Clin. Lab. 2020;66:XXX-XXX

ORIGINAL ARTICLE

Enhanced CDR1as Promotes the Development of Bladder Urothelial Carcinoma

Yan Yang [1, 2], Fancong Kong [2], Ying Cai [2], Qingqing Ding [2], Beisha Tang [1, 3]

[1] *Department of Neurology, Xiangya Hospital, Central South University, Changsha, Hunan, China*
[2] *Department of Neurology, Affiliated Hospital of Jining Medical University, Jining, Shandong, China*
[3] *National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan, China*

SUMMARY

Background: **The current study aimed to evaluate the expression of circular RNA (circRNA) CDR1as in bladder urothelial carcinoma and adjacent normal tissues, thereby laying the foundation for the research of bladder urothelial carcinoma.**
Methods: **The level of CDR1as was evaluated in the tissues of bladder urothelial carcinoma patients. ROC analysis was carried out to explore the possible diagnostic value of CDR1as.**
Results: **In the current study, we showed novel data that the expression of CDR1as in bladder urothelial carcinoma tissues was significantly higher than that in adjacent tissues. Furthermore, the expression of CDR1as was significantly higher in non-muscle invasive bladder cancer (NMIBC) and high-grade cancer tissues than in muscle invasive bladder cancer (MIBC) and low-grade cancer tissues. In patients with lymph node metastasis, the expression of CDR1as was also significantly higher. Moreover, the expression of CDR1as was higher in high-stage, high-grade bladder urothelial carcinoma than in low-stage and low-grade cancer tissues. Real time PCR analysis showed that there was no difference in the expression of CDR1as among the patients according to the grouping of gender (male vs. female), age (≤ 65 years vs. > 65 years), tumor size (≤ 3 cm vs. > 3 cm), and lymphatic invasion (LVI).**
Conclusions: **The current study for the first time showed upregulation of CDR1as may be involved in the development and progression of bladder urothelial carcinoma.**
(Clin. Lab. 2020;66:xx-xx. DOI: 10.7754/Clin.Lab.2019.191213)

Correspondence:
Dr. Beisha Tang
Department of Neurology
Xiangya Hospital
Central South University
Changsha
410008 Hunan
China
Email: dryangyan@mail.jnmc.edu.cn

Manuscript accepted December 12, 2019

KEY WORDS

CDR1as, bladder urothelial carcinoma, upregulation, oncogene

INTRODUCTION

Bladder urothelial carcinoma is a common tumor of the urinary system and is more common in the elderly (55 years and older) [1]. Ninety-five percent of bladder urothelial carcinomas are urothelial carcinomas, including adenocarcinoma, squamous cell carcinoma, small cell carcinoma, and other rare pathological types, often accompanied with a poor prognosis [2]. Because of the high recurrence rate of bladder urothelial carcinoma, proper patient monitoring is necessary to reduce patients' suffering and associated medical expenses [3-5]. However, the biological mechanism of its occurrence

has not yet been fully understood. There is still insufficient theoretical basis for how to prevent the occurrence and development of bladder urothelial carcinoma [6,7]. Therefore, studying the biological mechanism is essential for the therapy against bladder urothelial carcinoma. In recent years, circular RNAs (circRNAs), as a new class of non-coding RNAs, play an important role in regulating molecular mechanisms of cancer and have attracted widespread attention [8,9]. A growing number of studies confirm that circRNA can optimize the early diagnosis and treatment of disease [10-13]. As a circRNA, CDRlas is a relatively early and well-defined circRNA, and its sequence is densely distributed with multiple miRNA binding sites [14-16]. Exogenous CDR1as has been shown to cause midbrain damage in zebrafish by binding to miR-7 [17]. Studies have also found that CDR1as may be a novel tumor marker for predicting hepatocellular carcinoma [18,19]. However, the role of CDR1as in bladder urothelial carcinoma is poorly understood.

The current study aims to investigate the differential expression of CDR1as in bladder urothelial carcinoma and adjacent normal tissues, thereby laying the foundation for the research of bladder urothelial carcinoma.

