Exp Oncol 2014
36, 3, 196–201

Experimental ONCOLOGY

PECULIARITIES OF ANTIOXIDANT SYSTEM AND IRON METABOLISM IN ORGANISM DURING DEVELOPMENT OF TUMOR RESISTANCE TO CISPLATIN

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***Aim*: To study *in vivo* the peculiarities of changes of iron metabolism and antioxidant system in dynamics of growth of Guerin carcinoma with different sensitivity to cisplatin. *Materials and Methods*: In order to evaluate the content of metallothionein-1 (MT-1) in tumor homogenates and blood serum of rats with cisplatin-sensitive and cisplatin-resistant Guerin carcinoma the immunoenzyme method was used. The evaluation of ceruloplasmin activity, content of "free iron" complexes, superoxide and NO-generating activity of NADPH-oxidase and iNOS activity in neutrophils, blood serum and tumor homogenates was measured by EPR-spectroscopy. *Results*: Maximal accumulation of MT-1 in blood serum and tumor, more pronounced in resistant strain, at the border of latent and exponential phase of growth has been shown that is the evidence of protective role of this protein in the respect to the generation of free radical compounds. It has been determined that in animals with cisplatin-resistant strain of Guerin carcinoma, increase of "free iron" complexes is more apparent both on the level of tumor and organism on the background on increase of CP/TR ratio that is the consequence of organism antioxidant protection system disorder. *Conclutions*: Mentioned changes in metabolism of iron with its accumulation in tumor and further reprogramming of mitochondria metabolism and activity of NADPH-oxidase for non-transformed cells are favorable conditions for the formation of oxidative phenotype of tumor.**

***Key Words:* Guerin carcinoma, metallothionein-1, antioxidant system, transferrin, ceruloplasmin, "free iron" complexes, activity of NADPH-oxidase and iNOS neutrophils.**

Today, condition of antioxidant system (AOS) has significant place in the chain of insufficiently studied rearrangements occurring in the organism of host in the process of development of neoplasm. It has been proved that activation of oxidative processes, which cause disintegration of cellular membranes and destabilization of many elements of metabolism underlie pathological changes associated with malignant transformation of cell. The increase of oxidation of lipids, proteins and DNA becomes more intensive during progression of tumor process. It has been determined that tumor progression is accompanied with development of oxidative stress and this consequently requires effective mechanisms of protection from damaging impact of reactive oxygen species (ROS) and other free radical compounds. The system inactivating free radical compounds is AOS. By localization, antioxidant protection system is divided into the intra- and extracellular antioxidants. To the intracellular antioxidants are referred glutathione, α-tocopherol, Zn/Mg-SOD, catalase, glutathione peroxidase, ferritin and other. System of extracellular antioxidants consists of low-molecular substances (ascorbic and uric acids, bilirubin) and high-molecular compounds (extracellular superoxide dismutase, SH-groups of proteins, ceruloplasmin — CP and transferrin — TR). Development of tumors causes the activation of AOS of blood, in particular, involvement of CP and TR [1–4]. CP is known to be multifunctional copper-containing protein — oxidase of α_2-globulin fraction of blood plasma of mammals, which performs non-specific protection at exo- and endogenous damages. Also, it is connected with hematopoietic system playing role of the catalyst in transformation of Fe^{2+} in Fe^{3+} with its followed including in apo-transferrin. It has been determined that development of malignant process causes accumulation in organism of "free iron" (Fe^{2+}), which activates processes of lipid peroxidation (LPO) that causes in turn increase of antioxidant activity of blood serum, exactly activity of CP and TR [6–8]. By its origin, TR is single chain iron-containing glycoprotein of β-globulin fraction of blood serum, which plays the main role in transfer and regulation of iron metabolism in tissues and also impacts the antioxidant potential of blood. It should be mentioned that CP along with TR forms molecular system able to regulate content of iron ions (Fe^{2+}), total antioxidant activity of blood serum and oxidation of Fe^{2+} by CP. It has been showed that CP/TR (lactoferrin) ratio increases in cancer patients compared with indices of healthy people that is the evidence of significant content of Fe^{2+} as a pro-oxidant marker [4, 6–11]. Thus, complicated system of interaction between CP and TR, which is fully connected with transformation and redistribution of iron in organism with its further involvement in the processes of generation of ROS, proves once again the importance of this microelement in occurrence and development of malignant neoplasm.

