Original Article

Evaluation of miR-182/miR-100 Ratio for Diagnosis and Survival Prediction in Bladder Cancer

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Abstract

Background: Abnormal expression of microRNAs (miRNAs) plays an important role in development of several cancer types, including bladder cancer (BCa). However, the relationship between the ratio of miR-181/miR-100 and the prognosis of BCa has not been studied yet. The aim of this study was to evaluate the expression of miR-182, miR-100 and their clinical significance in BCa.

Methods: Upregulation of miR-182 and down-regulation of miR-100 were validated in tissue specimens of 134 BCa cases compared with 148 normal bladder epithelia (NBE) specimens using TaqMan-based real-time reverse transcription quantitative PCR (RT-qPCR). The diagnostic and prognostic evaluation of miR-182, miR-100, and miR-182/miR-100 ratio was also performed.

Results: miR-182 was upregulated in BCa and miR-100 was down-regulated in BCa compared with NBE ($P < 0.001$). The areas under receiver operating characteristic curves (AUCs-ROC) for miR-182 and miR-100 were 0.913 and 0.810, respectively. However, miR-182/miR-100 ratio increased the diagnostic performance, yielding an AUC of 0.981 (97.01% sensitivity and 90.54% specificity). Moreover, miR-182/miR-100 ratio was associated with pT-stage, histological grade, BCa recurrence and carcinoma *in situ* ($P < 0.05$ for all). Multivariate Cox regression analysis indicated that miR-182/miR-100 ratio was an independent prognostic factor for overall survival (Hazard ratio: 7.142; 95% CI: 2.106 – 9.891; $P < 0.01$). Furthermore, Kaplan-Meier curve analysis revealed that high-level of miR-182/miR-100 ratio was significantly correlated with shortened survival time for BCa patients ($P < 0.01$).

Conclusion: The miR-182/miR-100 ratio may serve as a novel promising biomarker for diagnosis and survival prediction in BCa. Further studies are needed to elucidate the role of miR-182/miR-100 ratio as a non-invasive diagnostic tool for BCa.

Keywords: Biomarker, bladder cancer, miR-182, miR-100, prognosis

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Introduction

Bladder cancer (BCa) is one of the most common malignant diseases and the leading cause of cancer-related death worldwide. Approximately, 90% of these tumors are confined to transitional cell carcinoma (TCC).[1] Despite the existence of appropriate therapies, such as surveillance cystoscopy and periodic imaging, the majority of these patients are continually under the risk of recurrence and progression. Extensive research has spurred a number of new diagnostic and prognostic markers based on novel molecular networks, but few of these tests are recommended for daily practice due to limited diagnostic/prognostic performance.[2] Therefore, well-characterized biomarkers for diagnosis/prognosis of BCa are strongly desired.

The miRNAs constitute an abundant class of single-stranded, small non-protein-coding RNAs 19 – 22 nucleotides long, which can negative-modulate gene expression by either partially or completely pairing with complementary sites within target messenger RNAs (mRNAs) of target genes and inducing transcriptional or post-transcriptional regulation.[3] Several studies have described that aberrant expression of miRNAs plays an important role in the initiation, development and metastasis of most cancers, such as chronic lymphocytic leukemia, and several solid cancers including liver cancer, gastric cancer, colorectal cancer, and prostate cancer.[4] Furthermore, recent studies have indicated that dysregulation of miRNAs can act as either tumor suppressors or oncogenes.[5] However, few reports have demonstrated miRNA expression profiling in BCa as diagnostic and prognostic biomarker. The high throughput Solexa sequencing technology allowing the detection of differentially expressed miRNAs in BCa and NBE has paved the way for employment of miRNAs as novel BCa biomarkers for diagnosis/prognosis.[6]

The miR-182 is widely established as a tumor oncogene, which has been demonstrated to be up-regulated in prostate, lung and endometrial and bladder cancer and activated by cellular proliferation, invasiveness and metastasis.[7,8] On the other hand, dysregulated expression of miR-100 has been reported in various types of cancers. In particular, miR-100 was found to be acting as a tumor suppressor by deregulation of its target genes in hepatocellular carcinoma, bladder cancer, epithelial ovarian cancer, and nasopharyngeal cancer. Furthermore, it can also act as an oncogene by accelerating tumorigenesis in renal cell carcinoma and acute myeloid leukemia.[9] However, the diagnostic and prognostic role of miR-182, miR-100 and the ratio of miR-182/miR-100 in BCa remain largely unknown.

