Research Article ISSN 2689-1077

Genetics & Molecular Medicine

The miR125b as Biomarkers in The Early Diagnosis of Bladder Cancer

Duran Canatan[1,6*], Özlem Yılmaz[1], Yonca Sönmez[2], Mehmet Baykara[3], Murat Savaş[4], Hasan Şenol Coşkun[5], Sema Sezgin Göksu[5], and Mehmet Rıfkı Aktekin[2]

[1]*Antalya Genetic Diseases Center, Antalya, Turkey.*

[2]*Akdeniz University, Faculty of Medicine, Department of Public Health, Antalya, Turkey.*

[3]*Lara Anadolu Hospital, Department of Urology, Antalya, Turkey.*

[4]*Memorial Hospital, Department of Urology, Antalya, Turkey.*

[5]*Akdeniz University, Medical Faculty, Department of Medical Oncology, Antalya, Turkey.*

[6]*Antalya Bilim University, Antalya, Turkey.*

***Correspondence:**
Prof. Dr. Duran Canatan MD, Antalya Bilim University, Çıplaklı Çıplaklı, Akdeniz Blv. No:290/A, 07190 Döşemealtı/Antalya Turkey, ORCID:0000-0001-8128-8269.

Received: 15 Mar 2022; **Accepted:** 17 Apr 2022; **Published:** 22 Apr 2022

Citation: Canatan D, Yılmaz O, Sönmez Y. The miR125b as Biomarkers in The Early Diagnosis of Bladder Cancer. Genet Mol Med. 2022; 4(1): 1-4.

ABSTRACT

Objective: *Bladder cancer (BCa) is the fifth most common cancer and the second most common urinary tract cancer in worldwide. BCa accounts for 3% of all cancers and is particularly common in developed countries.*

Material and methods: *Eighteen of the 20 patients included in the study were male, 2 were female, mean age was 65.1±12.1(range:37-75) years. The control group consisted of 19 male one female, mean age was 60.3±11.2 (range:40-70) years. A total of seven miRNAs, let-7c, miR-155 , miR-125b, miR-141 miR-145, miR 181 and miR 192 were evaluated in two groups.*

Results: *MiRNAs of bladder cancer patients and healthy individuals were compared according to endogenous miR 181 and miR 192 Delta CT values, miR125b values of bladder cancer cases were found to be significantly higher than healthy controls.(p=0.021) In our study, miR125b, which we found specific and sensitive in endogenous controls, was compatible with studies in the literature.*

As conclusion, we emphasize that mir125b can be used as a predictor in bladder cancer compared with the literature.

Keywords
Bladder cancer, Biomarker, MicroRNAs, mir125b.

Introduction
Bladder cancer (BCa) is the fifth most common cancer and the second most common urinary tract cancer worldwide. BCa accounts for 3% of all cancers and is particularly common in developed countries. In terms of prognosis, it is divided into muscle-invasive (MIBCa) and non-muscle-invasive (NMIBCa). MIBC type has a high recurrence rate after drug therapy and resection and has a poor prognosis [1-3].

Although traditional methods such as cystoscopy, urinary cytology and ultrasound sonography are widely used in diagnosis and screening, It has been published in recent years that micro RNA (miRNA) profiles in urine and blood are a good method for cancer screening. The high reproducibility, specificity and sensitivity of miRNA levels in body fluids indicate their potential use as biomarkers for BCa screening and diagnosis [4,5].

This study was carried out to use miRNAs as a biomarker in patients with BCa.

Material

Blood samples were collected from Department of Oncology of Akdeniz University, Department of Urology Antalya Training and Research Hospital and Lara Anadolu Hospital. Eighteen male and two female patients who were diagnosed with new bladder cancer and, nineteen male and one female were included as control to this study. As inclusion criteria, patients were newly diagnosed, and no treatment was started. As exclusion criteria was the patient's voluntary withdrawal. A total 5 cc of EDTA anti-coagulated blood samples were taken from patients and individuals of control group. An additional 5 cc whole blood samples were also taken from healthy individuals.

Methods

Approval was obtained from Faculty of Medicine Clinical Research Ethics Committee of Akdeniz University. Ethics committee approval number and date 471 and 17.08.2016.

MiRNA selection

A total of seven miRNAs, let-7c, miR-155 , miR-125b, miR-141 miR-145, miR 181 ve miR 192 have been selected according to literature review. Mir-181 and miR-192 were endogenous control according to their expression levels.