In recent time, significant attention was given to one more little-studied component of AOS of organism — metallothionein (MT). For instance, it has been showed that MT owing to significant content of SH-bonds is capable to change redox-condition and act as active interceptor of superoxide radicals [12–14]. For this reason, evaluation and analysis of the processes, which allow cells to regulate reaction to exo- and

Submitted: July 16, 2014.
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Abbreviations used: AOS – antioxidant system; CP – ceruloplasmin; EPR – electron paramagnetic resonance; iNOS – inducible NO-syntase; LPO – lipid peroxidation; MT – metallothionein; NADPH – nicotinamide adenine dinucleotide phosphate reduced; ROS – reactive oxygen species; TNF – tumor necrosis factor; TR – transferrin.

endogenous impacts at tumor process, have very important practical significance. On the example of antioxidant protection in development of malignant neoplasms in organism, conception of "tumor-host" interaction is confirmed. Study of AOS of organism (exactly chelators of metal ions with variable atomicity, MT-1) and parallel determination of "free iron" complexes and generation of ROS, especially taking into account sensitivity of malignant neoplasm to antitumor drugs, is insufficiently studied direction in oncology.

Taking into account mentioned above, the aim of our study was to analyze the peculiarities of iron metabolism and AOS in organism of host in dynamics of tumor drug resistance development.

MATERIALS AND METHODS

Animals and experimental model. Object of our study included 80 male rats with sensitive and resistant to cisplatin Guerin carcinoma. Guerin carcinoma was transplanted subcutaneously (2×10^6 cells/rat). Resistant strain was obtained through subsequent transplantations of Guerin carcinoma after carrying out of chemotherapy course with cisplatin to the rats. To obtain resistant tumor, 10 courses of chemotherapy were carried out. All animals were decapitated with adhering current bioethical norms. According with the aim of study, change in the level of metal-containing proteins in blood of animals was determined in different periods of growth of Guerin carcinoma — 1, 3, 5, 7, 17 and 23 days after transplantation. By conventional bioethics, 1–5 day of development of tumor is considered as latent growth period, 6–14 day is referred to the phase of exponential growth, and 23 day — to the terminal phase of tumor growth.

In blood serum and tumor tissue of experimental animals, content of the following metal-containing proteins was studied: MT-1 and TR [4]. Also, in blood and tumors of these animals, "free iron" complexes were determined [2]. Moreover, in blood of experimental rats, activity of CP and superoxide- and NO-generating activity of NADPH-oxidase, iNOS neutrophils was determined. In tumor tissue, level of superoxide radicals in mitochondria was registered. Mentioned above indices in tumor were studied on 7th, 14th and 23rd days after its transplantation, exactly in those periods, when changes may obtain essential significance.

Evaluation of the content of MT-1 in blood serum and tumor homogenates of rats with Guerin carcinoma by immunoenzyme method. Content of MT-1 in blood serum and tumor homogenate was determined by immunoenzyme method — IEM (ELISA) using appropriate kits of reagents: USCN (China) with the help of automatic biochemical and immunoenzyme analyzer Chem Well 2990. Samples of blood serum for IEM were obtained according with the recommendations indicated in instructions to the kits. All samples of blood serum had no signs of hemolysis. Homogenate of tumor tissue was obtained in physiological solution in the ratio of ⅓.

