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EXPRESSION OF E-CADHERIN IN DRUG RESISTANT HUMAN BREAST CANCER CELLS AND THEIR SENSITIVITY TO LYMPHOKINE-ACTIVATED LYMPHOCYTES ACTION

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Aim: **To analyze the correlation between the elevated sensitivity of drug resistant breast cancer cells to the action of lymphokine-activated lymphocytes (LAK) and expression of E-cadherin and other marker proteins by cancer cells and lymphocytes.** ***Methods:*** **Breast tumor explants were cultured with autologous lymphocytes in double diffusion chambers. The results were evaluated by morphological criteria of explants growth. Expression level of proteins on tumor cells was analyzed using immunohistochemical method on paraffin embedded sections, and by indirect immunofluorescence — on lymphocytes.** ***Results:*** **Significant decrease of E-cadherin expression and significant increase of nuclear antigen of proliferating cells expression have been detected on drug resistant malignant human breast tumor (DRHBT) cells compared with drug sensitive breast tumor (DSHBT) cells. Autologous LAK possessed the highest antitumor activity against DRHBT cells that was associated with high expression level of soybean lectin receptor.** ***Conclusion:*** **Malignant drug resistant tumors are characterized by reverse relation between E-cadherin expression level and their proliferative activity. Marked antitumor action of LAK against these tumors is associated with high expression level of soybean lectin receptor on the lymphocytes.**
Key Words: **E-cadherin, proliferative activity, malignant breast tumors, drug resistance, LAK.**

In our earlier studies we have demonstrated the presence of elevated sensitivity of drug resistant tumors to adoptive immunotherapy with the use of autologous IL-2 activated lymphocytes (LAK). In particular, such sensitivity was demonstrated *in vivo* and *in vitro* for B16 melanoma, transplanted MC-rhabdomyosarcoma, soft tissue sarcoma of locomotor system, endometrial and ovarian cancers [1–3], as well as for malignant human breast tumors [4]. However, the reason for elevated sensitivity of drug resistant tumors to immunotherapy, in particular, LAK-therapy, remains unknown, and the respective data are scarce [5].

To address this problem, we decided to analyze expression of E-cadherin by malignant human breast cancer cells. E-cadherin is a member of a family of calcium-dependent adhesion molecules mediating cell adhesion in different tissues, and a key regulator of adhesive properties of epithelial cells [6, 7]. Similarly to other members of the family, E-cadherin contains 5 extracellular domains one of which contains intercellular domain and can create complexes with α and β catenins and p120 protein [7, 8]. Due to formation of such complexes, E-cadherin possesses a number of regulatory functions, in particular, participates in morpho- and embryogenesis [9, 10]. Its multifunctionality leads to a special interest for researchers studying E-cadherin expression in different pathologies.

Altered expression of E-cadherin is shown to be related to formation of drug resistance of breast cancer cell lines and primary cell cultures, because E-cadherin, like other adhesion molecules, provides homotypic adhesion, the decrease of which promotes development of drug resistance [4, 11–13]. Also E-cadherin related decrease of adhesive properties is directly linked to survival of circulating tumor cells resistant to some cytostatic drugs. In particular, it has been shown that development of drug resistance of circulating prostate cancer cells is associated with the decrease of E-cadherin, β4-integrin, and γ-catenin expression, and with elevation of Bcl-2 expression [14].

It's necessary to note that decreased expression of E-cadherin in breast cancer cells and development of drug resistance are associated with a number of alterations on molecular, genetic and epigenetic levels (in particular, altered DNA methylation and *p53* transcription, altered expression of some antiapoptotic molecules, etc.) [12, 15, 16].

Possible mechanisms of influence of decreased E-cadherin expression on formation of drug resistance are poorly studied yet. It is could be explained partially by the involvement of E-cadherin in a number of various biological processes. In particular, it has been shown that E-cadherin interacts with EGF receptor and inhibits EGF-induced signals [17], but not the activity of other receptors [18]. The switch of E-cadherin to its isoforms, in particular, to N-cadherin, that is characteristic for breast cancer, may play a role in drug resistance formation [8, 19]. According to the recent data, decreased E-cadherin expression in epithelial tumors depends on mutation of genes coding E-cadherin, α- and β-catenins, p120, altered DNA methylation and transcription control [8].

Above mentioned findings made it important to examine the correlation of E-cadherin expression with the increased sensitivity of drug resistant breast tumor cells to action of LAK.

