South Russian Journal of Cancer. 2024. Vol. 5, No. 3. P. 76-90
https://doi.org/10.37748/2686-9039-2024-5-3-7
https://elibrary.ru/zovxqo
ORIGINAL ARTICLE

Urine transcriptomic profile in terms of malignant ovarian tumors

D. S. Kutilin[1]✉, F. E. Filippov[2], N. V. Porkhanova[2], A. Yu. Maksimov[1]

[1] National Medical Research Centre for Oncology, Rostov-on-Don, Russian Federation
[2] Clinical Oncology Dispensary No. 1, Krasnodar, Russian Federation
✉ k.denees@yandex.ru

ABSTRACT

Purpose of the study. Bioinformatic search for transcriptomic markers (based on metabolomic data) and their validation in the urine of serous ovarian adenocarcinoma patients.
Materials and methods. The study included 70 patients with serous ovarian adenocarcinoma and 30 conditionally healthy individuals. The search for metabolite regulator genes and gene regulator microRNAs was performed using the Random forest machine learning method. Ribonucleic acid (RNA) was isolated using the RNeasy Plus Universal Kits. The level of microRNA transcripts in urine was determined by real-time PCR. Differences were assessed using the Mann-Whitney test with Bonferroni correction.
Results. Using the Random forest method, metabolite-regulator gene (47 genes) and metabolite-regulator microRNA (613 unique microRNA) relationships were established. The identified microRNAs were validated by real-time PCR. Changes in the levels of microRNA transcripts were detected: miR-382-5p, miR-593-3p, miR-29a-5p, miR-2110, miR-30c-5p, miR-181a-5p, let-7b-5p, miR-27a-3p, miR-370-3p, miR-6529-5p, miR-653-5p, miR-4742-5p, miR-2467-3p, miR-1909-5p, miR-6743-5p, miR-875-3p, miR-19a-3p, miR-208a-5p, miR-330-5p, miR-1207-5p, miR-4668-3p, miR-3193, miR-23a-3p, miR-12132, miR-765, miR-181b-5p, miR-4529-3p, miR-33b-5p, miR-17-5p, miR-6866-3p, miR-4753-5p, miR-103a-3p, miR-423-5p, miR-491-5p, miR-196b-5p, miR-6843-3p, miR-423-5p and miR-3184-5p in the urine of patients compared to conditionally healthy individuals.
Conclusion. Thus, urine transcriptome profiling allowed both to identify potential disease markers and to better understand the molecular mechanisms of changes underlying ovarian cancer development.

Keywords: microRNAs, polymerase chain reaction, machine learning, bioinformatics, ovarian serous adenocarcinoma, urine, biomarkers

For citation: Kutilin D. S., Filippov F. E., Porkhanova N. V., Maksimov A. Yu. Urine transcriptomic profile in terms of malignant ovarian tumors. South Russian Journal of Cancer. 2024; 5(3): 76-90. https://doi.org/10.37748/2686-9039-2024-5-3-7, https://elibrary.ru/zovxqo

For correspondence: Denis S. Kutilin – Cand. Sci. (Biol.), Leading Researcher, Laboratory of Molecular Oncology, National Medical Research Centre for Oncology, Rostov-on-Don, Russian Federation
Address: 63 14 line str., Rostov-on-Don 344037, Russian Federation
E-mail: k.denees@yandex.ru
ORCID: https://orcid.org/0000-0002-8942-3733
SPIN: 8382-4460, AuthorID: 794680
Scopus Author ID: 55328886800

Compliance with ethical standards: the research study is carried out in compliance with the ethical principles set forth by World Medical Association Declaration of Helsinki, 1964, ed. 2013. The study was approved by the Committee on Biomedical Ethics at the National Medical Research Center for Oncology (extract from the minutes of the meeting No. 15 dated 06/14/2022). Informed consent was received from all participants of the study

Funding: this work was not funded. The work was performed with scientific equipment provided by the Central Research Institute of the National Medical Research Center for Oncology: https://ckp-rf.ru/catalog/ckp/3554742/

Conflict of interest: the authors declare that there are no obvious and potential conflicts of interest associated with the publication of this article

The article was submitted 19.07.2024; approved after reviewing 22.08.2024; accepted for publication 27.08.2024

Южно-Российский онкологический журнал. 2024. Т. 5, № 3. С. 76-90
https://doi.org/10.37748/2686-9039-2024-5-3-7
https://elibrary.ru/zovxqo
3.1.6. Онкология, лучевая терапия
ОРИГИНАЛЬНАЯ СТАТЬЯ

Транскриптомный профиль мочи при злокачественных новообразованиях яичника

Д. С. Кутилин[1]✉, Ф. Е. Филиппов[2], Н. В. Порханова[2], А. Ю. Максимов[1]

[1] ФГБУ «Национальный медицинский исследовательский центр онкологии» Министерства здравоохранения Российской Федерации, г. Ростов-на-Дону, Российская Федерация
[2] ГБУЗ «Клинический онкологический диспансер № 1» Министерства здравоохранения Краснодарского края, г. Краснодар, Российская Федерация
✉ k.denees@yandex.ru

РЕЗЮМЕ

Цель исследования. Биоинформатический поиск транскриптомных маркеров (на основании метаболомных данных) и их валидация в моче больных серозной аденокарциномой яичников.
Материалы и методы. В исследование было включено 70 пациенток с диагнозом серозная аденокарцинома яичников и 30 условно здоровых индивидуумов. Поиск генов-регуляторов метаболитов и микроРНК регуляторов генов осуществляли с использованием метода машинного обучения Random forest. Выделение рибонуклеиновой кислоты (РНК) производили с помощью набора RNeasy Plus Universal Kits. Уровень транскриптов микроРНК в моче определяли методом полимеразной цепной реакции (ПЦР) в режиме реального времени. Оценку различий проводили с использованием критерия Манна-Уитни с поправкой Бонферрони.
Результаты. С использованием метода Random forest были установлены взаимосвязи метаболит-ген регулятор (47 генов) и метаболит-микроРНК регулятор (613 уникальных микроРНК). Выявленные микроРНК были валидированы методом ПЦР в режиме реального времени. Обнаружено изменения уровня транскриптов микроРНК miR-382-5p, miR-593-3p, miR-29a-5p, miR-2110, miR-30c-5p, miR-181a-5p, let-7b-5p, miR-27a-3p, miR-370-3p, miR-6529-5p, miR-653-5p, miR-4742-5p, miR-2467-3p, miR-1909-5p, miR-6743-5p, miR-875-3p, miR-19a-3p, miR-208a-5p, miR-330-5p, miR-1207-5p, miR-4668-3p, miR-3193, miR-23a-3p, miR-12132, miR-765, miR-181b-5p, miR-4529-3p, miR-33b-5p, miR-17-5p, miR-6866-3p, miR-4753-5p, miR-103a-3p, miR-423-5p, miR-491-5p, miR-196b-5p, miR-6843-3p, miR-423-5p и miR-3184-5p в моче пациенток относительно условно-здоровых индивидуумов.
Заключение. Таким образом, транскриптомное профилирование мочи позволило как выявить потенциальные маркеры заболевания, так и лучше понять молекулярные механизмы изменений, лежащих в основе развития рака яичников.

Ключевые слова: микроРНК, полимеразная цепная реакция, машинное обучение, биоинформатика, серозная аденокарцинома яичника, моча, биомаркеры

Для цитирования: Кутилин Д. С., Филиппов Ф. Е., Порханова Н. В., Максимов А. Ю. Транскриптомный профиль мочи при злокачественных новообразованиях яичника. Южно-Российский онкологический журнал. 2024; 5(3): 76-90. https://doi.org/10.37748/2686-9039-2024-5-3-7, https://elibrary.ru/zovxqo

Для корреспонденции: Кутилин Денис Сергеевич – к.б.н., ведущий научный сотрудник лаборатории молекулярной онкологии, ФГБУ «Национальный медицинский исследовательский центр онкологии» Министерства здравоохранения Российской Федерации, г. Ростов-на-Дону, Российская Федерация
Адрес: 344037, Российская Федерация, г. Ростов-на-Дону, ул. 14 линия, д. 63
E-mail: k.denees@yandex.ru
ORCID: https://orcid.org/0000-0002-8942-3733
SPIN: 8382-4460, AuthorID: 794680
Scopus Author ID: 55328886800

Соблюдение этических стандартов: в работе соблюдались этические принципы, предъявляемые Хельсинкской декларацией Всемирной медицинской ассоциации (World Medical Association Declaration of Helsinki, 1964, ред. 2013). Исследование одобрено Комитетом по биомедицинской этике при ФГБУ «Национальный медицинский исследовательский центр онкологии» Министерства здравоохранения Российской Федерации (выписка из протокола заседания № 15 от 14.06.2022 г.). Информированное согласие получено от всех участников исследования

Финансирование: финансирование данной работы не проводилось. Работа выполнена с использованием научного оборудования ЦКП ФГБУ «Национальный медицинский исследовательский центр онкологии» Министерства здравоохранения Российской Федерации: https://ckp-rf.ru/catalog/ckp/3554742/

Конфликт интересов: все авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи

Статья поступила в редакцию 19.07.2024; одобрена после рецензирования 22.08.2024; принята к публикации 27.08.2024

INTRODUCTION

Ovarian cancer (OC) currently takes one of the leading positions in terms of morbidity and mortality in the world and the Russian Federation among gynecological malignancies [1, 2]. OC includes many subtypes of tumors, each of which has distinctive biological and clinical characteristics. According to the WHO classification, serous carcinoma, endometrioid carcinoma, mucinous carcinoma, light cell carcinoma, malignant Brenner tumor, serous-mucinous carcinoma, undifferentiated carcinoma and mixed epithelial carcinoma are distinguished [3, 4].