Evaluation of CP activity, content of TR, "free iron" complexes and superoxide- and NO-generating activity of NADPH-oxidase, iNOS neutrophils in blood of experimental animals by EPR. In blood of experimental animals, activity of CP and content of TR, quantity of "free iron" complexes and superoxide- and NO-generating activity of NADPH-oxidase, iNOS neutrophils were analyzed by method of electron paramagnetic resonance (EPR) at the temperature of liquid nitrogen 77 K. As anticoagulant, trilon B was used. Samples for the study with computerized spectrometer were prepared using special press-form with followed freezing in liquid nitrogen.

Determination of content of TR, "free iron" complexes and levels of superoxide radicals in tumor tissue of experimental animals by EPR. To determine these indices, 0.5 g of tumor tissue was placed in special press-forms and frozen in liquid nitrogen. After this, EPR was applied using spin trap — 1-hydroxy-2,2,6,6-tetramethyl-4-oxo-piperidine hydrochloride (TEMPONE-H) [4].

Statistical analysis. Significance of differences between indices of different groups was evaluated using Student's *t*-criterion. Differences were considered significant at $p<0.05$. Statistical processing of data was carried out using software STATISTICA 6.0.

RESULTS AND DISCUSSION

Studies showed that from 1st to 7th day after transplantation of tumor, gradual increase of MT-1 was observed in blood serum of animals. Maximal value of this index both in tumor and blood serum was registered on 7th day. It means that results of our studies prove that this metal-containing protein plays key role exactly in the end of latent and beginning of exponential stage of tumor growth protecting malignant neoplasm from reactive oxygen forms. Also, it has been confirmed by our data concerning generation of free radical compounds exactly in mentioned period of tumor growth that will be discussed below. At the peak of exponential tumor growth (14 days after transplantation), gradual decrease of MT-1 in tumor tissue and blood serum was observed. Mentioned changes were more pronounced in development of resistant tumor (Table 1).

These data give ground for assumption that MT-1 plays important role in forming of resistant phenotype in animals with Guerin carcinoma. Our view coincides with the results of some studies, which have showed that due to metallothioneins, cells are capable to acquire resistance, in particular, to the tumor necrosis factor (TNF) [15, 16]. Presence in molecule of MT sulfhydryl groups with nucleophilic properties gives them opportunity to bind not only metal ions, but also ROS [13, 14]. It has been determined that high levels of metallothioneins are found in the places of inflammation and malignant transformation of tissue. Data of literature show that MT plays significant role in occurrence and development of tumor process [16, 17]. On the example of patients with hepatocellular carcinoma, it has been shown that high expression of MT-1 in tumors correlates with low efficacy of chemotherapy with carboplatin [18]. It has been

shown that hyperexpression of MT is antiapoptotic and stimulates increase the proliferative activity of tumor cells. MT activates and specifically interacts with p50 (subunit of NFkB transcription factor) that causes the transactivation of the latter [14].

Table 1. Content of MT-1 in tumor tissue and blood serum of animals with Guerin carcinoma in different stages of its growth

Groups of animals by periods after transplantation	Content of MT-1, pg/ml			
	Sensitive strain		Resistant strain	
	serum	tumor	serum	tumor
Control (intact animals)	282.40±9.73	–	279.40±8.23	–
1 day	319.41±14.23	–	320.10±15.14	–
3 days	334.22±17.13*	–	350.12±19.80*	–
5 days	535.10±34.50*	–	750.34±21.18*	–
7 days	634.20±31.23*	740.33±23.14	820.10±32.14*	920.43±38.12*
14 days	424.12±9.24*	510.18±28.10*	506.92±15.52*	512.31±21.18*
23 days	423.64±13.51*	390.14±16.13*	399.61±10.21*	490.12±31.21*

Note: *p < 0.05 – significant regarding control values.