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In our previous experiment, the profiles of differentially expressed miRNAs were sequenced by the next generation high throughput Solexa sequencing technology in the BCa and NBE specimens.[6] The aim of this study was to validate changes in the levels of miR-182 and miR-100 in the tissue of BCa patients in comparison with NBE. We sought to determine whether miR-182/miR-100 could serve as a novel biomarker for early diagnosis and prognosis of BCa patients, as well as their clinical significance in BCa.

Materials and Methods

Patients and tissue samples

Human BCa tissues (n = 134) were derived from patients receiving transurethral bladder resection (TURBT) or radical cystectomy at the First Affiliated Hospital of Wenzhou Medical University and Renmin Hospital of Wuhan University between June 2007 and May 2011. The patients undergoing radiotherapy or chemotherapy before resection were excluded from this study. NBE (n = 148) mucosal tissues were recruited from genetically unrelated cancer-free individuals. Tumor fragments were immediately stored in liquid nitrogen after TURBT. The diagnoses of tumors were confirmed by pathological evaluation. All tumors were graded histologically according to the WHO 2004 grading scheme and staged according to the 2002 Union for International Cancer Control (UICC) TNM classification of BCa.[10] The distribution of selected clinical characteristics between the BCa and NBE groups is depicted in Table 1. No significant differences were found in terms of distribution of age or gender ($P = 0.813$ for age; $P = 0.567$ for gender), while a significant difference was found in terms of smoking status ($P < 0.05$). Written informed consent form was obtained from each patient, and this study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University and Renmin Hospital of Wuhan University.

RNA extraction

Total RNA was isolated from pulverized tissues using the TRIzol reagent (Invitrogen, Shanghai, China) according to the manufacturer's instructions. Total RNA concentration was quantified using a NanoDrop 2000 (NanoDrop Technologies, Wilmington, USA). The quality of RNA was confirmed by agarose gel electrophoresis. The 18S and 28S RNA bands were visualized under ultraviolet light. The purified total RNA samples were stored at -80°C for subsequent analysis.

Table 1. distribution of selected clinical-pathological variables between BCa cases and NBE subjects

Variables	BCa (n, %)	NBE (n, %)	*P*-value
Patients (Cases)	134	148	
Gender			0.148
Male	97 (72.3)	118 (79.7)	
Female	37 (27.6)	30 (20.3)	
Age (Years)			0.813
Median (Range)	65 (41~75)	66 (39~73)	
Smoking status (pack/month)			**0.023**
0	72 (53.7)	103 (69.6)	
0–15	38 (28.4)	28 (18.9)	
> 15	24 (17.9)	17 (11.5)	
Tumor size			- - -
< 3cm	79 (59.0)	- - -	
≥ 3cm	55 (41.0)	- - -	
Associated Cis			- - -
No	129 (96.3)	- - -	
Yes	5 (3.7)	- - -	
Clinical stage			- - -
Ta–T1	87 (64.9)	- - -	
T2–T4	47 (35.1)	- - -	
Histological grade			- - -
G1	48 (35.8)	- - -	
G2	44 (32.8)	- - -	
G3	42 (31.6)	- - -	
Lymph node status			- - -
N-	95 (70.8)	- - -	
N+	39 (29.2)	- - -	
Surgical procedure			- - -
TURBT	96 (71.6)	- - -	
Cystectomy	38 (28.4)	- - -	

