Expression of MDR proteins in breast cancer and its correlation with some clinical and pathological parameters*

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The aim of this work was to determine the expression of the multidrug resistance (MDR) proteins, namely MDR1 (P-glycoprotein), MRP1 (multidrug resistance-related protein) and LRP (lung resistance-related protein), in 87 samples of breast carcinoma. Detection of these proteins was provided by using indirect enzymatic immunohistochemistry. Our findings were compared with the other clinical and pathological parameters: expression of Her2/neu, estrogen receptor status (ER), progesteron receptor status (PR), histological grade and regional lymph node status. For statistical analysis, non-parametric two sided Mann-Whitney-U test was used. Majority of breast carcinoma specimens show positivity for these proteins. The MDR1 and MRP1 signal was found in the cytoplasm of cancer cells. The expression of LRP was detected in the cytoplasm close to the nuclear membrane. The samples were positive for MDR1 protein in 57%, for MRP1 in 84% and for LRP in 79%. Comparing our results with other clinical and pathological parameters, negative correlation between ER, PR and MDR1 expressions and histological grading status was found. No associations were observed between the MRP1 and LRP proteins and histological grading, as well as between the expression of three MDR proteins and the other clinicaly relevant parameters.

In conclusion, high frequency of expression of MDR proteins in breast carcinoma cells suggests, that these proteins might be an important factor of drug resistance in breast carcinoma. Nevertheles, the negative correlation between the histological grade of malignancy of tumor and the expression of ER, PR and MDR1 indicates possible influence of progressive tumor cell dediferentiation. However, this finding has to be confirmed in additional evaluations.

Key words: MDR1 protein, MRP1 protein, LRP protein, breast cancer, immunohistochemistry

Breast cancer is an extremely important disease in industrial countries of the world. Over the last 30 to 40 years, substantial progress has been made in the diagnosis and treatment of breast cancer. An effort has been made to detect risk factors and the genetic factors that contribute to the risk of breast cancer development. Similarly, molecular markers for the prediction of response to chemotherapy of already existing breast tumor were tried to be exactly established. Among them, the expression of steroid hormone receptors, growth-factor receptors and multidrug-resistance (MDR) proteins, seem to play an important role [1–3].

Breast cancer is often considered to be one of the most chemoresponsive solid tumors. Normal breast epithelial cells and their malignant counterparts are sensitive to estrogens, progesterones, and androgens. Estrogen primarily appears to stimulate normal ductal growth, whereas progesterone is responsible for lobulo-alveolar development. Expression of estrogen and progesterone receptors (ER and PR) is not universal in malignant epithelial breast cancer cells. Knowledge of steroid receptor content of a breast cancer is important as tu-

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mors with high ER or PR content are better differentiated, and patients with this tumors have better prognosis. Moreover, tumor concentration of ER and PR is strongly predictive of response to endocrine therapy [4]. Recent pharmacological treatment regimens of breast cancer include conventional chemotherapy on the basis of cytotoxic drugs, in steroid receptor-positive patients an endocrine therapy and an immunological-basing therapy in Her-2/neu-positive neoplasms of the breast [5]. The Her-2/neu belongs to the erbB family of polypeptide growth factor receptor system consisting of four separate receptors, designated erbB1, 2, 3, and 4 [6]. The protein consists of an extracellular domain functioning as a ligand receptor, a transmembrane domain, and an intracellular domain that serves as a tyrosine kinase. The Her-2/neu is either amplified and/or overexpressed in 20–30% of newly diagnosed breast cancers [7, 8].

Patients whose breast cancers have amplified and/or overexpressed Her-2/neu have a worse prognosis than patients with normal copies of Her-2/neu [9]. Furthermore, Her-2/neu amplification/overexpression may result in selective response or resistance to specific systemic therapies. Although it is not yet completely established, preliminary studies suggest that Her-2/neu amplification/overexpression may result in resistance to endocrine therapy (tamoxifen) and alkylating agents (cyclophosphamide, metothrexate, 5-fluorouracil), and relative sensitivity to doxorubicine-based therapy [10].