In most patients, rheumatoid arthritis is sporadic, usually detected late, and the overall 5-year survival rate is only 30–40 %. Early detection of OC is the most important factor in improving patient survival [6]. New methodological approaches, including modern molecular biology approaches, are needed for early detection and improved diagnosis of this disease. The application of genomics and metabolomics has opened a new chapter of research, which will allow the development of new tools for early diagnosis and monitoring of the course of oncological diseases. Advances in metabolomic approaches using liquid or gas chromatography combined with high-resolution mass spectrometry (MS) have opened new prospects for simultaneous detection and identification of biomarkers in biological samples [7].

Our earlier study [8] of the urine metabolomic profile by ultrahigh-performance liquid chromatography with mass spectrometric detection showed that patients with serous ovarian carcinoma have an imbalance in the content of certain fatty acids and their derivatives, acylcarnitines, phospholipids, amino acids and their derivatives, as well as some derivatives of nitrogenous bases. At the same time, 26 metabolites with abnormal concentrations in urine may have some potential as non-invasive biomarkers of breast cancer in women belonging to high-risk groups.

Thus, it was shown that 14 metabolites (kynurenine, phenylalanyl valine, lysophosphatidylcholine (18:3), lysophosphatidylcholine (18:2), alanyl leucine, lysophosphatidylcholine (20:4), L-phenylalanine, phosphatidylinositol (34:1), 5-methoxytryptophan, 2-hydroxymyristic acid, 3-oxocholic acid, lysophosphatidylcholine (14:0), indolacrylic acid, lysophosphatidylserine (20:4)) have significantly higher concentrations compared to conditionally healthy individuals. The content of 12 compounds, on the contrary, was reduced (L-beta-aspartyl-L-phenylalanine, myristic acid, decanoyl carnitine, aspartyl-glycine, malonylcarnitine, 3-hydroxybutyrylcarnitine, 3-methylxanthine, 2,6-dimethylheptanoyl carnitine, 3-oxododecanoic acid, N-acetylproline, L-octanoylcarnitine, capriloylglycine) [8].

The determination of a number of the above compounds with high accuracy in urine samples is a procedure that requires expensive equipment and is feasible only in a small number of medical institutions. In this regard, it is extremely important to switch to more accessible predictive markers, for example, transcriptomic data. In this regard, the level of microRNA transcripts in urine is of particular interest [9].

microRNAs are short non-coding RNAs that regulate gene expression by catalyzing the destruction of mRNA, or by inhibiting the translation of mRNA into protein. Mature microRNA is a single-stranded RNA of the order of 22 nucleotides in size, obtained from a primary transcript. microRNAs are transcriptional regulators and modulate gene expression by interacting with complementary nucleotide sequences of target mRNAs [10]. microRNAs make a significant contribution to the initiation and development of various molecular events, including the initiation of oncogenesis, progression, and metastasis of tumors, which makes microRNAs potential biomarkers for assessing the progression and prognosis of cancer [11]. The study of the microRNA-mRNA regulatory network is of great importance both for elucidating the molecular mechanisms underlying carcinogenesis and for creating a panel of new biomarkers.

The purpose of the study was the bioinformatic search for transcriptomic markers (based on metabolomic data) and their validation in the urine of patients with serous ovarian adenocarcinoma.

MATERIALS AND METHODS

The prospective study included 70 patients diagnosed with ovarian cancer (serous adenocarcinoma of low (n = 30) and high grade malignancy (n = 30), T1a – 4, T1b – 3, T1c – 5, T2a – 3, T2b – 5, T3a – 14, T3b – 6, T3c – 30) and 30 conditionally healthy volunteers (without any known pathologies) who make up the control group.

Urine samples were used as objects of research. Before the study, the patients gave informed consent to the scientific use of biological samples. Urine was collected before the start of treatment.

Evaluation of microRNA expression

500 µl of urine sample was mixed with 900 µl of QIAzol reagent (QIAGEN). Further isolation of total RNA was carried out using the RNeasy Plus Universal Kits kit according to the manufacturer's protocol. To identify mature microRNAs and small U6 RNAs, the method proposed by Balcells I. and co-authors was used [12]. The isolated total RNA was used in a reverse transcription reaction, which was performed simultaneously with polyadenylation of RNA using specific OC primers. Next, the obtained complementary DNA was detected using real-time polymerase chain reaction (PCR) (PCR-RV).

The design of specific oligonucleotide primers was carried out using the Balcells I. algorithm [12]. Several sets of oligonucleotides were selected for each micro-RNA, from which those characterized by the highest efficiency of reverse transcription and PCR were selected. The effectiveness of reverse transcription was evaluated by the values of threshold cycles (Ct) obtained by analyzing synthetic analogues of microRNA and mRNA (Biosan CJSC, Russia) taken at a known concentration. The efficiency of amplification (E) for each system was evaluated by constructing a calibration curve, using the dilution analysis of the corresponding RNAs isolated from clinical samples according to the protocol described above (the average value of E was 2.0). The stability of expression for the selection of reference genes was evaluated using the geNorm algorithm [13]. The initial list of proposed normalizers for microRNAs included: miR-191 (expression of this microRNA was the most stable in 13 compared tissues [14]); miR-23a (as a normalizer suitable for the analysis of cervical samples [15] and U6 (traditionally used as a separate standard for normalization of microRNA expression data). Using the geNorm algorithm, U6 was selected to normalize microRNA expression data.

A reverse transcription reaction was performed separately for each microRNA in one repeat. For reverse transcription, a reaction mixture containing 1x poly(A) buffer (BioLabs), 10 U/µl Reverse Transcriptase MMLV (Synthol), 0.1 mM dNTPs (Synthol), 0.1 mM ATP (BioLabs), 1 µM OC primer, 0.5 U/µl Poly(A) polymerase (BioLabs) and 1 mcg of total RNA. The reaction was carried out for 15 minutes. at 16 oC, 15 min. at 42 °C, the reverse transcriptase was then inactivated for 2 min. at 95 °C.

The change in the relative expression of microRNA was evaluated by PCR-RV. Amplification was performed in 20 µl of a PCR mixture containing 1x PCR buffer, 0.25 mM dNTPs, 2 mM MgCl2, 1 unit act. Taq-DNA polymerase, 500 nM of direct and reverse primers. OC-qPCR formulation of each sample was performed in three repeats. The resulting mixtures were incubated in a CFX 96 amplifier (Bio-Rad Laboratories, USA) according to the following program: 2 minutes 94 °C, 50 cycles: denaturation at 95 °C for 10 seconds, annealing and elongation – 63 °C for 30 seconds. The results corresponding to Ct > 40 were found to be negative.

The relative expression (RE) was calculated using the formula $RE = 2^{-\Delta\Delta Ct}$. The normalization of the results was carried out according to the reference locus and the expression level of the corresponding target microRNAs in the samples of the control group, sequentially according to the scheme given below:

1. Normalization by reference locus:
$\Delta C(t) = C(t_{target} - C(t)_{reference}$, where $C(t)_{reference}$ is C(t) of the reference locus.

2. Calculation of $E^{-\Delta C(t)}$ for each microRNA for each patient of the control group and the main group.

3. Calculation of the median $E^{-\Delta C(t)}$ for each locus for the control group and the main group.

4. Normalization for the control group and the result as a multiplicity of changes: $RE = E^{-\Delta C(t)median}$ of the main group $/E^{-\Delta C(t)median}$ of the control group (which is identical to $RE = E^{-\Delta\Delta C(t)}$ [16].

Statistical and bioinformatic data processing

The differences were assessed using the Mann-Whitney criterion for a threshold level of statistical significance of $p < 0.05$, and the Bonferroni correction was used to account for multiple comparisons. The data analysis was carried out in the Python programming language using the SciPy library [17].

The search for metabolite regulatory genes and microRNA regulators of genes was carried out using the Random forest machine learning method, which combines the Breiman bagging method and the random subset method. The result of the "ran-

dom forest" model is the predicted probability that the target gene or microRNA is the true regulator of a particular metabolite [18].