MATERIALS AND METHODS

Collection of specimens

In this study, sixty patients with bladder urothelial carcinoma were enrolled from December 2016 to April 2017, including 36 (60%) males and 24 (40%) females. The average age of the enrolled patients was 68.10 ± 7.47 years, of which 22 (37%) patients were ≤ 65 years old and 38 (63%) patients > 65 years old (Table 1). Sixty pairs of bladder urothelial carcinoma tissues and corresponding adjacent normal tissues were collected from December 2016 to April 2017. Specimens were taken from the surgical room by two designated urologists. Once the specimens were removed, they were immediately stored in RNAsaver (Beijing Solarbio Science & Technology Co., Ltd, Beijing, China) and stored at -80°C to minimize RNA degradation. The specimens were obtained by collecting typical cancer and adjacent tissues during total cystectomy. According to the two doctors in the pathology department of our hospital, the clinical pathological staging and grading were confirmed. Corresponding adjacent tissues were defined as normal bladder tissues at least 3 cm from the edge of the tumor and confirmed by pathological techniques to be free of cancer cell infiltration. All patients signed an informed consent, and the study was approved by the Ethics Committee of The First Affiliated Hospital of Jinzhou Medical University.

Real time-quantitative PCR

The purity of the total RNA was determined using an ultraviolet spectrophotometer. If the ratio of OD260/OD280 was between 1.8 and 2.1, the purity of the RNA was considered acceptable.

The total RNA was extracted by RNAVzol (Qiagen GmbH, Hilden, Germany). The reverse transcription was carried out by miScript II RT Kit (Qiagen GmbH, Hilden, Germany) according to the kit instructions. One milligram of cDNA was used for qPCR using SYBR green Master mix (Roche Diagnostics, Basel, Switzerland) on a Roche Lightcycler 480 (Roche Diagnostics, Basel, Switzerland). The reaction procedure was: 95°C 15 minutes, 40 cycles of 94°C 15 seconds, 55°C 30 seconds, and 70°C 30 seconds. Relative miRNA expression of CDR1as was normalized against the endogenous control, GAPDH, using the ΔΔCq method [20]. Each sample was repeated three times in a 96-well plate.

Statistical analysis

The data are represented as the mean ± standard deviation (SD). The two-tailed unpaired Student's *t*-tests were used for comparisons of two groups. The one-way ANOVA multiple comparison test (SPSS 20.0) followed by Tukey's post hoc test were used for comparisons of two or more groups. Receiver operating characteristic (ROC) curves were used to assess CDR1as as a biomarker, and the area under the curve (AUC) was reported (IBM Corp; IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY, USA). $p < 0.05$ was considered significant.

RESULTS

Expression of CDR1as in bladder urothelial carcinoma and adjacent tissues

First, we determined the expression of CDR1as in bladder urothelial carcinoma and adjacent tissues. Compared with the normal tissues adjacent to the cancer (1.00 ± 0.77), the expression of CDR1as in cancer tissues was significantly up-regulated (4.35 ± 2.68) (Figure 1).

Expression of CDR1as in bladder urothelial carcinoma with different pathological stages

According to the TNM staging system of the International Union Against Cancer (UICC), bladder urothelial carcinoma was classified into NMIBC and MIBC. The former included Tis, Ta, and T1; the latter included T2, T3, and T4. To investigate whether CDR1as was associated with the biological behavior of urothelial carcinoma progression, we investigated the expression of CDR1as among different stages in bladder urothelial cancer patients. Among the 60 patients, a total of 17 NMIBC patients showed the average expression of CDR1as was 1.00 ± 0.42, while a total of 43 MIBC patients showed the average expression of CDR1as was 3.38 ± 1.48 (Figure 2A). Furthermore, with the increase of bladder urothelial carcinoma grading, the relative expression of CDR1as was gradually enhanced in high grade patients (4.70 ± 2.55) compared to that in low grade patients (1.00 ± 0.16) (Figure 2B), indicating that

Table 1. General characteristics of patients.

