Overexpression levels of cripto-1 predict poor prognosis in patients with prostate cancer following radical prostatectomy

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Abstract. Overexpression of cripto-1 (CR-1), an epidermal growth factor-cripto-1/FRL-1/Cryptic family protein, has been reported in multiple types of malignancy. However, the clinical functions of CR-1 in prostate cancer (PCa) remain largely unclear. The objective of the present study was to investigate the association between CR-1 expression and the clinicopathological features and prognosis of PCa. CR-1 expression was evaluated in 138 PCa tissues and 67 benign prostate hyperplasia (BPH) tissues using immunohistochemistry. The association between the clinicopathological features of patients with PCa and CR-1 expression was analyzed using a χ^2 test. Receiver operating characteristic (ROC) curve and Cox regression model were used to analyze the association between CR-1 expression and biochemical recurrence (BCR)-free survival. It was revealed that the protein expression of CR-1 was markedly higher in PCa tissues than in BPH tissues. The mRNA expression of CR-1 in PCa tissue and cells was also significantly higher than in BPH tissue and the normal RWPE-1 prostate cell line (P<0.05). In addition, high CR-1 expression was significantly associated with prostate-specific antigen level (P=0.008), Gleason score (P=0.011) and lymph node metastasis (P=0.025) in patients with PCa. ROC curve indicated that patients with elevated expression of CR-1 exhibited shorter BCR-free survival (P<0.001). Furthermore, multivariate statistical analysis demonstrated that overexpression of CR-1 may be a novel predictor for prognosis of patients with PCa. Accordingly, the present study considered CR-1 to be a valuable predictor of poor prognosis and progression in PCa, and a potential therapeutic target for patients with PCa.

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Introduction

Prostate cancer (PCa), a type of malignant tumor, is a major cause of mortality in men. It is estimated that ~164,690 Americans will be diagnosed with PCa in 2018, with ~29,430 PCa-associated mortalities (1). PCa is considered to be the most important cancer type in males. The incidence of PCa varies greatly among different countries (2). Particularly, the incidence rate of PCa in China is considered to be relatively low worldwide. For Chinese males, in 2018, the five most common causes of cancer-related deaths were lung, liver, stomach, esophageal and colorectal cancer; prostate cancer was not included (3). Although the majority of patients with PCa initially respond to therapy following radical prostatectomy, many eventually experience biochemical recurrence (BCR) (4). As with other types of tumor, the molecular pathogenesis of PCa remains unclear. Furthermore, there are no entirely effective treatments for PCa. Therefore, it is important to identify novel PCa predictors that can be used to actively monitor the disease and determine the appropriate treatment (5).

Cripto-1 (CR-1), also termed teratocarcinoma-derived growth factor 1, is a member of the epidermal growth factor-cripto-1/FRL-1/Cryptic (EGF-CFC) family (6). CR-1 was originally isolated from NTERA2 human embryonic carcinoma cells (7). CR-1 protein consists of an extracellular signal sequence, EGF-like domain CFC-motif and glycosylphosphatidylinositol (GPI) (8). CR-1 has a role in fetal development and carcinogenesis. CR-1 is a receptor for transforming growth factor-β ligands and Nodal (9). The EGF-like domain contains an O-linked fucosylation site (10), and it has been reported that the residue threonine 88 is required for Nodal to activate CR-1 (11). In addition, the GPI anchor of CR-1 has a critical paracrine role (12). CR-1 expression is restricted in adults (13). By contrast, CR-1 may be re-expressed in patients with most types of cancers (14). CR-1 has an active role in modulating cancer cell proliferation and cancer progression (15,16). It has been reported that CR-1 expression is elevated in gastric cancer, lung cancer, breast cancer and esophageal carcinoma (17-20). Data indicate that CR-1 may regulate breast cancer in mice via the Wnt/β-catenin pathway (13,21,22). Furthermore, a previous study reported that CR-1 promotes tumor invasion and

metastasis via Nodal-dependent signaling, Nodal-independent signaling, Wnt signaling and Notch signaling pathways (23).

