

# Overexpression of BUB1B contributes to progression of prostate cancer and predicts poor outcome in patients with prostate cancer

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**Abstract:** BUB1 mitotic checkpoint serine/threonine kinase B (BUB1B) is a member of the spindle assembly checkpoint protein family, which has been proven to be associated with many kinds of cancers. The aim of this study was to investigate whether BUB1B was correlated with progression and prognosis in patients with prostate cancer (PCa) and how BUB1B regulated the proliferation, migration, and invasion of PCa cell lines. Compared to benign prostate cells and tissues, both messenger RNA and protein expressions of BUB1B were statistically increased in PCa cell lines and tumor tissues. In vitro studies revealed that BUB1B overexpression enhanced the proliferation, migration, and invasion ability of PCa cell lines, whereas depletion of BUB1B did not affect the cell functions. Microarray analysis showed the positive staining of BUB1B was upregulated in the higher Gleason score group, which also correlated with advanced clinicopathological stage, higher serum prostate-specific antigen, metastasis, overall survival, and prostate-specific antigen failure. Furthermore, the survival analysis indicated that high expression of BUB1B was an independent predictor for shorter biochemical recurrence-free survival, which had no effect on overall survival. BUB1B plays an important role in tumor growth and progression, which can lead to its use as a potential biomarker for the diagnosis and prognosis of PCa.

**Keywords:** prostate cancer, BUB1B, SAC, biochemical recurrence-free survival, prognosis

## Introduction

Prostate cancer (PCa) is one of the most frequently diagnosed solid tumors among males. Nearly 1,111,700 newly diagnosed PCa cases were estimated worldwide in 2012, and more than two-thirds occurred in developed countries.<sup>1</sup> The PCa incidence and mortality are increasing rapidly and constantly in the People's Republic of China.<sup>2,3</sup> The initiation and progression of PCa is a multistep process, which can be impacted by both genetic mutations in epithelial cells and alternation in epithelial–stromal interaction.<sup>4</sup> PCa can be clinically divided into different risk groups based on the levels of serum prostate-specific antigen (PSA), clinical tumor-node-metastasis (TNM) stage, and Gleason score.<sup>5</sup> PSA has been widely used as a biomarker for diagnosis and prognosis of PCa. However, it has limitations and cannot be used as a sole criterion.<sup>6</sup> Therefore, there is an urgent need to find a more accurate marker for PCa diagnosis and prognosis.

The spindle assembly checkpoint (SAC) protein family consists of multiple members that include Mad1, Mad2, Bub1, Bub3, Mad3/Bub1b, and Mps1. The main function of SAC is to maintain genome stability by delaying cell division until accurate chromosome segregation is guaranteed during mitosis and meiosis.<sup>7</sup> Mutations or abnormal expression of SAC proteins can contribute to cancer progression.<sup>8</sup>



BUB1B (BUB1 mitotic checkpoint serine/threonine kinase B) is a member of the SAC protein family. It is a key component of the mitotic checkpoint complex that comprises Cdc20 and other SAC proteins, including Mad2 and Bub3.<sup>9,10</sup> BUB1B interacts directly with Cdc20 and inhibits its activity to activate the anaphase-promoting complex/cyclosome, and therefore suppresses the onset of anaphase to ensure proper chromosome segregation.<sup>11</sup>

It has been reported that low expression of BUB1B contributes to initiation and progression of human colon adenocarcinomas and lung cancer.<sup>12,13</sup> However, a large number of reports have demonstrated that overexpression of BUB1B is associated with progression and recurrence of bladder cancer,<sup>14</sup> gastric cancer,<sup>15</sup> esophageal squamous cell carcinoma,<sup>16</sup> breast cancer,<sup>17</sup> hepatocellular carcinoma,<sup>18</sup> and some other cancers. Whether aberrant expression of BUB1B is associated with PCa is not clear. Herein, we report that overexpression of BUB1B is associated with tumor progression of PCa and poor prognosis of patients with PCa.

## Methods

### Patients and tissue samples

These procedures were approved and supported by the Research Ethics Committee of Guangzhou First People's Hospital, Guangzhou Medical University, People's Republic of China. Written informed consent was obtained from all patients and their relatives. All specimens were handled and made anonymous according to the ethical and legal standards. A tissue microarray (TMA) of 99 primary PCa tissues and 81 adjacent noncancerous prostate tissues was purchased from Shanghai Outdo Biotech Co, Ltd (Shanghai, People's Republic of China; catalog number: HPro-Ade180PG-01). None of the patients recruited in this study had chemotherapy or radiotherapy before the surgery.

The Taylor dataset (NCBI GEO accession no: GSE21032), an online PCa dataset with microarray for messenger RNAs (mRNAs) and miRNAs that included 150 primary PCa tissues and 29 adjacent noncancerous prostate tissues was downloaded from <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE21032>.<sup>19</sup> For the analysis of survival and follow-up, the date of prostatectomy was defined as the beginning of the follow-up period. The primary criterion was biochemical recurrence-free survival time. The other analysis criterion was overall survival time. All patients who died from diseases other than PCa or unexpected events were excluded from the case collection. The clinical features of all patients, which were used in our TMA and Taylor dataset, are listed in Table 1.

**Table 1** Clinical features of all patients

Clinical features	Experiment type	
	IHC	Taylor dataset
Benign tissue (cases)	81	29
Prostate cancer (cases)	99	150
Age, years		
Mean age	70.71±8.00	58.33±7.10
<66	26	124
≥66	73	26
Serum PSA (ng/mL)		
<4	–	24
4–10	–	81
≥10	–	42
Gleason score		
≤6	26	41
7	44	76
≥8	28	22
Pathological stage		
T2	70	86
≥ T3A	29	55

**Notes:** Mean age data presented as mean ± standard deviation. The “–” means there is a lack of relative information on patients in our cohort. Some patients had missing data for related items.

