Iran J Public Health, Vol. 50, No.4, Apr 2021, pp.772-779

Original Article

Expression of miR-451a in Prostate Cancer and Its Effect on Prognosis

Bo Fan, Xiaohua Jin, Qi Ding, Cheng Cao, Yi Shi, Hailiang Zhu, Wenjun Zhou

Department of Urology, Changshu Hospital Affiliated to Soochow University, Changshu No.1 People's Hospital, Changshu 215500, P.R. China

***Corresponding Author:** Email: fan_bo_111@163.com

(Received 15 Apr 2020; accepted 12 Jul 2020)

Abstract

Background: To investigate the expression of miR-451a in prostate cancer tissues and its effect on prognosis.

Methods: Each of 78 specimens of prostate cancer tissues and corresponding adjacent normal tissues were collected from patients in Changshu Hospital Affiliated to Soochow University, Changshu, China from Apr 2014 to Jun 2015. Real-time quantitative RT-PCR (qRT-PCR) was used to detect the expression of miR-451a in tissues. The relationship between the expression of miR-451a and clinical pathological parameters was analyzed. The median expression of miR-451a in the experimental group was used to distinguish the high and low expressions of miR-451a in the experimental group. Kaplan-Meier was used to analyze the survival of miR-451a high and low expression groups.

Results: The expressions of miR-451a in the patient's tissues and serum were decreased, and the correlation analysis found that they were positively correlated. ROC curve analysis showed that miR-451a had a high clinical value in the diagnosis of prostate cancer and the area under the curve was 0.921. The incidence of stage III+IV lymph node metastasis, Gleason score of >7 points and a serum Prostate-specific antigen (PSA) level of >20 ng/ml in patients of the low expression group increased significantly. The 5-yr survival rate of patients with low expression was significantly lower than that of those with high expression (P=0.005). MiR-451a was an independent factor affecting the prognosis of patients.

Conclusion: miR-451a is lowly expressed in prostate cancer, and patients with low expression have a poor prognosis.

Keywords: Prostate cancer; Expression level; Clinical pathology; Prognosis

Introduction

Prostate cancer is one of the most common solid tumors in males and the fifth leading cause of cancer death in males. According to the latest research data, in the United States in 2017, there were more than 160,000 new patients with prostate cancer and over 20,000 patients with prostate cancer died. The incidence of prostate cancer is still rising in most countries (1, 2). At present, patients with prostate cancer are mainly treated by radical prostatectomy in clinical practice. More than 25% of patients have recurrence during long-term follow-up, and prostate cancer is high-

ly susceptible to change over time. The differences in prognosis between patients with prostate cancer remain large, even though prostate cancer is early diagnosed (3-5). However, there is still a lack of clinical biomarkers for evaluating the prognosis of patients with prostate cancer. Therefore, to explore the pathogenesis of prostate cancer and to find more effective prognostic markers are of great significance for guiding the clinical treatment of prostate cancer and improving the prognostic survival of patients.

MicroRNAs (miRNAs) are non-coding RNA sequences consisting of 18-22 nucleotides. They have been found to extensively regulate intracellular molecular signaling pathways, and their abnormal regulation may lead to different cancers. Very stable in serum, plasma and urine, miRNA has become an important biomarker for the diagnosis, prognosis and monitoring of tumor progression (6). Previous study have found that miR-451a inhibits the proliferation and growth of cancer cells and enhances their activity against certain anticancer drugs (7). MiR-451a has been found to be abnormally expressed in thyroid cancer, gastric cancer and other malignant tumors, and its low expression is significantly correlated with the clinical pathological features of some malignant tumors. MiR-451a may be involved in the occurrence and development of various malignant tumors (8, 9). It is suspected that miR-451a may also be involved in the occurrence of prostate cancer, which plays a supporting role in the clinical prognostic evaluation of patients with prostate cancer.

Therefore, this study focused on the expression of miR-451a in prostate cancer tissues, and analyzed its effect on the prognosis of patients.

