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The deregulation of miR-133b is associated with poor prognosis in bladder cancer

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ABSTRACT

Background: MicroRNAs (miRNAs) are single-stranded, endogenous, non-coding RNAs that are increased or decreased in almost all cancer types, and they paly crucial roles in the tumorigenesis as well as development.
Materials and methods: 90 patients diagnosed with bladder cancer were enrolled in the present study. The bladder cancer tissues or adjacent normal tissues were obtained from the tumor area or adjacent normal zone. The expression level of miR-133b was examined by quantitative real-time polymerase chain reaction assay (qRT-PCR). Survival curves were displayed by the Kaplan-Meier method, and differences between two survival curves were calculated by the log-rank test.
Results: The expression levels of miR-133b in bladder tissues were significantly decreased when compared with the matched adjacent normal bladder tissues ($P < 0.05$). Moreover, miR-133b expression levels are significantly associated with lymphatic invasion ($P = 0.026$), distant metastasis ($P = 0.025$), tumor grade ($P = 0.038$), as well as the muscle invasion status ($P < 0.001$). The log-rank test indicated that patients with decreased miR-133b expression underwent poorer overall survival ($P = 0.007$). Furthermore, multivariate Cox regression analysis showed that the expression level of miR-133b ($P = 0.024$) was an independent factor for predicting the overall survival in patients with bladder cancer.
Conclusions: The present study showed that miR-133b might be associated with bladder cancer progression, and its down-regulation might be a biomarker for poor prognosis of bladder cancer.

1. Introduction

Bladder cancer ranks as seventh common cancer among male and the seventeenth common cancer among female worldwide [1]. In spite of recent advances in surgical methods and development of targeted therapies, the mortality rate of bladder cancer remains high. Therefore, the investigation of new suitable biomarkers for early diagnosis and prognosis is crucial for bladder cancer patients.

MicroRNAs (miRNAs) are endogenous, single-stranded, non-coding RNAs which participate in gene expression regulation. miRNAs bind with the 3′-UTRs of target mRNAs, repressing the expression of these genes at the translation level [2]. They have been demonstrated to play important roles in almost all biological processes, including proliferation, differentiation, angiogenesis, as well as metabolism. Furthermore, miRNAs act as critical players in tumorigenesis, and progression. Recently, miRNAs have been found to be associated with the invasiveness and metastatic potential of human cancer, and may have the potential to be diagnostic and prognostic markers for several types of cancer [3–6].

The expression of miR-133b has been found to be down-regulated in several types of cancer, including lung cancer, colorectal cancer, gastric cancer, esophageal squamous cell carcinoma(ESCC), as well as tongue squamous cell carcinoma (TSCC) [7–11]. Previously, Chen et al found that miR-133b expression level in bladder cancer tissues was significantly down-regulated than adjacent normal tissues [12], however, the clinical significance and prognostic value have not been

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investigated. Therefore, in the present study, we aimed to investigate the clinical significance as well as prognostic value of miR-133b in bladder cancer.

2. Materials and methods

Our present study was approved by the Review Board of University of Washington, and written informed consent was obtained from all included patients. Bladder cancer tissue specimens and matched adjacent non-malignant bladder tissue specimens were collected from 90 patients who underwent surgery in University of Washington from March 2008 to April 2016. All included patients had not received chemotherapy or radiotherapy before surgery. The tissues were frozen in liquid nitrogen until use immediately following surgical resection. The diagnosis was based on histopathological analysis of the resected tissues by two pathologist. The pathological stages of the bladder cancer patients were determined according to TNM staging. The cases with tumor invades muscular layer and external bladder structure would be recorded muscle-invasive and the cases with cancer distant metastases to liver, lung, bone, posterior urethra, prostate and rectum would be recorded as distant metastasis. The clinical and pathological data were shown in Table 1.

