CtBP2 overexpression is associated with tumorigenesis and poor clinical outcome of prostate cancer

Changwen Zhang, Shuanghui Li, Baomin Qiao, Kuo Yang, Ranlu Liu, Baojie Ma, Yan Liu, Zhihong Zhang, Yong Xu

Department of Urology, Second Hospital of Tianjin Medical University, Tianjin Institute of Urology, Tianjin, China

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Corresponding author:
Yong Xu, Zhihong Zhang
Department of Urology
Second Hospital
of Tianjin Medical University
Tianjin Institute of Urology
Tianjin 300211, China
E-mail:
xuyong8816@hotmail.com,
zhangzhihong8816@hotmail.com

Abstract

Introduction: The aim of the study was to evaluate the expression of CtBP2 in prostate cancer and to determine its relationship with clinicopathologic parameters.
Material and methods: The expression of CtBP2 in 119 prostate cancer tissues and 41 normal tissues was examined by qPCR and Western blot analysis, and the results were correlated with clinicopathologic parameters.
Results: CtBP2 expression in prostate cancer tissues was higher than that in normal samples. CtBP2 overexpression was closely correlated with serum prostatic specific antigen (PSA) (p = 0.018), advanced tumor stage (T3) (p = 0.025), higher Gleason scores (p = 0.019), positive extraprostatic extension (p = 0.012), positive vascular invasion (p = 0.011) and perineural invasion (p = 0.035). However, no significant association was found between CtBP2 abnormal expression and other parameters, including age (p = 0.776), positive lymph node (p = 0.872) and positive surgical margin (p = 0.37). Moreover, CtBP2 overexpression was significantly associated with poor clinical outcome of prostate cancer (p = 0.0168).
Conclusions: CtBP2 is overexpressed in prostate cancer, and its increased expression is closely associated with tumor progression and the outcome of prostate cancer.

Key words: prostate cancer, CtBP2, clinicopathologic parameters.

Introduction

Prostate cancer is one of the most common malignancies and the sixth leading cause of cancer death among males all over the world. It was reported that there were about 903,500 new cases of prostate cancer and 258,400 related cancer deaths in 2008 [1]. Thus far, prostate cancer has become one of the most shocking diseases threatening male health. For most primary prostate cancers, androgen ablation therapy is an effective strategy to retard the progression of the disease; however, only temporary remissions are produced. Once the cancer eventually transits to the androgen-refractory stage, no specific therapeutic method is available. Moreover, when the tumors progress to the metastatic stage, they essentially enter an incurable phase [2]. Therefore, it is urgently necessary to make improvements for early diagnostics and prevention of prostate cancer and seek a therapeutic target.

Many studies have demonstrated that C-terminal binding proteins (CtBPs) act as a transcriptional corepressor to regulate cancer cell survival and tumorigenesis by interacting with cellular binding partners of adenovirus E1A proteins in the nucleus [3–5]. CtBPs could mediate tumor cell migration and proliferation by epithelial-to-mesenchymal transition (EMT)-independent mechanisms in several cancer cells, e.g. colon, hepatocellular carcinoma and melanoma cancer cells [6–8]. Meanwhile, CtBPs also promoted the tumorigenesis and tumor progression in a manner independent of E1A binding CtBPs in colon cancer cells [3, 9]. Some studies indicated that CtBPs could be potential therapeutic targets for the treatment of several tumors [4, 5]. Moreover, a recent genome-wide association study (GWAS) revealed increasing susceptibility of CtBP2 to the risk for prostate cancer [10], but the molecular mechanisms responsible for CtBP2-mediated effects on the tumor have not been identified. We suspect that CtBP2 might play a role independently of E1A binding. Meanwhile, there are no pertinent studies on the relationship between CtBP2 and human prostate cancer.

In the current study, we found that CtBP2 was overexpressed in T3 stage prostate tumors and closely correlated with various clinical features and survival. Therefore, we inferred that CtBP2 might be a potential biomarker for tumorigenesis and a prognostic indicator of prostate cancer.

Material and methods

Patients

A total of 119 prostate tumor samples were obtained during radical retropubic prostatectomy from patients with prostate cancer at the Second Hospital of Tianjin Medical University from January 2005 to September 2011. The surgical approach and the resection of local nodes were performed according to the previous method [11, 12]. The tissues adjacent to prostate tumors were collected and used as a control, and all the tissues were pathologically examined. Approval for this study was obtained from the ethics committee of the Tianjin Medical University.

RNA extraction and reverse transcription reaction

Total RNA was extracted using the PureLink RNA Mini Kit (Invitrogen) following the instructions provided by the manufacturer. The quantity of RNA was determined by OD^{260} reading and further confirmed by ethidium bromide-stained agarose gel electrophoresis. After that, RNA (2.0 µg) was reversely transcribed to synthesize cDNA in a 20 µl reaction volume, which consisted of oligo-dT primer (1 µl/reaction), dNTP (1 µl of each), M-MLV (Promega) (1 µl/reaction), and RNase inhibitor (BioTeke) (1 µl/reaction). The reaction conditions were as follows: 65°C/5 min, 42°C/60 min, and 72°C/5 min.

