High expression of citron kinase predicts poor prognosis of prostate cancer

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Received January 14, 2019; Accepted July 11, 2019

DOI: 10.3892/ol.2020.11254

Abstract. Citron kinase (CIT) is a Rho-effector protein kinase that is associated with several types of cancer. However, the role of CIT in prostate cancer (PCa) is unclear. The current study utilized microarray data obtained from The Cancer Genome Atlas, which was analyzed via Biometric Research Program array tools. Additionally, reverse transcription-quantitative (RT-q)PCR was performed to compare the mRNA expression of CIT in PCa tissue and in benign prostatic hyperplasia. The protein expression of CIT was detected in a consecutive cohort via immunochemistry and CIT was screened as a potential oncogene in PCa. The results of RT-qPCR demonstrated that the mRNA expression of CIT was increased in PCa tissues. Furthermore, immunochemistry revealed that CIT protein expression was positively associated with age at diagnosis, Gleason grade, serum PSA, clinical T stage, risk group, lymph node invasion and metastasis. When compared with the low expression group, patients with a high CIT expression exhibited shorter survival rates, cancer specific mortalities (CSM) and biochemical recurrence (BCR). In addition, multivariate analysis revealed that CIT was a potential predictor of CSM and BCR. The results revealed that CIT is overexpressed during the malignant progression of PCa and may be a predictor of a poor patient prognosis.

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Abbreviations: PCa, prostate cancer; BPH, benign prostatic hyperplasia; CIT, citron kinase; ADT, androgen deprivation therapy; PR, prostatectomy; PSA, prostate specific antigen; CSM, cancer specific morality; BCR, biochemical recurrence

Key words: prostate cancer, citron kinase, prognosis

Introduction

Prostate cancer (PCa) is one of the most common cancers worldwide (1), with an incidence rate that has increased in China in recent years (2). Prostate specific antigen (PSA) screening is a primary method for the surveillance of PCa. However, PSA exhibits a low specificity, which leads to the incorrect diagnoses and treatment of patients with PCa (3). Therefore, the discovery and identification of new biomarkers are essential for monitoring patients with PCa.

Citron-kinase (CIT) comprises an amino-terminal serine/threonine kinase domain, which is highly conserved between insects and mammals (4). It has been revealed that CIT is critical for cytokinesis (5,6). CIT is also involved in the cleavage of the furrow and midbody, which is essential to cellular abscission (7-9). Furthermore, CIT phosphorylates the regulatory light chain of myosin II at the Ser 19/Thr 18 positions, consequently activating myosin II, which is the primary motor protein and responsible for cytokinesis (10).

In the current study, increased expression of CIT was identified as an oncogene by bioinformatic analysis. This result was verified by reverse transcription-quantitative (RT-q)PCR and immunochemistry. The aim of the current study was to assess the role of CIT in PCa and to determine the possibility of using CIT in the diagnosis and therapy of patients with PCa.

Materials and methods

Dataset gene expression analysis. mRNA expression profiles and associated PCa clinical datasets (PRAD_2015_02_24) from The Cancer Genome Atlas (TCGA) were downloaded from the University of California Santa Cruz cancer genome browser (https://xena.ucsc.edu/welcome-to-ucsc-xena/). The profile contained 52 cases of normal tissue and 499 cases of primary PCa tissue. Microarray data were normalized and compared using Biometric Research Program (BRB) array tools developed by Dr Richard Simon and Dr Yingdong Zhao (http://linus.nci.nih.gov/BRB-ArrayTools) (11). Differentially expressed genes (DEGs) were filtered by comparing cancer

and normal tissue, Gleason grades ≥7 and Gleason grades <7, PSA ≥10 ng/ml and PSA <10 ng/ml, Ta-2 and T3-4, regional lymph node metastasis (N1) and no regional lymph nodes metastasis (N0), and metastasis to distant organs (M1) and no distant metastasis (M0). DEGs were defined as a fold-change (FC) >1 and P<0.01. Volcano plots were established to visualize the genes that were screened.

Patients and tissues. To determine the expression of CIT mRNA in patients with PCa, fresh PCa tissue (n=35) and benign prostatic hyperplasia tissue (BPH; n=20) were collected from the First Affiliated Hospital of Chongqing Medical University (Chongqing, China). All samples were confirmed by pathological examination and subsequently stored in liquid nitrogen (-196˚C) for mRNA analysis. Patient characteristics are shown in Table I.

Formalin fixed paraffin embedded BPH (n=39) and PCa (n=271) samples were retrieved from the Pathology Department of the First Affiliated Hospital of Chongqing Medical University, Zigong Fourth People's Hospital and Zigong First People's Hospital from 2005 to 2017. None of the patients recruited into the present study received chemotherapy, radiation therapy androgen deprivation (ADT) or radical prostatectomy (RP) prior to enrollment. The use of tissue was approved by the Ethics committee of the First Affiliated Hospital of Chongqing Medical University (approval no. 2018-69), Zigong Fourth People's Hospital (approval no. 2018-32) and Zigong First People's Hospital (approval no. 2018-47).

