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ORIGINAL ARTICLE

Metabolomic profile of malignant ovarian tumors

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ABSTRACT

Purpose of the study. Investigate the metabolomic profile in tissues of patients with serous ovarian adenocarcinoma.
Materials and methods. The study included 100 patients with serous ovarian adenocarcinoma. Chromatographic separation of metabolites was performed on a Vanquish Flex UHPLC System chromatograph, which was coupled with an Orbitrap Exploris 480 mass spectrometer. Differences were assessed using the Mann-Whitney test with Bonferroni correction.
Results. In ovarian tumor tissue, 20 compounds had abnormal concentrations compared to normal tissue: increased levels of kynurenine, phenylalanylvaline, lysophosphatidylcholine (18:3), lysophosphatidylcholine (18:2), alanylleucine, L-phenylalanine, phosphatidylinositol (34:1), 5-methoxytryptophan, lysophosphatidylcholine (14:0), indoleacrylic acid and decreased levels of myristic acid, decanoylcarnitine, aspartylglycine, malonylcarnitine, 3-methylxanthine, 3-oxododecanoic acid, 2-hydroxymyristic acid, N-acetylproline, L-octanoylcarnitine and capryloylglycine.
Conclusion. A significant metabolic imbalance was found in ovarian tumor tissue, expressed in abnormal concentrations of fatty acids and their derivatives, acylcarnitines, amino acids and their derivatives, phospholipids and nitrogenous base derivatives. The concentrations of these 20 metabolites in tissues can serve as diagnostic markers of ovarian cancer. Thus, metabolomic tissue profiling allowed both to identify potential markers of the disease and to better understand the molecular mechanisms of changes underlying the development of this disease.

Keywords: metabolites, ultra-high performance liquid chromatography and mass spectrometry, ovarian serous adenocarcinoma, biomarkers

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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. 17 dated 06/28/2022). Informed consent was received from all participants of the study

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ОРИГИНАЛЬНАЯ СТАТЬЯ

Метаболомный профиль злокачественных опухолей яичника

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РЕЗЮМЕ

Цель исследования. Изучение метаболомного профиля в тканях у больных серозной аденокарциномой яичников.
Материалы и методы. В исследование было включено 100 пациенток с диагнозом серозная аденокарцинома яичников. Хроматографическое разделение метаболитов проводили на хроматографе Vanquish Flex UHPLC System, который был сопряжен с масс-спектрометром Orbitrap Exploris 480. Оценку различий проводили с использованием критерия Манна Уитни с поправкой Бонферрони.
Результаты. В опухолевой ткани яичника 20 соединений имели аномальную концентрацию по сравнению с нормальной тканью: обнаружено увеличение содержания кинуренина, фенилаланил-валина, лизофосфатидилхолина (18:3), лизофосфатидилхолина (18:2), аланил-лейцина, L-фенилаланина, фосфатидилинозитола (34:1), 5-метокситриптофана, лизофосфатидилхолина (14:0), индолакриловой кислоты и снижение содержания миристиновой кислоты, деканоилкарнитина, аспартил-глицина, малонилкарнитина, 3-метилксантина, 3-оксододекановой кислоты, 2-гидроксимиристиновой кислоты, N-ацетилпролина, L-октаноилкарнитина и каприлоилглицина.
Заключение. В опухолевой ткани яичника обнаружен значительный метаболомный дисбаланс, выраженный в аномальных концентрациях жирных кислот и их производных, ацилкарнитинов, аминокислот и их производных, фосфолипидов и производных азотистых оснований. Концентрации этих 20 метаболитов в тканях могут служить диагностическими маркерами рака яичников. Таким образом, метаболомное профилирование тканей позволило как выявить потенциальные маркеры заболевания, так и лучше понять молекулярные механизмы изменений, лежащих в основе развития данного заболевания.

Ключевые слова: метаболиты, ультравысокоэффективная жидкостная хроматография и масс-спектрометрия, серозная аденокарцинома яичника, биомаркеры

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INTRODUCTION

In the last decade, among oncogynecological diseases, ovarian cancer has occupied leading positions in terms of morbidity and mortality in the world and Russia [1, 2]. Malignant ovarian tumors are divided into many histological subtypes, each of which has distinctive biological and clinical characteristics. There are serous carcinoma, endometrioid carcinoma, mucinous carcinoma, light cell carcinoma, malignant Brenner tumor, serous-mucinous carcinoma, undifferentiated carcinoma and mixed epithelial carcinoma. Serous adenocarcinoma is the most common subtype [3, 4].

