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ORIGINAL RESEARCH

Association of doublecortin-like kinase 1 with tumor aggressiveness and poor biochemical recurrence-free survival in prostate cancer

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Background: Doublecortin-like kinase 1 (DCLK1) has been proven to be involved in numerous tumors, while its role in prostate cancer (PCa) is still unclear. This study aimed at investigating the expression pattern and prognostic value of DCLK1 in PCa.

Patients and methods: Real-time polymerase chain reaction and Western blot were employed to determine DCLK1 mRNA and protein levels in 25 paired fresh samples of PCa and benign prostatic hyperplasia (BPH) as well as in PCa cell lines. Immunohistochemistry (IHC) was also performed in 125 PCa and 65 BPH tissues to assess DCLK1 expression. Then, the association of DCLK1 expression with clinicopathological parameters and biochemical recurrence (BCR) after radical prostatectomy was statistically analyzed. In addition, the role of DCLK1 in PCa cell proliferation, migration, and invasion was evaluated by using MTT and transwell assays.

Results: The mRNA and protein levels of DCLK1 were markedly higher in the fresh samples of PCa than that in BPH. Consistently, IHC revealed increased expression of DCLK1 in PCa paraffin-embedded tissues compared with BPH. Moreover, increased DCLK1 expression was significantly associated with postoperative Gleason grading (P=0.012), pathological T stage (P=0.001), seminal vesicle invasion (P=0.026), and lymph node involvement (P=0.017), respectively. The Kaplan–Meier curve analysis demonstrated that high DCLK1 expression was associated with lower postoperative BCR-free survival (bRFS). Furthermore, multivariate Cox analysis showed that postoperative Gleason grading (P=0.018), pathological T stage (P<0.001), seminal vesicle invasion (P=0.012), lymph node involvement (P=0.014), and DCLK1 expression (P=0.014) were independent predictors of BCR. In vitro, the overexpression and knockdown of DCLK1 in PCa cell lines indicated that DCLK1 could promote cell proliferation, migration, and invasion.

Conclusion: Increased DCLK1 expression is associated with PCa aggressiveness and may independently predict poor bRFS in patients with PCa.

Keywords: prostatic neoplasm, DCLK1, BCR, prognosis, radical prostatectomy, biomarker

Introduction

The incidence of prostate cancer (PCa) in China is increasing steadily in recent years, with estimated 60,300 new cases and 26,600 deaths of PCa in 2015.[1] As a clinically heterogeneous multifocal disease, up to 40% of PCa treated by radical prostatectomy (RP) may undergo biochemical recurrence (BCR) at a long-term follow-up.[2] Although diagnostic tools, including prostate-specific antigen (PSA), pathological T stage, and Gleason score, were traditionally recognized as prognostic factors for PCa, their accuracies may have limitations.[3] Therefore, novel effective prognostic biomarkers for

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BCR after RP are required to provide valuable information on PCa risk, recurrence, and prognosis clinically.

Doublecortin-like kinase 1 (DCLK1) is a serine/threonine protein kinase that belongs to the family of microtubule-associated proteins. DCLK1 was initially reported to play a pivotal role in neuronal migration and neurogenesis.[4] Accumulating evidences have demonstrated the overexpression of DCLK1 in numerous human cancers, including colon,[5,6] pancreatic,[6] liver,[7] kidney,[8] and esophageal cancers.[9] DCLK1 has also been proposed as a marker of intestinal and pancreatic cancer stem cells (CSCs), and its overexpression is critical for cancer initiation, growth, stemness, epithelial–mesenchymal transition (EMT), and metastasis.[5–8] However, to the best of our knowledge, little is known about the association between DCLK1 and PCa; the expression and prognostic implications of DCLK1 in PCa have not yet been fully elucidated.

In the present study, quantitative real-time polymerase chain reaction (qRT-PCR), Western blot, and immunohistochemistry (IHC) were performed to assess DCLK1 expression in tissue samples and PCa cell lines. We then analyzed the association between DCLK1 expression and clinicopathological features of the patients who underwent RP. We, for the first time, investigated the role of DCLK1 in PCa cell proliferation, migration, and invasion. Overall, our study revealed that DCLK1 expression was significantly upregulated in PCa compared with benign prostatic hyperplasia (BPH). The increased DCLK1 expression was associated with PCa tumor aggressiveness and might efficiently predict poor BCR-free survival (bRFS) in patients after RP.

Table 1 Correlation between DCLK1 expression and clinicopathological parameters of the 125 patients with PCa

Variables	Total (%)	DCLK1 expression		*P*-value
		Low (%)	High (%)	
All cases	125 (100)	53 (100)	72 (100)	–
Age (years)				0.799
<68	62 (48.8)	27 (50.9)	35 (48.6)	
≥68	63 (51.2)	26 (49.1)	37 (51.4)	
Preoperative PSA (ng/mL)				0.066
<10	21 (16.8)	14 (26.4)	7 (9.7)	
10–20	47 (37.6)	18 (34.0)	29 (40.3)	
>20	57 (45.6)	21 (39.6)	36 (50.0)	
Postoperative Gleason grading				0.012
Grade group 1	53 (42.4)	30 (56.6)	23 (32.0)	
Grade groups 2 and 3	38 (30.4)	12 (22.6)	26 (36.0)	
Grade groups 4 and 5	34 (27.2)	11 (20.8)	23 (32.0)	
Pathological T stage				0.001
pT2	75 (60.0)	40 (75.5)	35 (48.6)	
pT3a	33 (26.4)	10 (18.9)	23 (32.0)	
pT3b	17 (13.6)	3 (5.6)	14 (19.4)	
Seminal vesicle invasion				0.026
Yes	17 (13.6)	3 (5.66)	14 (19.44)	
No	108 (86.4)	50 (94.34)	58 (80.56)	
Lymph node involvement				0.017
Yes	24 (19.2)	5 (9.4)	19 (26.4)	
No	101 (80.8)	48 (90.6)	53 (73.6)	
Positive surgical margin				0.909
Yes	23 (18.4)	10 (18.9)	13 (18.1)	
No	102 (81.6)	43 (81.1)	59 (81.9)	

