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METALLOPROTEINS DURING DEVELOPMENT OF WALKER-256 CARCINOSARCOMA RESISTANT PHENOTYPE

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The study was focused on the detection of changes in serum and tumor metal-containing proteins in animals during development of doxorubicin-resistant phenotype in malignant cells after 12 courses of chemotherapy. We found that on every stage of resistance development there was a significant increase in content of ferritin and transferrin proteins (which take part in iron traffick and storage) in Walker-256 carcinosarcoma tissue. We observed decreased serum ferritin levels at the beginning stage of the resistance development and significant elevation of this protein levels in the cases with fully developed resistance phenotype. Transferrin content showed changes opposite to that of ferritin. During the development of resistance phenotype the tumor tissue also exhibited increased 'free iron' concentration that putatively correlate with elevation of ROS generation and levels of MMP-2 and MMP-9 active forms. The tumor non-protein thiol content increases gradually as well. The serum of animals with early stages of resistance phenotype development showed high ceruloplasmin activity and its significant reduction after loss of tumor sensitivity to doxorubicin. Therefore, the development of resistance phenotype in Walker-256 carcinosarcoma is accompanied by both the deregulation of metal-containing proteins in serum and tumor tissue and by the changes in activity of antioxidant defense system. Thus, the results of this study allow us to determine the spectrum of metal-containing proteins that are involved in the development of resistant tumor phenotype and that may be targeted for methods for doxorubicin sensitivity correction therapy.

K e y w o r d s: Walker-256 carcinosarcoma, ferritin, transferrin, ceruloplasmin, 'free iron' complexes, reactive oxygen species, MMP-2 and MMP-9, thiol groups, doxorubicin.

The topic of tumor-organism interaction persists as a crucial issue in modern clinical and experimental oncology. This is primarily due to the fact that no data has been presented as yet that would describe the totality of the complex rearrangements in tumor and organism that occur during tumor growth, beginning with the formation of primary locus and through to the active generalization. The latter is clearly related to another unsolved issue of the modern oncology – the tumor resistance to cytostatics. Numerous studies prove the substantial differences in metabolism between transformed and normal tissue, especially in resistant neoplastic forms [1-3]. For example, it has been established that neoplastic growth is accompanied by disruptions in functional activity of numerous regulation systems, particularly those of energy, lipid, and protein metabolism, as well as antioxidant defense system [1-3].

Numerous problems concerning metalloprotein participation in attainment of resistance to cytostatics remain unsolved as yet. These problems are conceivably related to the fact that the metalloproteins are involved in two functionally opposite processes, namely, superoxide radical generation and antioxidant defense against them. The attainment of the resistant phenotype by a tumor leads to oxidative stress, according to current understanding. This stress serves to launch efficient mechanisms of defense against the damaging action of free-radical compounds. It is well known that iron and iron metabolism proteins play an important part in the realization of these processes, as together they are intended to ensure organism's basic biological functions. For instance, blood ferritin level is taken as to be correspondent to iron pool in tissues, and it can affect the cell cycle via microtubules, as it is located

terminally. Moreover, it serves to pass iron ions to mitochondria, which is an important factor in development of therapy-resistant malignant cells phenotype [7, 8]. The primary role of ceruloplasmin and transferrin in the organism antioxidant defense is played by inhibition of iron-induced peroxidation of extracellular matrix [8, 9]. That is why many modern studies are focused on the role that iron and proteins involved in regulation of its metabolism play in carcinogenesis and tumor development of wild-type and resistant malignant cell lines [10, 11]. Thus, despite the existence of some data describing the characteristics of status of certain iron-containing proteins, there is no evidence as to their systemic participation in formation of cytostatic-resistant cancer cells phenotype.

Due to all of the above, we aimed the present study to characterize the functional state of metalloproteins at tumor and organism levels during formation of Walker-256 carcinosarcoma doxorubicin-resistant phenotype, namely that of transferrin, ferritin, ceruloplasmin and matrix metalloproteinases 2 and 9 on *in vivo* models.