The overall five-year survival rate of ovarian cancer patients does not exceed 40 %, which is due to late diagnosis. To date, the sensitivity and specificity of the main diagnostic methods of this disease are insufficient to detect it at an early stage [5, 6]. New approaches are needed to improve diagnosis. Metabolomics methods based on high-resolution liquid chromatography and mass spectrometry (MS) open up new prospects for the detection and identification of biomarkers in the femtomolar and attomolar ranges.

So in the work of Y. Ahmed-Salim and co-authors analyzed the results of 32 publications in the field of metabolic research in ovarian cancer. Most studies have reported a violation of the regulation of phospholipids and amino acids: histidine, citrulline, alanine and methionine. At the same time, combinations of more than one metabolite as a panel in various studies achieved higher sensitivity and specificity for diagnosis than a single metabolite; for example, combinations of various phospholipids [7].

In [8], the role of histidine and citrulline in the development of ovarian cancer was confirmed, and new lipid compounds (lysophosphatidylcholine C16:1, phosphatidylcholine C32:2, C34:4 and C36:6) potentially involved in cancer metabolism were discovered.

However, such studies in ovarian cancer are not numerous compared to genomic and transcriptomic ones, and most of them were performed on equipment with lower resolution and a principle of operation different from Orbitrap technology [9], and biological fluids of patients, such as urine [10] or blood [11].

The purpose of the study was to study the metabolic profile of tissues of patients with serous ovarian adenocarcinoma in order to identify potential diagnostic markers of the disease.

MATERIALS AND METHODS

The study included 100 patients diagnosed with serous ovarian adenocarcinoma (T3a-c). Samples of normal and tumor tissue obtained at the stage of surgical treatment were used as objects of research. The average age of the patients was 54.2 years.

Analysis of metabolites by the HPLC-MS method

Surgical biopsies of tumor and normal ovarian tissue were used for analysis, which were stored in liquid nitrogen until the moment of metabolic and molecular genetic studies. The samples were homogenized at a temperature no higher than 4 °C. The homogenate was mixed with 600 µl of acetonitrile LC-MS (Merck, Germany)/methanol LC-MS (Merck, Germany) in a ratio of 3/1, was stirred for 15 minutes using a vortex and incubated for 15 hours at –20 °C. Proteins were precipitated by centrifugation at 16000 g 0 °C for 30 minutes. The supernatant was transferred to clean Eppendorf tubes. The solvent was evaporated at 45 °C for 4 hours on a SpeedVac vacuum evaporator (Eppendorf). The resulting dry precipitate was dissolved in 300 µl of 95 % acetonitrile LC-MS solution (Merck, Germany) with the addition of 0.1 % formic acid (Merck, Germany). To better dissolve the sediment, the samples were treated with ultrasound in an Elmsonic P 120 H ultrasonic bath (ELMA, Germany). Further, the samples were centrifuged for 30 min at 16000 g and the resulting supernatant was used for chromatomass spectrometric analysis.

Chromatographic separation of metabolites was performed on a Vanquish Flex UHPLC System Thermo Fisher Scientific chromatograph. The chromatograph was paired with the Orbitrap Exploris 480 mass spectrometer, which has an electrospray ionization source. A sample of metabolites in a volume of 2 µl was divided on a Hypersil GOLD™ C18 column (1.9 µm, 150 × 2.1 mm), eluents: A – 0.1 % formic acid LC-MS (Merck, Germany), B – acetonitrile LC-MS (Merck, Germany) containing 0.1 % formic

acid (Merck, Germany). The following elution gradient was used: 1 min – 5 % of eluent B, 15 min – linear gradient of eluent B from 5 to 95 %, 2 min – 95 % of eluent B, 0.5 min – change of eluent composition to 5 % of eluent B, 3 min – 5 % of eluent B. The flow of eluents is 200 µl/min.