2.1. RNA extraction and real-time PCR

Total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The concentration and purity of all RNA samples distant metastasis les were detected by NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies, Houston, TX, USA). NCodeTM SYBR ® Green miRNA qRT-PCR Kit (Invitrogen, Carlsbad, CA, USA) was used to synthesize specific cDNA of miR-133b and U6B(as an internal control), and perform qRT-PCR, which was analyzed with the DNA Engine Opticon 2 Real-Time Cycler (MJ Research Inc., Waltham, MA, USA) according to the manufacturer's instructions. Each sample was examined in triplicate and analyzed by the comparative threshold cycle (Ct) method. The expression levels of miR-133b were normalized to U6B. The primers were listed as the following: miR-133b forward primer: 5′-CTCAGCTT TGGTCCCCTTCAAC-3′, reverse primer: 5′-GTGCAGGGTCCGAGGT-3′ and U6 forward primer 5′-CGCTTCGGCAGCACATATAC-3′, reverse primer5′-CAGGGGCCATGCTAATCTT-3′.

Table 1
Relationship between clinicopathological features and miR-133b expression in bladder cancer.

Parameters	Number of cases	miR-133b expression		P value
		Low(n = 45)	High(n = 45)	
Age (y)				
< 65	49	26	23	0.672
≥ 65	41	19	22	
Sex				
Male	57	27	30	0.662
Female	33	18	15	
Lymphatic invasion				
Positive	31	21	10	0.026
Negative	59	24	35	
Distant metastasis				
Positive	11	9	2	0.025
Negative	79	36	43	
Muscle-invasive				
NMIBC (Ta, T1)	29	5	24	< 0.001
MIBC (T2, T3, T4)	61	40	21	
Tumor grade				
G1/G2	31	11	20	0.038
G3	59	34	25	

NMIBC: non-muscle-invasive bladder cancer, MIBC: muscle-invasive bladder cancer.

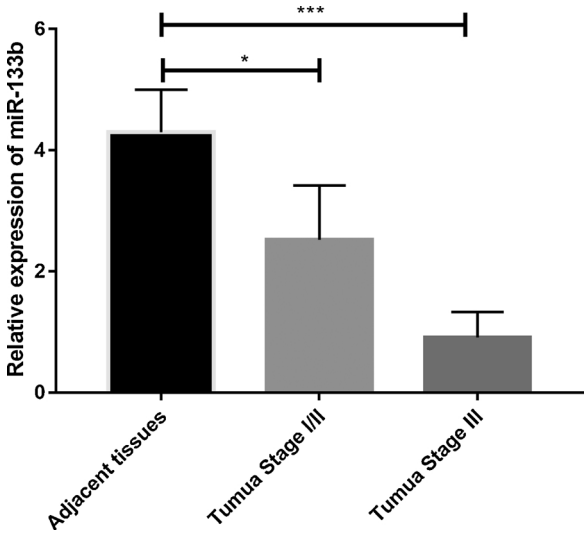

Fig. 1. Expression of miR-133b was downregulated in bladder cancer tissues. *: $P < 0.05$, ***: $P < 0.001$.

2.2. Statistical analysis

Student's *t*-test was used to determine the differences between two groups. The overall survival time (OS) was calculated from postoperative time to the death time. Survival curves were displayed by the Kaplan-Meier method, and differences between two survival curves were calculated by the log-rank test. Univariate Cox regression was performed on each clinical covariate to examine its influence on patient survival. Final multivariate models were based on step-wise addition. A Wald statistic of $P < 0.05$ was used as the criterion for inclusion in final multivariate models. Statistical analysis was conducted using the Statistical Package for the Social Sciences 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Significance was set at $p < 0.05$.

3. Results

3.1. miR-133b expression and its association with clinicopathological features

The analysis of miR-133b levels in matched bladder tissue specimens (shown in Fig. 1) indicated significantly decreased expression in the malignant specimens compared with the adjacent normal zones in both early ($P < 0.05$) and late stage ($P < 0.001$) of bladder cancer cases. Moreover, miR-133b expression is significantly associated with lymphatic invasion ($P = 0.026$), distant metastasis ($P = 0.025$), tumor grade ($P = 0.038$), and the muscle invasion status ($P < 0.001$). Details are presented in Table 1.