Materials and Methods

Experimental materials

The pathological data of 78 patients with prostate cancer who underwent surgical resection in Changshu Hospital Affiliated to Soochow University, Changshu, China from Apr 2014 to Jun 2015 were collected as an experimental group, and 40 healthy people who received examinations in the same period were enrolled as a control group. The tested specimens met the inclusion and exclusion criteria.

Inclusion criteria: 1) All specimens were diagnosed by histopathological diagnosis and stored in liquid nitrogen; 2) adjacent normal tissues were more than 3 cm away from the tumor lesion; 3) the clinical basic data of all patients were detailed with no deletions.

Exclusion criteria: 1) Patients who received radiotherapy, chemotherapy or related treatment before operation; 2) patients complicated with other systemic malignant tumors.

All the experimental contents were approved by the Medical Ethics Committee of Changshu Hospital Affiliated to Soochow University. All subjects were informed of the experimental contents and signed a complete informed consent form. The cancer tissues and adjacent tissues of the patients were collected and stored at -80 ℃. Serum of the patients and controls were collected for testing.

Experimental methods

Experimental reagents and instruments

Trizol (Wuhan Kehaojia Biotechnology Co., Ltd., 15596-026), UV spectrophotometer (Thmorgan Biotechnology Co., Ltd., MD3000), American ABI PCR instrument (Shanghai Aolu Biotechnology Co., Ltd., 9700), TransScript Green Two - Step qRT-PCR SuperMix (Beijing Quanshijin Biotechnology Co., Ltd., AQ301), Nuclease-Free Water (Xiamen Research Biotechnology Co., Ltd., P1193).

Real-time quantitative RT-PCR (qRT PCR) detection of expression level of miR-451a

The tissue specimens were taken out from the liquid nitrogen jar and cut to a size of approximately 1 mm^3. Trizol reagent was used for extracting total RNA from the tissue specimens, the UV spectrophotometer for detecting the purity and concentration of the total RNA extracted, agarose gel electrophoresis for detecting the integrity of the total RNA. One μg of the total RNA was taken for reverse transcription, and the

experimental procedure was carried out in strict accordance with the instructions. Reaction conditions were at 50°C for 15 min and at 85°C for 5 s. The ABI PCR thermal cycler was used to amplify the target gene. The PCR reaction system was 20 μl: 1 μl of cDNA, each 0.4 μl of upper and downstream primers, 0.4 μl of Passive Reference Dye (50x) (optional), 10 μl of 2×TransScript® Tip Green qRT-PCR SuperMix, and the system was supplemented to 20 μl with Nuclease-Free Water. The experiment was carried out with a three-step method. Reaction conditions were pre-denaturation at 94 °C for 30 s; denaturation at 94 °C for 5 s; annealing at 50-60 °C for 15 s, extension at 72 °C for 10 s, for a total of 40 cycles. U6 was used as an internal reference and the Ct value was recorded. $2^{-\Delta Ct}$ was used to express the relative expression of the gene. Independent experiments were repeated 3 times. The upstream sequence and downstream sequence of miR-451a were respectively 5'-TCCGATTGAGTCATTACCAT-3' and 5'-GTGCAGGGTCCGAGGT-3', and those of U6 were respectively 5'-CTCGCTTCGGCAGCACA-3' and 5-AACGCTTCACGAATTTGCGT-3'.

Follow-up methods

All 78 patients with prostate cancer were followed up for 5 yr by telephone. The follow-up was performed in Jan, Mar, Jun, Sep, and Dec of each year.

Statistical methods

SPSS20.0 statistical software (SPSS Inc., Ch36icago, IL, USA) was used for statistically analyzing the experimental data, GraphPad Prism7 (Beijing Huanzhong Ruichi Technology Co., Ltd., Beijing, China) for plotting the figures. Count data were expressed as %, and chi-square test was used for comparison between groups. Measurement data were expressed as (mean ± standard deviation), receiver operating characteristic curve (ROC) was used to plot the diagnostic value of miR-451a in prostate cancer, and independent sample *t* test was used for comparison between groups. Kaplan-Meier was used for survival analysis and Log-rank test was used for test. When $P<0.05$, the difference is statistically significant.