Characteristics		No. of patients
Gender	Male	36
	Female	24
Age	≤ 65 years	22
	> 65 years	38
Staging	NMIBC	17
	MIBC	43
Grading	Low grade	6
	High grade	54
LVI	None	36
	Yes	24
Tumor size	≤ 3 cm	24
	< 3cm	36
Lymph node	N-	38
	N+	22

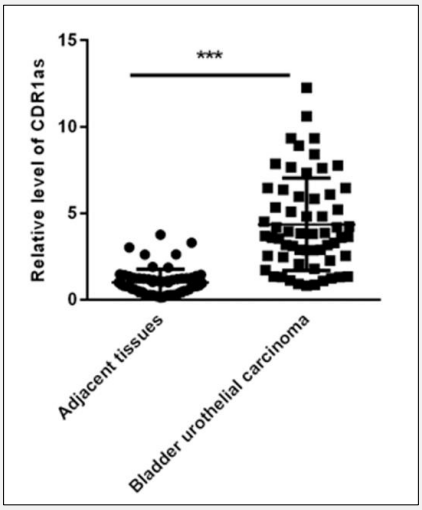

Figure 1. Real time PCR analysis showed that the expression of CDR1as in cancer tissues was significantly up-regulated compared to that in the normal tissues adjacent to the cancer.

CDR1as played an important role in the progression of bladder urothelial carcinoma.

The expression of CDR1as was increased along with lymph node metastasis

According to the TNM staging system of the 2009 International Anti-Cancer Alliance (UICC), lymph node metastasis was divided into N0, N1, N2, N3, and NX. In the current study, both NX and N0 were classified as N- (lymph node negative), while N1, N2, and N3 were all classified as N+ (lymph node positive). According to our data, the relative expression of CDR1as was lower in N- patients (1.00 ± 0.48), while the expression of CDR1as in N+ patients was higher (2.76 ± 1.08), and

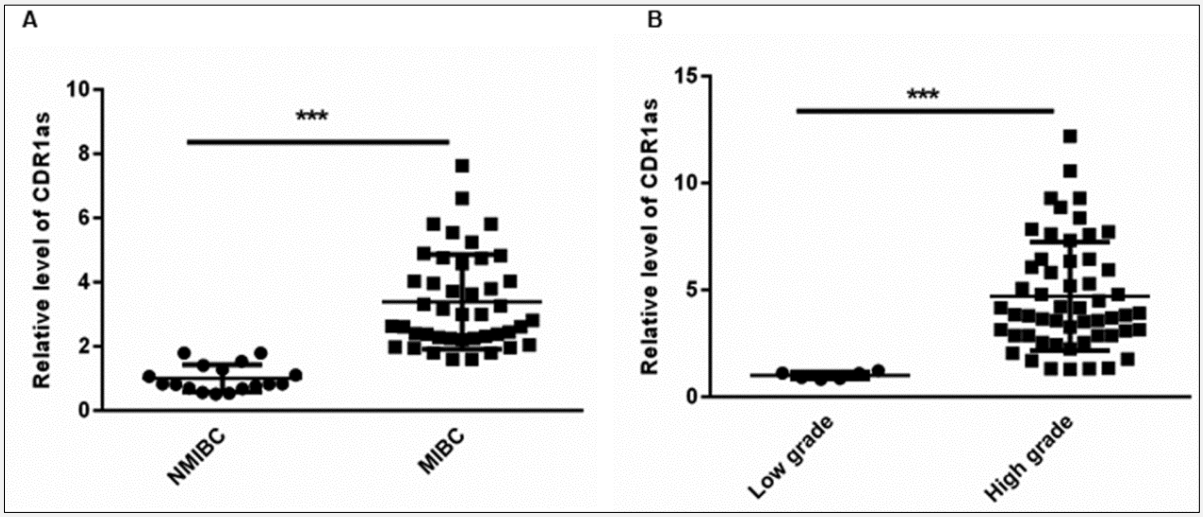

Figure 2. The expression of CDR1as was determined in bladder urothelial carcinoma with different pathological stages.

(A) The expression of CDR1as was increased in MIBC patients compared to that in NMIBC patients. (B) The relative expression of CDR1as was gradually enhanced with the increase of bladder urothelial carcinoma stating.

