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THE PROGNOSIS OF RESISTANCE TO PLATINUM DRUGS IN PATIENTS WITH SEROUS OVARIAN CANCER

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Summary. Kolesnik O. O., Svintsitsky V. S., Rybin A. I. **THE PROGNOSIS OF RESISTANCE TO PLATINUM DRUGS IN PATIENTS WITH SEROUS OVARIAN CANCER.** The cytogenetic study of peripheral blood lymphocytes was carried out in patients with the serous ovarian cancer divided into three groups: platinum refractory patients (1st group), platinum-resistant patients (2nd group) and platinum-sensitive (3rd group) patients. Aberrant cells met authentically more often ($p<0.05$) in 1st group (42,2±8,3%) than in 2nd group (32,5±6.3%) and 3rd one (16,5±4,4%). Different fragile spectra of chromosomes sites were discovered in patients with the serous ovarian cancer in all groups. We've found 14 fragile sites, 12 of which coincide with classified ones: 10 fragile sites are common ones, but 2 sites belong to the rare fragile sites. Regions of fragile sites we found were of the same localization with chromosomal rearrangements found in tumors and in the ovarian cancer contain genes involved in cancer development. Chromosomal instability and the presence of certain fragile sites of chromosomes can be used as markers of platinum-sensitivity in patients with the serous ovarian cancer.

Key words: ovarian cancer, treatment, platinum-resistance, prognosis, cytogenetic changes.

Реферат. Колесник Е. А., Свинцицкий В. С., Рыбин А. И. **ПРОГНОЗИРОВАНИЕ ПЛАТИНОРЕЗИСТЕНТНОСТИ У БОЛЬНЫХ РАКОМ ЯИЧНИКОВ.** Авторами проведено цитогенетическое исследование лимфоцитов периферической крови у больных раком яичников трех групп: платинорефрактерных пациентов (1 группа), платинорезистентных пациентов (2 группа) и платиночувствительных пациентов (3 группа). Аберрантные клетки встречались достоверно чаще ($P<0,05$) в 1 группе больных раком яичников(42,2±8,3%), чем во 2 группе больных(32,5±6,3%) и 3 группе больных(16,5±4,4%). Выявлены различные спектры фрагильных сайтов хромосом у больных раком яичников разных групп. Обнаружены 14 фрагильных сайтов, 12 из которых совпадают с классифицированными, 10 фрагильных сайтов являются общими фрагильными сайтами, а 2 фрагильных сайта принадлежат к редким фрагильным сайтам. Регионы фрагильных сайтов, найденных нами, совпадают по локализации с хромосомными перестройками, обнаруженными в опухолях при раке яичников и содержат гены, участвующие в развитии рака. Хромосомную нестабильность и наличие определенных фрагильных сайтов хромосом можно рассматривать, как маркеры платиночувствительности у больных раком яичников.

Ключевые слова: рак яичников, платинорезитентность, прогнозирование, цитогенетическая аномалии.

Реферат. Колесник О. О., Свінціцький В. С., Рибін А. І. **ПРОГНОЗУВАННЯ ПЛАТИНОРЕЗИСТЕНТНОСТІ У ХВОРИХ НА РАК ЯЄЧНИКІВ.** Авторами проведено цитогенетичне дослідження лімфоцитів периферичної крові у хворих на рак яєчників трьох груп: платінорефрактерних пацієнтів (1 група), платінорезістентних пацієнтів (2 група) і платіночутливих пацієнтів (3 група). Аберрантні клітини зустрічалися достовірно частіше ($P <0,05$) в 1 групі хворих на рак яєчників (42,2 ± 8,3%), ніж у 2 групі хворих (32,5 ± 6,3%) і 3 групі хворих (16 , 5 ± 4,4%). Виявлено різні спектри фрагільних сайтів хромосом у хворих на рак яєчників різних груп. Виявлено 14 фрагільної сайтів, 12 з яких збігаються з класифікованими, 10 фрагільної сайтів є загальними фрагільної сайтами, а 2 фрагільної

сайту належать до рідкісних фрагільних сайтів. Регіони фрагільних сайтів, знайдених нами, збігаються з локалізації з хромосомними перебудовами, виявленими в пухлинах при раку яєчників і містять гени, що беруть участь в розвитку раку. Хромосомну нестабільність і наявність певних фрагільних сайтів хромосом можна розглядати, як маркери платіночутливості у хворих на рак яєчників.

Ключові слова: рак яєчників, платінорезітентність, прогнозування, цитогенетичні аномалії.

