MANIFESTATIONS OF OXIDATIVE STRESS AND MOLECULAR DAMAGES IN OVARIAN CANCER TISSUE

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Indices of oxidative stress are recognized molecular markers and prognostic criteria for malignant transformation of tissue, but their value depends on the type of tumor and the stage of its development. The goal of this study was to clarify the relationship between the characteristics of the oxidative stress system including metal-associated ones and the cytotoxicity manifestations in neoplastically transformed human ovarian tissue. The highest level of Mn-superoxide dismutase activity (by 630%) and metallothionein protein (MT, 100%) has been estimated for the first time in malignant ovarian tissue compared to normal ovarian tissue. The researchers have also found a much higher level of oxy-radical formation (by 332%), a lower activity of catalase (by 49%) and a lower level of reduced glutathione (by 46%) and its redox index (0.84 versus 0.89 in the control) in tumor tissue. Under the relatively stable content of zinc, copper and cadmium in MTs, the content of zinc and especially copper in a form non-binding with MTs was significantly lower in the malignant tissue compared to normal one while the content of cadmium was higher. A discriminant analysis of all definable parameters revealed that the higher content of the products of oxidative destruction of proteins, lipids, fragmented DNA and the activity of cathepsin D, especially in its free form (by 235%), are the main characteristic signs of malignant ovarian tissue.

K e y   w o r d s: ovarian cancer, oxidative stress, apoptosis, metallothionein, glutathione, cathepsin D, copper, zinc.

Ovarian cancer dominates among the death causes of malignant tumors. In particular, according to the International Agency for Cancer Research, more than 165 thousand of newly diagnosed cases of ovarian cancer are registered each year over the world. This disease is the cause of death for more than 100 thousand women [1]. Currently there are no screening programs for precancerous and malignant ovarian pathology diagnostic, with the help of which specialists could have reduced the incidence and fatalities of this disease [2]. Therefore, the search and development of pathogenetically grounded methods of early diagnosis and treatment of ovarian pathology are the urgent issues of modern medical biochemistry, gynecology and oncology.

Ovarian cancer has an especially high ability to metastasize, which defines the clinical course of this disease [3, 4]. The main determinants of the invasiveness and metastatic potential of malignant cells include proteolytic enzymes involved in degradation of the membranes and extracellular matrix. Among this group of enzymes, an estrogen induced lysosomal aspartyl protease cathepsin D deserves special attention. It plays a central role in the catabolism of proteins, destruction of tissue architecture and indirect tumor progression via growth factors and p53 protein expression [4]. Cathepsin D is expressed and activated in cell lines of human breast and ovarian cancer, moreover, a high content of this enzyme is a recognized marker of an adverse prognosis [3,4]. Cathepsin D release from lysosomes may result from the oxidative destruction of membranes, but the relationships between these phenomena are not established [4, 5].

It is reported that in the pathological conditions in a cell, including malignant tumors resulted from a number of endogenous factors, such as mitochondrial dysfunction of malignant cells, the concentration of reactive oxygen species may significantly increase [6]. These species modulate the proliferative activity of cells, cause destructive changes in the biological membranes, cellular compartments etc. For example,

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HCC1937 cell line of human breast cancer contains a high level of hydrogen peroxide, which promotes the survival and resistance of cancer cells and inhibits their removal by apoptosis [7]. Similar results were obtained for other cancers as well [8], while the information on the molecular mechanisms of pro-oxidative changes in ovarian cancer is limited. A significant contribution to the development of oxidative stress may be caused by imbalance of the content of transition metals that are capable of generating radicals in the Fenton reaction and/or influencing the redox state of the cell by binding to cellular thiols [9]. Therefore, the goal of our study was to determine a functional state of the antioxidant defense system as well as the stress-sensitive and metal-binding thiols in conjunction with the cytotoxicity manifestations in malignant ovarian tissue samples and in ovarian tissue samples of people not affected by the relevant gynecologic pathology.