In the current study, immunohistochemistry (IHC) was used to determine the level of CR-1 in PCa and benign prostate hyperplasia (BPH) tissues. The findings revealed that the expression of CR-1 was higher in PCa tissues, compared with BPH. Subsequently, the association between CR-1 and clinicopathological parameters was investigated to identify its clinical function. Finally, the present study assessed whether CR-1 may be used as a novel predictor of prognosis in PCa following radical prostatectomy.

Table I. CR-1 expression in PCa tissues and BPH tissues.

CR-1	PCa tissues (%)	BPH tissues (%)	χ^2	P-value
Low	80 (57.97)	59 (88.06)	18.705	<0.001
High	58 (42.03)	8 (11.94)		

Data are expressed as no. (%). CR-1, cripto-1; PCa, prostate cancer; BPH, benign prostate hyperplasia.

Patients and methods

Patients and tissue samples. A total of 138 human PCa tissues and 67 BPH tissues were collected between January 2001 and June 2014 at Tianjin Institute of Urology (Tianjin, China). The clinicopathological data of the patients are summarized in Table I. Samples from patients with PCa were collected during radical prostatectomy. Matched adjacent BPH tissues were also obtained from patients with PCa. No patient had received radiotherapy or chemotherapy prior to surgery. No patient had any other type of tumor. The study was approved by the Ethics Committee of Tianjin Institute of Urology and patients signed written informed consent. Tissues were fixed in 10% formaldehyde solution at room temperature for 24 h and paraffin embedded. They were subsequently stained with hematoxylin for 10 min and eosin for 5 min at room temperature and observed for morphology using a light microscope (magnification, x200) for diagnosis by experienced pathologists. The clinicopathological parameters, including age, Gleason score, pre-operative prostate-specific antigen (PSA), clinical stage, lymph node metastasis and surgical margin status were carefully obtained from the records of the 138 patients with PCa. TNM, Gleason, tumor grade and clinical stage of the samples were assessed according to the 2002 Tumor-Node-Metastasis classification and the Gleason system for PCa (24,25). Serum PSA levels were detected postoperatively every three months during the first year and every six months from the second year (26). BCR was defined as two readings of serum PSA >0.2 ng/ml following radical prostatectomy. The survival status of patients with PCa was followed up for a maximum of 120 months post-operation. Follow-up data were primarily obtained by telephone and patient review. The average age of patients was 70 years old (range, 49-91 years). The ages of patients with BPH were matched to those of patients with PCa.

Cell culture. The human PCa cell lines PC-3 and LNCaP, and a normal prostate cell line (RWPE-1) were used in the present study. All the cell lines were obtained from the American Type Culture Collection. LNCaP cells were maintained in Eagle's Minimum Essential Medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (Invitrogen; Thermo Fisher Scientific, Inc.), 2 mM L-glutamine, 2% penicillin-streptomycin and 0.2% gentamicin. PC-3 cells were cultured in Ham's F12K medium (Gibco; Thermo Fisher Scientific, Inc.) with 2 mM L-glutamine adjusted to contain 1.5 g/l sodium bicarbonate (90%) and 10% fetal bovine serum. The RWPE-1 cell line was cultured in keratinocyte serum-free medium (Gibco; Thermo Fisher Scientific, Inc.). Cells were cultured at 37˚C with 5% CO_2.

Immunofluorescent (IF) staining and confocal microscopy. PC-3 and RWPE-1 cells were cultured on cover slips for 48 h. Cells were fixed in 4% paraformaldehyde for 15 min at room temperature. PC-3 and RWPE-1 cells were washed in PBS. Subsequently, cells were added in 0.5% Trixon for 5 min at room temperature. Following incubation with a rabbit polyclonal primary antibody against human CR-1 (cat. no. SAB1306280; Sigma-Aldrich; Merck KGaA; dilution 1:80) overnight at 4˚C. PC-3 and RWPE-1 cells were washed and incubated with a polyclonal secondary fluorescein-conjugated goat anti-rabbit IgG antibody (dilution, 1:200; cat. no., ZF-0311; OriGene Technologies, Inc.) in the dark at room temperature. DAPI was used to counterstain PC-3 cells for 5 min at room temperature. Following washing, the coverslips were placed in anti-fade solution (cat. no. AR1109; Boster Biological Technology). Images were captured using laser scanning confocal microscopy (magnification, x400).