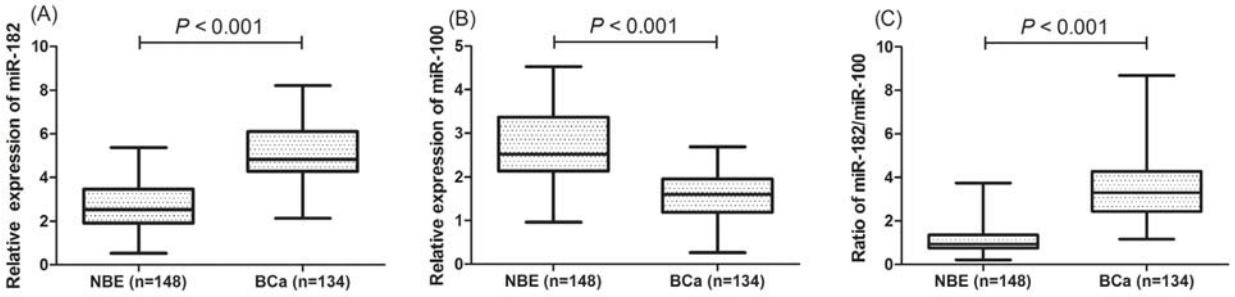

Figure 1. Box plot of selected expression level of miRNAs in BCa cases and NBE subjects. **(A)** The relative expression of miR-182; **(B)** The relative expression of miR-100; **(C)** The ratio of miR-182/miR-100. Boxes represent the 25th and 75th percentiles, and the bold lines indicate the median values. Statistically significant difference was determined using Mann–Whitney U test.

Real-time reverse transcription quantitative PCR (RT-qPCR) for miRNA

The expression of mature miRNAs in BCa and NBE tissues was quantified by TaqMan MicroRNA Assays (Applied Biosystems, USA). The cDNA was synthesized using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, USA) according to the manufacturer's protocol. Briefly, 7 μL reverse transcription master mix and 5 μL purified total RNA were mixed with 3 μL 5×RT primers (miR-182, miR-100 and RNU6B RNA) in a tube to 15 μL final volume. After gently mixing with ice and centrifuging, the mixture was reverse transcribed at 16°C for 60 min followed by an inactivation step at 95°C for 5 min and a holding step on ice. For RT-qPCR, each reaction included 1 μL primer and TaqMan probe Mix (Applied Biosystems, USA), 10 μL TaqMan Universal PCR Master Mix II (Applied Biosystems, USA) and 1.33 μL product from RT reaction. The above two-step reactions were carried out on the ABI Prism 7900 Sequence Detection System (Applied Biosystems, USA) according to the manufacturer's instruction. The expression of target miRNA was determined using the comparative Ct method ($2^{-\Delta Ct}$). The raw data were presented as the relative quantity of target miRNA, and normalized with RNU6B using the $2^{-\Delta Ct}$ method. At the beginning of the reaction, serial diluted cDNAs were used for exploring whether the PCR efficiency between target miRNA and RNU6B are equal. To overcome the limits of detection of RT-qPCR, all assays were performed in triplicate and a valid result required a threshold cycle (Ct) value of RNU6B within a range from 10 to 30.

Patient follow-up

All BCa patients have been followed up with phone calls at intervals of 3 months following the initial TURBT and we defined a time point of 60 months to investigate the overall survival (OS) which was calculated from the date of initial surgery to the date of the last follow-up or cancer-related death.

Statistical analysis

All statistical analyses were performed with SPSS ver. 19.0 (Chicago, IL, USA) and GraphPad Prism 5.0. Comparisons of miRNAs levels between groups were carried out using Kruskal–Wallis test or the Mann–Whitney U test. For each miRNA, ROC analyses were used to evaluate its predictive value of BCa, and the cutoff values were also calculated. According to the cutoff values of ROC curve, we defined the result of miRNAs below the cutoff value as low expression and above the cutoff value as high expression. The Chi-square or Fisher's exact probability test was performed to examine possible correlations between the levels of candidate miRNAs and clinicopathological parameters. OS curves were plotted by the Kaplan-Meier method and compared by log-rank test. The assessment of correlation between survival time and multiple clinicopathological variables was carried out by the Cox proportional hazards regression model. Univariate and multivariate Cox proportional hazard models were used to identify variables associated with OS in the group of BCa patients. When the significant variables associated with OS were obtained by univariate analysis, multivariate analysis was used for evaluating which variables were the most important in prediction of OS. A *P*-value less than 0.05 was considered as statistically significant.

Results

Expression levels of miR-182 and miR-100 validated by RT-qPCR

To validate whether expression of miR-182 and miR-100 differs between BCa and NBE tissues, the levels of these two miRNAs were first measured in 134 BCa and 148 NBE tissues by RT-qPCR normalized to RNU6B. A favorable Ct value lower than 30 was taken into consideration. Compared with the NBE tissues, the miR-182 was observed to be significantly upregulated in BCa tissues [4.84 (2.13 – 8.21) *vs.* 2.52 (0.53 – 5.38), $P < 0.001$] (Figure 1A) and the miR-100 down-regulated in BCa tissues [1.60 (0.27 – 2.69) *vs.* 2.51 (0.96 – 4.53), $P < 0.001$) (Figure 1B). Therefore, miR182/miR-100 ratio was found to be significantly upregulated in BCa tissues [3.30 (1.16 – 8.68) *vs.* 0.93 (0.21 – 3.75), $P < 0.001$] (Figure 1C).