**Abbreviations:** IHC, immunohistochemistry; PSA, prostate-specific antigen.

### Cell culture and plasmid transfections

Three human PCa cell lines (PC3, DU145, and LNCaP) and one benign prostate cell line (BPH-1) were used in this study. All of them were purchased from American Type Culture Collection (Manassas, VA, USA) and were maintained in the high-glucose Dulbecco's Modified Eagle's Medium (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum. The cells were cultured at 37°C in a 5% CO<sub>2</sub> humidified incubator. The BUB1B plasmid was constructed by inserting the cDNA into pcDNA3.1-HA plasmid (Invitrogen, Carlsbad, CA, USA); the small interfering RNA (siRNA) oligonucleotides for inhibiting BUB1B (siBUB1B) and the mock oligonucleotides (siNC) were purchased from Cell Signaling Technology (Danvers, MA, USA). Fugene HD transfection reagents (Promega, Fitchburg, WI, USA) were used for transfecting plasmids or siRNAs following the manufacturer's instructions.

### Cell viability assay

The CCK-8 assay kit was used to evaluate the proliferation of PCa cells. Approximately 2×10<sup>3</sup> cells were seeded into 96-well plates and cultured for 24, 48, and 72 hours. Cells were then incubated with 10 μL of CCK-8 (catalog number: C0038, Beyotime, Shanghai, People's Republic of China) for 2 hours at 37°C. The absorbance was measured at 450 nm wavelength using a spectrophotometer (Multiskan MK3, Thermo Scientific, Waltham, MA, USA). Data were

expressed as mean  $\pm$  standard deviation (SD) of three independent experiments.

### Wound-healing assay

The scratch wound-healing motility assay was performed to evaluate the migration ability of PCa cells. The transfections with BUB1B or negative control plasmids were carried out when the cell reached 80%–90% confluence. Twenty-four hours after the transfection, a scratch was made with a 10  $\mu$ L pipette tip. The cells were then returned to the incubator until the indicated time. Representative sites were photographed, and the cells that migrated from the wound edge were counted at each time point. Data were presented as mean  $\pm$  SD of three independent experiments.

### Transwell invasion assay

The CytoSelect Cell Migration and Invasion Kit (Cell Biolabs, Inc., San Diego, CA, USA) was used according to manufacturer's instructions. Twenty-four hours after the transfection, PCa cells were resuspended in a serum-free medium to a density of  $25 \times 10^4$ /mL. The cell suspension (200  $\mu$ L) was transferred to the upper chambers. The lower chamber contained 10% fetal bovine serum used as a chemoattractant. After incubation for 48 hours, cells migrated through the membrane and were fixed, stained, and quantified by counting nine independent symmetrical visual fields under a microscope. Data were presented as mean  $\pm$  SD of three independent experiments.

### Quantitative reverse transcription polymerase chain reaction analysis

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis was used to assess the expression of BUB1B in prostate cells. The mRNA was extracted from the cells using the TRIZOL reagent (Invitrogen), and the cDNA was synthesized from RNA templates. The PCR primers for BUB1B were: forward, 5'-CTTAGGGTGCAGCTGGATGT-3'; reverse, 5'-ACCCATCCCAGA AGACCTGT-3', and the primers for  $\beta$ -actin were: forward, 5'-AGCGAGCATCCCCAAAGTT-3'; reverse, 5'-GGGCACGAAGGCTCATCATT-3'. Gene expression was determined by the SYBR Green PCR mix (Toyobo Co, Ltd., Osaka, Japan) and performed on a MyiQ.2 two-color, real-time detection system (Bio-Rad, Hercules, CA, USA). Gene expression in each sample was normalized with  $\beta$ -actin internal controls. Relative quantification of target gene expression was evaluated using the comparative cycle threshold (Ct) method. Mean  $\pm$  SD was calculated from three independent experiments.

### Western blot analysis

The proteins were extracted for Western blot analyses 72 hours after transfection. Whole-cell extracts that contained 30  $\mu$ g proteins were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred onto Hybond nitrocellulose membranes (GE Healthcare, Piscataway, NJ, USA). The membranes were blocked with 10% skim milk in Tris-buffered saline–Tween 20 and probed with anti-BUB1B antibody (1:1,000, #4116, Cell Signaling Technology) or anti- $\beta$ -actin antibody (1:2,000, sc-47778, Santa Cruz, Dallas, TX, USA). The results were visualized using the SuperSignal West Pico chemiluminescent detection system (Pierce Biotechnology, Rockford, IL, USA).  $\beta$ -Actin was used as an internal loading control.