Mass spectrometric analysis was performed on an Orbitrap Exploris 480 (Thermo Fisher Scientific) mass spectrometer with an electrospray ionization source. The mass spectrometer was configured for priority ion detection in the m/z range from 67 to 1000 Da with a resolution of 60,000. The spectra were taken in the detection mode of positively charged ions. The time to remove one spectrum is 20 minutes. Additional MS settings were as follows: ion sputtering voltage = –3.5 kV; capillary temperature = 320 °C; sample heater temperature = 300 °C; protective gas = 35; auxiliary gas = 10 and radio frequency S-lens – 50.

For mass spectrometric peaks to be identified, compliance with specific metabolites from the Human Metabolome Database was established (http://www.hmdb.ca) and Metlin (Scripps Center for Mass Spectrometry, USA; http://metlin.scripps.edu). For this purpose, an accurately measured mass of the chemical compound was used. Bioinformatic analysis was performed using Compound Discoverer Software (Thermo Fisher Scientific, USA) and analysis of biochemical pathways using the KEGG PATHWAY Database.

Statistical 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 [12].

STUDY RESULTS

During the conducted metabolomic profiling, 100 samples of serous ovarian adenocarcinoma and 100 samples of conditionally normal ovarian tissues were analyzed. 750 metabolites were identified. For metabolites whose intensities in the mass spectra differed statistically significantly relative to normal tissue, P-value and FoldChange were determined (Table 1).

According to the data obtained, the metabolome of the tumor tissue of patients with serous ovarian carcinoma differed significantly from samples of normal ovarian tissue of the same patients. In the tumor tissue of the patients, 10 metabolites (kynurenine, phenylalanyl valine, lysophosphatidylcholine (18:3), lysophosphatidylcholine (18:2), alanyl leucine, L-phenylalanine, phosphatidylinositol (34:1), 5-methoxytryptophan, lysophosphatidylcholine (14:0), indolacrylic acid) had significantly higher concentrations In comparison with conditionally normal tissue, the concentration of 10 compounds (myristic acid, decanoyl carnitine, aspartyl-glycine, malonylcarnitine, 3-methylxanthine, 3-oxododecanoic acid, 2-hydroxymyristinic acid, N-acetylproline, L-octanoylcarnitine, caprylylglycine), on the contrary, was reduced.

Thus, it was found that the concentrations of myristic acid, 2-hydroxymyristic acid and 3-oxododecanoic acid in tumor tissue were statistically significantly ($p < 0.01$) reduced by 2.6 times, 4.8 times and 1.4 times, respectively, compared with normal tissue. The levels of decanoyl carnitine, malonylcarnitine and L-octanoyl carnitine in tumor tissue were statistically significantly ($p < 0.0001$) lower by 5.3 times, 1.5 times and 6.7 times, respectively, than in normal tissue. Statistically significantly ($p < 0.00000005$), the concentration of a number of phospholipids in tumor tissue in patients with ovarian cancer was increased relative to normal ovarian tissue: lysophosphatidylcholine (18:3) by 2.1 times, lysophosphatidylcholine (18:2) by 3.4 times, phosphatidylinositol (34:1) by 4.1 times and lysophosphatidylcholine (14:0) by 1.9 times. Statistically significant ($p < 0.01$) changes in the concentration of some amino acids and their derivatives were also found: an increase in the concentration of kynurenine by 6.1 times, phenylalanyl valine by 2.2 times, alanyl leucine by 1.6 times, L-phenylalanine by 1.8 times, 5-methoxytryptophan by 1.6 times and indolacrylic acid by 1.5 times relative to normal tissue, as well as a decrease in the concentration of N-acetylproline by 1.7 times, caprylylglycine by 1.5 times and aspartyl glycine by 5.0 times, respectively, relative to normal ovarian tissue. A change in the content of nitrogenous base derivatives in ovarian tumor tissue was also detected, i.e. a 2.3-fold decrease in the concentration of 3-methylxanthine ($p < 0.0001$).

DISCUSSION

The HPLC-MS method identified 750 metabolites of various classes, while the concentration of 10 metabolites in the tumor tissue was significantly increased compared to conditionally normal tissue, and the concentration of 10 compounds was lowered on the contrary.

Fatty acids and their derivatives

In the tumor tissue, the concentrations of most fatty acid derivatives – myristic acid, 2-hydroxymyristic acid and 3-oxododecanoic acid were reduced compared to conditionally normal tissue. Tumor cells are characterized by a profound restructuring of the metabolism of lipids and fatty acids. In some types of tumors, the utilization of fatty acids increases, while in others it is suppressed [13, 14].

