Benha Journal of Applied Sciences (BJAS) **print: ISSN 2356–9751**
Vol.(6) Issue(1) Part (2) (2021), (291-293) **online: ISSN 2356–976x**
http://bjas.bu.edu.eg

Urinary MicroRNA96 as Biomarkers for Bladder Cancer Detection

A.H.Attia[1] , O.A.Abdullah[1], M.A.Hassanien[2], S.K.Eliwa[1] and H.E.Ahmed[1]
[1]Biochemistry & Molecular Biology Dept., Faculty of Medicine, Benha Univ., Benha, Egypt
[2]Urology Dept., Faculty of Medicine, Benha Univ., Benha, Egypt
E-Mail:Heba@gmail.com

Abstract

Bladder malignancy is viewed as the fifth most basic disease with expanded disorder and demise. As of late, incessant examinations showed that microRNAs are arising as indicative biomarkers for bladder malignancy. Explicit miRNA profiles have been distinguished for a few examples from patients with bladder malignancy. MicroRNAs are noncoding RNA particles of around 23 nucleotides that assume significant parts in different strides during the movement of bladder malignant growth. Here, we audit the declaration of miRNAs and their organic capacities, guideline, and clinical ramifications in bladder malignant growth. Either downregulation or upregulation of miRNAs happens in bladder malignant growth through epigenetic changes or imperfections of the biogenesis mechanical assembly. Liberation of miRNAs is engaged with cell cycle capture, apoptosis, multiplication, metastasis, drug obstruction, and different capacities in bladder malignancy. Various miRNAs, have been related with tumor type, stage, or patient endurance, and miRNAs may be created as demonstrative or prognostic markers. A superior comprehension of the jobs of miRNAs in bladder malignant growth will reveal insight into the sub-atomic systems of bladder disease.

1. Introduction

Bladder disease (BC) is perhaps the most pervasive urologic malignancies around the world. It has been realized that BC is a heterogeneous sickness with a variable regular history; So, new markers are as yet required in clinical practice either for better determination and therapy [1].

At present, no atomic or hereditary biomarkers are generally consolidated into routine clinical practice [2]. Be that as it may, better comprehension of the sub-atomic modifications in BC will give the premise to consolidation of sub-atomic and hereditary biomarkers into clinical dynamic to direct administration. Clinical use of such novel atomic and hereditary ideas is the key base of presentation of the period of exactness medication into patient consideration [2].

Pee cytology is a fundamental methodology for the identification of urothelial neoplasia. It has for quite some time been realized that pee cytology is precise in the analysis of high-grade urothelial carcinoma. Notwithstanding, it conveys a much lower indicative yield for poor quality urothelial neoplastic injuries [3]. Numerous investigations have assessed the precision of pee cytology in the location of bladder malignant growth. Generally speaking, the announced affectability goes from 20% to 97.3%; explicitness goes from 74% to 99.5% [3].

Various urinary markers have been researched, with the point of diminishing recurrence of cystoscopy (4). A few are financially accessible, yet none has been embraced into routine norm of care, attributable to helpless affectability as well as cost. These markers may fill in as an adjunctive analytic test in situations where pee cytology is dubious [3].

miRNAs are endogenous, non-coding short RNA atoms ~22 nucleotides (nt) long that are richly communicated across organs and species. They are considered to manage the cleavage of target mRNAs or simply quell their interpretation at posttranscriptional level.

MiR-96 has a place with the miR-183 family [5]. The coding succession of miR-96 is situated in chromatin 7q32.2, between two protein-coding areas. miRNA-96 is one individual from the miR-183-96-182 group [6]. miR-96 has been discovered to be upregulated in different human diseases like bosom, lung, liver, colon, prostate, ovary, testis malignant growth and lymphoma, proposing the outflow of miR-96 was related with the movement of tumor [7].

The objective qualities of miR-96 incorporate the tumor silencer qualities FOXO1 and FOXO3a in bosom malignancy [8] and other approved focuses of miR-96 remember RECK for esophageal disease [16], EphrinA5 in Hepatocellular carcinoma [7], SAMD9 in non-little cell cellular breakdown in the lungs (NSCLC) [10].