### Immunohistochemistry analysis

The specimens were fixed in 10% neutral buffered formalin and were subsequently processed by gradient dehydration in ethanol, embedded in paraffin, and sectioned into 4  $\mu$ m thick sections for hematoxylin and eosin or immunohistochemistry staining with the DAKO EnVision System (Dako Diagnostics, Zug, Switzerland). The primary antibodies against BUB1B were used at a dilution of 1:200. Peroxidase-labeled antibodies and alkaline-phosphatase-labeled antibodies were employed to detect the specifically bound primary antibodies. Immunostaining was scored by two independent experienced pathologists who were blinded to the clinicopathological data and clinical outcomes of the patients. The scores of the two pathologists were compared, and any discrepant scores were resolved through reexamining the staining by both pathologists to achieve a consensus score. The number of positive staining cells in ten representative microscopic fields was counted and the percentage of positive cells calculated. Given the homogeneity of the staining of the target proteins, tumor specimens were scored in a semiquantitative manner. The percentage scoring of immunoreactive cells was separated into five groups as follows: 0 (0%), 1 (1%–10%), 2 (11%–50%), 3 (51%–80%), and 4 (>80%). The staining intensity was visually score and stratified as follows: 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). Final immunoreactivity scores (IRSS) were obtained for each case by multiplying the percentage and the intensity score.<sup>20</sup>

### Statistical analysis

The software of SPSS 13.0 for Windows (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Continuous variables were expressed as mean  $\pm$  SD. The Kaplan–Meier method was used for the survival analysis, and a

log-rank test was used to analyze the difference of survival times. The Student's *t*-test and chiquet trend test were used for ordinal data analysis. Cox regression analysis was used for the univariate and multivariate analyses. Differences were considered statistically significant when *P*-value was  $<0.05$ .

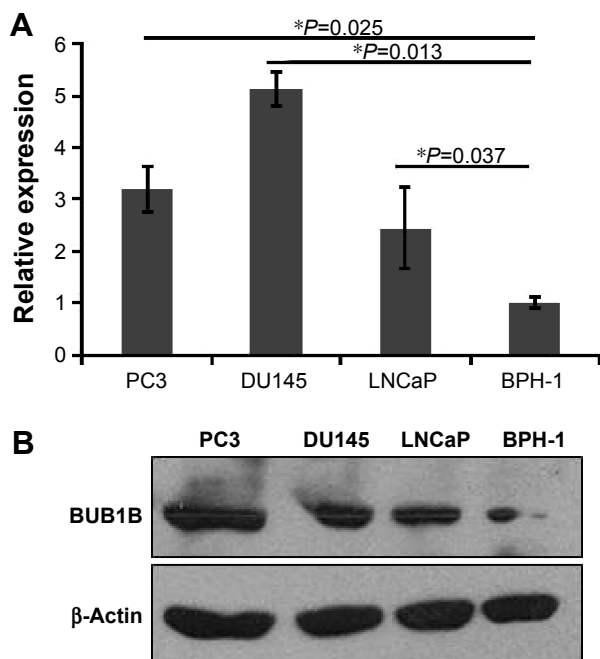
## Results

### Overexpression of BUB1B promotes cell proliferation and migration in PCa cells

To assess BUB1B expression in human PCa cells at the mRNA and protein levels, qRT-PCR and Western blotting analyses were performed. The results showed that the expression of BUB1B at both the mRNA and protein levels was higher in LNCaP, DU145, and PC3 cells than in BPH-1 cells ( $P<0.05$ , Figure 1). The results suggest that PCa cells express BUB1B at higher levels than benign prostate cells. To investigate the effects of abnormal BUB1B expression in PCa cells, siRNAs specific for BUB1B were used to deplete the expression of BUB1B in DU145 cells. In addition, DU145 cells were forced to overexpress BUB1B by transfection with the plasmid carrying human BUB1B cDNA. qRT-PCR and Western blotting analyses showed that siRNA transfection reduced expression of BUB1B in DU145 cells ( $P=0.020$ ,

Figure 2A and B). Interestingly, although the expression of BUB1B was reduced by siRNA transfection in DU145 cells, the growth and migration of the cells were not affected (Figure 2C–G). As seen in Figure 2C, the CCK-8 assay data showed that there was no significant difference between the siRNA group and blank vector group at 24, 48, and 72 hours ( $P=0.754$ ,  $0.178$ , and  $0.272$ ). The wound-healing assay and Transwell assay both showed similar results ( $P=0.645$ , Figure 2D and E,  $P=0.547$ , Figure 2F and G).

However, overexpression by transfection promoted cell growth and migration in DU145 cells (Figure 3). qRT-PCR and Western blotting analyses showed that cDNA transfection increased expression of BUB1B in DU145 cells ( $P=0.035$ , Figure 3A and B). As seen in Figure 3C, the CCK-8 assay data showed that the cell viability of BUB1B transfected DU145 cells was significantly more than that of blank vector group at 48 hours after transfection ( $P=0.035$ ), and the trend was enhanced over time ( $P=0.002$ , at 72 hours). As seen in Figure 3D and E, after 72 hours of scratching, the BUB1B overexpression DU145 cells showed more migration cells than those with blank vector ( $P<0.001$ ). Similar results were also obtained in the Transwell assay (Figure 3F and G), the upregulation of BUB1B could enhance the invasion ability compared with the negative control group ( $P<0.001$ ).