Materials and Methods

For this research we used the intraoperative samples taken from the tumor core (10×10 mm) of 15 newly diagnosed patients of reproductive age who had been operated for epithelial ovarian cancer at the Gynecological Department of the Ternopil Regional Oncological Dispensary. Cancer pathology was verified histologically. According to FIGO classification, all diagnosed patients had stage III disease. None of the operated oncologic female patients had been previously treated with platinum-based drugs (cisplatin/carboplatin/cycloplatam) or cyclophosphamide. Ovarian tissues of 15 females who died in the age of 22 to 35 years and were not affected by the relevant pathology during the sectional study, were taken as controls. The period from death to collection of samples was not more than six hours. All experimental studies were conducted in accordance with the rules of the National Congress on Bioethics (Kyiv, 2000) and the decision of the Commission on Bioethics of the Ternopil State Medical University (N 3, 2013).

All the procedures on tissues were carried out at 4 °C. All the reagents, except those specified below, were produced by "Synbias", "chemically pure" grade.

To determine the parameters of the antioxidant defense system state, Ovarian tissue samples were homogenized (1:10 w:v) in 0.1 M pH 7.4 phosphate buffer containing 100 mM KCl, 1 mM EDTA and 0.1 mM PMSF to inhibit proteolysis. Homogenization was carried out at 4 °C using 12–15 strokes of

a motor driven Teflon Potter-Elvehjem homogenizer. To determine the content of oxyradicals, we prepared 10% tissue homogenate in HEPES-sucrose buffer, pH 7.4. The determination was conducted in the soluble fraction of homogenate, which had been received to determine superoxide dismutase (SOD) and catalase (CAT) as a result of centrifugation of homogenate within 10 min at 6000 g (S6), to determine the level of oxygen radicals – as a result of its centrifugation within 45 min at 12 000 g (S12) and to determine the concentration of metallothioneins – within 45 min at 16 000 g (S16).

Superoxide dismutase activity (SOD) (EC 1.15.1.1) was measured by a decrease in the rate of the reduction of Nitrotetrazolium blue in the presence of phenazine methosulfate and NADH [10]. To determine the activity of Mn-SOD, S6 fraction was previously kept at 0°C in the presence of 5 mM KCN for 60 min, which caused a total suppression of Cu, Zn-SOD activity with the following determination of activity by the method [11]. The activity of Cu, Zn-SOD was calculated by the difference in the activities of total SOD and Mn-SOD. Enzymatic activity was expressed in conventional units (CU). An enzyme's activity, which was able to cause a decrease in optical density in the process of the reduction of Nitrotetrazolium blue in 50% test sample per 1 mg of protein from the homogenate in soluble form, was taken as 1 CU.

Catalase activity (EC 1.11.1.6) was measured in S6 fraction by Aebi method [12], which is based on the decomposition of hydrogen peroxide with the catalase derived from the sample. The test mixture contained 50 mg of protein in 50 mM K-phosphate buffer, pH 7.0 in the presence of 15 mM of H_2O_2 in the total volume of 3.0 ml. The reaction was initiated by adding the appropriate volume of S6 fraction, and then the absorbance at 240 nm within a 60-second interval was measured. Enzymatic activity was calculated by the millimolar coefficient of the hydrogen peroxide's light absorbance ($\varepsilon = -0.04$ mM^{-1}·cm^{-1}) and expressed in µmol/(mg of homogenate soluble protein·min.).

The content of total glutathione was determined in the homogenate tissue after complete reduction of glutathione through the use of glutathione reductase (Sigma, USA) and with the help of Ellman's reagent [13]. The level of 5-trinitrobenzoic acid was monitored with a spectrophotometer at 412 nm. To determine the content of oxidized glutathione (GSSG), 2-vinylpyridine was added to the incubation mixture

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to a final concentration of 2% [14] 60 min before the determination, and the content of reduced glutathione (GSH) was calculated as the difference of concentrations between total glutathione and its oxidized forms. Redox index (RI) GSH was calculated as the ratio ([GSH] - [GSSG])/[GSH].