The diagnostic efficacy of miR-182, miR-100 and miR-182/ miR-100 ratio in BCa

ROC curve analyses were performed to evaluate the diagnostic accuracy of the miR-182, miR-100 and miR-182/miR-100 ratio. ROC curve analyses revealed that when the optimal cutoff values of miR-182, miR-100 and miR-182/miR-100 ratio were 3.910, 2.225 and 1.820, respectively, the area under the curve (AUC) values for them were 0.913 (95% CI: 0.806 – 0.961, $P < 0.001$; sensitivity = 87.31%; specificity = 85.81%), 0.810 (95% CI: 0.753 – 0.927, $P < 0.001$; sensitivity = 70.27%; specificity = 94.03%), and 0.981 (95% CI: 0.968 – 0.993, $P < 0.001$; sensitivity = 97.01%; specificity = 90.54%), respectively (Figure 2). It is suggested that

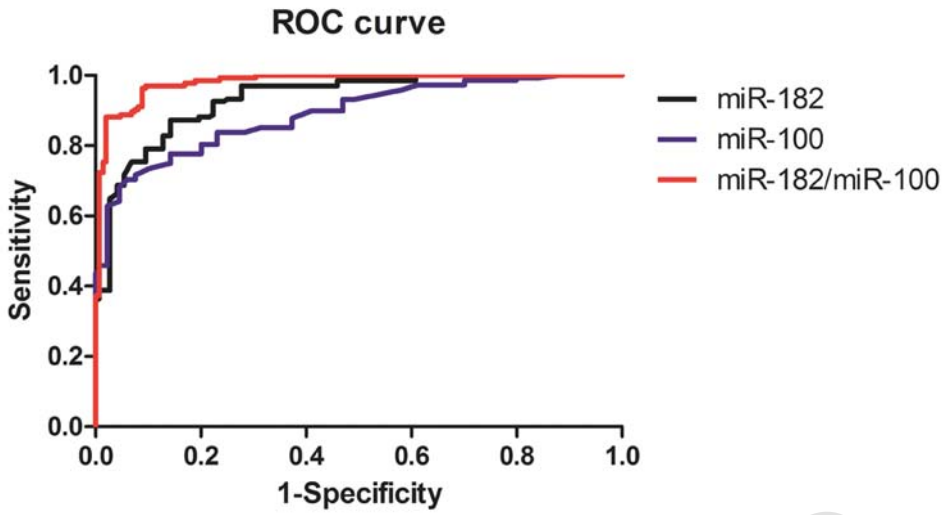

Figure 2. Receiver operating characteristics (ROC) curve analysis using tissue selected miRNAs for discriminating BCa patients. When the optimal cutoff values of miR-182, miR-100 and miR-182/miR-100 ratio were 3.910, 2.225 and 1.820, respectively, an area under the curve (AUC) values for them were 0.913, 0.810 and 0.981, respectively.

miR-182, miR-100 and miR-182/miR-100 ratio could accurately discriminate BCa patients from controls; however, the latter could enhance the sensitivity and the specificity.

Association between selected miRNAs and clinical characteristics of BCa patients

The relationships between the levels of selected miRNAs and clinicopathological features in BCa are summarized in Table 2. High levels of miR-182/miR-100 ratio were significantly associated with pT-stage ($P < 0.01$), histological grade ($P < 0.05$), BCa recurrence ($P < 0.05$) and carcinoma in situ ($P < 0.01$). However, there was no significant association between miR-182 or miR-100 expression and clinical characteristics in BCa patients ($P > 0.05$).

Cox proportional hazards regression models of risk factors associated with OS among BCa patients

The results of univariate and multivariate analysis are presented in Table 3. Gender, age, tumor size, recurrence, miR-182,

Table 2. Relationships between the expression of selected miRNAs and clinicopathological features in BCa.