Potential drug resistance mechanisms clinically active in breast cancer patients are those involved in the MDR phenotype [11, 12]. Among the others they include expression of the multidrug-resistance protein (MDR1/Pgp), multidrug-resistance-related protein (MRP1) and lung resistance-related protein (LRP). They have been detected with various frequencies in breast cancer specimens [13–16]. The MDR of tumor cells is believed to be the reason for failure of chemotherapy.

The latest discovered MDR protein is breast cancer-resistance protein (BCRP). Its level is undetectable by immunohistochemistry not only in specimens of breast cancer, but also in samples of many human tumors [17].

Our study was undertaken to investigate the expression of 6 proteins, all of which are involved in broad resistance to anticancer drugs. The studies were performed on a series of breast cancers with different grading. Furthermore, we studied if the expression of individual proteins influences each another by comparing their expression levels in newly diagnosed tumor specimens before chemotherapy.

Material and methods

Clinical samples. In this study we have used 87 samples of breast carcinoma. The samples were obtained from the Department of Pathological Anatomy, J.A. Comenius University, Jessenius Faculty of Medicine in Martin. In all the cases a standardized world-wide accepted uniform approach for the biopsy examination of the breast carcinoma specimen was used, including a panel of histological and immunohistochemical examinations. For the purposes of the histological grading a system recommended by ELSTON and ELLIS (1991) [18] was used. Patients and tumor characteristics are summarized in Table 1.

Table 1. Patients and tumor characteristics

Characteristics		No
All patients		87
Age	≤ 50	27
	≥ 51	59
	Unknown	1
Histological grade	1 – good	11
	2 – moderate /worse/	41
	3 – poor	35
Histo–pathol. type	Ductal	56
	Lobular	13
	Other	18
Her2/neu	Positive	19
	Negative	63
	Unknown	5
Estrogen receptor status /ER/	Positive	56
	Negative	31
Progesteron receptor status /PR/	Positive	32
	Negative	55
Involvement of regional lymph nodes	1	39
	2	39
	2x	1
	3	3
	3x	0
	4	3
	Unknown	2

Immunohistochemical detection of MDR1, MRP1, LRP, ER and PR. We have used indirect enzymatic immunohistochemical method. Formalin fixed, paraffin embedded tissue blocks were cut (7 μm) and attached to the slides. The slides were processed for immunohistochemistry.

Tissue sections were deparaffinized with xylene and rehydrated in decreasing ethanols to water. The slides were finally washed in phosphate-buffered saline containing 0.05% Tween-20 (PBS-Tw), pH 7.6. Endogenous peroxidase activity was blocked by 0.3% H_2O_2 (3% H_2O_2 for ER and PR) in methanol for 30 minutes at room temperature. According to the analyzed protein, sections were pretreated in citrate buffer solution and Target Solution in the microwave oven differently. The slides stained for MDR1 and LRP were pretreated in the microwave 2x5 minutes, MRP1 slides for 20 minutes, PR for 45 minutes and ER in Target Solution (DakoCytomation, Denmark) for 45 minutes. MDR1 and LRP staining procedure continued by blocking nonspecific staining with blocking serum (prediluted normal horse serum

– Vector Laboratories, USA) for 90 minutes in humidified chamber at room temperature. In the case of MRP1, ER and PR staining the blocking serum was omitted. The next step was application of primary antibodies. We have used the following antibodies: mouse anti-LRP, LRP-56 (BD Transduction Laboratories, USA), mouse anti-MDR1, clone UIC2 (Immunotech, France), mouse anti-MRP1, clone MRPm6 (Alexis, Canada), mouse anti-human estrogen receptor α, clone 1D5 (DakoCytomation, Denmark) and monoclonal anti-human progesteron receptor, clone 1A6 (Immunotech, France). Primary antibodies were applied overnight in humidified chamber at 4 °C.

After rinsing in PBS-Tw (3x5 minutes) the sections were subsequently incubated with the secondary antibodies: prediluted biotinylated horse antibody for MDR proteins (Vector Laboratories, USA), and biotinylated secondary antibody for ER and PR (DakoCytomation, Denmark) for 30 minutes at room temperature. The slides were washed with PBS-Tw and submitted to application of peroxidase-conjugated streptavidine: prediluted R.T.U. Vectastain for MDR proteins (Vector Laboratories, USA), and peroxidase-conjugated streptavidin for ER and PR (DakoCytomation, Denmark) for 30 minutes at room temperature. The sections stained for MDR proteins were then visualized with DAB, ER and PR immunostained sections with AEC. Slides were stream-rinsed with tap water, counterstained with hematoxylin for 2 minutes, washed in tap water, dried, mounted and coverslipped. Sections processed with omission of primary antibody served as a negative control of immunohistochemical procedure.

