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MOLECULAR PROFILE AND CELL CYCLE IN MCF-7 AND MCF-7/Dox CELLS EXPOSED TO CONVENTIONAL AND LIPOSOMAL FORMS OF DOXORUBICIN

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Aim: **To compare the molecular profile and cell cycle of sensitive and resistant to doxorubicin MCF-7 breast cancer cells upon exposition to conventional or liposome-encapsulated forms of doxorubicin.** ***Methods:*** **MTT-test, immunocytochemistry, flow cytometry.** ***Results:*** **In sensitive MCF-7 cells the exposure to conventional but not liposomal form of doxorubicin decreased metallothionein (MT) expression demonstrating activation of MT-detoxification system. In doxorubicin-resistant cells (MCF-7/Dox) MT expression drastically decreased. Conventional but not liposomal form of doxorubicin reduced the levels of expression of steroid hormones receptors on MCF-7 sensitive cells. The exposure of MCF-7 cells to conventional form of doxorubicin resulted in the decrease of p53 expression and the increase of Bcl-2 expression. In MCF-7/Dox cells neither conventional nor liposomal form of doxorubicin altered Bcl-2 expression. The exposure of MCF-7 but not MCF-7/Dox to doxorubicin in conventional form caused cell cycle arrest in G_0/G_1. Upon exposure to doxorubicin in liposomal form, cell cycle blockage in G_0/G_1 phase was observed in both sensitive and resistant sublines.** ***Conclusion:*** **The differential effects of the conventional and liposomal forms of doxorubicin in sensitive and resistant cells have been demonstrated. Exposure of MCF-7 and MCF-7/Dox cells to doxorubicin in liposomal form alters the molecular profile and cell distribution over cell cycle phases.**

Key Words: **MCF-7, doxorubicin, liposome.**

Anthracyclines are widely used for treating breast cancer (BC). Nevertheless, their cardiotoxicity is a cause of serious concern. Moreover, the resistance of the tumor to anthracyclines developed in the course of the treatment makes them inefficient for further therapy. Recently, it was suggested that both problems might be partially overcame using liposome-encapsulated anthracyclines. Liposome-encapsulated anthracyclines differ from their free counterparts in many respects, e. g. the ways of target delivery, pharmacokinetics, distribution in organs and tissues, toxicity and efficacy [1, 2]. Several studies suggest the partial reversion of cytostatic resistance using liposomal forms of doxorubicin. That is why liposome-encapsulated cytostatics are recommended for the treatment of the resistant forms of BC [3]. Meanwhile, we have not found out the data on the effects of liposome-encapsulated anthracyclines *in vitro* in cells with resistance phenotype in the literature.

The aim of our study was to compare the effects of doxorubicin in conventional and liposomal forms on molecular profile and cell cycle of MCF-7 cells sensitive and resistant to doxorubicin.

MATERIALS AND METHODS

Cell lines. For our studies, we used MCF-7 human BC cell line and its subline resistant to doxorubicin — MCF-7/Dox. MCF-7 cells were cultured in Iscove's Modified Dulbecco's Medium (Sigma, Germany) with 10% FBS (Sangva, Ukraine), at 37 °C and 5% of CO_2. The cells were reseeded twice a week with the density of 2–4 x 10^4 cells/cm^2, when cell layer covered 50% of the flask surface. MCF-7/Dox subline was obtained by growing original MCF-7 cells with increasing concentrations of doxorubicin in the range from 0.1 to 32 μg/ml. Doxorubicin was added twice a week at each cell passage. Every two months, the cells were assayed for their resistance using MTT technique. IC_{50} (50% inhibition of cell growth) for MCF-7 and MCF-7/Dox cells were 0.5 and 8 μg/ml of doxorubicin respectively. Therefore, MCF-7/Dox cells were 16 times as much resistant to doxorubicin as compared with their initial counterpart.

MTT-test. The sensitivity to doxorubicin and its liposomal form was assayed using standard MTT-coloremitric with 3-[4,5-dimethylthiasol-2–1]-2,5-diphenyltetrasolium bromide produced by Sigma, Germany. MCF-7 and MCF-7/Dox cells were cultured with liposomal or conventional forms of doxorubicin in the dose range of 1–32 μg/ml for 24 h [4].

Immunocytochemical analysis. The expression of surface and intracellular antigens was examined using immunocytochemical method with monoclonal antibodies to P-glycoprotein (P-gp), estrogen receptor (ER), progesterone receptor (PR), p53, Bcl-2, E-cadherin, metallothioneins (MT), Ki-67, cycline D1, pRb, c-myc, p21 (Dako Cytomation, Denmark) in described concentrations [5].

