3, C4-2, and LNPCa) at mRNA level than that in nonmalignant cell line, RWPE-1 ($P < 0.05$, Fig. 2A), among the malignant cell lines, RPS7 was highest expressed in PC3 cells ($P < 0.05$, Fig. 2A). Thus, PC3 cell lines were selected in the following experiments. We then transfected prevalidated RPS7 siRNA in PC3 cells. As shown in Fig. 2B, compared with the control, the level of RPS7 was decreased by 73% using RT-qPCR analysis $P <$ 0.001 and western blot analysis. Consequentially, cell growth was significantly suppressed in cells transfected with siRNA compared with the control, as reflected by the MTT assay ($P < 0.05$, Fig. 2C) and cell counting analysis ($P < 0.05$, Fig. 2D). Meanwhile, cell mobility was

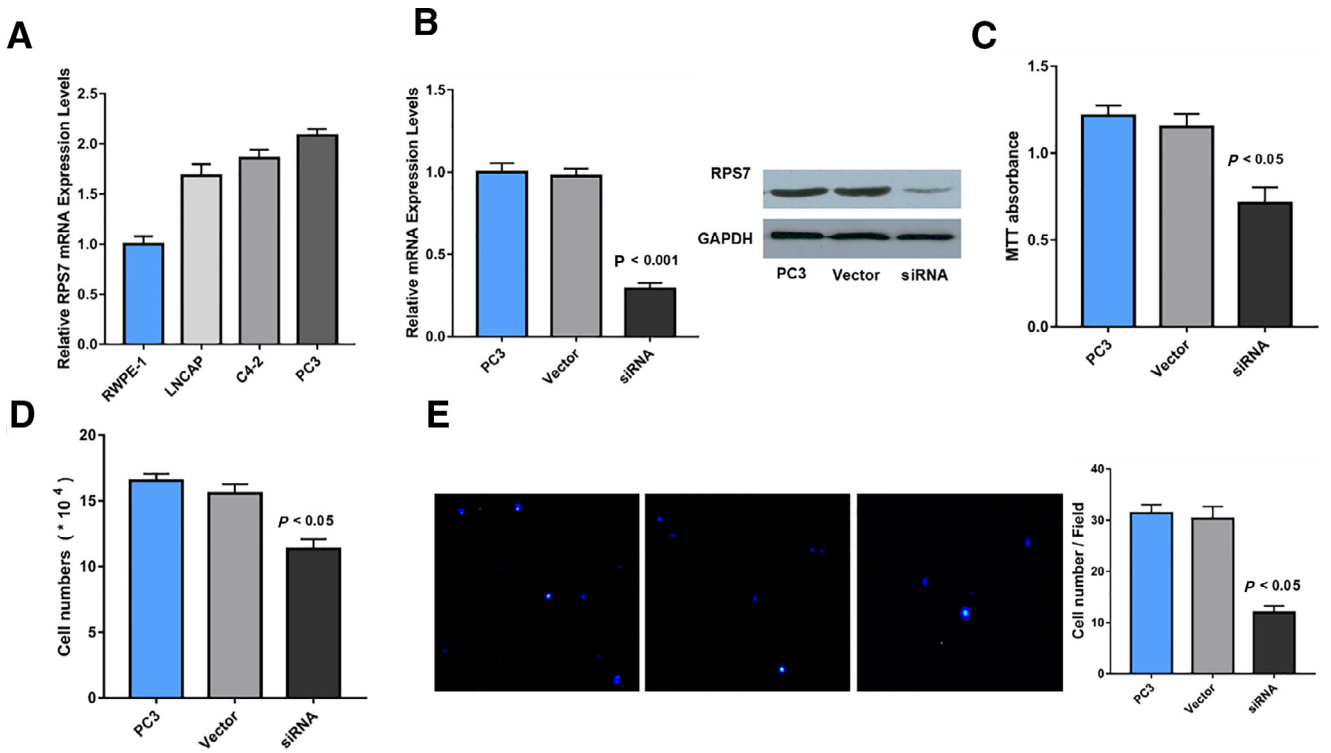

Fig. 2. Inhibition of RPS7 attenuates PCa cell growth and migration. (A). The mRNA expression of RPS7 in PCa by RT-qPCR analyses ($n = 6$). (B). The mRNA and protein level of RPS7 in PC3 cells transfected with its specific siRNA for 48 hours ($n = 6$). (C). MTT analyses for cell growth of PC3 cells transfected with its specific siRNA for 48 hours ($n = 6$). (D). Cell counting for cell growth of PC3 cells transfected with its specific siRNA for 48 hours ($n = 6$). (E). Transwell migration assay for cell invasion of PC3 cells upon antimony exposure ($n = 3$). BPH = benign prostatic hyperplasia; PCa = prostate cancer; qPCR = quantitative polymerase chain reaction; RPS7 = ribosomal small subunit protein 7.

