Int J Clin Exp Pathol 2017;10(5):5624-5629
www.ijcep.com /ISSN:1936-2625/IJCEP0049637

Original Article

Downregulation of miR-138-5p is associated with poor prognosis in human bladder cancer

Yunyan Wang, Gongcheng Wang, Lu Ji, Qing Sun, Bing Zhong, Junsong Meng

Department of Urology, Huai'an First People's Hospital, Nanjing Medical University, Huai'an, Jiangsu, China

Received January 24, 2017; Accepted February 23, 2017; Epub May 1, 2017; Published May 15, 2017

Abstract: Accumulating evidence suggested that microRNA (miRNA) plays an important role in the carcinogenesis of bladder cancer. MicroRNA-138-5p (miR-138-5p) was dysregulated in human cancers including bladder cancer. However, its clinical significance remains unclear. We recruited 126 patients with bladder cancer in the study. Real-time quantitative polymerase chain reaction (RT-qPCR) was conducted for detection of miR-138-5p levels in tumor tissues and paired normal tissues. The prognostic significance of miR-138-5p in bladder cancer was further analyzed. MiR-138-5p levels were significantly decreased in the tumor tissues compared with the non-cancerous tissues. In addition, low levels of miR-138-5p were strongly associated with various clinicopathological parameters including TNM stage and histological grade as well as Ki-67 staining intensity. Bladder cancer patients with lower miR-138-5p levels had worse 5 year overall survival and recurrence free survival rates. Finally, multivariate analysis demonstrated that miR-138-5p was an independent prognostic factor for bladder cancer. Overall, our study indicated that miR-138-5p might serve as a useful prognostic biomarker for patients with bladder cancer.

Keywords: Bladder cancer, miR-138-5p, prognosis

Introduction

Bladder cancer (BC) is one of the most prevalent fatal cancers around the world. In USA, about 74000 new cases and 16000 new deaths caused by BC are reported in 2014 [1, 2]. In China, BC is the most leading cause of cancer-related death of the urogenital system [3]. According to the depth of tumor invasion, nearly 75% of bladder cancer cases are classified as non-muscle invasive tumors while other cases are classified as muscle invasive tumors. Compared to non-muscle invasive tumors, muscle invasive tumors are more deadly and have a much poorer prognosis [4, 5]. Though great progress had been achieved in the treatment of this malignancy in the past decades, a number of BC patients were diagnosed at advanced stage and the clinical outcome for most BC patients remained dismal. Therefore, identification of effective biomarkers will help improve predicting prognosis of BC and developing more effective therapies for this disease.

MicroRNAs (miRNAs) are a subset of small non-coding RNAs (19-22 nucleotides in length) that inversely regulate gene expression by binding to the 3' untranslated region (UTR) of target mRNAs, inducing mRNAs degradation and/or impairing their translation [6, 7]. Increasing studies have shown that miRNAs function as either oncogenes or tumor suppressors and play critical roles in multiple biological processes [8, 9]. Aberrant expression of miRNAs has been observed in variety types of human cancers including BC [10, 11]. Huang et al. revealed that miR-206 level was greatly decreased in BC tissues and cell lines. Moreover, downregulation of miR-206 promoted cell proliferation, colony formation, migration, invasion of BC by targeting YRDC [12]. Xiu and colleagues demonstrated that up-regulated miR-137 expression was closely related with pM or pTNM stage in BC patients. Furthermore, loss of miR-137 expression significantly attenuated BC cell proliferation, migration and invasion *via* regulating PAQR3 [13].

Recent reports have shown that miR-138-5p played a tumor suppressive role in various cancers, such as BC [14], pancreatic cancer [15] and colorectal cancer [16]. However, the association between tissue miR-138-5p expression

Table 1. Relationship between tissue miR-138-5p expression and clinicopathological features of BC patients

Clinical variables	MiR-138-5p expression (n=126)		*P*-value
	Low (n=63)	High (n=63)	
Age			0.5861
≥60	36	39	
<60	27	24	
Sex			0.5573
Male	43	46	
Female	20	17	
Stage			<0.0001
Ta/T1	15	51	
T2/T3/T4	48	12	
Smoker			0.3281
No	16	21	
Yes	47	42	
Grade			0.0289
G1/G2	32	44	
G3	31	19	
M stage			0.1431
M0	54	59	
M1/M2/M3	9	4	
N stage			0.0679
N0	51	58	
N1/N2/N3	12	5	

and the prognosis of BC had not yet been elucidated. The aim of our study was to examine tissue miR-138-5p expression in clinical samples and evaluate its potential value as a novel biomarker for BC patients.