In the cases of ER and PR immunostaining procedure TRIS buffer and tap water were used instead of PBS-Tw.

Immunohistochemical detection of HER2/neu protein. For the immunohistochemical detection of the HER2/neu protein the HercepTestTM kit (DakoCytomation, Denmark) was used. In relation to the world-wide accepted agreement, all steps of the detection strictly follow the guidelines published in the manual of the kit producer.

Statistical analysis. Statistical evaluation was performed using non-parametric two sided Mann-Whitney-U test analysis. $P<0.05$ was considered to be significant.

Results

Screening for MDR1, MRP1 and LRP expression in clinical samples. Eightyseven (87) samples of breast carcinoma (Tab. 1) were immunohistochemically analyzed for MDR1, MRP1 and LRP proteins expression.

In the tissue of breast carcinoma we detected intracellular cytoplasmic localization of MDR1 protein (Fig. 1a – ductal type, 2a – lobular type). LRP protein, which is the main component of "vaults", was expressed in the cytoplasm close to the nuclear membrane (Fig. 1a – ductal type, 2b – lobular type). MRP1 protein similarly showed cytoplasmatic staining pattern (Fig. 1c – ductal type, 2c – lobular type).

The breast carcinoma samples were according the histo-pathological type divided into 3 groups: 1. ductal carcinoma (56 samples), 2. lobular carcinoma (13 samples), 3. other types (papillary, cribriform, mucinous, medullary and tubular carcinoma) (18 samples). The evaluation of expression of MDR1, MRP1 and LRP proteins was provided by three of us. We have distinguished four categories of quantity of these proteins: 3+ = high level, 90–100% of positive cells; 2+ = medium level, 10–90% of positive cells; 1+ = low level, up to 10% of positive cells; – = negative cells, 0% of positive cells. For statistical analysis as positive were considered only samples with high level (3+) and medium level (2+) protein expressions. Samples scored as 1+ or - were considered negative [19].

High level of MDR1 showed 21 (24%) samples of breast carcinomas. Twentynine (33%) samples expressed medium level, 20 (23%) displayed low level and in 17 (19%) samples no signal was observed. Totally, 50 (57%) breast carcinomas were MDR1 positive, the rest (37 = 43%) showed no MDR1 positivity.

MRP1 protein expressed the highest level in 28 (32%) cases. Medium concentrations were detected in 45 (51%) samples, 10 (11%) showed low concentration and 4 (5%) samples expressed no one positive cell. Majority of samples (73 = 84%) expressed high or medium levels of this protein. The signal was low or not present only in 14 (16%) samples and these samples were considered negative.

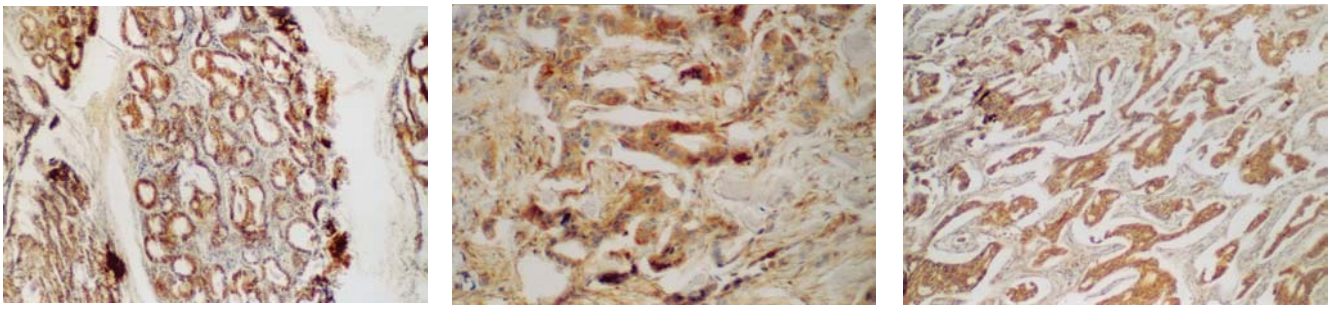
Strong LRP immunopositivity at the high level was observed in 19 (22%) tissue samples. Fortynine (56%) specimens showed medium level of LRP, 15 (17%) low and 4 (5%) samples expressed no LRP immunoreactivity. Totally, 68 (78%) cases were LRP positive and 19 (22%) specimens were negative.