Abbreviations: DCLK1, doublecortin-like kinase 1; PCa, prostate cancer; PSA, prostate-specific antigen.

Patients and methods

Patients and tissue samples

A total of 125 archived paraffin-embedded tissues from PCa patients after RP and 65 BPH tissues from transurethral resection of the prostate were obtained from the Department of Pathology, the Third Affiliated Hospital of Sun Yat-Sen University, between January 2008 and June 2010. None of the PCa patients had received chemotherapy, radiotherapy, or hormonal therapy prior to the RP. All the tissues were processed in a comparable manner and histologically reviewed by two pathologists. The tumors were staged by the American Joint Committee on Cancer 8th Edition Prostate Cancer Staging Classification[10] and graded according to the 2014 International Society of Urological Pathology guidelines.[11] The patients were followed up from the date of surgery to the date of death or the last follow-up, and BCR was defined as two consecutive serum PSA levels >0.2 ng/mL after RP.[12] Demographic and clinicopathological data of the patients were obtained from their medical records and reassessed (Table 1). Besides, for qRT-PCR and Western blot analysis, 25 paired fresh PCa and BPH specimens were collected randomly and stored in liquid nitrogen immediately after the surgery between January 2016 and December 2016. This study was approved by the Ethics Committee of the Third Affiliated Hospital of Sun Yat-Sen University (Number: [2015] 2-130), with a Research Registry Unique Identifying Number (researchregistry2902). Written consent from the study population was considered, but as this was a retrospective study where most of the materials were >5 years old and all data were analyzed anonymously, it was considered not needed.

IHC and scoring

Formaldehyde-fixed and paraffin-embedded specimens were cut into 4-μm serial sections. Briefly, tissue sections were deparaffinized in xylene, rehydrated with ethanol, and then incubated in 3% hydrogen peroxide for 10 minutes to halt endogenous peroxidase activity. After being heated in

0.01 M citrate buffer (pH=6.0) for 15 minutes at 100°C in a microwave oven to repair the tissue antigen, the sections were incubated overnight at 4°C with polyclonal rabbit anti-DCLK1 antibody (1:100, AP7219b; Abgent, San Diego, CA, USA), followed by secondary antibody incubation for 30 minutes at 37°C. The sections were semi-quantitatively scored according to a previous report[13] as follows: staining intensity was scored as 0 (no staining), 1 (faint/equivocal or focal staining), 2 (intermediate staining), and 3 (bright staining), multiplied by the percentage of cells at each immunostaining level, resulting in a total score range of 0–300. The total DCLK1 expression score of each section was defined as follows: 0, negative; 1–100, weak; 101–200, moderate; and 201–300, strong. For further analysis, the IHC result of each patient was finally considered as low DCLK1 expression (total expression score, 0–100) or high DCLK1 expression (total expression score, 101–300).

Cell lines and cell culture

Human PCa cell lines (LNCaP, PC3, DU145, and 22Rv1) and a prostatic normal epithelial cell line (RWPE-1) were obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). The PCa cells were cultured in Dulbecco's Modified Eagle's Medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS), 100 μg/mL streptomycin, and 100 U/mL penicillin in a humidified incubator at 37°C containing 5% CO_2, while RWPE-1 cells were cultured in a defined keratinocyte serum-free medium with growth supplement (Thermo Fisher Scientific) in the same condition mentioned above.

RNA extraction and qRT-PCR

Total RNA from tissues and cells was extracted by using TRIzol reagent (Invitrogen, Grand Island, NY, USA) following the manufacturer's instructions. The cDNA was synthesized from RNA samples with the Transcriptor First-Strand cDNA Synthesis Kit (Roche, Mannheim, Germany). qRT-PCR analysis was performed on LightCycler® 480 System (Roche) using an SYBR Green I Master Kit (Roche). A human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an endogenous control for sample normalization. The primers used were as follows: forward: 5′-CTGGGCT ACACTGAGCACC-3′ and reverse: 5′-AAGTGGTCGTT GAGGGCAATG-3′ for human GAPDH; and forward: 5′-CAGCAACCAGGAATGTATTGGA-3′ and reverse: 5′-CTCAACTCGGAATCGGAAGACT-3′ for human DCKL1. The relative DCKL1 mRNA levels were analyzed by calculating the $2^{-\Delta\Delta Ct}$ values.