Immunohistochemistry (IHC) staining. CR-1 staining was performed on all 138 PCa tissues and 67 BPH tissues. Paraffin-embedded blocks (4-μm thick) were deparaffinized and rehydrated in 100 and 80% alcohol, each for 5 min, subsequently 0.3% hydrogen peroxide in methanol was added to the tissues for 15 min at room temperature to block endogenous peroxidase activity. Slides were washed in PBS (three times for 3 min each), whereby antigen retrieval was conducted in citrate buffer (pH 6.0; cat. no. P0081; Beyotime Institute of Biotechnology;) for 10 min at 100˚C. Following three more PBS washes (3 min each), the slides were stained with a rabbit polyclonal antibody against human CR-1 (cat. no., SAB1306280; Sigma-Aldrich; Merck KGaA; dilution, 1:80) for 2 h at 37˚C, and washed again with PBS (three times for 3 min each). Subsequently, the slides were incubated with horseradish peroxidase (HRP) universal IgG antibody polymer (cat. no., PV-9000; OriGene Technologies, Inc.; dilution, 1:200) for 30 min at 37˚C, followed by three PBS washes (3 min each). Each slide was treated with 50 μl diaminobenzadine working solution (DAB HRP color development kit; cat. no. P0202; Beyotime Institute of Biotechnology) at room temperature for 3-10 min, followed by a final wash in PBS. All sections were counterstained with haematoxylin for 1-2 min at room temperature for the purpose of enabling the morphology of the tissue to be observed using a light microscope (magnification, x200). A slide without the addition of the primary

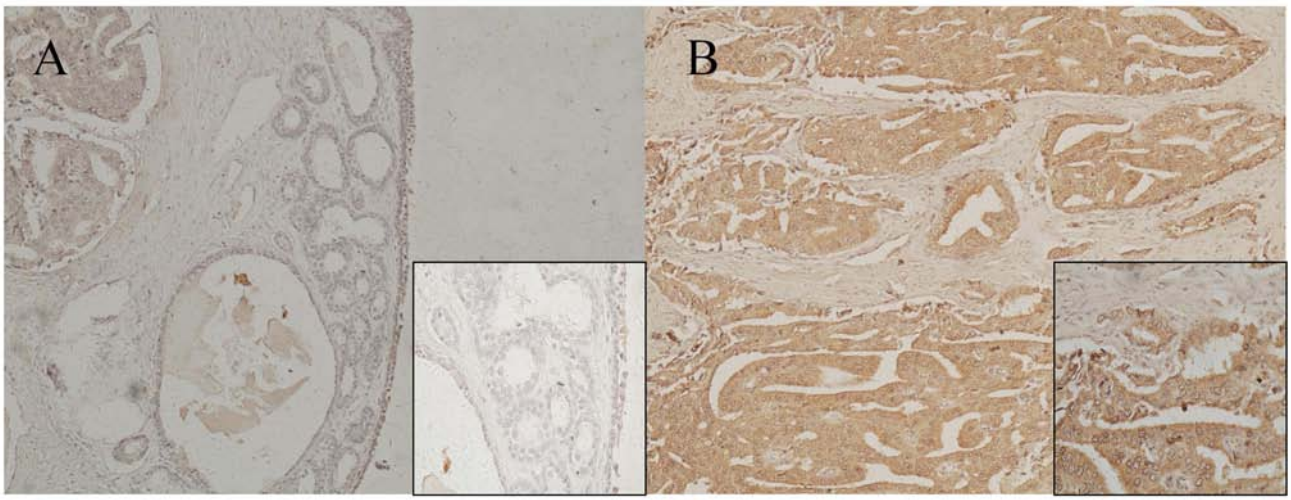

Figure 1. Immunohistochemistry analysis of CR-1 expression. (A) Low expression (0-4) of CR-1 in adjacent non-tumor BPH tissues. (B) High expression (5-12) of CR-1 in PCa tissues. Magnification, x100. Insert magnification, x400. CR-1, cripto-1; PCa, prostate cancer; BPH, benign prostate hyperplasia.

antibody was used as a negative control. All stained slides were re-examined by two experienced pathologists that were blinded to patient clinical information. Stained cells were scored according to the staining area and staining intensity (27). Staining intensities for CR-1 were graded on a 0-3 scale: 0, no staining; 1, weak staining; 2, moderate staining and 3, strong staining. The staining areas were scored on a 0-4 scale: 0, 0-20% positive cells; 1, 21-40% positive cells; 2, 41-60% positive cells; 3, 61-80% positive cells; 4, >80% positive cells. The two scores were multiplied to calculate a subjective score. CR-1 expression levels were defined as low expression (0-4) and high expression (5-12).