Materials and Methods

The experiments were performed in vivo on animals bearing Walker-256 carcinosarcoma with induced resistance to doxorubicin. The experimental animals were subjected to 12 courses of doxorubicin chemotherapy in order to obtain the doxorubicin-resistant tumor. Twenty female rats (150-200 g) were injected with Walker-256 carcinosarcoma cell line. The animals were assigned to control or therapy (experimental) group. Rats of the therapy group received 1.5 mg/kg of body mass intraperitoneal injections of doxorubicin starting with the 4 day after tumor transplantation. The animals received 5 injections of the drug. The antitumor effect was assessed 2 days after the final injection by comparing the carcinosarcoma volume in therapy group I to that of control. The Walker-256 carcinosarcoma was inhibited by 65 ± 5%. Tumor tissue was then excised from therapy group I animals and used to prepare suspended tumor cells that were injected into 10 female rats. These animals were also subjected to a course of chemotherapy as described above. The compound's antitumor effect was evaluated after the 4, 8, and 12 courses of therapy (therapy group II). Four courses of chemotherapy resulted in tumor growth inhibition by 30.0 ± 2.7%, 8 courses – by 9.0 ± 1.4%. There was virtually no inhibition of tumor growth by doxorubicin after the 12 course (0% inhibition). Every chemotherapy course was paralleled by Walker-256 carcinosarcoma passage to animals never subjected to doxorubicin treatment. The number of passages was the same for both the parental tumor and the resistance-conditioned tumor. The results obtained on every pass of resistance-conditioning (that is, on course 4, 8 and 12 of doxorubicin treatment) were compared to those of the animals that had not been subjected to doxorubicin treatment (0 course). All of the animals were euthanized by decapitation preceded by Sedazin injection (Pulawy, Poland).

Active forms of ceruloplasmin (CP), transferrin content (Tr), and 'free iron' complexes were assayed in serum and tumor tissue of animals by electron paramagnetic resonance (EPR) at liquid nitrogen temperature (77 K). EDTA-2Na was used as anticoagulant.

To this end, 0.25 ml of serum or whole blood sample was put in a mold and frozen. EPR spectra for CP have g = 2.05, and for Tr g = 4.25. Levels of CP and Tr active forms were evaluated by EPR spectra of those proteins. Tr content was also determined for tumor tissue of Walker 256 carcinosarcoma. 0.5 g of tumor tissue was excised and frozen in a mold by dipping in liquid nitrogen.

In addition, 'free iron' complexes signal was also registered. These are the decompartmentalized iron ions that appear as a result of lipid peroxidation (LPO), increased membrane permeability, and destruction of iron-containing proteins [6, 9]. 'Free iron' complexes spectrum was g = 2.2. All of the samples were assayed on computerized spectrometer P-1307 (Russia). The levels of metalloproteins' activity and content were expressed in arbitrary units (a.u.).

Ferritin (Fr) content was evaluated in serum and tumor tissue of rats bearing Walker-256 carcinosarcoma by ELISA with corresponding reagent kits (Uscer, China) on automatic biochemical and immunoenzyme analyzer ChemWell 2900 (USA). Serum samples for ELISA were obtained in accordance with kit instructions.

All serum samples were free from hemolysis, were centrifuged at 1500 rpm (g = 1.5) and stored at -20 °C. Tumor tissue supernatant was obtained in normal saline at 1:3 ratio. The samples were centrifuged at 1000 rpm and stored at -20 °C.

Reactive oxygen species (ROS) generation and thiol group content in tumor tissue of animals bearing Walker-256 carcinosarcoma was evaluated

on Beckman Coulter EPICS® XL Flow Cytometer via non-enzymatic cell suspension method with addition of 0.2% EDTA and normal saline mix. The changes in cellular ROS generation levels were assayed with CM-H2DCFDA dye followed by flow cytometer data analysis.

Thiol group content in Walker-256 carcinosarcoma tumors was assayed with Cell Tracker Green (CMFDA, USA) [12] dye. This dye interacts with intracellular non-protein thiol compounds, such as glutathione, which is a major component of detoxication system.

The concentration of active forms of MMP-2 and MMP-9 matrix metalloproteinanses was evaluated in serum and tumor tissue samples of Walker-256 carcinosarcoma-bearing animals by zymography in polyacrylamide gel (with gelatin added as a substrate) based on protein SDS-electrophoresis. The gel was washed, and MMP-2 and MMP-9 active forms were visualized as discolored bands on bluish background. Their localization was verified against molecular mass standards (Sigma, Germany), and was found to be correspondent to the molecular mass of the respective enzyme (72 and 92 kDa, accordingly). Proteolytic activity was evaluated by measurments of lytic area, as compared to the Sigma kit standards for MMP-2 and MMP-9. The results were analyzed in TotalLab 1.01 program [13].