Patients were sub-divided into a high-risk group (HR-group) when one of the following criteria was met: i) Gleason grades ≥8; ii) T2c-T4 tumor or iii) PSA level ≥20 ng/ml (12). Patients that exhibited local invasion and metastasis were considered to have aggressive PCa (13). The Gleason score was evaluated according to the guidelines conducted by World Health Organization and the International Society of Urological Pathology (14,15). Moreover, patients with PCa were stratified into three grades including low, middle and high grade, which determined by a Gleason sum <5, between 5 to 7, and >7, respectively (16).

Patients who received RP were followed-up by a telephone call and patients who received ADT were monitored via continuous serum PSA surveillance (in 6-month intervals). The follow-up time of patients receiving ADT was 14.6±8.2 months with 54.2% patients being followed-up for more than one year. The follow-up time of patients receiving RP was 25±20.3 months, with 66.3% patients being followed-up for more than one year. Due to the different therapies administered and the follow-up methods used, follow-up outcomes were stratified to cancer-specific mortality (CSM) for patients receiving RP and biochemical recurrence (BCR) for patients receiving ADT. BCR was defined when patients exhibited a PSA level ≥0.2 ng/ml on at least two consecutive postoperative occasions, as described previously (17).

Immunohistochemistry. Tissues from the patients were fixed in 10% buffered formalin at room temperature for 2 days, and then were transferred to 70% ethanol overnight. The infiltrated tissues were embedded into paraffin blocks. A single 3-μm section was cut from each block. Immunochemistry and the assessment of immunoreactivity were performed as described previously (18). The sections were incubated with primary

Table I. Characteristics of prostate cancer patients.

Items	N (%)
Sample type	
Aggressive PCa	131 (48.34)
Primary PCa	140 (51.66)
Origin	
The First Affiliated Hospital of Chongqing Medical University	156 (57.56)
The Zigong No. 4 People's Hospital	53 (19.56)
The Zigong No. 1 People's Hospital	62 (22.88)
Age, years	
<70	25 (9.23)
70-79	148 (54.61)
≥80	98 (36.16)
Gleason score	
<7	88 (32.47)
7	89 (32.84)
≥8	94 (34.69)
PSA level	
<4	34 (12.55)
4-9.9	35 (12.92)
10-19.9	41 (15.13)
≥20	161 (59.40)
pT stage	
≤T2	150 (55.35)
T3	75 (27.68)
T4	46 (16.97)
pN stage	
N0	255 (94.10)
N1	16 (5.90)
pM stage	
M0	254 (93.73)
M1	17 (6.27)
Therapy	
ADT	88 (32.47)
PR	183 (67.53)
BCR after ADT	
No	15 (17.05)
Yes	68 (77.27)
Loss	5 (5.68)
CSM after RP	
No	137 (74.86)
Yes	35 (19.13)
Loss or death for other cause	11 (6.01)

PSA, prostate-specific antigen; BCR, biochemical recurrence; ADT, androgen deprivation therapy; RP, radical prostatectomy; CSM, cancer-specific morality; PCa, prostate cancer.

