Int J Clin Exp Med 2019;12(7):8172-8180
www.ijcem.com /ISSN:1940-5901/IJCEM0093378

Original Article

Correlation between miRNA-21 expression and diagnosis, metastasis and prognosis of prostate cancer

Guanqun Ju[1*], Jie Lian[2*], Zhijun Wang[1*], Wanli Cao[1], Jianhai Lin[1], Yao Li[1], Lei Yin[1]

[1]Department of Urology, Shanghai Changzheng Hospital, Shanghai City, China; [2]Department of Plastic and Reconstructive Surgery, Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine, Shanghai City, China. [*]Equal contributors and co-first authors.

Received March 5, 2019; Accepted May 13, 2019; Epub July 15, 2019; Published July 30, 2019

Abstract: Objective: To find out the correlation between miRNA-21 expression and diagnosis, metastasis and prognosis of prostate cancer. Methods: Eighty-eight patients with prostate cancer (62 cases underwent radical prostatectomy), 84 patients with benign prostatic hyperplasia and 80 healthy subjects were enrolled in this study. The relative expression level of miRNA-21 in the blood and its predictive value in diagnosis, metastasis and prognosis of prostate cancer were observed. Results: The expression of miRNA was significantly higher in the prostate cancer group than in the prostatic hyperplasia group and the healthy group (both $P < 0.001$), but there was no significant difference between the prostatic hyperplasia group and the healthy group. Prostate cancer patients with Gleason score > 7 points had higher relative expression of miRNA than patients with Gleason score ≤ 7 points ($P < 0.05$). The relative expression of miRNA was the highest in T4 group than in other T staging groups (all $P < 0.001$), was higher in N1 group than in N0 group, and was higher in M1 group than in M0 group (both $P < 0.05$). In terms of the risk levels, the relative expression of miRNAs in the low risk group was significantly lower than that in the intermediate risk and high risk groups (both $P < 0.001$). Conclusion: The relative expression of miRNA-21 in the blood of patients with prostate cancer is increased, and related to the malignant degree and biochemical recurrence of tumors. So, it is a valuable indicator for the diagnosis and prediction of prostate cancer.

Keywords: miRNA-21, prostate cancer, diagnosis, prognosis, predictive value

Introduction

Prostate cancer is one of the most common malignant tumors in males, and ranks the first in the incidence of tumors in the Western countries. Latest research showed that about 20% of newly-diagnosed tumors in the United States were prostate cancer [1]. The incidence of prostate cancer in China is also increasing [2]. In recent years, a large number of screening and diagnosis techniques for prostate cancer have been improved, such as detection of prostate specific antigen density and robot-assisted radical prostatectomy. But for patients who are unsuitable for surgery or have surgical contraindications, androgen deprivation therapy is the only method, which can become ineffective after 1-2 years of treatment with poor prognosis [3]. Studies reported that about 30% patients with prostate cancer had bone metastases, and most patients would eventually develop bone metastases [4, 5]. The severity of bone metastases could play a prognostic role in prognosis [6]. Prostate specific antigen (PSA) has a poor specificity for early diagnosis since it is also elevated in patients with benign prostatic hyperplasia or prostatitis, so positron emission tomography-computed tomography is often used for early diagnosis of metastases [7, 8]. But it is unfavorable due to high costs and a large amount of radiation [9]. So, biomarkers with relatively high diagnostic specificity and low costs are required clinically for early screening and determination of metastasis.

Biomarkers for prostate cancer are presently investigated in many studies, among which miRNA is the most common one [10]. The expression of miRNA in tumor tissues is closely related to the occurrence and development of tumors. It is often highly expressed in many solid tumors, and plays an important role in the process of tumor proliferation and differentiation, so miRNA becomes a promising predictor

Table 1. Risk levels of prostate cancer

	Low risk	Intermediate risk	High risk
Gleason score	≤ 6	7	≥ 8
PSA (ng/mL)	< 10	10-20	> 20
T staging	≤ T2a	T2b	≥ T2c

Note: PSA: prostate specific antigen.