**Figure 1** The expression of BUB1B in cell lines.

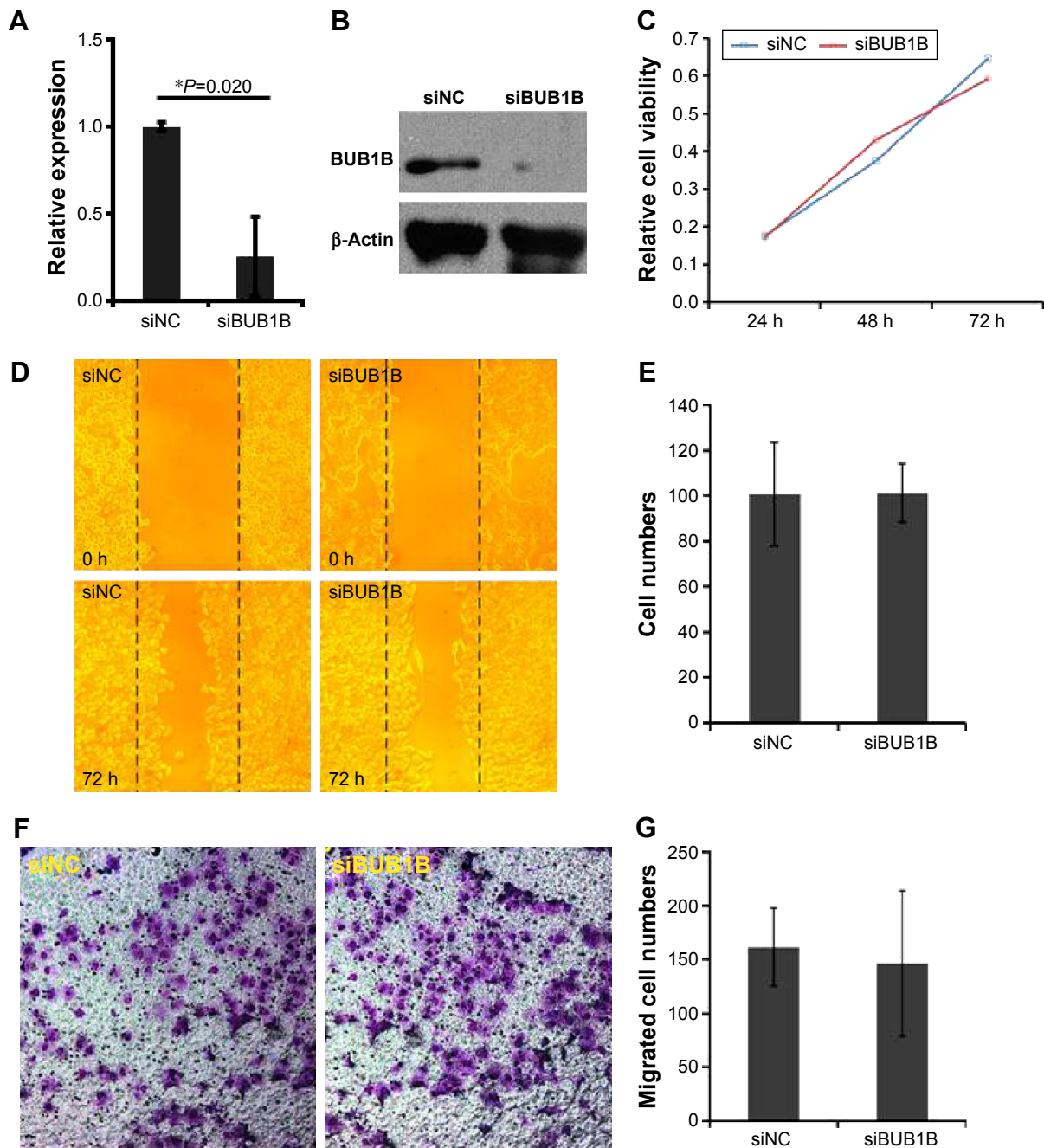
**Notes:** (A) The relative expression of the mRNA level using qRT-PCR.  $*P<0.05$ .

(B) The protein expression level by Western blot.

**Abbreviations:** BUB1B, BUB1 mitotic checkpoint serine/threonine kinase B; mRNA, messenger RNA; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

### BUB1B expression is associated with the clinicopathological characteristics of PCa and tumor recurrence time of patients with PCa

To assess BUB1B expression in PCa at the protein level, immunohistochemistry analysis was performed to examine the expression pattern and localization of BUB1B in the TMA that included 99 PCa and 81 adjacent noncancerous prostate tissues (Figure 4A). The results showed that most PCa tissues with Gleason score higher than 7 (20 out of 26, 76.9%) and a Gleason score of 7 (25 out of 44, 56.8%) highly expressed BUB1B. In contrast, high BUB1B-expressing cells were less frequently found in those cells with a Gleason score lower than 7 (eleven out of 26 samples, 42.3%) (Figure 4B). The results indicate that expression of BUB1B is positively associated with the progression of PCa. Since the TMA data did not contain more clinicopathological characteristics, such as PSA level, overall survival, metastasis, we used the Taylor dataset, an online PCa dataset, for the analysis of survival and follow-up. Analyses of the online Taylor dataset also revealed that PCa showed a higher expression level of BUB1B than the benign tissues (PCa =  $5.54 \pm 0.31$  vs benign =  $5.30 \pm 0.12$ ,  $P<0.001$ , Table 2).