Till this day, condition of extracellular high-molecular AOS of blood at development of malignant neoplasm remains insufficiently studied. As stated above, exactly such proteins, as TR and CP in blood plasma, form AOS that regulates concentration of renewed iron ions [4, 18]. The main role in antioxidant protection of organism is performed by these proteins due to the inhibition of iron-induced peroxidation of the components of extracellular environment (i.e., due to the oxidase activity of CP and iron-binding and transport function of TR). First of all, we draw attention to the one of the proteins, which participates in iron metabolism, exactly — TR. According with data of literature, decrease of TR in dynamics of tumor growth in 2.5–3 times on the background of simultaneous increase of CP in 1.5–2 times occurs in blood plasma of animals [19].

Results of our studies of TR content in blood of experimental animals with Guerin carcinoma (sensitive and resistant strains) showed that almost on the first day of tumor growth, its level in blood starts gradually decreasing compared with control (Table 2). In tumor tissue of sensitive to cisplatin strain of Guerin carcinoma, level of TR remained on the same level both in latent and exponential growth phases. In the end of terminal growth phase, insignificant decrease of this index was observed that may be connected with the processes of necrobiotic changes and necrosis in tumor. The same tendencies concerning content of TR were observed in blood serum and tumor of resistant strain.

Table 2. Content of TR in tumor tissue and blood of animals with Guerin carcinoma in different stages of its growth

Groups of animals by periods of transplantation	TR level (RU)			
	Sensitive strain		Resistant strain	
	serum	tumor	serum	tumor
Control (intact animals)	0.93±0.07	–	0.88±0.1	–
1 day	0.67±0.06	–	0.82±0.21	–
3 days	0.55±0.04*	–	0.64±0.12	–
5 days	0.48±0.12*	–	0.58±0.10	–
7 days	0.35±0.03*	0.15±0.05	0.47±0.12*	0.16±0.05
14 days	0.24±0.05*	0.15±0.09	0.39±0.20*	0.14±0.12
23 days	0.16±0.07*	0.10±0.05*	0.21±0.04*	0.12±0.05*

Note: *p < 0.05 – significant regarding control values.

It should be mentioned that recent publications on mechanisms of metal metabolism in organism point at significant role of TR in transfer and accumulation of iron. For instance, on the example of cell line of small cell lung carcinoma NCL-H510, expression of this protein on their surface was detected by method of immune fluorescence [20]. Also, it has been determined that synthesis of TR 10 times increases during cell proliferation. Thus, it has been proved in study that hyperexpression of TR induces cellular proliferation, but fully depends on regulatory system of iron metabolism (autocrine mechanism of regulation) [20]. Other studies *in vivo* showed that at presence in organism of malignant process, decrease of content of TR and decrease of iron concentration in blood serum occurs [21–23]. It results in disorder of iron transfer from blood serum to ferritin in tumor cells that is the evidence of accumulation and storage of iron ions in tumor cells. Since another one metal-containing protein, which is a part of extracellular high-molecular AOS of organism and indirectly participates in the processes of recovery and oxidation of iron in blood, is CP, we decided to study activity of this protein in host blood, as well as to analyze CP/TR ratio in dynamics of tumor growth as marker of reserve possibility of AOS (Table 3).

Table 3. Activity of CP and CP/TR ratio in blood of animals with Guerin carcinoma in different stages of its growth

Groups of animals by periods of transplantation	CP activity (RU)			
	Sensitive strain		Resistant strain	
	CP	CP/TR	CP	CP/TR
Control (intact animals)	0.86 ± 0.09	0.92	0.88 ± 0.1	0.85
1 day	1.94 ± 0.11	1.49*	0.48 ± 0.06	0.58*
3 days	5.51 ± 0.09*	10.01*	0.50 ± 0.04	0.78
5 days	7.21 ± 0.16*	15.02*	0.69 ± 0.09	1.18
7 days	7.03 ± 0.10*	20.08*	1.13 ± 0.25*	2.40
14 days	5.79 ± 0.12*	24.12*	1.8 ± 0.18*	4.61
23 days	5.99 ± 0.15*	37.43*	2.76 ± 0.31	13.14

Note: *p < 0.05 – significant regarding control values.