Flow cytometry. For the cell cycle analysis, cell suspension was washed and fixed on ice with 70% ethanol. Afterwards the samples were centrifugued at 300 x g for 5 min and the pellet was resuspended in 400 μl of physiological saline. 50 μl of RNAse and

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Abbreviations used: BC – breast cancer; ER – estrogen receptor; MT – metallothionein; P-gp – P-glycoprotein; PR – progesterone receptor. FBS – fetal bovin serum; IC – ingibitory concetration; MTT-3-[4,5-dimethylthiasol-2–1]-2,5-diphenyltetrasolium bromide; Dox – doxorubicin.

10 μl of propidium iodide were added. The samples were analyzed on "Partec" flow cytometer (Germany) [6].

RESULTS AND DISCUSSION

Phenotypic patterns of sensitive and resistant MCF-7 cells exposed to conventional or liposome-encapsulated doxorubicin. In our previous research, we have shown that the formation of doxorubicin resistance phenotype in human MCF-7 cells was accompanied by the changes in receptor status, cells adhesion properties and proliferation activity. We have also shown that the systems of metallothionein detoxication and ATP-dependent P-glycoprotein are directly involved in resistance to doxorubucine in MCF-7 cells [7].

The phenotypic patterns of MCF-7 cells and their resistant counterparts exposed to different forms doxorubicin are summarized in Fig. 1.

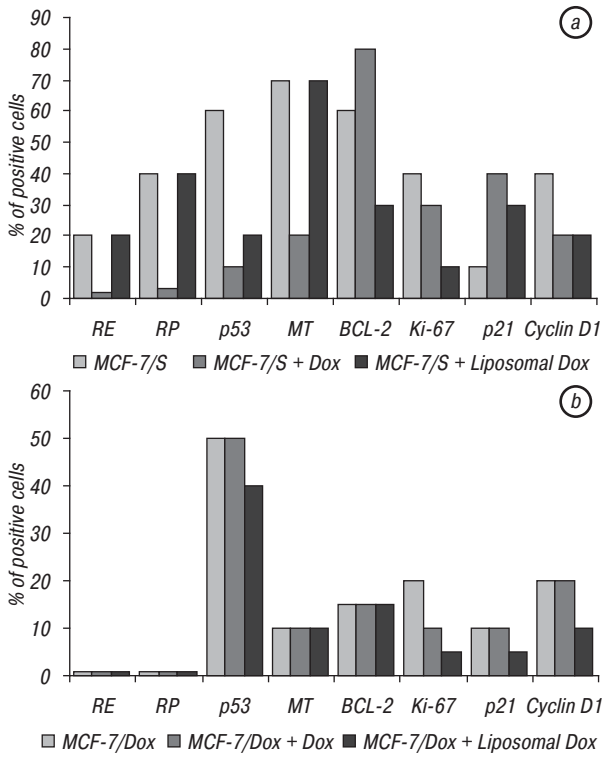

Fig. 1. Phenotype of MCF-7 cells exposed to conventional or liposomal forms of doxorubicin. *a*, Initial MCF-7 cells. *b*, Doxorubicin-resistant cells

Intracellular MT system is an important component of cell protection against cytostatic toxic effects. MT detoxifying effects are provided by their binding electrophylic anti-tumor cytostatics, as in free state MT are nucleophylic compounds [8, 9]. In our research, MT expression was detected in the majority (70%) of sensitive MCF-7 cells. The incubation of MCF-7 sensitive cells with doxorubicin in conventional form decreased the amount of MT-positive cells by 50% while MT expression upon exposure to liposomal form of doxorubicin remained unchanged (Fig. 1, *a*). The development of resistance to doxorubicin was accompanied by the decrease of the MT expression level by 60% and the same low level of expression remained upon exposure of resistance cells to conventional or liposomal forms of doxorubicin (Fig. 1, *b*). Therefore, MT directly participates in detoxification of the free form of doxorubicin in sensitive MCF-7 cells.

According to the data from numerous clinical observations [10], the absence of steroid receptor expression found in ⅓ of BC patients correlates with low sensitivity of tumors to anti-tumor therapy. Earlier we have shown that in MCF-7 cells resistant to doxorubicin the level of ER and PR expression significantly dropped. Significant decrease of the expression level of steroid hormones receptors was observed upon culturing of original MCF-7 cells with doxorubicin in conventional form, while cultivation with its liposomal form did not trigger such changes (see Fig. 1, *a*).