Introduction. Ovarian cancer (OC) is one of the most aggressive types of cancer pathology. Worldwide this disease is diagnosed in 70-75% of cases as later stages, when the prognosis are not favourable. In these cases, the main expectation assigned to adjuvant chemical therapy after cytoreductive operations. According to some authors, the prognosis of these patients with widespread ovarian cancer is largely determined by the effectiveness of the 1st stage of the combined treatment. Sensitivity to platinum drugs in 1st line chemotherapy of the serous OC significantly affects to the prognosis of the disease. Hence, the search for pathogenetically based marker of tumor sensitivity to platinum drugs is a component of the development of personalized treatment of ovarian cancer. Depending on the time of disease progression, there are the following types of tumors: platinum refractory (tumor progresses during first-line chemotherapy with the inclusion of platinum drugs), platinum-resistant (tumor progresses within 6 months after the end of chemotherapy with inclusion of platinum drugs) and platinum-sensitive (tumor progresses more than 6 months after the end of chemotherapy) [2; 5]. Fragile sites of chromosomes are regions of genome that are predisposed to break of DNA double chain in response to external oncogenic or replicative stress. Oncogenic replicative stress is initiated by mutations in genes of protein-kinases, responsible for DNA damage that causes chromosomal instability in these regions, also in these regions there are about half of all known cancer-associated genes [4]. There are some chromosomal markers of resistance to platinum drugs [6; 9]. *The aim* of this work is the studying of cytogenetic abnormalities on peripheral blood lymphocytes in patients with ovarian cancer with different levels of sensitivity to platinum drugs.

Materials and methods of research. We examined 189 patients with ovarian serous carcinoma of stage III-IV after the optimal, suboptimal and non-optimal cytoreductive surgery and adjuvant courses of chemotherapy with platinum drugs. Groups were formed depending on the patient's sensitivity to platinum drugs. The first group of platinum-resistant tumors included patients with recurrent disease within 6 months after the end of treatment (59 patients), second group (platinum-refractory tumors) included patients with progression of the disease during the platinum chemotherapy (44 patients), and the third group included platinum-sensitive tumors, patients after the treatment (86 patients) without relapse during 6 months of observation. Blood sampling for conducting cytogenetic studies were performed by venipuncture of the cubital vein before operation interventions and therapy. For the cultivation of lymphocytes of peripheral blood using semi-micro [6; 7]. For the cultivation of peripheral blood lymphocytes used medium 199, which don't contain folate and folic acid. This is very important for identifying fragile sites of chromosomes. Colouring of the samples was carried out by the method of GTG- and GAG-colouring. Chromosomal analysis were performed with the help of karyotyping system "MetaSystems" (Germany) using the program "Ikaros". It was analyzed at least 100 metaphase plates for each patient. Chromosome analysis of lymphocytes include: counting the number of chromosomes in metaphase plates, identification of chromosomes, the determination of frequency of aberrant cells (percentage to total number of investigated metaphases), characterization of chromosome aberrations. Chromosome analysis was performed in accordance with the criteria of ISCN [8]. Take into account chromatine and chromosomal aberrations and karyotypic abnormal cells (polyploidy, aneuploidy). Also take into account fragile sites of chromosomes. Fragile site has appeared to be identified as a space or heterogeneity in the structure of chromosomes. The received digital material was subjected to statistical analysis. The differences at $p<0.05$ was considered as reliable.

The research results. In the study of karyotype of peripheral blood lymphocytes of patients with the serous OC were observed differences in the amount and spectrum of chromosomal aberrations between the groups (tab.1). The comparison of the total number of aberrant cells indicated that most of the aberrations was discovered in the group of patients with platinum-refractory tumors (42.2±8.3%)/ In the group of patients with platinum-resistant tumors this figure

amounted to 32.5±6.3%. And in group of patients with platinum-sensitive tumors it was 16.5±4.4%. Chromosomal aberration type and aneuploidy encountered most frequently in the groups of patients with platinum-resistant and platinum-refractory tumors. Aberrations of the chromosomal type were mainly represented by deletions of different parts of chromosomes with rearrangements, as well as decentrique. Aberration chromatide type was observed less often and were presented chromatide breaks and achromatic spaces. Very often aneuploidy cells possessed extra chromosomes with a variety of unknown changes, also frequent discovery were cells with allotriploid and allotetraploid karyotype. Chromosomal instability in cancer patients is typical not only for tumor cells but also peripheral blood lymphocytes. It's likely that there is a transformation of the genome of lymphocytes fragments of tumor DNA [11]. The third part of the examined patients with ovarian cancer had a blood tumor DNA and p53 gene mutation that is associated with low survival rate of patients [12]. There are several genetic pathways of ovarian cancer: mutations in tumor suppressor genes (BRCA1, BRCA2, TP53, RB1), activation of oncogenes (BRAF, KRAS, NM1), interruptions in cell cycle control (ABL1, CCND1, CDK), mutations in genes of DNA-repair (BRIP1, ERCC1, XRCC2, RAD51, CDK-genes), mutations in genes of steroid hormones(PGR, ESR1, CYP3A4, CYP19A1, SRD5A2) [1, 12]. Mutations in genes of DNA-repair directly promote the increasing of aberrations number of chromosomal and chromatide type because of insufficient activity of repair enzymes, and disturbances in interaction with repairing DNA. Mutations of genes controlling the cell cycle lead to violations of the mitotic spindle and the appearance of polyploid cells and aneuploidy.