The statistical analysis was done with MS Excel 2010 instuments. Frequency distribution tables for ROS and thiol groups were analyzed in FCS Express V3, ModFit V3.2 program for cytometric data processing. The significance of differences between various groups was determined with Student's *t*-test. The correlation coefficient was calculated according to Pearson. The differences were considered significant at $P < 0.05$. The statistical processing was done with STATISTICA 6.0 programming kit.

Results and Discussion

It has been established by now that tumor locus formation followed by endogenous intoxication of organism leads to misbalancing in regulation of essential trace elements, including accumulation of 'free iron' (Fe^{2+}). This may result in lipid peroxidation (LPO), and in changes in pro- to antioxidant qualities of blood serum that contains Cp and Tr [4, 8, 9]. It is worth mentioning that CP and Tr together constitute a molecular system able to regulate reduced iron ions (Fe^{2+}) content and total antioxidant activity of serum [8, 9].

According to our data, in the initial stages of formation of the resistant malignant cells phenotype their growth inhibition diminishes progressively by 30 and 9% (after 4 and 8 courses of chemotherapy), and serum and tumor tissue Tr content of experimental animals increases gradually in comparison to wild type line cells (Table 1). In example, rat serum Tr content after 4 courses of chemotherapy was elevated 3.7 times, and tumor Tr content 1.7 times in comparison to those of wild type line. The increase of serum and tissue Tr content was even more profound (1.33 and 1.6 times, accordingly) after 8 courses of chemotherapy, when growth inhibition was by 9%. The changes in this factor were different for 12 doxorubicin courses, when tumor reaction to further cytostatic interventions was virtually absent. For instance, we found significantly decreased serum Tr levels in experimental animals in comparison to the previous stage of resistance formation ($P < 0.05$), and it was significantly increased in tumor tissue, that may be connected to the enhanced requirements of resistant malignant cells in iron pooling in order to provide it for metabolism (Table 1).

Doxorubicin toxicity as described in publications affects various metabolic pathways, which results in inhibition of metalloprotein synthesis, including that of ceruloplasmin [10, 11]. Nevertheless, our data indicate that doxorubicin resistance formation in animals bearing Walker-256 carcinosarcoma is accompanied by 2.8-times increase in blood active ceruloplasmin content after 4 courses of chemotherapy, and by 4-times increase after 8 courses, in comparison to those figures for the wild-type line. The opposite pattern of the changes in these parameters was observed after 12 courses of chemotherapy only. It decreased noticeably if compared to the former stages. On the other hand, we observed structural changes in ceruloplasmin, namely, the loss of sialic acids that may impair the activity of this copper-carrying protein [8-10]. The data signifying simultaneous Tr and CP activity decrease in blood of the experimental animals on the background of fully formed cytostatic-resistant tumor phenotype may be related to changes in ceruloplasmin ferroxidase activity [8-10]. The changes in activity of these metalloproteins may be tied to appearance of doxorubicin-resistant cellular populations, the phenotype of which is characteristically more aggressive. Changes in functional state of CP and Tr in blood of animals with well-developed cytostatic-insensitive aggressive tumor phenotype leads to disruptions in

Table 1. Transferrin (Tr), ceruloplasmin (CP), and ferritin (FR) changes in tissues of Walker-256 carcinosarcoma-bearing rats during formation of resistance to doxorubicin (M ± m, n = 3)

Metalloproteins	Control		Chemotherapy course number					
			4		8		12	
	Blood	Tumor	Blood	Tumor	Blood	Tumor	Blood	Tumor
Tr, a.u.	0.48 ± 0.17	0.39 ± 0.15	1.80 ± 0.17*	0.51±0.8*	2.40 ± 0.14 *	0.82 ± 0.10*	0.76 ± 0.15*	0.96 ± 0.09*
CP, a.u.	0.6 ± 0.2	–	1.70 ± 0.25*	–	2.40 ± 0.17*	–	0.90 ± 0.11*	–
FR, ng/ml	28.62 ± 2.00	6.0 ± 0.1	4.65 ± 1.16*	37.84±5.03*	2.35 ± 0.85*	52.12 ± 9.41*	56.9 ± 3.12*	390.10 ± 17.19*

Note: *$P < 0.05$ changes are statistically significant in comparison to control

iron homeostasis that result in gradual accumulation of 'free iron' complexes.