Table 2. Clinical data of patients with prostate cancer

Item		Number of cases (n, %)
Age (year)	≥ 65	32 (36.4)
	< 65	56 (63.6)
Gleason score (points)	> 7	55 (62.5)
	7	8 (9.1)
	< 7	25 (28.4)
PSA (ng/mL)	≤ 10	27 (30.7)
	11-20	51 (58.0)
	> 20	10 (11.3)
T staging	T1	19 (21.6)
	T2	35 (39.8)
	T3	24 (27.3)
	T4	10 (11.3)
N staging	N0	74 (84.1)
	N1	14 (15.9)
M staging	M0	80 (90.9)
	M1	8 (9.1)
Risk levels	Low risk	16 (18.2)
	Intermediate risk	15 (17.0)
	High risk	57 (64.8)

Note: PSA: prostate specific antigen.

of tumor diagnosis and prognosis [11, 12]. A study has shown that miRNA has a predictive effect in prostate cancer [13]; moreover, positive androgen receptor can bind to miRNA-21 in patients with prostate cancer, resulting in high expression of miRNA-21, thereby promoting tumor growth [14]. Besides, miRNA-21 is stably expressed in body fluid such as blood, urine and sputum, etc. [15]. Therefore, miRNA-21 in peripheral blood was measured in this study to investigate its effect on diagnosis, metastasis and prognosis of prostate cancer.

Materials and methods

General data

A total of 88 patients with prostate cancer admitted to Biomedicine Research, Second Military Medical University from January 2015 to September 2016 were included, aged from 46 to 72 years old, with an average age of 63.02 ± 8.81 years. Among them, 62 patients underwent radical prostatectomy. Additionally, 84 patients with benign prostatic hyperplasia (an average age of 64.43 ± 8.39 years) treated in Biomedicine Research, Second Military Medical University during the same period; 80 healthy subjects (an average age of 64.36 ± 8.12 years) underwent physical examination were selected as controls. Patients who underwent radical prostatectomy were followed for 2 years. The present study was approved by the Ethics Committee of Biomedicine Research, Second Military Medical University. Written informed consent was obtained from all the subjects.

Inclusion & exclusion criteria

Patients were eligible if they were diagnosed with prostate cancer or benign prostatic hyperplasia [16, 17], aged between 18 to 75 years old.

Patients were excluded if they had incomplete clinical data; had severe heart, liver, or kidney diseases; had mental disorders or cerebrovascular diseases; were difficult or inconvenient to follow up; had other cancers or a non-primary prostate cancer.

Extraction of miRNA

The Trizol kit used in this study was purchased from Molecular Research Center, USA. Upstream primers and downstream primers were synthesized by Guangzhou Ruibo, China [18]. The miRNA was reverse transcribed into cDNA with the use of reverse transcription kit (Fernentas, USA) by RT-PCR, which was then used as a template to amplify the DNA. The expression of miRNA-21 in serum samples was quantitatively detected by the fluorescence probe method [19]. Specific procedures were as follows. Firstly, 2 mL of peripheral venous blood was collected from each subject on an empty stomach the next morning after fasting and water deprivation at 10 p.m., added with EDTA anticoagulants to maintain the integrity of the cells, and gently mixed, so that the blood cells were in full contact with the anticoagulant. Secondly, the treated tube was placed in a refrigerator at 4°C, and the plasma was separated within 2 hours and centrifuged for 10 min using a high-speed centrifuge (Shanghai Biotech, China).

Table 3. Relative expression of miRNA

Group	Cases	miRNA
Prostate cancer group	88	2.65 ± 0.69
Benign prostatic hyperplasia group	84	1.51 ± 0.49
Healthy group	80	1.48 ± 0.48
F value		105.34
P value		0.000
P value (prostate cancer group vs. benign prostatic hyperplasia group)		0.000
P value (prostate cancer group vs. healthy group)		0.000
P value (benign prostatic hyperplasia group vs. healthy group)		0.830

Thirdly, the supernatant liquid was taken out, placed in an Eppendorf tube, and centrifuged again for 10 min. Next, the twice-centrifuged samples were placed in a refrigerator at -80°C, with a volume of 500 μL each. Lastly, the miRNA-21 was extracted and purified using the Trizol kit. The upstream primer sequences were 5'-GCGTAGCTTATCAGACTGATGTTG-3' (miR-21) and 5'-TCGCTTCGGCAGCACA-3' (U6). Circulatory system 25 μL: SYBR Premix (2×) 12.5 μL, 0.5 μL of upstream and downstream primer of the target gene, 2.0 μL of cDNA template, 9.5 μL of ddH_2O; reaction conditions: 4 min of predenaturation at 94°C, 40 s at 95°C, 30 s at 60°C, and 30 s at 72°C, for 35 cycles, plus another 1 min at 72°C for extend. The PCR amplification products were detected by agarose electrophoresis. The relative expression was analyzed by $2^{-\Delta\Delta C(T)}$ method using the expression of U6 snRNA as standard. The relative expression of miRNA-21 was determined [20].

Determination of PSA

Fasting venous blood (5 mL) was collected from each patient in the morning and determined by Elisa with the use of an automated biochemical analyzer. Patients with PSA > 20 ng/mL and ≤ 20 ng/mL were assigned into two different subgroups.

