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ORIGINAL ARTICLE

Diagnostic and prognostic potentials of AK126393 in bladder cancer

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Summary

Purpose: *To elucidate the diagnostic and prognostic potentials of lncRNA AK126393 in bladder cancer.*

Methods: *The expression levels of AK126393 in 60 matched bladder cancer tissues and paracancerous tissues were determined. In addition, AK126393 level in bladder cancer patients with different tumor staging (stage I-II and stage III-IV) was detected as well. Receiver operating characteristic (ROC) was introduced for assessing the diagnostic potential of AK126393 in bladder cancer. Based on the cut-off value of AK126393 in the enrolled 60 bladder cancer patients, they were assigned into high and low expression groups, respectively. Correlation between AK126393 level and pathological indexes of bladder cancer patients was analyzed by chi-square test. By collecting 5-year follow-up data, Kaplan-Meier method was conducted to evaluate survival influenced by AK126393. Moreover, Cox regression model was applied for analyzing potential factors affecting the prognosis of bladder cancer patients.*

Results: *AK126393 was downregulated in bladder cancer tissues than in paracancerous ones. Its level remained lower in bladder cancer patients with stage III-IV relative to those with stage I-II. ROC illustrated the diagnostic potential of AK126393 in bladder cancer (AUC=0.8647, diagnosis threshold=2.03, sensitivity=76.7%, specificity=96.7%, Youden index=0.734). Besides, lower level of AK126393 was observed in bladder cancer patients with stage III-IV, lymph node metastasis or high-level differentiation. Kaplan-Meier curves demonstrated worse prognosis in bladder cancer patients expressing low level of AK126393. Cox regression analysis showed that AK126393 level, TNM staging, lymph node metastasis and tumor differentiation were independent risk factors influencing the prognosis of bladder cancer.*

Conclusions: *AK126393 is downregulated in bladder cancer and closely linked to high rate of metastasis, advanced stage and poor prognosis. AK126393 may serve as diagnostic and prognostic hallmark in bladder cancer.*

Key words: *bladder cancer, AK126393, prognosis*

Introduction

Bladder cancer is the most common malignancy in the urinary system. The incidence of bladder cancer ranks fourth in Europe and the United States, showing an increased trend and younger onset in recent years [1]. Smoking and long-term exposure to industrial chemical products are the major risk factors for bladder cancer, which are able to change the genetic phenotype in individuals. Nowadays, both epigenetic and genetic changes are believed to be involved in the occurrence and development of tumors [2]. Abnormal activation of a series of pathways has been observed in bladder cancer, which are responsible for affecting various aspects of tumor cell behaviors [3]. Notably, these tumor-associated pathways have heterogeneity in different subtypes of bladder cancer. The occurrence and progression of bladder cancer are complex and chronic, involving multiple factors and

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steps [4]. In-depth understanding of the mechanism of bladder cancer provide new ideas for the prevention and treatment.

Long non-coding RNA (lncRNA) is a kind of RNA molecule with a transcript length of over 200 nucleotides. It does not encode proteins and is able to regulate gene expressions at pre-transcriptional, post-transcriptional and translational levels. LncRNAs are widely distributed in mammals [5]. The extensive involvement of lncRNAs in tumors has been identified. The alteration of lncRNA is closely linked to the process of chromatin remodeling and transcriptional regulation of tumors [6,7]. Liu et al [8] suggested that GAS5 is downregulated in bladder cancer tissues and cell lines. Knockdown of GAS5 is able to accelerate the proliferation in bladder cancer cells. Serving as an oncogene, H19 in bladder cancer may be utilized as a tumor hallmark providing a potential therapeutic value [9].

LncRNA AK126393 locates on the chromatin 10q26. Based on the high-throughput analysis, AK126393 is downregulated in colorectal cancer as a tumor-suppressor gene [10]. Additionally, AK126393 is lowly expressed in primary liver cancer, which is closely related to tumor prognosis [11]. In this article, we elucidated the potential diagnostic and prognostic values of AK126393 in bladder cancer. Factors affecting the prognosis of bladder cancer were also identified.

Methods

Baseline characteristics

Sixty bladder cancer patients were enrolled. Their tumor tissues and paracancerous tissues (at least 5 cm away from the tumor boundary) were surgically resected, quickly frozen in liquid nitrogen and preserved at -80°C. Clinical data of enrolled patients were recorded. None of them had pre-operative anti-tumor treatment. Briefly, enrolled bladder cancer patients were 37-75 years old (54.9 ± 7.5), including 38 males and 22 females. According to TNM staging, 29 cases were in stage I-II and 31 in stage III-IV. A total of 34 bladder cancer patients showed lymph node metastasis. 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 the Third Medical Center of PLA General Hospital.