FR is also a well-known iron-carrying protein that plays a major part in iron metabolism. Although its main function is iron storage, it can also participate in antioxidant defenses, if free radical compounds are accumulated within cell [4]. It had been established that FR has an important role in cellular defense against cytotoxic effects of H_2O_2 [14]. It had also been demonstrated by others that doxorubicin may affect cytosolic and mitochondrial iron homeostasis. This leads to increased mitochondrial ferritin expression, which acts as detoxicant under doxorubicin-induced toxicity [15].

According to our data, the animals bearing Walker-256 carcinosarcoma had decreased blood serum FR levels from the beginning of resistance formation 6.4 times in comparison to wild type line, and 12.7 times at later stages (Table 1). This decrease on the starting stages of resistance induction may conceivably be explained by inactivation of iron release from FR. It had been proposed that the latter is accumulated in lysosomes, which inhibits their function and results in gradual degradation of FR [11]. It is also worth noting that serum FR level under absent reaction of tumor to cytostatic is double that of control. These changes may reflect the appearance of a resistant cell strain at a certain stage of aggressive tumor phenotype formation with new functional characteristics associated with increased level of this iron-carrying protein in blood serum.

The FR changes were different in tumor tissue of Walker-256 carcinosarcoma-bearing animals during formation of doxorubicin-resistant tumor phenotype (Table 1). We observed gradual statistically significant increase in these protein's tissue content along the process of treatment with cytostatic. Still, it should be mentioned that with no tumor sensitivity the FR level increased maximally in comparison to previous data. The results obtained correspond well with that of other publications concerning FR accumulation in tumor tissue as doxorubicin resistance develops, which leads to more profound proliferative activity of the neoplastic tissue. This gives ground for some researchers to assume the possibility of application of FR level evaluation as a marker of tumor progression [7, 16, 17]. Therefore, the imbalance we found in levels of a number of metalloproteins in Walker-256 carcinosarcoma cells under prolonged doxorubicin effect is the result of disruption in mechanisms aimed to defend cells against the damaging impact of cytostatics.

The problem of those metalloproteins' participation in regulation of redox mechanisms in tumor tissue during the process of formation of aggressive phenotype of neoplastic tissue is poorly studied as yet. Nevertheless, it has been established that changes in redox balance towards oxidation is a favorable condition for intensification of proliferation.

We investigated a number of indices that allow evaluating the intensity of such processes in tumor tissue: 'free iron' complexes, ROS, thiol groups, MMP-2 and MMP-9.

In particular, we demonstrated gradual increase in 'free iron' complexes content in tumor tissue of the experimental animals at every stage of aggressive neoplasy phenotype formation under effect of the cytostatic compound.

This index was at its maximum when tumor tissue showed no response to doxorubicin treatment (aggressive malignant cell line phenotype fully developed) (Table 2).

It is well known that increased 'free iron' complexes content initiates Fenton and Haber-Weiss reactions and starts generation of free-radical compounds followed by damage to multiple metabolic pathways. According to published data, 'free iron' accumulation in tumor is a condition that determines the oxidative phenotype of proliferating cells [3-7].

Our studies of changes in ROS generation in tumor tissue of experimental animals during formative processes of doxorubicin-resistant tumor cell phenotype demonstrated the statistically significant ($P < 0.05$) increase in this index. These data may reflect the synergistic relation between heightened free-radical compounds generation and increased 'free iron' complexes amount ($r = 0.39$) in comparison to wild-type line (Table 2).

The maximal significant increase in ROS generation during development of resistance ($P < 0.05$) was observed after 12 courses of the chemotherapy (Table 2). Thus, the results confirm that during the development of Walker-256 carcinosarcoma's resistance to doxorubicin ROS generation is initially on the dynamic rise. This rise may signify the tumor's adaptation to cytostatic effects, as the mechanism of action of doxorubicin involves damage to DNA structure and dysfunction of mitochondria, which in turn become the main producers of unregulated amounts of superoxide radicals in cells.

The increased ROS generation may also result in changes to thiol branch of antioxidant defense system. It is known that a number of thiol enzymes are indirectly involved in antioxidant defense by spontaneous reactions with peroxides and free-radical compounds. According to published data, free thiol group content is on the rise prior to the manifestation of disease. This may reflect glutathione system activation associated with progression of neoplasy [17]. We had previously demonstrated the important part that thiol groups play in mechanisms of formation of resistance to a cytostatic [18].

As redox balance in tumor tissue is upset during carcinosarcoma's development and thiol groups content is on the rise, we aimed to establish the correlation between thiol groups content and ROS generation in tumor tissue [17].