Pathological diagnosis of prostate cancer

The prostate cancer pathological grading was conducted with the use of Gleason grading system (5 grades and 10 points in total) [21]. Tumor morphological structure was graded from 1 to 5 (1 point for grade 1 and 5 points for grade 5), and differentiated degree of tumor was also graded from 1 to 5 (1 point for grade 1 and 5 points for grade 5). The sum of the two scores should be between 2 and 10, and patients with Gleason > 7 points and ≤ 7 points were assigned into two different subgroups.

TNM staging system

The TNM staging of malignant tumors was determined by comprehensive analyses of the results of examinations, such as digital rectal examination, chest X-ray, CT, nuclear magnetic resonance imaging, bone scan and positron emission tomography-computed tomography [16]. T describes the size of the primary tumor and whether it has invaded nearby tissues, including T1a, T1b, T1c, T2a, T2b, T2c, T3a, T3b and T4. Patients were assigned into T1, T2, T3, T4 subgroups respectively according to T staging in this study. As for N staging, N0 and N1 represented the presence and absence (respectively) of regional lymph node metastasis. In terms of M staging, no distant metastasis was classified as M0, and metastasis to distant organs was classified as M1.

Risk levels of prostate cancer

Patients were assigned into low risk, intermediate risk and high risk groups respectively according to Gleason scores, tumor stages (T staging) and PSA levels [22]. See **Table 1**.

Follow up

Regular outpatient follow-ups were carried out in 62 patients who had underwent radical prostatectomy. Serum PSA was tested 1 month after surgery, and every three months thereafter. Biochemical recurrence was observed during the 2-year follow-up, which was defined as two consecutive PSA levels above 0.2 ng/mL after the presence of the lowest PSA level [23].

Statistical analyses

Data were processed with the use of SPSS 17.0 statistical software. Continuous variables are expressed as mean ± standard deviation (mean ± sd). Variables with normal distribution and

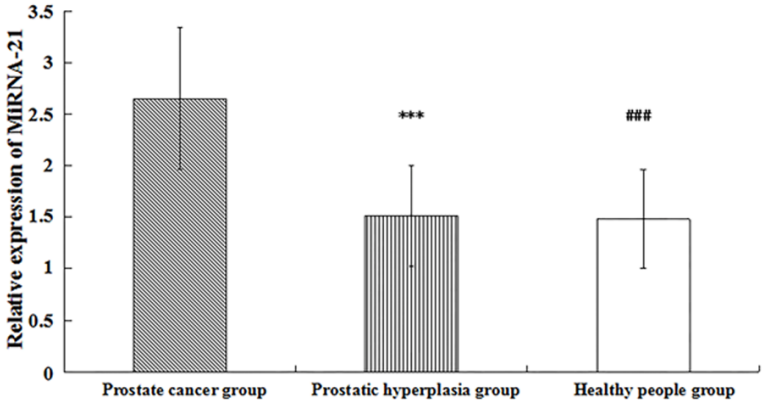

Figure 1. Relative expression of miRNA. Compared with the prostate cancer group, ***P < 0.001, ###P < 0.001.

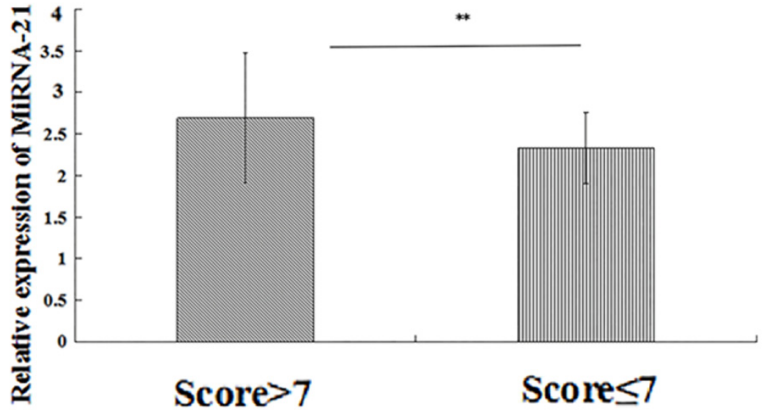

Figure 2. Relative expression of miRNA in the Gleason score subgroups. **P < 0.01.