The determination of protein carbonyls (PC) was conducted due to their ability to form 2,4-dinitrophenylhydrazones under the homogenate incubation of ovarian tissue samples in the presence of 0.1 M 2,4-dinitrophenylhydrazine in HCl, 2 M. The light absorbance was registered at 370 nm against the control, and the content of phenylhydrazone was calculated using a molar extinction coefficient of $2.1 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$ [15]. Lipid peroxidation was characterized by the products of interaction between deproteinized homogenate supernatant after precipitation of proteins with trichloroacetic acid (with final concentration of 5%) from ovarian tissue samples and 2-thiobarbituric acid (TBA). The formation of TBA-reactive substance (TBARS) was calculated by the intensity of the absorption of a pink-colored complex at 532 nm by the molar extinction coefficient of the complex equal to $\varepsilon = 1.56 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$ [16].

The content of oxygen radicals in S12 fraction of the ovarian tissue was evaluated using the non-fluorescent derivative, dihydrorhodamine, which is converted to the fluorescent dye, rhodamine-123, after a reaction with reactive oxygen species. The fluorescence signal was detected by using a f-max fluorescence plate-reader [excitation = 485 nm, emission = 538 nm] immediately and after 20 min and used to determine the rate of ROS formation [17] and expressed in relative fluorescence units (RFU) per 1 mg of protein.

The content of metallothioneins (MT) in S16 fraction of ovarian tissue was evaluated by the content of thiol groups (MT-SH) by the method of Viarengo et al. [18] with 5,5′-dithio-bis-2-nitrobenzoic acid (DTNB, Sigma, USA) after the chloroform-ethanol extraction of MT, and calculated assuming that 1 mol of MT contains the same amount of SH-groups as 20 moles of GSH [18]. The content of metals in the fraction of MT was determined after gel-permission chromatography on Sephadex G-50 [19]. MT content by its content of metals (MT-Me) was calculated by the modified Hamilton equation taking in account the stoichiometric nature of these metals' binding: m (metallothioneins) = 0.5 (ν(Zn, Cd)·M (MT)/7 + ν(Cu)·M(MT)/12) (µg), where ν – the quantity of metal in MT, µmol/g of tissue; M (MT) – MT molar mass (7000 g/mol), 7 and 12 – the number of the according ions that bind to a molecule of MT under complete saturation [20].

DNA damage was determined by the content of the fragmented deproteinized DNA in the total DNA by the method of alkaline precipitation of 10% tissue homogenate in 50 mM Tris-EDTA buffer, pH 8.0, containing 0.5% sodium dodecyl sulfate (Sigma, USA). The supernatant contains damaged DNA molecules when the pellet contains protein and a whole DNA. DNA content in the supernatant and in the pellet was determined by the Hoescht dye in the presence of 0.4 M NaCl, 4 mM of sodium cholate and 0.1 M Tris (pH 9.0) at the excitation wave (ex.) = 360 nm and emission (em.) = 450 nm [21]. The content of fragmented DNA was expressed as a percentage to the total DNA in the sample.

The enzymatic activity of cathepsin D (EC 3.4.23.5) was determined spectrophotometrically by the formation of acid-soluble products of hemoglobin enzymatic hydrolysis [22]. The reaction mixture contained 50% tissue homogenate in 0.25 M of sucrose solution and, as a substrate, 1% solution of bovine hemoglobin (Sigma, USA) in 0.1 M acetate buffer (pH 5.0). The enzymatic reaction was stopped by adding 10% solution of trichloracetic acid up to a final concentration of 2%. To determine the total activity of cathepsin D, the ovarian tissue homogenate sample was previously treated with 1% solution of Triton X-100 (Sigma, USA) for 10 min at 37 °C. The control sample was incubated at 4 °C for 30 min before adding 10% trichloracetic acid solution. The activity of cathepsin D was calculated by the difference in the optical density of experimental and control samples at 280 nm wave length and expressed as nmol of tyrosine/(min×g of tissue).