Results

Comparison of expression level of miR-451a between experimental group and control group

The expression of miR-451a in the tissues of patients with prostate cancer was detected, and the results showed that miR-451a was lowly expressed in the cancer tissues of patients with prostate cancer, and was lower than that of the adjacent tissues $P<0.001$). Further detection of the expression of miR-451a in the serum of patients showed that miR-451a was also lowly expressed in the serum of patients with prostate cancer, and the correlation analysis showed that the expression of miR-451a in the serum was positively correlated with that in tissues. Moreover, ROC curve analysis showed that miR-451a had a high clinical value in the diagnosis of prostate cancer (Fig. 1).

Relationship between miR-451a expression and clinical pathological features

The median expression of miR-451a divided patients into high and low expression groups, and the clinicopathological data of the two groups were compared. The incidence of stage III+IV lymph node metastasis, Gleason score of >7 points and a serum PSA level of >20 ng/ml in patients of the low expression group increased significantly (Table 1).

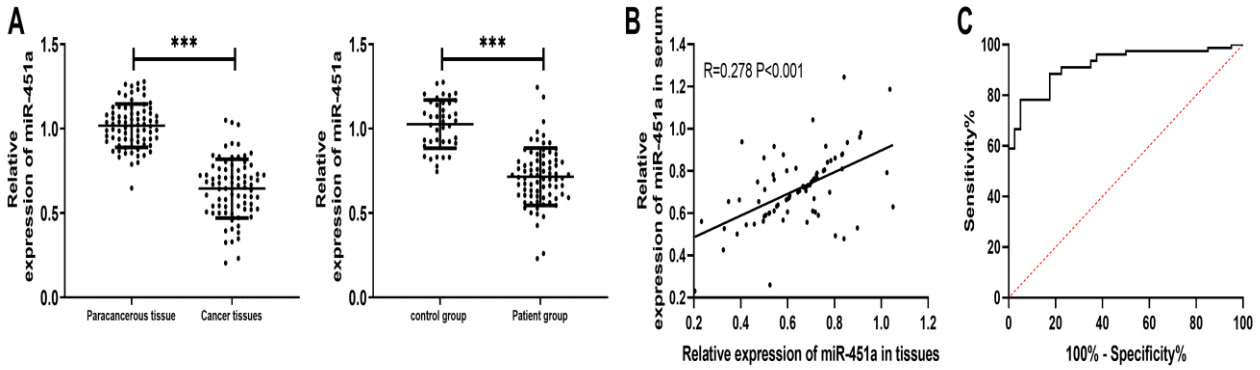

Fig. 1: The expression of miR-451a in patients with prostate cancer

A. The expression of miR-451a decreased significantly in the patient tissues and serum. B. The correlation analysis showed that the expression of miR-451a in the tissues was positively correlated with that in serum. C. ROC curve analysis showed that the area of miR-451a under the curve of prostate cancer and normal subjects was 0.921. When the cut-off value was 0.817, the optimal specificity was 95.00% and the optimal sensitivity was 78.21%. * * * P<0.001

Table 1: Relationship between miR-451a expression and clinical pathological features [n(%)]

Item		***miR-451a***		χ^2	***P***
		High expression group (n=39)	Low expression group (n=39)		
Age (yr)					
	≤50 (n=22)	12(54.55)	10(45.45)	0.253	0.615
	＞ 50 (n=56)	27(48.21)	29(51.79)		
Clinical stage				8.690	0.003
	Stage Ⅰ+Ⅱ (n=37)	25（64.10）	12（30.77）		
	StageⅢ+Ⅳ (n=41)	14（355.90）	27（69.23）		
Lymph node metastasis				7.389	0.007
	Yes (n=38)	13（33.33）	25（64.10）		
	No (n=40)	26（66.67）	14（35.90）		
Gleason score				4.994	0.025
	≤7 points (n=55)	32(58.18)	23(41.82)		
	＞7 points (n=23)	7(30.43)	16(69.57)		
Serum PSA level (ng/ml)				6.478	0.011
	≤20 (n=47)	29(61.70)	18(38.30)		
	＞ 20 (n=31)	10(32.26)	21(67.74)		

Survival analysis

In order to observe the survival value of miR-451a in prostate cancer patients, we followed up the patients for 5 yr and found that 36 patients died within 5 yr, with a survival rate of 53.84%. Further observation of the 5-yr survival rate in the high and low expression groups showed that the 5-yr survival rate in the low expression group was significantly lower than that in the high expression group (*P*=0.005), as shown in Fig. 2.