Quantitative polymerase chain reaction with SYBR Green

For each quantitative polymerase chain reaction (qPCR) assay, a standard curve was constructed to calculate the estimated copy numbers of the sample mRNAs. A single reaction was prepared for the tissue cDNA along with each serial dilution using the Go Taq qPCR Master Mix (Promega). In order to verify the specific amplification with the primers, each PCR reaction also included a negative control. Primer sequences were as follows: CtBP2 (forward primer: 5'-ATCCACGAGAAGGTTCTAAACGA-3', reverse primer: 5'- CCGCACGATCACTCTCAGG-3'); GAPDH (forward primer: 5'-GAAGGTGAAGGTCGGAGT-3', reverse primer: 5'-GAAGATGGTGATGGGATTTC-3'). The real time quantitative PCR was run on an Eco Real-Time PCR System (Illumina). The cycling conditions were one cycle of denaturation at 94°C/5 min, followed by 45 three-segment cycles of amplification (94°C/20 s, 60°C/20 s, 72°C/20 s) (the fluorescence was automatically measured), and one three-segment cycle of product melting (94°C/1 min, 60°C/30 s, 94°C/30 s). The melting curve was constructed for each primer to verify the presence of one gene-specific peak and the absence of primer dimer. All samples were amplified in duplicate and the mean ± SE was used for further analysis.

Western blot analysis

Tissue samples were harvested into lysis buffer (Solarbio, China). Protein concentration determinations were performed with the Lowry protein assay (Solarbio, China) according to the instructions provided by the manufacturer. Equal amounts of protein (30–50 µg/lane) were subjected to 10% SDS-PAGE and processed for Western blot analysis as described previously [13]. The intensities of autoradiogram in Western blots were quantified with Image J (rsbweb.nih.gov/ij), and the quantified data of each protein were normalized with GAPDH.

Antibodies used were anti-GAPDH (1 : 8,000) and anti-CtBP2 (1 : 100) (obtained from Santa Cruz Biotechnology, USA)

Statistical analysis

The independent *t*-test was used to test the difference of the CtBP2 expression between the tumor and normal tissues. The χ^2 test was used to assess the associations between the abnor-

mal expression of CtBP2 and clinical features. The survival rates of the patients with abnormal CtBP2 expression were estimated by Kaplan-Meier survival analysis. In all statistical analyses, $p < 0.05$ was considered statistically significant.

Results

In order to assess the biogenic role of CtBP2 in prostate cancers, we examined the expression of CtBP2 in tumor and normal tissues by RT-qPCR and Western blot analysis. The results showed that the mRNA level of CtBP2 was increased 2-fold in tumor tissues compared to normal tissues (Figure 1 A, $p = 0.012$), and a similar result was also observed in the protein level (Figure 1 B), consist with previous study [10], suggesting that CtBP2 upregulation might be closely involved in the tumorigenesis and tumor progression of prostate cancer.

The main purpose of our study is to assess the clinical significance of abnormal expression of CtBP2 with prostate cancer. As we known, the level of serum PSA was an effective and widely used biomarker for prostate cancer, so we analyzed the relationship between the level of serum PSA and abnormal expression of CtBP2 in tumor tissues of patients with prostate cancer. First of all, we searched for an appropriate cut-off of CtBP2 abnormal expression, above which it was considered as overexpressed and below as low-expressed, and the mean level was used. We found that CtBP2 overexpression was closely correlated with increased serum PSA level ($p = 0.018$) (Table I), suggesting that CtBP2 might be a potential biomarker for prostate cancer. Subsequently, we focused our attention on the relationship between the abnormal CtBP2 expression and the other clinicopathologic parameters of prostate cancer patients. The results indicated that CtBP2 upregulation was closely correlated with advanced tumor stage (T3) ($p = 0.025$), higher Gleason scores ($p = 0.019$), positive extraprostatic extension ($p = 0.012$), positive vascular invasion ($p = 0.011$) and perineural invasion ($p = 0.035$), whereas no significant association was found between CtBP2 abnormal expression and age ($p = 0.776$), positive lymph node ($p = 0.872$) and positive surgical margin ($p = 0.37$) (Table I).

In order to evaluate the potential effect of high CtBP2 expression on the clinical outcome of patients with prostate cancer, we finally plotted the Kaplan-Meier survival curves. The result showed that CtBP2 upregulation was closely associated with poor survival of patients with prostate cancer ($p = 0.040$) (Figure 1 C).