To determine the content of copper and zinc in the ovarian tissue and MT, the tissue samples and MT were digested in 65% pure nitric acid in a ratio of 1:5 (weight:volume) and determined using atomic absorption spectrophotometer C115 (Lomo, Russia). The content of cadmium was analyzed by atomic absorption spectrophotometer C-600 and expressed in µg, or µmol to nmol per 1 g of the wet tissue [19].

The results of the measurements are presented as means ± standard deviation (M ± SD) for 15 samples of the malignant and control ovarian tissue. If the data was not normally distributed according to the Lilieford test, it had been transformed using the Box-Cox method [23]. For the data that were not normally distributed even after the transformation,

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non-parametric tests (Kruskall-Wallis ANOVA and Mann-Whitney U-test) were performed. Differences were considered significant if the probability of Type I error was less than 0.05. The relationship between biochemical parameters of the ovarian tissue samples was evaluated using the principal component analysis (the value greater than 0.7 was considered as a probable factorial weight), discriminant analysis and correlation analysis (Pearson's correlation coefficient r under the probability of the value $P < 0.05$). All statistical calculations were performed with Statistica v 10.0 and Excel for Windows-2007.

Results and Discussion

The results of an evaluation of antioxidant defense system state (Table 1) show that Mn-SOD activity in the malignant ovarian tissue is higher (by 630%) than in controls, and the activity of Cu, Zn-SOD does not differ between the two groups. The catalase activity (by 49%) and reduced glutathione level (by 46%) are lower in the malignant tissue. At the same time, the intensity of the oxyradicals formation (by 332%), the concentration of TBARS (by 100%) and protein carbonyls (by 71%) and the lowest concentration of oxidized glutathione (by 17%) are higher in the tumor tissue compared to the control samples. Moreover, the redox index of glutathione is equal to 0.89 in control and 0.84 in the malignant tissue samples.

Analysis of the metals content in the ovarian tumors samples evidences that the content of copper (by 76%) and zinc (by 47%) is lower while the content of cadmium (by 1322%) is higher than in the comparison group (Table 2). Therefore, the ratio of the concentrations of zinc, copper and cadmium in the tumor and the control tissue (µmol/g of tissue) is fundamentally different: it is equal to 1.990:0.029:0.003 in control, and 1.050:0.007:0.040 in the malignant tissue.

The analysis of metals content in metallothioneins (Table 2) evidences that the differences between the groups, although they are presumable for copper and zinc, are substantially less than in the tissue. Thus, the content of zinc within the metallothioneins is higher in the malignant tissue than in control. However, the content of copper and cadmium in the composition of metallothioneins taken from the tumor tissue is less than one atom per molecule of this metal-binding protein [24]. From the comparison of the content of metals in the tissue and accumulated in the metallothioneins, we see that the massive imbalance of metals can be observed in the malignant ovarian tissue, including almost two times less content of zinc and five times less content of copper in non-binding form, whereas the content of such potentially toxic form of cadmium is nearly 40 times higher. The total content of the metal-binding form of metallothioneins (MT-Me) is commensurate

T a b l e   1. Biochemical parameters in the malignant and control ovarian tissues (M ± SD, n = 15)

Parameter	Groups	
	Control tissue	Malignant tissue
MT-SH content, µg·g⁻¹ of tissue	7.6 ± 0.9	15.3 ± 1.8*
MT-Me content, µg·g⁻¹ of tissue	6.2 ± 0.5	6.8 ± 0.8
Reduced glutathione content, µmol·g⁻¹ of tissue	5.0 ± 0.5	2.7 ± 0.5*
Oxidized glutathione content, µmol·g⁻¹ of tissue	0.6 ± 0.1	0.5 ± 0.1*
Cu,Zn-SOD activity, CU·mg⁻¹ of protein	1.5 ± 0.3	1.4 ± 0.2
Mn-SOD activity, CU·mg⁻¹ of protein	1.3 ± 0.2	9.5 ± 0.8*
Catalase activity, mmol·min⁻¹·mg⁻¹ of protein	27.2 ± 5.8	18.2 ± 1.5*
TBARS content, nmol·g⁻¹ of tissue	18.0 ± 2.1	35.8 ± 4.3*
Protein carbonyls level, nmol·g⁻¹ of tissue	1.4 ± 0.3	2.4 ± 0.3*
Oxyradicals content, RFU·mg⁻¹ of protein	0.34 ± 0.05	1.47 ± 0.27*
Total activity of cathepsin D, nmol·min⁻¹·g⁻¹ of tissue	3.7 ± 0.5	8.5 ± 0,9*
Free cathepsin D activity, nmol·min⁻¹·g⁻¹ of tissue	1.7 ± 0.3	5.7 ± 0.8*
Content of fragmented DNA in total DNA, %	3.4 ± 0.6	6.8 ± 0.5*

