Original Article

Prostate Cancer Detection in Patients with Total Serum Prostate-specific Antigen Levels of 4–10 ng/mL: Diagnostic Efficacy of MicroRNA-141

Abdulkadir Çat, Tülay Köken, Mustafa Karalar[1]

Departments of Clinical Biochemistry and [1]Urology, School of Medicine, Afyon Kocatepe University, Afyon, Turkey

Abstract

Objective: The purpose of this small pilot study was to evaluate the utility of microRNA-141 (*miR-141*) as a biomarker for detecting prostate cancer (PCa) in patients with total serum prostate-specific antigen (PSA) levels of 4–10 ng/mL, which is referred to as the "gray zone." **Materials and Methods:** Eleven PCa patients, 23 benign prostatic hyperplasia (BPH) patients with PSA levels of 4–10 ng/mL, and 16 healthy controls were enrolled in this study. Total RNA was extracted from serum samples, and the level of *miR-141* was analyzed by quantitative reverse transcription polymerase chain reaction. **Results:** The circulating *miR-141* level was significantly higher in PCa patients than in BPH patients and healthy controls (fold change [mean ± standard deviation], 0.528 ± 0.083 for PCa, 0.297 ± 0.038 for BPH, and 0.262 ± 0.025 for controls; $P < 0.05$). Receiver operating characteristic curve revealed that the serum *miR-141* yielded an area under the curve of 0.751, with 72% sensitivity and 92% specificity in discriminating patients with PCa from BPH patients with total serum PSA levels in the gray zone. **Conclusion:** The present results indicate that *miR-141*expression is significantly increased in the peripheral blood of patients with PCa compared with BPH patients and healthy individuals. We think that *miR-141* may guide clinicians during the decision phase of patients with PCa and BPH in the PSA gray zone.

Keywords: *Benign prostatic hyperplasia, biomarker, microRNA-141, prostate cancer*

Introduction

Serum prostate-specific antigen (PSA) is currently the best screening test for prostate cancer (PCa). A limitation of PSA testing has been its relative lack of specificity within the intermediate range (4.0–10.0 ng/mL), a diagnostic "gray zone." Individuals with PSA <4.0 ng/ml are generally considered at low risk for developing cancer, while those with PSA >10.0 ng/ml are considered at high risk. For men who fall in the gray zone, it is more difficult to make the distinction between malignant and benign conditions.[1,2] When managing these patients, clinicians are faced with the dilemma of recommending a repeat biopsy or further PSA monitoring. Biopsy shows a positive predictive value for cancer of approximately 25%.[3] Repeated biopsy increases the cancer detection rate up to 10%–30% depending on the technique.[4,5] Nevertheless, biopsy commonly causes a vast variety of complications and may fail in detecting cancer.[6,7]

Therefore, there is a requirement for novel methods to improve PCa detection, in particular, for men in the gray zone. Furthermore, it is desired to develop novel diagnostic tools with minimal invasiveness to avoid the complications associated with biopsy. Accumulating data suggest that small noncoding RNAs such as microRNAs (miRNAs) can be utilized as potential biomarkers for the diagnosis and prognosis of various types of cancer. miRNAs are regulatory, nonprotein-coding, 9–25 nucleotide long RNA molecules that regulate the expression of a variety of genes by sequence-specific base pairing with the 3' untranslated regions of the target messenger RNA (mRNA), resulting in mRNA degradation or inhibition of translation. Specific miRNAs have been found to have key regulatory roles in a variety of oncogenic processes, including angiogenesis,[8] metastasis,[9] differentiation,[10] proliferation,[11] and apoptosis.[12] Owing to their distinct patterns of expression associated with cancer type and their extreme stability and presence in the blood, miRNAs are considered to be highly promising cancer biomarkers.[13]

The number of papers reporting that circulating miRNAs could serve as

How to cite this article: Çat A, Köken T, Karalar M. Prostate cancer detection in patients with total serum prostate-specific antigen levels of 4–10 ng/mL: Diagnostic efficacy of MicroRNA-141. Clin Cancer Investig J 2017;6:10-4.