Obtained results prove that along with decrease of content of TR (Table 2), increase of CP activity occurs (Table 3) in blood of animals with sensitive and resistant Guerin carcinoma that is the evidence of disorder of iron transport in host organism. After all, ratio CP/TR increases, and it may cause the increase of "free iron" concentration. Thus, as activity of CP and TR alters, disorder of balance oxidant/antioxidant occurs with further development of oxidative stress. However, values of CP/TR in different stages of tumor growth were lower in blood of animals with resistant strain, than in blood of animals with sensitive strain. It can be explained by the fact that as resistance of cisplatin forms, changes in transportation of the component of CP, copper, occurs due to the series of proteins-transporters. Moreover, according to the data of literature, key protein-transporter of copper CTR1 plays important role not only in transporting of copper, but also cisplatin. Authors of these studies showed that, on the one hand, copper is required for transportation of platinum inside cell, and, on the other hand, it competes with platinum for the sites of binding [24]. Series of studies on cellular lines of breast and ovarian

cancer resistant to cisplatin have showed alteration in expression of series of proteins-transporters of copper: CTR1, ATP7A, ATP7B, which also are involved in transportation of cisplatin and its derivatives. Direct correlation between delivery of copper and cisplatin in cells and decrease of transportation of these metals in tumor with phenotype resistant to cisplatin has been determined [25]. Clinical studies showed direct correlation between increase of concentrations of copper and CP in blood serum of patients with breast, ovarian, lung, and intestine cancers and progression of tumor process [26]. For this reason, we have made an assumption that low levels of CP activity in blood of animals with resistant to cisplatin strain (regarding indices of sensitive strain) are connected with disorder of transportation of copper as a result of decrease of expression of proteins-transporters CTR1, ATP7A, ATP7B.

It has been determined that at malignant transformation, which is accompanied with disorder of iron metabolism in organism, misbalance of system of antioxidant protection with involvement of metal-containing proteins CP and TR is observed. Total antioxidant activity of serum concerning Fe^{2+}-induced LPO is mainly depended on content of these proteins. It should be mentioned that exactly TR binds iron ions Fe^{3+} that prevents their renewal and participation in Fenton/Haber — Weiss reaction, which indirectly causes release of free radical compounds with further damage of many metabolic processes [9, 10]. It has been shown that interaction between metals (first of all iron) and hydrogen peroxide results in formation of the most toxic form of ROS. It damages DNA, RNA as well as carbohydrates, amino acids and lipids. Moreover, presence of ions of these metals may cause spontaneous and induced oxide modification of proteins. It is known that these changes acquire higher sensitivity to proteolysis that causes invasion of tumor in surrounding tissues [10].

For the full understanding of changes of CP and TR as metal-containing proteins, as well as important components of AOS, we have studied quantity of "free iron" complexes in dynamics of tumor growth both in tumor tissue and peripheral blood.

Obtained results showed that increase of "free iron" complexes in tumor tissue of both strains in dynamics of development of malignant neoplasms was accompanied with simultaneous increase of the rate of generation of superoxide radicals (Table 4). However, stronger increase of level of superoxide radicals was observed in resistant tumors, especially 23 days after transplantation. It demonstrates that for resistant tumors, more active generating of superoxide radicals is typical compared with sensitive tumors that in turn causes more apparent destabilization of iron-containing proteins. Moreover, we have determined direct correlation between increase of rate of generating of superoxide radicals and number of "free iron" complexes in sensitive ($r = 0.98$) and resistant ($r = 0.79$) tumors.

