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Prognostic value of microRNA-20b expression level in patients with prostate cancer

Rongkui Zhang[1], Fuwei Li[2], Yuchong Wang[3], Ming Yao[4] and Changliang Chi[5]

[1]Department of Radiology, The First Hospital of Jilin University, [2]Operating Room, China-Japan Union Hospital of Jilin University, [3]Department of Magnetic Resonance, Jilin Province FAW General Hospital, [4]Department of Radiology, Jilin Cancer Hospital and [5]Department of Urology Surgery, The First Hospital of Jilin University, Changchun, Jilin, P.R. China

Summary. Background. miR-20b is a member of the miR-106a-363 gene cluster located in the mammalian X chromosome, the larger miR-17 family, and the miR-17-92 and miR-106b-25 gene clusters. Previous studies have indicated that miR-20b may function as oncogene or tumor suppressor in different types of cancers. The present study analyzed the association between miR-20b and clinicopathological characteristics of patients with prostate cancer.

Methods. A total of 127 pairs of prostate cancer tissue samples and adjacent prostate tissue samples were collected from April 2013 to March 2018. The associations between miR-20b expression levels and clinicopathological factors were assessed using the χ^2-test. Survival was estimated using the Kaplan-Meier method, and the differences in survival according to miR-20b expression were compared using the log-rank test. Prognostic values of miR-20b expression and clinical outcomes were evaluated by Cox regression analysis.

Results. The relative expression of miR-20b in prostate cancer tissues was significantly higher than that in adjacent noncancerous prostate tissues (P<0.001). miR-20b expression was observed to be significantly associated with Gleason score (P<0.001), lymph node metastasis (P<0.001), and TNM stage (P=0.002). The log-rank test indicated that patients with increased miR-20b expression experienced poor overall survival (P=0.037). Multivariate Cox regression analysis showed that miR-20b expression level (HR=2.181, 95% CI: 1.772-9.021, P=0.016) was an independent factor in predicting the overall survival of prostate cancer patients.

Conclusion. The present study demonstrated that tissue miR-20b expression level could be a promising biomarker of prognosis in prostate cancer.

Key words: MicroRNA-20b, Multivariate Cox regression analysis, Expression, Prognosis, Prostate cancer

Introduction

Prostate cancer ranks as the second highest cause of cancer-associated mortality among male population in Western countries (Bray et al., 2018). It is a type of heterogeneous disease which can present as either indolent or aggressive. Although most patients present with localized prostate cancer which is potentially curable by radical prostatectomy or radiotherapy, most patients relapse biochemically and eventually develop castration resistant prostate cancer (CRPC) (Buyyounouski et al., 2012; Ost et al., 2015; Hoang et al., 2017). Therefore, it is necessary to find sensitive prognostic factors to differentiate high-risk and low-risk prostate cancer patients to guide treatment.

MicroRNAs (miRNAs) are a class of single stranded, about 20 to 24 nucleotides, non-coding RNAs,

Offprint requests to: Changliang Chi, 71 Xinmin Street, Changchun 130021, Jilin, China. e-mail: wangweifengdl@163.com

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Abbreviations. CRPC, castrated resistant prostate cancer; miRNAs, MicroRNAs; 3'-UTR, 3'-untranslated region.

which are associated with the post-transcriptional regulation of gene expression. miRNAs have been reported to play important roles in development, proliferation, apoptosis, and differentiation (Ke et al., 2003; Brown and Sanseau, 2005). Numerous studies have demonstrated that miRNAs may act as tumor suppressors or oncogenes in various types of cancers (Croce and Calin, 2005; Fabbri et al., 2007; Gartel and Kandel, 2008).

Previous studies have indicated that miR-20b may function as an oncogene or tumor suppressor in different types of cancers. It plays the role of oncogene in breast cancer (Zhou et al., 2014), T-cell leukemia (Landais et al., 2007), colorectal cancer (Zhu et al., 2014), and gastric cancer (Xue et al., 2015), however, it plays the role of tumor suppressor in bladder cancer (Park et al., 2015), and papillary thyroid carcinoma (Hong et al., 2016). The expression level, cellular and molecular mechanisms of miR-20b in prostate cancer have also been investigated. In the study by Guo J et al, miR-20b was found to be strongly expressed in prostate cancer tissues compared with adjacent normal prostate tissues. Notably, knockdown of miR-20b expression exhibits an anti-tumor effect in vitro. miR-20b acts as an oncogene that performs a critical role in the growth and migration of prostate cancer cell by targeting PTEN (Guo et al., 2017). The present study analyzed the association between miR-20b and clinicopathological characteristics of patients with prostate cancer.