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR). An EasyPure® kit (Beijing Transgen Biotech Co., Ltd.) was used to extract the total RNA from prostate tissues and cells. The total RNA was subsequently used for cDNA synthesis with TransScript® SuperMix (Beijing Transgen Biotech Co., Ltd.). The reactions were carried out at 25˚C for 10 min, 42˚C for 30 min and 85˚C for 5 sec. The expression of CR-1 was quantified using a SYBR-Green kit (cat. no. 4387406; Thermo Fisher Scientific, Inc.). Each experiment was performed in triplicate. Samples were denatured at 95˚C for 10 min, followed by 45 cycles at 95˚C for 15 sec, 60˚C for 30 sec and 72˚C for 15 sec. The primers were synthesized by Sangon Biotech Co., Ltd. Human U6 and β-actin served as the control for CR-1. The primer sequences were as follows: CR-1 sense, 5'-GGAATTTGCTCGTCCATCTC-3' and antisense, 5'-ACCGTGCCAGCATTTACAC-3'; U6 sense, 5'-CTCGCTTCGGCAGCACA-3' and antisense, 5'-AACGCTTCACGAATTTGCGT-3'; β-actin sense, 5'-CTCTTCCAGCCTTCCTTCCT-3' and antisense, 5'-ACTCCTGCTTGCTGATCCAC-3'. The CR-1 levels were analyzed using the $2^{-\Delta\Delta Cq}$ method (28).

Western blot analysis. Total proteins were extracted in RIPA cell lysis buffer (cat. no. P0013B; Beyotime Institute of Biotechnology) from PCa and BPH tissues. The concentration of the proteins was measured using an enhanced bicinchoninic acid protein assay kit (cat. no. P0009; Beyotime Institute of Biotechnology). A total of 30 μg protein was subjected to a 10% SDS-PAGE gel and then transferred onto nitrocellulose membranes (Pall Life Sciences). Following blocking with 5% skimmed milk in TBS-Tween-20 (TBST) for 2 h at room temperature, the membranes were incubated with mouse monoclonal anti-CR-1 (cat. no. sc-376448; 1:500; Santa Cruz Biotechnology, Inc.) and mouse monoclonal anti-β-actin (cat. no. TA811000; 1:400; OriGene Technologies, Inc.) at 4˚C overnight. Following washing with TBST three times, the membranes were incubated with anti-mouse secondary antibodies (cat. no. ASS1007, 1:2,000; Abgent, Inc.), conjugated with HRP for 1 h at room temperature. Finally, the proteins were detected using an enhanced chemiluminescence kit (cat. no. P0018AM; Beyotime Institute of Biotechnology).

Statistical analyses. Data were analyzed using SPSS software version 17.0 (SPSS, Inc.) and GraphPad Prism 5.0 software (GraphPad Software, Inc.). Quantitative data were compared using Student's t-test. χ^2 was used to determine the association of CR-1 with clinicopathological parameters. Receiver operating characteristic (ROC) curve was generated and log-rank test was used to monitor BCR. The effect of clinicopathological parameters on survival was assessed by Cox regression analysis. Multivariate analysis was conducted based on results of univariate analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

CR-1 expression is elevated in PCa tissues and cell lines. CR-1 expression in all tissues was determined by IHC (Fig. 1). The expression of CR-1 was elevated in 42.03% of PCa tissues (58/138); however, expression was elevated in only 11.94% of the BPH tissues (8/67) (Table I). Therefore, CR-1 expression was significantly higher in PCa tissues compared with BPH tissues ($P<0.05$). The expression of CR-1 was increased in PC-3 and LNCaP cells compared with