Taken together, 85 (98%) samples were positive to at least one MDR protein and only 2 (2%) were negative. Exact results concerning the positivity in all three proteins (in total number of tissue samples and percentage) are shown in Table 2.

Comparison of MDR1, MRP1 and LRP expression with other predictor markers. The following clinicaly relevant predictor parameters were determined and compared with MDR1, MRP1 and LRP expression: Her2/neu, estrogen re-

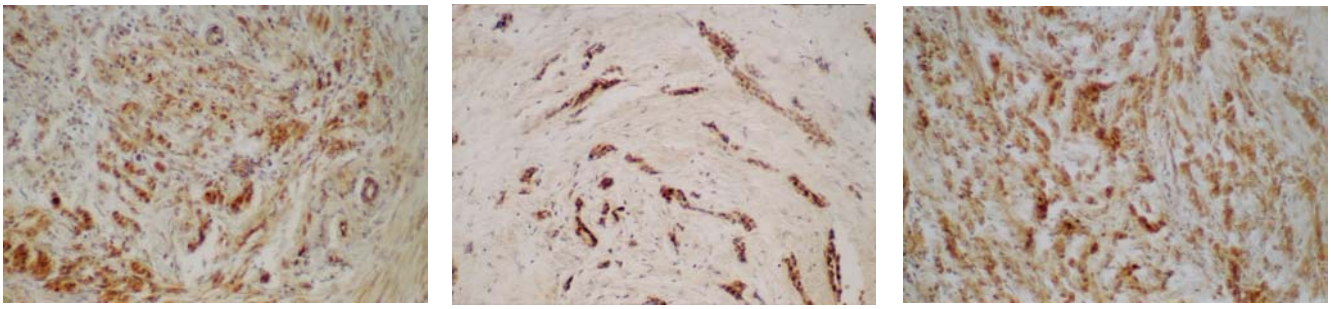
Table 2. Various levels of MDR1 (%), MRP1 (%) and LRP (%) proteins in 87 samples of breast carcinoma tissue. The majority of samples expressed MDR1, MRP1 and LRP positivity.

Quantity of expression	MDR1	MRP1	LRP
+++	21 (24)	28 (32)	19 (22)
++	29 (33)	45 (51)	49 (56)
+	20 (23)	10 (11)	15 (17)
–	17 (19)	4 (5)	4 (5)
Number of positive samples	50 (57)	73 (84)	68 (78)
Number of negative samples	37 (42)	14 (16)	19 (22)



a b c

Figure 1. Expression of MDR proteins in ductal type of breast cancer was immunohistochemically detected by using of monoclonal antibodies. To detect the MDR1 protein we have used the monoclonal antibody UIC2 (1a), LRP-56 was used for LRP protein (1b), and MRPm6 for detection of MRP1 protein (1c). MDR positive cells show brown staining.
Magnifications: 1a – 4x10, 1b – 10x10, 1c – 4x10.



a b c

Figure 2. Lobular type of breast carcinoma with immunohistochemical staining of MDR proteins. The monoclonal antibodies used for this staining were the same as in the case of ductal type of mammary carcinoma. MDR1 (2a), LRP (2b) and MRP1 (2c) immunopositive cells are represented by brown colour.
Magnifications: 2a – 10x10, 2b – 10x10, 2c – 10x10.

ceptor status (ER), progesteron receptor status (PR) and tumor grade. The comparison of these parameters was evaluated in 87 clinical samples of all histo-pathological types of breast cancer tissue.

The expression parameters of Her2/neu protein was available in 82 samples of breast carcinomas tested. To establish the relationship between Her2/neu and MDR1, MRP1, LRP, ER and PR protein expressions we compared 63 Her2/neu negative versus 19 Her2/neu positive samples (the expression of Her2/neu has not been detected in 5 samples). However, when the expression of individual proteins in Her2/neu negative and Her2/neu positive tumor samples were statisticaly determined, the analysis did not reveal the correlation in Her2/neu and evaluated protein expressions (data not shown).