Material and methods

Patients and tissue samples

A total of 127 pairs of prostate cancer tissue samples and adjacent prostate tissue samples were collected from 127 patients with prostate cancer who underwent surgical resection (open radical prostatectomy or laparoscopic radical prostatectomy) at the First Hospital of Jilin University from April 2010 to March 2014. No patients had undergone preoperative endocrine therapy, chemotherapy or radiotherapy. All resected prostate cancer tissue samples and adjacent prostate tissue samples were immediately stored in liquid nitrogen at -80°C before use. The present study was approved by the Ethics Committee of Jilin University. All patients provided written informed consent. The clinical information of the 127 patients with prostate cancer is summarized in Table 1.

RNA extraction and reverse transcription-quantitative PCR

Total RNA was extracted from collected cells using TRIzol reagent (Invitrogen). Then, 2 μg of RNA was first polyadenylated with polyA polymerase and reverse-transcribed into cDNA with a poly(T) adapter primer using ImProm-II™ Reverse Transcription System (Promega) according to the manufacturer's protocols. RT-qPCR was performed using FastStart Universal SYBR Green Master (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's protocols. The amplification conditions were as follows: 95°C for 10 s, 40 cycles of 95°C for 10 s, and 60°C for 40 s, and dissociation at 95°C for 60 s, 55°C for 30 s, and 95°C for 30 s. The results were calculated with the comparative threshold cycle (Ct) method and U6 snRNA, which was used as an internal control. The primers used in this study were obtained from RiboBio (Guangzhou, China). The primer sequences were shown as follows(6): miR-20b forward, 5'-GAGGACGGAACCGGAAAC3', and reverse, 5'-ACCUGCACUAUGAGCACUUUGUU-3'; U6 forward, 5'-CGCTTCACGAATTTGCGTGT CAT-3' and reverse, 5'-GCTTCGGCAGCACATAT ACTAAAAT-3'.

Statistical analyses

Differences between two groups were compared with the Student's t-test. The associations between miR-20b expression levels and clinicopathological factors were assessed using the χ^2–test. Survival was estimated using the Kaplan-Meier method, and the differences in survival according to miR-20b expression were compared using the log-rank test. The prognostic value of miR-20b expression was determined by univariate

Table 1. Relationship between miR-20b expression and clinicopathological characteristics of patients with prostate cancer.

Variables	Cases (n)	miR-20b expression		P value
		High level (n=66)	Low level (n=61)	
Age (years)				
<60	56	25	31	0.156
≥60	71	41	30	
Family history of prostate cancer				
Yes	31	17	14	0.837
No	96	49	47	
Preoperative PSA level(ng/ml)				
<10	68	38	30	0.377
≥10	59	28	31	
Positive margin				
Yes	33	19	14	0.545
No	94	47	47	
Gleason score				
<8	59	18	41	<0.001
≥8	68	48	20	
Type of Surgery				
Laparoscopic radical prostatectomy	97	46	51	0.094
Open radical prostatectomy	30	20	10	
Lymph node metastasis				
Yes	46	39	7	<0.001
No	81	27	54	
TNM stage				
I+II	57	21	36	0.002
III	70	45	25	

and multivariate analysis. Statistical analysis was performed using SPSS 18.0 (SPSS, Inc., Chicago, IL, USA). Graphs were built using GraphPad Prism 5.0 software (GraphPad Software Inc., La Jolla, CA, USA). $P<0.05$ was considered to indicate a statistically signifcant difference.