Table 1.

CHROMOSOMAL ABNORMALITIES OF PERIPHERAL BLOOD LYMPHOCYTES IN PATIENTS WITH OVARIAN CANCER DEPENDING ON SENSITIVITY TO PLATINUM DRUGS

Group	*Number of metamorphoses*	*Abberant cells, %*	*Frequency of chromosomal type of aberration, %*	*Frequency of chromatide type of aberration, %*	*Frequency of aneuploidy (hyperploidy) %*
I platinum-resistant(59)	5900	32,5±6,3	9,6±3,3	4,5±1,9	19,4±4,5
II platinum refractory (44)	4400	42,2±8,3	8,5±3,1	5,4±2,3	28,3±6,3
III platinum-sensitive (86)	8600	16,5±4,4	4,5±1,7	2,5±0,8	9,5±2,3

During the study of chromosomes fragile sites in patients with OC we found the following fragile sites: in the group with platinum refractory ovarian cancer – FRA (1)(q1.2); FRA (1)(p3.3-3.4), FRA (3)(q2.3-2.4), FRA (4)(q3.1-3.2), FRA (8)(q24), FRA (5)(q2.3-3.1), FRA (7)(q31-32), FRA (17)(q12-13); in the group with platinum-resistant ovarian cancer - FRA(1)(q1.2); FRA (5)(q3.1); FRA (7)(q3.1); FRA (8)(q2.4); FRA (17)(q1.2-1.3); in the group with platinum-sensitive ovarian cancer - FRA(6)(q2.3-2.4), FRA(3)(p14-15), FRA(3)(q21), FRA (11)(q2.2-2.3), FRA (12)(q2.3-2.4) (table.2). It was identified 14 sites in patients with the serous OC, 12 of these sites may match with the classified by the location on the chromosome. 10 fragile sites are concern to common fragile sites (detected by replicative stress), and 2 fragile sites belong to the rare fragile sites (identified in the folates deficiency) (Fig. 1, 2). The majority of detected fragile sites are generic, such fragile sites are identified in the external conditions (exposure to mutagens) or internal (oncogenic) replicative stress. Fragile common sites are areas of the genome, susceptible to breaks in double strands of DNA. When cancer breaks in the regions total fragile sites occur in the early stages. Oncogenic replicative stress is caused by mutations in the genes of protein-kinase that is responsible for DNA damage leading to chromosomal instability in the regions of common fragile sites.

In the regions of common fragile site for cancer is the initiation of chromosomal instability with a massive local accumulation of chromosomal rearrangements in single points (chromotripsis or destruction of chromosomes), amplification of oncogenes, deletion of tumor suppressors, integration of the virus into the genome [6, 11].