3.2. Decreased miR-133b expression predicts the favorable prognosis of bladder cancer

Bladder cancer tissues expressing miR-133b at the level less than the median expression level were assigned to the low expression group (n = 45), and these samples with expression above the median value were assigned to the high expression group (n = 45). The median postoperative follow-up time was 39.4 ± 19.2 months. Using kaplan-Meier survival plots and log-rank analyses, we evaluated the association of miR-133b expression with overall survival. The log-rank test indicated that patients with decreased miR-133b expression experienced poor overall survival ($P = 0.007$, shown in Fig. 2). In addition, we also detect the effect of low expression of miR-133b and prognosis in both NMIBC and MIBC. It was found that low expression of miR-133b would demonstrate a poor prognosis in both NMIBC ($P = 0.013$) and MIBC

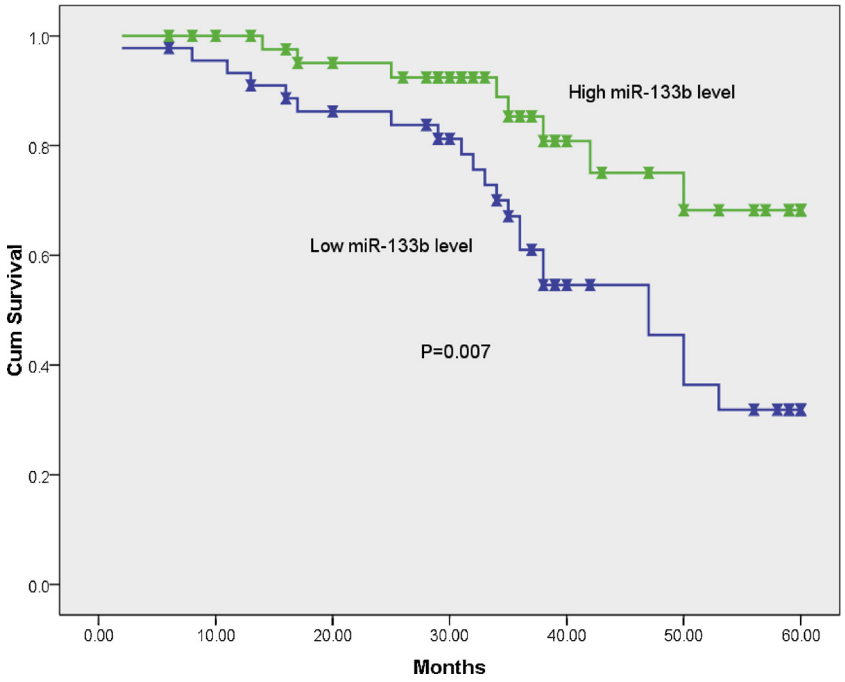

Fig. 2. Kaplan–Meier survival curves in bladder cancer patients after surgery according to miR-133b expression (n = 90).

Table 2
Cox proportional regression analysis for assessing the correlation of miR-133b expression with the prognosis of bladder cancer.

Variables	Hazard ratio	95 % CI	P value
Sex	0.543	0.265–2.882	0.631
Age	1.623	0.725–3.754	0.113
Lymphatic invasion	2.872	1.821–8.012	0.017
Distant metastasis	3.617	2.192–10.287	0.007
Muscle-invasive	2.828	1.219–6.918	0.031
Tumor grade	3.192	2.011–14.922	0.005
miR-133b expression	2.165	1.233–7.991	0.024

($P < 0.001$). To determine the possibility of miR-133b as an independent risk factor for poor prognosis, both clinicopathological factors and the level of miR-133b expression were evaluated by multivariate Cox regression analysis. Results showed that the muscle-invasive status ($P = 0.031$), lymphatic invasion ($P = 0.017$), distant metastasis ($P = 0.007$), tumor grade ($P = 0.005$), and miR-133b expression level ($P = 0.024$) were independent factors in predicting the OS of bladder cancer patients (shown in Table 2).

4. Discussion

Bladder cancer ranks as the fourth most common malignancy among male population around the world. The pathological staging of bladder cancer mainly depends on transurethral resection of bladder tumor. Approximately two-thirds of bladder cancer are low-grade or non-muscle-invasive bladder cancer (NMIBC), and about one-third of bladder cancer are high-grade or muscle-invasive bladder cancer (MIBC) [13]. For local MIBC, radical cystectomy is the standard treatment option; however, 6% of patients with organ-confined, lymph node-negative bladder tumors will locally relapse, and 13% will have a new distant metastasis after radical cystectomy [14]. In clinical practice, we found that patients with the same pathological stage might have different prognosis, therefore, we need new better prognostic markers to estimate the risk of cancer progression among bladder cancer patients (Fig. 3).

miRNAs have been demonstrated to play important roles in almost all biological processes, including proliferation, differentiation, angiogenesis, as well as metabolism. Furthermore, miRNAs play oncogenes or tumor suppressor genes roles, depending on the type of cancer tissue [15,16]. There is increasing evidence that the expression levels of miRNA are associated with the prognosis of tumors and they might be used as biomarkers of tumor prognosis [17–20].