Results

miRNA-20b expression level in prostate cancer and normal prostate tissues

Using the qRT-PCR method, miR-20b was detected in all the 127 pairs of prostate cancer tissues and adjacent noncancerous prostate tissues. As shown in Fig. 1, the relative expression of miR-20b in prostate cancer tissues was significantly higher (2.312 fold) than that in adjacent noncancerous prostate tissues (P=0.0005). After normalization to U6 expression levels, the median level of miR-20b in prostate cancer tissues was used as a cut-off point to divide all 127 patients into two groups: high miR-20b group (n=66, patients who expressed miR-20b at levels more than the cut-off value) and low miR-20b group (n=61, patients who expressed miR-20b at levels less than the cut-off value).

The association between miR-20b expression and clinical characteristics of patients with prostate cancer

Table 1 summarized the association between miR-20b expression and clinicopathologic variables in patients with prostate cancer. By statistical analysis, miR-20b expression was observed to be significantly associated with Gleason score (P<0.001), lymph node metastasis (P<0.001), and TNM stage (P=0.002). However, there were no significant differences between miR-20b expression and other variables of patients, including age(P=0.156), family history of prostate cancer (P=0.837), preoperative PSA level (P=0.377), type of surgery(P=0.094), and positive margin (P=0.545).

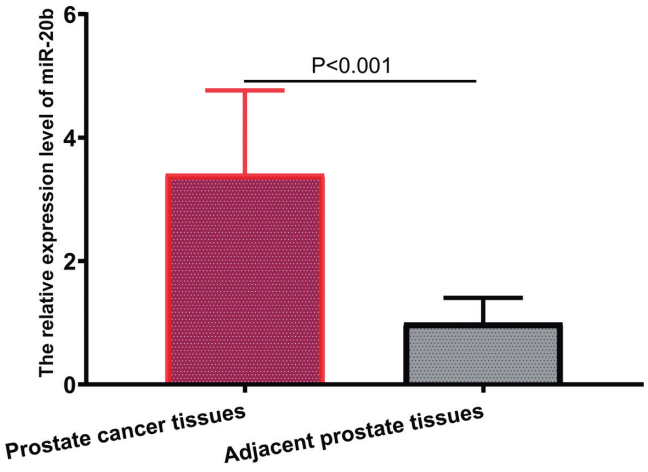

Fig. 1. miRNA-20b expression level in prostate cancer tissues and normal prostate tissues.

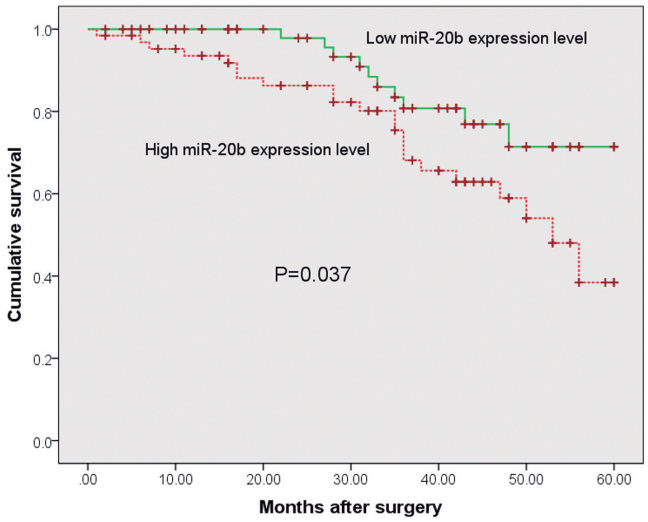

Fig. 2. Survival rate analysis of miR-20b expression levels in prostate cancer.

Table 2. Univariate and multivariate analysis of overall survival in prostate cancer patients.