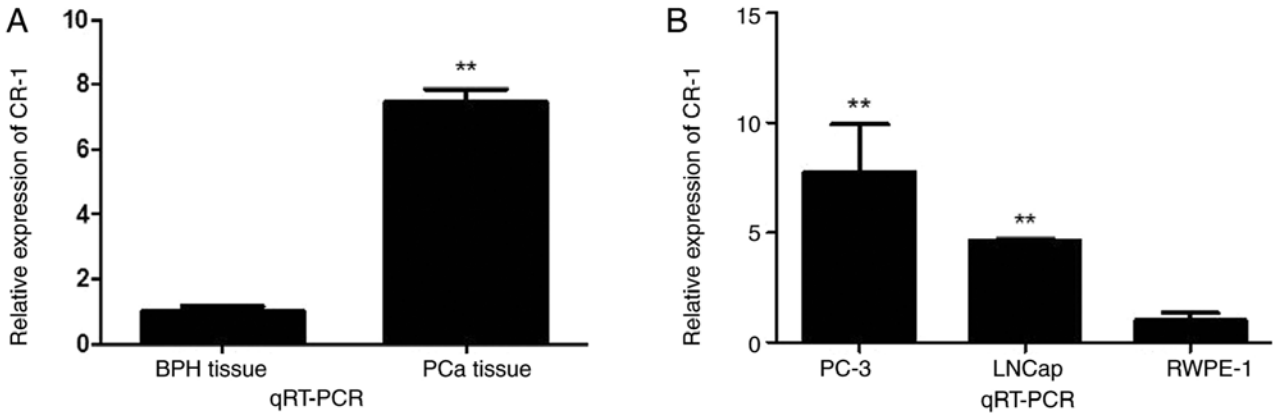

Figure 2. Relative expression of CR-1 mRNA in PCa and BPH tissues and in prostate cells. (A) CR-1 levels in BPH tissues were regarded as 100%, and PCa tissues were compared with BPH tissues. CR-1 mRNA was significantly increased in PCa tissues compared with BPH tissues. $^{**}P<0.05$ vs. BPH tissue. (B) Relative expression of CR-1 mRNA in prostate cells. CR-1 levels in RWPE-1 cells were regarded as 100% and RWPE-1 was compared with PC-3 and LNCap. CR-1 mRNA was significantly increased in PC-3 and LNCaP compared with RWPE-1. $^{**}P<0.05$ vs. RWPE-1. CR-1, cripto-1; PCa, prostate cancer; BPH, benign prostate hyperplasia; RT-qPCR, reverse transcription-quantitative PCR.

