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Original Article

Down-regulation of microRNA-29b is correlated with unfavorable prognosis in human prostate cancer

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Abstract: Objective: In the present study, we aimed to investigate the expression level of miR-29b and its clinical significance in prostate cancer. Materials and methods: A total of 142 patients with prostate cancer who underwent tumor resection during December 2006 to February 2015 were enrolled in our study. The expression of miR-29b was measured using quantitative real-time PCR (qRT-PCR). The chi-squared test was used to determine the clinic pathological significance of miR-29b expression in prostate cancer patients. Differences in the survival rates of patients were determined by the Kaplan-Meier method and log-rank test. A Cox proportional hazard regression analysis was used for multivariate analyses of prognostic values. Results: Compared with normal prostate tissues, prostate cancer tissues had significantly decreased miR-29b expression (P<0.001). Low miR-29b expression was closely correlated with the Gleason score (P=0.015) and clinical stage (P=0.013). Kaplan-Meier analyses showed that the 5-year overall survival of the group with low miR-29b expression was significantly shorter than that of the group with high miR-29b expression (P=0.013), and the multivariate analysis with Cox's proportional hazard model confirmed that low miR-29b expression levels were an independent predictor of poor prognosis in prostate cancer patients (hazard ratio=2.784; 95% CI: 1.563-9.049, P=0.017). Conclusions: Our study provides evidence that miR-29b acts as a tumor suppressor in prostate cancer and might be a potential marker for indicating the prognosis of prostate cancer.

Keywords: Prostate cancer, microRNA-29b, overall survival, prognosis

Introduction

Prostate cancer is one of the most common and malignant tumors in males worldwide and is the second leading cause of deaths in men [1]. Despite efforts to improve therapeutic strategies, the average survival of prostate cancer patients has improved only slightly in the past decades. Currently, there is no curative treatment for androgen-independent prostate cancer. Therefore, new approaches to the diagnosis, prognostic evaluation and therapy used for prostate cancer must be explored by using the genetic and epigenetic changes involved in this cancer [2].

MicroRNAs (miRNAs), which are noncoding RNAs 20-22 nt in length, play important roles in many aspects of human cancers [3]. They are involved in regulating the development and maintenance of undifferentiated or incompletely differentiated cell types [4, 5]. Accumulating evidence shows that microRNAs may take part in tumor angiogenesis, invasion, and metastasis. They can act as oncogenes or anti-oncogenes depending on their target genes, which may provide insight into the diagnosis and prognosis of human cancers [6, 7].

The miR-29 family consists of miR-29a, miR-29b, and miR-29c, differing by only two or three bases. Recently, overwhelming evidence has suggested that aberrant expression of the members of the miR-29 family is involved in multiple cancers [8, 9]. As a member of the miR-29 family, miR-29b is generally recognized as a fundamental regulator of the epithelial-mesenchymal transition (EMT), a pathway involved in cancer metastasis and chemoresistance [10]. Some researchers have suggested that miR-29b has a potential tumor suppressive function. For example, the study by Wang et al demonstrated that miR-29b served as a tumor metastasis suppressor in NSCLC; miR-

Table 1. Association between miR-29b expression and patients' clinicopathologic features

Clinicopathologic variables	N	miR-29b level		*P* value
		Low (n=50)	High (n=52)	
Age (years)				
≤65	47	20	27	0.241
>65	55	30	25	
Preoperative PSA				
≤10	61	25	36	0.069
>10	41	25	16	
Tumor lesion number				
Solitary	43	17	26	0.113
Multiple	59	33	26	
Gleason score				
≤7	62	24	38	0.015
>7	40	26	14	
Clinical stage				
I-II	35	11	24	0.013
III-IV	67	39	28	

29b suppressed NSCLC cell metastasis by directly inhibiting matrix metalloproteinase 2 (MMP2) expression [11]. In contrast, other studies have identified that miR-29b is an oncogenic miRNA. A previous study has shown that miR-29b was up-regulated in highly metastatic human breast cancer and that miR-29b contributed to cancer cell growth, migration, invasion, and anti-apoptosis activity [12]. In this study, we aimed to investigate the expression level and clinical significance of miR-29b in prostate cancer.

Methods and materials

Patients and tissue samples

A total of 142 prostate cancer patients who underwent tumor resection during December 2006 to February 2015 at Linyi People's Hospital were enrolled in our study. All the patients' diagnoses were confirmed based on the histopathological examination, for which the exclusion criteria included receiving chemotherapy, endocrine therapy, or radiotherapy prior to surgery. The mean age of the patients was 61.3 years, the mean Gleason score was 7.3, and the mean preparatory PSA level was 7.6 ng/mL. The mean PSA level in BR was 3.5 ng/mL. The control group consisted of 20 surgical specimens from patients who underwent retropubic prostate resection to treat benign prostate hyperplasia. The mean age of these patients was 65 years. Resected specimens were snap frozen in liquid nitrogen and stored at -80°C for qRT-PCR. Follow-ups were conducted by telephone. Patients' clinic pathological information, such as age, PSA level, Gleason score, and clinical stage, is shown in **Table 1**. This study was approved by the Research Ethics Committee of Linyi People's Hospital. Written informed consent was obtained from all of the patients. All specimens were handled anonymously according to ethical and legal standards.