Both indices were gradually increasing as the doxorubicin resistant tumor phenotype formation progressed. They achieved the respective maximums with mutual correlation ($r = 0.31$) when the tumor ceased to respond to doxorubicin treatment (Table 2).

Moreover, we established high level of direct correlation ($r = 0.43$) between ROS genearation and tumor FR content during resistant line formative process, which may signify the important role of FR in malignant neoplasm's antioxidant defense against damage by free-radical compounds.

Increased ROS generation itself against the background of high 'free iron' complexes' concentrations influences activation of redox-regulated matrix metalloproteinases (MMP) [19]. The ability of matrix metalloproteinases, MMP-9 and MMP-9 (gelatinases) in particular, to modify extracellular

Table 2. Redox state indices in tumor tissue during development of doxorubicin resistance (M ± m, n = 3)

Redox state of tumor tissue (a.u.)	Control	Chemotherapy course number		
		4	8	12
'Free iron' complexes	0.17 ± 0.09	0.20 ± 0.09	0.51 ± 0.11*	0.70 ± 0.14*
ROS	5.20 ± 0.17	17.12 ± 1.03*	23.16 ± 2.11*	180.12 ± 10.14*
Thiol groups	19.1 ± 1.2	80.11 ± 4.15*	110.11 ± 6.18*	560.10 ± 24.11*

Note: *P < 0.05 changes are statistically significant in comparison to control

matrix proteins allows them to provide for cancer cells regulatory properties and participate in barrier breaching during invasion and metastasis, which is an important factor in cancer cell survivability. It is possible that they are involved also in resistance development to environmental damage [4, 19].

We found that serum and tumor tissue of wild-type Walker-256 carcinosarcoma-bearing rats was virtually free of MMP-2 active forms, which are linked to invasiveness and metastasis properties. On the other hand, MMP-9 concentration, perceived as a marker for angiogenesis, was measured to be 2.2 ± 0.8 μg/g in tumor tissue and 120.0 ± 30.0 ng/ml in serum (Fig. 1).

The serum and tumor tissue concentrations of MMP-2 and MMP-9 in active state increased with each consecutive course of the doxorubicin therapy, reaching maximal levels after course 12 (Fig. 1).

Therefore, the comnination of changes to functional state of metalloprotein's, 'free iron' complexes, and matrix metalloproteinases' activity gives evidence of the important role of essential trace elements in development of cancer cells' resistant phenotype (Fig. 2 and 3).

Rapid decrease of blood serum Tr level and CP activity and corresponding increase in indices of metalloproteins and matrix metalloproteinases in tumor loci on the background of total doxorubicin insensitivity is a favorable condition for activation of proliferation and for development of malignant strain with enhanced invasiveness and metastasis potential [20].

The presented data concerning total insensitivity of tumor to doxorubicin allow us to postulate hypothetical mechanisms of changes in levels of 'free iron' complexes and proteins related to their metabolism, namely ceruloplasmin, transferrin, MMP-2, and MMP-9 in blood serum of experimental animals, as well as ROS and thiol groups in tumor tissue (Fig. 4).

Consequently, we suppose that total loss of tumor sensitivity to doxorubicin is accompanied by decreased blood ceruloplasmin oxidase activity as a result of complex formation between the cytostacic and Cu^{2+} ions in ceruloplasmin catalytic center [3]. These changes lead to blocked Fe^{2+} to Fe^{3+} oxidation and, correspondingly, inactivation of Fe^{3+} binding to transferrin, which manifests as its decreased blood level and increased tumor tissue level. Along with this, highly reactive decompartmentalized iron ('free iron' complexes) levels in tumor tissue rise, which may lead to initiation of Fenton reaction and related increase in ROS generation. This results in higher serum and tumor tissue concentrations of redox-dependent gelatinases and thiol group content. In tumor tissue, besides increased 'free iron' complexes concentrations, growing FR concentrations are also detected with statistical significance. It plays an antioxidant and accumulative role, and this is confirmed by results of correlation analysis over the mentioned parameters (r = 0.77).