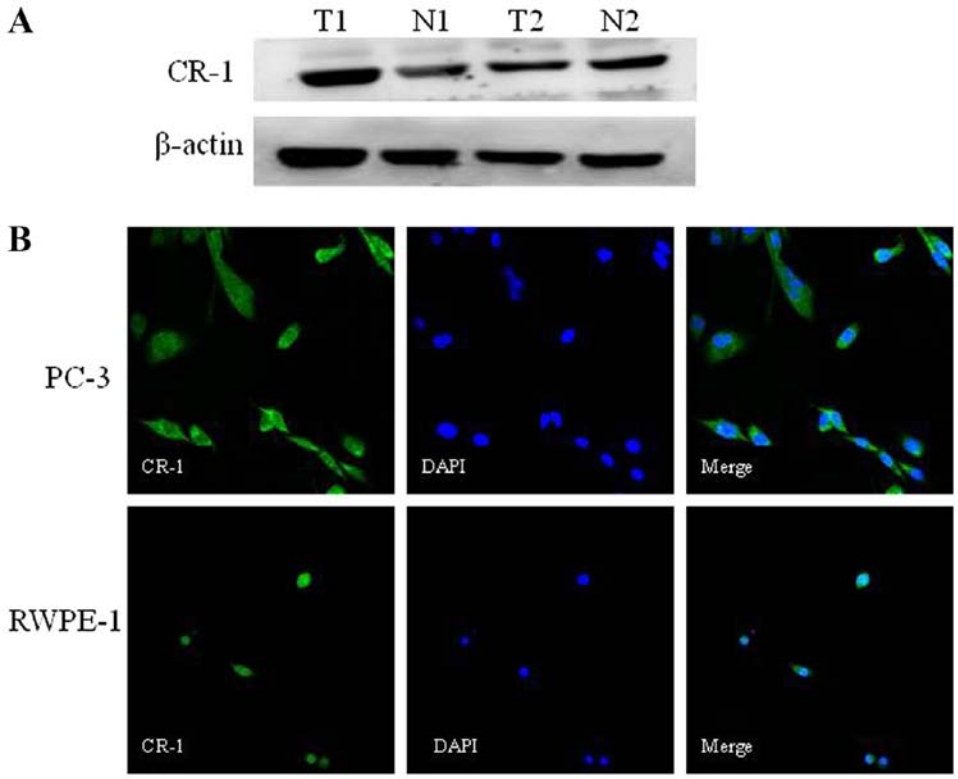

Figure 3. CR-1 gene expression in prostate tissues and PC-3 cells. (A) CR-1 levels in PCa and non-cancerous BPH tissues were detected using western blot analysis, with β-actin as the internal reference. T represents PCa tissues and N represents non-cancerous BPH tissues and the numbers represent tissues from 2 different patients. (B) Immunofluorescence staining for CR-1 protein in PC-3 cells and RWPE-1 cells. PC-3 and RWPE-1 cells were immunostained for CR-1 (green). Nuclei were visualized by DAPI staining (blue). CR-1 protein is mainly located in the cytoplasm of cells (magnification, x400). CR-1, cripto-1; PCa, prostate cancer; BPH, benign prostate hyperplasia.

that in RWPE-1 cells ($P<0.05$; Fig. 2). Additionally, to further determine the CR-1 expression in PCa and BPH tissues, CR-1 protein was extracted for western blot analysis. The results confirmed that CR-1 expression levels in PCa were higher compared with that in BPH (Fig. 3A), where T represents PCa tissues, N represents non-cancerous BPH tissues and the different numbers represent tissue from two different patients. In addition, IF staining was also performed on PC-3 and RWPE-1 cells, demonstrating that CR-1 was located in the cytoplasm (Fig. 3B).

Association between CR-1 expression and clinical parameters. To understand the association between CR-1 expression and clinical features of patients with PCa, χ^2 analysis was performed. IHC was used to assess the CR-1 expression in samples from 138 patients with PCa, which revealed that

Table II. Association of CR-1 expression with characteristics of 138 patients with PCa.

Characteristic	n	Overexpression (%)	Low expression (%)	χ^2	P-value
Age (years)					
<70	84	36 (42.86)	48 (57.14)	0.005	0.945
≥70	54	22 (40.74)	32 (59.26)		
Surgical margin status					
Presence	12	4 (33.33)	8 (66.67)	0.111	0.739
Absence	126	54 (42.86)	72 (57.14)		
Serum PSA level (ng/ml)					
<10	57	32 (56.14)	25 (43.86)	6.981	0.008
≥10	81	26 (32.10)	55 (67.90)		
Gleason score					
<7	67	36 (53.73)	31 (46.27)	6.416	0.011
≥7	71	22 (30.99)	49 (69.01)		
T stage					
T_1	99	40 (40.40)	59 (59.60)	0.180	0.671
T_2/T_3	39	18 (46.15)	21 (53.85)		
Lymphatic metastasis					
Presence	19	3 (15.79)	16 (84.21)	5.040	0.025
Absence	119	55 (46.22)	64 (53.78)		

Data are expressed as no. (%). PCa, prostate cancer; CR-1, cripto-1; PSA, prostate-specific antigen; T stage, tumor stage.

