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Original Article

MiR-675 is over-expressed in patients with prostate cancer

Jian Lang[1*], Jiang Lin[2*], Qin Chen[2], Yang Xiang[3], Wei-Bin Wang[1], Zhao-Qun Deng[2], Feng-Qin Han[1], Ying-Zhao Liu[2], Jun Qian[2], Yu-Quan Zhou[1], Wei Qian[2]

[1]Cardiothoracic & Urology Surgery, [3]Department of Pathology, Bingtuan Sishi Hospital, Yining, Xinjiang, People's Republic of China; [2]Zhenjiang Key Laboratory of Experimental Hematology, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu, People's Republic of China. [*]Equal contributors and co-first authors.

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Abstract: Abnormal expression of *miR-675* has been found in various human tumors. However, the expression pattern of *miR-675* in prostate cancer patients and its clinical significance remain largely elusive. In the present study, we evaluated the expression status of *miR-675* gene in 83 prostate cancer patients and 40 benign prostatic hyperplasia (BPH) cases using real-time quantitative PCR. The results revealed that *miR-675* was significantly over-expressed in prostate cancer patients compared with BPH controls ($P<0.001$). No significant difference between *miR-675* over-expressed and low-expressed cases could be observed in age, differentiation grade, tumor size, lymph nodes stage, metastasis status, Gleason score or serum prostate-specific antigen (PSA) level. Receiver operating characteristic curve (ROC) analysis of *miR-675* expression showed a great performance in distinguishing prostate cancer patients from controls with an area under the ROC curve (AUC) of 0.933 ($P<0.001$). Our data suggest that the over-expression of *miR-675* is a frequent event and might be a valuable diagnostic biomarker combination with PSA in prostate cancer patients.

Keywords: *miR-675*, over-expression, prostate cancer

Introduction

As one of the most prevalent cancers and the sixth leading cause of cancer-related death worldwide [1-3], prostate cancer has been a growing public health problem. Although its incidence is higher in developed countries than in developing counties, the incidence and mortality from this cancer in China have increased rapidly in recent years [4]. Prostate cancer patients diagnosed in early stage can be treated by radical prostatectomy, however, majority of patients are detected at the metastatic stage that the optimal treatment time is lost [5]. To overcome the limitations of the current diagnostic methods, more sensitive and more specific biomarkers are strongly needed to explore for the early diagnosis and the identification of prostate cancer [6-8].

MicroRNAs (miRNAs) are a group of endogenous, small (~22 nucleotide), non-coding RNA molecules that are critical in regulating the expression of numerous genes involved in the initiation of cancers [9, 10]. MiRNAs modulate gene expression mainly through binding to the 3'-untranslated region (3'UTR) of target genes, leading to mRNA degradation or translational suppression [11]. Several investigations have demonstrated that a few miRNAs are dysregulated in prostate cancer and affect cell proliferation, cell growth, cell cycle arrest, disease progression, metastasis and relapse [12-17].

Abnormal expression of *miR-675* has been observed in a variety of human tumors such as colorectal cancer, hepatocellular cancer, gastric cancer, breast cancer, bladder cancer, glioma and lung cancer [18-27]. Recently, it was shown that the abnormal expression of *miR-675* was present in the metastatic prostate cancer cell line and repressed prostate cancer metastasis by targeting TGFBI [28]. However, so far, the expression pattern of *miR-675* in prostate cancer patients and its clinical significance have not been studied. Therefore, the present

Table 1. Clinicopathological characteristics and expression of *miR-675* in prostate cancer

Patient's parameters	Status of *miR-675* expression in prostate cancer		
	High (n=41)	Low (n=42)	*P*
Median age, years (range)	76 (30-88)	76.5 (54-90)	0.859
Differentiation grade			0.145
G1	12	13	
G2	13	6	
G3	16	23	
pT			0.939
T1	3	4	
T2	11	9	
T3	26	28	
T4	1	1	
pN			0.591
N0	18	16	
N1	23	26	
pM			0.133
M0	29	23	
M1	12	19	
Gleason score			0.274
<7	25	19	
=7	2	5	
>7	14	18	
Serum PSA (ng/ml)			0.447
≤20	7	10	
>20	34	32	

PSA, prostate-specific antigen.

study was aimed to investigate the status of *miR-675* expression and further analyzed its clinical implications in prostate cancer patients.

Materials and methods

Patients and samples

A total of 83 formalin-fixed and paraffin-embedded (FFPE) tissue samples of prostate cancer patients and 40 FFPE tissue samples of benign prostatic hyperplasia (BPH) were collected from the Bingtuan Sishi Hospital in present study after written informed consent was signed. None of the prostate cancer patients recruited had ever received any chemical treatment or physical therapy before surgery. Clinicopathological features of patients including age at diagnosis, differentiation grade, pTNM status, Gleason score, and serum PSA level were listed in **Table 1**. This study was approved by the Ethics Committee Board of Bingtuan Sishi Hospital.