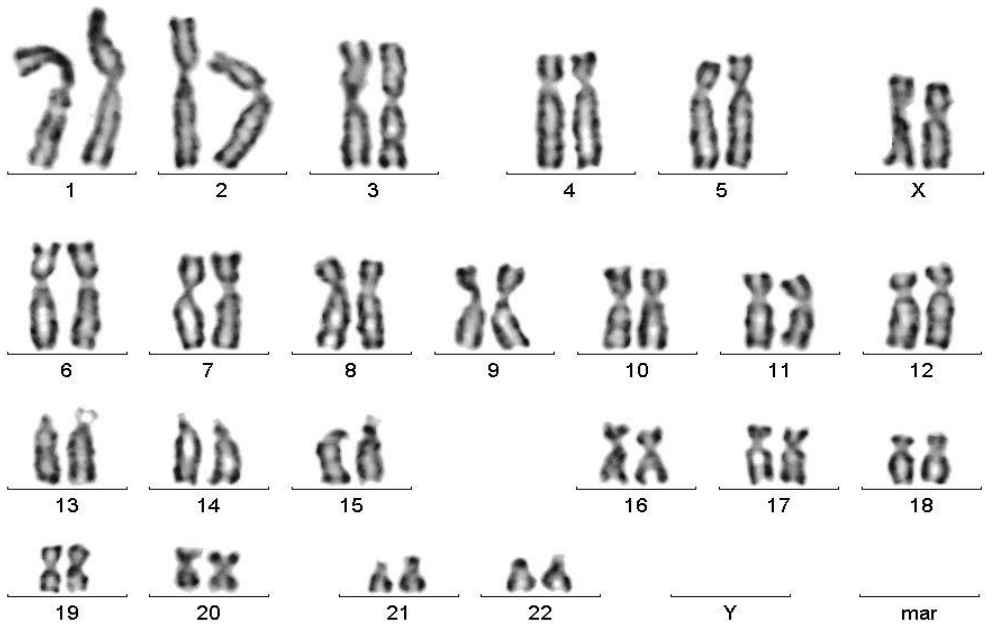

Fig. 1. The karyotype of a patient with ovarian cancer 46,XX; FRA (1)(q1.2), FRA(3)(q2.3-2.4)

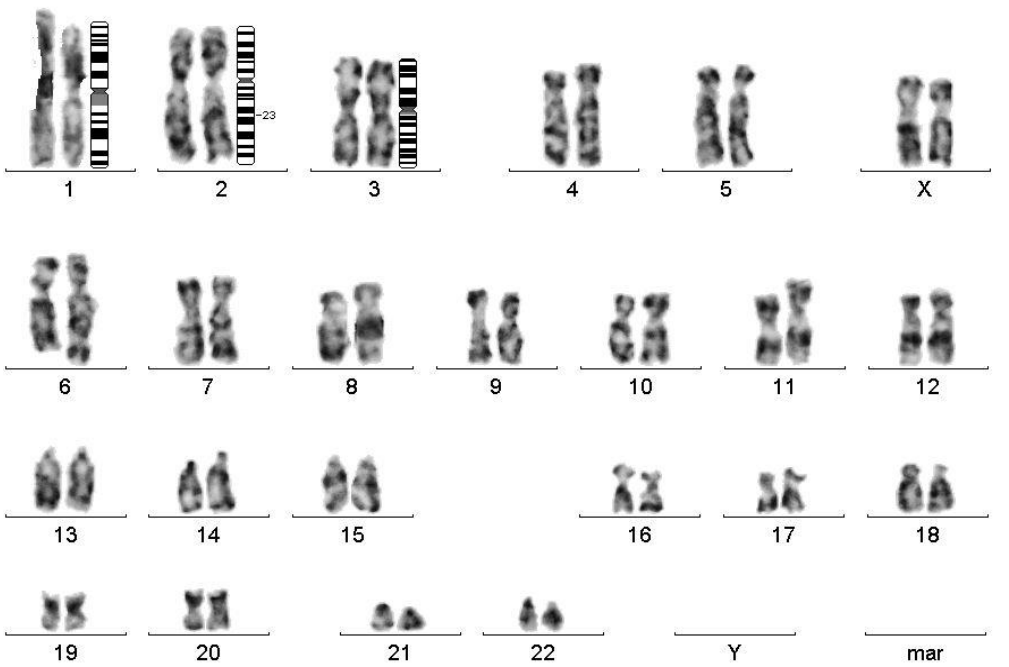

Fig. 2. The karyotype of a patient with ovarian cancer 46,XX; FRA(6)(q2.3-2.4)

Chromosomal localization of some fragile sites we found coincides with the location of known cytogenetic markers of platinum-resistance in ovarian cancer such us chromosomal regions 1q (chromosome insert), 8q22-qter (chromosome insertion) [7]. The majority of detected fragile

sites coincide with known cytogenetic abnormalities in tumors in ovarian cancer (tab.2).

Table 2.

FRAGILE SITES FOUND IN PATIENTS WITH OVARIAN CANCER

Chromosomal fragile region	Classified fragile site	The type of fragile site	Locuses loss of heterozygosity in tumors with OC	Genes associated with the OC	The presence of in patients with ovarian cancer
1q1.2	FRA1J	General			PR, PRS
1p3.3-3.4					PR
3p1.4-15	FRA3B	General	3p, 3p1.4	FHIT	PS
3q2.1	FRA3F	General	insertions 3q1.3-2.4		PS
3q2.3-2.4			insertion and amplification 3q2.3, 3q2.4-2.5		PR
4q3.1-3.2	FRA4C	General	deletions 4q2.2-3.1		PR
5q3.1	FRA5C	General		TCF7	PR, PRS
6q2.3-2.4	FRA6E	General	deletions 6q2.4-2.7		PS
7q3.1	FRA7G	General	7q3.1	MET, WNT2	PR, PRS
7q3.2	FRA7H	General	7q3.2		PR
8q2.4	FRA8C	General	insertion and amplification 8q2.4		PR, PRS
11q2.2-2.3	FRA11G	General	1q22-23	CBL6, ZBTB16	PS
12q2.3-2.4	FRA12C FRA12D	Rare	deletions 12q23ter		PS
17q1.2-1.3		Rare	deletions 17q12-21	BRCA1	PR, PRS