Western blot

Total proteins were extracted by using radioimmunoprecipitation assay (Cell Signaling Technology, Danvers, MA, USA) containing protease inhibitors. Protein levels were measured by Bradford method with the BCA Protein Assay Kit (Thermo Fisher Scientific). Equal amounts of 40 μg respective tissue proteins were separated by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then transferred to polyvinylidene fluoride membranes (Millipore Corporation, Billerica, MA, USA). After blocked by 5% nonfat milk for 1 hour, the membranes were incubated overnight at 4°C with primary antibodies, including anti-DCLK1 antibody (1:1,000; Abgent AP7219b), anti-GAPDH antibody (1:2,000; Cell Signaling Technology 2118S), monoclonal mouse anti-E-cadherin antibody (1:2,000, 60335-1-Ig; Proteintech Group, Rosemont, IL, USA), monoclonal rabbit anti-N-cadherin antibody (1:2,000, D4R1H; Cell Signaling Technology), and monoclonal rabbit anti-vimentin antibody (1:2,000, D21H3; Cell Signaling Technology). Horseradish peroxidase-conjugated secondary antibodies were used to detect the primary antibodies. Protein bands were finally visualized by using FluorChem M system (ProteinSimple, San Jose, CA, USA).

Overexpression and silencing of DCLK1

The siRNA specifically targeting DCLK1 (si-DCLK1-1: 5′-CGGAGAUCGAUACUUCAAAGG-3′, si-DCLK1-2: 5′-CAGAGGUGCGAGAGAAUAAGG-3′) and scrambled nucleotide used as a negative control were commercially constructed (GenePharma, Shanghai, China). The plasmids were constructed as previously described[14] to create transient tumor cells overexpressing DCLK1. The cells were plated and cultured until cell density reached 70%–80% prior to plasmids or siRNA transfection using Lipofectamine™ 3000 Transfection Reagent (Thermo Fisher Scientific) following the manufacturer's protocol. The assays were conducted 48 hours posttransfection.

Cell proliferation assay

The effects of DCLK1 overexpression and inhibition on PCa cell proliferation were examined by MTT assay (KeyGEN BioTECH, Nanjing, China). The cells transfected with plasmids or siRNA were seeded in 96-well plates at a density of 3,000 cells/well. The optical density was measured by using an automatic microplate reader (Infinite M200, TECAN, Männedorf, Switzerland) at 490 nm.

In vitro cell migration and invasion assays

The 24-well transwell chamber (Corning Incorporated, Corning, NY, USA) coated without or with Matrigel® was used to measure the ability of in vitro cell migration and invasion; 200 µL cell suspension (3×10^4 cells) was loaded into the upper chamber, and 0.5 mL medium supplemented with 10% FBS was added into the lower chamber. After 24 hours, the invaded cells were fixed, stained, and visualized by microscope (Olympus Optical, Tokyo, Japan). Five randomly selected fields were counted per chamber, and the results were repeated at least thrice in triplicate.

Statistical analysis

Statistical analysis was performed by using Statistical Package for the Social Science Version 20.0 (IBM Corporation, Armonk, NY, USA). Continuous parametric variables were presented as mean ± SD and compared by using Student's *t*-test. The association between DCLK1 expression and clinicopathological variables was examined by Spearman correlation coefficient. The bRFS was analyzed by using the Kaplan–Meier method followed by log-rank test. Univariate and multivariate analyses using Cox proportional hazard regression model were employed to identify independent prognostic predictors of BCR. All tests of significance were two-sided, and $P<0.05$ indicated statistical significance.

Results

Increased DCLK1 expression in PCa fresh samples

DCLK1 mRNA expression was assessed in 25 fresh PCa samples and paired BPH samples by qRT-PCR assay. On average, the relative DCLK1 mRNA level was significantly higher in tumor tissues than in BPH tissues ($P<0.001$; Figure 1A). Western blot analysis was performed simultaneously in the 25 pairs of fresh samples. Consistent with the results of qRT-PCR assay, DCLK1 protein expression was observed to be significantly elevated in PCa tissues compared with BPH ($P<0.001$; Figure 1B and C).

DCLK1 expression in PCa cell lines

As shown in Figure 2, DCLK1 mRNA expression was markedly increased in PCa cell lines compared with the normal epithelial cell line RWPE-1 ($P<0.01$; Figure 2A). Similarly, Western blot indicated that metastatic PCa cell lines (LNCaP, PC3, and DU145) had higher DCLK1 protein expression level than RWPE-1 and localized PCa cell 22Rv1, with the highest level in PC3 ($P<0.05$; Figure 2B and C).

DCLK1 enhances PCa cell proliferation, migration, and invasion

To confirm the role of DCLK1 during PCa development, we altered its expression by the transfection of eukaryotic