Comparing ER positive samples with PR positivity resulted in strong correlation in ER and PR coexpression. In 56 ER positive tumors a number of 31 (55%) was found to be positive also for PR expression. In ER negative tumor samples (n=31), the only one PR positive tissue was detected (Tab. 3). This difference was found as statisticaly significant ($p<0.0001$).

Coexpressions of ER or PR and individual MDR proteins (MDR1, MRP1, LRP) were also evaluated. The results indicated that if comparing ER positive and ER negative samples, no correlation in ER and MDR proteins was determined. In PR negative tumor samples (n=55) the concomitant expression of MDR1, MRP1 and LRP has been found in 22, 46 and 38 samples, respectively. In PR positive tumors (n=32) the positive expression of MDR1, MRP and LRP has been found in 22, 27 and 30 tissue samples, respectively. As shown in Table 4 the only statisticaly significant correlation has been found ($p=0.0073$) in PR and concomitant LRP expressions.

Comparison of three MDR protein coexpressions (MDR1, MRP1, LRP) as well as MDR1 versus ER, PR and Her2/neu expressions resulted in only one significant difference between MDR1 positive (n=50) and negative (n=37) tumor samples concomitantly expressing LRP. Positive expression of LRP has been found in 46 MDR1 positive and in 22 MDR1 negative tumors (92% vs 59%), the difference which was

Table 3. Estrogen receptor (ER) and progesterom receptor (PR) coexpressions in all (n=87) breast carcinoma samples evaluated.

Receptor	PR^+	PR^-	p-value	OR (95% CI)[1]
ER^+ n=56 (64.4%)	31 (55%)	25 (45%)	p<0.0001[2]	0.03 (0.003-0.21)
ER^- n=31 (35.6%)	1 (4%)	30 (96%)		

[1]OR calculated when p<0.05; [2]Chi-squared statistic for 2 degrees of freedom

Table 4. Correlation of LRP expression with ER and PR. The only statisticall significant finding was observed in PR and LRP coexpression.

	LRP^+	LRP^-	p-value	OR (95% CI)[1]
ER^+ n=56 (64.4%)	45 (80%)	11 (20%)	p=0.59[2]	
ER^- n=31 (35.6%)	23 (74%)	8 (26%)		0.15 (0.032-0.69)
PR^+ n=32(55) (63%)	38 (69%)	17 (31%)	p=0.0073[2]	
PR^- n=55(32) (37%)	30 (93.8%)	2 (6.2%)		

[1]OR calculated when p<0.05; [2]Chi-squared statistic for 2 degrees of freedom

statisticaly significant (p=0.0003). The results are shown in Table 5.

Statisticaly significant differences were found also in tumor grade and ER, PR and MDR1 protein expressions. The number of samples with positive expression of all three proteins mentioned above decreased progressively with the increase of tumor grade. In ER expression 100% of grade 1 tumors were found to be positive. Expression of ER was positive only in 88% of grade 2 and 48% of grade 3 tumors. Similarly, 64% of grade 1 tumor samples expressed PR, whereas no more than 46% and 20% of grade 2 and grade 3 tumors were positive for PR, respectively. The expression of MDR1 protein followed the tendency of previous two proteins. The percentage of MDR1 positive tumors progressively decreased from 82% in grade 1 to 61% and 46% in grade 2 and 3 tumor samples, respectively. The exact numbers of tissue tumoral samples and statistical correlations are shown in Tables 6 and 7.

When the expression of MDR proteins was compared with the involvement of regional lymph nodes we did not find correlation in these two parameters. In all cases (n=87) the regional lymph nodes were involved, with no respect to MDR status (data not shown).

Table 5. Correlation of co-expression of MDR1 and LRP, MRP, Her2/neu, ER and PR. The only statistical significance has been found in MDR1 and LRP coexpression.