**Figure 2** Depletion of BUB1B by siRNA has no effect on the proliferation and migration of DU145 cells.

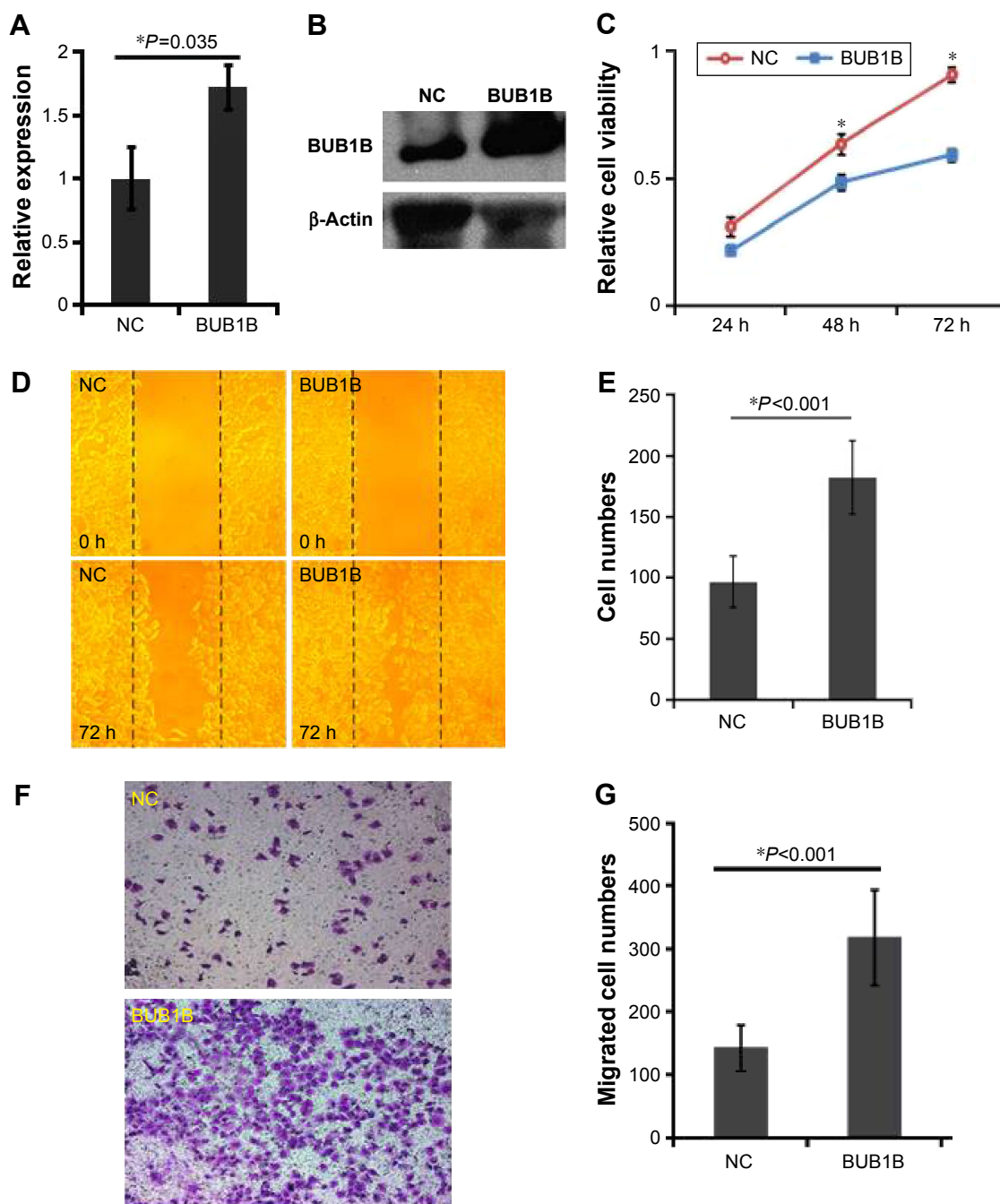
**Notes:** (A and B) qRT-PCR and Western blot analysis for BUB1B expression after siRNA transfection. (C) The growth curve of DU145 cells. (D and E) Wound-healing assay. (F and G) Transwell analysis to detect cell migration.

**Abbreviations:** BUB1B, BUB1 mitotic checkpoint serine/threonine kinase B; h, hours; qRT-PCR, quantitative reverse transcription polymerase chain reaction; siBUB1B, small interfering RNA oligonucleotides for inhibiting BUB1B; siNC, small interfering RNA mock oligonucleotides; siRNA, small interfering RNA.

In addition, immunohistochemistry staining showed that BUB1B was mainly located in the epithelial compartment (Figure 4C). The stromal compartment only had weak or negative staining in the nucleus. In the epithelial compartment, BUB1B was mainly located in the cytoplasm of PCa cells as well as in the luminal and basal cells of adjacent noncancerous prostate tissues. Compared with the adjacent

noncancerous prostate tissues, the expression level of BUB1B in PCa cells was significantly higher (IRS: PCa =  $4.18 \pm 1.83$  vs benign =  $3.53 \pm 1.43$ ,  $P=0.011$ , Figure 4D).

Detailed analyses of the association between expression of BUB1B and the clinicopathological features of PCa revealed that increased expression of BUB1B in PCa tissues was significantly correlated with higher Gleason score