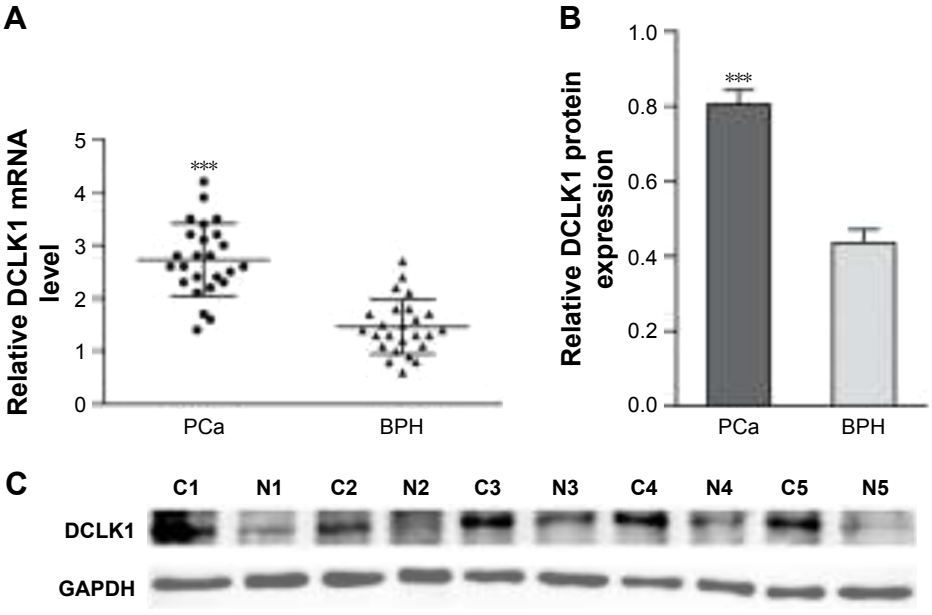

Figure 1 qRT-PCR and Western blot analysis of DCLK1 expression in fresh PCa (n=25) and BPH (n=25) tissues.

Notes: (**A**) Relative DCLK1 mRNA level was significantly higher in PCa tissues compared with BPH. Horizontal lines represent mean with SD. (**B**) Relative protein expression level of DCLK1/GAPDH detected by Western blot was markedly increased in PCa tissues. (**C**) Representative Western blot of DCLK1 protein in five paired PCa (C) and BPH (N) tissues. Data are presented as the mean ± SD; ***$P<0.001$.

Abbreviations: BPH, benign prostatic hyperplasia; DCLK1, doublecortin-like kinase 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; qRT-PCR, quantitative real-time polymerase chain reaction; PCa, prostate cancer.

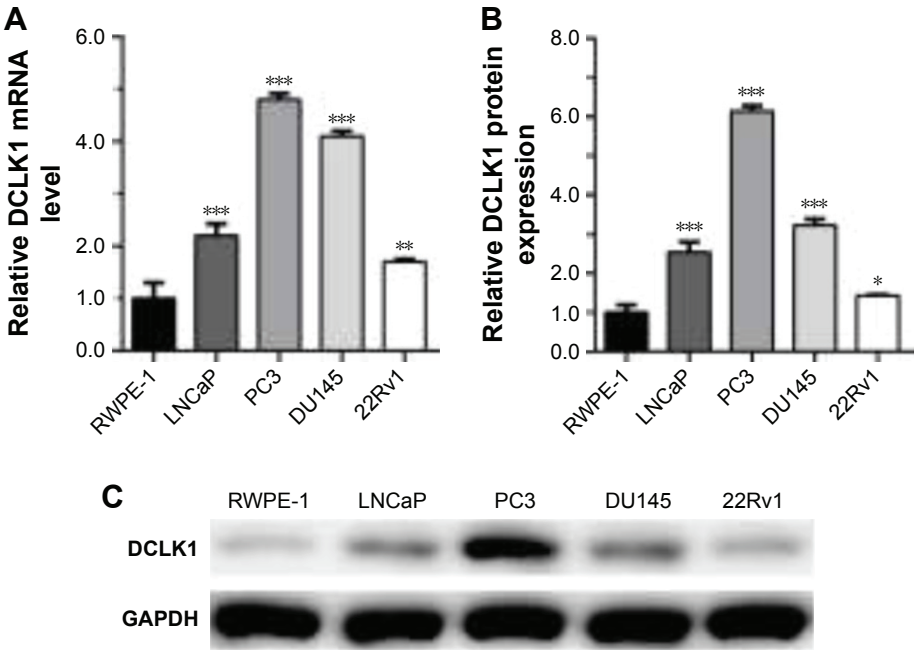

Figure 2 qRT-PCR and Western blot analysis of DCLK1 expression in PCa cell lines.
Notes: (**A** and **B**) Relative DCLK1 mRNA and protein expression levels were markedly increased in PCa cell lines (LNCaP, PC3, DU145, and 22Rv1) compared with the normal epithelial cell RWPE-1. (**C**) Representative Western blot of DCLK1 protein in cell lines. Data are presented as the mean ± SD; *$P<0.05$, **$P<0.01$, and ***$P<0.001$.
Abbreviations: DCLK1, doublecortin-like kinase 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; qRT-PCR, quantitative real-time polymerase chain reaction; PCa, prostate cancer.

overexpression of plasmid and specific siRNA in two PCa cell lines 22Rv1 and PC3, respectively. Overexpression and knockdown of DCLK1 were confirmed by Western blot analysis (Figures 3A and 4A). MTT assays showed that DCLK1 overexpression promoted 22Rv1 cell proliferation, while the knockdown exhibited a contrary effect in PC3 cells ($P<0.001$; Figures 3B and 4B). In accordance with MTT assays, transwell assays demonstrated that increased DCLK1 enhanced 22Rv1 cell migration and invasion, whereas downregulation inhibited them in PC3 cells ($P<0.001$; Figures 3C and 4C). Taken together, there is an obvious positive regulation of proliferation, migration,