Note. Here and Table 2: *differences compared with data for the intact ovarian tissue are presumable, $P < 0.05$

T a b l e 2. Content of copper, zinc and cadmium in the ovarian tissue and metallothioneins (M ± SD, n = 15)

Parameter	Control	Ovarian cancer
Cu, μg·g^{-1} of tissue	1.83 ± 0.19	0.44 ± 0.06*
Zn, μg·g^{-1} of tissue	129.5 ± 21.4	68.3 ± 19.0*
Cd, μg·g^{-1} of tissue	0.32 ± 0.04	4.55 ± 0.52*
Cu-MT, nmol·g^{-1} of tissue	2.2 ± 0.2	1.6 ± 0.2*
Zn-MT, nmol·g^{-1} of tissue	9.2 ± 1.2	10.9 ± 1.5*
Cd-MT, nmol·g^{-1} of tissue	2.0 ± 0.2	1.8 ± 0.2
Cu : Zn : Cd (MT)	1.0 : 4.3 : 0.9	1.0 : 7.0 : 1.1

in the tissue samples from both groups, but the total content of metallothioneins protein determined by the content of thiols (MT-SH), is twice as high as in the malignant tissue compared to the intact tissue.

The signs of cytotoxicity are determined in the tumor tissue: an increased (compared to control) level of DNA fragmentation (by 100%) and cathepsin D activity, both of its total (by 130%) and free forms (by 235%).

Using the method of principal component analysis (a subtype of the multivariate data analysis) allowed us to quantitatively prove the degree of the relationships between the changes of individual indicators in the tissue samples of comparison groups. As shown in Fig. 1, 63.47% of the absolute values belong to Factors 1 and 2. By their values, MT-SH form a common cluster with the parameters of oxidative stress, cytotoxicity and cadmium, which are localized opposite to the group of essential metals, such as zinc and copper, catalase and reduced glutathione concerning Factor 1 (Fig. 1). This arrangement proves the affinity of the consistencies of the values changes within the cluster and their opposite character between the two clusters. With the help of stepwise discriminant analysis, the biochemical markers were defined, which differentiate the studied tissue samples with high reliability (F(10.13) = 606.95, $P < 0.001$). In particular, the characteristic signs of the malignant ovarian tissue include high activity values of free cathepsin D, the level of fragmented DNA, TBARS and protein carbonyls, as well as the low content of reduced glutathione in the tissue.

The concordance increase in Mn-SOD, MT-SH and oxyradicals levels (r(Mn-SOD/oxyradicals) = 0.91, $P < 0.001$; r (MT-SH/oxyradicals) = 0.85, $P < 0.001$) in the malignant tissue indicates the alarm function of reactive oxygen species under the overexpression of these stress-dependent proteins

[25]. It is believed that such coordination may be based on the oxyradicals induced activation of MAP kinase pathway (MAPK) and a number of sensitive to oxidative stress transcription factors including Nrf2. The last one is assigned to the induction regulation of the cytoprotective genes block through binding to the antioxidant-responsive promoter element [26] and contributes to balancing the level of reactive oxygen species and therefore, the regulation of the transformed cells proliferation. The data on the role of metallothioneins and SOD overexpression in tumor cells is conflicting. Mitochondrial Mn-SOD, as a powerful antioxidant factor, plays a crucial role in cancer development. Many types of tumor cells are marked with the low protein and Mn-SOD activity, but some forms of tumors have a high level of both expression and activity of this enzyme, which can display different types and stages of cancer development [27]. Overexpression of both Mn-SOD and Cu, Zn-SOD as a result of gene therapy inhibits the growth of breast cancer cells *in vitro* and *in vivo* [28].