Table 4. Relative expression of miRNAs in T-staged subgroups

Group	Cases	miRNA
T1	19	2.31 ± 0.55
T2	35	2.33 ± 0.45
T3	24	2.56 ± 0.62
T4	10	3.80 ± 0.45
F		27.631
P		0.000

Note: Comparison between T1 group and T2 group: t = 0.149, P = 0.883; comparison between T3 group and T4 group: t = 5.695, P = 0.000; comparison between T1 group and T3 group: t = 1.137, P = 0.176; comparison between T1 group and T4 group: t = 7.133, P = 0.000; comparison between T2 group and T3 group: t = 1.640, P = 0.106; comparison between T2 group and T4 group: t = 9.044, P = 0.000.

homogeneity of variance was compared with the use of t tests, denoted by t, while variables not conforming to normal distribution and homogeneity of variance was compared using rank sum tests, denoted by Z. Comparisons among the groups were performed using the Mann-Whitney U tests. Count data are analyzed with the use of a Pearson chi-square test and a Fisher's exact probability test, denoted by χ^2. Difference is statistically significant when P < 0.05.

Results

Clinical data

A total of 252 subjects were enrolled in the study, including 88 patients with prostate cancer, 84 patients with benign prostatic hyperplasia and 80 healthy subjects, and their mean ages were 63.02 ± 8.81 years, 64.43 ± 8.39 and 64.36 ± 8.12 years, respectively, without significant difference in age (P > 0.05). See **Table 2**.

Relative expression of miRNAs in the three groups

The expression of miRNA was significantly higher in the prostate cancer group than in the prostatic hyperplasia group and the healthy group (both P < 0.001), but was not statistically different between the benign prostatic hyperplasia group and the healthy group (P > 0.05). See **Table 3** and **Figure 1**.

Relative expression of miRNA in the Gleason score subgroups

The relative expression of miRNA was compared in prostate cancer patients with different Gleason scores; patients with Gleason score > 7 points had higher expression (2.69 ± 0.78) than patients with score ≤ 7 points (2.33 ± 0.43), with statistical significance (P = 0.007). See **Figure 2**.

Relative expressions of miRNA in PSA subgroups

The relative expression of miRNA was compared in prostate cancer patients with diffe-

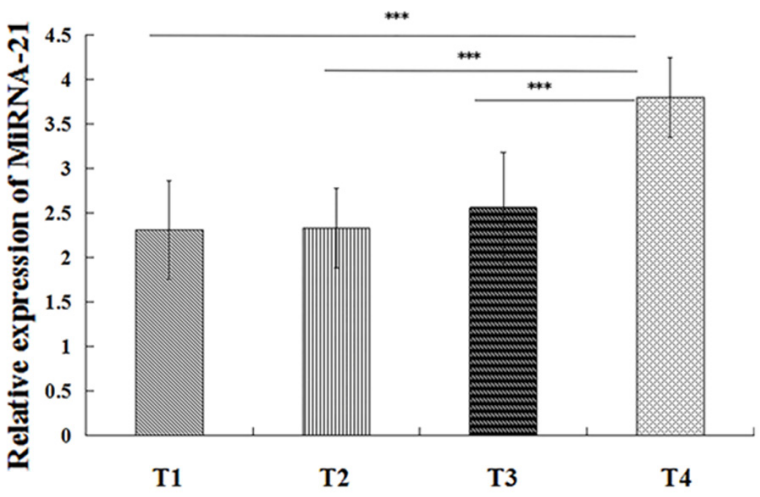

Figure 3. Relative expression of miRNAs in the T-staged subgroups. ***P < 0.001.

Table 5. Relative expression of miRNAs in N-staged and M-staged subgroups

Group		Cases	miRNA	t	P
N staging	N0	74	2.46 ± 0.64	3.037	0.003
	N1	14	3.05 ± 0.75		
M staging	M0	80	2.41 ± 0.54	12.853	0.000
	M1	8	3.98 ± 0.29		

rent PSA levels; patients with PSA > 20 ng/mL had higher expression (2.70 ± 0.73) than patients with PSA ≤ 20 ng/mL (2.49 ± 0.67), but without statistical significance (P = 0.204).

Relative expression of miRNAs in the T staging subgroups

The relative expression of miRNA was the highest in T4 group than in other T staging groups (all P < 0.001); pairwise comparisons of the other 3 groups were not statistically significant (all P > 0.05). See **Table 4** and **Figure 3**.

Relative expression of miRNAs in N staging and M staging subgroups

The relative expression of miRNA was higher in N1 group (regional lymphatic metastasis) than in N0 group (no regional lymphatic metastasis), and was higher in M1 group (metastasis to distant organs) than in M0 group (no distant metastasis) (both P < 0.01). See **Table 5**, **Figures 4** and **5**.

Relative expression of miRNAs in risk level subgroups

The relative expression of miRNAs in the low risk group was significantly lower than that in the intermediate risk and the high risk groups (both P < 0.05), but there was no significant difference between the intermediate risk group and the high risk group (P > 0.05). See **Table 6** and **Figure 6**.