**Figure 3** Overexpression of BUB1B promotes the proliferation and migration of DU145 cells.

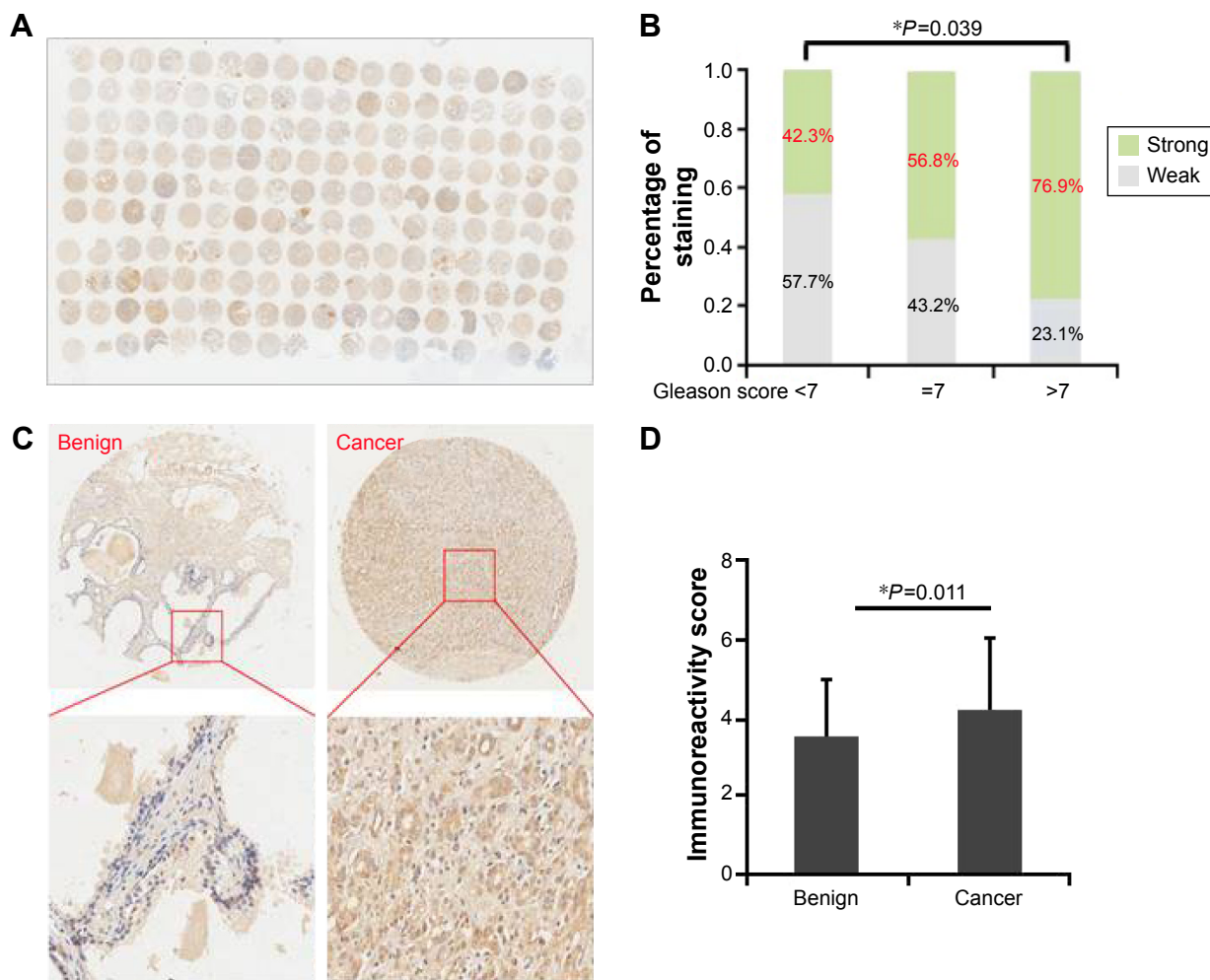
**Notes:** (A and B) qRT-PCR and Western blot analysis for BUB1B expression. (C) The growth curve of DU145 cells. (D and E) Wound-healing assay. (F and G) Transwell analysis to detect cell migration. \* $P < 0.05$ .

**Abbreviations:** BUB1B, BUB1 mitotic checkpoint serine/threonine kinase B; h, hours; NC, negative control; qRT-PCR, quantitative reverse transcription polymerase chain reaction.

( $P=0.017$ ) and advanced clinicopathological stage ( $P=0.032$ ). No association between expression levels of BUB1B with the age of patients was found. Similarly, analyses of the online Taylor dataset revealed that upregulation of BUB1B expression occurred more frequently in PCa patients with high serum PSA ( $>10$  ng/mL,  $P=0.038$ ), high Gleason score ( $P=0.016$ ), and persons with metastasis ( $P < 0.001$ ). It was

also associated with shorter overall survival ( $P=0.006$ ) and PSA failure ( $P=0.003$ ) time, but not with age or pathological stage of the patients (Table 2).

We then used the Kaplan–Meier method to analyze the association of BUB1B expression levels with the biochemical recurrence-free time and the overall survival time of patients with PCa. The median of BUB1B expression in all PCa



**Figure 4** Immunohistochemical staining for BUB1B expression in PCa and adjacent noncancerous tissues.

**Notes:** (A) The whole scanned picture of the tissue array. (B) The percentage of strong (green column) and weak BUB1B staining (gray column) in different groups by the level of Gleason score (<7, =7, and >7). \* $P < 0.05$ . (C) Immunostaining of benign and cancer tissues. Magnification of the upper panel is  $\times 50$ ; the bottom panel is  $\times 200$ . (D) Statistical analyses showing higher IRS in cancerous tissues than in adjacent noncancerous tissues (IRS: PCa =  $4.18 \pm 1.83$  vs benign =  $3.53 \pm 1.43$ ,  $P = 0.011$ ).

**Abbreviations:** BUB1B, BUB1 mitotic checkpoint serine/threonine kinase B; IRS, immunoreactivity score; PCa, prostate cancer.

tissues of the Taylor dataset was used as the cutoff to divide all PCa tissues into high ( $n=74$ ) and low ( $n=74$ ) BUB1B expression groups. As shown in Figure 5, the biochemical recurrence-free time of PCa patients with high BUB1B expression levels was shorter than those with low BUB1B expression levels ( $P < 0.001$ , Figure 5A). However, no correlation of the overall survival time of PCa patients with BUB1B expression levels was seen (Figure 5B).

In addition, the Cox proportional hazards regression model was used for the univariate and multivariate analyses (Table 3). The univariate and multivariate analyses indicated that the upregulation of BUB1B (hazard ratio [HR] = 3.482 and 2.209,  $P = 0.001$  and 0.045, respectively) was an independent predictor for shorter biochemical recurrence-free survival, together with higher Gleason score (HR = 3.351 and 2.228,  $P = 0.000$  and 0.000, respectively), higher preoperative PSA

(HR = 1.005 and 1.005,  $P = 0.010$  and 0.007, respectively), and advanced pathological tumor stage (HR = 5.221 and 3.137,  $P = 0.000$  and 0.009, respectively). However, the upregulation of BUB1B could not predict shorter overall survival both in univariate and multivariate analyses ( $P = 0.273$  and 0.676, respectively).