Variable	Univariate analysis			Multivariate analysis		
	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
Age	1.033	0.773-2.559	0.182	-	-	-
Family history of prostate cancer	1.981	0.591-3.283	0.275	-	-	-
Preoperative PSA level	1.778	1.023-5.921	0.029	1.682	1.339-6.882	0.019
Positive margin	2.012	1.721-8.266	0.031	1.955	1.482-7.291	0.024
Gleason score	3.008	1.923-10.665	0.009	2.834	1.729-13.553	0.011
Type of Surgery	0.821	0.527-2.384	0.632	-	-	-
Lymph node metastasis	3.192	2.102-15.668	0.005	3.259	1.924-14.449	0.003
TNM stage	3.104	1.558-12.293	0.007	3.726	2.904-11.883	0.005
miR-20b expression level	2.189	1.775-10.921	0.019	2.181	1.772-9.021	0.016

Tissue miR-20b expression level predicts prognosis of prostate cancer patients

Using kaplan-Meier survival plots and log-rank analyses, we evaluated the association of miR-20b expression with overall survival. The log-rank test indicated that patients with increased miR-20b expression experienced poor overall survival (P=0.037, shown in Fig. 2). To determine the possibility of miR-20b as an independent risk factor for poor prognosis, both clinicopathological factors and the level of miR-20b expression were evaluated by multivariate Cox regression analysis. Results showed that miR-20b expression level (HR=2.181, 95% CI: 1.772-9.021, P=0.016) was an independent factor in predicting the overall survival of prostate cancer patients (shown in Table 2).

Discussion

At present, radical prostatectomy (including open radical prostatectomy, laparoscopic radical prostatectomy, and robot-assisted radical prostatectomy) and external radiation therapy are the main methods of treatment for localized prostate cancer or oligometastatic prostate cancer. Orchiectomy and endocrine therapy (luteinizing hormone releasing hormone, and non-steroidal antiandrogenic drugs) are the most basic treatment for prostate cancer (Cornford et al., 2017; Mottet et al., 2017). In recent years, targeted therapy and immunotherapy have also made considerable progress (Higano, 2014; Zumsteg et al., 2016; Catton et al., 2018; Morgans, 2018). Therefore, it is necessary to find sensitive prognostic factors to differentiate high-risk and low-risk prostate cancer patients to guide treatment.

miR-20b is a member of the miR-106a-363 gene cluster located in the mammalian X chromosome, the larger miR-17 family, and the miR-17-92 and miR-106b-25 gene clusters (Khuu et al., 2016). Previous studies have indicated that miR-20b may function as oncogene or tumor-suppressor in several types of cancers (Zhou et al., 2014; Zhu et al., 2014; Ahmad et al., 2015; Park et al., 2015; Xue et al., 2015; Hong et al., 2016). Moreover, the expression level, cellular and molecular mechanisms of miR-20b in prostate cancer have also been investigated. In the study by Guo et al. miR-20b was found to be strongly expressed in prostate cancer tissues compared with adjacent normal prostate tissues. Notably, knockdown of miR-20b expression exhibits an anti-tumor effect in vitro. miR-20b acts as an oncogene that performs a critical role in the growth and migration of prostate cancer cell by targeting PTEN (Guo et al., 2017). In the present study, using qRT-PCR method, miR-20b was detected in all the 127 pairs of prostate cancer tissues and adjacent noncancerous prostate tissues. We found that the relative expression of miR-20b in prostate cancer tissues was significantly higher than that in adjacent noncancerous prostate tissues. miR-20b expression was observed to be significantly associated with Gleason score, lymph node metastasis, and TNM stage. These results implied that the expression level of miR-20b may be associated with the disease progression of prostate cancer. Using kaplan-Meier survival plots and log-rank analyses, we evaluated the association of miR-20b expression with overall survival. The log-rank test indicated that patients with increased miR-20b expression experienced poor overall survival. To determine the possibility of miR-20b as an independent risk factor for poor prognosis, both clinicopathological factors and the level of miR-20b expression were evaluated by multivariate Cox regression analysis. Results showed that miR-20b expression level was an independent factor in predicting the overall survival of prostate cancer patients, which was similar to a previous study (Hoey et al., 2019).

In conclusion, our study found that high expression of tissue miR-20b was related to poor prognosis and could be a promising biomarker of clinicopathologic features in prostate cancer. Our study will help to add a new dimension to the study of molecular mechanisms of prostate cancer and will also provide new directions for cancer prognosis and treatment.

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Ethics approval and consent to participate. All procedures performed in this study involving human participants were approved by Ethics Committee of The First Hospital of Jilin University and were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Moreover, written informed consent was obtained from each participants.

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Competing interests. The authors declare that they have no competing interests.

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