MATERIALS AND METHODS

The study has been performed on clinical materials obtained from the patients with benign (n = 12) and

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Abbreviations used: DRHBT – drug resistant human breast tumor cells; DSHBT – drug sensitive breast tumor cells; LAK – lymphokine-activated lymphocytes.

malignant (n = 20; diagnosis — infiltrative carcinoma) breast tumors cured in the Department of Surgery of Kyiv Hospital № 1 (Kyiv, Ukraine). Tumor tissue samples (tumor explants and tumor tissue sections) were obtained from surgically resected specimens. Peripheral blood lymphocytes (PBL) from these patients have been analyzed. The study was performed in accordance with international and state rules for bioethics.

To obtain lymphokine-activated lymphocytes (LAK) from PBL (3×10^6 cells/ml), cells were incubated with recombinant interleukin-2 (RIL-2; 1000 MU/ml) (BIOTECH, Russia) for 2 h at 37 °C in the athmosphere of 5% CO_2, and washed twice.

Antitumor action of activated and nonactivated lymphocytes toward tumor explants was studied by their co-cultivation for 5 days in double diffusion chambers. After cultivation the preparations were fixed in spirit-formol (Makrochim, Ukraine) for 1 h, stained with Carazzi's hematoxylin (Fluka, Switzerland) for 20 min, passed through the spirits of increased concentration and treated with xylene; then preparations are covered by Canadian balsam and examined by microscopy (JENAVAL microscope, Germany). Evaluation of antitumor activity of lymphocytes was done on the basis of morphological patterns of explant's growth (ability of cell to migrate from explant, formation of monolayer of different density, and of cell conglomerates, etc.).

Individual sensitivity of tumor explants to chemotherapeutic drugs (doxorubicine (0.02 mg/ml, Ebewe, Austria), cyclophosphane (0.006 mg/ml, Olanpharm, Latvia), 5-fluorouracil (0.006 mg/ml Ebeve, Austria), metothrexate (0.005 mg/ml, Teva Pharmaceutical Industries LTD, Israel) was determined by cultivation of explants in diffusion chambers in culture medium supplemented with mentioned drugs as described in [20].

To determine the expression of E-cadherin, p53 (DacoCytomation, Denmark), and nuclear antigen of proliferating cells (mAb IPO-38, IEPOR NASU, Kiev, Ukraine) in tumor cells, paraffin sections of tumors were analyzed by immunohistochemistry with the use of respective monoclonal antibodies, and secondary complex EnVision (DacoCytomation, Denmark). Expression of lectin from soybean, coral bean, lentil and PHA receptors (SMI Lectinotest, Ukraine) on lymphocytes was studied by immunocytochemistry. The level of expression of mentioned markers was evaluated by semiquantitative method where integral index of expression level was determined by the sum of scores < for the percent of stained cells (score 1 — ⩽ 10%, score 2 — 10–30%, score 3 — 30–45%, score 4 — 45–60%, score 5 — ⩾60%) and staining intensity (score 1 — low, score 2 — medium, score 3 — high) [21]. Expression of CD54, CD11b, and nuclear antigen of proliferating cells on lymphocytes was studied by the method of indirect immunofluorescence with the use of respective monoclonal antibodies (IEPOR NASU) at the dilution of 40 μg/ml.

RESULTS AND DISCUSSION

By determination of individual sensitivity of malignant tumors to mentioned chemopreparations it has been shown that the tumors of 15 patients are resistant to one, two, three or four cytostatics, in most cases — to doxorubicine and methotrexate. Only in 5 cases, the tumors were sensitive to all four drugs. So, the patients with malignant tumors were distributed into two groups — with drug resistant malignant human breast tumors (DRHBT) and drug sensitive breast tumors (DSHBT), and the third group (the patients with benign breast tumors (BBT)) served as a control.

In all groups, the expression of E-cadherin, nuclear antigen of proliferating cells and p53 by tumor cells has been studied. Evaluation of E-cadherin expression has demonstrated that in DRHBT cells it was significantly lower than in DSHBT or BBT cells (3.3 ± 0.7 *vs* 4.8 ± 0.5 and 5.9 ± 0.9, respectively) (Table 1). Fig. 1 presents the level of E-cadherin expression in each tumor case. In DRHBT group an integral index of E-cadherin expression level reached only score 3 in 60% of cases, while in DSHBT or BBT groups it was significantly higher.