RNA extraction, reverse transcription and real-time PCR

Total RNA was extracted from frozen prostate cancer tissues and control tissues using the mirVana miRNA Isolation Kit (Ambion, Austin, TX, USA) according to the manufacturer's instructions. Reverse transcription (RT) was performed using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions; 10 ng of total RNA was utilized for the RT reactions. qRT-PCR was performed using the TaqMan microRNA assay (Applied Biosystems, Foster City, CA, USA). RNU-6B was used as an endogenous control, and the relative quantification of miR-29b expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The primers used for RT-PCR were as follows: miR-29b, forward: 5'-ACACTCCAGCTGGGUAGCACCAUUUGAAAUC-3', reverse: 5'-TGGTGTCGTGGAGTCG-3'; U6, forward: 5'-CTCGCTTCGGCAGCACA-3', reverse: 5'-CTCGCTTCGGCAGCACA-3'.

Statistical analysis

All statistical analyses were performed using the SPSS 18.0 software package (SPSS, Chicago, IL, USA). The chi-squared test was used to determine the clinic pathological significance of miR-29b expression in prostate cancer patients. Differences in patient survival were determined by the Kaplan-Meier method and log-rank test. A Cox proportional hazard regression analysis was used for multivariate analyses of prognostic values. P<0.05 was consid-

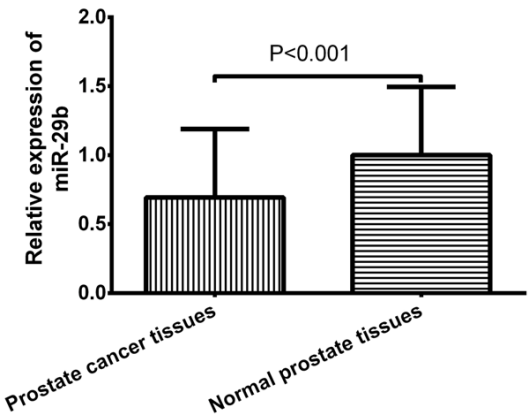

Figure 1. qRT-PCR detection of relative miR-29b expression in prostate cancer tissues and normal prostate tissues.

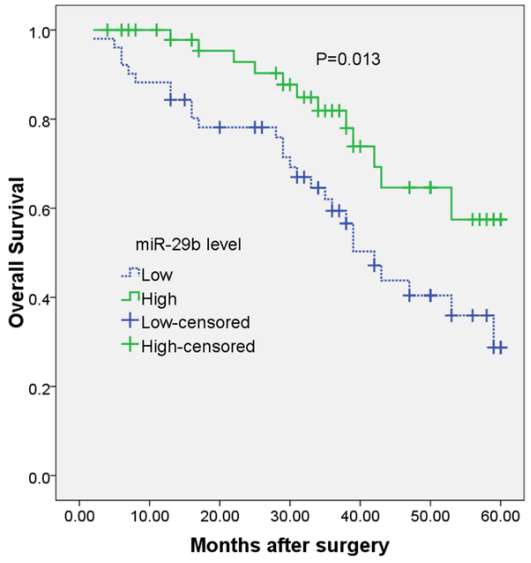

Figure 2. Overall survival analysis according to the Kaplan-Meier method for miR-29b expression (log-rank test, P=0.013).

ered to indicate a statistically significant difference.

Results

MiR-29b was down-regulated in prostate cancer tissues

To identify the role of miR-29b in the development of prostate cancer, we analyzed the expression level of miR-29b in 142 pairs of prostate cancer tissues and normal prostate tissues by real-time quantitative PCR. Compared to normal prostate tissues, a significant down-regulation of miR-29b was observed in prostate cancer tissues (P<0.001, shown in **Figure 1**).

Correlation of miR-29b expression with the clinic pathologic features of patients with prostate cancer

To further investigate the correlation of miR-29b with various clinic pathologic features of patients with prostate cancer, all patients were divided into two groups, a high miR-29b expression group (n=72) and a low miR-29b expression group (n=70), according to the median value of miR-29b in all prostate cancer tissues. Then, the correlation of miR-29b expression with the clinic pathologic features of prostate cancer patients was statistically analyzed. As shown in **Table 1**, low miR-29b expression was closely correlated with the Gleason score (P=0.015) and clinical stage (P=0.013). However, there was no significant correlation between miR-29b expression and other clinic pathologic factors.