2. Subject and methods

This study was completed between July 2018 and July 2020 after endorsement of the examination plot by the exploration moral advisory group of Benha Faculty of Medicine and acquiring educated assent from the included subjects. The investigation included 70 subjects of both sex chose from Urology Department, Faculty of Medicine, Benha University Hospital.

The subjects were sorted into 2 gatherings: bladder disease gathering: included 50 patients, analyzed as bladder malignancy patients by clinical, radiological assessments and control gathering: included 20 people, age and sex coordinated, with histopathologically typical urothelium.

All patients were exposed to full history taking with regard for: exceptional propensities including, tobacco smoking and numerous examinations, demonstrative cystoscopy and biopsy for histopathology. Quantitative converse record polymerase chain response (qRT-PCR) for discovery of miRNAs articulation levels.

Voided pee tests were gotten from all people. Pee tests were gathered utilizing sterile pee assortment cups, fixed promptly and set on ice put away at - 80oc and centrifuged at 2,500–4,000 xg for 15–20 min. Extraction of miRNA from pee pellet tests: utilizing the PureLink® RNA Mini Kit (Ambion, USA) as per maker's guidelines. cDNA amalgamation was finished utilizing RevertAid First

Strand cDNA Synthesis Kit (Thermo Scientific, USA) as per producer's guidelines. qRT-PCR for recognition of miR-96 utilizing Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific, USA) as per maker's instructions.

Statistical analysis

The collected data were summarized in terms of mean ± StandardDeviation (SD) and range for quantitative data; and frequency andpercentage for qualitative data. Comparisons between cases and control were carried out using the student T- test, to compare quantitative data between two groups and Chi- squared (χ^2) test, to compare proportions of two or more groups. Pearson correlation was used to estimate the correlation between miRNA-96, miRNA-126 and age of the studied group. The corresponding test statistics were calculated and the corresponding P-values were obtained. P-value 0.05 was considered statistically significant, while P-value > 0.05 was considered statistically non-significant. Analysis is performed using the Statistics Program for Social Sciences (SPSS) and Microsoft Office Excel is used for the data processing and data analysis.

3. Results

70 patients were included in the study. 50 patients were diagnosed with bladder cancer while the remaining 20, having no evidence of malignancy, were included as controls.

Demographic data of studied groups

The baseline characteristics of the study population are presented in Table-1. There was insignificant difference between cases and control groups regarding age and gender (p=0.1; 0.7 respectively)

Table (1) Demographic data of the studied groups.

		Cases group	Control group	p- value
Age	mean±SD	57.4±6.45	61.0± 8.9	0.1
Sex				0.7
Male	N (%)	44 (88%)	17 (85%)	
Female	N (%)	6 (12%)	3 (15%)	

Xpression level of miRNA 96 of studied groups microRNA 96, were studied and their expression was analyzed in urine samples. The expression of miR-96 was significantly higher (p< 0.001) in bladder cancer tissues as compared to controls Table (2).

Table (2) Difference between bladder cancer group and control group regarding miR 96 relative expression.

Study Group MiR-96 (Log)	Control group	Bladder cancer group	T- Test	P-value
Mean	4.2	5.2	6.3	< 0.001
± SD	± 0.5	± 0.9		

SD: Standard deviation, P 0.05 ≤ significant, P > 0.05 non-significant, analysis done by independent samples Student T test.

4. Discussion

Bladder disease is as yet the most well-known dangerous tumor amongmales in Egypt, some African and Middle East nations [10]. As per the National Cancer Institute in Cairo, Egypt, it comprises 30.3%, all things considered [11]. The yearly passing rate from this sickness is huge and consistently there is an expansion in its occurrence all around the world [12].