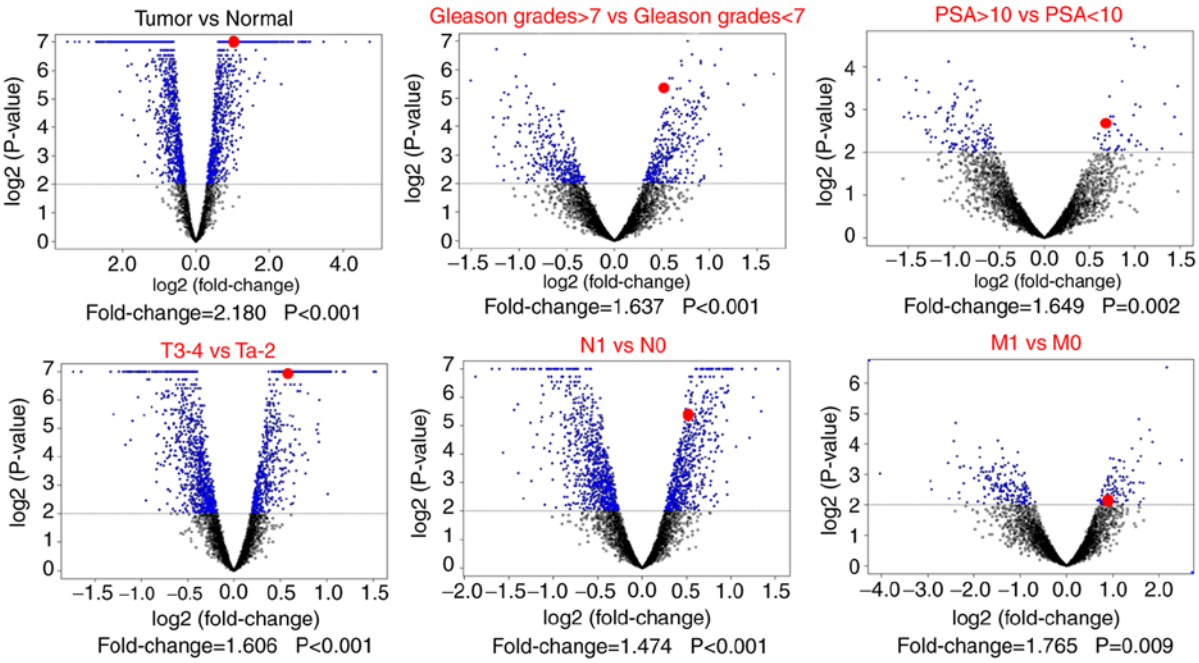

Figure 1. CIT is screened as a PCa-associated gene from the TCGA database. The fold-changes (log2 scale) of gene expression between different parameters were plotted on the y-axis and the P-values (log2 scale) of the FDR-corrected t-test were plotted on the x-axis. The screening of DEGs was based on the TCGA cohort (PRAD_2015_02_24) and is indicated by blue dots (P<0.01). CIT is indicated by red dots (P<0.01). CIT, citron kinase; PCa, prostate cancer; TCGA, The Cancer Genome Atlas; DEGs, differentially expressed genes; FDR, false discovery rate; PSA, prostate specific antigen.

antibody (1:50; cat. no. YT0931; ImmunoWay Biotechnology Company) at 4˚C overnight. CIT immunoreactivity was scored by multiplying the staining intensity by the percentage of area stained. Intensity was scored as follows: 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining). The percentage of area stained was defined as follows: 0 (no staining), 1 (1-25% of cells stained), 2 (26-50% of cells stained), 3 (51-75% of cells stained), 4 (>75% of cells stained). A high expression of CIT (H-CIT) was defined as 6-12, whereas a low expression of CIT (L-CIT) was defined as 0-5 (18). CIT immunohistochemical staining was scored under a light microscope independently by two experienced pathologists (LY and ZT) who were blinded to patient clinical information.

RT-qPCR. The isolation of total RNA and RT-qPCR were performed as described previously (19). All samples were amplified in triplicate. To calculate the expression of CIT mRNA in samples, GAPDH was used as reference gene. The following primers were used in RT-qPCR: CIT forward, 5'-ACCATAGCTGAGTTACAGGAGC-3' and reverse, 5'-GTCCCCGGTTGCTTTCTCT-3'; GAPDH forward, 5'-TGGAAGGACTCATGACCACA-3' and reverse, 5'-TTCAGCTCAGGGATGACCTT-3'.

Statistical analyses. Statistical analyses were performed using SPSS 20.0 software (IBM Corp.) and Prism 5.0 software (GraphPad Software, Inc.). Comparison between groups was made by unpaired t-tests or Kruskal-Wallis test. The association between CIT expression and the clinicopathological parameters of patients with PCa was analyzed using a χ^2 test. Follow-up outcomes were stratified to CSM for patients that received RP or BCR for patients that received ADT. The Kaplan-Meier method and a log-rank test were established to plot survival curves. Univariate and multivariate Cox regression analysis by backward selection were used to evaluate the prognostic significance of CIT for predicting BCR and CSM. The experiments were repeated 3 times and the data were presented as mean ± standard error. P<0.05 was considered to indicate a statistically significant difference.

Results

CIT is screened as an oncogene in PCa. A total of 3,279 DEGs were filtered from the TCGA profile when comparing normal prostate gland tissue with PCa tissue. A further screening was performed by dividing groups according to Gleason grades, serum PSA levels and tumor, node and metastasis (TNM) stages (Fig. 1). A total of 30 DEGs were identified to be significant in all of these comparisons (Table II). Significantly high expression of CIT mRNA was exhibited in PCa samples (FC=2.180; P<0.001) and in patients with Gleason grades ≥7 (FC=1.637; P<0.001), serum PSA levels ≥10 ng/ml (FC=1.649; P=0.002), T3-T4 (FC=1.606; P<0.001), positive lymph node invasion (LNI; FC=1.474; P<0.001) and distant metastasis (FC=1.765; P=0.009). The results indicate that CIT may be a potential PCa-associated oncogene.

Expression of CIT is increased in PCa. The expression of CIT mRNA was increased in PCa when compared with BPH (Fig. 2A). The immunoreactivity of CIT is presented in Fig. 2B. None and low staining were detected in BPH and low-grade PCa, whereas moderate and strong staining was detected in middle- and high-grade PCa. The staining scores of CIT were significantly increased in primary and aggressive PCa, compared with BPH (P<0.001; Fig. 2C). Additionally, compared with the non-HR-group, CIT expression was

Table II. The differential expression of citron kinase mRNA in the Cancer Genome Atlas mRNA expression profiles (PRAD_2015_02_24).