Therefore, the data of the complex study demonstrate that a newly aggressive phenotype of cancer cell strain develops in animals bearing Walker-256 cercinosarcoma after 12 courses of doxorubicin chemotherapy. This phenotype differs in its functional characteristics from the original cell line. The observed changes in ratios of metallopro-

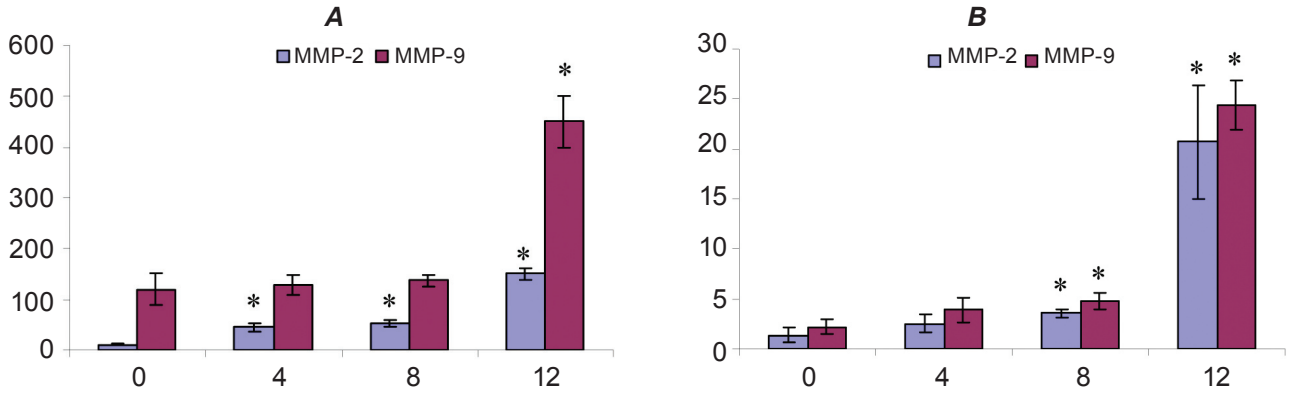

*Fig. 1. Changes in MMP-2 and MMP-9 concentrations of blood serum (A) and tumor tissue (B) of Walker-256 carcinosarcoma-bearing animals in process of developing aggressive doxorubicin-resistant tumor phenotype. X-axis: chemotherapy course number; Y-axis: active MMP concentration (ng/ml of serum, μg/g of tumor tissue); * $P < 0.05$ changes are significant in comparison to control*

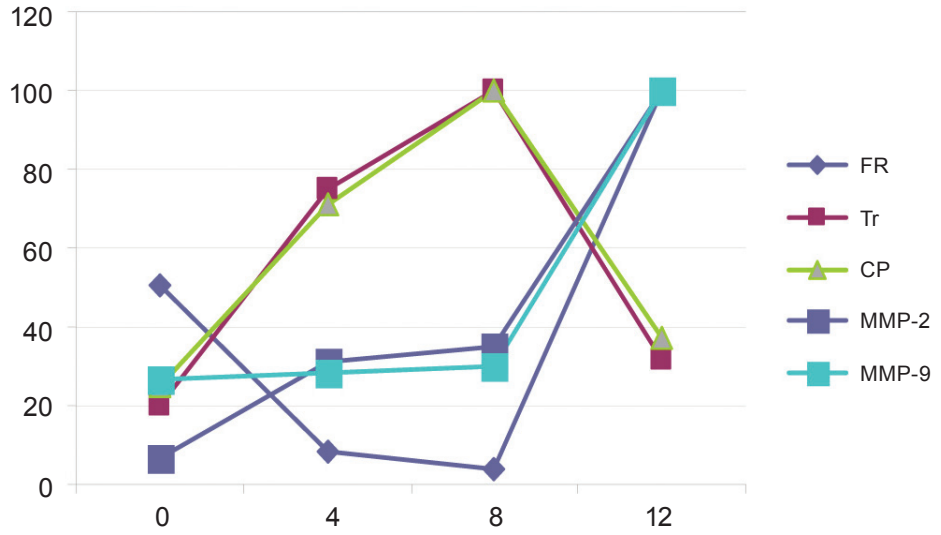

Fig. 2. Ferritin (FR), transferrin (Tr), ceruloplasmin (CP), MMP-2, and MMP-9 levels in blood of Walker-256 carcinosarcoma-bearing animals during development of doxorubicin-resistant phenotype. X-axis: chemotherapy course number (0-12); Y-axis: parameter value as percentage of its maximal level