LASSO (Least Absolute Shrinkage and Selection Operator), a penalized logistic regression in the R programming language in the Rstudio shell, was used to select minimal sets of microRNAs The importance of variables was determined by counting the number of bootstrap models with a non-zero coefficient of the variable [19].

Table 1. Metabolites and genes regulating metabolic pathways

Metabolites*	Regulator genes	Enzymes
Kinurenin	*KYNU, KMO, KYAT3, IDO*	kynureninase, kynurenine-3-monooxygenase, kynurenine aminotransferase 3, indolamine-pyrrole-2,3-dioxygenase
Phenylalanine-Valine	*PAH*	Phenylalanine hydroxylase
Myristic acid	*PPARA, PPARGC1A, CYP4Z1, IYD, FASN, PLA2G5, LGALS13*	fatty acid synthase, beta-ketoacyl synthase domain, calcium-dependent phospholipase A2, soluble lectin 13 binding galactoside
Lysophosphatidylcholine (18:3), (18:2), (20:4), (14:0)	*PLA2G2A, PLB1, LPCAT1*	phospholipase A2, lysophospholipase, LPC-acyltransferase
Decanoyl carnitine	*ACADM, ACADS, CROT*	Acyl-CoA dehydrogenase, Carnitine octanoyltransferase (Carnitine O-Octanoyltransferase)
Malonyl carnitine	*CPT1, CPT1A, ACADM*	palmitoyl-CoA transferase, malonyl-CoA decarboxylase
Alanine-Leucine	*GAL, PGA3*	galanin, pepsinogen A
3-hydroxybutyrylcarnitine	*ACADM, CRAT*	3-hydroxyacyl CoA dehydrogenase, carnitine-O-acetyltransferase
3-methylxanthine	*PDE4D*	cAMP-specific 3',5'-cyclic phosphodiesterase 4D
L-Phenylalanine	*PAH, DDC*	phenylalanine hydroxylase, DOPA decarboxylase
Phosphatidylinositol (34:1)	*PIK3CA, PIK3CB, PIK-3C2A, PLCB1, PIGL*	phosphatidylinositol-3-kinase, 1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase beta-1, N-acetylglucosaminylphosphatidylinositol-de-N-acetylase
2,6 dimethylheptanoyl carnitine	*ACADM, CRAT*	3-hydroxyacyl CoA dehydrogenase, carnitine-O-acetyltransferase
5-methoxytryptophan	*TPH1*	tryptophanhydroxylase
3-oxododecanoic acid	*FASN, OXSM*	3-oxoacyl synthase, fatty acid synthase
2- hydroxymyristic acid	*NMT1*	n-myristoyl transferase 1
3-oxocholic acid	*FABP6*	gastropine
Indolylacrylic acid	*KYAT1*	kynurenine aminotransferase 1 (kynurenine aminotransferase 1)
N-acetylproline	*APEH*	N-acylpeptide hydrolase
L-octanoylcarnitine	*CROT, COT, CPT2, CPT1*	Carnitine-O-octanoyltransferase, carnitine-O-palmitoyltransferase 2(Carnitine O-octanoyltransferase, Carnitine O-palmitoyltransferase 2)
Capriloyl glycine	*ACADM, ODC1, GLYATL1*	3-hydroxyacyl CoA dehydrogenase, ornithine decarboxylase 1, Glycine N-acyltransferase
Lysophosphatidylserine	*GPR34, PLA1A*	lysophosphatidylserine new receptor 1, phospholipase a1

Note: * – the list of metabolites based on the data of the article [8]

Table 2. Metabolites, regulatory genes of metabolic pathways and microRNAs interacting with them

	Metabolites	Regulator genes	microRNA
1	Kinurenin	*KYNU, KMO, KYAT3, IDO1*	***KMO***:miR-30b-3p, miR-153-5p, miR-149-3p, miR-363-5p, miR-624-3p, miR-937-5p, miR-1233-3p, miR-1238-3p, miR-1972, miR-3200-5p, miR-4319, miR-3689a-5p, miR-3689b-5p, miR-4478, miR-3689e, miR-4695-5p, miR-4724-5p, miR-664b-3p, miR-5684, miR-6758-5p, miR-6780a-5p, miR-6799-5p, miR-6856-5p, miR-6867-5p, miR-6883-5p, miR-6894-5p, miR-6894-5p, miR-7106-5p, miR-7106-5p, miR-1273h-5p, miR-12122. ***KYNU***:miR-30a-3p,miR-200c-3p, miR-382-5p, miR-382-5p, miR-2117, miR-3654, miR-4652-3p, miR-4743-3p, miR-6739-3p, miR-6879-3p, miR-6885-3p, miR-10397-5p, miR-4638-5p, miR-30a-3p, miR-200c-3p, miR-382-5p, miR-382-5p, miR-2117, miR-3654, miR-4652-3p, miR-4743-3p, miR-6739-3p, miR-6879-3p, miR-6885-3p, miR-10397-5p. ***KYAT3***: miR-5692c, miR-5692b, miR-5692c, miR-5692b, miR-5692c, miR-5692b. ***IDO1***: miR-593-3p, miR-891a-3p, miR-5683, miR-6728-3p
2	Phenylalanine-Valine	*PAH*	miR-23a-3p, miR-4502, miR-12132
3	Myristic acid	*PPARA, PPARGC1A, CYP4Z1, IYD, FASN, PLA2G5, LGALS13*	***IYD:*** miR-760, miR-29a-5p, miR-208a-5p, miR-30b-3p, miR-184, miR-195-3p, miR-320a-3p, miR-373-5p, miR-483-3p, miR-551b-5p, miR-643, miR-646, miR-1224-5p, miR-320b,miR-922, miR-1202, miR-1205, miR-1287-3p, miR-513c-3p, miR-1321, miR-3144-3p, miR-3152-5p, miR-3185, miR-3191-5p, miR-3199, miR-514b-5p, miR-4279, miR-3663-5p, miR-3681-5p, miR-3689a-3p, miR-3689b-3p, miR-4429, miR-4452, miR-3689c, miR-4531, miR-4533, miR-3972, miR-3976, miR-451b, miR-4731-5p, miR-4796-3p, miR-4799-3p, miR-5003-3p, miR-5195-3p, miR-5588-5p, miR-6509-3p, miR-6737-5p, miR-6737-3p, miR-6752-3p, miR-6764-3p, miR-6779-5p, miR-6780a-5p, miR-6824-3p, miR-6829-5p, miR-6830-5p, miR-6849-5p, miR-6849-3p, miR-6882-5p, miR-6894-3p, miR-7106-5p, miR-7844-5p, miR-8052, miR-8069, miR-8078, miR-146a-5p, miR-607, miR-3614-5p, miR-4482-3p, miR-197-3p, miR-744-3p, miR-3187-3p, miR-3652, miR-4420, miR-4430, miR-4633-5p, miR-4642, miR-4781-3p, miR-5698, miR-6499-3p, miR-6787-3p, miR-6843-3p, miR-6848-3p, miR-588, miR-4423-5p, miR-6501-5p. ***CYP4Z1:*** miR-2110, ***FASN:*** miR-30c-5p, ***LGALS13:*** miR-4650-3p. ***PLA2G5:*** miR-765, miR-3682-3p, miR-4533,miR-2467-3p,miR-4786-3p,miR-1253,miR-3191-5p,miR-6847-5p,miR-11181-3p,miR-3916. ***PPARA:***miR-181a-5p, miR-181b-5p, miR-20b-5p, miR-181d-5p, miR-22-3p, miR-140-5p, miR-372-3p, miR-330-5p, miR-331-3p, miR-345-3p, miR-520d-3p, miR-551b-5p, miR-619-5p, miR-622, miR-2113, miR-665, miR-939-3p, miR-1976, miR-3116, miR-3183, miR-4251, miR-3690, miR-550b-2-5p, miR-4436a, miR-4443, miR-4515, miR-4717-5p, miR-4723-5p, miR-4745-5p, miR-4749-5p, miR-4755-3p, miR-5591-5p, miR-6126, miR-6131, miR-6134, miR-6505-3p, miR-6734-3p, miR-6744-3p, miR-6753-3p, miR-6766-5p, miR-6791-5p, miR-6805-3p, miR-6817-5p, miR-6852-5p, miR-6873-3p, miR-6880-5p, miR-7151-3p, miR-8071, let-7b-5p, let-7e-5p, miR-224-3p, miR-302a-3p, miR-326, miR-335-3p, miR-429, miR-511-5p, miR-8085, miR-10394-5p, miR-10524-5p, miR-9851-5p, miR-7107-3p, miR-7110-3p, miR-7155-3p, miR-7158-3p, miR-7976, miR-1233-5p, miR-4651, miR-6757-5p, miR-6778-5p, miR-27a-5p, miR-34a-5p, miR-130b-5p, miR-196b-5p, miR-607, miR-1249-5p, miR-3689d, miR-5006-5p, miR-6756-5p, miR-6788-5p, miR-6797-5p, miR-6851-5p. ***PARGC1A:*** let-7a-5p, let-7b-5p, let-7c-5p, let-7e-5p, miR-23b-3p, miR-138-5p, miR-409-5p, miR-487a-3p, miR-193b-3p, miR-4458, miR-6884-5p, miR-23a-3p, miR-193a-3p, miR-485-3p, miR-3666, miR-3681-3p, miR-211-5p, miR-485-5p, miR-342-5p, miR-452-5p, miR-511-5p, miR-508-5p, miR-573, miR-659-3p, miR-764, miR-1825, miR-2116-3p, miR-2682-3p, miR-3929, miR-4436a, miR-4649-5p, miR-4664-5p, miR-4713-5p, miR-4728-3p, miR-122b-3p, miR-4768-5p, miR-4769-3p, miR-5003-5p, miR-5006-3p, miR-5011-5p, miR-5591-3p, miR-5685, miR-6124, miR-6740-3p, miR-6818-3p, miR-6833-3p, miR-6845-3p, miR-6892-3p, miR-7110-5p, miR-7703, miR-7850-5p, miR-8075, miR-8485, miR-148a-5p, miR-214-3p, miR-222-3p, miR-9898, miR-6083
4	Lysophosphatidylcholine	*PLA2G2A, PLB1, LPCAT1*	***LPCAT1***: miR-27a-3p, miR-370-3p, miR-4739, miR-4768-3p, miR-4783-3p. ***PLB1***: miR-3162-5p, miR-4529-3p, miR-4740-5p. ***PLA2G2A***: miR-765, miR-3652, miR-6134, miR-6745, miR-6756-5p, miR-6769a-5p, miR-6785-5p, miR-6769b-5p, miR-7847-3p