	Tumor vs. normal		Gleason ≥7 vs. Gleason <7		PSA ≥10 vs. PSA <10		T3-4 vs. Ta-2		N1 vs. N0		M1 vs. M0	
Gene name	FC	P	FC	P	FC	P	FC	P	FC	P	FC	P
CIT	2.18	<0.001	1.64	<0.001	1.65	<0.001	1.61	<0.001	1.47	<0.001	1.77	0.010
STAC	1.55	<0.001	-1.78	<0.001	-2.63	<0.001	-1.45	<0.001	-1.85	<0.001	-3.72	<0.001
HELLS	1.35	<0.001	1.41	<0.001	1.66	<0.001	1.43	<0.001	1.42	<0.001	1.75	0.010
RIC3	-1.40	<0.001	-1.38	<0.001	-1.56	0.010	-1.38	<0.001	-1.42	<0.001	-2.21	<0.001
C8orf46	-1.54	<0.001	-1.30	0.010	-1.59	0.010	-1.29	<0.001	-1.32	<0.001	-1.87	0.010
PTN	-1.89	<0.001	-1.75	<0.001	-2.04	0.010	-1.73	<0.001	-1.89	<0.001	-2.65	0.010
NTF3	-2.00	<0.001	-1.26	0.010	-1.49	<0.001	-1.17	<0.001	-1.24	<0.001	-1.73	<0.001
PAGE4	-2.19	<0.001	-2.22	<0.001	-2.78	<0.001	-2.05	<0.001	-2.65	<0.001	-5.16	<0.001
BMPER	-2.25	<0.001	-1.91	<0.001	-2.67	<0.001	-1.46	<0.001	-1.84	<0.001	-3.01	<0.001
RSPO2	-2.27	<0.001	-1.85	<0.001	-2.28	<0.001	-1.73	<0.001	-1.94	<0.001	-3.11	<0.001
FXYD1	-2.31	<0.001	-1.58	<0.001	-1.97	0.010	-1.36	<0.001	-1.75	<0.001	-2.55	0.010
RNF112	-2.41	<0.001	-1.74	<0.001	-2.21	<0.001	-1.68	<0.001	-1.87	<0.001	-3.00	<0.001
PROK1	-2.42	<0.001	-2.13	<0.001	-2.40	<0.001	-2.01	<0.001	-2.40	<0.001	-3.73	<0.001
C20orf200	-2.55	<0.001	-1.67	<0.001	-1.83	<0.001	-1.55	<0.001	-1.68	<0.001	-2.32	<0.001
ANO4	-2.82	<0.001	-1.72	<0.001	-2.23	<0.001	-1.79	<0.001	-1.99	<0.001	-2.64	<0.001
GSTM5	-2.96	<0.001	-1.53	<0.001	-2.01	<0.001	-1.44	<0.001	-1.75	<0.001	-2.27	0.010
B3GALT2	-3.08	<0.001	-1.69	<0.001	-2.01	<0.001	-1.48	<0.001	-1.95	<0.001	-2.40	<0.001
ADRA1D	-3.19	<0.001	-2.05	<0.001	-1.98	0.010	-1.63	<0.001	-1.97	<0.001	-2.86	<0.001
NDP	-3.30	<0.001	-1.72	<0.001	-2.04	<0.001	-1.48	<0.001	-1.71	<0.001	-2.58	<0.001
HIF3A	-3.55	<0.001	-1.78	<0.001	-2.42	<0.001	-1.67	<0.001	-2.06	<0.001	-2.91	<0.001
SMOC1	-4.19	<0.001	-1.72	<0.001	-2.30	<0.001	-2.03	<0.001	-2.48	<0.001	-3.21	<0.001
LDB3	-4.28	<0.001	-1.80	<0.001	-2.06	0.010	-1.64	<0.001	-2.00	<0.001	-3.00	<0.001
LOC572558	-4.35	<0.001	-2.29	<0.001	-2.46	<0.001	-2.15	<0.001	-2.57	<0.001	-3.98	<0.001
PPARGC1A	-4.42	<0.001	-1.60	<0.001	-2.08	<0.001	-1.60	<0.001	-1.98	<0.001	-2.43	0.010
HRNBP3	-4.54	<0.001	-2.17	<0.001	-2.48	<0.001	-2.11	<0.001	-2.72	<0.001	-5.42	<0.001
SRD5A2	-4.57	<0.001	-1.98	<0.001	-2.17	0.010	-2.18	<0.001	-2.74	<0.001	-5.26	<0.001
COL4A6	-4.95	<0.001	-1.84	<0.001	-2.01	0.010	-1.78	<0.001	-1.98	<0.001	-3.32	<0.001
LGR6	-6.41	<0.001	-1.78	<0.001	-2.44	<0.001	-1.59	<0.001	-2.02	<0.001	-3.45	<0.001

FC, fold-change; P, P-value; PSA, prostate specific antigen.

significantly increased in the HR-group (P<0.001; Fig. 2D). As presented in Table III, the percentage of patients with H-CIT was significantly associated with Gleason grades (P=0.001), serum PSA levels (P=0.001), T stages (P<0.001), lymph node invasion (P=0.032) and metastasis (P=0.021). These results were consistent with those of the aforementioned bioinformatic analysis.