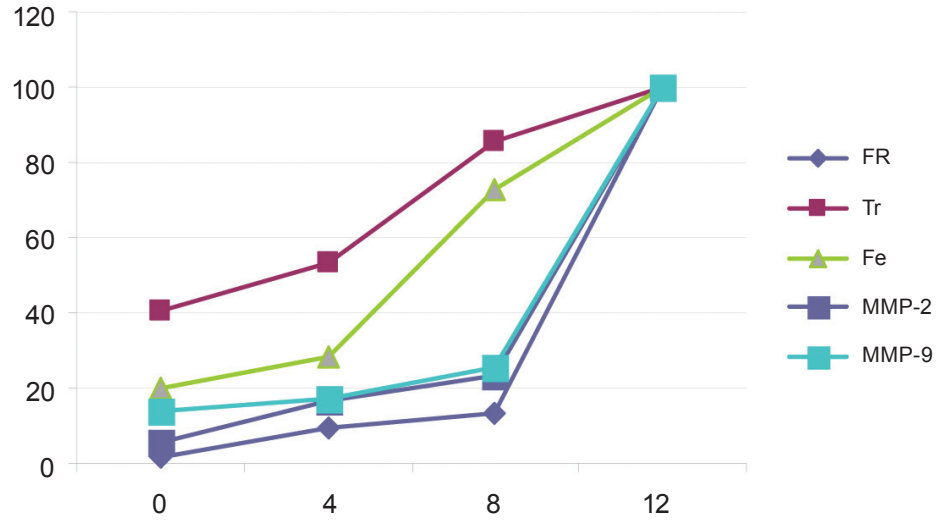

Fig. 3. Ferritin (FR), transferrin (Tr), 'free iron' complexes (Fe), MMP-2, and MMP-9 levels in blood of Walker-256 carcinosarcoma-bearing animals during development of doxorubicin-resistant phenotype. X-axis: chemotherapy course number (0-12); Y-axis: parameter value as percentage of its maximal level

teins give grounds to assume their roles as major components of maintenance of normal homeostasis that may be involved in mechanisms responsible for development of drug resistance. This allows to consider levels of ferritin, transferring, ceruloplasmin activity and blood concentrations of active forms of gelatinases as extratumor markers of malignant cells' sensitivity to effects of cytostatics.

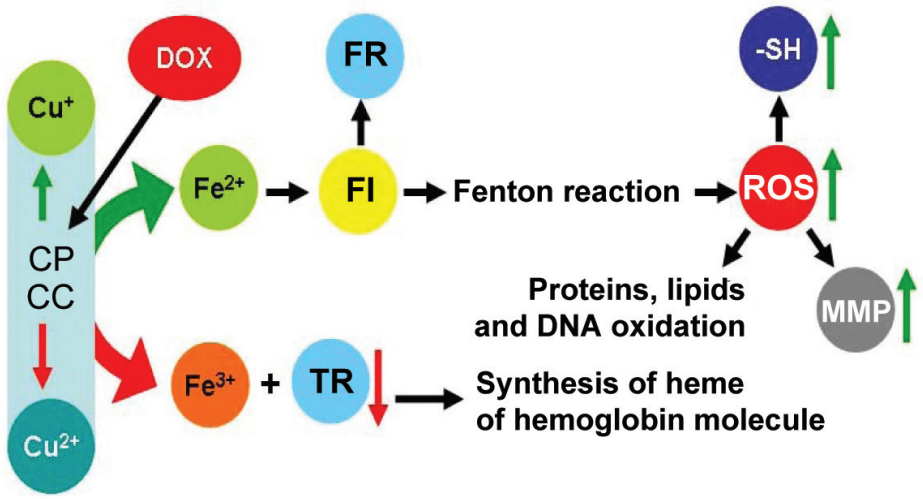

Fig. 4. Schematic representation of changes in iron and iron-related protein metabolism under total tumor insensitivity to doxorubicin. Legend: DOX, doxorubicin; CP-CC, ceruloplasmin catalytic center; FI, 'free iron'; FR, ferritin; ROS, reactive oxygen species; SH, thiol groups; MMP, matrix metalloproteinases; TR, transferrin; red arrow – iron ions oxidation blocked, their binding to transferrin inhibited as a result of loss of ceruloplasmin ferroxidse activity; green arrow – sped up Fe^{2+} accumulation, 'free iron' complexes increase, Fenton reaction initiated