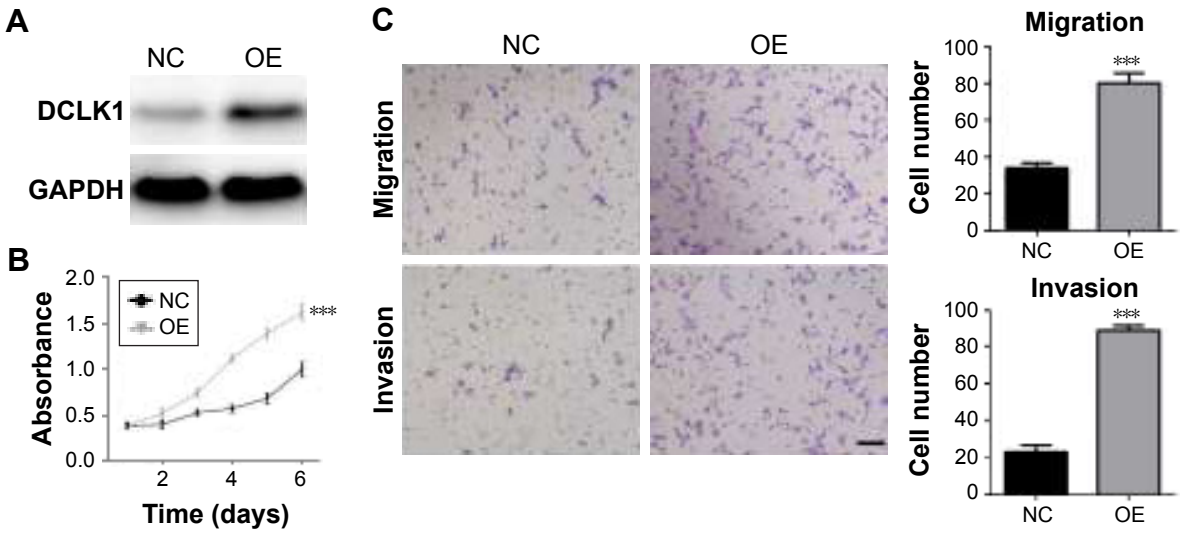

Figure 3 DCLK1 overexpression enhances cell proliferation, migration, and invasion in 22Rv1 cells.
Notes: (**A**) Western blot analysis confirmed DCLK1 overexpression in 22Rv1 cells. (**B**) MTT assays showed that DCLK1 overexpression promoted 22Rv1 cell proliferation. (**C**) Transwell assays demonstrated that increased DCLK1 enhanced 22Rv1 cell migration and invasion (magnification ×100). Data are presented as the mean ± SD; ***$P<0.001$.
Abbreviations: DCLK1, doublecortin-like kinase 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NC, negative control; OE, overexpression.