CIT is a risk factor for poor outcomes in patients with PCa. In IHC, the protein level of CIT expression was significantly upregulated in BCR patients (P<0.001; Fig. 3A) and the recurrence time of patients with H-CIT was significantly decreased compared with L-CIT (P=0.013; Fig. 3B). Further multivariate analysis demonstrated that the independent value of H-CIT [hazard ratio (HR)=1.090-4.231; P=0.027] and LNI (HR=1.002-4.294; P=0.049) was significant for BCR prediction (Table IV).

The results also revealed that the expression of CIT was increased in CSM patients (Fig. 3C). The Kaplan-Meier survival curve revealed that patients with H-CIT exhibited shorter survival times compared with patients with L-CIT (P<0.001; Fig. 3D). Multivariate analysis also revealed that the independent risk factors of CSM were CIT (HR=2.408-12.802; P=0.000), Gleason grades (HR=1.148-5.068; P=0.020) and T stages (HR=1.815-8.085; P<0.001; Table V).

Discussion

A previous study of CIT in PCa demonstrated that the loss of CIT inhibited the proliferation of LNCaP and C4-2B cells (20), however the limited number of cell types available and lack of investigation in a clinical setting restricted the study. The current study screened CIT as a potential oncogene in PCa. CIT was highly expressed in PCa samples and was associated