MiRNA extraction and measurement from blood samples, the measurement methods of the four available miRNAs in patients and healthy individuals were performed as indicated in our previous study [6]. Plasma samples were separated from 2 cc EDTA anti-coagulated blood using a refrigerated centrifugate at 2000xg for 15 minutes. for all patients and healthy individuals. MiRNA isolation from plasma was performed using mirVana™ miRNA Isolation Kit, with phenol (Invitrogen by Thermo Fisher Scientific, Cat. no: AM1560). The concentration of miRNA was measured using Qubit™ microRNA Assay Kit (Invitrogen by Thermo Fisher Scientific, Cat. No: Q32880) and Qubit-3 Fluorometer and noted as ng/µl units. MiRNA samples, that were in the suitable concentration, converted to cDNA using TaqMan™ Advanced miRNA cDNA Synthesis Kit (Invitrogen by Thermo Fisher Scientific, Cat. no: A28007) and Veriti Thermal Cycler (Applied Biosystems by Thermo Fisher Scientific). Two aliquots of cDNA samples were stored at -20°C until the sufficient numbers of samples were collected to study. TaqMan™ Advanced miRNA assay, TaqMan™ Fast Advanced Master Mix (Applied Biosystems by Thermo Fisher Scientific, Cat. No: A25576 and Cat. No: 4444557 respectively), cDNA and adequate nuclease-free water were mixed to reach a total volume of 20 µl according to the test protocol. Reaction was carried out in duplicates in a 96 well-plate using StepOnePlus™ Real-Time PCR system (Catalog No: 4376598, Thermo Fisher Scientific) [6].

MiRNA expression levels were evaluated using Treshhold cycle (Cт) values. Cт values were automatically exported from the system to an excel file. The mean Cт values of the duplicated samples were calculated and ΔCт values were defined by taking into account of the Ct values of endogenous controls miR 181 and miR 192. The ΔCт values of the individuals with PCa were compared to those of the healthy control group.

Statistical Method

Data were evaluated with SPSS (Statistical Package for the Social Sciences) version 23.0 (SPSS Inc., Chicago, IL, USA) program. Receiver study characteristic (ROC): ROC curve analysis was performed to determine the sensitivity and specificity and diagnostic efficacy of miRNAs [7].

Results

Eighteen of the 20 patients included in the study were male and 2 of them were female, mean age was 65.1 ± 12.1 (range:37-75) years. The control group consisted of 19 male one female, mean age was 60.3 ± 11.2 (range:40-70) years.

The pathology results of the patients were evaluated according to the muscle invasion status; While 12 patients (60%) had muscle invasion (MIBCa), 4 patients (20 %) had NMBCa and 4 patients (20 %) had prostatic adenocarcinoma. In tumor staging; twelve patients were in stage 8 and nine patients in stage 4. While lymph node metastasis was present in two patients, it was absent in eighteen patients, while 17 patients did not have extensive metastases, three patients had extensive metastases (Table 1).

MiRNAs of bladder cancer patients and healthy individuals were compared according to endogenous MiRNA 181 Delta CT values, miR125b values of bladder cancer cases were found to be significantly higher than healthy controls (p=0.021) (Table 2). MiRNAs of patients with bladder cancer and healthy individuals were compared according to endogenous MiRNA 192 Delta CT values; No significant miRNA was found (Table 3).

ROC Analysis

As a result of group comparisons, the diagnostic decision-making properties of miR125b, which had a significant difference between bladder cancer and healthy controls, were analyzed by Receiver Operating Characteristics (ROC) curve analysis.

The closer the value of area under the curve (AUC) was to 1.00, the more important was the miRNA that reflected the significant difference between bladder cancer and healthy controls. In the ROC analysis curve, AUC was significantly high for Delta181CT miR-125b (0.850). The sensitivity and specificity values of Delta181CT miR-125b. with an optimal cut-off value of 0.07532512650. were 75.0% and 100.0%. respectively.

Discussion

In recent years, circulating microRNA (miRNA) profiles have been identified as biomarkers in cancer screening.

A panel of 7 miRNAs (miR-6087, miR-6724-5p, miR-3960, miR-1343-5p, miR-1185-1-3p, miR-6831-5p, and miR-4695-5p) in 486 samples with BCa has been studied that this panel of seven can be a biomarker for the specific and early detection of bladder cancer [4].

The effects of miR-125b-5p on metastasis and in vitro apoptosis

Table 1: Pathological diagnosis and stage metastasis status of bladder cancer cases.