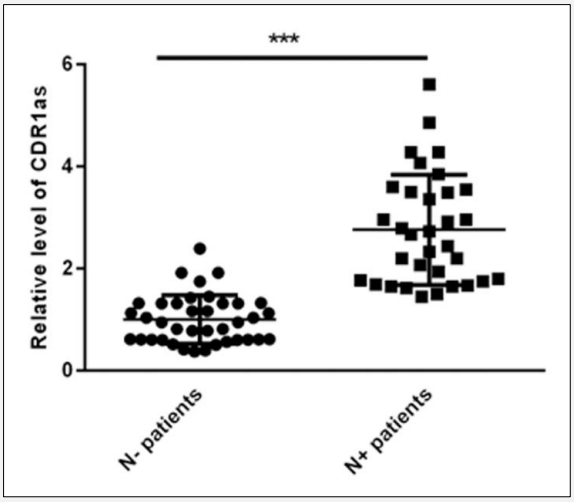

Figure 3. The relative expression of CDR1as was increased in N+ patients compared to that in N- patients.

the difference was statistically significant (Figure 3), suggesting that in bladder urothelial carcinoma, CDR1as could enhance the invasiveness of the tumor and promote lymph node metastasis of the tumor.

Other clinical features and the expression of CDR1as

Additionally, we also analyzed the expression of CDR1as according to other clinical pathological features, such as gender (male vs. female: 3.44 ± 2.09 vs.

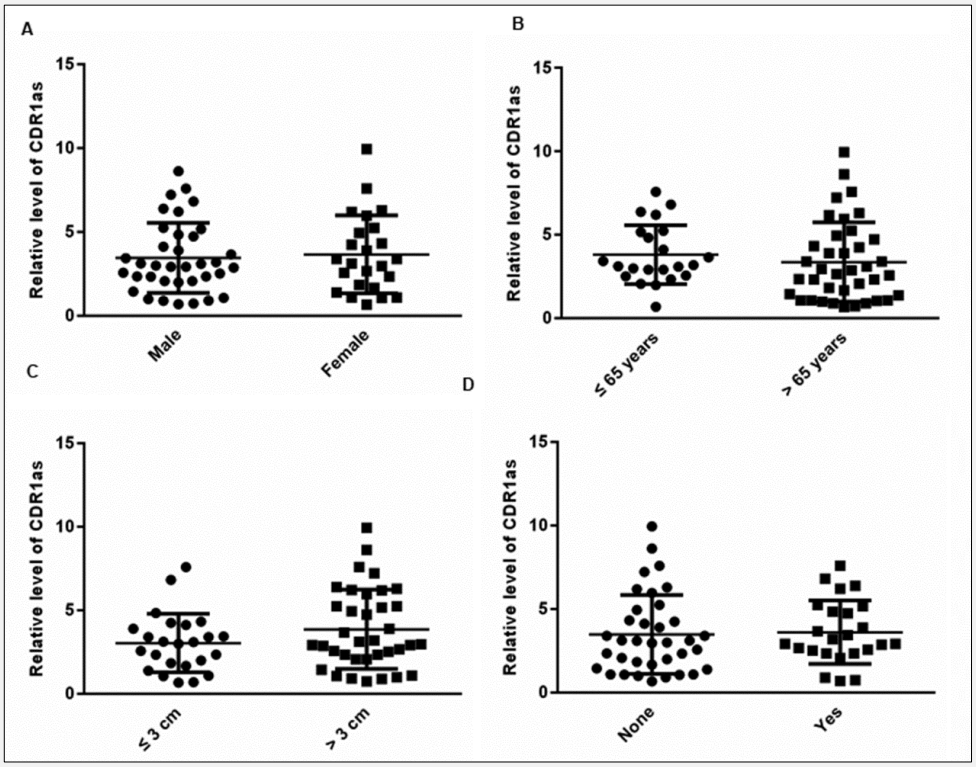

Figure 4. The expression of CDR1as was also analyzed according to other clinical pathological features.

Real time PCR analysis showed that there was no difference in the expression of CDR1as among the patients according to the grouping of gender (male vs. female) (A), age (≤ 65 years vs. > 65 years) (B), tumor size (≤ 3 cm vs. > 3 cm) (C), and lymphatic invasion (LVI) (D).