RNA extraction and reverse transcription

Total RNA was extracted from FFPE samples using the RecoverAll™ Total Nucleic Acid Isolation Kit (Ambion, catalog no: AM 1975) according to the manufacturer's instructions, and reverse transcription was subsequently performed using miScript Reverse Transcription Kit (Qiagen, catalog no: 218161) according to the manufacturer's protocol.

Real-time quantitative PCR

Real-time quantitative PCR (RQ-PCR) was carried out to detect *miR-675* according to the manufacturer's instructions using the miScript SYBR green PCR kit (Qiagen, catalog no: 218075) on a 7500 Thermo cycler (Applied Biosystems, CA, USA). The *miR-675*-specific forward primer was 5'-GCGGAGAGGGCCCACAG-3' and the reverse primer was the manufacturer-provided miScript universal primer. 20 μL of reaction volume contained 1× miScript universal primer, 1× QuantiTect SYBR Green PCR Master Mix, and 1.0 μM of the specific forward primer. The RQ-PCR running protocol was composed of an initial denaturation step of 95°C for 15 min, followed by an amplification program of 40 cycles for 15 s at 94°C, 30 s at 55°C, 34 s at 72°C to collect data before a melting program of one cycle at 95°C for 15 s, 60°C for 60 s, 95°C for 15 s and 60°C for 15 s. U6 was used for normalization, and the data was analyzed by the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

Statistical analyses of experimental data were implemented on SPSS 17.0 software package (SPSS, Chicago, IL). In order to compare the difference of categorical variables, Pearson Chi-square analysis or Fisher exact test were used. Comparisons of continuous variables between two different groups were determined by the Mann-Whitney's U test. Receiver operating characteristic curve (ROC) and area under the ROC curve (AUC) were applied to evaluate the value of *miR-675* expression in discriminating prostate cancer patients from controls. A

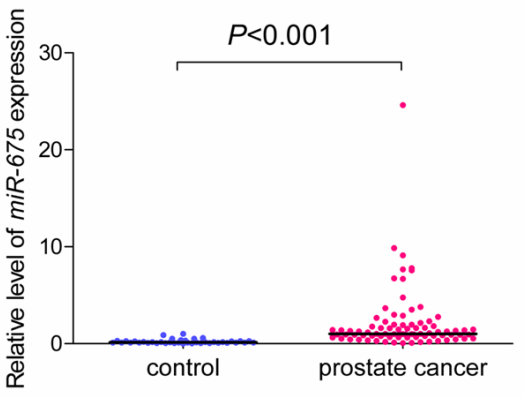

Figure 1. Levels of *miR-675* expression in controls and in prostate cancer patients.

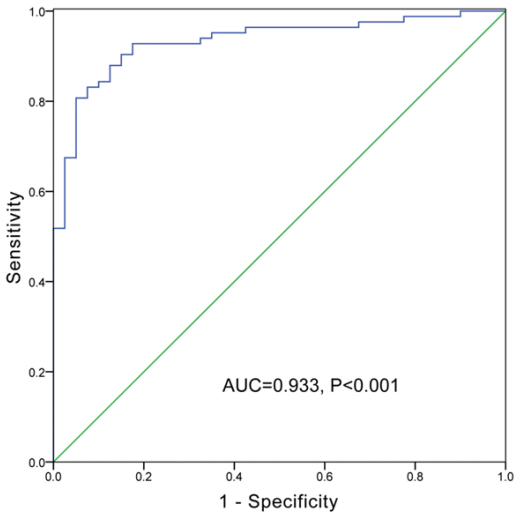

Figure 2. ROC curve of *miR-675* over-expression for distinguishing prostate cancer patients from controls.

two-tailed value of $P<0.05$ was determined as statistically significant.

Results

MiR-675 expression in prostate cancer patients and controls

In this study, we analyzed *miR-675* expression level in 83 prostate cancer samples and 40 controls using RQ-PCR. The result revealed that the relative expression level of *miR-675* in the controls tissues was 0.02-1.00 (median 0.14), while that in prostate cancer tissues was 0.07-20.62 (median 1.01). A significant up-regulation in *miR-675* expression was found in prostate cancer cases ($P<0.001$, **Figure 1**).

Association between miR-675 expression and clinical characteristics of prostate cancer patients

To elucidate whether the over-expression of *miR-675* was related to the clinical progression of prostate cancer, we linked the *miR-675* expression to clinicopathologic parameters in prostate cancer cases. A cohort of 83 patients with prostate cancer was divided into two groups according to the median of 1.01, namely, those who expressed *miR-675* at levels more than the median were defined as high *miR-675* expression (*miR-675*^{high}) and those with *miR-675* expression levels less than the median were defined as low *miR-675* expression (*miR-675*^{low}). No significant difference between *miR-675*^{high} and *miR-675*^{low} cases could be observed in age, differentiation grade, tumor size, lymph nodes stage, metastasis status, Gleason score or serum PSA level ($P>0.05$, **Table 1**). Furthermore, there was no significant difference in the quantitative level of *miR-675* expression between patients with metastasis or without metastasis.