Postoperative relative expression of miRNAs

Among the 62 cases underwent radical prostatectomy in the prostate cancer group, 4 of them died during the 2-year follow-up period, and the other 58 patients were assigned into the postoperative recurrence group (24 cases, presence of recurrence) and the normal postoperative group (34 cases, no recurrence). The relative expression of miRNA-21 was determined by collecting venous blood samples at the last follow-up; the expression in the postoperative recurrence group (2.86 ± 0.60) was significantly higher than that in the normal postoperative group (2.09 ± 0.62, P < 0.001). See **Figure 7**.

Discussion

Inhibition of miRNA plays an important role in regulation of differentiation, proliferation and apoptosis of cells, as well as in the development of cancers. The occurrences of more than 50% human malignant tumors are associated with miRNA genes [24, 25]. Prostate cancer as one of the most common malignant tumors in males has an increasing incidence in China [2]. Therefore, low-cost biomarkers with high specificity are needed for early screening, metastatic determination, diagnosis, postoperative recurrence and prognosis. Among them, miRNA-21 was shown to have abnormal expression in a variety of malignant tumors [26, 27]. The reason might be that malignant tumors can affect the expression of miRNA-21. So, the present study detected the expression levels of miRNA-21 in patients with prostate cancer to provide a new idea in diagnosis of prostate cancer and prediction of biochemical recurrence.

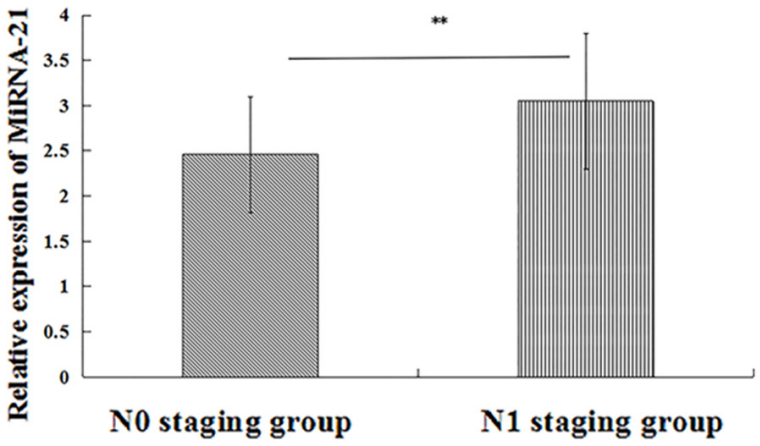

Figure 4. Relative expression of miRNAs in the N-staged subgroups. **P < 0.01.

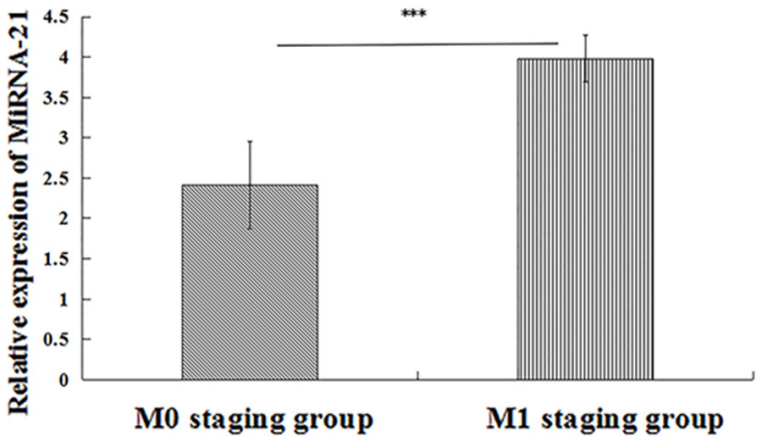

Figure 5. Relative expression of miRNAs in the M-staged subgroups. ***P < 0.001.

Table 6. Relative expression of miRNAs in risk-level subgroups

Group	Cases	miRNA
Low risk group	16	1.93 ± 0.33
Intermediate risk group	15	2.63 ± 0.98
High risk group	57	2.71 ± 0.57
F		20.009
P		0.000

Note: Comparison between low-risk group and intermediate risk group: t = 2.631, P = 0.018; the comparison between low-risk group and high-risk group: t = 5.206, P = 0.000; comparison between intermediate risk group and high-risk group: t = 0.300, P = 0.768.