Such contradictory information also concerns the estimation of the MT role in cancer cells. MT are low-molecular weight, cysteine-rich (up to 30% of amino acids), metal-keeping and stress proteins, which chelating zinc, cadmium and copper in cells. Because of the presence of three elements in the promoter of MT genes, metal-responsive, antioxidant-responsive and glucocorticoid- responsive, they are induced by metals, many stress factors and pro-oxidants and in vitro inhibit peroxide-radical processes [29-31]. The formation of the joint MT cluster with the parameters of oxidative stress in the ovarian tissue in the diagram obtained by the principal component analysis (Fig. 1), and the similarity of MT-Me index in the two groups evidences that under ovarian cancer MT function is connected to scavenge of re-

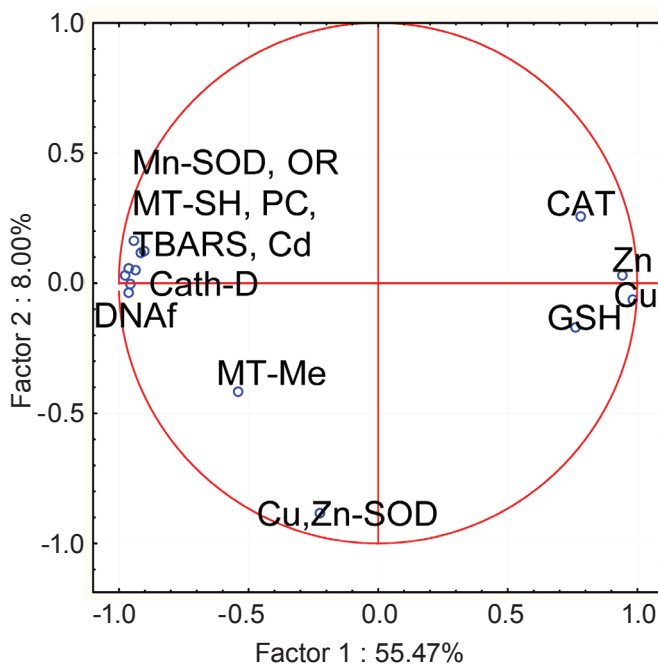*ISSN 2409-4943. Ukr. Biochem. J., 2015, Vol. 87, N 5*

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Fig. 1. The multivariate analysis of the metals content and the parameters of the oxidative stress system and apoptosis in the malignant and control ovarian tissues. Legend: MT-SH, content of metallothioneins by the level of thiols in the protein; MT-Me, content of metallothioneins by the level of metals (copper, zinc and cadmium) in the protein; Cu, Zn, Cd, metal content in the tissue; GSH, reduced glutathione content; PC, protein carbonyls level; TBARS, TBA-reactive substance content; OR, oxyradicals content; Cu,Zn-/Mn-SOD, Cu,Zn-/Mn superoxide dismutase activity; DNAf, DNA fragmentation; CAT - catalase activity; Cath-D, cathepsin D

active oxygen species (r (MT-SH/oxyradicals) = 0.85, $P < 0.001$) more, than to binding of metals, which had been noted by us under thyroid pathology [32, 33]. At the same time, we know that more than 74% of carcinomas have lower MT level than normal mucosa. However, overexpression of MT under colon cancer and high protein content specified by immunochemical method (i.e. metal-binding form of MT-Me), under breast cancer is considered a sign of unfavorable prognosis of the disease, including the formation of metastases, and in the use of chemotherapy [34]. At least 10 genes encoding MT, are associated with breast cancer [34]. However, under testicular cancer, the high levels of MT defined immunochemically, is the evidence of a positive prognosis for the use of chemotherapy [35]. In addition, the isoforms composition of MT may determine the mechanism of cell death, moreover, MT-3 expression leads to the inhibition of the apoptosis and necrosis scenario development [36]. Thus, coordinated activation of Mn-SOD and increase of MT content, which is served as radicals scavenger, can not be interpreted uniquely in the studied pathology. Therefore, simultaneous increase in activity and/or