Bladder malignancy patients at beginning phase could be treated by revolutionary medical procedure, which may improve the patients' life quality and guess. In any case, those patients at cutting edge stage may just be treated by chemotherapy, radiotherapy and focused on treatment, and the clinical adequacy is very unique among various people. Accordingly, the early finding of bladder malignant growth is firmly associated with the clinical result, showing that the approval of compelling biomarkers for early determination of bladder disease is crucial to the clinical administration of such patients [13].

The pathogenesis of malignant growth is a mind boggling measure; different viewpoints influence its beginning and advancement. A normally held view is that the imbalanced articulation of oncogenes and tumor silencer qualities adds to tumor cell expansion, and intrusion [14].

The current techniques for bladder disease finding are pee cytologyand cystoscopy. Pee cytology is a technique with 95% explicitness butlow affectability, particularly in poor quality tumors. Cystoscopy is the current highest quality level strategy for bladder malignancy identification, however it is an invasiveand costly system with low explicitness and affectability in detectingflat CIS tumors [6].

Accordingly, there have been heaps of endeavors in the field to discover touchy, and explicit sub-atomic markers for bladder malignant growth [15]. It has been proposed that miRNAs are helpful in this regard [16]. In the previous few years, collective exploration has shown the significant jobs of miRNAs in the advancement of BC [17].

MiRNAs are a class of exceptionally saved little RNAs that tight spot the 3'- UTR area of its objective quality and manage the statement of target qualities. The association of miRNAs in quality administrative cycles and their suggestion in a few illnesses, including malignancy, makes them appealing for determination, anticipation, and

treatment in clinical application [18]. The expanding number of studies exploring miRNA articulation profiles explicit to bladder disease demonstrate the developing revenue in looking for explicit miRNAs to work as analytic biomarkers [15].

Ongoing examinations showed that miR-96 was habitually expanded in a few human malignant growths. MiR-96 has been accounted for to apply an oncogenic impact in non-little cell cellular breakdown in the lungs, esophageal malignancy, hepatocellular carcinoma, bosom disease [5]. The objective qualities of miR-96 incorporate the tumor silencer qualities FOXO1 and FOXO3a in bosom malignancy [19] and other approved focuses of miR-96 remember RECK for esophageal disease [18], EphrinA5 in Hepatocellular carcinoma [19], SAMD9 in non-little cell cellular breakdown in the lungs (NSCLC) [17].

It very well may be reasoned that the huge expansion in miR-96 articulation level in bladder malignancy patients contrasted with the controls proposes its part as a tumor diagnostic markers.

5. Conclusion

It could be concluded that the significant increase in miR-96 expression level in bladder cancer patients compared to the controls suggests its role as a tumor diagnostic markers.

References

[1] K. Saginala, A. Barsouk, J.S.Aluru, Epidemiology of bladder cancer. Med Sci [Internet]. Mar 13 [cited 2021 Feb 25], Vol.8(1),PP.11–25,2020.

[2] K.D.Davis , N. Aghaeepour, A.H.Ahn , Discovery and validation of biomarkers to aid the development of safe and effective pain therapeutics: challenges and opportunities. Nat Rev Neurol [Internet]. Jul 1 [cited 2021 Feb 25], Vol.16(7),PP.381–400,2020.

[3] R.T.Vollmer , B. Case, A. Aprikian, Accuracy of Urine Cytology and the Significance of an Atypical Category. Am J Clin Pathol [Internet]. Nov 1 [cited 2021 Feb 26], Vol.132(5),PP.785–93,2009.

[4] V. Yutkin, B. Nisman, D.Pode, Can urinary biomarkers replace cystoscopic examination in bladder cancer surveillance? Expert Rev Anticancer Ther [Internet]. Jun 10 [cited 2021 Feb 26], Vol.10(6),PP.787–90,2010.

[5] Z. Yang, Z. Liu,The Emerging Role of MicroRNAs in Breast Cancer., Journal of Oncology. Hindawi Limited, Vol.70(8), pp415-425, 2020.