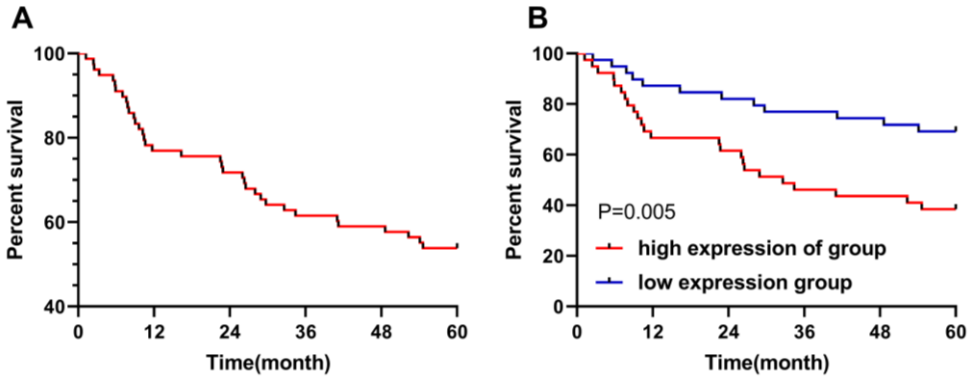

Fig. 2: The relationship between miR-451a and patient survival
A. Patients' 5-year survival rate. B. The 5-year survival rate was significantly reduced in miR-451a low expression group.

Cox regression analysis

Cox regression was used to analyze the independent factors affecting the prognosis of patients. By collecting clinical data, univariate analysis found that clinical staging, lymph node metastasis, Gleason score and miR-451a expression were factors affecting the prognosis of patients. Multi-factor Cox regression analysis was further performed by LR method and it was found that miR-451a was an independent factor affecting the prognosis of patients (Table 2).

Table 2: Cox regression analysis

Item	*Univariate Cox regression*			*Multi-factor Cox regression*		
	P value	HR	95CI%	*P* value	HR	95CI%
Age (Yr) (≤50 *VS.*＞50)	0.190	1.738	0.761~3.970			
Clinical stage (I+II *VS.* III+IV)	0.026	2.195	1.096~4.394	0.185	1.651	0.786~3.467
Lymph node metastasis (Yes *VS.* No)	0.043	0.500	0.256~0.979	0.291	0.676	0.328~1.397
Gleason score (≤7 *VS.* ＞7)	0.042	2.006	1.025~3.927	0.225	1.552	0.763~3.159
Serum PSA level (ng/ml) (≤20 *VS.* ＞20)	0.279	1.438	0.745~2.779			
MiR-451a (high expression *VS.* low expression)	0.007	2.607	1.301~5.226	0.007	2.607	1.301~5.226

Discussion

MiRNAs are abnormally expressed in a variety of human malignant tumors and specific in different cancerous tissues. It is found that the expression of miRNAs can be clearly quantified in specimens of tissues resected from the prostate. The detection of miRNAs expression is considered to be a promising prognostic indicator that improves the predictive rate of the biochemical recurrence of prostate cancer. For example, miR-135B, hsa-miR-96 and miR-221 will be the first early warning signal for recurrence after radical prostatectomy (10,11). Nowadays, miR-21 and miR195 are highly or lowly expressed in prostate cancer tissues. Their abnormal expressions are related to the prognosis of prostate cancer patients, and can be used as potential targets for the prognostic evaluation and treatment of prostate cancer (12,13). miR451a is a promising biomarker that inhibits the migration and invasion of tumor cells by regulating ATF2 in non-small cell lung cancer (NSCLC), and its abnormal expression may be related to NSCLC (14). miR451a, which may also be a potential biomarker and therapeutic target for the diagnosis and prognosis of gastric cancer, provides a new research direction for the diagnosis and treatment of gastric cancer (15). However, there is little research on the expression of miR-451a in prostate cancer tissues and its correlation with prognosis and outcome. Therefore, this study focuses on this to provide clinical reference data for the prognostic evaluation of prostate cancer.