MiR-133b is a type of muscle-specific microRNA; it takes part in the formation of cardiocytes and the expression of myocardium ion channels by regulating target genes. The expression level of miR-133b was down-regulated in many types of human cancers, such as lung cancer, colorectal cancer, as well as esophagus cancer [7,8,11,21]. Functionally, miR-133b targets oncogenic FSCN1 in esophageal squamous cell carcinoma, pro-survival molecules MCL-1 and BCL2L2 in lung cancer, and MET proto-oncogene in colorectal cancer cells affecting cell proliferation and invasion [7,8,21]. In this study, it was found that miR-133b level was associated with distant metastasis muscle-invasive status and it indicated that miR-133b might play important role in the regulation of cancer cellular migration. As reported in previous studies, up-regulation of miR-133b inhibits invasion, migration in vitro and bone metastasis in vivo in prostate cancer [22], breast cancer [23] and non-small cell lung cancer [24]. It could be conjectured that miR-133b might be also associated with the cellular migration of bladder cancer cells and we would like to conduct relevant studies to prove it.

Previously, Chen et al found that miR-133b expression level in bladder cancer tissues was significantly down-regulated when compared with adjacent normal bladder tissues ($P < 0.01$); and miR-133b low expression level was significantly associated with tumor grade ($P < 0.01$). Furthermore, miR-133b may play a critical role in the proliferation, invasion and metastasis of bladder cancer cells through regulating the expression of Bcl-w and Akt1 [12]. However, the clinical significance and prognostic value have not been investigated by now. In the present study, the analysis of miR-133b levels in matched bladder tissue specimens indicated significantly decreased expression in the malignant specimens compared with the adjacent normal zones. Moreover, miR-133b expression is significantly associated with lymphatic invasion, distant metastasis, tumor grade, and the muscle invasion status. Using kaplan-Meier survival plots and log-rank analyses, we evaluated the association of miR-133b expression with overall survival. The log-rank test indicated that patients with decreased miR-133b

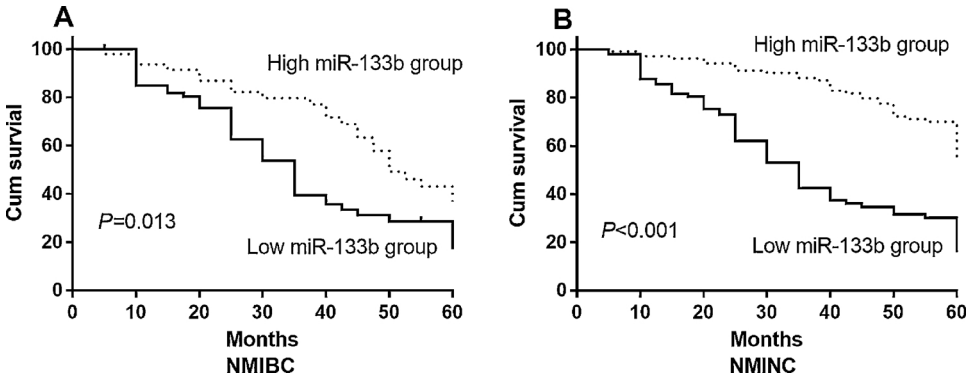

Fig. 3. Kaplan–Meier survival curves in bladder cancer patients after surgery according to miR-133b expression in both NMIBC (n = 29) and MIBC (n = 61).

expression experienced poor overall survival. To determine the possibility of miR-133b as an independent risk factor for poor prognosis, both clinicopathological factors and the level of miR-133b expression were evaluated by multivariate Cox regression analysis. Results showed that miR-133b expression level were independent factors in predicting the OS of bladder cancer patients.

In conclusion, this study showed that miR-133b may contribute to the progression of bladder cancer and its down-regulation may be independently associated with poor prognosis of bladder cancer, suggesting that miR-133b might serve as a promising biological marker for further risk stratification in the management of bladder cancer.

Disclosure of conflict of interest

None.

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