Table 2. (Continuation) Metabolites, regulatory genes of metabolic pathways and microRNAs interacting with them

	Metabolites	Regulator genes	microRNA
5	Decanoyl carnitine	*ACADM, ACADS, CROT*	***CROT***: miR-33a-5p, miR-373-3p, miR-33b-5p, miR-17-5p, miR-500a-5p, miR-501-5p, miR-1250-3p, miR-4659b-3p, miR-219b-5p, miR-4795-3p, miR-6807-3p, miR-6867-5p, miR-522-3p, miR-4325, miR-5004-3p, miR-6833-5p, miR-221-3p. ***ACADM***: miR-4437, miR-5580-3p, miR-6529-5p, miR-3184-5p, miR-4704-3p. ***ACADS***: miR-484
6	Malonyl carnitine	*CPT1A, ACADM*	***ACADM***: miR-4437, miR-5580-3p, miR-6529-5p, miR-3184-5p, miR-4704-3p. ***CPT1A***: miR-653-5p, miR-328-3p, miR-6866-3p, miR-1296-3p, miR-1322, miR-6883-5p, miR-7-2-3p, miR-335-3p, miR-520a-3p, miR-4310, miR-4287, miR-6718-5p, miR-6785-5p, miR-6869-3p, miR-7856-5p, miR-93-5p, miR-4293, miR-4322, miR-4707-3p, miR-24-3p, miR-6849-3p
7	Alanine-Leucine	*GAL, PGA3*	***GAL***: miR-922, miR-4742-5p, miR-4753-5p, miR-4436b-3p, miR-5004-5p, miR-5089-3p, miR-15b-5p, miR-138-1-3p, miR-302d-5p, miR-6810-5p, miR-3976. ***PGA3***: miR-2467-3p, miR-1909-5p, miR-6743-5p, miR-1913, miR-2115-5p, miR-4646-5p, miR-5006-5p, miR-6857-5p, miR-11399, miR-5008-5p, miR-4649-3p,miR-423-5p, miR-3679-5p, miR-423-3p, miR-1296-3p, miR-3126-5p, miR-6759-3p, miR-3180, miR-6763-5p, miR-769-3p, miR-3139, miR-5571-5p, miR-6768-5p, miR-761, miR-3151-5p, miR-18a-5p, miR-4672, miR-6873-3p, miR-6875-3p, miR-3156-5p, miR-6771-5p, miR-6879-5p, miR-3945
8	3-hydroxybutyrylcarnitine	*ACADM, CRAT*	***ACADM***: miR-4437, miR-5580-3p, miR-6529-5p, miR-3184-5p, miR-4704-3p. ***CRAT***: miR-936, miR-1207-5p, miR-6764-5p, miR-7150, miR-10392-3p
9	3-methylxanthine	*PDE4D*	***PDE4D***: miR-18a-5p, miR-31-5p, miR-148a-3p, miR-301a-3p, miR-148b-3p, miR-875-5p, miR-6766-3p, miR-26a-5p, miR-103a-3p, miR-107, miR-139-5p, miR-362-3p, miR-339-5p, miR-18b-5p, miR-448, miR-487a-3p, miR-4429, miR-203a-3p, miR-211-3p, miR-124-3p, miR-149-5p, miR-99b-3p, miR-372-3p, miR-373-5p, miR-520a-3p, miR-520d-3p, miR-625-5p, miR-641, miR-1301-3p, miR-449c-5p, miR-1266-5p, miR-1321, miR-1912-3p, miR-2114-5p, miR-3125, miR-3187-5p, miR-4261, miR-4280, miR-3646, miR-3689a-5p, miR-3689b-5p, miR-3922-5p, miR-4446-3p, miR-3689d, miR-3689e, miR-4492, miR-4502, miR-4511, miR-3977, miR-4646-5p, miR-4675, miR-4698, miR-4741, miR-4756-5p, miR-4768-3p, miR-5193, miR-1295b-5p, miR-5589-3p, miR-6500-3p, miR-548az-5p, miR-6504-5p, miR-6511a-5p, miR-6512-5p, miR-6809-5p, miR-6809-3p, miR-6829-5p, miR-6839-5p, miR-6859-5p, miR-5787, miR-6077, miR-6796-3p, miR-6860, miR-7114-5p, miR-7151-3p, miR-8080, miR-8081, miR-8086, miR-195-5p, miR-3136-5p, miR-6080, miR-6888-5p, miR-340-5p, miR-4439, miR-3148, miR-6857-3p, miR-497-5p
10	L-Phenylalanine	*PAH, DDC*	***PAH***: miR-23a-3p, miR-4502, miR-12132. ***DDC***: miR-875-3p, miR-3166, miR-4502, miR-3158-3p

STUDY RESULTS

Bioinformatic analysis of the relationship of the urine metabolomic profile with gene and microRNA expression. Using the Random forest machine learning method implemented in the R programming language, the analysis of the metabolomic data from the article [8] was carried out, as well as the Human Metabolome Database (HMDB, https://hmdb.ca/metabolites).

At the first stage, the metabolite-enzyme and enzyme-gene regulator relationships were established. The results are presented in table 1.

The results of the metabolite-gene-microRNA relationship are presented in Table 2 and Fig. 1–2. It can be seen that the content of metabolites detected in urine is regulated by a complex network of rna and microRNA interactions. For two metabolites, myristic acid and phosphatidylinositol, 237 and 143 micro-RNAs were detected, respectively, regulating the content of these substances in biological fluids.

Thus, bioinformatic analysis has determined a list of 613 unique microRNAs involved in the regulation of the concentration of 21 metabolites. Of the 613 microRNAs, only the microRNAs with the maximum

Table 2. (Continuation) Metabolites, regulatory genes of metabolic pathways and microRNAs interacting with them