In this study, the expression of miR-451a in the patient's tissues and serum was detected, and the results showed that the expression of miR-451a was decreased in both serum and tissues, suggesting that miR-451a could be a potential diagnostic indicator for prostate cancer. ROC curve analysis showed that the detection of miR-451a in serum had higher diagnostic value in prostate cancer. Correlation analysis showed that miR-451a expression in serum was positively correlated with that in tissues, suggesting that the detection of serum expression could also feedback the severity of patients' condition. In order to observe the relationship between miR-451a and survival, we followed up the patients for 5 yr and found that patients with prostate cancer had a survival rate of 53.84%, which was consistent with previous research results (16,17). The survival rate of patients with high and low expressions further showed that the 5-yr survival rate of patients with low expression was significantly reduced. Moreover, Cox regression showed that miR-451a was an independent factor affecting the prognosis of patients with prostate cancer.

Heterochromatin protein 1γ (HP1γ) and oncoprotein c-Myc are overexpressed in prostate cancer. The overexpression of HP1γ predicts poor prognosis in patients with prostate cancer, and that of c-Myc is related to the recurrence and metastasis of prostate cancer cells. On one hand, the decrease in the expression of miR-451a reverses the apoptosis of cancer cells due to HP1γ deletion, and the overexpression of miR-451a inhibits the survival of prostate cancer cells by down-regulating the expression of c-Myc (18). MiR-451a may be involved in the proliferation and apoptosis of prostate cancer cells. On the other hand, it has been found that miR-451a inhibits the migration of cancer cells and the ERK 1/2 signaling pathway and reverses the epithelial mesenchymal transition (EMT) of hepatocellular carcinoma by directly down-regulating the expression of c-Myc in hepatocellular carcinoma (19). EMT, which plays a crucial role in the invasion, metastasis and recurrence of tumor, inhibits its miRNAs loss and induces its occurrence in prostate cancer, thereby leading to tumor progression, metastasis and recurrence (20). Therefore, it is hypothesized that miR-451a may be similarly expressed in prostate cancer. It may inhibit the migration and proliferation of prostate cancer cells and the survival of cancer cells by regulating c-Myc and HP1γ, and can reverse prostate cancer EMT. In addition, family factor is currently considered to be one of the risk factors for prostate cancer. When a first-degree relative has prostate cancer, the risk of illness is doubled (21,22). Prostate cancer patients with a Gleason score of ≥8 points and/or PSA ≥20 ng/μL have a higher

prognostic risk, and this type of patient is highly relapsed after radical prostatectomy, with high mortality (23,24). This also verifies our findings from the side. The low expression of miR-451a in prostate cancer is related to the Gleason score ≥8 points and/or PSA≥20 ng/μL and family history, which may be able to predict the poor prognosis of patients with prostate cancer to some extent.

There are still shortcomings in this study. On one hand, due to limited experimental conditions, one-way and multi-factor prognostic analysis was not performed on the expression level of miR-451a and clinical pathological parameters in this study. In addition, the expression level of miR-451a only in prostate cancerous tissues was detected in this study. That of miR-451a in fresh tissue specimens or other samples such as patient serum should be increased in subsequent experiments. On the other hand, the results of this study indicated that miR-451a may be involved in the occurrence and progression of prostate cancer and its low expression may indicate a poor prognosis in patients with prostate cancer. Other researchers are expected to further study miR-451a and explore whether it can be a new therapeutic target for prostate cancer, or assess the prognostic risk of patients with prostate cancer.

Conclusion

MiR-451a is lowly expressed in prostate cancer, and patients with low expression have a poor prognosis.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

No funding was received in this study.

Conflict of interest

The authors declare that there is no conflict of interest.

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