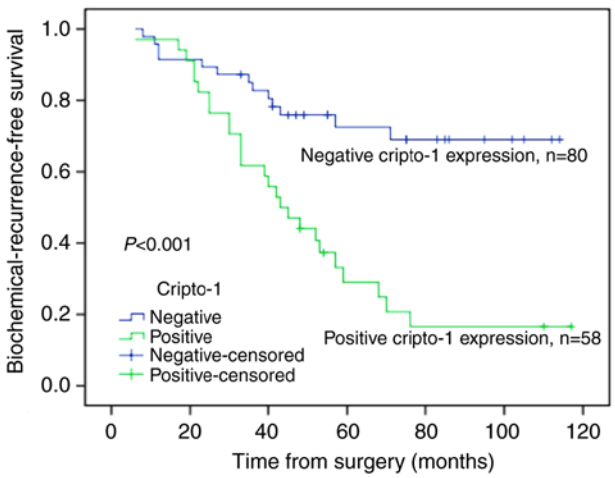

Figure 4. The BCR-free survival of patients with PCa was estimated by Kaplan-Meier analysis. Patients with high CR-1 expression showed significantly shorter BCR-free survival compared with those with low CR-1 expression (P<0.001). CR-1, cripto-1; PCa, prostate cancer; BPH, benign prostate hyperplasia; BCR, biochemical recurrence.

CR-1 expression was decreased in 57.97% of patients with PCa (80/138) and was increased in 42.03% of patients with PCa (58/138). Overexpression of CR-1 was significantly associated with the pre-operative PSA level (P=0.008), Gleason score (P=0.011) and lymph node metastasis (P=0.025). However, there was no association between CR-1 and age, surgical margin status or clinical stage. The association of CR-1 with clinicopathological parameters in patients with PCa is presented in Table II.

CR-1 expression and prognosis in patients with PCa. In the current study, the association between CR-1 overexpression and BCR was assessed with a ROC curve, which was generated using the Kaplan-Meier method. The data demonstrated that patients with high and low CR-1 expression had different BCR-free survival times. Statistical analysis revealed that overexpression of CR-1 was associated with shorter BCR-free survival (P<0.001; Fig. 4).

Role of CR-1 expression in PCa prognosis by Cox univariate and multivariate analysis. To confirm the prognostic factors associated with BCR-free survival of patients with PCa, several factors were assessed by univariate and multivariate analysis. Univariate analysis revealed that elevated CR-1 expression [hazard ratio (HR)=3.670; 95% confidence interval (CI), 1.874-7.186; P<0.001)], Gleason score (HR=4.382; 95% CI, 1.997-9.614; P<0.001) and lymph node metastasis (HR=2.612; 95% CI, 1.149-5.939; P=0.022) were significantly associated with BCR. However, age, surgical margin status, preoperative PSA levels and clinical stage were not significantly associated with PCa prognosis.

Furthermore, multivariate analysis revealed that CR-1 expression (HR=3.175; 95% CI, 1.247-8.084; P=0.015) and lymph node metastasis (HR=3.627; 95% CI, 1.229-10.699; P=0.020) were independent prognostic indicators in patients with PCa (Table III).

Discussion

In the present study, CR-1 expression was increased in PCa compared with BPH, which is consistent with previous

Table III. Univariate and multivariate analysis of prognostic factors and CR-1 expression with BCR-free survival in PCa.

	Univariate analysis		Multivariate analysis	
Prognostic factors	HR (95% CI)	P-value	HR (95% CI)	P-value
CR-1 expression (high vs. low)	3.670 (1.874-7.186)	<0.001	3.175 (1.247-8.084)	0.015
Age (years) (≥60 vs. <60)	1.363 (0.690-2.691)	0.373		
Surgical margin status (yes vs. no)	1.804 (0.876-3.717)	0.110		
PSA level (≥10 ng/ml vs. <10 ng/ml)	1.193 (0.632-2.253)	0.587		
Gleason score (≥7 vs. <7)	4.382 (1.997-9.614)	<0.001		
T stage (T_1 vs. T_2/T_3)	1.860 (0.958-3.611)	0.067		
Lymph node metastasis (yes vs. no)	2.612 (1.149-5.939)	0.022	3.627 (1.229-10.699)	0.020

PCa, prostate cancer; CR-1, cripto-1; PSA, prostate-specific antigen; T stage, tumor stage; HR, hazard ratio; CI, confidence interval; BCR, biochemical recurrence.

studies (29,30). CR-1 mRNA expression in PCa and BPH tissues was detected using RT-qPCR. The results revealed that CR-1 mRNA expression was higher in PCa tissues than in BPH tissues, following radical prostatectomy. In addition, CR-1 protein expression was also higher in PCa tissues following radical prostatectomy, as determined by western blot analysis. Nevertheless, additional clarification of whether CR-1 overexpression affects prognosis in male patients with PCa before and after radical prostatectomy is required.