	Metabolites	Regulator genes	microRNA
11	Phosphatidylinositol (34:1)	*PIK3CA, PIK3CB, PIK3C2A, PLCB1, PIGL*	***PIGL***: miR-4651, miR-5087, miR-6499-3p, miR-6739-3p, miR-6764-5p, miR-212-5p, miR-659-3p, miR-3189-3p, miR-3934-3p, miR-378g, miR-4519, miR-6819-5p. ***PIK3C2A***: miR-503-5p, miR-301b-3p, miR-6838-5p, miR-23a-5p, miR-29a-5p, miR-23b-5p, miR-510-5p, miR-1264, miR-2113, miR-1286, miR-3619-3p, miR-4423-3p, miR-4436a, miR-4484, miR-1343-3p, miR-6074, miR-6760-3p, miR-6867-5p, miR-212-5p, miR-150-5p, miR-378a-5p, miR-518a-5p, miR-1224-3p, miR-764, miR-6821-3p. ***PIK3CA***: let-7i-5p, let-7e-5p, miR-19a-3p, miR-19b-3p, let-7g-5p, miR-152-3p, miR-202-5p, miR-4429, miR-198, miR-548e-5p, miR-548o-3p, miR-2114-5p, miR-4430, miR-4493, miR-4659b-3p, miR-122b-5p, miR-4803, miR-5006-3p, miR-6797-3p, miR-1972, miR-2116-5p, miR-3157-5p, miR-3191-5p, miR-514b-5p, miR-4303, miR-4277, miR-3606-5p, miR-3614-3p, miR-3679-3p, miR-676-5p, miR-378g, miR-4446-5p, miR-4477b, miR-4486, miR-4652-3p, miR-6819-5p, miR-6857-5p, miR-6868-3p, miR-6893-3p, miR-7162-3p, miR-10526-3p, miR-12126, miR-139-5p, miR-422a. ***PIK3CB***: miR-23b-3p, miR-362-5p, miR-3666, miR-3064-5p, miR-4465, miR-199a-3p, miR-199b-3p, miR-212-5p, miR-150-5p, miR-6504-5p, miR-204-3p, miR-671-5p, miR-1263, miR-3646, miR-4430, miR-4682, miR-5093, miR-6165, miR-6715a-3p, miR-7850-5p, miR-9500, miR-130b-5p, miR-3619-5p, miR-32-3p, miR-623, miR-542-5p, miR-548j-5p, miR-544b, miR-3614-5p, miR-3652, miR-548aw, miR-5703, miR-8077, miR-2117. ***PLCB1***: miR-103a-3p, miR-107, miR-423-5p, miR-3129-5p, miR-139-5p, miR-124-3p, miR-138-1-3p, miR-302c-5p, miR-876-5p, miR-1244, miR-1322, miR-548s, miR-4267, miR-3692-3p, miR-4433a-3p, miR-4436a, miR-3978, miR-4647, miR-4659a-3p, miR-4670-3p, miR-5194, miR-548az-3p, miR-6783-3p, miR-6860, miR-7151-5p, miR-8056, miR-8063, miR-502-3p
12	2,6 dimethylheptanoyl carnitine	*ACADM, CRAT*	***ACADM***: miR-4437, miR-5580-3p, miR-6529-5p, miR-3184-5p, miR-4704-3p. ***CRAT***: miR-936, miR-1207-5p, miR-6764-5p, miR-7150, miR-10392-3p
13	5-methoxytryptophan	*TPH1*	***TPH1***: miR-320a-3p, miR-450a-2-3p, miR-320b, miR-2110, miR-4435, miR-5693, miR-5702, miR-6830-5p, miR-12118
14	3-oxododecane acid	*FASN*	miR-30c-5p
15	2-hydroxymyristic acid	*NMT1*	***NMT1***: miR-181a-5p, miR-214-3p, miR-491-5p, miR-432-5p, miR-922, miR-1202, miR-1205, miR-1972, miR-2110, miR-2682-3p, miR-3160-5p, miR-3176, miR-4303, miR-4291, miR-4447, miR-3972, miR-4667-5p, miR-4690-3p, miR-4700-5p, miR-23b-3p, miR-615-3p
16	3-oxocholic acid	*FABP6*	***FABP6***: miR-208a-5p, miR-330-5p, miR-196b-5p, miR-3180-3p, miR-3181, miR-4278, miR-3689f, miR-4754, miR-4786-3p, miR-5190, miR-5195-3p, miR-6745, miR-6751-5p, miR-6769a-5p, miR-6771-5p, miR-6792-5p, miR-6821-5p, miR-7156-3p, miR-10226, miR-10392-5p
17	Indolylacrylic acid	*KYAT1*	***KYAT1***: miR-423-5p, miR-6842-5p, miR-597-3p, miR-4710, miR-6741-5p, miR-6796-5p, miR-4447, miR-193b-3p
18	N-acetylproline	*APEH*	miR-1289
19	L-octanoylcarnitine	*CROT, CPT2*	***CROT***: miR-33a-5p, miR-373-3p, miR-33b-5p, miR-17-5p, miR-500a-5p, miR-501-5p, miR-1250-3p, miR-4659b-3p, miR-219b-5p, miR-4795-3p, miR-6807-3p, miR-6867-5p, miR-522-3p, miR-4325, miR-5004-3p, miR-6833-5p, miR-221-3p. ***CPT2***: miR-433-3p, miR-6843-3p, miR-6848-3p, miR-208a-5p, miR-6742-3p, miR-34a-5p
20	Capriloyl glycine	*ACADM, ODC1, GLYATL1*	***ACADM***: miR-4437, miR-5580-3p, miR-6529-5p, miR-3184-5p, miR-4704-3p. ***ODC1***: miR-423-5p, miR-3184-5p, miR-7973, miR-193b-3p. ***GLYATL1***: miR-1207-5p, miR-4668-3p, miR-4742-3p, miR-4999-5p, miR-664b-3p, miR-6846-3p, miR-6893-3p
21	Lysophosphatidylserine (20:4)	*GPR34, PLA1A*	***PLA1A***: miR-3153, miR-7110-3p, miR-6754-5p, miR-6887-3p. ***GPR34***: miR-3193, miR-2909, miR-4738-5p, miR-486-3p, miR-6808-5p

interaction strength with the mRNA of the genes regulating the content of metabolites were selected. The final list contained 91 microRNAs, presented in Table 3.

Features of the content of microRNA transcripts in the urine of patients with serous ovarian adenocarcinoma

The generated list of 91 microRNAs regulating the activity of 37 genes was used for validation by real-time PCR on urine samples of patients and conditionally healthy volunteers.

A statistically significant ($p < 0.005$) change in the transcript level of 47 microRNAs relative to conditionally healthy volunteers was found in the urine of patients with serous ovarian adenocarcinoma (Fig. 3).

A significant increase ($p < 0.05$) in the level of miR-382-5p by 1.9 times, miR-593-3p by 3.4 times, miR-29a-5p by 2.6 times, miR-2110 by 2.5 times,

Table 3. The final list of microRNAs involved in the regulation of the concentration of metabolites

	Metabolites	microRNA
1	Kinurenin	***KMO***: miR-30b-3p, miR-153-5p, miR-149-3p, miR-363-5p. ***KYNU***: miR-30a-3p, miR-200c-3p, miR-382-5p, miR-382-5p. ***KYAT3***: miR-5692c, miR-5692b, miR-5692c. ***IDO1***: miR-593-3p, miR-891a-3p.
2	Phenylalanine-Valine	***PAH:*** miR-23a-3p, miR-4502, miR-12132
3	Myristic acid	***IYD***: miR-760, miR-29a-5p. ***CYP4Z1***: miR-2110, ***FASN***: miR-30c-5p, ***LGALS13***: miR-4650-3p. ***PLA2G5***: miR-765, miR-3682-3p. ***PPARA***: miR-181a-5p, miR-181b-5p, miR-20b-5p. ***PPARGC1A***: let-7a-5p, let-7b-5p, let-7c-5p
4	Lysophosphatidylcholine	***LPCAT1***: miR-27a-3p, miR-370-3p, miR-4768-3p. ***PLB1***: miR-3162-5p, miR-4529-3p. ***PLA2G2A***: miR-765, miR-3652
5	Decanoyl carnitine	***CROT***: miR-33a-5p, miR-373-3p, miR-33b-5p, miR-17-5p. ***ACADM***: miR-4437, miR-5580-3p, miR-6529-5p. ***ACADS***: miR-484
6	Malonyl carnitine	***ACADM***: miR-4437, miR-5580-3p, miR-6529-5p. ***CPT1A***: miR-653-5p, miR-328-3p, miR-6866-3p
7	Alanine-Leucine	***GAL***: miR-922, miR-4742-5p, miR-4753-5p. ***PGA3***: miR-2467-3p, miR-1909-5p, miR-6743-5p
8	3-hydroxybutyrylcarnitine	***ACADM***: miR-4437, miR-5580-3p, miR-6529-5p
9	3-methylxanthine	***PDE4D***: miR-18a-5p, miR-31-5p, miR-148a-3p
10	L-Phenylalanine	***PAH***: miR-23a-3p. ***DDC***: miR-875-3p, miR-3166
11	Phosphatidylinositol (34:1)	***PIGL***: miR-4651, miR-5087, miR-6499-3p. ***PIK3C2A***: miR-503-5p, miR-301b-3p. ***PIK3CA***: let-7i-5p, let-7e-5p, miR-19a-3p. ***PLCB1***: miR-103a-3p, miR-107, miR-423-5p
12	2,6 dimethylheptanoyl carnitine	***ACADM***: miR-4437, miR-5580-3p, miR-6529-5pю ***CRAT***: miR-936, miR-1207-5p
13	5-methoxytryptophan	***TPH1***: miR-320a-3p, miR-450a-2-3p, miR-320b
14	3-oxododecanoic acid	***FASN:*** miR-30c-5p
15	2-hydroxymyrystine acid	***NMT1***: miR-181a-5p, miR-214-3p, miR-491-5p
16	3-oxocholic acid	***FABP6***: miR-208a-5p, miR-330-5p, miR-196b-5p
17	Indolylacrylic acid	***KYAT1***: miR-423-5p, miR-6842-5p, miR-597-3p
18	N-acetylproline	***APEH:*** miR-1289
19	L-octanoylcarnitine	***CROT***: miR-33a-5p, miR-373-3p, miR-33b-5p, miR-17-5p. ***CPT2***: miR-433-3p, miR-6843-3p
20	Capriloyl glycine	***ACADM***: miR-4437, miR-5580-3p, miR-6529-5p. ***ODC1***: miR-423-5p, miR-3184-5p. ***GLYATL1***: miR-1207-5p, miR-4668-3p
21	Lysophosphatidylserine (20:4)	***PLA1A***: miR-3153, miR-7110-3p. ***GPR34***: miR-3193, miR-2909