PSA is often used for post-measurement prediction in early diagnosis and screening of prostate cancer, but the expression of PSA also increases in patients with prostatic hyperplasia or prostatitis, so its specificity for early diagnosis is poor [7]. We compared patients with prostate cancer, patients with benign prostatic hyperplasia and healthy subjects in this study. It was found that the expression of miRNA-21 in the prostate cancer group was significantly higher than that in the prostatic hyperplasia group and the healthy group, and there was no difference between the prostatic hyperplasia group and the healthy group. It is similar to the results of previous studies that investigated the abnormal expression of miRNA-21 in patients with various kinds of malignant tumors [26, 27].

PSA, Gleason scores, and clinical staging were often used for risk assessments in previous studies of metastasis in patients with prostate cancer [28]. A latest study suggested that patients with prostate cancer who had a PSA ≤ 20 ng/mL and a Gleason score ≤ 7 had a lower risk of bone metastases, and were not required for bone scan, vice versa [21]. Previous studies have found that prostate cancer is prone to metastasis when patients have a Gleason score between 8 to 10 points [29]. Therefore, the present study set the boundary of PSA to 20 ng/mL, and Gleason score to 7 points. We found that the relative expression of miRNA-21 in prostate cancer patients with a Gleason score > 7 points was significantly higher than that in patients with a score ≤ 7 points. However, there was no difference in the relative expression of miRNA-21 between patients with PSA ≤ 20 ng/mL and those with PSA > 20 ng/mL. This study found that the increase in the relative expression of miRNA-21 was associated with tumor invasion and proliferation in patients with Gleason score > 7. In the comparison between TNM staging subgroups, the relative expression

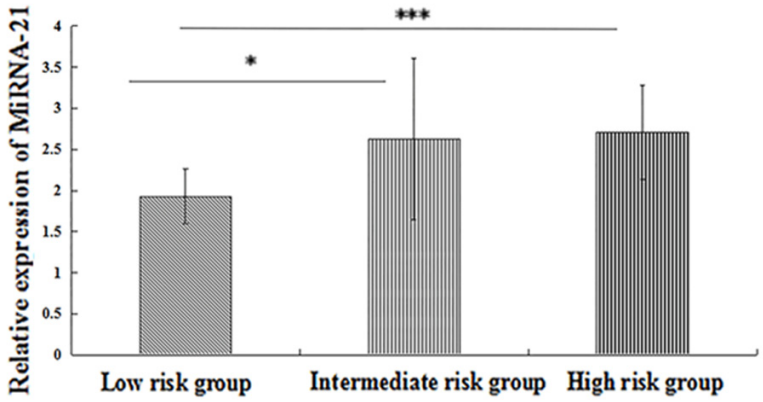

Figure 6. Relative expression of miRNAs in the risk-level subgroups. *P < 0.05, ***P < 0.001.

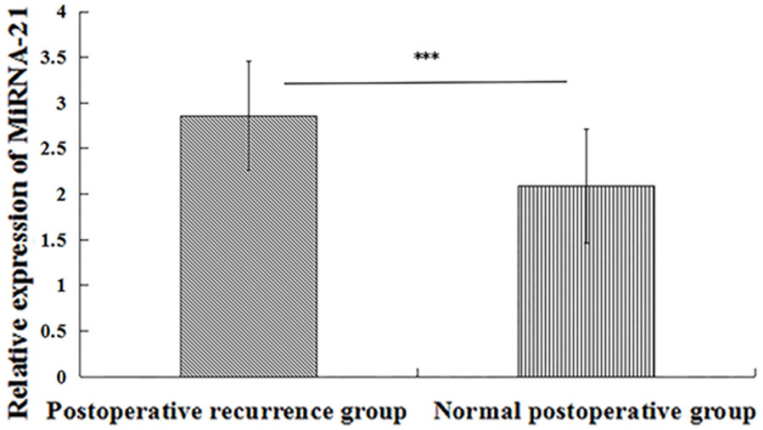

Figure 7. Postoperative relative expression of miRNAs. ***P < 0.001.

of miRNA was the highest in T4 group than in other T staging groups, higher in N1 group (regional lymphatic metastasis) than in N0 group (no regional lymphatic metastasis), and higher in M1 group (metastasis to distant organs) than in M0 group (no distant metastasis). The increase of tumor volume, differentiation of cancer tissues, as well as invasion and metastasis of surrounding tissues could result in increased expression of miRNA-21 [11, 12]. Patients were assigned into the low risk, intermediate risk and high risk groups respectively according to the Gleason scores, tumor stages (T staging) and PSA levels. Significantly elevated relative expression was found in the intermediate risk group and the high risk group when compared with the low risk group, which suggests that the risk level is positively associated with the malignant degree of the tumor and the relative expression of miRNA-21 [30]. In terms of postoperative biochemical recurrence, the relative expression of miRNA-21 in the postoperative recurrence group was higher than that in the normal postoperative group, indicating that the recurrence and proliferation of tumors could lead to an elevation in the relative expression of miRNA-21. This study suggests that the relative expression of miRNA-21 is positively associated with the malignant degree and disease progression, which is consistent with the results of previous studies of miRNA-21 in other tumor histology [31].