## Discussion

Although the therapies for PCa have improved and the survival rate for early PCa patients has increased nowadays, the prognosis of late-stage PCa is still poor. The current diagnostic biomarkers still fail to predict the progression of PCa. Therefore, it is of great interest to develop novel biomarkers for estimation of recurrence and metastasis potential. Herein, we report that overexpression of BUB1B in PCa cells promoted proliferation and migration of the cells.

**Table 2** Association of BUB1B expression with the clinicopathological characteristics of PCa

Clinical features	IRS of BUB1B in our TMA cohort			BUB1B expression in Taylor dataset		
	Case	$\bar{x}\pm s$	P-value	Case	$\bar{x}\pm s$	P-value
Tissues						
Benign	81	3.53±1.43	0.011	29	5.30±0.12	<0.001
Cancer	99	4.18±1.83		150	5.54±0.31	
Age						
<66 years	26	3.88±1.61	0.347	124	5.53±0.31	0.380
≥66 years	71	4.28±1.91		26	5.59±0.32	
Serum PSA						
<4 (ng/mL)	–	–	–	24	5.50±0.25	0.768
≥4 (ng/mL)	–	–	–	123	5.52±0.27	
<10 (ng/mL)	–	–	–	105	5.48±0.21	0.038
≥10 (ng/mL)	–	–	–	42	5.60±0.35	
Gleason score						
<8	70	3.93±1.62	0.017	117	5.47±0.21	0.016
≥8	26	4.92±2.17		22	5.71±0.42	
Pathological stage						
<T3A	70	3.93±1.62	0.032	86	5.47±0.20	0.054
≥T3A	27	4.81±2.20		55	5.57±0.34	
Metastasis						
No	–	–	–	122	5.46±0.19	<0.001
Yes	–	–	–	28	5.87±0.49	
Overall survival						
Alive	–	–	–	131	5.49±0.23	0.006
Die	–	–	–	19	5.88±0.55	
PSA failure						
Negative	–	–	–	104	5.45±0.19	0.003
Positive	–	–	–	36	5.66±0.38	

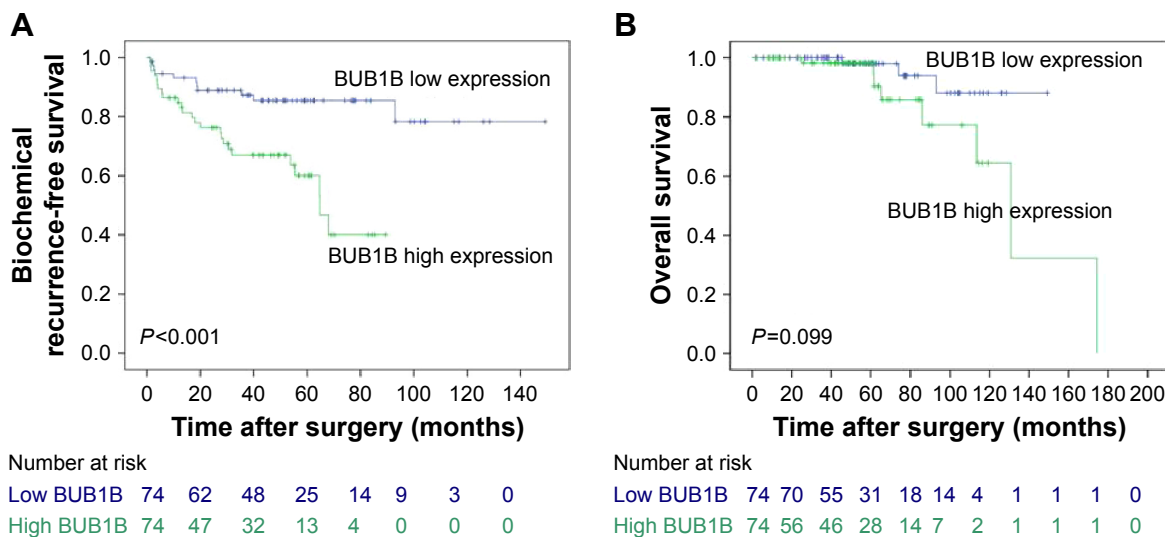
**Notes:** The “–” means there is a lack of relative information on patients in our cohort. Some patients had missing data for related items.

**Abbreviations:** BUB1B, BUB1 mitotic checkpoint serine/threonine kinase B; IRS, immunoreactivity score; PCa, prostate cancer; PSA, prostate-specific antigen; TMA, tissue microarray.

Overexpression of BUB1B was more frequently found in advanced PCa and predicted short recurrent time of PCa. Statistical analyses of the online dataset revealed that the expression level of BUB1B was associated with high Gleason scores and poor clinicopathological features. Together, the results suggest that the overexpression of BUB1B has the potential to serve as a biomarker for PCa prognosis.