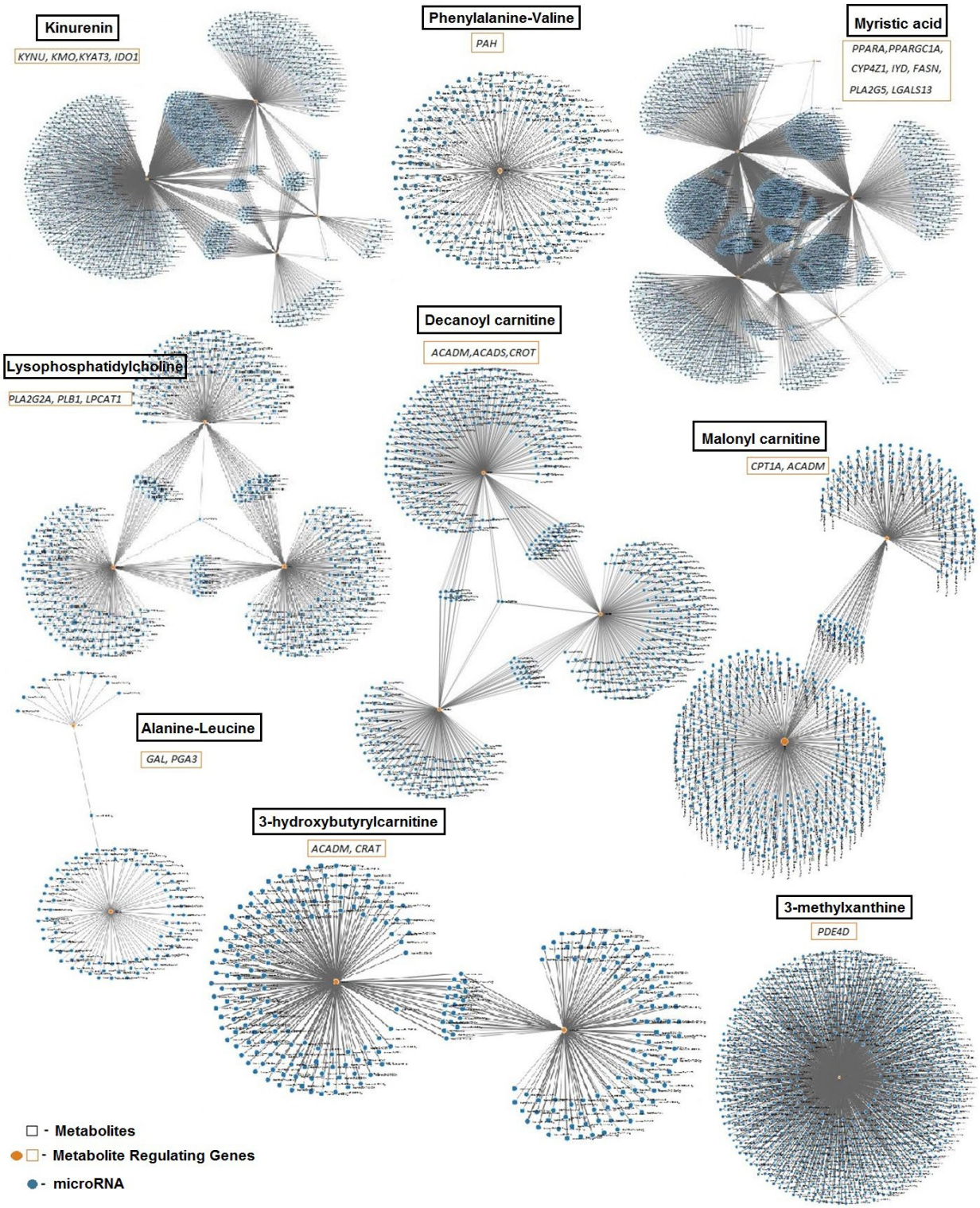

Fig. 1. Metabolites, regulatory genes of metabolic pathways and microRNAs interacting with them

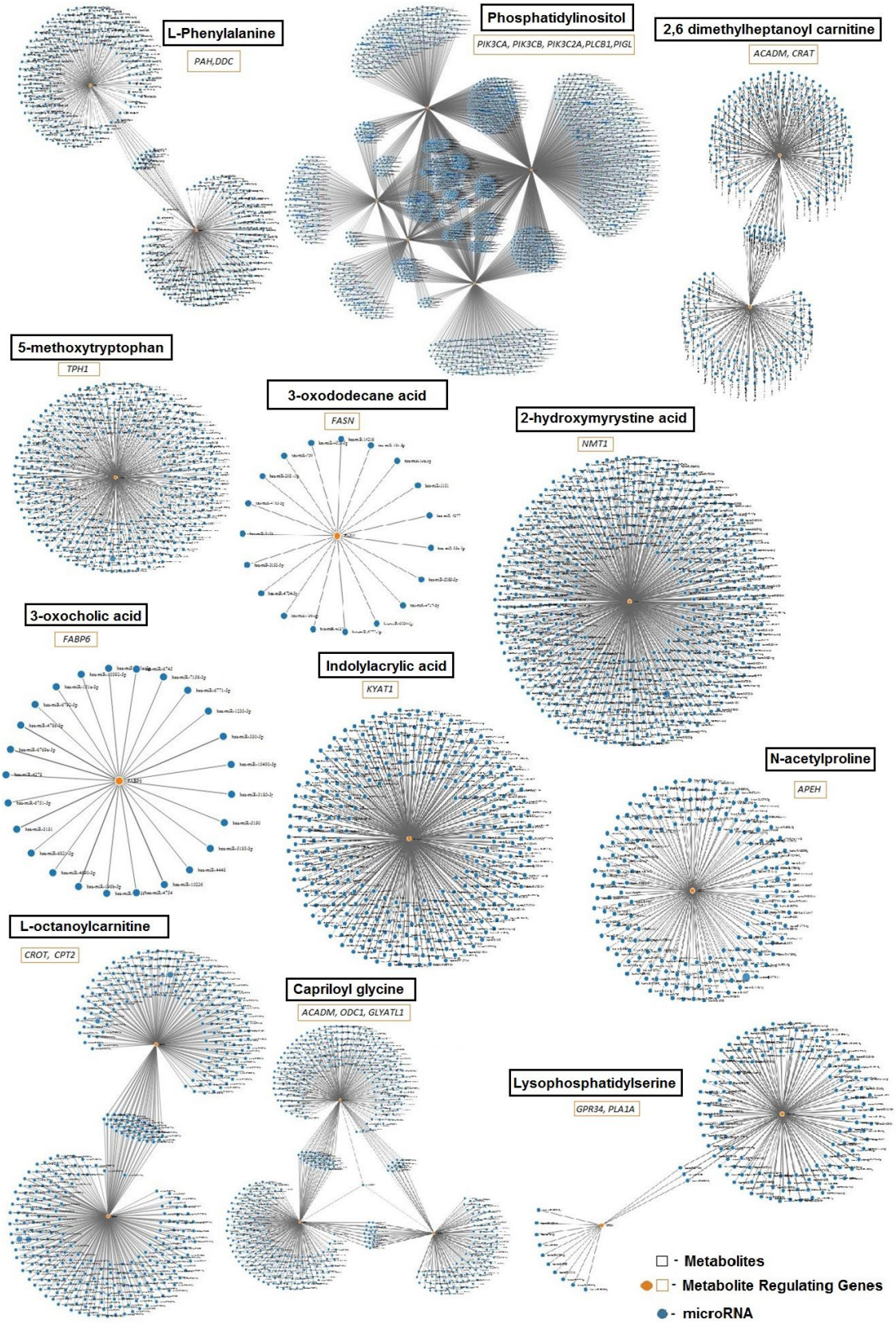

Fig. 2. Metabolites, regulatory genes of metabolic pathways and microRNAs interacting with them

miR-30c-5p by 2.9 times, miR-181a-5p by 2.6 times was found, let-7b-5p 2.6 times, miR-27A-3p 1.9 times, miR-370-3p 2.6 times, miR-6529-5p 2.5 times, miR-653-5p 2.2 times, miR-4742-5p 2.4 times, miR-2467-3p 2.6 times, miR-1909-5p 3.5 times, miR-6743-5p 4.9 times, miR-875-3p 2.3 times, miR-19a-3p 4.9 times, miR-208a-5p 2.6 times, miR-330-5p 3.2 times, miR-1207-5p by 3.5 times, miR-4668-3p by 4.2 times, miR-3193 is 2.6 times higher than their level in the urine of conditionally healthy individuals.