Clinical parameters	N	miR-182		*P*-value	miR-100		*P*-value	miR-182/miR-100 ratio		*P*-value
		Low (n = 51)	High (n = 83)		Low (n = 80)	High (n = 54)		Low (n = 47)	High (n = 87)	
Gender				0.715			0.668			0.221
Male	97	36 (37.1)	61 (62.9)		59 (60.8)	38 (39.2)		31 (32.0)	66 (68.0)	
Female	37	15 (40.5)	22 (59.5)		21 (56.8)	16 (43.2)		16 (43.2)	21 (56.8)	
Age (years)				0.734			0.563			0.810
≥ 60	58	23 (39.7)	35 (60.3)		33 (56.5)	25 (43.5)		21 (36.2)	37 (63.8)	
< 60	76	28 (36.8)	48 (63.2)		47 (58.8)	29 (41.2)		26 (34.20)	50 (65.8)	
Tumor size				0.485			0.170			0.399
< 3cm	79	32 (40.5)	47 (59.5)		51 (64.6)	28 (35.4)		30 (38.0)	49 (62.0)	
≥ 3cm	55	19 (34.5)	36 (65.5)		29 (52.7)	26 (47.3)		17 (31.0)	38 (69.0)	
Clinical stage				0.967			0.278			**0.009**
Ta–pT1	87	33 (37.9)	54 (62.1)		49 (56.3)	38 (43.7)		20(23.0)	67(77.0)	
T2–pT4	47	18 (38.3)	29 (61.7)		31 (65.9)	16 (34.1)		27(57.4)	20(42.6)	
Histological grade				0.716			0.528			**0.010**
G1	48	20 (41.6)	28 (58.3)		26 (54.2)	22 (45.8)		26 (54.2)	22 (45.8)	
G2	44	17 (38.6)	27 (61.4)		29 (65.9)	15 (34.1)		15 (34.1)	29 (65.9)	
G3	42	14 (33.3)	28 (66.7)		25 (59.5)	17 (40.5)		10 (23.8)	32 (76.2)	
Associated Cis				0.927			0.989			**0.008**
No	129	49 (38.9)	80 (62.0)		77 (59.7)	52 (40.3)		46(35.7)	83(64.3)	
Yes	5	2 (40.0)	3 (60.0)		3 (60.0)	2 (40.0)		1(20.0)	4(80.0)	
Recurrence				0.572			0.569			**0.042**
No	88	35 (39.8)	53 (60.2)		51 (57.9)	37 (43.1)		39(44.3)	49(55.7)	
Yes	46	16 (34.8)	30 (65.2)		29 (63.0)	17 (37.0)		8(17.4)	38(82.6)	

Table 3. Cox proportional hazards regression models of risk factors associated with overall survival among bladder cancer patients (n = 134)

Variables	Univariate analysis			Multivariate analysis[a]		
	HR[b]	95% CI[c]	*P*-value	HR[b]	95% CI[c]	*P*-value
Gender	1.216	0.894–1.653	0.212	- - -	- - -	- - -
Age (years)	1.148	0.678–1.945	0.608	- - -	- - -	- - -
Tumor size	0.759	0.457–1.261	0.287	- - -	- - -	- - -
Clinical stage	2.906	1.175–7.190	**0.021**	5.701	1.106–7.891	**0.015**
Histological grade	2.427	1.050–5.608	**0.038**	3.252	1.136–9.311	**0.028**
Recurrence	1.025	0.988–1.064	0.194	- - -	- - -	- - -
Associated Cis	1.774	1.178–2.816	**0.015**	2.765	1.097–6.967	**0.031**
miR-182	1.045	0.945–1.155	0.389	- - -	- - -	- - -
miR-100	0.886	0.602–1.304	0.539	- - -	- - -	- - -
miR-182/miR-100 ratio	4.206	1.657–10.674	**0.0025**	7.142	2.155–23.669	**0.0013**

[a]Backward Wald test was used for variables screened, $P < 0.05$ was chosen as a criterion for significance; [b]HR, hazard ratio; [c] CI, 95% confidence interval.

and miR-100 were not significant predictive factors for the prognosis of BCa patients as determined by univariate analysis ($P > 0.05$). However, high levels of miR-182/miR-100 ratio, pT-stage, histological grade, and carcinoma *in situ* were significant predictive factors for the prognosis of BCa patients ($P < 0.05$ for all, Table 3). In the multivariate Cox analysis of OS, miR-182/miR-100 ratio, pT-stage, histological grade, and carcinoma in situ were independent predictive risk factors for the prognosis of BCa patients after adjustment for gender, age, and tumor size (HR: 7.142, $P = 0.0013$; HR: 5.701, $P = 0.015$; HR: 3.252, $P = 0.028$; HR: 2.765, $P = 0.031$, respectively, Table 3).