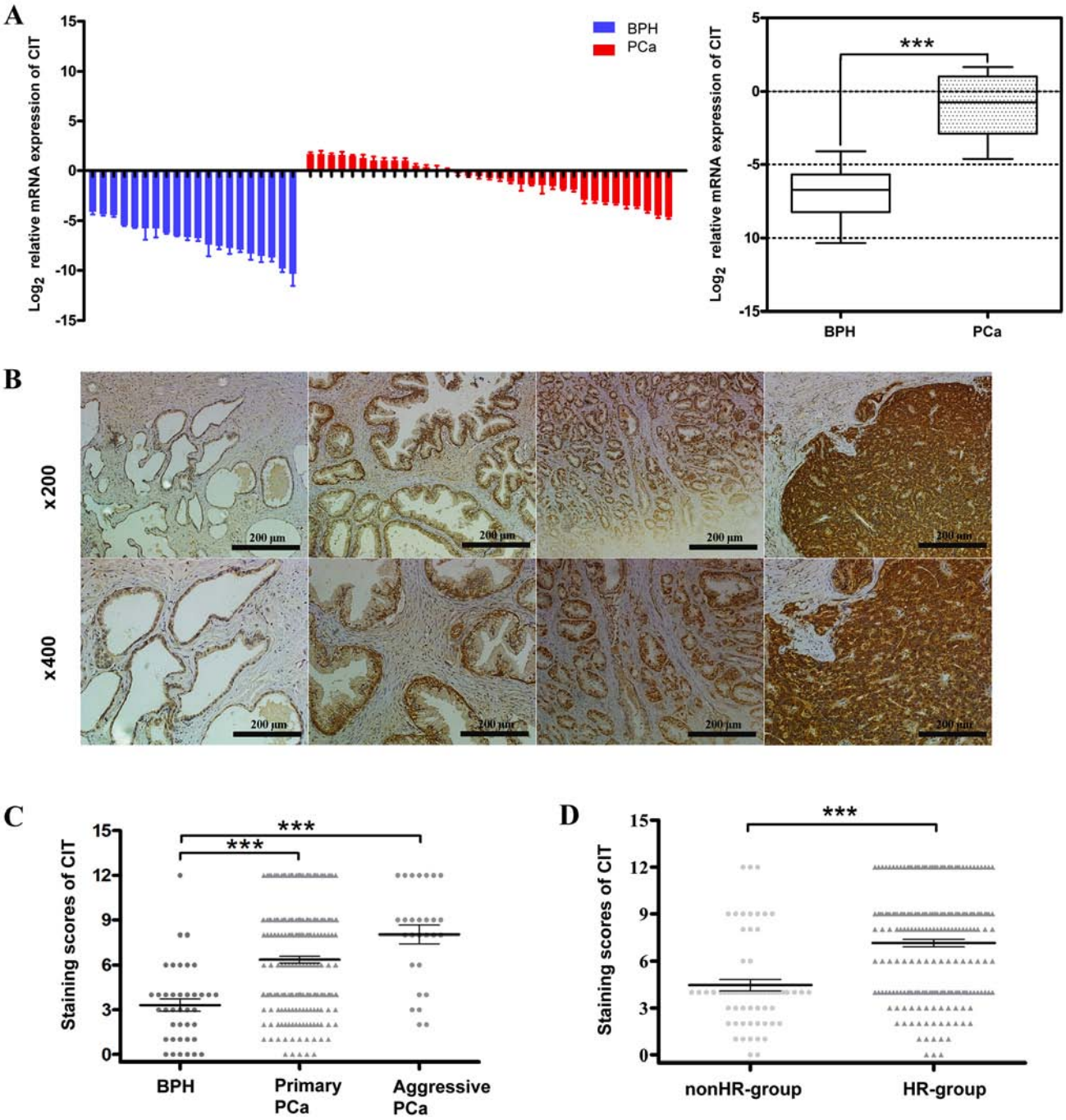

Figure 2. CIT expression is increased in PCa. (A) CIT mRNA was extracted from 35 cases of fresh PCa and 20 cases of BPH. The results of reverse transcription-quantitative PCR revealed that the mRNA expression of CIT was increased in PCa samples. Data are presented as the SEM. The overall comparison between PCa and BPH is presented in the box plot with the median result, in which the bottom and top of the boxes represent the maximum and minimum value, respectively. (B) The slides for IHC were cut from formalin fixed paraffin embedded tissue obtained from 39 cases of BPH and 271 cases of PCa. Representative images of IHC indicate CIT staining. No staining was present in BPH tissue; light staining was exhibited in low-grade PCa, moderate staining was revealed in middle-grade PCa and strong staining was indicated in high-grade PCa. Each image was captured at a respective magnification of x200 and x400, respectively. Compared with BPH, there was a significant increase in primary and aggressive PCa, whereas no statistically significant difference was observed between primary and aggressive PCa (C) The expression of CIT in HR-group was also higher than nonHR-group (D). Error bars represent the SEM. The data in A and D were analyzed using an unpaired t-test, and the data in C were analyzed using Kruskal-Wallis test ***P<0.001. CIT, citron kinase; PCa, prostate cancer; BPH, benign prostatic hyperplasia; IHC, immunohistochemistry; SEM, standard error of the mean; HR, high risk.

with Gleason scores, serum PSA levels, T stage and risk groups. Furthermore, patients with a high CIT expression were more likely to exhibit an increased BCR and CSM compared with those with a low CIT expression. Additionally, the high expression of CIT was determined to be a risk factor for BCR and CSM in patients with PCa.

Cytokinesis is the final stage of cell division, in which two daughter cells are separated (21). Resolving the midbody during the final stage of abscission serves an important role in cytokinesis (5). Failure to complete cytokinesis may lead to tetraploidy and the presence of multiple centrosomes, which has been proposed to promote tumorigenesis (22).

Table III. Correlation between CIT and clinical parameters of prostate cancer patients.

Parameters	No. (%)	Low CIT expression	High CIT expression	P-value
Gleason scores				0.001
<7	88	52 (59.09)	36 (40.91)	
≥7	183	69 (37.70)	114 (62.30)	
Serum PSA (ng/ml)				0.001
<10	69	43 (62.32)	26 (37.68)	
≥10	202	78 (38.61)	124 (61.39)	
pT stage				<0.001
Ta-T2	150	87 (58.00)	63 (42.00)	
T3-T4	121	34 (28.10)	87 (71.90)	
LNI				0.032
N0	255	118 (46.46)	137 (53.94)	
N1	16	3 (18.75)	13 (81.25)	
Metastasis				0.021
M0	254	118 (46.46)	136 (53.54)	
M1	17	3 (17.65)	14 (82.35)	

PCa, prostate cancer; BPH, benign prostatic hyperplasia; PSA, prostate-specific antigen; LNI, lymph node invasion; CIT, citron kinase.