There was also a significant decrease ($p < 0.05$) in the level of miR-23a-3p by 20.0 times, miR-12132 by 4.0 times, miR-765 by 1.8 times, miR-181b-5p by 4.0 times, miR-4529-3p by 1.8 times, miR-33b-5p by 3.1 times, miR-17-5p by 4.6 times, miR-6866-3p by 1.7 times, miR-4753-5p by 14.3 times, miR-103a-3p by 19.6 times, miR-423-5p by 3.0 times, miR-491-5p by 1.7 times, miR-196b-5p by 5.0 times, miR-6843-3p 2.3 times, miR-423-5p 4.6 times and miR-3184-5p 2.6 times relative to their urine levels in conditionally healthy individuals.

Thus, the microRNA profile miR-382-5p, miR-593-3p, miR-29a-5p, miR-2110, miR-30c-5p, miR-181a-5p, let-7b-5p, miR-27a-3p, miR-2110, miR-30c-5p, miR-181a-5p, let-7b-5p, miR-27a-3p, miR-370-3p, miR-6529-5p, miR-653-5p, miR-4742-5p, miR-2467-3p, miR-1909-5p, miR-6743-5p, miR-875-3p, miR-19a-3p, miR-208a-5p, miR-330-5p, miR-1207-5p, miR-4668-3p, miR-3193, miR-23a-3p, miR-12132, miR-765, miR-181b-5p, miR-4529-3p, miR-33b-5p, miR-17-5p, miR-6866-3p, miR-4753-5p, miR-103a-3p, miR-423-5p, miR-491-5p, miR-196b-5p, miR-6843-3p, miR-423-5p and miR-3184-5p are differential for patients and conditionally healthy individuals.

DISCUSSION

In our study, using machine learning methods, links were established between metabolites that changed the concentrations of relatively healthy donors and genes encoding proteins involved in the synthesis and degradation of these metabolites, as well as links between metabolite regulatory genes and microRNA regulators of these genes.

Bioinformatics analysis has identified a list of 613 unique micro-RNAs involved in the regulation of the concentration of 21 metabolites. Of the 613 microRNAs, only the microRNAs with the maximum interaction strength with the mRNA of the genes regulating the content of metabolites were selected. The final list contained 91 micro-RNAs, of which 47 changed the level of their transcripts in urine (validated by PCR).

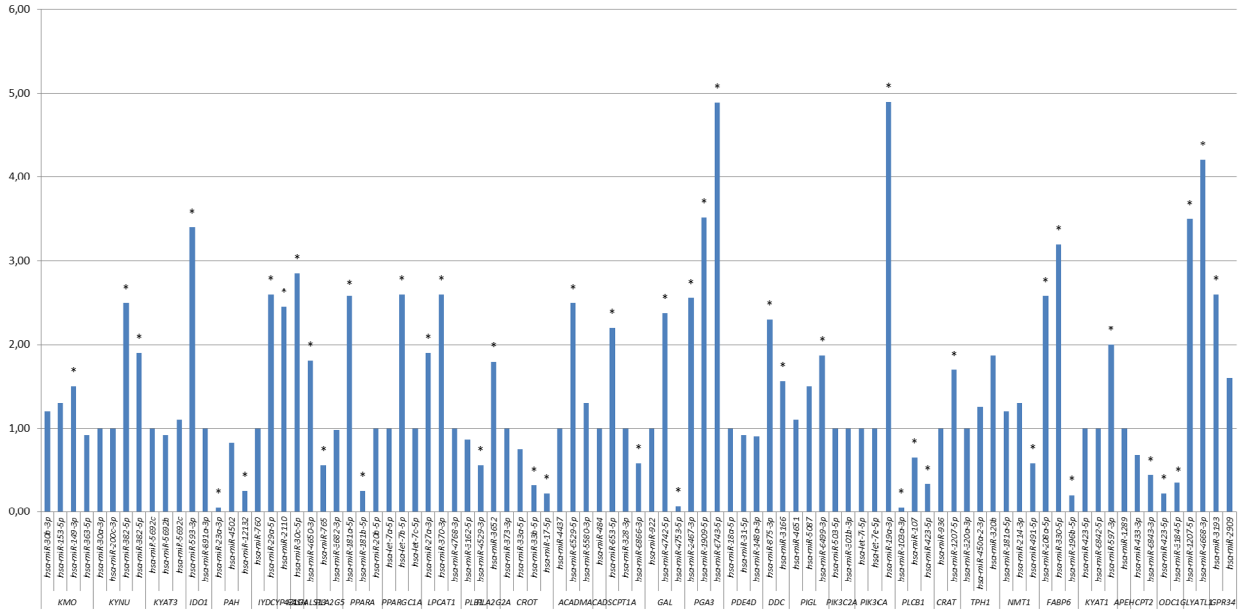

Fig. 3. Changes in the level of 91 micro-RNA transcripts in the urine of patients with serous ovarian adenocarcinoma
Note: * – statistically significant ($p < 0.05$) change in the transcript level relative to the control group

The level of transcripts miR-382-5p, miR-593-3p, miR-29a-5p, miR-2110, miR-30c-5p, miR-181a-5p, let-7b-5p, miR-27a-3p, miR-370-3p changed most significantly in patients with OC miR-6529-5p, miR-653-5p, miR-4742-5p, miR-2467-3p, miR-1909-5p, miR-6743-5p, miR-875-3p, miR-19a-3p, miR-208a-5p, miR-330-5p, miR-1207-5p, miR-4668-3p, miR-3193, miR-23a-3p, miR-12132, miR-765, miR-181b-5p, miR-4529-3p, miR-33b-5p, miR-17-5p, miR-6866-3p, miR-4753-5p, miR-103a-3p, miR-423-5p, miR-491-5p, miR-196b-5p, miR-6843-3p, miR-423-5p and miR-3184-5p relative to their urine levels in conditionally healthy individuals.

According to a number of authors, changes in the expression level of some of these microRNAs are associated with serous ovarian cancer: hsa-miR-382-5p, hsa-miR-27a-3p, hsa-miR-1207-5p, hsa-miR-423-5p [20], hsa-miR-593-3p [21], hsa-miR-29a-5p [22, 23] and hsa-miR-30c-5p [24, 25] and hsa-miR-30a-5p [26].

Nevertheless, the micro-RNA panel we identified (miR-382-5p, miR-593-3p, miR-29a-5p, miR-2110, miR-30c-5p, miR-181a-5p, let-7b-5p, miR-27a-3p, miR-370-3p, miR-6529-5p, miR-653-5p, miR-4742-5p, miR-2467-3p, miR-1909-5p, miR-6743-5p, miR-875-3p, miR-19A-3p, miR-208a-5p, miR-330-5p, miR-1207-5p, miR-4668-3p, miR-3193, miR-23A-3p, miR-12132, miR-765, miR-181b-5p, miR-4529-3p, miR-33b-5p, miR-17-5p, miR-6866-3p, miR-4753-5p, miR-103a-3p, miR-423-5p, miR-491-5p, miR-196b-5p, miR-6843-3p, miR-423-5p and miR-3184-5p) is unique and in this combination in literary sources not represented.

Obviously, transcriptomic imbalance begins in tissues and leads to a metabolic imbalance, which eventually affects the composition of body fluids, including urine.

Modern clinical oncogynecology has a serious need for effective biomarkers, changes in the levels of which can serve as evidence of the occurrence of a malignant process. Non-invasive and inexpensive PCR analysis of micro-RNA in urine makes it a particularly attractive screening tool. The application of this approach may allow for frequent testing of women belonging to high-risk groups and ensure long-term patient monitoring.

CONCLUSION

Bioinformatics analysis revealed a list of 613 unique microRNAs involved in the regulation of 21 metabolites. At the same time, the level of transcripts of 38 microRNAs (miR-382-5p, miR-593-3p, miR-29a-5p, miR-2110, miR-30c-5p, miR-181a-5p, let-7b-5p, miR-27a-3p, miR-370-3p, miR-6529-5p, miR-653-5p, miR-4742-5p, miR-2467-3p, miR-1909-5p, miR-6743-5p, miR-875-3p, miR-19a-3p, miR-208a-5p, miR-330-5p, miR-1207-5p, miR-4668-3p, miR-3193, miR-23a-3p, miR-12132, miR-765, miR-181b-5p, miR-4529-3p, miR-33b-5p, miR-17-5p, miR-6866-3p, miR-4753-5p, miR-103a-3p, miR-423-5p, miR-491-5p, miR-196b-5p, miR-6843-3p, miR-423-5p and miR-3184-5p) urine has diagnostic potential in ovarian cancer and is the basis for further research.. Thus, transcriptomic profiling of urine made it possible both to identify potential markers of the disease and to better understand the molecular mechanisms of changes underlying the development of OC.