The *Bub1b* gene is located at chromosome 15q15. It encodes a protein of 1,050 amino acid residues. The BUB1B protein has three structure domains, the highly conserved sequence of N-terminal domain, the kinase domain at the C-terminal region, and the sequence between the two domains.<sup>21</sup> The function of BUB1B in mitosis includes activation, maintenance, and silencing the SAC as well as regulating chromosome-spindle attachment, and it is also required for controlling mitotic timing. Depletion of BUB1B shortens mitotic duration.<sup>21–24</sup> Aberrant expression or mutations of BUB1B can cause aneuploidy. Mice lacking one functional *Bub1r* (counterpart of human *Bub1b*) allele are more prone to develop lung and colon cancer when treated with carcinogen azoxymethane (AOM) than wild-type mice.<sup>25</sup> Deletion of one *Bub1r* allele increases spontaneous cancer in mice bearing an *Apc-min* mutant alleles.<sup>26</sup>

Although not frequently found, several mutations of *Bub1b* have been shown to be partially associated with cancers of various organ origins, such as M40T in colorectal cancer, Q363R in breast cancer, and E390D in Wilms tumor.<sup>27</sup> However, both increased and decreased expression levels of BUB1B have been found to be associated with

**Figure 5** High BUB1B expression is linked to poor prognosis in patients with PCa.

**Notes:** (A) Kaplan–Meier analysis of the biochemical recurrence-free time of PCa patients with high BUB1B expression levels was shorter than those with low BUB1B expression levels. (B) The overall survival time of patients with PCa was not correlated to BUB1B expression levels.

**Abbreviations:** BUB1B, BUB1 mitotic checkpoint serine/threonine kinase B; PCa, prostate cancer.



**Table 3** Prognostic value of BUB1B expression for the biochemical recurrence-free survival in univariate and multivariate analyses by Cox regression

Factors	Biochemical recurrence-free survival		Overall survival	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Univariate				
BUB1B	3.482 (1.665–7.283)	0.001	1.947 (0.592–6.412)	0.273
Gleason score	3.351 (2.379–4.720)	0.000	1.200 (0.537–2.679)	0.657
Preoperative PSA	1.005 (1.001–1.008)	0.010	1.006 (1.001–1.011)	0.014
Pathological tumor stage	5.221 (2.559–10.655)	0.000	0.623 (0.154–2.523)	0.507
Multivariate				
BUB1B	2.209 (1.016–4.847)	0.045	1.690 (0.145–19.754)	0.676
Gleason score	2.228 (1.481–3.352)	0.000	1.671 (0.564–4.952)	0.354
Preoperative PSA	1.005 (1.001–1.010)	0.007	1.005 (1.000–1.010)	0.056
Pathological tumor stage	3.137 (1.323–7.441)	0.009	0.542 (0.081–3.635)	0.528

**Abbreviations:** BUB1B, BUB1 mitotic checkpoint serine/threonine kinase B; CI, confidence interval; PSA, prostate-specific antigen.

human cancer. On the one hand, approximately 31% of human colon adenocarcinomas show a decreased level of BUB1B, compared with adjacent noncancerous tissues.<sup>12</sup> Colon cancer with reduced BUB1B mRNA levels is more likely to have lymph node metastasis and short relapse-free survival time after the surgery.<sup>28</sup> On the other hand, more than 50% of gastric cancers show a high expression of BUB1B, which correlates with DNA aneuploidy and poor prognosis.<sup>15</sup> Similarly, ~45% of hepatocellular carcinomas have BUB1B overexpression.<sup>18</sup> In pancreatobiliary tumors, BUB1B expression independently predicts poor prognosis, especially in small tumors.<sup>29</sup> There are only a few reports focused on the expression of BUB1B in PCa. A panel of seven genes that include BUB1B are differentially expressed in PCa after the patients received docetaxel and androgen-deprivation treatments, which have prognostic values for advanced PCa.<sup>30</sup> In addition, it has been proposed that Ki67 and expression of BUB1B may serve as sensitive markers for identifying clinically insignificant PCa.<sup>31</sup>

Herein, we demonstrated that the expression of BUB1B was higher in malignant prostate tissues than in nonmalignant prostate tissues. Although the overexpression of BUB1B increased cell proliferation and migration, depletion of BUB1B did not affect both cell proliferation and migration. The results demonstrate the dosage-dependent activity in the gain-of-function experiments. However, in the loss-of-function setting, we did not see any obvious effect due to depletion of BUB1B expression. It is possible that other redundant counterparts may compensate the loss of BUB1B. Further investigation is needed to unravel the culprits.

Our data derived from the TMA show that a high percentage of PCa with a Gleason score >7 had strong BUB1B expression, which was consistent with the results derived from statistical analyses of the online Taylor dataset. Both

analyses showed the association of high BUB1B expression with clinicopathological properties, high serum PSA level, and high Gleason score. In addition, it is also associated with metastasis and short biochemical recurrence and survival time. Furthermore, analyses of the data with the Cox proportional hazards regression model revealed that the overexpression of BUB1B can be an independent biomarker for PCa prognosis.

Bioinformatics analyses of the Taylor dataset revealed that the expression of BUB1B positively correlated with 166 genes and negatively correlated with 44 genes with a correlation coefficient >0.5 (data not shown). Most of them were involved in the regulation of cell division process, including mitosis, cell cycle, organelle fission, nuclear division, and chromosome segregation. Pathway analyses showed that these genes are mainly involved in the p53, cell cycle, DNA replication, and Wnt pathways. Further analyses are needed to determine how BUB1B regulates these pathways and how aberrant BUB1B expression leads to deregulation of these signaling pathways.

## Conclusion

Our report suggests that the overexpression of BUB1B in PCa cells promotes cell proliferation and migration. Furthermore, high expression levels of BUB1B in PCa are associated with poor clinicopathological features of PCa and predict poor outcomes of patients with advanced PCa. The results suggest that the overexpression of BUB1B is a potential biomarker for PCa diagnosis and prognosis.

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## Disclosure

The authors report no conflicts of interest in this work.

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