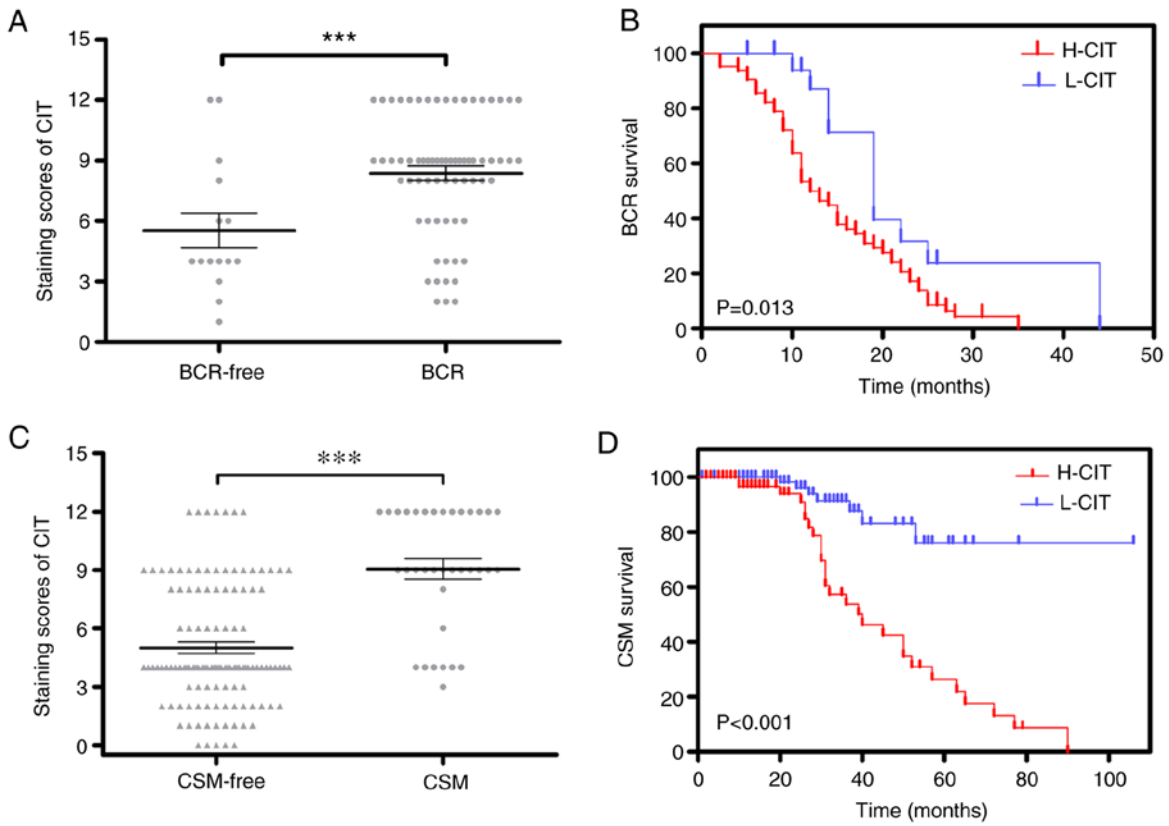

Figure 3. Prognostic value of CIT in PCa. (A and C) As revealed by the results of immunohistochemistry, the protein level of CIT was increased in BCR and CSM patients. Error bars represent the standard error of the mean. ***P<0.001. (B and D) Kaplan-Meier survival analysis revealed the survival time of BCR and CSM patients, with a high or low CIT expression. Data were analyzed using a log-rank test. CIT, citron kinase; PCa, prostate cancer; BCR, biochemical recurrence; CSM, cancer specific mortality.

Pihan *et al* (23) observed that centrosomes were structurally and numerically abnormal in the majority of patients with PCa. Furthermore, bladder cancer samples frequently contain a number of centrosomes that are significantly increased as a result of cytokinesis failure (24). CIT is specifically required during the late stages of cytokinesis for the organization

Table IV. Univariate and Multivariate Cox regression analysis for BCR.

	Univariate		Multivariate	
Variables	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
CIT (low vs. high)	2.231 (1.137-4.377)	0.020	2.147 (1.090-4.231)	0.027
Gleason score (<8 vs. ≥8)	2.309 (1.148-4.644)	0.019	1.561 (0.663-3.676)	0.404
Serum PSA level (<10 vs. ≥10 ng/ml)	2.634 (1.124-6.171)	0.026	2.238 (0.949-5.277)	0.066
T stage (Ta-2 vs. T3-4)	1.021 (0.614-1.699)	0.935	1.034 (0.601-1.782)	0.903
LNI (N0 vs. N1)	2.181 (1.060-4.489)	0.034	2.074 (1.002-4.294)	0.049
Metastasis (M0 vs. M1)	1.225 (0.623-2.409)	0.556	0.515 (0.237-1.118)	0.094

CIT, citron kinase; PSA, prostate-specific antigen; BCR, biochemical recurrence; LNI, lymph node invasion.

Table V. Univariate and multivariate Cox regression analysis for cancer-specific morality.