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

TRIzol (Invitrogen, Carlsbad, CA, USA) was applied for isolating total RNA. QRT-PCR was conducted at 95°C pre-denaturation for 5 min, 95°C denaturation for 10 s and 60°C annealing for 30 s, for a total of 35 cycles. Primer sequences were listed as follows: AK126393: forward: 5'-TTGCGTGCCTAAGATTGGGT-3', reverse: 5'-AAGCTACGCCACACTGACAT-3'; glyceraldehyde 3-phosphate dehydrogenase (GAPDH): forward: 5'-GAGGCTGGGAACCTTAAGGT-3', reverse: 5'-AGGGCCGCTGGTCAGAAGTT-3'.

Postoperative follow-up

Through outpatient review, telephone and e-mail communication, follow-up was conducted once at the first month postoperatively, followed by one time every three months in the postoperative first year, and once every six months in the second year. From the third year on, follow-up was conducted once a year, until the final follow-up on date. All patients completed the follow-up and their prognostic data were recorded for at least 5 years.

Statistics

SPSS 20.0 (IBM, Armonk, NY, USA) was used for data analyses. Measurement data were expressed as mean ± standard deviation (x±SD) and analyzed by the independent *t*-test. Counting data were analyzed by x^2 test. Chi-square analysis was conducted to compare the relationship between AK126393 level and pathological indexes of bladder cancer patients. Kaplan-Meier method was introduced for survival analysis, followed by Log-rank test for comparing differences between two curves. Multivariate Cox regression was conducted to validate factors influencing survival in bladder cancer. P<0.05 showed statistically significant difference.

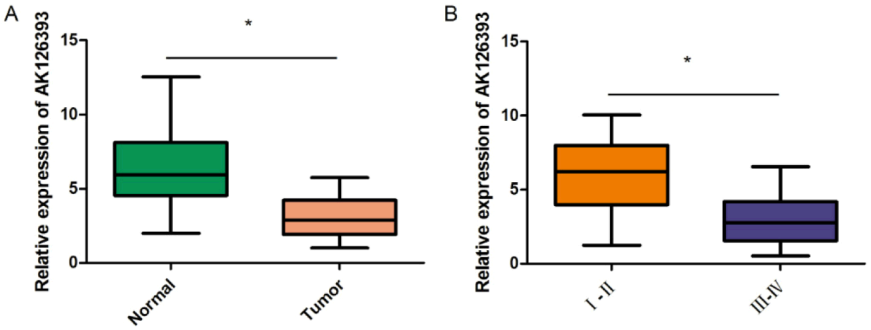

Figure 1. AK126393 was downregulated in bladder cancer. **A:** AK126393 levels in bladder cancer tissues and paracancerous tissues. **B:** AK126393 levels in bladder cancer patients with stage I-II and stage III-IV. *p<0.05.

Results

AK126393 was downregulated in bladder cancer

QRT-PCR data revealed lower abundance of AK126393 in bladder cancer tissues relative to paracancerous ones (Figure 1A). Based on tumor staging, it was shown that AK126393 level was higher in bladder cancer patients with stage I-II relative to those with stage III-IV (Figure 1B). The above findings demonstrated that AK126393 may be a tumor-suppressor gene in bladder cancer.

Diagnostic potential of AK126393 in bladder cancer

Differential expression of AK126393 in bladder cancer tissues and normal tissues has been identified. Thereafter, we speculated that AK126393 may be a diagnostic hallmark in bladder cancer. ROC curves were depicted and AUC was calculated as 0.8647 (p<0.001, 95%CI: 0.789-0.861). Moreover, cut-off (2.03), specificity (76.7%), sensitivity (96.7%) and Youden index (0.734) were calculated based on the depicted ROC. The data indicated an increased susceptibility to bladder cancer once AK126393 level was higher than 2.03 (Figure 2).

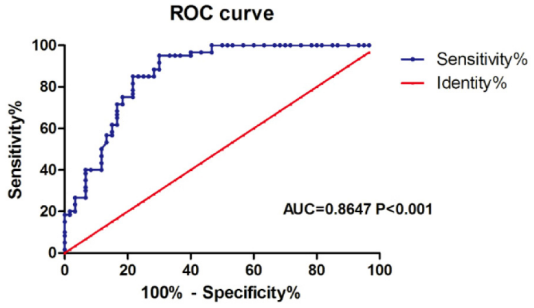

Figure 2. Diagnostic potential of AK126393 in bladder cancer.

Correlation between AK126393 level and pathological indexes of bladder cancer patients

Based on the cut-off value of AK126393, 60 bladder cancer patients were divided into high expression (n=27) and low expression (n=33) group. Chi-square analysis illustrated that AK126393 level was not correlated to age, gender and tumor size (p>0.05), while it was closely linked to TNM staging, lymph node metastasis and tumor differentiation in bladder cancer patients (p<0.05). In particular, AK126393 level was markedly reduced in bladder cancer patients with stage III-IV, lymphatic metastasis or high-level differentiation (Table 1).

Prognostic potential of AK126393 in bladder cancer

Postoperative follow-up for bladder cancer patients was conducted for 5 years. Kaplan-Meier curves depicted worse survival in bladder cancer patients expressing low level of AK126393, indicating that AK126393 was favorable for the prognosis of bladder cancer (HR=3.995, p=0.0456, Figure 3).