Table 4. Number of "free iron" complexes and rate of generating of superoxide radicals in tumor tissue of Guerin carcinoma in different stages of its growth

Groups of animals by periods after transplantation	"Free iron" complexes (RU)		Rate of generating of superoxide radicals in tumor tissue (nmol/g of fresh tissue·min)	
	Sensitive strain	Resistant strain	Sensitive strain	Resistant strain
7 days	0.19 ± 0.08	0.21 ± 0.07	0.50 ± 0.1	0.51 ± 0.23
14 days	0.61 ± 0.05*	0.68 ± 0.04*	0.51 ± 0.1	0.63 ± 0.18
23 days	1.95 ± 0.08*	2.00 ± 0.08*	0.56 ± 0.09	0.64 ± 0.11*

Note: *$p < 0.05$ – significant regarding control values.

At the same time, number of "free iron" complexes in peripheral blood of the same animals in dynamics of malignant process was studied. It is well-known that emergence of "free iron" in blood is stipulated by activation of LPO and damage of membranes, hemolysis, destruction of iron-containing proteins, transformation of Fe^{3+}-transferrin in apo-transferrin: Fe^{3+}-TR $\xrightarrow{e^-}$ Fe^{2+} + apo-TR, as well as release of deposited iron from ferritin. It is also known that ions Fe^{2+} play important role in LPO, and iron "overload" of organism causes damage of cellular structures. Low-molecular iron complexes are promoters of free radical reactions, and emergence of "free iron" pool in blood causes the formation of ·OH and $O_2^{\cdot-}$.

Our studies have determined increase of "free iron" complexes in peripheral blood of animals in dynamics of growth of both tumor strains (Figure).

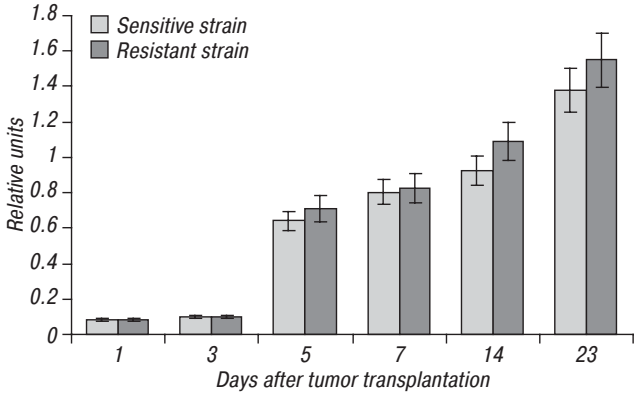

Figure. Number of "free iron" complexes in peripheral blood of rats with sensitive and resistant Guerin carcinoma in dynamics of tumor growth

It should be mentioned that in peripheral blood of intact animals, "free iron" complexes were not detected. Mentioned increase of level of superoxide radicals and "free iron" complexes in experimental animals are the evidence of development of oxidative stress. Moreover, we have studied rate of generating of superoxide radicals by NADPH-oxidase and activity of iNOS neutrophils depending on phases of growth of Guerin carcinoma of both strains. Gradual increase of these indices from the beginning of tumor growth compared with their values in corresponding controls has been determined (Table 5). As seen from Table 5, changes of activity of NADPH-oxidase and iNOS neutrophils in blood of animals with Guerin carcinoma in dynamics of growth of both sensitive and resistant strains are unidirectional. However, in blood of animals with resistant strain, these indices are slightly higher, than in animals with sensitive tumor strain.

Table 5. Rate of generating of superoxide radicals by NADPH-oxidase and iNOS neutrophils of rat blood in dynamics of Guerin carcinoma growth

Groups of animals by periods after transplantation	Activity of NADPH-oxidase of neutrophils (nmol/10^3 cells·min)		Activity of iNOS neutrophils (nmol/10^3 cells·min)	
	Sensitive strain	Resistant strain	Sensitive strain	Resistant strain
Control (intact animals)	0.28 ± 0.05	0.29 ± 0.04	1.60 ± 0.02	1.76 ± 0.04
1 day	0.70 ± 0.03*	0.77 ± 0.02*	2.01 ± 0.07	2.04 ± 0.05
3 days	0.98 ± 0.09*	0.96 ± 0.09*	2.15 ± 0.11*	2.11 ± 0.12*
5 days	1.08 ± 0.09*	1.12 ± 0.08*	2.95 ± 0.15*	3.55 ± 0.15*
7 days	2.12 ± 0.03*	2.09 ± 0.01*	3.71 ± 0.20*	3.80 ± 0.16*
14 days	1.95 ± 0.02*	2.35 ± 0.18*	4.12 ± 0.10*	3.95 ± 0.15*
23 days	1.98 ± 0.05*	2.46 ± 0.19*	3.79 ± 0.06*	4.16 ± 0.21*

