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MicroRNA-508-3p Level in the Blood of Kidney Cancer Patients

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As of today, no marker for kidney cancer (KC) was discovered. Over the last decade, it was found a few microRNAs demonstrating the aberrant expression in various biological fluids of KC patients. Among of them miR-508-3p is one of the potential KC markers.
The aim of our study was to establish the expression of miR-508-3p in the blood of patients with KC and healthy subjects.
Levels of miR-508-3p were quantified in the blood samples of 67 patients with renal cell carcinoma and 64 controls using real-time PCR. The level of this microRNA appeared significantly lower in KC patients compared to controls. The cut-off level of miR-508-3p in KC is 12.7 relative units. Sensitivity of miR-508-3p level for KC in our study is 82,% and specificity – 89,6%.
In conclusion, miR-508-3p level in blood can be considered as a possible diagnostic marker of kidney cancer.
Key words: *kidney cancer, microRNA, miR-508-3p, marker.*

Widespread use of highly specific tumor markers for cancer screening enables clinicians to detect tumors of different histological nature and localization, as well as to monitor the disease.

Depending on the biological environment in which they are defined, tumor markers can be divided into different groups: tissue, blood and urine markers [1, 4]

Unlike many diseases in oncourology, no marker of kidney cancer (KC) was found. Over the past decade, the experts made many attempts to establish the diagnostic value of microRNA expression in kidney tissue, blood and urine of KC patients. Generally accepted, that through convenience in sampling blood and urine are considered as promising test material providing the possibility to use it for markers screening.

Previously, Q. Zhai and colleagues first provided the evidence of the diagnostic value of miR-508-3p as a tissue and blood marker for renal cell carcinoma (RCC). This conclusion was made by analyzing the expression of miR-508-3p in tumor biopsies and blood samples of 10 patients with KC and healthy volunteers. The authors summarized that to establish the true value of miR-508-3p as biomarker for RCC requires further study [13].

We have conducted a pilot study with a similar design and definition of the expression of miR-508-3p in the blood of 28 patients with KC and 27 healthy subjects and found that the expression of this microRNA in KC patients was significantly higher compared to the controls [2]. However, given the small number of patients, these results stimulated us to perform the study with more patients. Results of this study are provided in the present manuscript.

MATERIALS AND METHODS

We have collected 67 blood samples from KC patients in the period between 2013–2017. All patients in KC group were diagnosed with kidney cancer verified by the results of preoperative biopsies and/or postoperative histopathological studies. Control samples were taken from 64 persons without CK. The collected samples were stored prior to RNA extraction at -25 °C.

Total RNA was purified from the samples followed by reverse transcription and TaqMan-based real-time PCR to determine the level of miR-508-3p. RNA extraction and microRNA quantification were performed at the Department of General and Molecular Pathophysiology, Bogomoletz Institute of Physiology NAS of Ukraine. Data were analyzed using statistical methods [6, 12].

Total RNA purification from blood samples

Total RNA was isolated from the blood samples using mirVana PARIS kit (Ambion, USA) according to the protocol suggested by the manufacturer. RNA concentration was measured using a spectrophotometer NanoDrop ND1000 (NanoDrop Technologies, USA).

Reverse transcription and real-time PCR

Reverse transcription was performed using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, USA), specific looped primers for each microRNA and 10 ng of total RNA. Quantitative Real-Time PCR was performed using TaqMan MicroRNA Assays (Applied Biosystems, USA): U6 snRNA (as endogenous control) and hsa-miR-508-3p. Amplification was performed on the 7500 Fast Real-Time PCR (Applied Biosystems, USA). The data were analyzed using software 7500 Fast Real-time PCR (Figure 1) [2].