Table 1. Correlation between AK126393 level and clinical parameters of bladder cancer patients

Variables	*n*	*AK126393*		*p*
		High level (n=27)	*Low level (n=33)*	
Sex				
Male	38	17	21	0.957
Female	22	10	12	
Age, years				
<55	41	18	23	0.802
≥55	19	9	10	
TNM stage				
I-II	29	23	6	<0.001
III-IV	31	4	27	
Lymph node metastasis				
No	26	16	10	0.024
Yes	34	11	23	
Tumor size, cm				
<2	33	18	15	0.1
≥2	27	9	18	
Pathological differentiation				
Low	20	14	6	0.006
Medium /High	40	13	27	

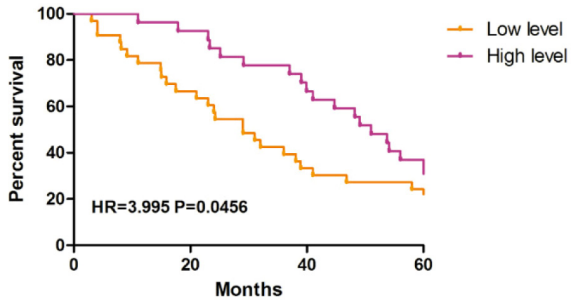

Figure 3. Prognostic potential of AK126393 in bladder cancer.

Table 2. Cox regression analysis on factors influencing survival of bladder cancer patients

Variables	*HR (95%CI)*	*p*
TNM stage	2.044(1.931-2.949)	0.018
Lymph node metastasis	1.713(1.312-3.148)	0.031
Pathological differentiation	2.861(1.971-4.713)	0.039
AK126393	2.911(1.923-3.188)	0.021

TNM stage: Stage I-II considered as the control; Lymph node metastasis: Non-metastatic patients considered as the control; Pathological differentiation: Low-level differentiation considered as the control; AK126393: High-level AK126393 considered as the control. HR=hazard ratios; CI=confidence interval.

Cox regression analysis on factors influencing the prognosis in bladder cancer

Cox regression model was applied to analyze potential influence of AK126393 level, age, gender, TNM staging, lymph node metastasis, tumor size and tumor differentiation on the prognosis of bladder cancer. The results showed that low level of AK126393, stage III-IV, lymph node metastasis and moderate to high level of differentiation were independent risk factors for the prognosis of bladder cancer ($p<0.05$, Table 2).

Discussion

Bladder cancer is the most common malignant tumor of the urinary system. Its incidence ranks high and tends to increase recently [12]. Therapeutic strategies for bladder cancer have been greatly developed. Surgery is preferred in early- and middle-stage bladder cancer. Nevertheless, advanced bladder cancer patients are not suitable for receiving surgical procedures. Chemotherapy and radiotherapy are not sensitive for them, leading to high rates of recurrence and mortality [13,14]. Therefore, molecular hallmarks for detecting early-stage bladder cancer are urgently required [15].

LncRNAs participate in post-transcriptional processing of RNAs by recognizing complementary sequences [16]. Most mammalian genes express their antisense transcripts, and these types of lncRNAs exert a major advantage in regulating the dynamics of RNAs [17]. In-depth studies have elucidated the critical functions of lncRNAs in cellular behaviors [18,19]. Through the formation of RNA-protein complex, lncRNAs target downstream genes and influence their biological functions [20]. Notably, lncRNAs are extensively involved in tumorigenesis and tumor metastasis [21].

Differentially expressed lncRNAs may be crucial mediators in tumor progression. Zhu et al [22] uncovered 3,324 differentially expressed lncRNAs and 2,120 differentially expressed mRNAs (>2-fold alteration) by microarray analysis on four matched bladder cancer tissues and parancancerous ones. Among them, a total of 110 lncRNAs were remarkably differentially expressed (>8-fold alteration). Ying et al [23] reported that MEG3 is downregulated in 27/31 bladder cancer tissues, and its level was negatively correlated to the mRNA level of the autophagy marker LC3-II. Similarly, our findings demonstrated that AK126393 was downregulated in bladder cancer, especially in those with stage III-IV, lymph node metastasis or high-level differentiation. Survival analysis suggested negative correlation between AK126393 level and survival in bladder cancer patients. Meanwhile, the diagnostic potential of AK126393 in bladder cancer was proved by introducing ROC.

Furthermore, potential factors influencing the prognosis of bladder cancer were analyzed by applying Cox regression model. AK126393 level, TNM staging, lymph node metastasis and tumor differentiation were independent risk factors influencing the prognosis of bladder cancer. Collectively, AK126393 was believed to be a promising target for predicting the diagnosis and prognosis in bladder cancer.

Conclusions

AK126393 is downregulated in bladder cancer and its low level is closely linked to poor prognosis. AK126393 may serve as diagnostic and prognostic hallmark in bladder cancer.

Conflict of interests

The authors declare no conflict of interests.

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