No	Pathological Diagnosis	Stage	T Stage	N Stage	M Stage
1	Muscle-invasive BCa (MIBCa)	4	PTa	N0	M0
2	Muscle-invasive BCa (MIBCa)	3	PT3a	N0	M1
3	Muscle-invasive BCa (MIBCa)	4	PT2c	N1	M0
4	Prostatic adenocarcinoma	3	PT3a	N0	M0
5	Non invasive BCa (NMIBCa)	3	PT4a	N0	M0
6	Muscle-invasive BCa (MIBCa)	3	PTa	N0	M0
7	Muscle-invasive BCa (MIBCa)	4	PTa	N0	M0
8	Muscle-invasive BCa (MIBCa)	4	PTa	N0	M0
9	Muscle-invasive BCa (MIBCa)	3	PT2a	N0	M0
10	Prostatic adenocarcinoma	4	PTa	N0	M0
11	Muscle-invasive BCa (MIBCa)	3	PTa	N0	M1
12	Muscle-invasive BCa (MIBCa)	3	PT3a	N1	M0
13	Non invasive BCa (NMIBCa)	3	PT2c	N0	M0
14	Non invasive BCa (NMIBCa)	4	PT3a	N0	M0
15	Non invasive BCa (NMIBCa)	4	PT4a	N0	M0
16	Prostatic adenocarcinoma	3	PTa	N0	M0
17	Muscle-invasive BCa (MIBCa)	4	PTa	N0	M0
18	Muscle-invasive BCa (MIBCa)	4	PTa	N0	M0
19	Muscle-invasive BCa (MIBCa)	3	PT2a	N0	M0
20	Prostatic adenocarcinoma	3	PTa	N0	M1
	Muscle-invasiveBCa (MIBC):12 Non invasive BCa (NMIBCa):4 Prostatic adenocarcinoma: 4	Stage3:12 Stage 4:8	PTa:10 PT2a: 2 PT2c:2 PT3a:4 PT4a:2	N0: 18 N1:2	M0:17 M1:3

Table 2: MiRNA comparison of bladder cancer and healthy subjects (Delta181CT).

miRNA			
	Mean ± SD†	**Median**	**p**
Let7c			
Bladder ca (n=16)	-0.01108992894 ± 2.132113235488	-0.20952510850	0.251*
Control (n=18)	-0.94377718800 ± 2.475510002142	-0.88966629050	
Mir125b			
Bladder ca (n=16)	0.73719220944 ± 3.374166494455	1.82729053500	**0.02[1]†**
Control (n=5)	-1.58331383060 ± 1.023017110199	-1.73000000000	
Mir141			
Bladder ca (n=12)	-6.51934893967 ± 5.162217582719	-5.94573402400	0.157†
Control (n=13)	5.47854681408 ± 6.549562558008	-3.18200000000	
Mir145			
Bladder ca (n=16)	-1.75522553138 ± 3.556352176895	-1.23654842400	0.559*
Control (n=4)	-2.84025000000 ± .815786481460	-2.68000000000	
Mir155			
Bladder ca (n=13)	3.62428992831 ± 3.540826312263	3.93939018200	0.096*
Control (n=7)	1.09412547486 ± 1.783293348932	1.48800000000	

* Independent samples t test. † Mann-Whitney U testi

in 52 samples with BCa have been shown that miR-125b-5p is downregulated in BCa tissues and cell lines. Patients with low miR-125b-5p expression showed significant correlations with distant metastasis, tumor size and lymph node metastasis, and 5-year survival was found to be lower in these patients [8].

MiR-125b is involved in the regulation of NF-KB, p53, PI3K/Akt/mTOR, ErbB2, Wnt and other signaling pathways, thereby controlling cell proliferation, differentiation, metabolism, apoptosis, drug resistance and tumor immunity [9].

The effect of human trophoblast cell surface antigen 2 (Trop-2),/miR-125b axis on proliferation and migration of BCa cells wase evaluated in vitro study. The importance of the trop-2/miR-125b axis in the diagnosis and prognosis of BC has been noted and proposed as drug delivery targets for innovative therapeutic approaches [10].