However, the sample size of this study was small. Further multi-center studies with larger sample size should be carried out to investigate the predictive value of miRNA plus PSA in the diagnosis and prognosis of prostate cancer.

In conclusion, the relative expression of miRNA-21 in the blood of patients with prostate cancer is increased, and related to the malignant degree and biochemical recurrence of tumor. So, it is a valuable indicator in diagnosis and prediction of prostate cancer.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (81702895) and Basic Key Project of Shanghai Science and Technology Commission (14JC1491200).

Disclosure of conflict of interest

None.

Address correspondence to: Yao Li and Lei Yin, Department of Urology, Shanghai Changzheng Hospital, No. 415 Fengyang Road, Shanghai City 200003, China. Tel: +86-021-81886813; E-mail: liyao85ui@163.com (YL); yinlei8866@163.com (LY)

References

[1] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016; 66: 7-30.

[2] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.

[3] Schweizer MT and Yu EY. AR-signaling in human malignancies: prostate cancer and beyond. Cancers (Basel) 2017; 9.

[4] Perez-Lopez R, Lorente D, Blackledge MD, Collins DJ, Mateo J, Bianchini D, Omlin A, Zivi A, Leach MO, de Bono JS, Koh DM and Tunariu N. Volume of bone metastasis assessed with whole-body diffusion-weighted imaging is associated with overall survival in metastatic castration-resistant prostate cancer. Radiology 2016; 280: 151-160.

[5] Satoh T and Ishiyama H. Current diagnosis and the multidisciplinary approach to the treatment of bone metastases in patients with prostate cancer. Nihon Rinsho 2016; 74: 129-136.

[6] An H, Tao N, Li J, Guan Y, Wang W, Wang Y and Wang F. Detection of prostate cancer metastasis by whole body magnetic resonance imaging combined with bone scintigraphy and PSA levels. Cell Physiol Biochem 2016; 40: 1052-1062.

[7] Cary KC and Cooperberg MR. Biomarkers in prostate cancer surveillance and screening: past, present, and future. Ther Adv Urol 2013; 5: 318-329.

[8] Kintzele L and Weber MA. Imaging diagnostics in bone metastases. Radiologe 2017; 57: 113-128.

[9] Wei RJ, Li TY, Yang XC, Jia N, Yang XL and Song HB. Serum levels of PSA, ALP, ICTP, and BSP in prostate cancer patients and the significance of ROC curve in the diagnosis of prostate cancer bone metastases. Genet Mol Res 2016; 15.

[10] Madu CO and Lu Y. Novel diagnostic biomarkers for prostate cancer. J Cancer 2010; 1: 150-177.

[11] Peralta-Zaragoza O, Deas J, Meneses-Acosta A, De la O-Gómez F, Fernández-Tilapa G, Gómez-Cerón C, Benítez-Boijseauneau O, Burguete-García A, Torres-Poveda K, Bermúdez-Morales VH, Madrid-Marina V, Rodríguez-Dorantes M, Hidalgo-Miranda A, Pérez-Plasencia C. Relevance of miR-21 in regulation of tumor suppressor gene PTEN in human cervical cancer cells. BMC Cancer 2016; 16: 215.

[12] Wang LJ, He CC, Sui X, Cai MJ, Zhou CY, Ma JL, Wu L, Wang H, Han SX and Zhu Q. MiR-21 promotes intrahepatic cholangiocarcinoma proliferation and growth in vitro and in vivo by targeting PTPN14 and PTEN. Oncotarget 2015; 6: 5932-5946.

[13] Giusti I and Dolo V. Extracellular vesicles in prostate cancer: new future clinical strategies? Biomed Res Int 2014; 2014: 561571.

[14] Ribas J, Ni X, Haffner M, Wentzel EA, Salmasi AH, Chowdhury WH, Kudrolli TA, Yegnasubramanian S, Luo J, Rodriguez R, Mendell JT and Lupold SE. miR-21: an androgen receptor-regulated microRNA that promotes hormone-dependent and hormone-independent prostate cancer growth. Cancer Res 2009; 69: 7165-7169.