Materials and methods

Patients and tissues acquisition

This study collected tumor tissues from 126 patients diagnosed and confirmed with BC. The corresponding adjacent normal tissues were also collected and used as controls. Fresh tissue samples were stored at -80°C until further analysis. No patient had accepted any preoperative chemotherapy or radiotherapy before surgery. The tumors were assessed according to OMS 2004 grading scheme for the grade and the 2002 UICC TNM classification for the stage. Patients who had smoked more than 100 cigarettes in the lifetime were defined as smokers. The clinicopathological data from the patients with BC were summarized in **Table 1**.

The duration of follow-up for overall survival (OS) was calculated from the date of surgery to the date of death or last follow-up, and the duration of follow-up for recurrence free survival (RFS) was calculated from the date of surgery to the date of recurrence or death or last follow-up. The potential association between Ki-67 staining status and miR-138-5p levels was also assessed. The staining intensity of Ki-67 was evaluated by determining the percentage of positive cells stained. The tissue sections with more than 50% positive cells were classified as high expression.

RNA extraction and quantitative real-time PCR analysis

Total RNA was isolated from 126 pairs of BC primary tumor samples and adjacent noncancerous tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA concentration and purity were measured using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Reverse transcription was performed using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed using the Maxima SYBR Green qPCR Kit (Thermo Scientific) on an ABI PRISM 7000 Fluorescent Quantitative PCR System (Applied Biosystems) in accordance with the manufacturer's instructions. Each experiment was repeated in triplicate for controlling technical variance. RNU6B was used as a control to normalize miR-138-5p expression. The $2^{-\Delta\Delta Ct}$ method was used to quantify the relative fold-changes of miR-138-5p expression.

Statistical analysis

Difference of tissue miR-138-5p levels between matched tumor tissues and non-malignant samples was compared with Mann-Whitney U test. The relations between tissue miR-138-5p expression and clinical features were examined using the Chi-square test. Furthermore, the Kaplan-Meier analysis was performed to construct survival curves. Log-rank test was used to assess the association between tissue miR-138-5p expression and OS as well as RFS. Multivariate analyses were performed to evaluate independent prognostic factors of OS and RFS using the Cox proportional hazards regression model. All statistical analyses were processed with SigmaPlot 13.0 (Systat, Chicago,

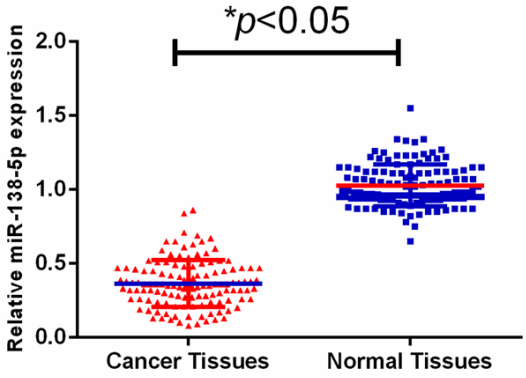

Figure 1. The relative expression level of miR-138-5p between BC tissues and adjacent normal tissues.