The potential role of miR-125b expression levels has been investigated in bladder cancer and disease pathogenesis. The expression level of miR-125b was measured by quantitative polymerase chain reaction (qPCR) in 40 bladder cancer and normal

Table 3: MiRNA comparison of bladder cancer and healthy subjects (Delta 192CT).

miRNA			
	Mean ± SD†	**Median**	**p**
Let7c			
Bladder ca (n=18)	3.76090879089 ± 2.152102234939	3.97873338050	0.573*
Control (n=11)	2.87751182700 ± 4.775456322256	2.86214447000	
Mir125b			
Bladder ca (n=17)	4.76169449306 ± 2.923455542150	4.76171836900	0.929†
Control (n=4)	3.50100000000 ± 5.367380615036	5.98500000000	
Mir141			
Bladder ca (n=8)	-1.94696925223 ± 4.101863283672	-1.99785232500	0.526†
Control (n=9)	-2.30704042156 ± 10.237201367556	2.93885421800	
Mir145			
Bladder ca (n=8)	2.26906507900 ± 2.836289144083	2.54104614300	0.883*
Control (n=9)	1.99600000000 ± 5.068822545720	3.56300000000	
Mir155			
Bladder ca (n=8)	7.49227880815 ± 3.482352226583	7.94336128200	0.372*
Control (n=9)	5.81863133671 ± 4.626845409180	7.62983894300	

* Independent samples t test. † Mann-Whitney U testi

tissues. MiR-125b expression was found to be lower in bladder cancer samples than in adjacent normal tissues ($P < 0.05$) [11]. In our study, miR125b, which we found specific and sensitive in endogenous controls, was compatible with studies in the literature.

Limitations

The small number of cases in our study was the most important limitation. It is planned to increase the number of patients and to evaluate the pathological diagnoses in detail in future validation studies.

Conclusion

As conclusion, we emphasize that mir125b can be used as a predictor in bladder cancer when compared with the literature.

Main Points

Bladder cancer (BCa) is the fifth most common cancer worldwide. Although traditional methods such as cystoscopy, urinary cytology and ultrasound sonography are widely used in diagnosis, screeing is very imprtant for early diagnosis Micro RNAs profiles in urine and blood are a good method for cancer screening in recent years. We found miR125b as specific and sensitive, it, can be used as a predictor in bladder cancer.

Acknowledgement

The current study was funded by Republic of Turkey, Ministry of Science and Industry, KOSGEB Antalya Directorate within the scope of the project entitled “MicroRNA kits in the early diagnosis of cancer” conducted by AGTC Özel Genetik Sağlık Hizm. Tur. San. Tic. Ltd. Şti. Grand Number: 0080785533

References

1. Li Q, Wang H, Peng H, et al. 3MicroRNAs: Key Players in Bladder Cancer. Mol Diagn Ther. 2019; 23: 579-601.
2. Enokida H, Yoshino H, Matsushita R, et al. The role of microRNAs in bladder cancer. Investig Clin Urol. 2016; 57: 60-76.
3. Taheri M, Shirvani-Farsani Z, Ghafouri-Fard S, et al. Expression profile of microRNAs in bladder cancer and their application as biomarkers. Biomed Pharmacother. 2020; 131: 110703.
4. Usuba W, Urabe F, Yamamoto Y, et al. Circulating miRNA panels for specific and early detection in bladder cancer. Cancer Sci. 2019; 110: 408-419.
5. Liu X, Liu X, Wu Y, et al. MicroRNAs in biofluids are novel tools for bladder cancer screening. Oncotarget. 2017; 8: 32370-32379.
6. Canatan D, Özlem Y, Sönmez Y, et al. Circulating microRNAs as Potential Non-invasive Biomarkers for Breast Cancer Detection. Acta Biomed 2021; 92: e2021028.
7. Hajian-Tilaki K. Receiver Operating Characteristic (ROC) Curve Analysis for Medical Diagnostic Test Evaluation. Caspian J Intern Med. 2013; 4: 627-635.
8. Liu S, Chen Q, Wang Y. MiR-125b-5p suppresses the bladder cancer progression via targeting HK2 and suppressing PI3K/AKT pathway. Hum Cell. 2019; 33: 185-194.
9. Wang Y, Zeng G, Jiang Y. The Emerging Roles of miR-125b in Cancers. Cancer Manag Res. 2020; 12: 1079-1088.
10. Avellini C, Licini C, Lazzarini R, et al. The trophoblast cell surface antigen 2 and miR-125b axis in urothelial bladder cancer Oncotarget. 2017; 8: 58642-58653.
11. Zhao X, He W, Li J, et al. MiRNA-125b inhibits proliferation and migration by targeting SphK1 in bladder cancer. Am J Transl Res. 2015; 7: 2346-2354.