Discussion

It was verified that CtBPs predominantly functioned as transcriptional corepressors by interact-

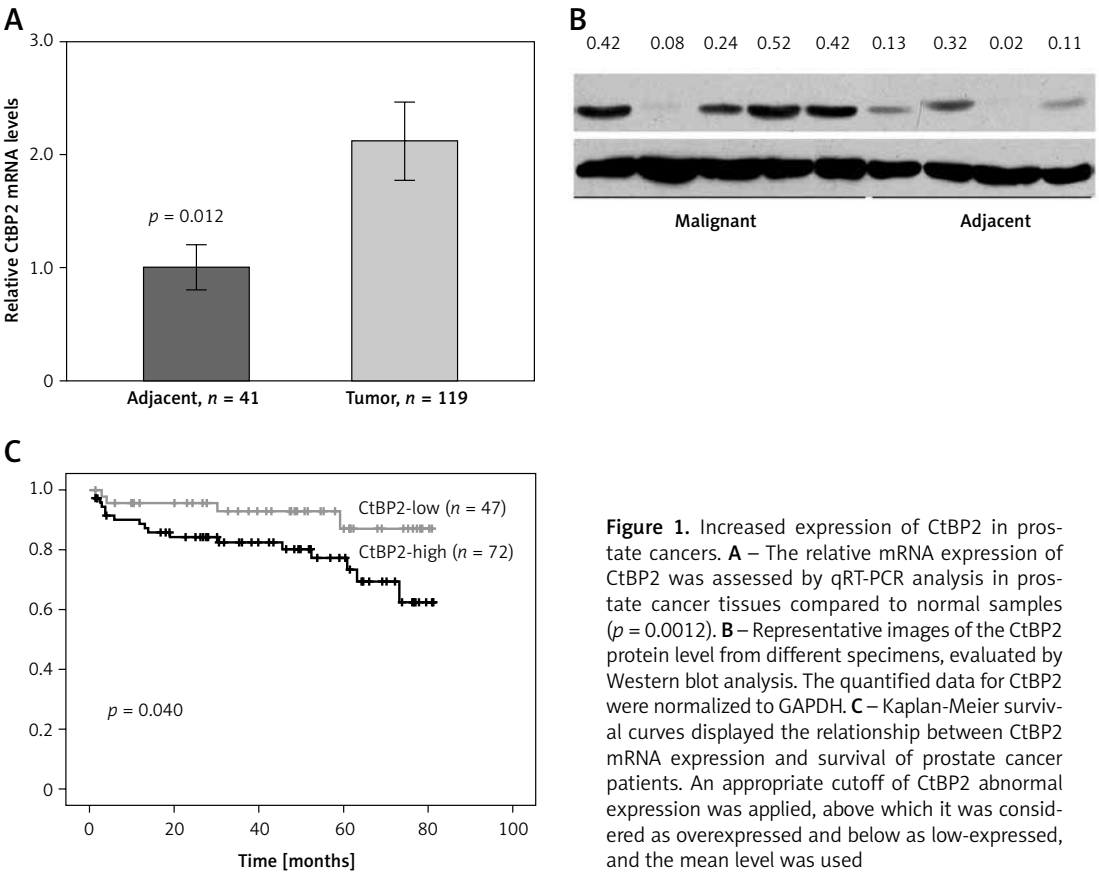

Figure 1. Increased expression of CtBP2 in prostate cancers. **A** – The relative mRNA expression of CtBP2 was assessed by qRT-PCR analysis in prostate cancer tissues compared to normal samples ($p = 0.0012$). **B** – Representative images of the CtBP2 protein level from different specimens, evaluated by Western blot analysis. The quantified data for CtBP2 were normalized to GAPDH. **C** – Kaplan-Meier survival curves displayed the relationship between CtBP2 mRNA expression and survival of prostate cancer patients. An appropriate cutoff of CtBP2 abnormal expression was applied, above which it was considered as overexpressed and below as low-expressed, and the mean level was used

Table I. Relationship between CtBP2 expression and clinicopathologic features

Features	Variables	Number	Overexpression, *n* (%)	Low expression, *n* (%)	Value of *p*
Age [years]	≥ 70	69	41 (59.4)	28 (40.6)	0.776
	< 70	50	31 (62)	19 (38)	
T status	T2	46	22 (47.8)	24 (52.2)	0.025
	T3	73	50 (68.5)	23 (31.5)	
Serum PSA	< 4	3	1 (33.3)	2 (66.7)	0.018
	4–110	35	15 (42.9)	20 (57.1)	
	> 10	81	56 (69.1)	25 (30.9)	
Gleason score	< 7	39	17 (43.6)	22 (56.4)	0.019
	7	14	8 (57.1)	6 (42.9)	
	> 7	66	47 (71.2)	19 (28.8)	
Lymph node	Positive	39	24 (61.5)	15 (38.5)	0.872
	Negative	80	48 (60)	32 (40)	
Extraprostatic extension	Positive	60	43 (71.7)	17 (28.3)	0.012
	Negative	59	29 (49.2)	30 (50.8)	
Surgical margin	Positive	49	32 (65.3)	17 (34.7)	0.37
	Negative	70	40 (57.1)	30 (42.9)	
Vascular invasion	Positive	40	26 (65)	14 (35)	0.011
	Negative	79	46 (58.2)	33 (41.8)	
Perineural invasion	Positive	84	57 (67.9)	27 (32.1)	0.035
	Negative	35	15 (42.9)	20 (57.1)	