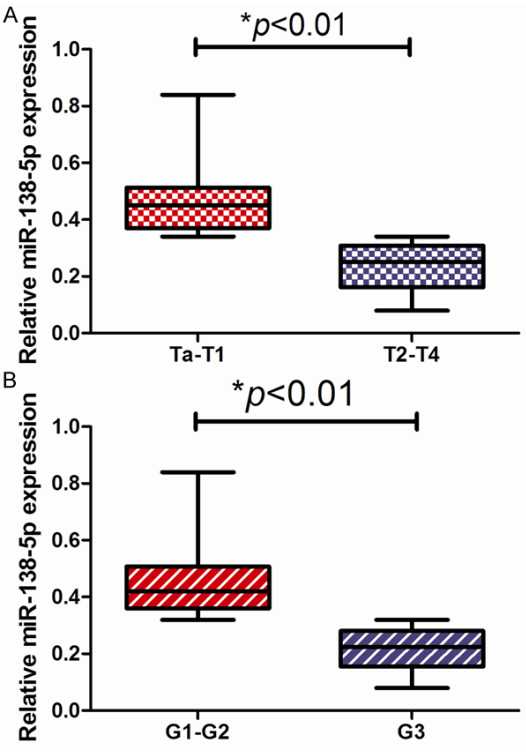

Figure 2. Association of the relative miR-138-5p expression with the clinical features of BC patients. A. The association of miR-138-5p expression with TNM stage of tumor tissues. B. The association of miR-138-5p expression with grade of tumor tissues.

IL, USA) and GraphPad Prism 6 (GraphPad Software Inc., San Diego, California, USA). A *P*-value less than 0.05 was defined statistically significant.

Ethics statement

Our study was approved by Huai'an First People's Hospital and prior signed informed consent was collected from all study participants.

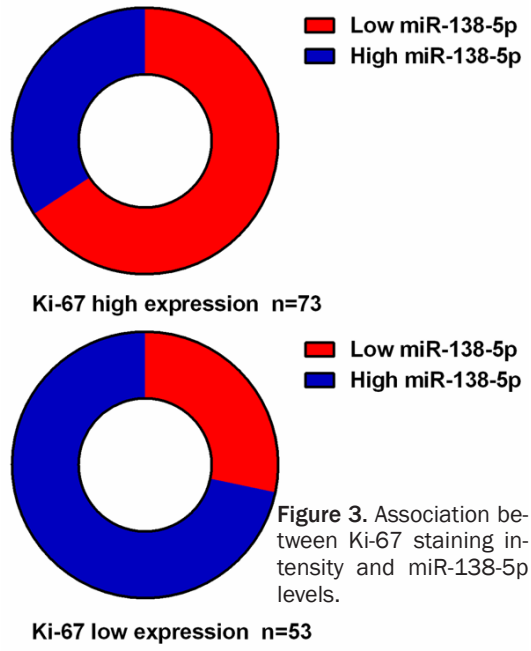

Figure 3. Association between Ki-67 staining intensity and miR-138-5p levels.

All specimens were handled and made anonymous according to the ethical and legal standards.

Results

MiR-138-5p was significantly decreased in bladder cancer tissues

To further assess tissue miR-138-5p expression levels in patients with BC, we measured the levels of miR-138-5p in 126 paired BC primary tumor tissues and the adjacent non-neoplastic tissues. As shown in **Figure 1**, a significant difference in tissue miR-138-5p expression between BC tumor samples and corresponding non-cancerous tissues was observed ($P<0.05$). In addition, tissue miR-138-5p expression in BC patients with higher TNM stage was remarkably down-regulated compared to those with lower TNM stage ($P<0.01$, **Figure 2A**). Moreover, tissue miR-138-5p expression in BC patients with poorly differentiated tumors was dramatically under-expressed compared to those with well or moderately differentiated tumors ($P<0.01$, **Figure 2B**).

Correlation between tissue miR-138-5p expression and clinical characteristics of BC patients

To investigate the relation between tissue miR-138-5p levels and clinicopathological parameters of the BC patients, we divided 126 tumor