Discriminative capacity of miR-675 expression

ROC curve analysis showed that the *miR-675* expression level could serve as a potential biomarker for discriminating prostate cancer cases from controls. It was indicated that the AUC of prostate cancer was 0.933 (95% confidence interval 0.888-0.977; $P<0.001$) (**Figure 2**). The cutoff value was that point closest to both maximum sensitivity and specificity. ROC curve analysis suggested that the sensitivity and specificity were 80.7% and 95.0% respectively at the cutoff value of 0.60.

Discussion

MiRNAs play critical roles in various physiological processes including cell proliferation, differentiation, cell death, apoptosis, migration and metabolism [29, 30]. Increasing evidence also demonstrated that *miR-675* was aberrantly expressed in various cancers and this abnormal expression was involved in the initiation and the progression in a variety of cancers. It was revealed that *miR-675* was over-expressed in primary colorectal cancer patients and colon cancer cell lines and regulated colorectal cancer development by down-regulation of its target gene *RB* [18]. Hernandez et al disclosed that *miR-675* up-regulation enhanced prolifera-

tive capacity and mediated the down-regulation of Twist1 and Rb in hepatocellular carcinoma [19]. In gastric cancer, it was indicated that *miR-675* was up-regulated and promoted cell proliferation, migration, invasion and metastasis in vitro and in vivo [21]. Zhuang et al also revealed that *miR-675* was over-expressed and modulated human gastric cancer cell proliferation by targeting tumor suppressor RUNX1 in gastric cancer [22]. Vennin et al reported that over-expression of H19/*miR-675* could promote breast cancer cell proliferation and migration in vitro, and accelerating tumor growth and metastasis in vivo [23]. Abnormally enhanced *miR-675* expression increased bladder cancer growth by regulating p53 activation [25]. However, *miR-675* was demonstrated to be down-regulated in non-small cell lung cancer and its down-regulation promoted tumor progression and development by targeting protumorigenic GPR55 [27]. Together with these studies, it implied that the expression pattern of *miR-675* and its role were different in different type of cancers. However, the pattern of *miR-675* expression and its correlation with clinical features of prostate cancer patients have rarely been elaborated.

In the current study, we analyzed the expression status of *miR-675* and clinical implications in 83 prostate cancer tissues and 40 BPH controls using RQ-PCR. This study demonstrated for the first time that *miR-675* was significantly up-regulated in prostate cancer patients than in BPH controls tissues, which might suggest that *miR-675* might be functioned as an oncomiRNA and might contribute to the tumorigenesis of prostate cancer. However, no significant correlation was found between the high *miR-675* expression with the age, differentiation grade, tumor size, lymph nodes stage, metastasis status, Gleason score or serum PSA level of prostate cancer patients ($P>0.05$). Although Zhu et al showed that *miR-675* was down-regulated in the metastatic prostate cancer cell line [28], we did not found this phenomenon in prostate cancer patients, which may due to the small amount of our study cohort. Therefore, further study on a larger number of prostate cancer patients is needed to explore the role of *miR-675* over-expression in the metastasis of prostate cancer.

Currently, PSA is still the most commonly used molecular marker for the diagnosis and screening of prostate cancer. However, PSA levels can become elevated not only in prostate cancer but also in non-cancerous conditions such as BPH and prostatitis [31, 32]. Therefore, more sensitive and more specific biomarkers are strongly needed for the diagnosis of prostate cancer. Epigenetic changes are increasingly regarded as key events in the development of cancer. It is well known that genetic and epigenetic mechanisms both contribute to the initiation and progression of prostate cancer [33]. Compared with genetic alterations, epigenetic changes appear to be more frequent and recurrent [34], which may make them useful as potential biomarkers. MiRNAs are one of epigenetic events and play critical roles in various cancers. In this study, we demonstrated that ROC analyses of *miR-675* expression provided good discriminatory results between prostate cancer tissues and BPH tissues. Therefore, as a member of miRNAs, *miR-675* may be a promising biomarker used for more accurate diagnosis in prostate cancer combination with PSA.

In a word, our results suggest that *miR-675* is frequently and significantly over-expressed and the over-expression of *miR-675* might be a valuable diagnostic biomarker combination with PSA in prostate cancer patients.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Wei Qian, Zhenjiang Key Laboratory of Experimental Hematology, Affiliated People's Hospital of Jiangsu University, 8 Dianli Road, Zhenjiang 212002, Jiangsu, People's Republic of China. Fax: +86-511-85234387; E-mail: dwqian@yahoo.com; Dr. Yu-Quan Zhou, Cardiothoracic & Urology Surgery, Bingtuan Sishi Hospital, 56 Xinhua West Road, Yining 835000, Xinjiang, People's Republic of China. Fax: +86-999-8023886; E-mail: 1784604129@qq.com

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