Address for correspondence:
Dr. Tülay Köken, Department of Clinical Biochemistry, School of Medicine, Afyon Kocatepe University, Ali Çetinkaya Campuse B Block, Afyon 03200, Turkey.
E-mail: tkoken1967@yahoo.com

Access this article online

Website: www.ccij-online.org

DOI: 10.4103/ccij.ccij_16_17

Quick Response Code:

noninvasive biomarkers for PCa detection is increasing. In this small pilot study, we analyzed the expression of *miR-141* in the serum of PC patients, of benign prostatic hyperplasia (BPH) individuals with total serum PSA levels of 4–10 ng/mL, and of asymptomatic men to evaluate its diagnostic value.

Materials and Methods

Patient population

From January 2014 to October 2014, serum total PSA concentrations were measured in male patients who visited the outpatient Clinic of Urology, Afyon Kocatepe University Hospital, for voiding difficulty. Patients with intermediate PSA levels between 4.1 and 10.0 ng/mL were enrolled prospectively. Patients with overt urinary tract infection, acute urinary retention, medical therapy that might affect serum PSA levels, transurethral invasive surgery, and known PCa were excluded from the study. The study population consisted of fifty patients aged between 50 and 80 years. Of the 34 patients with intermediate levels of PSA, pathologic examination revealed 11 patients with PCa and 23 patients with BPH. After obtaining approval from the Clinical Research Ethics Committee of Afyon Kocatepe University (2013/15-178) and informed consent from all patients, blood samples were drawn. Blood samples were centrifuged to separate and collect serum. Serum samples were stored at −80°C until RNA extraction. Total PSA levels were measured by electrochemiluminescence immunoassay on a Modular Cobas e 411 (Roche Diagnostics GmbH, Mannheim, Germany).

RNA isolation, complementary DNA synthesis, and real-time quantitative polymerase chain reaction

RNA was isolated using the High Pure miRNA Isolation Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions.

Complementary DNA synthesis

Isolated RNA samples were reverse-transcribed into complementary DNA (cDNA) in 10 µl final reaction volumes using the Universal cDNA Synthesis Kit II (Exiqon, Cat. No 203301). An artificial RNA (kit-based RNA spike-in) was added to each reverse transcription reaction as a control to confirm that the reverse transcription and amplification occurred with equal efficiency in all samples. Two-microliter total RNA was added to 8 µL of the reverse transcriptase (RT) Master Mix containing reaction buffer. Enzyme mix and spike-in were incubated at 42°C for 60 min. RT was then heat-inactivated at 95°C for 5 min, and the reactions were cooled down and stored at −20°C. Negative controls excluding template from the reverse transcription reaction were included and profiled as for the test samples.

MicroRNA quantitative reverse transcription polymerase chain reaction

cDNA was assayed according to the protocol for miRCURY LNA Universal RT miRNA polymerase chain reaction (PCR) (Exiqon, Cat. No. 203403). Duplicate qPCR reactions were performed in a final volume of 10 µL containing 5 µL of PCR SYBR green Master Mix, 1 µL of specific PCR primer (Exiqon), and 4 µL of cDNA template. Reactions were run on a LightCycler 480 Real-Time PCR System (Roche, Mannheim, Germany) in 96-well optical plates. After a polymerase activation step at 95°C for 10 min, the samples were cycled 40 times at 95°C for 10 s and 60°C for 60 s. The expression level of miRNAs was performed based on absolute quantification analysis using the second derivative method with the LightCycler 480 software from Roche allowing constant pressure values to be calculated. The expression of *miR-141* in the serum was normalized to the expression of U6 small nuclear RNA (RNU6B). The fold change was calculated with the equation $2^{-\Delta\Delta Ct}$.

Statistical analysis

All of the analyses were performed using the statistical software package SPSS version 20.0. The levels of *miR-141* in each group were defined as the mean ± standard deviation (SD). Mann–Whitney U-test or Kruskal–Wallis test was used to determine the statistical significance of the *miR-141* levels in different groups. Receiver operating characteristic (ROC) curves were used to assess *miR-141* as a biomarker, and the area under the curve (AUC) was reported. A two-tailed $P < 0.05$ was considered statistically significant.