Protein	ER^+	ER^-	P-value	OR (95%CI)[1]
$MDR1^+$ n=50 (57.5%)	36 (72%)	14 (28%)	P=0.11[2]	
$MDR1^-$ n=37 (42.5%)	20 (54.1%)	17 (45.9%)		
	PR^+	PR^-	p-value	OR (95%CI)[1]
$MDR1^+$	22 (44%)	28 (56%)	p=0.18[2]	
$MDR1^-$	11 (22%)	26 (78%)		
	Her^+	Her^-	p-value	OR (95%CI)[1]
$MDR1^+$	10 (20%)	40 (80%)	p=0.45[2]	
$MDR1^-$	10 (27%)	27 (73%)		
	MRP^+	MRP^-	p-value	OR (95%CI)[1]
$MDR1^+$	40 (80%)	10 (20%)	p=0.65[2]	
MDR1-	31 (83%)	6 (17%)		
	LRP^+	LRP^-	p-value	OR (95%CI)[1]
$MDR1^+$	46 (92%)	4 (8%)	p=0.0003[2]	7.84 (2.33–26.41)
$MDR1^-$	22 (59%)	15 (40%)		

Table 6. Number of patients in different grading with individual expression of ER, PR and MDR1 proteins. The number of samples with positive expression of all three proteins decreased progressively with the increase of tumor grade.

Grading	1	2	3
ER^+	11 (100%)	32 (88%)	13 (48%)
ER^-	0	9	22
PR^+	7 (64%)	19 (46%)	7 (20%)
PR^-	4	22	28
$MDR1^+$	9 (82%)	25 (61%)	16 (46%)
$MDR1^-$	2	16	19

Table 7. Statistical correlation of ER, PR and MDR1 protein expressions and grading of the tumors (gr.1–3)

Grading	ER positive	PR positive	MDR1 positive
gr. 1 vs gr. 2	P=0.17	P=0.49	P=0.29
gr. 2 vs gr. 3	P=0.0004	P=0.02	P=0.25
gr. 1 vs gr. 3	P=0.0002	P=0.01	P=0.045

Discussion

Chemotherapy resistance is a major problem in the therapy of patients with cancer. Although breast cancer is considered to be one of the most chemosensitive solid tumors, complete responses are rare and most of the initially responsive tumors relapse and develop MDR. A wide range of cellular and molecular mechanisms are involved in MDR of cancer cells. Besides the others, the overexpression of MDR proteins has been suggested to influence the chemosensitivity of the cells of hematologic as well as solid tumors.

In the present study, MDR1, MRP1 and LRP protein expressions were examined in relation to Her2/neu, ER status, PR status, tumor grade and regional lymph node involvement.

MDR1, MRP1 and LRP expressions were detected in 50 (57%), 73 (84%) and 68 (78%) out of 87 samples of breast carcinomas, respectively. If only ductal type was taken in consideration, MDR1, MRP1 and LRP have been found to be positive in 34 (61%), 46 (82%) and 47 (84%) out of 56 tumor samples, respectively. The difference in MDR protein expressions between the sum of all breast carcinomas (n=87) and ductal type (n=56) were statisticaly no significant (not shown).

The percentage of MDR1-, MRP1- and LRP-staining tumors was consistent with findings of the other researchers. Some of them found 55% [15] and 57% [20] breast carcinomas to be positive for MDR1. MRP1 was positive in 80% breast carcinomas [21] and 83% [22] and 88% breast carcinomas were found to be positive for LRP [23]. However, some other immunohistochemical findings revealed different expressions of MDR proteins in breast carcinomas. For example, FANEYTE et al [24] found no membrane-bound staining for MDR1 in the tumor cells of 80 chemotherapy-naive tumors and YU et al [25] observed 41% of MDR1 positive breast carcinoma samples. MRP1 expression was detected to be 62% in prechemotherapy samples and 88% in postchemotherapy breast cancer tumors [15]. The same protein has been detected by RT-PCR in breast cancer cells, but it was not observed with immunohistochemistry at all [24]. The expression of LRP was 65% in breast carcinoma samples and incresased to 97% in samples from postchemotherapy patients [15]. Taken together, the percentage of MDR (MDR1, MRP1, LRP) protein-positive breast cancer samples vary significantly across the studies and the impact of their expression on clinical outcome of breast cancer patients remains open.