CR-1 expression was significantly elevated in PC-3 and LNCaP cells compared with RWPE-1 cells. In addition, CR-1 was highly expressed in patients with PCa compared with BPH, as demonstrated by IHC. Analysis of the association between CR-1 expression and clinicopathological parameters revealed that CR-1 overexpression was significantly associated with pre-operative PSA level, Gleason score and lymph node metastasis in PCa. However, there was no association between CR-1 and age, surgical margin status and clinical stage. The findings indicated that CR-1 may have a critical role in the development of PCa.

Other studies have reported that various genes are associated with the prognosis of patients with PCa, including abnormal spindle microtubule assembly (ASPM), C-X-C motif chemokine ligand 12 (CXCL12), epithelial cell transforming sequence 2 (Ect2), a four-long non-coding RNA (lncRNA) signature (RP11-108P20.4, RP11-757G1.6, RP11-347I19.8 and LINC01123) and pleomorphic adenoma gene like-2 (PLAGL2) (31-35); however, research concerning the upregulation of CR-1 and prognosis in PCa has been limited. It has been reported that ASPM may have a critical role in PCa progression, and be an indicator of poor prognosis in patients with PCa (36). Goltz *et al* (37) reported that CXCL12 methylation associated with programmed death-ligand 1 expression was a prognostic predictor of BCR in patients with PCa following radical prostatectomy. Guo *et al* (33) demonstrated that elevated levels of Ect2 may be an independent prognostic biomarker of poor BCR-free survival; therefore, Ect2 levels may be a novel biomarker for PCa diagnosis or prognosis. In another study, a novel four-lncRNA signature was useful for survival prediction in patients with PCa (38). Furthermore, PLAGL2 overexpression was associated with PCa progression, and may be a predictor of poor prognosis (35).

ROC curve analysis demonstrated that overexpression of CR-1 was associated with poor clinical prognosis in PCa. Univariate analysis indicated that CR-1 had a significant effect on BCR-free survival, which was further validated in multivariate analysis. Patients with PCa with high CR-1 expression exhibited shorter BCR-free survival compared with patients with low CR-1 expression. The data demonstrated that CR-1 may be an important predictor of PCa for BCR-free survival. Additionally, CR-1 expression and lymph node metastasis were independent prognostic indicators in PCa. Therefore, these results suggested that CR-1 has potential to become a new promising prognostic indicator for patients with PCa.

The present study had several limitations. First, to study the function of a gene, besides overexpression, knockdown of its expression is also important. PC-3 cells exhibited high CR-1 expression in the present study, and therefore may be considered a good model for conducting future knockdown experiments. Second, mRNA levels of CR-1 in PC-3 cells were detected using RT-qPCR, however its protein expression should be also confirmed using western blot analysis. Third, further studies are required to investigate the molecular mechanisms between CR-1 expression and PCa. Furthermore, due to the limited sample size, future studies with larger sample sizes are required to verify these results.

The present study provides clinical evidence that CR-1 is overexpressed in PCa tissues. It has been shown that overexpression of CR-1 was identified to be a poor prognostic factor for BCR in patients with PCa. CR-1 detection may change the diagnostic and therapeutic approach in patients with PCa. These data suggest that CR-1 may be a novel factor in the design of future treatment strategies for PCa and in predicting the prognosis of patients with PCa following radical prostatectomy.

In conclusion, CR-1 was expressed at low levels in BPH tissues while CR-1 mRNA and protein were upregulated in PCa. The current study revealed that overexpression of CR-1 was associated with poor prognosis of patients with PCa and may serve a role in PCa progression. Consequently, CR-1 expression may be a novel biological target for personalized therapy in patients with PCa.

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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

RL and YX conceived and designed this study. YL performed the experiments and wrote the manuscript. JW and TY were responsible for data collection and performed the experiment. YL and TY analyzed and interpreted the data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The Second Hospital of Tianjin Medical University and informed consent was obtained from each patient prior to their involvement in the study.

Patient consent for publication

The study participants provided consent for the data in the present study to be published.

Competing interests

The authors declare that they have no competing interests.

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