References

1. Reid BM, Permuth JB, Sellers TA. Epidemiology of ovarian cancer: a review. Cancer Biol Med. 2017 Feb;14(1):9–32. https://doi.org/10.20892/j.issn.2095-3941.2016.0084
2. Malignant neoplasms in Russia in 2018 (morbidity and mortality). Edited by A. D. Kaprin, V. V. Starinsky, G. V. Petrova. Moscow: P. A. Herzen MNIOI – Branch of the National Medical Research Radiological Center, 2019, 250 p.
3. Tsandekova M.R., Porkhanova N.V., Kutilin D.S. Molecular characterization of serous ovarian adenocarcinoma: implications for diagnosis and treatment. Modern problems of science and education. 2020;(1):55. (In Russ.). https://doi.org/10.17513/spno.29428, EDN: LTMXTL
5. Meinhold-Heerlein I, Fotopoulou C, Harter P, Kurzeder C, Mustea A, Wimberger P, et al. The new WHO classification of ovarian, fallopian tube, and primary peritoneal cancer and its clinical implications. Arch Gynecol Obstet. 2016 Apr;293(4):695–700. https://doi.org/10.1007/s00404-016-4035-8
6. Rooth C. Ovarian cancer: risk factors, treatment and management. Br J Nurs. 2013 Sep 12;22(17):S23–30. https://doi.org/10.12968/bjon.2013.22.Sup17.S23

7. Swiatly A, Plewa S, Matysiak J, Kokot ZJ. Mass spectrometry-based proteomics techniques and their application in ovarian cancer research. J Ovarian Res. 2018 Oct 1;11(1):88. https://doi.org/10.1186/s13048-018-0460-6
8. Veenstra TD. Metabolomics: the final frontier? Genome Med. 2012 Apr 30;4(4):40. https://doi.org/10.1186/gm339
9. Guskova ON, Alliluev IA, Verenikina EV, Polovodova VV, Rogozin MA, Myagkova TYu, et al. Changes in urine metabolite concentration as a minimally invasive marker of ovarian serous adenocarcinoma. Russian Journal of Biotherapy. 2023;22(3):43–50. (In Russ.). https://doi.org/10.17650/1726-9784-2023-22-3-43-50, EDN: KRLBXC
10. Schmidt DR, Patel R, Kirsch DG, Lewis CA, Vander Heiden MG, Locasale JW. Metabolomics in cancer research and emerging applications in clinical oncology. CA Cancer J Clin. 2021 Jul;71(4):333–358. https://doi.org/10.3322/caac.21670
11. Dimitriadi TA, Burtsev DV, Dzhenkova EA, Kutilin DS. MicroRNAs as markers of progression of precancerous lesions to cervical cancer. Modern problems of science and education. 2020;(1):99. (In Russ.). https://doi.org/10.17513/spno.29529, EDN: SPESSH
12. Abdelsattar ZM, Wong SL, Regenbogen SE, Jomaa DM, Hardiman KM, Hendren S. Colorectal cancer outcomes and treatment patterns in patients too young for average-risk screening. Cancer. 2016 Mar 15;122(6):929–934. https://doi.org/10.1002/cncr.29716
13. Balcells I, Cirera S, Busk PK. Specific and sensitive quantitative RT-PCR of miRNAs with DNA primers. BMC Biotechnol. 2011 Jun 25;11:70. https://doi.org/10.1186/1472-6750-11-70
14. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002 Jun 18;3(7):RESEARCH0034. https://doi.org/10.1186/gb-2002-3-7-research0034
15. Peltier HJ, Latham GJ. Normalization of microRNA expression levels in quantitative RT-PCR assays: identification of suitable reference RNA targets in normal and cancerous human solid tissues. RNA. 2008 May;14(5):844–852. https://doi.org/10.1261/rna.939908
16. Shen Y, Li Y, Ye F, Wang F, Wan X, Lu W, et al. Identification of miR-23a as a novel microRNA normalizer for relative quantification in human uterine cervical tissues. Exp Mol Med. 2011 Jun 30;43(6):358–366. https://doi.org/10.3858/emm.2011.43.6.039
17. Kutilin DS, Dimitriadi SN, Vodolazhsky DI, Frantsiyants EM, Kit OI. Effect of thermal ischemia-reperfusion on expression of apoptosis-regulating genes in the renal tissue of patients with renal cell carcinoma. Nephrology (Saint-Petersburg). 2017;21(1):80-86. (In Russ.). https://doi.org/10.24884/1561-6274-2017-21-1-80-86, EDN: XVGWWP
18. Jones E, Oliphant E, Peterson P. SciPy: Open source scientific tools for python. 2001.
19. Ding J, Li X, Hu H. TarPmiR: a new approach for microRNA target site prediction. Bioinformatics. 2016 Sep 15;32(18):2768–2775. https://doi.org/10.1093/bioinformatics/btw318
20. Tsandekova MR, Porkhanova NV, Kit OI, Kutilin DS. Minimally invasive molecular diagnosis of high-grade and low-grade serous adenocarcinoma of the ovary. Oncogynecology. 2021;(4(40):35–50. (In Russ.). https://doi.org/10.52313/22278710_2021_4_35, EDN: ACKKXS
21. Li Y, Yao L, Liu F, Hong J, Chen L, Zhang B, et al. Characterization of microRNA expression in serous ovarian carcinoma. Int J Mol Med. 2014 Aug;34(2):491–498. https://doi.org/10.3892/ijmm.2014.1813
22. Han Y, Zheng Y, You J, Han Y, Lu X, Wang X, et al. Hsa_circ_0001535 inhibits the proliferation and migration of ovarian cancer by sponging miR-593-3p, upregulating PTEN expression. Clin Transl Oncol. 2023 Oct;25(10):2901–2910. https://doi.org/10.1007/s12094-023-03152-2
23. Resnick KE, Alder H, Hagan JP, Richardson DL, Croce CM, Cohn DE. The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol Oncol. 2009 Jan;112(1):55–59. https://doi.org/10.1016/j.ygyno.2008.08.036
24. Kwon JJ, Factora TD, Dey S, Kota J. A Systematic Review of miR-29 in Cancer. Mol Ther Oncolytics. 2019 Mar 29;12:173–194. https://doi.org/10.1016/j.omto.2018.12.011
25. Wu Q, Li G, Gong L, Cai J, Chen L, Xu X, et al. Identification of miR-30c-5p as a tumor suppressor by targeting the m6 A reader HNRNPA2B1 in ovarian cancer. Cancer Med. 2023 Feb;12(4):5055–5070. https://doi.org/10.1002/cam4.5246
26. Zhou J, Gong G, Tan H, Dai F, Zhu X, Chen Y, et al. Urinary microRNA-30a-5p is a potential biomarker for ovarian serous adenocarcinoma. Oncol Rep. 2015 Jun;33(6):2915–2923. https://doi.org/10.3892/or.2015.3937
27. Gasparri ML, Casorelli A, Bardhi E, Besharat AR, Savone D, Ruscito I, et al. Beyond circulating microRNA biomarkers: Urinary microRNAs in ovarian and breast cancer. Tumour Biol. 2017 May;39(5):1010428317695525. https://doi.org/10.1177/1010428317695525

Information about authors:

Denis S. Kutilin ✉ – Cand. Sci. (Biol.), Leading Researcher, Laboratory of Molecular Oncology, National Medical Research Centre for Oncology, Rostov-on-Don, Russian Federation
ORCID: https://orcid.org/0000-0002-8942-3733, SPIN: 8382-4460, AuthorID: 794680, Scopus Author ID: 55328886800

Fedor E. Filippov – MD, oncologist of the Department of Oncogynecology, Clinical Oncology Dispensary No. 1, Krasnodar, Russian Federation

Natalya V. Porhanova – Dr. Sci. (Med.), Associate Professor of the Department of Oncology, oncologist, Clinical Oncology Dispensary No. 1, Krasnodar, Russian Federation
SPIN: 2611-4840, AuthorID: 589928

Aleksey Yu. Maksimov – Dr. Sci. (Med.), Professor, Deputy General Director, National Medical Research Centre for Oncology, Rostov-on-Don, Russian Federation
ORCID: https://orcid.org/0000-0002-9471-3903, SPIN: 7322-5589, AuthorID: 710705, Scopus Author ID: 56579049500

Contribution of the authors:

Kutilin D. S. – concept and design of the study, conducting the experiment, bioinformatics analysis, writing the manuscript;
Filippov F. E. – conducting the experiment, writing the manuscript;
Porkhanova N. V. – statistical data processing;
Maksimov A. Yu. – editing the manuscript.