Table 1. Expression of E-cadherin, p53 and nuclear antigen of proliferating cells in human benign and malignant breast tumor cells (semiquantative analysis)

Groups of patients	Integral score of expression level (M ± m)		
	E-cadherin	p53	Nuclear antigen of proliferating cells
Patients with benign neoplasms (n = 12)	5.9 ± 0.9	5.2 ± 1.8	4.2 ± 0.8
Patients with drug resistant malignant breast tumors (n = 15)	3.3 ± 0.7*,**	3.4 ± 0.7	6.5 ± 0.8**
Patients with drug sensitive malignant breast tumors) (n = 5)	4.8 ± 0.5	4.2 ± 0.8	5.4 ± 0.5

*The difference between the groups of drug sensitive and resistant tumors is statistically significant ($p < 0.05$); **the difference between the groups of benign and malignant drug resistant tumors is statistically significant ($p < 0.05$).

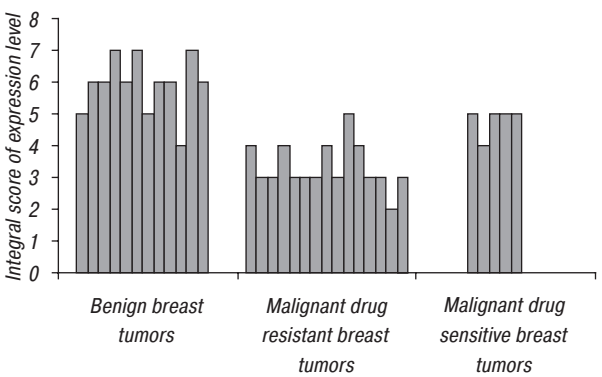

Fig. 1. E-cadherin expression in human breast tumor cells (semiquantitative analysis)

The levels of nuclear antigen of proliferating cells expression were significantly different between BBT cells and DRHBT cells (6.5 ± 0.8 and 4.2 ± 0.8, respectively), while in DSHBT group this index showed only a tendency to be higher (see Table 1, Fig. 2). Thus, DRHBT cells were characterized by low level of E-cadherin expression and high level of nuclear antigen of proliferating cells expression.

There were no significant differences in expression levels of p53 between the groups. However, individual variations in each group were rather different.

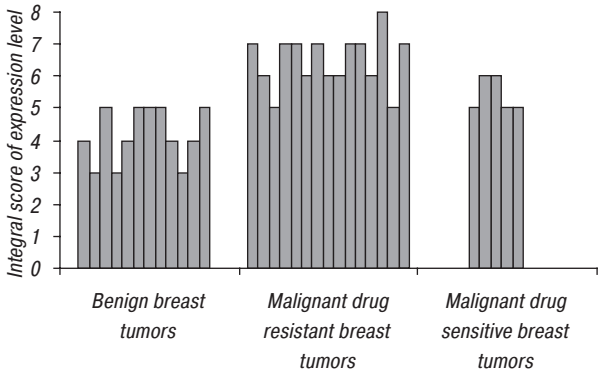

Fig. 2. Expression level of nuclear antigen of proliferating cells in human breast tumor cells (semiquantitative analysis)

We have studied antitumor activity of LAK towards tumor explants from the patients of all groups taking into account data on expression of E-cadherin and nuclear antigen of proliferating cells. The most pronounced activity of LAK was directed against DRHBT. DRHBT explants growth was significantly suppressed, while in control BBT and DSHBT groups, formation of monolayer of various densities was observed. In DRHBT group only single cells or no cells migrated, or in some cases tumor destruction was observed (Fig. 3, *a*, *b*).

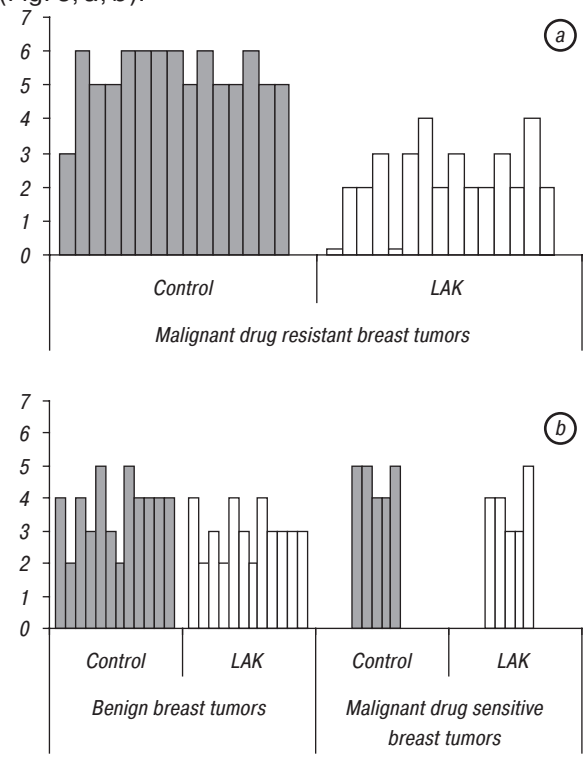

Fig. 3. Morphological analysis of the growth of explants from drug resistant (*a*) and sensitive (*b*) malignant tumors and benign breast tumors upon the action of LAK: 1 — no migration; tumor destruction; 2 — no migration; 3 — migration of single cells; 4 — initial stage of monolayer formation; 5 — monolayer of low density; 6 — monolayer of medium density