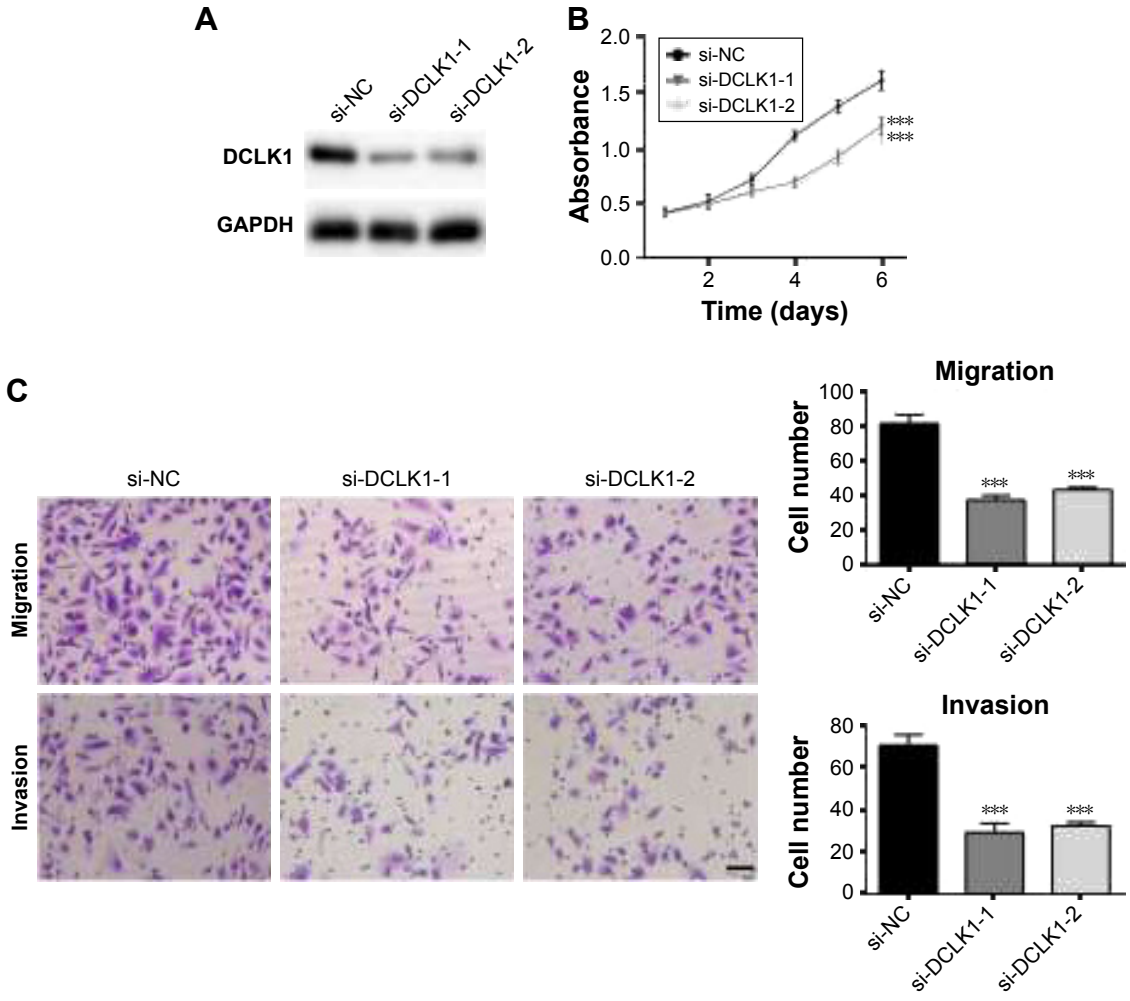

Figure 4 DCLK1 downregulation inhibits cell proliferation, migration, and invasion in PC3 cells.
Notes: (**A**) Western blot analysis confirmed DCLK1 downregulation in PC3 cells. (**B**) MTT assays showed that DCLK1 downregulation inhibited PC3 cell proliferation. (**C**) Transwell assays demonstrated that decreased DCLK1 inhibited PC3 cell migration and invasion (magnification ×100). Data are presented as the mean ± SD; ***$P<0.001$.
Abbreviations: DCLK1, doublecortin-like kinase 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NC, negative control.

and invasion by DCLK1 expression in PCa cells. Considering that DCLK1 played an important role during EMT in various other tumors, we further evaluated the effects of DCLK1 on EMT markers in PCa cell lines. The Western blot analysis demonstrated that DCLK1 knockdown increased the protein expression of E-cadherin and reduced the expression of N-cadherin and vimentin in PC3 cells ($P<0.001$; Figure 5A and C), while the overexpression exhibited a contrary effect in 22Rv1 cells ($P<0.001$; Figure 5A and B). The results indicated that DCLK1 may promote tumor aggressiveness via the EMT mechanism in PCa.

Overexpressed DCLK1 in PCa tissues compared with BPH as determined by IHC

Positive immunoreactivity for DCLK1 was observed in the cytoplasm and nuclei of epithelial cells at various intensities and distribution levels (Figure 6A). The positive expression rates of DCLK1 in PCa and BPH tissues were 92.8% (116 of 125) and 52.3% (34 of 65; $P<0.001$), respectively. Of the 116 PCa samples with positive DCLK1 expression, 35.2% (44 of 125) cases exhibited weak staining, 42.4% (53 of 125) moderate staining, and 15.2% (19 of 125) strong staining (Figure 6B). In contrast, 38.5% (25 of 65) BPH samples demonstrated weak staining, 12.3% (8 of 65) moderate staining, and 1.5% (1 of 65) strong staining (Figure 6B). The rate of patients with high DCLK1 expression (total expression score=101–300) was significantly higher in PCa than in BPH (57.6% vs 13.8%; $P<0.001$).

Association between DCLK1 expression and clinicopathological parameters of PCa patients

As demonstrated in Table 1, DCLK1 expression was found to be significantly correlated with postoperative Gleason grading ($P=0.012$), pathological T stage ($P=0.001$), seminal vesicle

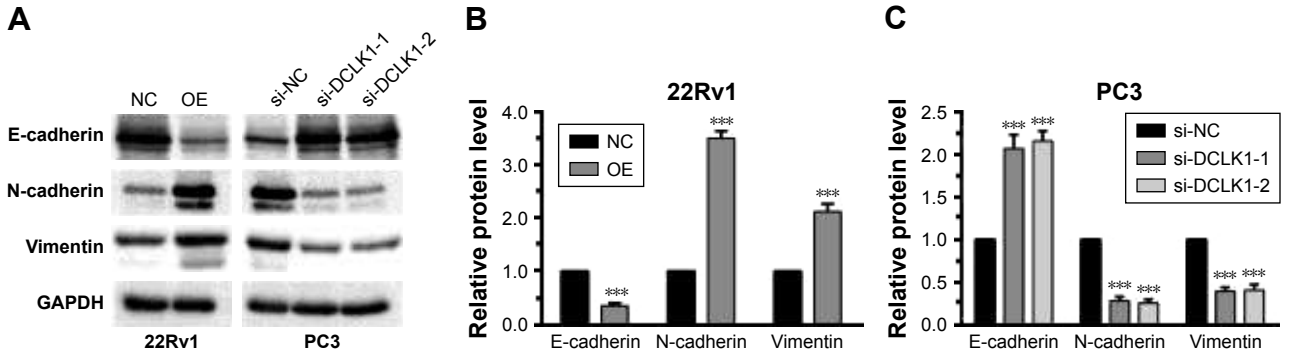

Figure 5 Western blot analysis of markers of EMT in 22Rv1 and PC3 cells after transfected with plasmid or siRNA.
Notes: (**A**) Representative Western blot bands of EMT-associated proteins. (**B**) DCLK1 overexpression inhibited the protein expression of E-cadherin but upregulated the expression of N-cadherin and vimentin in 22Rv1 cells. (**C**) DCLK1 knockdown increased the protein expression of E-cadherin while reduced the expression of N-cadherin and vimentin in PC3 cells. Data are presented as the mean ± SD; ***$P<0.001$.
Abbreviations: DCLK1, doublecortin-like kinase 1; EMT, epithelial–mesenchymal transition; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NC, negative control; OE, overexpression.

invasion (P=0.026), and lymph node involvement (P=0.017). However, no significant correlation was observed between DCLK1 expression and age (P=0.799), preoperative PSA (P=0.066), as well as surgical margin status (P=0.909).