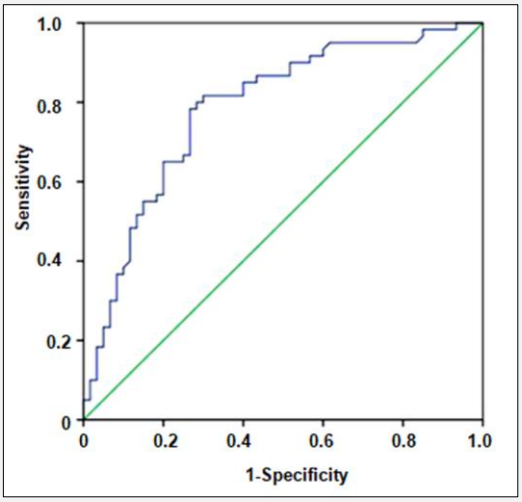

Figure 5. ROC curve analysis showed that CDR1as can contribute to the early diagnosis of bladder urothelial carcinoma.

3.66 ± 2.33), age (≤ 65 years vs. > 65 years: 3.81 ± 1.77 vs. 3.37 ± 2.38), tumor size (≤ 3 cm vs > 3 cm: 3.03 ± 1.75 vs. 3.86 ± 2.37), and lymphatic invasion (LVI None vs. Yes: 3.48 ± 2.39 vs. 3.61 ± 1.90). Our data showed that there was no statistically significant difference in the relative expression of CDR1as between the groups (Figure 4).

The diagnostic value of CDR1as was assessed by ROC curve analysis

To assess whether CDR1as can be used as a diagnostic marker for bladder urothelial carcinoma, the ROC curve was used in this study. A total of 60 patients with adjacent normal tissues served as controls. As shown in Figure 5, the area under the curve was 0.798 (95% CI: 0.756 - 0.892, $p < 0.001$). The sensitivity and specificity of CDR1as were 0.803 and 0.835, respectively, and the false positive rate and false negative rate of CDR1as were 0.267 and 0.217, respectively. The Youden index was assessed to be 0.517. Hence, CDR1as can contribute to the early diagnosis of bladder urothelial carcinoma.

DISCUSSION

CircRNAs have been discovered in recent years as special endogenous non-coding RNAs, which have gradually become a hot spot in RNA research [8,21,22]. Numerous studies have shown that circRNAs play an important regulatory role in the development and progression of cancer [23-25]. The key role of CDR1as has been extensively reported in different circumstances [19,26,27]. For instance, it has been reported that CDR1as can affect miRNAs and can be degraded by miR-671 [28]. Subsequent studies revealed that CDR1as contains more than 70 conserved sites to bind to miR-7 [29]. In addition, CDR1as expressed higher levels in neural tissues, while overexpression of CDR1as in zebrafish embryos reduces the size of the midbrain and is closely related to the loss of miR-7 function [31]. However, no study has been performed on the role of CDR1as in bladder urothelial carcinoma patients.

In the current study, we showed novel data that the expression of CDR1as in bladder urothelial carcinoma tissues was significantly higher than that in adjacent tissues. Furthermore, the expression of CDR1as was significantly higher in NMIBC and high-grade cancer tissues than in MIBC and low-grade cancer tissues. In patients with lymph node metastasis, the expression of CDR1as was also significantly higher. It is therefore possible to speculate that CDR1as can influence the expression of target proteins to promote the occurrence and progression of bladder urothelial carcinoma. Of course, the study of downstream mechanisms still requires future experiments to verify.

Moreover, our data initially confirmed the expression of CDR1as was higher in high-stage, high-grade bladder urothelial carcinoma than in low-stage and low-grade cancer tissues. These data revealed the potential role of CDR1as as a marker for predicting the development and progression of bladder urothelial carcinoma. ROC analysis also showed that CDR1as could differentiate bladder urothelial carcinoma tissues from normal adjacent tissues with the sensitivity and specificity of 0.783 and 0.733. This experiment also laid a solid foundation for the future research of CDR1as as a potential biomarker of bladder urothelial carcinoma patients.

Finally, the trial also has some shortcomings: First, we only verified the stable expression of CDR1as in bladder urothelial carcinoma and adjacent tissues, and analyzed the relationship between CDR1as and some clinicopathological features of 60 patients. A larger sample size is needed for the next step of verification. Secondly, we only explored the potential link between CDR1as and lymph node metastasis. Due to the short follow-up time, the patients' prognosis should be closely followed to study whether there is a close relationship between the expression of CDR1as and the biological behavior of distant metastasis.

CONCLUSION

In summary, the current study, for the first time, showed upregulation of CDR1as may be involved in the development and progression of bladder urothelial carcinoma.

Declaration of Interest:

We declare no conflicts of interest.

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