Myristic acid ($CH_3(CH_2)_{12}COOH$, FoldChange 0.38, p = 0.0000241) is a saturated fatty acid with an aliphatic long chain, present in almost all living organisms [15]. Abnormal levels of myristic acid can increase the risk of tumors [16]. It is involved in the implementation of several antitumor mechanisms,

Table 1. The difference between the metabolomic profile of tumor tissue and normal in patients with serous ovarian adenocarcinoma

Metabolites	m/z	FoldChange, tumor/normal tissue	*p*-value
1. Fatty acids and their derivatives			
Myristic acid	231.2	0.38	0.00002410
2-hydroxymyristic acid	267.2	0.21	0.00001000
3-oxododecanoic acid	237.1	0.74	0.01000060
2. Acylcarnitines			
Decanoyl carnitine	316.2	0.19	0.000000003
Malonyl carnitine	230.1	0.65	0.000100002
L-octanoylcarnitine	288.2	0.15	0.000004011
3. Phospholipids			
Lysophosphatidylcholine (18:3)	518.3	2.05	0.000000002
Lysophosphatidylcholine (18:2)	521.3	3.40	0.000000051
Lysophosphatidylcholine (14:0)	468.3	1.89	0.000000003
Фосфатидилинозитол (34:1)	430.8	4.11	0.000000001
4. Aminoacids and their derivatives			
Alanine-Leucine	185.1	1.55	0.000900000
Phenylalanine-Valine	265.2	2.15	0.000100000
L-Phenylalanine	166.1	1.84	0.000000002
Kinurenin	209.1	6.07	0.000001000
Aspartyl-glycine	208.1	0.20	0.000012400
5-methoxytryptophan	217.1	1.61	0.000004200
Indolylacrylic acid	171.1	1.49	0.010189400
N-acetylproline	140.1	0.59	0.000085630
Capriloyl glycine	202.1	0.65	0.010212890
5. Derivatives of nitrogenous bases			
3-methylxanthine	167.1	0.44	0.000100000

such as the production of myristoleic acid, which causes apoptosis, and in the synthesis of ceramides de novo. According to a number of authors, the content of myristic acid in biological fluids and tissues is inversely associated with the risk of colorectal cancer. However, the mechanisms underlying this relationship have not been fully studied [17-20].

2-hydroxymyristic acid ($C_{14}H_{28}O_3$, FoldChange 0.21, p = 0.00001) is a fatty acid containing an aliphatic chain carrying a hydroxyl substituent at position 2, is a derivative of myristic acid. The physiological function of hydroxy fatty acids remains largely unknown. They have been shown to play a specific role in signaling to cells [21]. 2-Hydroxymyristinic acid is metabolically activated in cells to form 2-hydroxymyristoyl-CoA, a potent inhibitor of myristoyl-CoA [22]. Currently, the main mechanisms by which 2-hydroxylation of fatty acids is associated with metabolic adaptation and tumor growth remain unclear [23].

3-oxododecanoic acid ($C_{12}H_{22}O_3$, FC = 0.74, p = 0.01000060) is a fatty acid that is a 3-oxo derivative of decanoic acid. In the human body, 3-oxododecanoic acid participates in a number of enzymatic reactions [24]. Keto-fatty acids are often reported as artifacts of fatty acid oxidation, but relatively rarely as natural fatty acids. 3-Keto-fatty acids, found as secondary components of animal tissues, are usually intermediates of β-oxidation.

For the beta-oxidation of fatty acids by mitochondria, the presence of carnitine, an important cofactor of metabolic processes, is an indispensable condition. There are more than 1,000 types of acylcarnitines in the human body, the general function of which is to transport acyl groups of organic acids and fatty acids from the cytoplasm to the mitochondria so that they can be broken down during beta oxidation to produce energy [25]. This is one of the most efficient ways of energy production in cells, therefore, tissues with high energy consumption mainly depend on the utilization of fatty acids [26].