Results

As shown in Table 1, no significant differences in age were observed between PCa patients, BPH patients, and healthy control individuals. Serum PSA levels were significantly higher in PCa patients and BPH patients than in healthy control individuals ($P < 0.001$). However, there were no significant differences between PCa patients and BPH patients.

To understand the potential value of serum *miR-141* in the diagnosis of PCa, the levels of *miR-141* in the 11 patients with PCa, 23 patients with BPH, and 40 healthy individuals were determined using quantitative

Table 1: Characteristics of the 50 patients in three groups

Characteristics	Control	BPH	PCa
Total number	16	23	11
Age (year)	60±1.9	66±1.3	66±2.0
PSA (ng/ml)	0.86±0.11	6.77±0.29*	7.32±0.31*

Data are expressed as mean±SD. *P>0.001 versus controls.
BPH: Benign prostatic hyperplasia, PCa: Prostate cancer, PSA: Prostate-specific antigen, SD: Standard deviation

RT-PCR [Figure 1]. The expression of *miR-141* was determined relative to the endogenous control, RNU6, in the peripheral blood. Values were expressed as the mean (±SD) fold difference in gene expression. The mean relative expression of *miR-141* was 0.528 ± 0.083 for PC patients, 0.297 ± 0.038 for BPH patients, and 0.262 ± 0.025 for healthy controls. The serum *miR-141* level was increased in the PCa group compared to both the BPH group and the healthy controls ($P < 0.05$).

Subsequently, ROC curves were generated to assess the power of *miR-141* to distinguish patients with PCa from patients with BPH. Based on the ROC analysis, blood *miR-141* was able to distinguish PCa patients from BPH patients [AUC, 0.751; 95% confidence interval, 0.561–0.854; $P < 0.05$; Figure 2]. At the optimal cutoff value (0.421), the sensitivity and specificity were 72% and 92% for *miR-141*. While false-positive results occurred at a rate of 8.7%, the negative predictive values remained at 87.5% for cancer detection in patients with "gray zone" PSA levels and prior negative biopsies.

Discussion

A large number of miRNAs have been found to be abnormal in PCa, and many of them contribute to tumorigenesis and progression.[14-16] The present study evaluated the levels of *miR-141*, a highly expressed miRNA in the tissues of PCa,[16-18] in the peripheral blood of patients with PCa or BPH, and in healthy individuals, to test the feasibility of using peripheral blood *miR-141* as a potential novel biomarker for PCa in patients who fall in the gray zone.

miR-141 is a member of the *miR-200* family. The *miR-200* family is composed of five members (*miR-200a*, *miR-200b*, *miR-200c*, *miR-141*, and *miR-429*) that are clustered and expressed as two separate polycistronic pri-miRNA transcripts, *miR-200b-200a-429* and *miR-200c-141*, located on human chromosomes 1 and 12, respectively.[19]

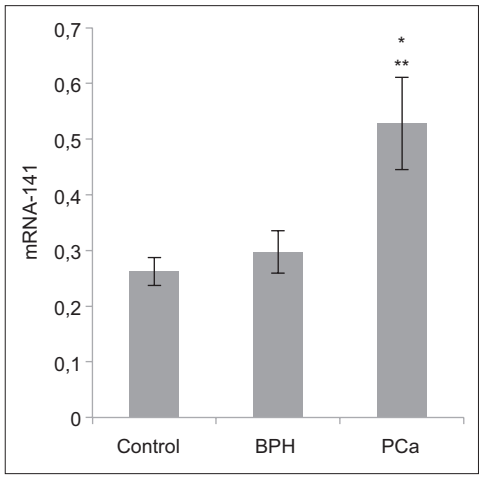

Figure 1: Serum microRNA-141 levels in different groups of patients within prostate-specific antigen "gray zone." BPH: Benign prostatic hyperplasia, PCa: Prostate cancer. *$P > 0.05$ versus controls. **$P > 0.05$ versus BPH