Predictive value of DCLK1 expression on BCR

The median follow-up was 6.4 years with a maximum follow-up of 9.6 years. During the follow-up period, 41.7% (30 of 72) PCa patients with high DCLK1 expression demonstrated BCR, while the BCR rate in patients with low DCLK1 expression was 13.2% (7 of 53; P=0.001). As demonstrated in Table 2, both univariate and multivariate Cox proportional hazard analyses indicated that postoperative Gleason grading, pathological T stage, seminal vesicle invasion, lymph node involvement, and DCLK1 expression were independent predictors of BCR after RP. Since DCLK1 expression was strongly associated with postoperative Gleason grading, pathological T stage, seminal vesicle invasion, and lymph node involvement, data were further examined by stratified models, aiming at confirming predictive value independent of the parameters mentioned above. We also performed the Cox regression analysis according to the subgroups of the associated parameters (data not shown). The P-values for DCLK1 expression in BCR prediction in these stratified factors were 0.038 (postoperative Gleason grading), 0.013 (pathological T stage), 0.013 (seminal vesicle invasion), and 0.016 (lymph node involvement), respectively, which suggested that DCLK1 was an independent predictor of BCR. As shown in Figure 6C, patients with high DCLK1 expression had remarkably lower bRFS than those with low DCLK1 expression. The 5-year bRFS rates for patients with high DCLK1 expression and those with low DCLK1 expression were 58.9% and 88.4%, respectively (P<0.001).

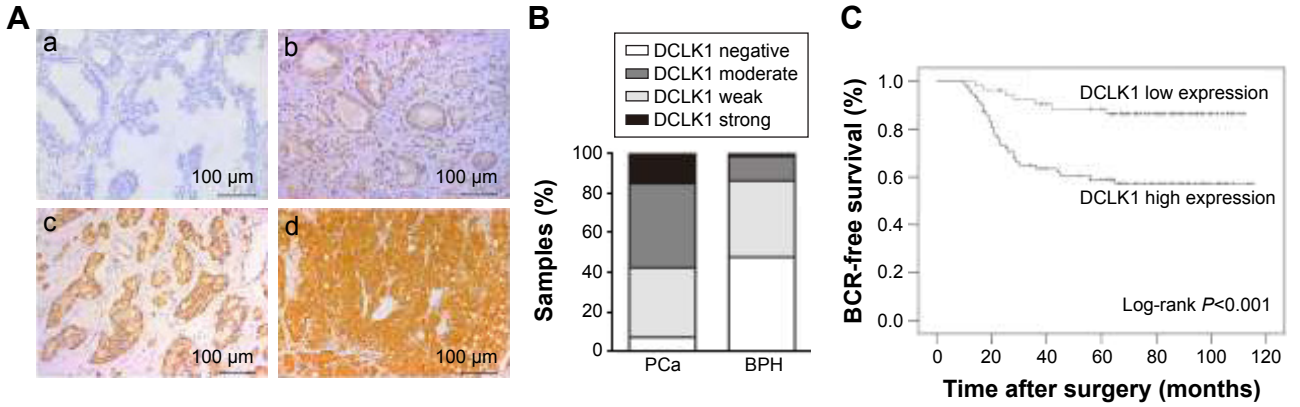

Figure 6 DCLK1 expression determined by IHC and its correlation with BCR-free survival.
Notes: (**A**) Representative images of DCLK1 staining in BPH and PCa: (a) BPH tissue showed no DCLK1 staining; (b–d) PCa tissues showed faint/equivocal or focal (b), intermediate (c), and bright (d) DCLK1 staining, respectively (magnification ×200). (**B**) Distribution of DCLK1 total expression score for PCa (n=125) and BPH (n=65) tissues. (**C**) Kaplan–Meier survival curves of BCR-free survival based on DCLK1 expression in patients with PCa.
Abbreviations: BCR, biochemical recurrence; BPH, benign prostatic hyperplasia; DCLK1, doublecortin-like kinase 1; IHC, immunohistochemistry; PCa, prostate cancer.

Table 2 Univariate and multivariate analyses of prognostic factors for biochemical recurrence

Variables	Univariate		Multivariate	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (years)		0.550	–	–
<68	1 (reference)			
≥68	1.22 (0.64–2.33)			
Preoperative PSA (ng/mL)		0.689	–	–
<10	1 (reference)			
10–20	1.29 (0.46–3.58)	0.626		
>20	1.52 (0.57–4.11)	0.404		
Postoperative Gleason grading		0.039		0.018
Grade group 1	1 (reference)		1 (reference)	
Grade groups 2 and 3	1.82 (0.79–4.22)	0.161	2.67 (1.11–6.45)	0.029
Grade groups 4 and 5	2.83 (1.27–6.30)	0.011	3.19 (1.39–7.34)	0.006
Pathological T stage		<0.001		<0.001
T2	1 (reference)		1 (reference)	
T3a	4.86 (2.14–11.03)	<0.001	3.87 (1.60–9.32)	0.003
T3b	17.90 (7.22–44.38)	<0.001	19.96 (6.91–57.65)	<0.001
Seminal vesicle invasion		<0.001		0.012
No	1 (reference)		1 (reference)	
Yes	4.68 (2.34–9.39)		2.54 (1.23–5.28)	
Lymph node involvement		<0.001		0.014
No	1 (reference)		1 (reference)	
Yes	3.86 (1.98–7.55)		2.40 (1.20–4.83)	
Positive surgical margin		0.849	–	–
No	1 (reference)			
Yes	0.92 (0.38–2.20)			
DCLK1 expression		0.001		0.014
Low	1 (reference)		1 (reference)	
High	4.00 (1.75–9.12)		2.98 (1.25–7.14)	

Abbreviations: DCLK1, doublecortin-like kinase 1; HR, hazard ratio; PSA, prostate-specific antigen.

