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ORIGINAL ARTICLE

ADAMTS9-AS2: a potential diagnostic and prognostic hallmark in prostate cancer

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Summary

Purpose: *To explore the expression level and prognostic value of ADAMTS9-AS2 in prostate cancer (PCa).*

Methods: *ADAMTS9-AS2 levels in 110 paired PCa tissues and adjacent normal tissues were detected by quantitative real-time polymerase chain reaction (qRT-PCR). The relationship between ADAMTS9-AS2 level and clinical parameters of PCa was analyzed. ROC (receiver operating characteristics) curves were depicted for assessing the diagnostic value of ADAMTS9-AS2 in PCa. Through collecting 5-year follow-up data of PCa patients, survival analysis was performed by Kaplan-Meier method. Finally, Cox regression model was used to analyze factors affecting outcomes of PCa patients.*

Results: *ADAMTS9-AS2 was downregulated in PCa tissues than in adjacent normal ones. Its level was lower in PCa tissues with clinical stage III+IV or tumor size ≥3cm compared to those with stage I+II or tumor size <3cm. ROC curves verified the diagnostic value of ADAMTS9-AS2 in PCa (AUC=0.902, cut-off value=0.40, sensitivity=90.00%, specificity=79.09%, Youden index=0.6909). Kaplan-Meier method and log-rank test uncovered worse prognosis in PCa patients expressing low level of ADAMTS9-AS2. Clinical stage, tumor size and ADAMTS9-AS2 level were independent factors influencing prognosis of PCa.*

Conclusions: *ADAMTS9-AS2 is downregulated in PCa and its low level is unfavorable to the disease prognosis. ADAMTS9-AS2 may be utilized as a potential diagnostic and prognostic hallmark of PCa.*

Key words: *prostate cancer, ADAMTS9-AS2, prognosis*

Introduction

Prostate cancer (PCa) is the leading malignancy in males, and it is also the second fatal malignancy in developed countries [1]. Most of PCa cases can enjoy great therapeutic efficacy after radical resection and combined strategies in the early disease [2]. As a result, diagnosis of PCa as early as possible is of significance in tumor treatment and achieving tumor progression [3,4].

Long non-coding RNA (lncRNA) is a non-coding RNA with longer than 200 nucleotides. LncRNA-encoded genes are widely distributed in genomes and exert extensive biological functions [5,6]. Only a small part of RNAs synthesized by biological DNAs could translate into proteins. Actively transcribed PCa cells produce a large amount of lncRNAs, some of which may have high specificity of PCa and serve as an ideal biomarker for early diagnosis of PCa [7]. Lee et al [8] screened out several lncRNAs that are differentially expressed in PCa, including AK024556 (SPRY4-IT1), XLOC_007697, LOC100287482, XLOC_005327, XLOC_008559 and XLOC_009911. These lncRNAs are closely linked to the occurrence and progression of PCa, serving as diagnostic markers.

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ADAMTS9-AS2 is an antisense transcript of the tumor-suppressor gene ADAMTS9, which is located on human chromosome 3p14.1 pairing with ADAMTS9. ADAMTS9 is an important anti-angiogenic factor that inhibits tumor progression and metastasis by regulating fibroblast growth factor and vascular endothelial growth factor [9]. It is reported that ADAMTS9-AS2 is markedly downregulated in glioma tissues, and negatively correlated with tumor grade and prognosis. Overexpression of ADAMTS9-AS2 is able to block the migratory ability in glioma cells [10]. In this article, we first detected the expression level of ADAMTS9-AS2 in paired PCa tissues. Subsequently, its prognostic and diagnostic values in PCa were further analyzed.

Methods

Subjects and tissues

A total of 110 paired PCa tissues and adjacent normal ones were surgically resected from PCa patients and their clinical data were recorded, including age, Gleason score, clinical stage, tumor size, and tumor differentiation. Tissues were quickly frozen in liquid nitrogen and placed at -80℃ and signed the informed content form. Patients and their families in this study have been fully informed and signed the informed consent form. This study was approved by Ethics Committee of Affiliated Hospital of Nantong University (Decision no.CN-107-069502).

Quantitative real-time polymerase chain reaction (qRT-PCR)

PCa tissues were lysed for isolating total RNA using TRIzol method (Invitrogen, Carlsbad, CA, USA). RNA was reversely transcribed into complementary DNA (cDNA) using Primescript RT Reagent (TaKaRa, Otsu, Japan), and applied for RT-PCR using the All-in-OneTM miRNA qRT-PCR kit (Gene Copoeia Inc, Rockville, MD, USA). PCR system included: 10 μL of SYBR Green I mix (Rox), 1 μL of forward primer, 1 μL of reverse primer, 5 μL of ddH_2O and 3 μL of cDNA. Glyceraldheyde 3-phosphate dehydrogenase (GAPDH) was used as the internal reference. Primer sequences were listed as follows: AD-AMTS9-AS2: F: 5'-TCTGTTGCCCATTTCCTACC-3' and R: 5'-CCCTTCCATCCTGTCTACTCTA-3';GAPDH: F: 5'-GGACCAATACGACCAAATCCG-3' and R: 5'-AGCCACATCGCTCAGACAC-3'.