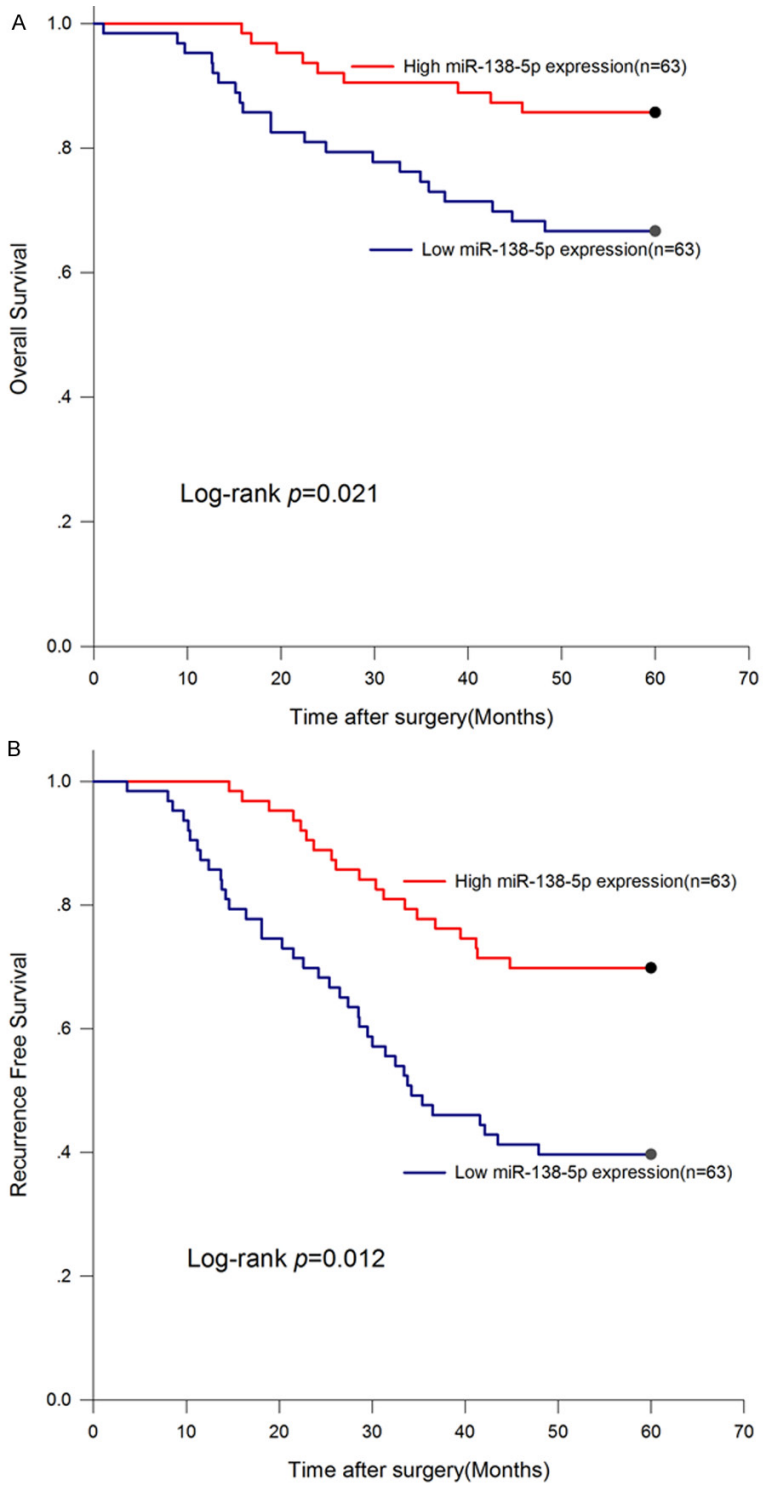

Figure 4. Kaplan-Meier curves of overall survival (A) and recurrence free survival (B) in BC patients.

samples into two groups: low expression group (n=63) and high expression group (n=63). The median value of tissue miR-138-5p expression level was used as a cut-off point. Tissue miR-138-5p expression was closely associated with some clinical variables, including TNM stage (P<0.0001) and grade (P=0.0289). However, tissue miR-138-5p expression was not found to be correlated with sex, age, smoker, M stage and N stage (**Table 1**, all P>0.05).