Discussion

Although PSA remains the most generally used biomarker for PCa screening and diagnosis, its sensitivity and specificity are limited, which may lead to false-positive diagnoses and excessive treatment.[15] Localized PCa patients with similar clinicopathological parameters and therapeutic regimen may yet present different clinical outcomes, such as BCR and tumor metastasis. In other words, as the heterogeneous genetic background of PCa, its biological and clinical behavior cannot be reliably predicted by conventional diagnostic or prognostic markers.[16] Therefore, more efforts are required to seek novel and effective biomarkers of initiation, progression, and prognosis in PCa.

To date, the expression of DCLK1 in PCa has been previously mentioned in only two publications.[13,17] The IHC analyses on human multicancer tissue microarrays performed by Sureban et al[17] showed that minimal or no DCLK1 immunoreactivity was detected in normal prostate, while overexpressed DCLK1 was found in the cytoplasm of PCa epithelial cells. In addition, Roudier et al[13] revealed that DCLK1 was upregulated at the protein level in unpaired ERG + primary PCa and metastatic castration-resistant PCa. However, the association between DCLK1 expression and the clinicopathological features of PCa patients, as well as the prognostic implication of DCLK1 in PCa, has not yet been clarified.

In the present study, we found that DCLK1 expression was markedly elevated in PCa tissues compared with BPH. Similarly, DCLK1 expression was remarkably increased in PCa cell lines than in normal prostatic epithelial cell. Although PC3 and DU145 demonstrated similar DCLK1 mRNA level, their protein expression showed a divergence, which may resulted from different processes of posttranslational modification. In PCa cell lines, DCLK1 enhanced cell proliferation, migration, and invasion, and its expressions in metastatic cells were significantly increased compared with localized cells, suggesting that DCLK1 may promote PCa metastasis. We also found that upregulated DCLK1 was associated with advanced clinicopathological features in patients with PCa. Furthermore, we identified DCLK1 as an independent predictor of BCR after RP. These results suggest that the upregulation of DCLK1 indicates biologically more

aggressiveness and may be an underlying initiating factor of PCa development. Accordingly, the expression level of DCLK1 may contribute to differentiating PCa from BPH and accurately identifying patients who would benefit from early intervention.

The molecular mechanisms that account for the association between DCLK1 overexpression and PCa aggressiveness and BCR are unclear. However, as a marker of intestinal and pancreatic CSCs, DCLK1 has been proposed to be critical for cancer initiation, growth, EMT, and metastasis of various other cancers.[5–8] Strikingly, the critical signaling pathways NF-κB, WNT, PTEN/PI3K/AKT, and NOTCH that are involved in both CSCs and EMT have been revealed to be regulated by DCLK1 expression in cancer models.[5,17–19] Previously, the presence of CSCs has also been demonstrated in PCa, which can initiate tumor by differentiating into heterogeneous lineages of cancer cells that comprise the whole tumor.[20] Furthermore, direct inhibition of WNT, NOTCH, PTEN/PI3K/AKT, and other cell signaling pathways has resulted in tumor suppressive effect via lowering the population of prostate CSCs.[21,22] To be note, BCR represents an elevated PSA value, and the expression of PSA is principally activated by androgen and regulated by the androgen receptor (AR) signaling at the transcriptional level. Accumulating researches have indicated that AR is a downstream regulatory factor of the critical cell signaling pathways mentioned above. For instance, one coactivator that remarkably influences the transcriptional activity of human AR is β-catenin, which is a pivotal molecule in the canonical WNT signaling pathway.[23] In addition, Yang et al[24] found that the WNT signaling pathway could modulate the levels of human AR-encoded protein through an ubiquitin-mediated degradation mechanism controlled by AKT/protein kinase B signaling. Moreover, the PI3K-AKT-mTOR pathway is thought to be dominant over AR signaling in PCa cells,[25] and both AKT activation and PTEN loss are associated with BCR following RP.[26,27] In the present study, we have also proved that DCLK1 could regulate the markers of EMT in PCa cells. Taken together, we speculated that DCLK1 may promote tumor initiation, growth, progression, and metastasis of PCa by the enhancement of CSCs and EMT properties, and upregulated DCLK1 might promote BCR via AR signaling activated by WNT or PTEN/PI3K/AKT signaling pathway. However, further investigations are still required to verify the abovementioned underlying mechanisms.

There are certain limitations in our study. First, it was just conducted as a retrospective analysis of the PCa cases from one single institution. Second, the sample volume of 125 PCa patients is relatively small as we strictly selected the participants to control the potential heterogeneity. However, it is enough to guarantee statistical significance. Third, research on the role of DCLK1-induced oncogenesis in PCa was absent in this study. Therefore, further studies are needed to elucidate the molecular mechanisms of DCLK1 expression in the tumorigenesis and progression of PCa.

Conclusion

In summary, we, for the first time, explored the expression pattern of DCLK1 in PCa and its correlation with clinicopathological features, as well as its prognostication of BCR after RP. Our data suggested that overexpressed DCLK1 in PCa tissues was significantly correlated with unfavorable clinicopathological characteristics and bRFS of patients. DCLK1 might serve as a potential biomarker for risk stratification and prognosis of patients with PCa, and it may help clinicians to render more rational and efficient therapeutic strategies for PCa patients.

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Disclosure

The authors report no conflicts of interest in this work.

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