МЕТАЛОПРОТЕЇНИ В ПРОЦЕСІ ФОРМУВАННЯ РЕЗИСТЕНТНОГО ФЕНОТИПУ КАРЦИНОСАРКОМИ УОКЕР-256 У ЩУРІВ

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Проведено дослідження функціонального стану металовмісних протеїнів у сироватці крові та пухлині щурів у процесі формування резистентного фенотипу злоякісно трансформованих клітин під час проведення 12 курсів хіміотерапії з доксорубіцином. Встановлено, що на всіх етапах формування резистентності до доксорубіцину, в пухлинній тканині тварин із карциносаркомою Уокер-256 відбувається істотне підвищення вмісту протеїнів – трансферину та феритину, що задіяні у транспорті та депонуванні заліза порівняно із тваринами, яким доксорубіцин не вводили. У сироватці крові цих тварин виявлено зниження рівня феритину на початковому етапі формування резистентності до доксорубіцину та істотне підвищення за сформованого резистентного фенотипу пухлини. Протилежний характер змін виявлено щодо вмісту трансферину. Крім того, у процесі розвитку резистентності до доксорубіцину в пухлинній тканині зростає рівень «вільного заліза», який вірогідно корелює з підвищенням генерації АФК та концентрацією активних форм матриксних металопротеїназ – ММП-2 та ММП-9. Поряд із цим поступово збільшується вміст непротеїнових SH-груп у пухлині. У сироватці крові на початку формування резистентності відбувається значне зростання активних форм церулоплазміну і різке їх зниження у разі повної втрати пухлиною чутливості до доксорубіцину. Отже, феномен формування резистентності карциносаркоми Уокер-256 супроводжується як дерегуляцією металовмісних протеїнів у сироватці крові та пухлинній тканині, так і зміною активності системи антиоксидантного захисту. Таким чином, внаслідок проведеного дослідження окреслено коло металовмісних протеїнів, які задіяні у формуванні резистентного фенотипу пухлин. Вони можуть слугувати мішенню для засобів корекції, що спрямовані на подолання резистентності до доксорубіцину.

Ключові слова: карциносаркома Уокер-256 щурів, феритин, трансферин,

церулоплазмін, комплекси «вільного заліза», активні форми кисню, ММП-2 та ММП-9, SH-групи, доксорубіцин.

МЕТАЛЛОПРОТЕИНЫ В ПРОЦЕССЕ ФОРМИРОВАНИЯ РЕЗИСТЕНТНОГО ФЕНОТИПА КАРЦИНОСАРКОМЫ УОКЕР-256 У КРЫС

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Исследования направлены на определение изменений металлосодержащих протеинов в сыворотке крови и опухоли животных в процессе формирования резистентного фенотипа злокачественно трансформированных клеток, в результате проведения 12 курсов химиотерапии с доксорубицином. Установлено, что на всех этапах формирования резистентности к цитостатику в опухолевой ткани животных с карциносаркомой Уокер-256 происходит существенное повышение уровней протеинов, которые задействованы в транспорте и депонировании железа – ферритина и трансферрина. Параллельно, в сыворотке крови этих животных выявлено снижение уровня ферритина в начале формирования резистентности и существенное повышение этого протеина при отсутствии торможения роста опухоли цитостатиком. Противоположный характер изменений выявлен со стороны активности трансферрина. Кроме того, в процессе развития резистентности к доксорубицину в опухолевой ткани увеличивается количество комплексов «свободного железа», которое коррелирует с повышением АФК и концентрацией активных форм ММП-2 и ММП-9. Наряду с этим постепенно увеличивается содержание SH-групп в опухоли, особенно при полном торможении ее роста, что может свидетельствовать об активных пролиферативных процессах в злокачественном новообразовании. В сыворотке крови этих животных в начале формирования резистентности к цитостатику происходит значительное увеличение активности церулоплазмина и резкое его снижение при полной потере чувствительности опухоли к доксорубицину. Таким образом, феномен формирования резистентности карциносаркомы Уокер-256 сопровождается как дерегуляцией металлосодержащих протеинов в сыворотке крови и опухолевой ткани, так и изменением активности системы антиоксидантной защиты. В результате проведенного исследования очерчен круг металлосодержащих протеинов, которые задействованы в формировании резистентного фенотипа опухоли. Они могут служить мишенью для средств коррекции, направленных на преодоление резистентности к доксорубицину.

Ключевые слова: карциносаркома Уокер-256, ферритин, трансферрин, церулоплазмин, комплексы «свободного железа», активные формы кислорода, ММП-2 и ММП-9, SH-группы, доксорубицин.

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