	Univariate		Multivariate	
Variables	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
CIT (low vs. high)	5.316 (2.314-12.213)	<0.001	5.553 (2.408-12.802)	<0.001
Gleason score (<8 vs. ≥8)	1.764 (0.875-3.556)	0.108	2.412 (1.148-5.068)	0.020
Serum PSA, ng/ml (<10 vs. ≥10)	1.452 (0.628-3.355)	0.383	0.869 (0.341-2.173)	0.751
T stage (Ta-2 vs. T3-4)	2.977 (1.504-5.895)	0.002	3.831 (1.815-8.085)	0.000
LNI (N0 vs. N1)	4.584 (1.570-13.384)	0.005	0.684 (0.181-2.586)	0.576
Metastasis (M0 vs. N0)	3.032 (1.570-13.384)	0.134	1.134 (0.288-5.645)	0.878

CIT, citron kinase; PSA, prostate-specific antigen; LNI, lymph node invasion; CI, confidence interval.

and function of the midbody (7,25). The overexpression of CIT kinase-active mutants causes the dysregulation of cytokinesis, which results in the production of multinucleate cells (26). Therefore, the disrupted function of CIT may contribute to cytokinesis failure, leading to the progression of cancer. Madhavan *et al* (27) revealed that the activation of the CIT/kinesin family member kinesin like protein KIF14 (KIF14) axis, where CIT localizes to the central spindle via the kinesin-3 motor, KIF14, is involved in the carcinogenesis of retinoblastoma.

Various kinases have been demonstrated to be intimately involved in processes and to contribute to tumor cell proliferation and survival (28). Certain kinases are considered to be oncogenic due to their transforming capacity, including BRAF in colon carcinoma and ALK in neuroblastoma (29,30). In addition, Rho-associated protein kinase serves an essential role in the metastasis and proliferation of breast cancer and hepatocellular carcinoma (31,32). The knockdown of CIT directly inhibits the proliferation of breast cancer and hepatocellular carcinoma cells (33,34). Since a previous study determined that CIT is an essential kinase that targets Rho-associated kinases (including ROCK and ROK) (27), it seems likely that CIT serves an important role in these cancers by interacting with Rho signaling. Previous studies have also revealed that Rho signaling factors are involved in the invasion of PCa cells (35,36), such that CIT may also participate in the regulation of Rho signaling, which serves a key role in the progression of PCa.

Currently, the main clinical signatures of patients with PCa include TNM stage PSA levels and Gleason scores (37). The results of the current study revealed that a high expression of CIT was positively associated to a high T stage, serum PSA level and Gleason score. Furthermore, CIT was determined to be an independent predictor of BCR and CSM. These data indicated that CIT may serve as a potential marker of PCa and may compensate for these clinical signatures. Currently, ADT is one of the primary methods of treatment for patients with PCa (38). However, certain patients that receive ADT will still advance to castration-resistant PCa and suffer from a poor prognosis (39). Although recent studies have determined that the glucocorticoid receptor can be targeted to improve anti-androgen therapy (40,41), new targets in the process of castration resistance should be explored. In the current study, patients with a high CIT expression exhibited shorter PSA recurrence time, which implies that CIT may serve a role in androgen-resistant PCa.

However, the number of PCa samples was limited in the current study and the mechanism of CIT in PCa also needs to be further elucidated. More patient samples should therefore be utilized in further study and the interaction between CIT and the Rho pathway should be determined in PCa cell lines.

In conclusion, the results of the current long-term retrospective study indicated that CIT is an independent indicator of CSM and BCR. CIT may therefore be a potential biomarker of PCa in the future. Although further study is required to assess the function and mechanism of CIT in PCa, it may still serve as a biomarker to improve the survival of patients with PCa.

Acknowledgements

The authors would like to thank Professor Fangzhou Song (Department of Biochemistry & Molecular Biology, Molecular Medicine & Cancer Research Center, Chongqing Medical University, Chongqing, PR China) and Professor Xiaoni Zhong (Department of Health Statistics and Information Management, School of Public Health and Management, Chongqing Medical University, Chongqing, China.) for helpful suggestions. Thanks to Dr. Yutao Zhang (Department of Pathology, Zigong First People's Hospital) and Dr. Yu Li (Department of Pathology, the First Affiliated Hospital of Chongqing Medical University) for the supports of pathological information and immunohistochemical assessment.

Funding

Funding was received from: National Natural Science Foundation of China (grant no. 30972999); Nature Science Foundation of Chongqing (grant no. Cstc2016shms-ztzx0 054 and cstc2015jcyjBX0045); the Science and technology planning project of Yuzhong District (grant no. 20150111); the Health and Family Planning Commission Foundation of Chongqing Municipal (grant no. Cstc2012gg-yyjs10043); the Health Bureau of Chongqing (grant no. 20132082) and the Chongqing Education Commission (grant no. CYS16125).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JD and JL analyzed the data and made major contributions to writing the manuscript. YQ, YJL and YL performed the experiments and wrote the initial draft of the manuscript. JH, WW, LM and HL analyzed the data and contributed to revising the article. DW and QY contributed to the design of the study and provided final approval of the manuscript. WJ and YLia contributed to the design of the study and assisted with writing the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics committee of the First Affiliated Hospital of Chongqing Medical University, Zigong Fourth People's Hospital and Zigong First People's Hospital approved the use of these samples for the educational purposes of this research. The consent from patients or patients' families was obtained verbally.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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