LncRNA CASC2 Plays an Indicative Role in Diagnosing Bladder Cancer

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Abstract

Background: Bladder cancer is a common cancer of urinary system, with high incidence and mortality. LncRNA *CASC2* as a tumor suppressor has been reported to be involved in many human tumors. In this study, we aimed to explore the diagnostic value of *CASC2* for bladder cancer patients.

Methods: qRT-PCR was used to detect the expression level of *CASC2* in 140 bladder cancer patients and 90 healthy volunteers. The differences of *CASC2* expression between the cancer group and healthy group were analyzed using student's t test. The correlation of *CASC2* expression with clinical characteristics of the bladder cancer patients was estimated with Chi-square test. In addition, ROC curve was plotted to evaluate the diagnostic value of *CASC2* for bladder cancer patients.

Results: Serum *CASC2* level was lower in bladder cancer patients than that in healthy group (P<0.05). The expression level of *CASC2* was significantly associated with histological grade (P=0.000), TNM stage (P=0.000), and lymph node metastasis (P=0.001). The area under the ROC curve (AUC) was 0.864 and the optimal cutoff value was 0.955, suggesting the diagnostic value of *CASC2* for bladder cancer. The diagnostic sensitivity was 77.8% and specificity was 85.7%.

Conclusion: *CASC2* may be a novel biomarker for early diagnosis of bladder cancer.

Background

Bladder cancer is a most common cancer in urogenital system around the world [1, 2]. Moreover, its morbidity is significantly higher in males than that in females [3]. A variety of risk factors have been confirmed for bladder cancer, including tobacco smoking, exposure to certain chemicals in the working, as well as genetic factors [4]. At present, the treatments for bladder cancer have been significantly improved [5], but the prognosis of the patients are not obviously improved, due to the delay in early diagnosis [6, 7]. In bladder cancer, the 5-year survival rate of the patients with early stages is more than 75%, but the 5-year survival rate of patients with advanced stages is very low [8]. Thus, it is crucial to explore effective biomarkers to achieve early diagnosis of bladder cancer.

Long non-coding RNAs (LncRNAs) are group of non-coding RNAs, with the length of more than 200 nucleotides [9]. Despite of limited protein coding ability, lncRNAs can regulate the expression of genes at both the transcriptional and post-transcriptional levels [10]. LncRNAs are involved in various physiological processes, such as cell apoptosis, proliferation, differentiation, and the dysregulation of lncRNAs may lead to human diseases, especially cancer [11]. The abnormal expression expression of lncRNAs is observed in many human tumors, such as esophageal squamous cell carcinoma, thyroid carcinoma, etc [12, 13]. Cancer susceptibility candidate 2 (*CASC2*) is is located in 10q26 chromosome, belonging to lncRNA family [14]. LncRNA *CASC2* was firstly confirmed as a tumor suppressor in endometrial cancer [15]. Then, the abnormal expression of *CASC2* has been observed in a number of cancers [16]. In bladder cancer, it was reported that the expression of *CASC2* was down-regulated, and

could affect the biological behaviors of bladder cancer cells [14]. However, the diagnostic performance of *CASC2* expression for bladder cancer patients had been rarely reported in the published articles.

In this study, we aimed to detect the serum level of *CASC2* in bladder cancer patients and its functional roles in caner progression. In addition, we investigated the diagnostic performance of serum *CASC2* for bladder cancer.

Methods

Study subjects

A total of 140 bladder cancer patients were recruited from Huaihe Hospital of Henan University, and they were all diagnosed by pathologists. 90 healthy volunteers were recruited as the control group. In the healthy group, no one had been diagnosed with any malignancies. This study was approved by the ethics committee of Huaihe Hospital of Henan University. The written consents were obtained from all the subjects. The clinical features of the patients were listed in Table 1, including age, gender, tumor number, tumor size, histological grade, TNM stage, and lymph node metastasis.