Follow-up

Every patient was followed-up through outpatient visit, telephone call or E-mail for at least 5 years. The follow-up was conducted once at the first month postoperative, three months in the first year, six months in the second year and once per year thereafter. The follow-up rate achieved 100%.

Statistics

SPSS 22.0 (IBM, Armonk, NY, USA) was used for data analyses. Measurement data were expressed as mean±standard deviation (x±s). Differences between two groups were compared by the t-test. Diagnostic value of ADAMTS9-AS2 in PCa was assessed by ROC curves, while its prognostic value was evaluated by Kaplan-Meier method, followed by log-rank test. Potential factors influencing prognosis of PCa were analyzed by Cox regression model. $P<0.05$ was considered as statistically significant.

Results

ADAMTS9-AS2 was downregulated in PCa

QRT-PCR data showed that ADAMTS9-AS2 was downregulated in PCa tissues relative to adjacent normal ones (Figure 1). It is suggested that ADAMTS9-AS2 may be a tumor-suppressor gene alleviating the progression of PCa.

Relationship between ADAMTS9-AS2 level and clinical parameters of PCa patients

To elucidate the clinical significance of ADAMTS9-AS2 in PCa, clinical parameters of PCa patients were recorded and showed that ADAMTS9-AS2 level was not correlated to age, Gleason score and tumor differentiation of PCa ($p>0.05$). However, ADAMTS9-AS2 level was much lower in PCa tissues with stage III +IV or tumor size ≥3cm than those with stage I+II or <3cm ($p<0.05$, Table 1).

Diagnostic value ofADAMTS9-AS2 in PCa

Since ADAMTS9-AS2 was differentially expressed in PCa tissues and normal ones, we speculated the diagnostic potential of ADAMTS9-AS2 in PCa. ROC curves depicted that AUC was 0.902 ($p<0.001$, 95%CI: 0.855-0.93). The cut-off value was 0.4 (sensitivity=90.00%, specificity=79.09%, Youden index=0.6909). It is suggested that the onset risk of PCa increased with ADAMTS9-AS2 level ≤0.4 (Figure 2).

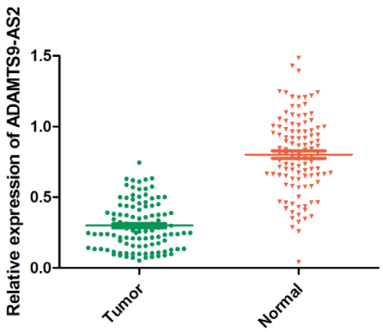

Figure 1. ADAMTS9-AS2 was downregulated in PCa. ADAMTS9-AS2 was downregulated in PCa tissues (n=110) relative to adjacent normal ones detected by qRT-PCR.

Prognostic value ofADAMTS9-AS2 in PCa

Based on the cut-off value of ADAMTS9-AS2, 110 PCa patients were assigned into high-level group (n=29) and low-level group (n=81). Kaplan-Meier curves based on 5-year postoperative follow-up data revealed worse prognosis in low-level group than that of high-level group (HR=1.974, p=0.009, Figure 3) showing that low level of ADAMTS9-AS2 was unfavorable to the prognosis of PCa.

Cox regression analysis on factors influencing survival of PCa

Potential factors that may affect survival of PCa were analyzed by Cox regression model. The mortality risk of PCa with clinical stage III+IV, tumor size ≥3cm and low level of ADAMTS9-AS2 was 1.781, 1.551 and 1.974 times higher than those with stage I+II, tumor size < 3cm and high level of ADAMTS9-AS2 (Table 2). Therefore, clinical stage, tumor size and ADAMTS9-AS2 level were independent factors influencing the prognosis of PCa.

Discussion

The incidence of PCa varies in different races and regions. The morbidity and mortality of PCa in European and American males rank first and second, respectively [11]. In addition, its incidence is on the rise in developing countries as well. PCa is characterized with insidious onset, high rate of metastasis, drug sensitivity and poor prognosis, and the 5-year survival of PCa is lower than 30% [12].

Plenty of evidence has shown the critical functions of lncRNAs in human diseases, especially malignant diseases [13]. LncRNAs are able to regulate tumor cell behaviors, suggesting that lncRNAs may serve as biological hallmarks and therapeutic targets of tumors [14]. It is also reported that differentially expressed lncRNAs are associated with tumorigenesis and tumor prognosis [15,16].