It is known that anti-tumor activity of the majority of anti-tumor cytostatics is associated with induction of apoptosis in tumor cells. Therefore, we have attempted to analyze the expression of p53 as well as anti-apoptotic Bcl-2 protein in sensitive and resistant MCF-7 cells exposed to doxorubicin in conventional or liposomal form. The expression level of p53 protein in doxorubicin-resistant MCF-7 cells was practically the same as in the initial cell line. The exposure of MCF-7 cells to doxorubicin in conventional form triggered the decrease of p53 expression and the increase of Bcl-2 expression (see Fig. 1, *a*). Neither conventional nor liposomal form of doxorubicin altered Bcl-2 expression in doxorubicin-resistant MCF-7 cells (see Fig. 1, *b*).

Analysis of the expression profile of the above mentioned as well other studied proteins (Fig. 1, *a*, *b*) suggests that the most prominent effects of doxorubicin are evident mostly in the original cell line. The differential effects of the conventional and liposomal forms of this drug in sensitive and resistant cells are also evident.

Analysis of cell cycle traverse in MCF-7 and MCF-7/Dox lines exposed to conventional or liposome-encapsulated doxorubicin. It is known that the mechanism of doxorubicin activity is in DNA binding and inhibition of nucleic acids synthesis. Doxorubicin is a phase-specific anti-tumor drug with maximal sensitivity demonstrated in S and G_2 phases of cell cycle. It is worth noting that cell phase distribution in MCF-7 and MCF-7/Dox lines was practically identical (Table, Fig. 2, *a*, *b*). Previously we have shown that the changes in cell distribution over cell cycle phases in course of prolonged cultivation of MCF-7 and MCF-7/Dox cells with doxorubicin and cysplatine were identical [7]. In our research, exposure of MCF-7 original line to doxorubicin in conventional form for 24 h caused cell cycle arrest in G_0/G_1 (Fig. 2, *c*). In contrast, conventional form of doxorubicin did not trigger changes in cell distribution over cell cycle phases of MCF-7/Dox cells in 24 h of cultivation (Fig. 2, *d*). Upon exposure of both sensitive and resistant lines to doxorubicin in liposomal form, cell cycle blockage in G_0/G_1 phase was observed (Table, Fig. 2, *e*, *f*). Such changes in cell distribution over cell cycle phases triggered the changes in the level of expression of proteins regulating cell cycle. During arrest of cell cycle in G_0/G_1 phase observed under the influence of conventional doxorubicin on MCF-7 cells and the influence of liposomal doxorubicin on MCF-7 and MCF-7/Dox sublines, the

simultaneous changes in the expression level of proteins regulating cell cycle took place. In particular, in MCF-7 and MCF-7/Dox cells the decrease of cycline-D1, pRb and c-myc expression level was observed, as well as the increase of p21expession (see Fig. 1). According to the data described in the literature, the increase of p21 expression causes suppression of activity of cycline D-Cdk 4,6 and cycline E-Cdk 2 complexes and, as a result, cells are arrested in G_0/G_1 phase [11]. The changes in the expression levels of proteins regulating cell cycle upon the exposure to doxorubicin in conventional and liposomal forms suggest the decrease of cell proliferation activity (see Fig. 1, Table).

Table. Cell cycle distribution of MCF-7 and MCF-7/Dox exposed to conventional or liposomal forms of doxorubicin

	MCF-7 control sample	MCF-7 conventional Dox	MCF-7 liposomal Dox	MCF-7/ Dox control sample	MCF-7/Dox conventional Dox	MCF-7/Dox liposomal Dox
G_0/G_1	55.11	70.4	69.19	61.05	64.93	77.51
S	28.85	11.93	20.79	27.25	20.55	7.6
G_2/M	16.04	17.67	10.02	11.73	14.52	14.89

Thus, we have characterized the molecular profile and cell cycle traverse of sensitive and resistant human BC lines exposed to doxorubicin in conventional and liposomal forms. It was shown that the conventional form of doxorubicin triggers significant changes in original MCF-7 cells, namely the decrease in metallothioneins, p53 protein and steroid hormones receptors and the increased level of anti-apoptotic Bcl-2 protein. The culture of MCF-7 cells with doxorubicin in conventional form resulted in the arrest of cell cycle in G_0/G_1 phase with the accompanying changes in the level of proteins, regulating cell cycle and the decrease of cells proliferation activity. In contrast, the exposure of MCF-7/Dox resistant line to doxorubicin in conventional form does not trigger the changes of molecular profile. The distribution of cells over cell cycle phases remains identical to that in control sample. Culturing of the cells with liposomal form of the drug causes insignificant decrease of p53 expression level in MCF-7/Dox cells. Upon exposure to doxorubicin in liposomal form, the blockage of cell cycle in G_0/G_1 phase was observed