Note: *$p < 0.05$ – significant regarding control values.

To determine connection between indices of iron metabolism and indices of oxidative stress, we have carried out study using correlation analysis. Results have showed that increase of "free iron" complexes in peripheral blood is accompanied by activation of generating superoxide radicals by NADPH-oxidase with prevalence in resistant tumors (r = 0.59 at growth of sensitive and r = 0.70 — resistant tumor). This fact points at the most essential disorders in iron metabolism in organism of tumor host at development of resistant tumor. It has been determined that as number of "free iron" complexes in peripheral blood of animals increases, activity of iNOS neutrophils also significantly increases (r = 0.50 at sensitive and r = 0.76 at resistant tumor). Also, we have proved direct correlation between content of powerful antioxidant MT-1 and activity of NADPH-oxidase of neutrophils of sensitive (r = 0.67) and resistant (r = 0.51) tumor. Similarly, direct correlation between level of MT-1 and activity of iNOS of neutrophils of sensitive (r = 0.67) and resistant (r = 0.65) strains has been determined. There is an idea in literature that MT-1 performs protective role from damaging action of superoxide radicals due to the SH-bonds and cysteine as components of this metal-containing protein [17]. On the example of Guerin carcinoma, it has been shown that increase of content of MT-1 in tumor tissue of resistant strain directly correlates with high level of generation of superoxide radicals in it (r = 0.72). This coefficient was lesser in sensitive tumor and equaled r = 0.59. Obtained results demonstrate that MT-1 in the process of Guerin carcinoma growth plays important role in antioxidant protection of transformed cells from destructive impact of free radical compounds.

Thus, in animals with sensitive and resistant to cisplatin Guerin carcinoma, change of condition of AOS components was determined in dynamics of tumor growth: content of MT-1, TR and activity of CP that points at disorder of iron metabolism in host organism.

In conclusion, on the model of Guerin carcinoma with sensitive and resistant to cisplatin strains, misbalance of regulatory proteins of iron metabolism has been shown: MT-1, TR and CP in dynamics of development of tumor process on the level of complicated system of interaction between tumor and organism. It has been determined that in the initial stages of development of neoplasm, changes in two functionally coupled components of pro- and AOS takes place in blood serum and tumor tissue of animals: significant increase of generating superoxide radicals and simultaneous increase of MT-1. It has been determined that in dynamics of tumor growth in animals of both strains, increase of "free iron" complexes both in tumor tissue and blood serum takes place on the background of significant increase of CP/TR ratio that shows disorder of functioning of host AOS which participates in the recovery processes and iron oxidation. In animals with strain resistant to cisplatin, mentioned changes were more pronounced that was manifested by increase of number of "free iron" complexes in tumor and blood of animals with further activation of generating superoxide radicals. It has been shown that tumor cells are capable to alter metabolism of iron via its accumulation in malignant neoplasm with followed reprogramming of mitochondria metabolism and activity of NADPH-oxidase of non-transformed cells contributing the increase of generating superoxide radicals. Mentioned changes are favorable for the formation of oxidative phenotype and appropriate conditions for the progression of tumor lesion.

ACKNOWLEDGEMENT

The study is performed in terms of the topic № 2.2.5.361 "Molecular epidemiology of metal-containing proteins in tumor-host interactions" of the target research program "Functional genomics and metabolomics in system biology" (2012–2016).

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