markedly inhibited in siRNA transfected cells compared to the control as revealed by the transwell migration assay ($P < 0.05$, Fig. 2E). All these results demonstrated that RPS7 played an important role in modulating tumor formation and progression of PCa.

3.3. RPS7 promoted cell migration via epithelial-mesenchymal transition

EMT has been initially defined as a complex molecular program that regulates cell morphology and function during the embryogenesis development [14]. Recently, many evidences have demonstrated that EMT was closely associated with both PCa metastatic progression and treatment resistance, underlining that EMT is a significant event responsible for triggering tumor metastatic process [15,16]. We have demonstrated that RPS7 could promote PCa cell migration. When we inhibited RPS7 expression with prevalidated RPS7 siRNA $P < 0.001$, the mRNA expression of Snail, N-cadherin was markedly decreased $P < 0.001$, while E-cadherin was up-regulated $P < 0.05$ (Fig. 3A), and similar results of protein level was assessed by western blot analysis (Fig. 3B), indicating that RPS7 could promote EMT in PCa.

4. Discussion

Ribosomal proteins are essential in the process of translation and crucial for ribosome assembly and subsequent ribosome biogenesis [8,17]. Recently, sparse literature showed that abnormal expression of ribosomal genes or proteins, crucially contributed to tumorigenesis and tumor progression, e.g., RPL11, RPS19, RPS21, and RPS24 [18,19]. RPS7, which is an important component of the 40s small subunit of ribosomes, was reported to repress tumor cell proliferation in ovarian and colorectal cancer [9,10]. In current study, we found that RPS7 was higher expressed in PCa, compared with BPH tissues, and the overexpression of RPS7 was closely associated with the development of PCa. Silencing of RPS7 attenuated cell proliferation and migration, indicating RPS7 might be a potential biomarker for PCa.

Recently, the important role of EMT in tumor biology has evoked, e.g., promoting tumor metastasis, conferring cancer stem cell abilities, and mediating drug resistance in several cancers such as breast and pancreatic cancer [20,21], is a multistep process involving many molecular and cellular changes, including the down-regulation of epithelial protein markers, such as E-cadherin, Desmoplakin, Occludin, and Cytokeratins, and the up-regulation of mesenchymal protein markers, such as N-cadherin, Twist1, Slug, and Vimentin, which endow the cell with increased motility and migration [21,22]. Till now, several key mechanisms in driving EMT in cancers. It was reported that EMT could be activated by EMT-related transcription factors, including Snail, Slug, Zeb1, and Twist [23,24]. In addition, the tumor microenvironment was demonstrated to play an important role in eliciting EMT, and some extracellular molecules, such as TGF-β, FGF, EGF, IL-6, and Wnt, and their related pathways are detected to induce EMT [24–26]. Epigenetic regulation is also considered as a key effector, e.g., epigenetic modification of HIC1 promoter, the histone methyltransferase of MMSET/WHSC1, deregulation of miRNA (such as miR-200 family, miR-203, and miR-205), abnormal expression of long noncoding RNAs (such as SChLAP1, PlncRNA-1, and SChLAP1) [27–32]. In current study, we found that RPS7 could induce EMT to promote PCa migration by activating the level of Snail.