Table 1
Association of *CASC2* expression with clinicopathological features of bladder cancer patients

Clinicopathologic features	N = 140	*CASC2* expression		χ^2	*P* Value
		Low(74)	High(66)		
Age (years)				1.693	0.193
< 50	76	44	32		
≥ 50	64	30	34		
Gender				0.387	0.534
male	81	41	40		
female	59	33	26		
Tumor number				0.032	0.857
single	52	28	24		
multiple	88	46	42		
Tumor size				3.205	0.073
< 3.6 cm	79	47	32		
≥ 3.6 cm	61	27	34		
Histological grade				42.455	0.000
low	64	53	11		
high	76	21	55		
TNM stage				19.378	0.000
I-II	70	24	46		
III-IV	70	50	20		
Lymph node metastasis				10.413	0.001
Positive	69	46	23		
Negative	71	28	43		

After a 12 h-fasting, 5 mL peripheral blood was collected from all the bladder cancer patients and healthy volunteers using a blood collection tube with EDTA. Then serum specimens were isolated from blood through centrifugation, and stored at -80℃ until used.

RNA extraction and quantitative real-time PCR

Total RNA was extracted from serum samples using Trizol reagents (Invitrogen, Carlsbad, CA) according to the manufacture's illustration. Then the first strand cDNA was synthesized by SuperScript III® (Invitrogen). In this study, the expression of *CASC2* was detected by qRT-PCR with SYBR Green method. *GAPDH* was employed as the internal reference. The primers of *CASC2* and *GAPDH* were as follows: *CASC2* forward: 5'-GCACATTGGACGGTGTTTCC-3', reverse: 5'-CCCAGTCCTTCACAGGTCAC-3'; *GAPDH* forward: 5'-AGAAGGCTGGGGCTCATTTG-3', reverse: 5'-AGGGGCCATCCACAGTCTTC-3' [16]. The relative expression level of *CASC2* was normalized to *GAPDH* and calculated using $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

In this study, all statistical analyses were performed with software of SPSS 19.0 (SPSS Inc., Chicago, IL) and GraphPad Prism 5 (GraphPad Software, Inc., USA). The expression of *CASC2* was expressed as mean ± SD, and student's T test was used to analyze the differences of the *CASC2* expression between the tumor and healthy samples. The bladder cancer patients were divided into two groups according to the average expression of *CASC2*. The relationship between serum *CASC2* expression and various clinical characteristics of bladder cancer patients was assessed with Chi-square tests. To determine the diagnostic value of serum *CASC2* in bladder cancer patients, receiver operating characteristic (ROC) analysis was performed. *P* values < 0.05 were considered statistically significant.

Results

The expression level of *CASC2* in bladder cancer tissues

QRT-PCR was used to detect the expression level of *CASC2* in bladder cancer patients and healthy volunteers. As showed in Fig. 1, the expression of *CASC2* was significantly lower in bladder cancer patients than that in healthy group ($P < 0.05$).

The association of *CASC2* expression with the clinical features of bladder cancer patients

Chi-square test was used to detect the correlation between *CASC2* expression level and the clinical data of bladder cancer patients. The results showed that *CASC2* expression was significantly associated with histological grade ($P = 0.000$), TNM stage ($P = 0.000$), and lymph node metastasis ($P = 0.001$). Meanwhile, *CASC2* expression had no significant correlation with the age, tumor number or tumor size (all $P > 0.05$) (Table 1).

The diagnostic value of *CASC2* in bladder cancer patients

ROC analysis was performed to estimate the diagnostic value of *CASC2* for patients with bladder cancer. The results were showed in Fig. 2. From the results, we could see that *CASC2* could distinguish bladder cancer patients from the healthy volunteers with the area under the curve (AUC) value of 0.864. The diagnostic sensitivity was 77.8%, and the specificity of 85.7%. The cutoff value of *CASC2* expression for bladder cancer diagnosis was 0.955.