To analyze whether some proteins expressed on LAK surface were involved in LAK interaction with tumor cells, expression levels of some lectin receptors (soybean, coral bean, lentil, PHA) and CD54 and CD11b were examined (Table 2). There were no significant differences in CD54, CD11b, receptors for lectins (coral bean, lentil, PHA) expression levels between the studied groups. However, lymphocytes from DRHBT group were characterized by significantly higher expression level of soybean lectin receptor than in DSHBT and BBT groups (47.5 ± 5.4 *vs* 35.3 ± 4.5; 27.5 ± 3.4, respectively). It could be supposed that soybean receptor may be involved in antitumor activity of LAK.

So, DRHBT cells were characterized by the reverse relation between expression levels of E-cadherin and nuclear antigen of proliferating cells. Marked antitumor action of LAK against these tumors was associated with elevated expression of soybean lectin receptor.

The aim of the present work was to examine if there any correlation between E-cadherin and the expression of some other markers with elevated sensitivity of resistant tumor cells to the action of LAK. Expression of a number of markers in lymphocytes themselves has been studied as well. It was shown that decreased E-cadherin expression was the most marked alteration of DRHBT cells compared with DSHBT and BBT cells.

It is known that decrease of E-cadherin expression is accompanied by dissociation of E-cadherin/catenin complex leading to altered activity of some signaling pathways (Wnt, PKD-1, Int-2 etc.), nuclear accumulation of β-catenin, and activation of transcription factors (NF-κB, Ap-1 and other) [22–25]. Finally, all mentioned intracellular processes that reflect molecular changes in cells upon the decrease of E-cadherin expression, are accompanied by elevated proliferation of tumor cells [26], which is in accordance with our data.

Unfortunately, there are just few studies on a relation between tumor cells proliferation and antitumor action of LAK, but we consider the marked proliferation of drug resistant tumor cells as a factor favoring LAK activity.

There are interesting data showing that disturbed intercellular adhesion promote antibody-dependent cell cytotoxicity. These data were obtained with the use of anti-adhesive antibodies and intestinal carcinoma on a model of spheroid formation. The use of these antibodies positively regulated antibody-dependent cell cytotoxicity mediated by FcγRIII receptor [27]. There, its looks reasonable to use anti-adhesive drugs (in particular SHE78-7 monoclonal antibodies) in therapy of some tumors [28].

Elevated expression of soybean lectin receptor in lymphocytes from patients with drug resistant tumors may be related to promotion of antitumor activity of LAK.

Table 2. Expression of CD54, CD11b, and lectins receptors by LAK from the patients with benign and malignant breast tumors

Groups of patients	Percent of cells expressing the marker protein (M ± m)					
	CD54	CD11b	Lectins receptors			
			Soybean	Coral bean	Lentil	PHA
Patients with benign neoplasms (n = 12)	12.2 ± 3.4	55.2 ± 6.3	28.2 ± 3.1	27.5 ± 3.4	3.5 ± 1.2	76.2 ± 2.2
Patients with drug resistant malignant breast tumors (n = 15)	16.4 ± 4.4	58.3 ± 5.3	47.5 ± 5.4*	31.8 ± 2.7	3.2 ± 1.4	70.0 ± 3.4
Patients with drug sensitive malignant breast tumors (n = 5)	15.1 ± 4.0	40.2 ± 7.4	35.3 ± 4.5	26.3 ± 5.1	3.0 ± 1.6	66.7 ± 2.5

*The difference between the groups of benign and malignant tumors is statistically significant ($p < 0.05$).

In is known that N-acetyl-D-galactosamine — carbohydrate present on a surface of a number of epithelial tumors — serves as a ligand for soybean lectin receptor. That is why the data on a role of N-glycosylation for E-cadherin-mediated cell adhesion are of special interest [13]. One may suppose that interaction between soybean receptor on lymphocytes with its ligand on tumor cells may promote their antitumor action.

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