Prognostic values of selected miRNAs in different BCa subgroups

To establish survival curves, continuous expression levels of miR-182, miR-100 and miR-182/miR-100 ratio were converted to a dichotomous variable, using their cutoff values from ROC curve analyses as a threshold, respectively. Kaplan-Meier method was employed to analyze the OS times of 134 BCa patients between high expression and low expression of the selected miRNAs. As shown in Figures 3A and 3B, BCa patients with different OS times could not be distinguished by miR-182 or miR-100 alone ($P > 0.05$). Inversely, however, miR-182/miR-100 ratio was more sensitive for predicting prognosis in subgroups of BCa patients ($P < 0.01$, Figure 3C). Moreover, the median survival time of BCa patients with low (n = 47) and high (n = 87) level of miR-182/miR-100 ratio was 60 and 45 months, respectively.

Discussion

In our previous study, sequence tags analysis showed that 33 miRNAs were upregulated and 41 miRNAs were downregulated in BCa compared with NBE. Likewise, we also identified 13 miRNAs in both BCa and NBE libraries, such as miR-182 and miR-100.[6] Three main findings were elucidated in our retrospective exploratory investigation: firstly, miR-182 was observed to be significantly elevated in BCa tissues in comparison with the NBE group. However, the expression levels of miR-100 were statistically lower in BCa samples. Secondly, miR-182/miR-100 ratio could serve as a novel biomarker for diagnosis and survival prediction in BCa. Finally, miR-182/miR-100 ratio retained a higher prognostic value than other clinicopathological parameters such as pT-stage, grading, or carcinoma *in situ*. In summary, our findings suggested that miR-182/miR-100 ratio could be applied to establish precise risk stratification in the present system. Furthermore, our knowledge about the clinical significance of miR-182/miR-100 ratio could also provide reliable information to deepen our insights of miRNAs in the mechanism of BCa.

The majority of altered miRNAs in clinical BCa tissues have been reported and identified in several genome-wide profiling studies.[11] It is noteworthy that there are some discrepancies in these reported investigations.[12] TaqMan probe-based RT-qPCR was performed to detect the levels of selected miRNA expression in our bladder specimens. It is an advanced technology, with robust reliability and reproducibility, which may explain some discrepancies in comparison to previous studies.

The miR-182 was shown to be upregulated in clinical BCa tissues in previous studies.[13] Dysregulation of miR-182 was also identified in other tumors. The miR-182 was reported to directly antagonize FOXO3 and inhibit microphthalmia-associated transcription factor, thus inducing its frequent amplification and melanoma metastasis.[14] Further studies demonstrated that miR-182 is also highly expressed in p53+ colon cancer cells (HCT116) after treatment with the DNA-damaging and p53-inducing agent Adriamycin.[15] Moreover, in malignant glioma and lung cancer, significant association was found between high expression of miR-182 and the poor overall-survival.[16] In accordance with their previous studies, we found that miR-182 was also significantly upregulated in BCa tissues compared with the NBE group. However, high levels of miR-182 were not associated with shorter OS of BCa patients. The discrepancies may be due to the different sample characteristics and procedures of miRNA preparation employed in our study design.

The miR-100, one of the oldest known animal miRNAs, has been demonstrated to be associated with the initiation and progression of cancers. It should be noted that miR-100 can serve as either a tumor promoter or suppressor depending on