Discussion

Bladder cancer is a fatal disease, with high metastasis ability [17]. There are various available treatments for bladder cancer patients, including radiation therapy, immunotherapy, radical cystectomy, and postoperative instillation of chemotherapy [18, 19]. However, the clinical outcomes of the patients have not been significantly improved. The prognosis of the patients is closely correlated with the tumor stages. Early diagnosis is very important for the prognosis of bladder cancer patients. Currently, the early detection of bladder cancer mainly dependents on cystoscopy and voided urinary cytology. However, the clinical values are limited due to invasive and uncomfortable procedures, low sensitivity and high cost [20, 21]. Therefore, the novel molecular biomarkers are in urgent need for early diagnosis of bladder cancer. In this study, we aimed to explore a novel biomarker to achieve the early diagnosis of bladder cancer.

CASC2 is a novel lncRNA in human genome, and it was firstly discovered by Baldinu, P et al. in human endometrial cancer [15]. The abnormal expression of *CASC2* is found in many human cancers, and it can act as a tumor suppressor [22]. For instance, Wang et al. found that in glioma tissues and cell lines the expression level of *CASC2* was down-regulated [23]. The study of He et al. found the expression of *CASC2* was lower in non-small cell lung cancer (NSCLC) tissues and was correlated with tumor size and tumor stage [24]. The study group of Cao et al. indicated that *CASC2* expression was down-regulated in human renal cell carcinoma (RCC) tissues and cell lines [25]. The expression patterns of *CASC2* showed close association with cancer progression, which might be a potential biomarker for management of cancers [26, 27]. However, the clinical significance of *CASC2* has been rarely investigated in bladder cancer.

In this study, we found that the expression level of *CASC2* was significantly lower in bladder cancer patients than that in healthy volunteers. Moreover, the expression of *CASC2* was negatively correlated with histological grade, TNM stage, and lymph node metastasis. The down-regulation of *CASC2* might contribute to malignant progression of bladder cancer. The study performed by Li et al. indicated that the bladder cancer patients with low expression of *CASC2* were more likely to undergo malignant disease progression and postoperative recurrence [28]. Pei er al. reported that the enforced expression of *CASC2* could remarkably inhibit cell growth, migration and invasion, and promote cell apoptosis in bladder cancer cells in vitro. *CASC2* might play anti-tumor action in bladder cancer through inhibiting Wnt/β-catenin pathway [14]. Both of the published articles might explain the results obtained in our study.

Given its distinct expression profile in bladder cancer, we hypothesized that serum *CASC2* might be a potential diagnostic biomarker for the cancer. Thus, ROC analysis was performed to detect the diagnostic value of *CASC2* in bladder cancer patients. From the ROC curve, we could see that the *CASC2* expression could discriminate between bladder cancer patients and healthy controls with the sensitivity of of 77.8%, specificity of 85.7%, and the AUC of 0.864. Serum *CASC2* might be a potential non-invasive biomarker for early detection of bladder cancer. However, several limitations should be stated in current study. Firstly, the sample size was relatively small that might reduce the statistical power of our results. Second, the molecular mechanisms underlying the anti-tumor action of *CASC2* in progression of bladder cancer was

not explored in our study. In addition, all the individuals in control group were healthy, our results only confirmed that serum *CASC2* could distinguish bladder cancer patients from the healthy individuals. Whether serum *CASC2* could discriminate between bladder cancer patients and other malignancies cases remained unclear. Therefore, well-designed studies with a larger sample are required to verify and improve our conclusion.

Conclusions

In conclusion, serum lncRNA *CASC2* is down-regulated in bladder cancer, and shows close association with aggressive cancer progression. Serum *CASC2* may be a potential biomarker for early detection of bladder cancer.

List Of Abbreviations

Long non-coding RNAs (LncRNAs)

Cancer susceptibility candidate 2 (*CASC2*)

Receiver operating characteristic (ROC)

Area under the curve (AUC)

Non-small cell lung cancer (NSCLC)

Renal cell carcinoma (RCC)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Huaihe Hospital of Henan University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials All data generated or analysed during this study are included in this published article.

Competing interests The authors declare that they have no competing interests.