As we known, PCa is a hormone-associated cancer, and deregulated androgen signaling is 1 of the most important factors for tumor progression [12]. Previous studies demonstrated that androgens might affect EMT by suppressing the

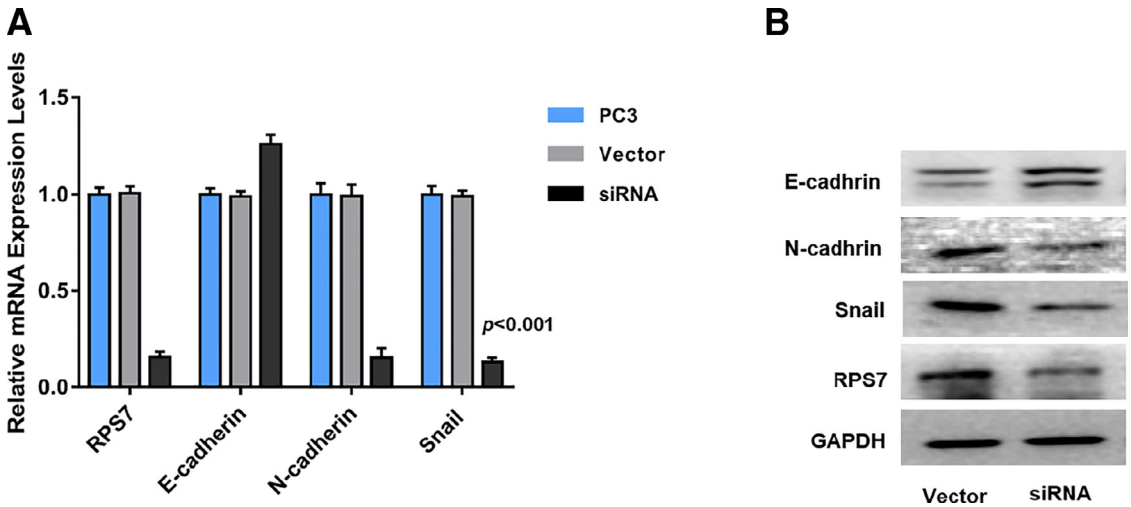

Fig. 3. RPS7 regulates cell migration via promoting EMT. (A). The mRNA expression of RPS7, E-cadherin, N-cadherin, and Snail in PC3 cells transfected with its specific siRNA for 48 hours ($n = 6$). (B). Western blot analyses of the protein levels of RPS7, E-cadherin, N-cadherin, and Snail in PC3 cells transfected with its specific siRNA for 48 hours. EMT = epithelial-to-mesenchymal transition; RPS7 = ribosomal small subunit protein 7.

expression of E-cadherin in PCa epithelial cells [33]. However, increasing evidence showed that androgen deprivation promoted EMT in both normal and tumor prostate tissues [34,35]. In addition, the expression of N-cadherin and ZEB1 were both enhanced by androgen withdrawal in the LNPCa cells, meanwhile, ZEB1 functioned as a transcriptional suppressor of androgen receptor (AR), by which EMT was intervened through a bidirectional negative feedback loop of AR [34,36]. Though the data involving the function of androgens on EMT are conflicting, we think that AR maintenance is necessary for EMT suppression, for its inhibition might attenuate EMT in androgen independent PCa cells.

5. Conclusions

To summarize, we deciphered here a novel role and mechanism of RPS7 in PCa. The results showed that RPS7 is higher expressed in PCa, compared with the BPH tissues, and the overexpression of RPS7 is closely associated with poor outcome of PCa patients with radical prostatectomy. In addition, the inhibition of RPS7 with specific RPS7 siRNA could markedly attenuate prostate tumor growth and migration. Mechanistic data revealed that inhibition of RPS7 could up-regulate the epithelial protein marker, E-cadherin, and down-regulate the mesenchymal protein markers, such as N-cadherin and Snail. These combined data indicate that RPS7 is a newly verified tumor promoter, and promotes cell migration by targeting EMT pathway. Thus, RPS7 induced EMT signaling might be a novel and promising molecular strategy, and inhibition of RPS7-EMT signaling might represent a prospective approach toward limiting prostate tumor progression, which will contribute to the multifactorial repertoire of individualized PCa care.

Conflicts of interest

The authors declare no conflicts of interest.

Authors' contributions

Conception and design: Z. Zhang, C. Zhang.

Development of methodology: C. Zhang, Y. Wen.

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): C. Zhang, Z. An, Z. Zhang, B. Qiao.

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Zhang.

Writing, review, and/or revision of the manuscript: C. Zhang, Z. Zhang.

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C. Zhang, Z. Zhang.

Study supervision: Z. Zhang.

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