Statistical data analysis

The data were analyzed using the following statistical methods. Kolmogorov-Smirnov test was used to check the normality of data distribution, Levene's test – to evaluate the

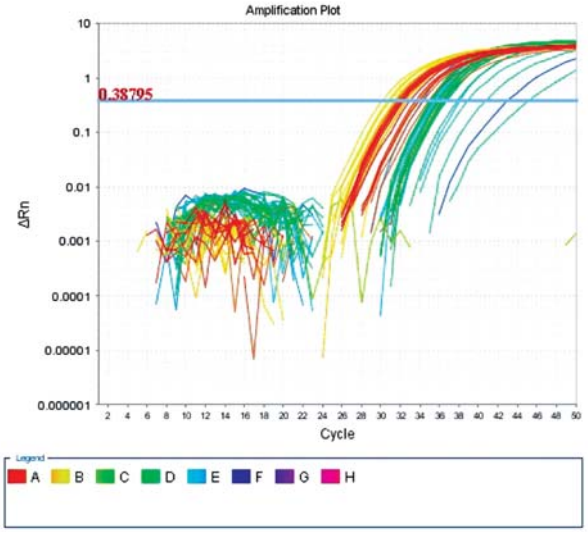

Fig. 1. Intensity of fluorescence during real-time PCR

Table 1

Descriptive statistics of miR-508-3p level in patients with KC and in the control group

	n	Mean	Standard deviation	95% confidence intervals		min	max
				Lower	Upper		
Kidney cancer	67	7,5	4,2	5,6	8,7	1,3	22,5
Controls	64	23,8	3,7	20,7	25,3	18,2	28,3

homogeneity of variance between the groups, one-way ANOVA was used to compare means between homogenic groups, otherwise we used Welch and Brown-Forsythe tests.

RESULTS AND DISCUSSION

We have found that the values of miR-508-3p levels were normally distributed according to Kolmogorov-Smirnov test (Z=1,364; p=0,068). Results of Levene's test (L=9,273; p=0,003) indicated that the variances in tested groups are heterogenic, and to compare the means we have used robust Welch and Brown-Forsythe tests.

Visualization of results are presented in Fig. 2 and table 1.

The results of robust (Welch and Brown-Forsythe) tests are presented in Table 2. According to results of both tests, level of miR-508-3p is significantly lower in KC patients compared to controls (p=0,01).

The results of miR-508-3p quantification in the blood of KC patients and controls indicate its high classification ability: the specificity of miR-508-3p for KC is 89,6% and sensitivity – 82,8%.

By calculating the Youden index (J = max (sensitivity + specificity)) cut-off value (cut-off) was calculated for the determination of specificity and sensitivity of miR-508-3p as a marker. According to our results, cut-off is 12,3±0,4 in relative units with a sensitivity of 82,8% and specificity of 89,6%.

Given these results and the fact that the level of studied microRNA was significantly lower in KC patients compared to controls, 12,3±0,4 relative units, that is 12.7 relative units can be considered as the cut-off level of miR-508-3p for kidney cancer. All cases of marker expression lower than this limit can be considered KC with the 82,8% accuracy (sensitivity).

There is no specific microRNA able to classify patients as KC or control. Our previous and present results demonstrate that miR-508-3p is potentially such marker. Results of the present study indicate that in cases the level of miR-508-3p in blood within 7,5±4,2 is associated with a high risk of KC, in cases its level is within 23,8±3,7 and higher, with high probability (89,6%) the patient has not KC. The interval from 7,5±4,2 (11,7) to 23,8–3,7 (20,1) is the so-called «gray zone» when the probability of presence of KC varies within 5–10%, however, it is impossible to predict the histological structure of the tumor, and patients need the monitoring of miR-508-3p level over time. The level of miR-508-3p in patient's blood higher than 20,1 relative units likely indicates a lack of KC.

Studies of recent decade found other microRNAs that could be considered as a marker of kidney cancer. L.M. Wulfken et al. in 2011 reported that miR-1233 is overexpressed in patients with KC [11]. The sensitivity of this marker reaches 77,4%, specificity – 37,6%. M. Redova et al. found that the simultaneous quantification of miR-451 and miR-378 in serum enables detection of KC in patients with sensitivity of 81% and specificity of 83% [8].

H. Tusong et al consider miR-21 and miR-106a as molecular markers for KC, since expression levels of these microRNAs in serum of KC patients significantly differs from that in controls [10].