Growing evidence has indicated that abnormal expression of *miR-141* is associated with tumorigenesis and carcinoma progression of various malignant tumors. For example, *miR-141* is overexpressed in biliary tract cancers,[20] bladder cancer,[21] nasopharyngeal carcinoma,[22] ovarian cancer,[23] colorectal cancer,[24] and nonsmall cell lung cancer[25] and acts as an oncogene. Furthermore, *miR-141*, as a tumor suppressor, is downregulated in numerous cancer types, such as renal cell carcinoma,[26] gastric cancer,[27] breast cancer,[28] hepatocellular carcinoma,[29] and pancreatic cancer.[30]

To date, many articles have reported a role of *miR-141* in PCa. Some researchers[16,31,32] identified *miR-141* to be upregulated and could be used to differentiate between metastatic and localized PCa,[33,34] whereas Kachakova *et al*.[18] reported downregulation of miR-141 in the plasma of PCa patients as compared to BPH samples. In addition, Mahn *et al*. suggested that *miR-141* levels in serum of PCa were too low for reliable testing.[35] A recent study also demonstrated that circulating *miR-141* levels were correlated with advanced PCa.[33] Li *et al*. have previously reported that serum expression of exosomal *miR-141* expression was upregulated in PCa compared with BPH or the healthy volunteers and was correlated with levels of whole-serum *miR-141*.[36] Liu *et al*. confirm that expression of *miR-141* was upregulated in patients with PCa. Furthermore, they suggested that *miR-141* may suppress the metastatic cascade at an early stage and that the overexpression of *miR-141* in PCa cells results in less metastasis.[37] Moreover, the feasibility of using peripheral blood *miR-141* as a potential novel noninvasive biomarker for PCa has not been studied in patients with total serum PSA levels in the gray zone. Therefore, we examined the expression of *miR-141* in 34 patients with total serum PSA levels in the gray zone and evaluated its diagnostic value.

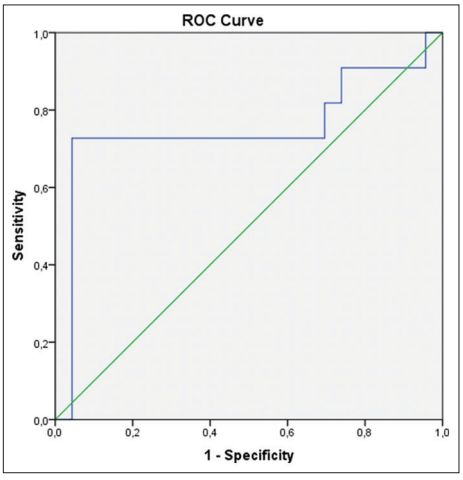

Figure 2: ROC curve analysis *miR-141* is able to distinguish PCa patients from BPH patients within prostate-specific antigen "gray zone" (AUC, 0.751; 95% CI, 0.561–0.854; $P < 0.05$). ROC: Receiver operating characteristic, *miR-141*: MicroRNA-141, PCa: Prostate cancer, BPH: Benign prostatic hyperplasia, AUC: Area under the curve, CI: Confidence interval

The results of the current study demonstrated that the expression of peripheral blood *miRNA-141* was significantly higher in patients with PCa than in BPH patients and healthy control individuals. In PCa patients, the *miR-141* cutoff of 0.421 showed a low proportion (8.7%) of false-positive results and a low proportion (27.3%) of false-negative results in discriminating from BPH. Plasma *miR-141* could distinguish PCa cases from BPH cases with an AUC value of 0.751, which indicates that serum *miR-141* could be a potential biomarker for diagnosing PCa in patients who fall in the gray zone.

Our study has some limitations. First, the sample size was small for a clinical study, i.e., further studies are required to confirm our data. Second, it would be necessary to study the function of *miR-141* in other disease patients. Another explanation for the *miR-141* results could be that this miRNA plays a role in various pathophysiological processes.

Conclusion

The results of the current study demonstrated that the expression of peripheral blood *miR-141* was significantly higher in patients with PC than in BPH patients and healthy control individuals. The ROC analysis revealed that peripheral blood *miR-141* was able to discriminate patients with PC from BPH patients with total serum PSA levels in the gray zone.