Correlation of miR-29b expression with the survival rate of prostate cancer patients

To further investigate the correlation of miR-29b expression with the survival rate of prostate cancer patients, Kaplan-Meier analyses were performed. As shown in **Figure 2**, the 5-year overall survival rate of the low miR-29b expression group was significantly shorter than that of high miR-29b expression group (P=0.013), indicating that the down-regulation of miR-29b might be correlated with poor survival in prostate cancer patients. The multivariate analysis with Cox's proportional hazard model confirmed that low miR-29b expression levels were an independent predictor of poor prognosis for the prostate cancer patients (hazard ratio=2.784; 95% CI: 1.563-9.049, P= 0.017, shown in **Table 2**).

Discussion

Prostate cancer is one of the most prevalent cancers occurring among men. Genetic and environmental factors have been demonstrated to be involved in its pathogenesis [13]. Despite the recent advances in cancer diagnosis and treatment, the dismal survival rate of high-risk prostate cancer represents one of the major challenges in clinical practice. The unmet demand for accurate early prognosis, which

Table 2. Multivariate analyses of parameters associated with overall survival of 102 patients with prostate cancer

Variable	Hazard ratio	95% CI	*P*-value
Age	1.469	0.894-2.056	0.107
Preoperative PSA	0.561	0.254-1.337	0.354
Tumor lesion number	1.564	0.878-2.449	0.553
Gleason score	2.419	1.045-5.779	0.046
Clinical stage	3.875	1.664-10.056	0.009
miR-29b expression	2.784	1.563-9.049	0.017

could guide the therapeutic strategy for treating prostate cancer, has highlighted the importance of developing new diagnostic and prognostic approaches.

MicroRNAs are endogenous small non-coding RNAs with the capacity to regulate gene expression post-transcriptionally. The miRNA-29 family consists of miR-29a, miR-29b, and miR-29c, among which miR-29b is the most highly expressed and is found at two genomic loci. Recently, numerous studies have demonstrated that the aberrant expression of miR-29b is common in the majority of human cancers [10]. Some researchers have suggested that miR-29b has a potential tumor suppressive function. For example, the study by Wang et al demonstrated that miR-29b served as a tumor metastasis suppressor in NSCLC; miR-29b suppressed NSCLC cell metastasis by directly inhibiting matrix metalloproteinase 2 (MMP2) expression, showing that miR-29b might be a novel therapeutic candidate target for slowing NSCLC metastasis [11]. In contrast, other studies have identified that miR-29b is an oncomiR. A previous study has shown that miR-29b was up-regulated in highly metastatic human breast cancer and that miR-29b contributed to cancer cell growth, migration, invasion, and anti-apoptosis [12].

The clinical significance and prognostic value of miR-29b have also been investigated in several types of cancer. Yang et al found that miR-29b expression was correlated with lymph node metastasis and advanced tumor stage in oral squamous cell carcinoma. Furthermore, a multivariate analysis revealed that miR-29b expression was significantly correlated with recurrence and indicated poor survival [14]. In the study by Xu et al, elevated expression of miR-29b was found in both renal cell carcinoma (RCC) tissues and cell lines. High expression levels of miR-29b were significantly associated with TNM stage (P=0.026) and the overall survival (P=0.009) in RCC. These data suggest that miR-29b acts as an oncogenic miRNA and that it might be a potential marker for determining the prognosis of RCC [15]. Flavin et al showed that miR-29b was significantly down-regulated in serous ovarian carcinomas and was associated with reduced disease-free survival [16]. The findings from Hong et al revealed that the miR-29 family might play crucial roles in the development and progression of human osteosarcoma. In particular, the serum levels of miR-29a and miR-29b might estimate the prognosis of patients with this malignancy [17].

In the present study, we investigated the expression level and clinical significance of miR-29b in prostate cancer. First, we analyzed the expression level of miR-29b in 142 pairs of prostate cancer tissues and normal prostate tissues by real-time quantitative PCR. Compared with normal prostate tissues, a significant down-regulation of miR-29b was observed in prostate cancer tissues. Then, the correlation of miR-29b expression with clinical pathologic features of patients with prostate cancer were statistically analyzed. Low miR-29b expression was closely correlated with the Gleason score and clinical stage. To further investigate the correlation of miR-29b expression with the survival rate of prostate cancer patients, Kaplan-Meier analyses were performed. We found that the 5-year overall survival of the low miR-29b expression group was significantly shorter than that of the high miR-29b expression group, indicating that the down-regulation of miR-29b might be correlated with poor survival in prostate cancer patients. Furthermore, the multivariate analysis with Cox's proportional hazard model confirmed that low miR-29b expression levels were an independent predictor of poor prognosis for prostate cancer patients.

In conclusion, our study provides evidence that miR-29b acts as a tumor suppressor in prostate cancer and might be a potential marker for indicating the prognosis of prostate cancer. More studies, however, are needed to confirm our findings.

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Disclosure of conflict of interest

None.

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