ADAMTS9-AS2 is identified as crucial in mediating many types of malignant tumor cells. Liu et al

Table 1. Relationship between ADAMTS9-AS2 level and clinical parameters of PCa patients

Variables	*n*	*x±s*	*t*	*p*
Age (years)				
<60	51	0.27±0.019	1.761	0.081
≥60	59	0.33±0.024		
Gleason score				
<6	62	0.30±0.020	0.393	0.695
≥6	48	0.29±0.025		
Clinical stage				
I +II	56	0.35±0.023	3.377	0.001
III +IV	54	0.25±0.019		
Tumor size, cm				
<3	58	0.33±0.021	2.220	0.029
≥3	52	0.26±0.023		
Pathological grade				
Low	50	0.28±0.021	0.959	0.340
Medium+High	60	0.28±0.024		

Table 2. Cox regression analysis on factors influencing survival of PCa

Variables	*HR (95%CI)*	*p*
Clinical stage (I +II ,III +IV)	1.781(1.213-5.108)	0.041
Tumor size (<3cm, ≥3cm)	1.551(1.001-2.733)	0.034
ADAMTS9-AS2 (High, Low)	1 974 (1.185-3.289)	0.009

HR=hazard ratios, CI=confidence interval

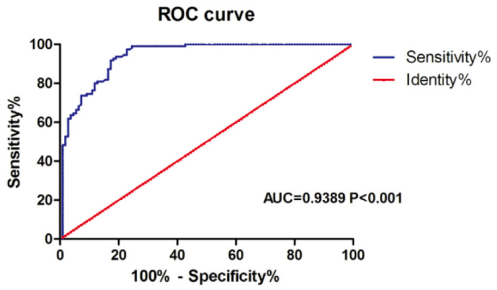

Figure 2. Diagnostic value of ADAMTS9-AS2 in PCa. ROC curves verified the diagnostic value of ADAMTS9-AS2 in PCa (AUC=0.902, cut-off value=0.4, sensitivity=90.00%, specificity=79.09%, Youden index=0.6909).

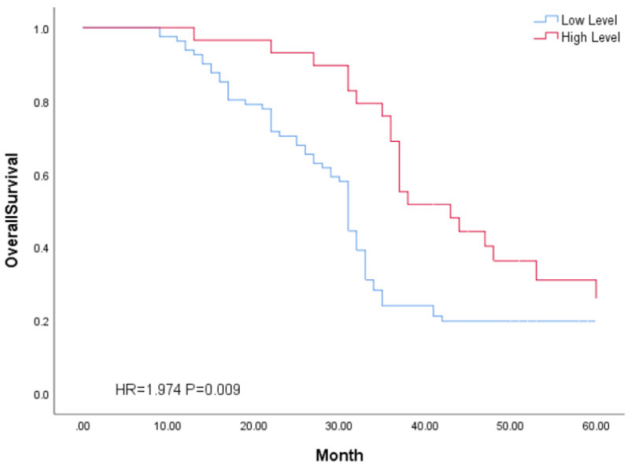

Figure 3. Prognostic value of ADAMTS9-AS2 in PCa. Kaplan-Meier curves revealed worse prognosis in ADAMTS9-AS2 low-level group than in ADAMTS9-AS2 high-level group (HR=1.974, p=0.009).

[17] reported that ADAMTS9-AS2 is downregulated in lung cancer. Overexpression of ADAMTS9-AS2 suppresses the proliferative and migratory abilities, and induces apoptosis in lung cancer cells. In glioma cells, ADAMTS9-AS2 is downregulated as a tumor-suppressor gene [18]. Consistently, our findings demonstrated that ADAMTS9-AS2 was downregulated in PCa tissues, and it stops the progression of PCa.

Current studies maintain that lncRNAs could be utilized as non-invasive hallmarks for diagnosing PCa. For example, de Kok et al [19] pointed out that PCA3 is markedly upregulated in PCa tissues (n=31) than in non-tumor tissues (11 cases of normal prostate tissues and 5 cases of benign prostate hyperplasia (BPH). In the meantime, the diagnostic efficacy of PCA3 is up to 98% (CI: 96-100%). PlncRNA-1 is found highly expressed in normal prostate tissues and BPH, posing a certain diagnostic value in PCa [20]. In this article, ROC curves confirmed the diagnostic value of ADAMTS9-AS2 in PCa.

Furthermore, our analysis uncovered that ADAMTS9-AS2 level was closely linked to clinical stage and tumor size in PCa. Cox regression analysis indicated that clinical stage, tumor size and ADAMTS9-AS2 level were independent factors influencing the prognosis of PCa. Our results proved that ADAMTS9-AS2 was a novel hallmark for diagnosing and predicting the survival of PCa patients.

Conclusions

ADAMTS9-AS2 is downregulated in PCa and its low level is unfavorable to the disease prognosis. ADAMTS9-AS2 may be utilized as a potential diagnostic and prognostic hallmark of PCa.

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Conflict of interests

The authors declare no conflict of interests.

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