Financial support and sponsorship

This work received financial support from Afyon Kocatepe University Scientific Research Project Commission (research grant: 13-TUS-13), Afyonkarahisar, Turkey.

Conflicts of interest

There are no conflicts of interest.

References

1. Catalona WJ, Smith DS, Ratliff TL, Dodds KM, Coplen DE, Yuan JJ, *et al.* Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. N Engl J Med 1991;324:1156-61.
2. Carter HB. Prostate cancers in men with low PSA levels – Must we find them? N Engl J Med 2004;350:2292-4.
3. Norberg M, Egevad L, Holmberg L, Sparén P, Norlén BJ, Busch C. The sextant protocol for ultrasound-guided core biopsies of the prostate underestimates the presence of cancer. Urology 1997;50:562-6.
4. Walz J, Graefen M, Chun FK, Erbersdobler A, Haese A, Steuber T, *et al.* High incidence of prostate cancer detected by saturation biopsy after previous negative biopsy series. Eur Urol 2006;50:498-505.
5. Busby JE, Evans CP. Determining variables for repeat prostate biopsy. Prostate Cancer Prostatic Dis 2004;7:93-8.
6. Lawrentschuk N, Fleshner N. The role of magnetic resonance imaging in targeting prostate cancer in patients with previous negative biopsies and elevated prostate-specific antigen levels. BJU Int 2009;103:730-3.
7. Loeb S, Carter HB, Berndt SI, Ricker W, Schaeffer EM. Complications after prostate biopsy: Data from SEER-Medicare. J Urol 2011;186:1830-4.
8. Suárez Y, Sessa WC. MicroRNAs as novel regulators of angiogenesis. Circ Res 2009;104:442-54.
9. Chan SH, Wang LH. Regulation of cancer metastasis by microRNAs. J Biomed Sci 2015;22:9.
10. Maebayashi T, Abe K, Aizawa T, Sakaguchi M, Ishibash N, Fukushima S, *et al.* Solitary pulmonary metastasis from prostate cancer with neuroendocrine differentiation: A case report and review of relevant cases from the literature. World J Surg Oncol 2015;13:173.
11. Hwang HW, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer 2006;94:776-80.
12. Pileczki V, Cojocneanu-Petric R, Maralani M, Neagoe IB, Sandulescu R. MicroRNAs as regulators of apoptosis mechanisms in cancer. Clujul Med 2016;89:50-5.
13. Krutovskikh VA, Herceg Z. Oncogenic microRNAs (OncomiRs) as a new class of cancer biomarkers. Bioessays 2010;32:894-904.
14. Porkka KP, Pfeiffer MJ, Waltering KK, Vessella RL, Tammela TL, Visakorpi T. MicroRNA expression profiling in prostate cancer. Cancer Res 2007;67:6130-5.
15. Kelly BD, Miller N, Sweeney KJ, Durkan GC, Rogers E, Walsh K, *et al.* A circulating MicroRNA signature as a biomarker for prostate cancer in a high risk group. J Clin Med 2015;4:1369-79.
16. Mitchell PS, Parkin RK, Kroh EM, Fritz BR, Wyman SK, Pogosova-Agadjanyan EL, *et al.* Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci U S A 2008;105:10513-8.
17. Lodes MJ, Caraballo M, Suciu D, Munro S, Kumar A, Anderson B. Detection of cancer with serum miRNAs on an oligonucleotide microarray. PLoS One 2009;4:e6229.
18. Kachakova D, Mitkova A, Popov E, Popov I, Vlahova A, Dikov T, *et al.* Combinations of serum prostate-specific antigen and plasma expression levels of let-7c, miR-30c, miR-141, and miR-375 as potential better diagnostic biomarkers for prostate cancer. DNA Cell Biol 2015;34:189-200.
19. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, *et al.* The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol 2008;10:593-601.
20. Kim J, Ryu JK, Lee SH, Kim YT. MicroRNA 141 expression is a potential prognostic marker of biliary tract cancers. Gut Liver 2016;10:836-41.
21. Zhou H, Tang K, Xiao H, Zeng J, Guan W, Guo X, *et al.* A panel of eight-miRNA signature as a potential biomarker for predicting survival in bladder cancer. J Exp Clin Cancer Res 2015;34:53.
22. Zhang L, Deng T, Li X, Liu H, Zhou H, Ma J, *et al.* MicroRNA-141 is involved in a nasopharyngeal carcinoma-related genes network. Carcinogenesis 2010;31:559-66.
23. Gao YC, Wu J. MicroRNA-200c and microRNA-141 as potential diagnostic and prognostic biomarkers for ovarian cancer. Tumour Biol 2015;36:4843-50.
24. Sun Y, Liu Y, Cogdell D, Calin GA, Sun B, Kopetz S, *et al.* Examining plasma microRNA markers for colorectal cancer at different stages. Oncotarget 2016;7:11434-49.
25. Mei Z, He Y, Feng J, Shi J, Du Y, Qian L, *et al.* MicroRNA-141