The association between miR-138-5p levels and Ki-67 staining intensity

Ki-67 staining intensity was regarded as an important prognostic factor for BC. Thus the correlation between miR-138-5p levels and Ki-67 staining intensity was assessed. Seventy-three BC patients had high Ki-67 expression while 53 patients had low expression. Our results showed that the percentage of cases with low expression of miR-138-5p was significantly higher in carcinomas with high Ki-67 expression (48/73 cases, 65.8%) than in those cases with low Ki-67 expression (15/53 cases, 28.3%) (**Figure 3**).

Correlation between tissue miR-138-5p expression and survival

Kaplan-Meier analysis and log-rank test were used to analyze the association between tissue miR-138-5p level and patient survival. We demonstrated that the BC patients in low tissue miR-138-5p expression group had shorter overall survival (P=0.021, **Figure 4A**) and higher risk of recurrence (P=0.012, **Figure 4B**).

Multivariate analysis of the effect on OS and RFS with the Cox proportional hazards model

Multivariate Cox regression analysis revealed that TNM stage (OS: RR=3.17; 95% CI, 1.48-

Table 2. Multivariate analyses for OS and RFS by Cox regression model

Parameters	Risk Ratio	95% CI	*P* value
Overall survival			
Grade (G3 *vs* G1/G2)	1.96	1.12-2.87	0.036
Stage (T2/T3/T4 *vs* Ta/T1)	3.17	1.48-4.82	0.009
MiR-138-5p expression (Low *vs* High)	2.55	1.34-3.81	0.017
Recurrence free survival			
Grade (G3 *vs* G1/G2)	2.33	1.28-3.46	0.023
Stage (T2/T3/T4 *vs* Ta/T1)	3.84	1.62-6.12	0.004
MiR-138-5p expression (Low *vs* High)	2.96	1.41-4.62	0.011

4.82; *P*=0.009; RFS: RR, 3.84; 95% CI, 1.62-6.12; *P*=0.004), tissue miR-138-5p expression (OS: RR, 2.55; 95% CI, 1.34-3.81; *P*=0.017; RFS: RR, 2.96; 95% CI, 1.41-4.62; *P*=0.011) and grade (OS: RR, 1.96; 95% CI, 1.12-2.87; *P*=0.036; RFS: RR, 2.33; 95% CI, 1.28-3.46; *P*=0.023) were strongly correlated with unfavorable OS and RFS, suggesting that TNM stage, grade and tissue miR-138-5p expression were identified to be independent risk factors for OS/RFS (all *P*<0.05, **Table 2**).

Discussion

BC is one of the most common cancer types and has become a significant public health issue throughout the world [17, 18]. In this study, we found that the expression levels of tissue miR-138-5p were dramatically down-regulated in BC tumor samples in comparison with adjacent normal tissues. In addition, reduced tissue miR-138-5p expression was positively correlated with worse clinical outcome and poorer prognosis. Furthermore, tissue miR-138-5p level was confirmed to be a significant and independent prognostic factor for BC. Our results suggested that miR-138-5p might exert a tumor suppressor function in BC. In accordance with our findings, Yang et al. provided *in vitro* and *in vivo* evidence to show that enhanced miR-138-5p expression repressed tumor cell proliferation and invasion *via* negatively regulating BIRC5, which was a validated oncogene in BC [14]. Taken together, deletion of miR-138-5p might involve in the tumorigenesis of BC.

To the best of our knowledge, current available studies support a tumor suppressive role for miR-138-5p in all types of cancer analyzed so far, indicating the biological function of miR-138-5p is highly conserved and it might play an important role in carcinogenesis and cancer progression. Yu and colleagues showed that miR-138-5p was markedly down-regulated in both primary tumor samples and cell lines of pancreatic cancer. Additionally, elevated expression of miR-138-5p greatly inhibited cancer cell proliferation *in vitro* and suppressed tumorigenicity *in vivo* through regulating FOXC1 [15]. Similarly, it was reported that a significant decreased miR-138-5p expression was detected in human colorectal tumor tissues and associated with poorer overall survival. Moreover, loss of miR-138-5p promoted colorectal cancer cell growth and carcinogenesis *via* upregulating programmed cell death ligand 1 [16].