contents of SOD and MT stress-related protein and parameters of prooxidant changes are observed in the tumor tissue. The reasons for the accumulation of the reactive oxygen intermediates can be both the depleted pool of glutathione and the imbalance between SOD activation and the inhibition of catalase activity, as evidenced by the negative correlation between these parameters ($r = -0.78$, $P < 0.001$) and the results of a multivariate analysis. Interrelations between SOD activities: catalase is six times higher in the malignant tissue than in control, which shows an insufficiently effective removal of hydrogen peroxide – a product of superoxide anion dismutation. It is commonly known, that with the participation of redox-active metals, mostly iron, hydrogen peroxide converts into highly reactive a hydroxyl radical [25]. In turn, the accumulation of oxygen radicals contributes to high proliferative capacity of the malignant cells.

The level of reduced glutathione and, even more, its redox index, refers to the prognostic signs of tumor growth because it largely controls the redox balance in cells, which, in turn, affects gene expression, cell differentiation, proliferation and

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apoptosis [37]. It is believed that the elevated level of glutathione indicates cellular resistance to chemotherapy with the help of cisplatin [38]. It is reported about the elevated glutathione level in the ovarian tumor tissue or its variability depending on the stage of tumor development (with an increase of the level in the process of tumor growth), while such characteristic is not observed under cervical cancer, or the reduction of glutathione is estimated in the process of tumor development [39]. Therefore, disregarding the large number of explorations, their results are ambiguous and, moreover, not correlated with other characteristics of the tissue. In our study, we clearly revealed common patterns of the changes of glutathione, copper and zinc levels in the ovarian tumor tissue (Fig. 1). These results indicate the irreversible exhaustion of glutathione, probably due to the removal of its essential ligands - zinc and copper - from the tissue and minimizing of its redox index. The reduction of copper content in the tissue can be assessed as a positive sign because the chelation and removal of copper and other essential metals as well as glutathione is considered as an effective component in limiting tumor progression and angiogenesis and inflammation related to it [40], while the higher content of zinc, copper and glutathione in the malignant tissue indicates tumor progression [41]. Identifying destructive changes of proteins, lipids and DNA evidences of a significant degree of tissue damage, despite the activation of stress-sensitive proteins.

An increased content of cadmium in a non-binding with MTs form occurred to be another damaging factor in the malignant tissue. An elevated level of cadmium was observed in the urine of women with breast cancer [42]. Our results let us estimate the relation between the level of cadmium in the ovarian tissue and the manifestations of oxidative damage to proteins and DNA:

$Cd = -5.44 + 3.6 \times PC^* - 0.02 \times TBARS - 0.12 \times OR + 0.33\ DNAf^*$, $R^2 = 0.90$, $F_{(4.19)} = 41.5$, $P < 0.001$ (*parameter makes a presumable contribution to the mathematical model). Thus, it's obvious that an increase in cadmium content in ovaries became one of the factors of the radical-mediated pathological process and tumor growth.

Lysosomes are considered to be the most vulnerable cell targets for oxyradicals [43]. However, under the positive scenario, they induce autophagy – a strictly regulated lysosomal pathway, which ensures the degradation of cytoplasmic structures and the cancer cells' death [43]. Regarding cathepsin D, its increased expression and release from lysosomes may both launch the apoptotic cascade with the death of damaged cells, functioning not as a protease, but as a signal factor, and inhibit apoptosis promoting malignant growth [44]. Moreover, peculiarly cathepsin D plays a key role in the progression of a number of malignant tumors [4, 44] and is a recognized independent marker of the unfavorable prognosis for various types of tumors [44, 45]. We have shown that cathepsin D relates to determining factors of the comparison group differentiating in the malignant ovarian tissue.