promotes the proliferation of non-small cell lung cancer cells by regulating expression of PHLPP1 and PHLPP2. FEBS Lett 2014;588:3055-61.
26. Nakada C, Matsuura K, Tsukamoto Y, Tanigawa M, Yoshimoto T, Narimatsu T, *et al.* Genome-wide microRNA expression profiling in renal cell carcinoma: Significant down-regulation of miR-141 and miR-200c. J Pathol 2008;216:418-27.
27. Zhou X, Wang Y, Shan B, Han J, Zhu H, Lv Y, *et al.* The downregulation of miR-200c/141 promotes ZEB1/2 expression and gastric cancer progression. Med Oncol 2015;32:428.
28. Madhavan D, Peng C, Wallwiener M, Zucknick M, Nees J, Schott S, *et al.* Circulating miRNAs with prognostic value in metastatic breast cancer and for early detection of metastasis. Carcinogenesis 2016;37:461-70.
29. Wu SM, Ai HW, Zhang DY, Han XQ, Pan Q, Luo FL, *et al.* MiR-141 targets ZEB2 to suppress HCC progression. Tumour Biol 2014;35:9993-7.
30. Zhao G, Wang B, Liu Y, Zhang JG, Deng SC, Qin Q, *et al.* MiRNA-141, downregulated in pancreatic cancer, inhibits cell proliferation and invasion by directly targeting MAP4K4. Mol Cancer Ther 2013;12:2569-80.
31. Haldrup C, Kosaka N, Ochiya T, Borre M, Høyer S, Orntoft TF, *et al.* Profiling of circulating microRNAs for prostate cancer biomarker discovery. Drug Deliv Transl Res 2014;4:19-30.
32. Yaman Agaoglu F, Kovancilar M, Dizdar Y, Darendeliler E, Holdenrieder S, Dalay N, *et al.* Investigation of miR-21, miR-141, and miR-221 in blood circulation of patients with prostate cancer. Tumour Biol 2011;32:583-8.
33. Brase JC, Johannes M, Schlomm T, Fälth M, Haese A, Steuber T, *et al.* Circulating miRNAs are correlated with tumor progression in prostate cancer. Int J Cancer 2011;128:608-16.
34. Nguyen HC, Xie W, Yang M, Hsieh CL, Drouin S, Lee GS, *et al.* Expression differences of circulating microRNAs in metastatic castration resistant prostate cancer and low-risk, localized prostate cancer. Prostate 2013;73:346-54.
35. Mahn R, Heukamp LC, Rogenhofer S, von Ruecker A, Müller SC, Ellinger J. Circulating microRNAs (miRNA) in serum of patients with prostate cancer. Urology 2011;77:1265.e9-16.
36. Li Z, Ma YY, Wang J, Zeng XF, Li R, Kang W, *et al.* Exosomal microRNA-141 is upregulated in the serum of prostate cancer patients. Onco Targets Ther 2015;9:139-48.
37. Liu C, Liu R, Zhang D, Deng Q, Liu B, Chao HP, *et al.* MicroRNA-141 suppresses prostate cancer stem cells and metastasis by targeting a cohort of pro-metastasis genes. Nat Commun 2017;8:14270.