There are some limitations to our study. Firstly, the clinical value of tissue miR-138-5p should be studied in a larger cohort of patients with BC. Secondly, it would be more convenient and easier for BC patients to evaluate miR-138-5p expression level in serum, plasma, urine or saliva compared with tissue. Finally, the underlying mechanisms of miR-138-5p in initiation and progression of BC may need deeper exploration.

In summary, our study showed that tissue miR-138-5p expression was remarkably reduced in BC tissues. Moreover, tissue miR-138-5p level was significantly correlated with worse clinical outcome and shorter survival of BC patients, indicating that miR-138-5p might play a crucial role in the progression of BC and serve as a promising marker for BC prognosis.

Acknowledgements

This study was supported by a grant from Jiangsu Planned Projects for Postdoctoral Research Fund (1501142B).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Junsong Meng, Department of Urology, Huai'an First People's Hospital, Nanjing Medical University, 6 Beijing West Road, Huai'an 223300, Jiangsu, China. Tel: +86-517-84922412; E-mail: junsongmeng@163.com

References

[1] Siegel R, Ma J, Zou Z. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.

[2] Micheli A, Francisci S, Krogh V, Rossi AG, Crosignani P. Cancer prevalence in Italian cancer registry areas: the ITAPREVAL study. ITAPREVAL working group. Tumori 1999; 85: 309-369.

[3] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.

[4] Babjuk M, Bohle A, Burger M, Capoun O, Cohen D, Comperat EM, Hernandez V, Kaasinen E, Palou J, Roupret M, van Rhijn BW, Shariat SF, Soukup V, Sylvester RJ, Zigeuner R. EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016. Eur Urol 2017; 71: 447-461.

[5] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[6] Lai EC. Micro RNAs are complementary to 3' UTR sequence motifs that mediate negative post-transcriptional regulation. Nat Genet 2002; 30: 363-364.

[7] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011; 144: 646-74.

[8] McManus MT. MicroRNAs and cancer. Semin Cancer Biol 2003; 13: 253-258.

[9] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[10] Wijnhoven BP, Michael MZ, Watson DI. MicroRNAs and cancer. Br J Surg 2007; 94: 23-30.

[11] Zhang C, Wang C, Chen X, Yang C, Li K, Wang J, Dai J, Hu Z, Zhou X, Chen L, Zhang Y, Li Y, Qiu H, Xing J, Liang Z, Ren B, Yang C, Zen K, Zhang CY. Expression profile of microRNAs in serum: a fingerprint for esophageal squamous cell carcinoma. Clin Chem 2010; 56: 1871-1879.

[12] Huang B, Zhai W, Hu G, Huang C, Xie T, Zhang J, Xu Y. MicroRNA-206 acts as a tumor suppressor in bladder cancer via targeting YRDC. Am J Transl Res 2016; 8: 4705-4715.

[13] Xiu Y, Liu Z, Xia S, Jin C, Yin H, Zhao W, Wu Q. MicroRNA-137 upregulation increases bladder cancer cell proliferation and invasion by targeting PAQR3. PLoS One 2014; 9: e109734.

[14] Yang R, Liu M, Liang H, Guo S, Guo X, Yuan M, Lian H, Yan X, Zhang S, Chen X, Fang F, Guo H, Zhang C. miR-138-5p contributes to cell proliferation and invasion by targeting survivin in bladder cancer cells. Mol Cancer 2016; 15: 82.

[15] Yu C, Wang M, Li Z, Xiao J, Peng F, Guo X, Deng Y, Jiang J, Sun C. MicroRNA-138-5p regulates pancreatic cancer cell growth through targeting FOXC1. Cell Oncol (Dordr) 2015; 38: 173-81.

[16] Zhao L, Yu H, Yi S, Peng X, Su P, Xiao Z, Liu R, Tang A, Li X, Liu F, Shen S. The tumor suppressor miR-138-5p targets PD-L1 in colorectal cancer. Oncotarget 2016; 7: 45370-45384.

[17] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5-29.

[18] Kaplan AL, Litwin MS, Chamie K. The future of bladder cancer care in the USA. Nat Rev Urol 2014; 11: 59-62.