Thus, a multi-marker analysis of stress-sensitive processes in the ovarian tumors allowed to determine the amount of features, under which the pathological changes in the malignant tissue are compounded – among them are the discoordination of the stress-dependent proteins activities, the imbalance of the sub-cellular distribution of copper, zinc and cadmium, as well as the activation and release of lysosomal cathepsin D.

ПРОЯВИ ОКИСНОГО СТРЕСУ ТА МОЛЕКУЛЯРНИХ УШКОДЖЕНЬ У РАКОВІЙ ТКАНИНІ ЯЙНИКІВ

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Показники окисного стресу є визнаними молекулярними маркерами та прогностичними критеріями злоякісного переродження тканини, проте їх виявлення залежать від типу пухлин і стадій їх розвитку. Метою дослідження було з'ясувати взаємозв'язок між характеристиками системи окисного стресу, у тому числі й метал-асоційованими, та проявами цитотоксичності в онкотрансформованій тканині яйників людини. Вперше встановлено вищий рівень Mn-супероксиддисмутазної активності цитозолю (на 630%) та протеїну металотіонеїну (МТ, на 100%) у трансформованій тканині порівняно з неура-

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женою тканиною яйників. У пухлинній тканині значно вищий рівень утворення оксирадикалів (на 332%), нижча активність каталази (на 49%) та нижчий вміст відновленого глутатіону (на 46%) та його редокс індексу (0,84 проти 0,89 у контролі). За відносно стабільного вмісту цинку, купруму та кадмію у складі МТ, вміст цинку та, особливо, купруму у недепонованій формі істотно нижчий у трансформованій тканині, а вміст кадмію вищий. Дискримінантний аналіз всіх досліджуваних показників виявив, що підвищений вміст продуктів окисного ураження протеїнів, ліпідів, фрагментованої ДНК та активність катепсину Д, особливо його вільної форми (вище на 235%) належить до головних характеристичних ознак онкотрансформованої тканини яйників.

К л ю ч о в і с л о в а: рак яйників, окисний стрес, апоптоз, металотіонеїни, глутатіон, катепсин Д, купрум, цинк.

ПРОЯВЛЕНИЯ ОКИСЛИТЕЛЬНОГО СТРЕССА И МОЛЕКУЛЯРНЫХ ПОВРЕЖДЕНИЙ В РАКОВОЙ ТКАНИ ЯИЧНИКОВ

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Показатели окислительного стресса являются признанными молекулярными маркерами и прогностическими критериями злокачественного перерождения ткани, однако их выраженность зависит от типа опухоли и стадий ее развития. Целью исследования было выяснение взаимосвязи между характеристиками окислительного стресса, в том числе и металлассоциированными, и проявлениями цитотоксичности в онкотрансформированной ткани яичников человека. Впервые установлен более высокий уровень активности Mn-супероксиддисмутазы (на 630%) и протеина металлотионеина (МТ, на 100%) в трансформированной ткани по сравнению с таковой у лиц с нетрансформированной тканью яичников (контроль). В опухолевой ткани уровень образования оксирадикалов (на 332%) значительно выше, а активность каталазы (на 49%), содержание восстановленного глутатиона (на 46%) и его редокс индекс – ниже (0,84 по сравнению с 0,89 в контроле). При относительно стабильном содержании цинка, меди и кадмия в составе МТ в опухолевой и здоровой ткани, содержание цинка и, особенно, меди в недепонированной форме в трансформированной ткани существенно ниже, чем в контроле, а содержание кадмия выше. Дискриминантный анализ всех исследуемых показателей выявил, что повышенное содержание продуктов окислительной деструкции протеинов, липидов, фрагментированной ДНК и активность катепсина Д, особенно его свободной формы (выше на 235%) относится к главным характеристическим признакам онкотрансформированной ткани яичников.

К л ю ч е в ы е с л о в а: рак яичников, окислительный стресс, апоптоз, металлотионеины, глутатион, катепсин Д, медь, цинк.

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