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Authors' contributions H.Z. design of the work; X.L. the acquisition, analysis, W.T. interpretation of data; L.G. the creation of new software used in the work; Z.Y. and X.B. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Figures

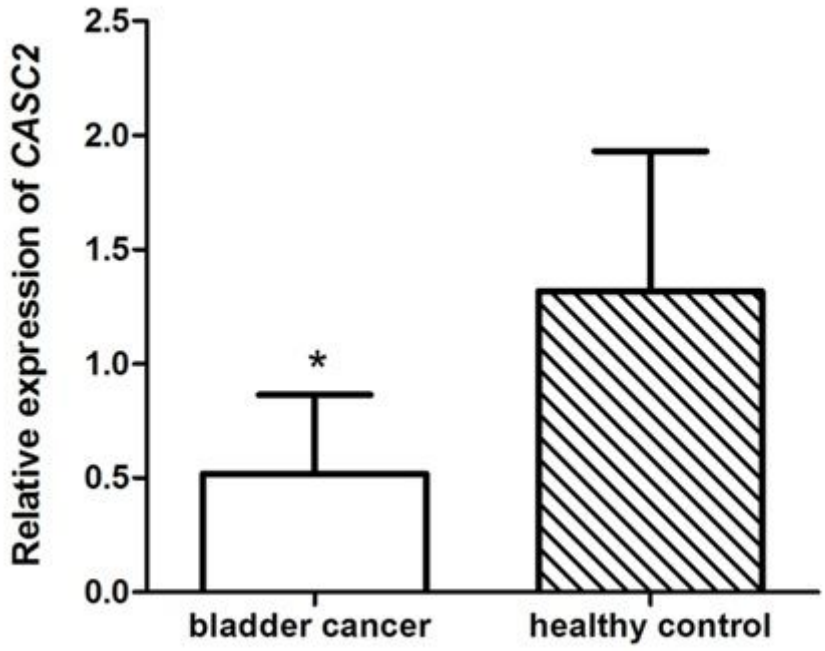

Figure 1

CASC2 expression level between bladder cancer tissues and healthy tissues. The levels of CASC2 were significantly decreased in bladder cancer patients, compared to the healthy controls.

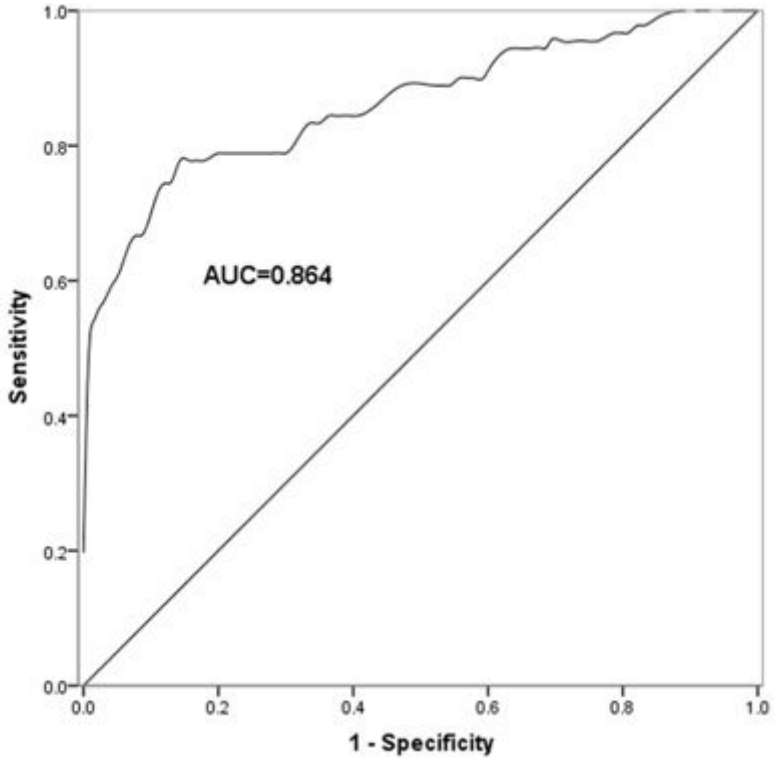

Figure 2

ROC analysis was performed to estimate the diagnostic performance of serum CASC2 for bladder cancer patients.