According to R. Nofech-Mozes et al, higher level of microRNA-194 is a positive prognostic factor in treatment of KC and small renal tumors patients [5].

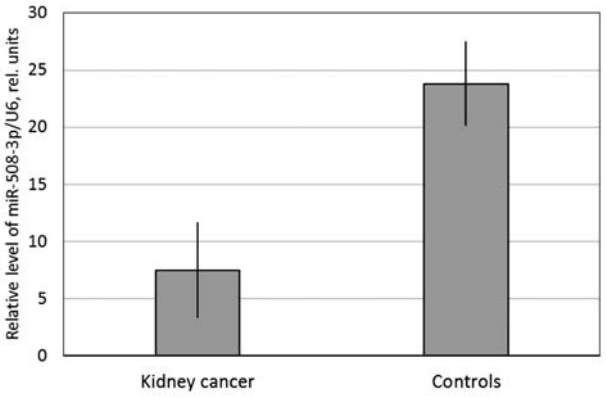

Fig. 2. Relative level of miR-508-3p in the blood of KC patients compared to controls.
Data are presented as mean ± standard deviation

Table 2

Results of robust tests for comparison of miR-508-3p means in KC patients and controls

Test	Criteria	p
Welch	54,46	0,01
Brown-Forsythe	55,28	0,01

Diagnostic and prognostic value of miR-21 in patients with KC was investigated by Arezoo Rasti et al. The researchers concluded that this microRNA is involved in apoptosis, proliferation of cancer cells in KC patients, and its expression can be a prognostic factor for the disease [7].

L. Gu et al. found that increased expression of miR-21, miR-1260b, miR-210, miR-100, miR-125b, miR-221, miR-630, and miR-497 is associated with progression of KC in while downregulation of miR-106b, miR-99a, miR-1 826, miR-215, miR-217, miR-187, miR-129-3p, miR-23b, miR-27b and miR-126 is also a sign of worsening prognosis for patients [3].

S. Samaan et al. describe miR-210 as a criterion for survival prognosis of KC patients [9].

In summary, our results and data from other research centers demonstrate that microRNAs are promising markers in the diagnostics of kidney cancer, and some of microRNAs will be validated as KC markers. Although, in order, to validate it, large multi-center studies with multiple patients are required. The further investigation of this problem in Ukraine requires improvement of research facilities in universities and clinics.

CONCLUSIONS

1. miR-508-3p (miR-508-3p) can be considered as a diagnostic marker of kidney cancer in the blood with the specificity of 89.6% and sensitivity of 82,8%.

2. To confirm the diagnostic value of the expression of miR-508-3p kidney cancer should be further multicenter studies involving more patients.

Рівень miR-508-3p у крові хворих на рак нирки

О.О. Строй

Станом на сьогодні універсальний маркер раку нирки (РН) відсутній. Протягом останнього десятиліття було встановлено декілька мікроРНК, які демонструють аберантну експресію у різних біологічних середовищах хворих на РН. В якості одного з таких потенційних маркерів розглядається miR-508-3p.
Метою дослідження було встановлення експресії miR-508-3p у сироватці крові хворих на РН та здорових досліджуваних.
Після аналізу рівнів експресії miR-508-3p у сироватці крові 67 хворих на РН та у 64 здорових досліджуваних встановлено, що рівень експресії зазначеної мікро-РНК при РН суттєво нижчий, ніж у контрольній групі. Порогове значення (cut-off) рівня miR-508-3p при РН становить 12,7 у.о.
Чутливість miR-508-3p у діагностиці РН у дослідженні становить 82,8%, специфічність – 89,6%.
Отже, рівень miR-508-3p у крові можна розглядати в якості потенційного діагностичного маркера раку нирки.