Cancer is a pathological condition characterized by high energy consumption. Glucose and glutamine as energy substrates are considered a distinctive feature of tumor cells, and the metabolic switch that allows their use in almost anaerobic conditions is known as the Warburg effect [27]. The canonical interpretation of the Warburg effect implies that cells bypass the mitochondrial respiratory chain to synthesize ATP even with sufficient oxygen supply [28]. However, it is obvious that the Warburg effect needs to be considered in a more general metabolic context, which also includes the utilization of fatty acids in accordance with the effectiveness of these substrates in terms of ATP output. Metabolic flexibility is a phenomenon observed in different types of cancer and within the same type of cancer at different stages of progression. Carnitine-induced fatty acid oxidation plays a critical role in the production of NADH, FADN2, NADPH, and ATP, which can contribute to the development of tumors [29].

Acylcarnitines

Metabolic reprogramming of tumor cells regulates the content of acylcarnitines with different chain lengths in order to create a balance between production, energy consumption and synthesis of metabolic intermediates to meet the requirements of rapid proliferation [30]. Acylcarnitines have cytotoxicity and immunomodulatory properties that can be used by the tumor for growth and survival in situ [31]. Thus, a change in the level of malonylcarnitine is associated with the risk of developing breast cancer.

Malonylcarnitine is a metabolite that accumulates with a specific violation of fatty acid oxidation caused by a violation of the intake of long-chain acylcarnitine esters into the mitochondria and insufficiency of the mitochondrial respiratory chain with a deficiency of complex 11 and malonyl-CoA decarboxylase [32].

L-octanoylcarnitine is a physiologically active form of octanoylcarnitine [33], which is found in deficiency of medium chain acyl-CoA dehydrogenase (MCAD). L-octanoylcarnitine is involved in lipid peroxidation (HMDB: HMDB0000791), fatty acid metabolism (HMDB: HMDB0000791), mitochondrial beta oxidation of short-chain saturated fatty acids (HMDB: HMDB0000791) and lipid transport (HMDB: HMDB0000791). Changes in its concentrations have been recorded in blood and faeces in colorectal cancer, Crohn's disease and ulcerative colitis [34].

Decanoyl carnitine is classified as an acylcarnitine with a medium chain length. A change in the concentration of decanoyl carnitine was found in renal cell carcinoma and breast cancer [35].

The study of fluctuations in the content of acylcarnitines can contribute to a better understanding of the mechanisms of oncological diseases and the development of methods for their diagnosis and treatment.

Phospholipids

In this study, an increase in the concentration of lysophosphatidylcholines and phosphatidylinositol was observed in ovarian tumor tissue. Lysophospholipids are secreted by various types of cells, including tumor cells. These chemical compounds play an important role in the development, activation, and regulation of the immune system [36]. Changes in the composition and content of phospholipids and lysophospholipids have previously been shown in prostate cancer and are considered as potential biomarkers [37]. Lysophospholipids function as signaling molecules through their specific membrane receptors. In addition, some of the lysophospholipids have tumor-promoting activity and are therefore called "oncolipids" [38]. Recent studies have shown that phospholipids are candidates for PH biomarkers. Several comprehensive prospective studies of lipids have been conducted, such as lysophosphatidylcholines, phosphatidylcholines, ceramides and sphingomyelins, the concentrations of which differ in patients with rheumatoid arthritis compared with healthy ones [39, 10].

Lysophosphatidylcholines, also called lysolecithins, are a class of chemical compounds formed from phosphatidylcholines by the enzyme phospholipase A2. Lysophosphatidylcholines are the most common phospholipids in the blood and key lipids in various pathophysiological conditions such as inflammation, endothelial activation and atherogenesis [40]. Among other properties, they act as a signaling molecule released by apoptotic cells to attract phagocytes, which then phagocytize apoptotic cells [41].

Phosphatidylinositols are minor phospholipids of the inner membrane layer of eukaryotic cells, important components of intracellular signaling pathways. Phosphatidylinositol is a substrate for a variety of signaling kinase molecules that can attach a phosphate group to inositol. The main biological functions of phosphatidylinositols are a membrane stabilizer (HMDB: HMDB0009799) and a molecular messenger (signaling molecule (HMDB: HMDB0009799)). Phosphatidylinositols are involved in such important signaling pathways and processes as fatty acid metabolism (HMDB: HMDB0009799), lipid peroxidation (HMDB: HMDB0009799), apoptosis, cell adhesion [42], cell migration and proliferation [43]. Their content increases in the blood (HMDB: HMDB0009799) in a number of oncological diseases, including breast cancer, colorectal cancer and stomach cancer [44].