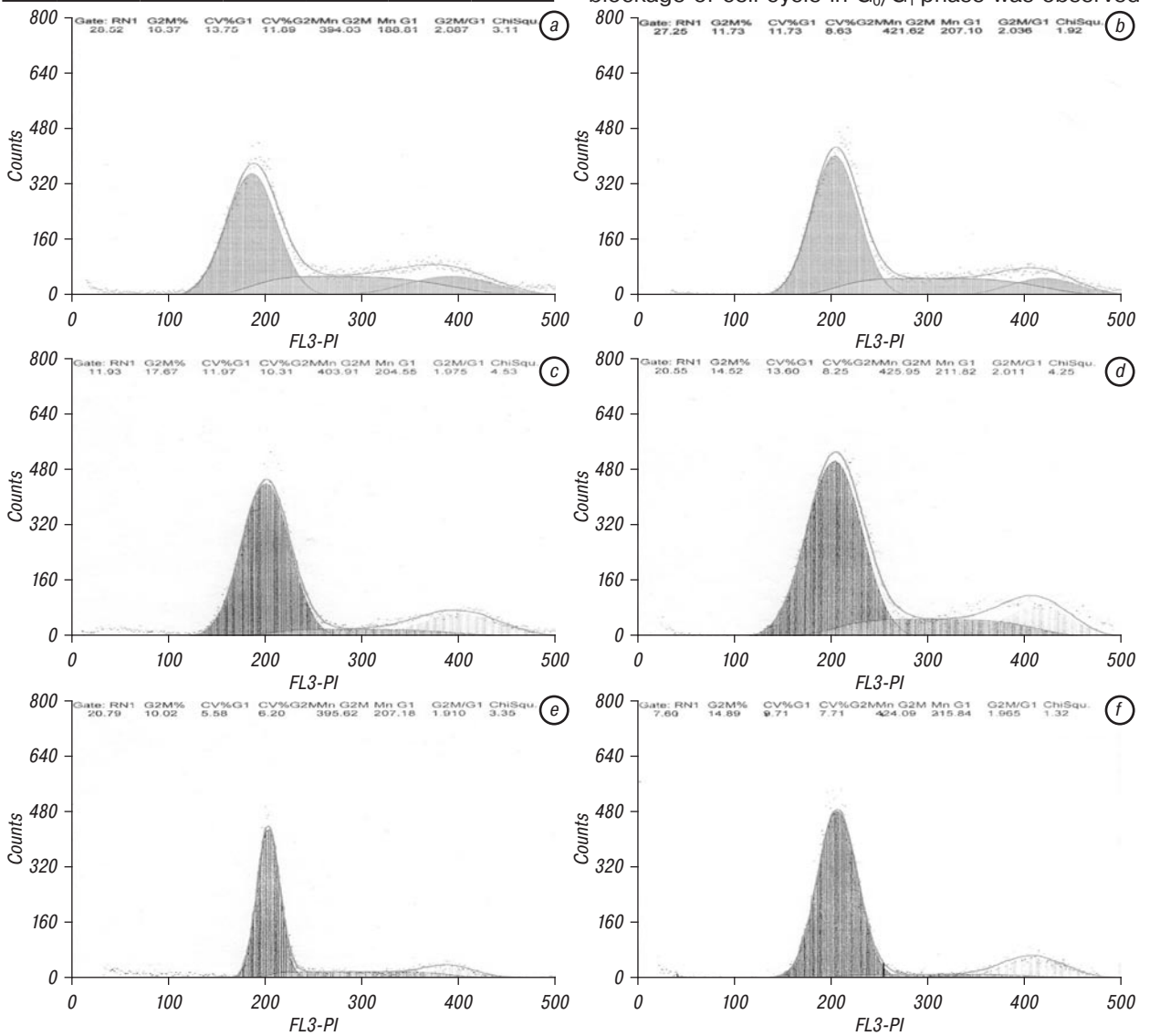

Fig. 2. Differences in cell cycle distribution in MCF-7 and MCF-7/Dox cells exposed to conventional or liposomal form of doxorubicin. *a*, MCF-7; *b*, MCF-7/Dox cells; *c*, MCF-7 cells after exposure to conventional form of doxorubicin; *d*, MCF-7/Dox cells after exposure to conventional form of doxorubicin; *e*, MCF-7 cells after exposure to liposomal doxorubicin; *f*, MCF-7/Dox cells after exposure to liposomal doxorubicin

in both sensitive and resistant cells. Therefore, we suggest that the effects of doxorubicin in liposomal form are directly associated with the blockage of cell cycle of sensitive and resistant MCF-7 cells.

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