ing with cellular binding partners of adenovirus E1A proteins. There are two similar but distinct isoforms, CtBP1 and CtBP2 [14]. Thus far, two distinct functions relating to CtBPs have been identified. First, they act as transcriptional corepressors to regulate cancer cell survival and tumorigenesis in the nucleus [4, 5]. Second, CtBPs were reported to play a major role in the fission of Golgi and endocytic membranes within the cytoplasm [5, 15, 16]. It was reported that CtBPs mediated tumor cell proliferation and migration by epithelial-to-mesenchymal transition (EMT)-independent mechanisms or by acting as an apoptosis antagonist in several cell lines, e.g., colon, hepatocellular carcinoma and melanoma cancer cells [6, 7, 9]. Additionally, several studies have demonstrated that E1A mutants in the CtBPs-binding motif (PLDLS) enhanced transformation of primary rodent epithelial cells, and cells transformed by the mutant E1A were also highly tumorigenic and metastatic, suggesting that the tumorigenic and tumor progressive role of CtBPs could occur independently of E1A binding [3, 5–7]. Moreover, a recent study proved that CtBP2 was a potential risk for prostate cancer [10]. In order to assess the biogenic role of CtBP2 in prostate cancers, we examined the expression of CtBP2 in tumor, the results showed that CtBP2 was high expressed in prostate cancer tissues compared to the normal tissues. These findings, in parallel to a previous study revealing that the expression of CtBP2 was a powerful predictor of prostatic malignancy [10], suggest that CtBP2 upregulation might be closely involved in the tumorigenesis of prostate cancer.

Vast numbers of studies have demonstrated that prostate specific antigen (PSA) was widely used for monitoring tumorigenesis, tumor progression, residual disease after operation and tumor recurrence of prostate cancer during follow-up, since serum PSA was considered as a sensitive biomarker of prostate cancer in the 1980s [17, 18]. In our study, we found that CtBP2 overexpression was closely correlated with increased serum PSA level, suggesting that CtBP2 might be a potential biomarker for prostate cancer. Though a previous study had presented similar ideas [10], strictly clinical evidence is insufficient.

Additionally, we also found that CtBP2 upregulation was closely correlated with advanced tumor stage (T3), higher Gleason scores, positive extraprostatic extension, positive vascular invasion, perineural invasion and poor outcome of patients.

A few recent studies reported that abnormal expression of CtBPs was involved with the progression of several cancer cells, e.g., breast, lung and colon cancer cells [6, 19]. All these data mean that high level of CtBP2 might be closely associated with the tumorigenesis and tumor progression of prostate cancer.

It was pointed out that CtBPs promoted hypoxia-induced migration of cancer cells by activating upon metabolic stress, such as hypoxia, and repressing epithelial and proapoptotic genes. Additionally, CtBPs promoted cell survival by maintaining the mitotic fidelity in breast cancer cells [20]; however, inhibition of CtBP expression sensitized breast cancer cells to diverse chemotherapeutic agents [21]. Thus, CtBPs have been proposed as potential therapeutic targets for the treatment of several tumors [4, 5]. Moreover, a recent genome-wide association study (GWAS) discovered that CtBP2 was a biomarker for prostate cancer [10]. Overexpression of CtBP2 activated the PI3 kinase signaling pathway to promote the progression of a p53-null human colon cancer cell line [6, 9, 22]. Although previous studies and our data demonstrated a fundamental role in tumorigenesis and tumor progression, the molecular mechanism of CtBP2 is still unclear, especially in prostate cancer. We suspect that CtBP2 might play a role independently of E1A binding during this process, and further study is needed.

In conclusion, our data suggested that CtBP2 was overexpressed in prostate cancer, and its increased expression was closely correlated with clinicopathologic parameters, e.g., serum prostatic specific antigen (PSA), advanced tumor stage (T3), higher Gleason scores, positive extraprostatic extension and perineural invasion. Moreover, the overexpression of CtBP2 was also associated with poor clinical outcome of prostate cancer. Therefore, CtBP2 might be a potential biomarker for tumorigenesis and a prognostic indicator of prostate cancer.

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Conflict of interest

The authors declare no conflict of interest.

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