[6] C.Z.Zhu , H.N.Ting , K.H.Ng , A review on the accuracy of bladder cancer detection methods [Inter net]. Journal of Cancer. Ivyspring International Publisher; [cited 2021 Feb 26] ,Vol. 10,PP. 4038–44,2019.

[7] X. Yang, Q. Zhang, M. Zhang, Serum microRNA signature is capable of early diagnosis for non-small cell lung cancer. Int J Biol Sci [Internet]. [cited 2021 Feb 27], Vol.15(8),PP.1712–22,2019.

[8] C. Li, K. Zhang, J. Chen, MicroRNAs as regulators and mediators of forkhead box transcription factors function in human cancers [Internet]. Oncotarget. Impact Journals LLC; [cited 2021 Feb 27] ,Vol. 8,PP. 12433–50,2017.

[9] C.T.Yeh, J.Y.Ho , K.F.Ng , T.C. Chen, OncomiR miR-96 and miR-182 promote cell proliferation and invasion through targeting ephrinA5 in hepatocellular carcinoma. Mol Carcinog [Internet]. Apr 1 [cited 2021 Feb 27], Vol.55(4):,PP.366–75,2016.

[10] L. Wu, X. Pu, Q. Wang, miR-96 induces cisplatin chemoresistance in non-small cell lung cancer cells by downregulating SAMD9. Oncol Lett [Internet]. Feb 1 [cited 2021 Feb 27],Vol.11(2),PP.945–52,2016.

[11] N.Eissa , E.N.A El-Ghiet. , A.A.Shaheen , Characterization of pseudomonas species isolated from tilapia. undefined, Vol.60(8),PP.225-235, 2010;

[12] M.V.Khochikar ,Rationale for an early detection program for bladder cancer. In: Indian Journal of Urology [Internet]. Indian J Urol; [cited 2021 Feb 27],Vol. 25,PP. 218–25,2011.

[13] A.Fendler, C.Stephan, G.M.Yousef , G. Kristiansen, The translational potential of microRNAs as biofluid markers of urological tumours [Internet]. Nature Reviews Urology. Nature Publishing Group; [cited 2021 Feb 27], Vol. 13,PP. 734–52,2016.

[14] Zhou K, Liu M, Cao Y. New Insight into microRNA Functions in Cancer: Oncogene–microRNA–Tumor Suppressor Gene Network. Front Mol Biosci , Vol.4(JUL),PP.46,2017.

[15] B.J.Schmitz-Dräger , M.Droller , V.B.Lokeshwar , Molecular markers for bladder cancer screening, early diagnosis, and surveillance: The WHO/ICUD consensus [Internet], Vol. 94,PP. 1–24,2015.

[16] N. Dip, S.T.Reis , L.S. Timoszczuk, Stage, grade and behavior of bladder urothelial carcinoma defined by the microrna expression profile. J Urol. Nov, Vol.188(5),PP.1951–6,2012.

[17] X. Wang, Y.Zhou, Q.Gao , The Role of Exosomal microRNAs and Oxidative Stress in Neurodegenerative Diseases. Oxidative medicine and cellular longevity. NLM (Medline), vol 50(8),PP. 3232869. 2020

[18] D.A.Armstrong , A.B.Nymon , C.S.Ringelberg , Pulmonary microRNA profiling: implications in upper lobe predominant lung disease. Clin Epigenetics [Internet]. 2017 May 30 [cited 2021 Feb 27], Vol.9(1),PP. 314-324,2017.

[19] F. Gao, W.Wang, MicroRNA-96 promotes the proliferation of colorectal cancer cells and targets tumor protein p53 inducible nuclear protein 1, forkhead box protein O1 (FOXO1) and FOXO3a. Mol Med Rep [Internet]. 2015 Feb 1 [cited 2021 Feb 27],Vol.11(2),PP.1200–6,2015.

[20] J.Wang , J. Chen, S.Sen, MicroRNA as Biomarkers and Diagnostics. J Cell Physiol [Internet]. 2016 Jan 1 [cited 2021 Feb 27],Vol.231(1), Vol.25–30,2016.