Amino acids and their derivatives

An abundant supply of nutrients, such as amino acids, is necessary for the increased metabolic needs of tumor cells that maintain high proliferative activity [45].

The alteration of tryptophan metabolism in cancer via the kynurenine pathway has attracted widespread attention as a mechanism by which tumors can elude immune control [45].

Kynurenine (β-(o-aminobenzene)-α-aminopropionic acid) is an intermediate product of the enzymatic breakdown of tryptophan and the biosynthesis of nicotinic acid in the human body. During enzymatic oxidation, kynurenine is converted to 3-hydroxykynurenine. The pathway of L-tryptophan biotransformation with the formation of "kynurenine" metabolites plays an important role in the mechanisms of immunoregulation and "negative" control of immune inflammation [46].

In addition to the main pathways of tryptophan catabolism, there are secondary ones, one of them leads to indolylacrylic acid ($C_{11}H_9NO_2$, indolacrylate), the biological role of which in animals is still unclear [47]. Stimulating the production of indolacrylic acid can promote anti-inflammatory reactions and have therapeutic value [47]. In our study, the level of indolacrylic acid is elevated in ovarian tumor tissue. The production of indolacrylic acid may contribute to the development of anti-inflammatory reactions [47]. It has been shown to selectively affect breast cancer cells, but does not affect untransformed primary fibroblasts. In our study, an increase in indolacrylic acid was accompanied by an increase in the content of kynurenine.

5-methoxytryptophan ($C_{12}H_{14}N_2O_3$), which is an endothelial factor with anti-inflammatory properties, is synthesized from L-tryptophan by 2 enzymes: tryptophan hydroxylase-1 and hydroxyindole-O-methyltransferase [48]. It controls the migration and acti-

vation of macrophages by inhibiting NF-kB [49], and also regulates epithelial-mesenchymal transition and metastasis [50].

Changes in the metabolism of another aromatic amino acid, *phenylalanine* and its derivatives, are also associated with inflammation and immune activation. Neurauter G. et al showed that the concentration of phenylalanine in serum in patients with ovarian carcinoma correlates with the concentration of markers of immune activation and the development of oxidative stress [51].

We also found a decrease in the content of aspartyl glycine dipeptide in tumor tissue. This compound is probably a product of incomplete breakdown of proteins and peptides. It is known that some dipeptides have physiological or cellular signaling effects, although most of them are simply short-lived intermediates on the way to specific amino acid degradation pathways. Some dipeptides are also considered as biomarkers of diseases [52].

Concentrations of *N-acetyl-L-proline* ($C_7H_{11}NO_3$) and caprylylglycine also decrease. N-acetylproline is a biologically available N-terminal form of the proteinogenic alpha amino acid L-proline. N-terminal acetylation of proteins is a widespread and highly conserved process in eukaryotes, which is involved in the protection and stability of proteins [53]. A number of studies have shown the association of N-acetyl-L-proline with colorectal cancer [54] and metastatic melanoma [55]. Caprylylglycine is a lipid amino acid consisting of caprylic acid and glycine. Acylglycines are usually minor metabolites of fatty acids [55].

Nitrogenous base derivatives and steroids

In our study, a decrease in the concentration of 3-methylxanthine was found in ovarian tumor tissue. 3-methylxanthine ($C_6H_6N_4O_2$) is a methyl derivative of purine with a ketone group (3,7-dihydropurine-2,6-dione). Some evidence suggests that methylxanthines have antitumor effect [56]: they inhibit *PI3K/Akt/mTOR* and stimulate *PTEN*, promoting apoptosis and autophagy [57].

CONCLUSION

A significant change in metabolism was found in the ovarian tumor tissue, presented in abnormal concentrations of fatty acids and their derivatives, acylcarnitines, amino acids and their derivatives, phospholipids and derivatives of nitrogenous bases. Concentrations of these metabolites in tissues can serve as diagnostic markers of ovarian cancer. Thus, the metabolic profiling of tissues allowed both to identify potential markers of the disease and to better understand the molecular mechanisms of changes underlying the development of this disease.

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