Ключові слова: *рак нирки, мікроРНК, miR-508-3p, маркер.*

Уровень miR-508-3p в крови больных раком почки

А.А. Строй

По состоянию на сегодня универсальный маркер рака почки (РП) отсутствует. В течение последнего десятилетия было установлено несколько микроРНК, которые демонстрируют аберрантную экспрессию в различных биологических средах больных РП. В качестве одного из таких потенциальных маркеров рассматривается miR-508-3p.
Целью исследования было установление экспрессии miR-508-3p в сыворотке крови больных РП и здоровых испытуемых.
После анализа уровней экспрессии miR-508-3p в сыворотке крови 67 больных РП и у 64 здоровых испытуемых установлено, что уровень экспрессии указанной микроРНК при РП существенно ниже, чем в контрольной группе. Пороговое значение (cut-off) экспрессии miR-508-3p при РП составляет 12,7 у.е.
Чувствительность miR-508-3p при диагностике РП в исследовании составляет 82,8%, специфичность – 89,6%.
Таким образом, miR-508-3p можно рассматривать в качестве вероятного диагностического маркера крови рака почки.

Ключевые слова: *рак почки, микроРНК, miR-508-3p, маркер.*

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REFERENCES

1. Банира О.Б. Рак нирки: пошук універсального маркера / О.Б. Банира, О.О. Строй, О.В. Шуляк // Укр. мед. часопис. – 2012. – № 2 (88). – С. 55–61.
2. Строй О.О. Цінність мікро-РНК 508-3р у діагностиці раку нирки / О.О. Строй, О.Б. Банира, В.Є. Досенко та співавт. // Укр. мед. часопис. – 2012. – № 6 (92). – С. 118–121.
3. Gu L. MicroRNAs as prognostic molecular signatures in renal cell carcinoma: a systematic review and meta-analysis / L. Gu, H. Li, L. Chen et al. // Oncotarget. – 2015. – № 6 (32). – P. 32545–60.
4. Li M. Biomarkers for Renal Cell Carcinoma. Kidney Cancer: Principles and Practice, Primo N. Lara Jr., Jonasch Eric (Eds.) / M. Li, W.K/ Rathmell. – Springer, 2012. – P. 47–65.
5. Nofech-Mozes R. MicroRNA-194 is a Marker for Good Prognosis in Clear Cell Renal Cell Carcinoma / R. Nofech-Mozes, H.W. Khella, A. Scorilas et al. // Cancer Med. – 2016. – 5 (4). – P. 656–664.
6. Norman G.R. Biostatistics: The Bare Essentials, 3rd Edition / G.R. Norman, D.L. Streiner // PMKC-USA, 2008. – 393 p.
7. Rasti A. Diagnostic and prognostic accuracy of miR-21 in renal cell carcinoma: a systematic review protocol / A. Rasti, M. Mehrazma, Z. Madjd et al. // BMJ Open. – 2016. – Jan 4, № 6 (1). – P. e009667.
8. Redova M. Circulating miR-378 and miR-451 in serum are potential biomarkers for renal cell carcinoma / M. Redova, A. Poprach, J. Nekvindova et al. // J. Transl. Med. – 2012. – № 10 (1). – P. 55.
9. Samaan S. miR-210 is a prognostic marker in clear cell renal cell carcinoma / S. Samaan, H.W. Khella, A. Girgis et al. // J Mol Diagn. – 2015, Mar. – № 17 (2). – P. 136–144.
10. Tusong H. Functional analysis of serum microRNAs miR-21 and miR-106a in renal cell carcinoma / H. Tusong, N. Maolakuerban, J. Guan et al. // Cancer Biomark. – 2017. – № 18(1). – P. 79–85.
11. Wulfken L.M. MicroRNAs in renal cell carcinoma: diagnostic implications of serum miR-1233 levels / L.M. Wulfken, R. Moritz, C. Ohlmann et al. // PLoS One. – 2011. – № 6 (9). – P. e25787.
12. Zar J.H. Biostatistical Analysis, 5th Edition / J.Y. Zar. – New York: Prentice Hall, 2009. – 960 p.
13. Zhai Q. Identification of miR-508-3p and miR-509-3p that are associated with cell invasion and migration and involved in the apoptosis of renal cell carcinoma / Q. Zhai, L. Zhou, C. Zhao et al. // Biochem. BioKCys. Res. Commun. – 2012. – № 419 (4). – P. 621–626.

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