[15] Qu K, Lin T, Pang Q, Liu T, Wang Z, Tai M, Meng F, Zhang J, Wan Y, Mao P, Dong X, Liu C, Niu W and Dong S. Extracellular miRNA-21 as a novel biomarker in glioma: evidence from meta-analysis, clinical validation and experimental investigations. Oncotarget 2016; 7: 33994-4010.

[16] Mottet N, Bellmunt J, Bolla M, Briers E, Cumberbatch MG, De Santis M, Fossati N, Gross T, Henry AM, Joniau S, Lam TB, Mason MD, Matveev VB, Moldovan PC, van den Bergh RCN, Van den Broeck T, van der Poel HG, van der Kwast TH, Rouviere O, Schoots IG, Wiegel T and Cornford P. EAU-ESTRO-SIOG guidelines on prostate cancer. Part 1: screening, diagnosis, and local treatment with curative intent. Eur Urol 2017; 71: 618-629.

[17] Schroeck FR, Hollingsworth JM, Hollenbeck BK, Jacobs BL, Suskind AM, Sarma AV and Wei JT. Differential adoption of laser prostatectomy for treatment of benign prostatic hyperplasia. Urology 2013; 81: 1177-1182.

[18] Fang F, Chang RM, Yu L, Lei X, Xiao S, Yang H and Yang LY. MicroRNA-188-5p suppresses tumor cell proliferation and metastasis by directly targeting FGF5 in hepatocellular carcinoma. J Hepatol 2015; 63: 874-885.

[19] Yang L, Feng Y, Qi P, Xu S and Zhou Y. Mechanism of serum miR-21 in the pathogenesis of familial and triple negative breast cancer. J Biol Regul Homeost Agents 2016; 30: 1041-1045.

[20] Sazanov AA, Kiselyova EV, Zakharenko AA, Romanov MN and Zaraysky MI. Erratum to: plasma and saliva miR-21 expression in colorectal cancer patients. J Appl Genet 2017; 58: 239.

[21] Chien TM, Lu YM, Geng JH, Huang TY, Ke HL, Huang CN, Li CC, Chou YH, Wu WJ and Huang SP. Predictors of positive bone metastasis in newly diagnosed prostate cancer patients. Asian Pac J Cancer Prev 2016; 17: 1187-1191.

[22] Na YQ, Ye ZQ, Sun YH and Sun G. Guidelines for the diagnosis and treatment of urological diseases in China (2014 edition). People's Medical Publishing House, 2014.

[23] Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, van der Kwast T, Mason M, Matveev

V, Wiegel T, Zattoni F and Mottet N. EAU guidelines on prostate cancer. Part II: treatment of advanced, relapsing, and castration-resistant prostate cancer. Eur Urol 2014; 65: 467-479.

[24] Aquino-Jarquin G. Emerging role of CRISPR/Cas9 technology for microRNAs editing in cancer research. Cancer Res 2017; 77: 6812-6817.

[25] Tang S, Wu WK, Li X, Wong SH, Wong N, Chan MT, Sung JJ and Yu J. Stratification of digestive cancers with different pathological features and survival outcomes by microRNA expression. Sci Rep 2016; 6: 24466.

[26] Fang H, Xie J, Zhang M, Zhao Z, Wan Y and Yao Y. miRNA-21 promotes proliferation and invasion of triple-negative breast cancer cells through targeting PTEN. Am J Transl Res 2017; 9: 953-961.

[27] Yang Y, Guo JX and Shao ZQ. miR-21 targets and inhibits tumor suppressor gene PTEN to promote prostate cancer cell proliferation and invasion: an experimental study. Asian Pac J Trop Med 2017; 10: 87-91.

[28] Pound CR, Partin AW, Eisenberger MA, Chan DW, Pearson JD and Walsh PC. Natural history of progression after PSA elevation following radical prostatectomy. JAMA 1999; 281: 1591-7.

[29] Hussain A, Aly A, Daniel Mullins C, Qian Y, Arellano J and Onukwugha E. Risk of skeletal related events among elderly prostate cancer patients by site of metastasis at diagnosis. Cancer Med 2016; 5: 3300-3309.

[30] Hatley ME, Patrick DM, Garcia MR, Richardson JA, Bassel-Duby R, van Rooij E and Olson EN. Modulation of K-Ras-dependent lung tumorigenesis by MicroRNA-21. Cancer Cell 2010; 18: 282-293.

[31] Tao T, Wang Y, Luo H, Yao L, Wang L, Wang J, Yan W, Zhang J, Wang H, Shi Y, Yin Y, Jiang T, Kang C, Liu N and You Y. Involvement of FOS-mediated miR-181b/miR-21 signalling in the progression of malignant gliomas. Eur J Cancer 2013; 49: 3055-3063.