Overexpression of HER2/neu has independent prognostic significance in early breast cancer and may also predict response to hormonal and cytotoxic therapies. Retrospective evidence strongly suggests that HER2/neu overexpression is associated with decreased disease-free and overall survival in breast cancer [3]. In our study, Her2/neu expression was detected in 19 (23%) out of 82 evaluated breast carcinomas. If 53 samples of ductal type carcinomas were evaluated separately 17 (32%) were found as Her2/neu positive.

Next, we compared the clinical parameters of the patients with the expression of Her2/neu and MDR proteins tested. No correlation was found between Her2/neu and MDR1, MRP1 and LRP expressions in our set of breast carcinoma samples. These results were in a good concordance with the observations of BURCOMBE et al [26] who did not find relationship between Her2/neu and other biological markers (ER, PR, Ki-67 expressions) in primary breast cancer samples. As the greatest value of Her2/neu lies in a negative prediction of tumor agressivity and response to therapies, this lack of correlation should be judged as an indirect evidence of little impact of MDR proteins on clinical outcome in breast cancer. However, there exists information that at least MDR1 expressions were affected by drug exposure [27] and MRP1 might be an important negative factor in breast cancer patients [21]. In that case the overexpression of these MDR proteins is fully independent on Her2/neu status and adds to negative prognosis of tumor development.

The expression of ER and PR in breast tumor samples was detected in 71% and 45%, respectively. Comparing ER positive samples with PR positivity resulted in 55% coexpression in ER and PR. This statisticaly significant difference was also recently found by others [26].

Similarly as in previous comparison with Her2/neu expression, lack of correlation between MDR1, MRP1, LRP and ER positivity was found. When the correlation of MDR proteins with PR was evaluated, significant positive coexpression of PR and LRP has been only detected. On the other hand, strong negative correlation between tumor grade and ER and PR expression has been found. In grade 1 tumors, 11 samples (100%) and 7 tumors (64%) expressed ER and PR, respectively. The expression progressively decreased in grade 2 and grade 3 tumors. The amount of ER positive samples in grade 2 tumors (n=41) decreased to 88% and in grade 3 tumors (n=35) to 48%. PR expressions decreased to 62% in grade 2 and 12% in grade 3 tumors. All these expression diminutions were statistically significant ($p<0.001$). Our findings are in good correlation with previously published results where ER in breast carcinomas correlated negatively with histologic grade, lymph node metastasis and TNM stage. Moreover, high proliferative activity and the absence of ER were considered as a high grade malignancy of breast carcinoma [28]. However, in recently published paper the relative survival advantage of ER positive tumor disappears after 5 years of survival and women with ER and PR negative tumors have better long-term survival outcomes [29].

Similarly as in ER and PR expressions, negative correlation between MDR1 expression and tumor grade has been observed. This difference has achieved the statistical significancy if MDR1 expressions in grade 1 and grade 3 tumors were evaluated ($p=0.045$). We consider this observation interesting, as the expression of MDR1 was usually found to be independent of grade [30, 31]. Moreover, MDR1 expression is not associated with ER status [31]. However, there exist quite important number of papers where no correlation of

MDR1 expression and cancer stage and grade [32, 25] or response to chemotherapy [16] has been detected. Additionally, estradiol is thought to regulate MDR1 expression in breast cancer. It increases the cytoplasmic concentration of MDR1 in MCF7 (ER positive) cells through the stimulation of ERalpha [33]. In the same way, progesterone specifically regulates the activity of the mdr1b promoter and this response is directed solely by the A form of the progesterone receptor [34]. Taken together, the results of our analysis indicate, that the decrease of ER and PR in higher grade breast carcinomas should result in progressive MDR1 expression decreases.

In conclusion, our data demonstrate that the expression of MDR1, MRP1 and LRP was not affected by Her2/neu positivity or negativity in breast carcinomas. In addition, the expression of all MDR proteins tested did not correlate either with the expression of ER or the involvement of regional lymph nodes. We have found the only positive correlation in PR/LRP and MDR1/LRP coexpression which was difficult to explain. We consider that the most important finding in this study is decreasing expression of ER and PR with increasing tumor grade. The loss of these receptors could be explained by progressive dediferentiation of tumor cells. Moreover, decreased expression of MDR1 has also been observed. Despite of some speculative explanations of this fact by the positive regulation of MDR1 expression through ER or PR, the elucidation and final approval of this finding needs additional experiments.

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