PS – platinum-sensitive tumors
PR – platinum-refractory tumors
PRS – platinum-resistant tumors

Discussion. When analyzing the cytogenetic abnormalities, identified in various groups of patients with ovarian cancer, we discovered the highest number of aberrant cells in the group platinum-refractory tumors (42.2±8.3%), compared with the group of platinum-resistant tumors (32.5±6.3%) and platinum-sensitive tumors (16.5±4.4%). Significant increase in the number of chromosomal aberrations in peripheral blood lymphocytes of patients with OC may indicate serious violations in the body of the patient, particularly in immune and reparative systems and poor prognosis. In the group of platinum-sensitive tumors we observed good survival rate of patients and a more favourable prognosis.

When studying the spectrum of chromosomes fragile sites of peripheral blood lymphocytes of patients with OC we discovered some fragile sites that met both in a group with platinum-refractory tumors and in the group with platinum-resistant tumors such as ((FRA(1)(q1.2); FRA (5)(q3.1); FRA (7)(q3.1); FRA (8)(q2.4); FRA (17)(q1.2-1.3)). In the group with platinum-sensitive tumors we observed another spectrum of fragile sites: FRA(6)(q2.3-2.4), FRA(3)(p14-15), FRA(3)(q21), FRA (11)(q2.2-2.3), FRA (12)(q2.3-2.4). Some chromosomal regions of fragile sites, we found coincides in location with cytogenetic markers of platinum-refractory detected in tumors (1q, 8q22-qter). Regions fragile sites we found, are of the same localization like chromosomal rearrangements that has been found in the serous OC. In the regions of common fragile sites the initiation of chromosomal instability with a massive local accumulation of chromosomal rearrangements in single points (chromotripsis or destruction of chromosomes) takes place. Our investigation discovered 14 fragile sites totally in examined patients. But 12 of these sites coincide with classified by location on chromosome. However 10 fragile sites are common fragile sites, and 2 fragile site belongs to the rare fragile sites. In the regions of fragile sites the genes are present involving in the process of carcinogenesis in OC such as: the tumor suppressors FHIT and BRCA 1, MET proto-oncogene, the cell cycle control protein gene ZBTB16, TCF7,

WNT2, CBL6. Chromosomal instability and the presence of certain fragile sites of chromosomes can be seen as markers of platinum refractory in patients with ovarian cancer.

Conclusions: 1. We identified different spectra of chromosomes fragile sites in patients with the serous ovarian cancer. In the group of patients with platinum-refractory tumors we discovered the following fragile sites: FRA (1)(q1.2); FRA (1)(p3.3-3.4), FRA (3)(q2.3-2.4), FRA(4)(q3.1-3.2), FRA(8)(q24), FRA (5)(q2.3-3.1), FRA (7)(q31-32), FRA (17)(q12-13). In the group of patients with platinum-resistant tumors were found such fragile sites as: FRA(1)(q1.2); FRA (5)(q3.1); FRA (7)(q3.1); FRA (8)(q2.4); FRA (17)(q1.2-1.3). In the group of platinum-sensitive tumors the completely different spectrum of the chromosome fragile sites was observed: FRA (6)(q2.3-2.4), FRA (3)(p14-15), FRA (3)(q21), FRA (11)(q2.2-2.3), FRA (12)(q2.3-2.4).

2. We found reliable positive correlation between the presence of cytogenetic abnormalities in patients with the serous ovarian cancer and the sensitivity of malignant ovarian tumor to chemotherapy with platinum drugs. Aberrant cells met authentically more often ($p<0.05$) in the group platinum-refractory patients with ovarian cancer (42.2±8.3%) than in platinum-resistant patients (32.5±6.3%) and the group of platinum-sensitive patients (16.5±4.4%).

3. We identified 14 fragile sites, 12 of which coincide with classified: 10 fragile sites are common fragile sites, and 2 fragile sites belong to the rare fragile sites. Regions of the fragile sites we found are of the same localization like chromosomal rearrangements that has been found in the serous ovarian cancer. These regions contain genes involved in the development of ovarian cancer.

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