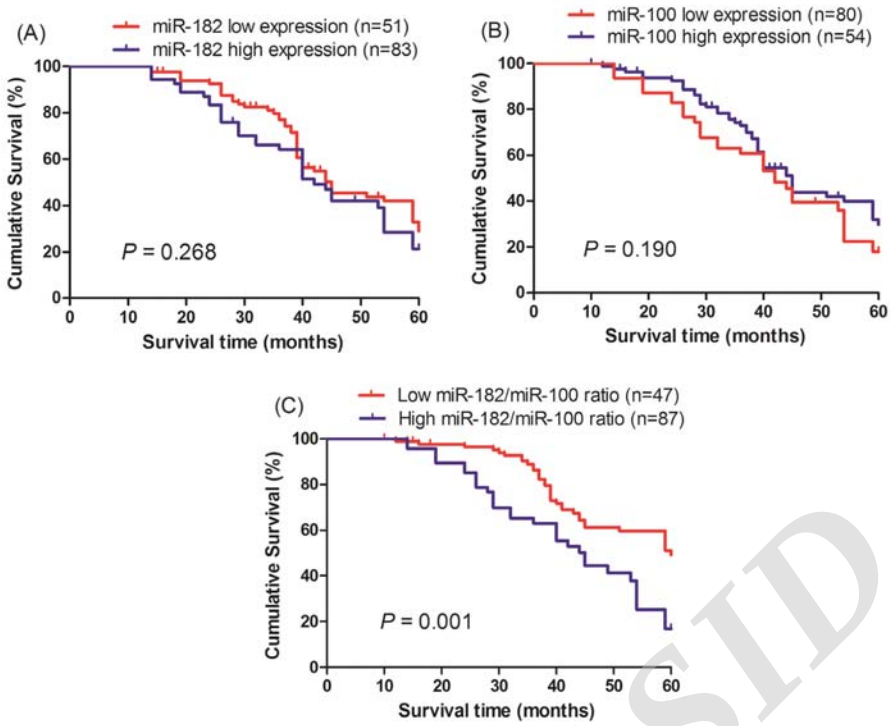

Figure 3. Survival analysis of 134 BCa patients by Kaplan–Meier method. **(A)** miR-182 for survival analysis of BCa patients; **(B)** miR-100 for survival analysis of BCa patients; **(C)** Ratio of miR-182/ miR-100 for BCa patients. Note: the overall survival rate in patients with high level of miR-182/ miR-100 ratio was significantly worse than that in patients with low level of miR-182/ miR-100 ratio

the type of cancer examined.[9] Downregulation of miR-100 has been validated in hepatocellular carcinoma, oral squamous cell carcinoma, nasopharyngeal cancer, ovarian cancer, and bladder cancer, whereas miR-100 is reported to be upregulated in medulloblastomas, pancreatic cancer, acute myeloid leukemia, gastric cancer, and prostate cancer.[17–19] Additionally, recent studies have indicated that the lower levels of miR-100 could serve as an independent poor prognostic factor for human epithelial ovarian cancer.[20] Other studies have suggested that decreased miR-100 expression is statistically associated with stage, lymph node metastasis, and shorter OS of cervical carcinoma patients.[21] In accordance with previous studies, our data also demonstrated that miR-100 expression was also significantly decreased in a large number of clinical BCa cases. Similarly, decreased miR-100 expression was not associated with shorter OS of BCa patients.

Recently, miR-21/miR-205 ratio was demonstrated to have the ability to discriminate between invasive and non-invasive BCa with a high diagnostic performance. Furthermore, miR-21/ miR-205 ratio in case of urine samples was at least 6-fold higher in high grade BCa patients compared with low grade.[22] Other investigations have also revealed that the ratios of miR-126/miR-152 and miR-182/miR-152 were both significantly upregulated in urine samples of BCa patients compared with healthy donors.[23] In this study, we identified miR-182/miR-100 ratio, which was significantly upregulated in BCa specimens compared with the NBE group, yielding a higher diagnostic performance for separation of BCa patients from the control groups. Moreover, miR-182/miR-100 ratio was significantly associated with several clinicopathological parameters, such as pT-stage, grading, BCa recurrence and carcinoma in situ, and retained a higher prognostic value in comparison with miR-182 and miR-100 alone. Therefore, miR-182/miR-100 ratio could be used as a novel prognostic indicator, distinguishing patients who are more likely to be at higher risk of recurrence and should receive more aggressive therapy. However, it is difficult to conduct a direct comparison of the present study and the data described previously, because of the different resources of RNA as well as the sample characteristics and methods incorporated in the investigation of the miRNA composition.

In conclusion, our study suggested that miR-182/miR-100 ratio could serve as a novel potential biomarker for diagnosis and survival prediction in BCa. A relatively small sample size of BCa patients may constitute a limitation of the present study, which could lead to the lack of power and the consequent imprecision. Therefore, more samples from multicenter studies will be needed to validate our results. To extend the clinical application of our data, further studies are needed to elucidate the role of miR-182/ miR-100 ratio as a non-invasive diagnostic tool for BCa.

Author's contribution

Zhanguo Chen, Lili Wu contributed equally to this work.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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Abbreviations

BCa: bladder cancer; NBE: normal bladder epithelia; RT-qPCR: real-time reverse transcription quantitative PCR; miRNA: microRNA; OS: overall survival

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