Expression of glucose transporter GLUT-1 and estrogen receptors ER-α and ER-β in human breast cancer

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We attempted to describe a GLUT-1 expression in breast cancer and characterize correlation between GLUT-1 and ERs α and β expression as well as correlate this with clinicopathologic features.

Sixty-nine patients were involved in the study. GLUT-1, ER-α and ER-β immunocytochemistry was performed using the streptavidin-biotin method.

Thirty-seven (53.6%) out of total 69 were GLUT-1 positive. Of GLUT-1 positive 45.3% were ER-α-positive, whereas 81.3% of ER-α-negative were GLUT-1 positive. Statistically significant correlation was observed between GLUT-1 and ER-α expression status but neither between GLUT-1 and ER-β nor with clinicopathologic features. No statistically significant correlation was found between expression level (expressed as immunocytoreactive score) of GLUT-1, ER-α and ER-β.

Since most of ER-α-negative (81.3%) were GLUT-1 positive and significant correlation exists between the two receptors it is reasonable to assume that some functional relation might exists between the expression of two receptors.

Key words: GLUT-1, ER-α, ER-β, breast cancer, immunohistochemistry

Malignant cells of different types show increased glucose uptake, which is believed to be mediated by expanding family of glucose transporters (GLUTs). GLUT-1 – the human erythrocyte glucose transporter, belongs to this family and is one of at least seven members that are marked by Arabic numerals according to time and site of detection. A significant increase in GLUT-1 mRNA has been detected in tumors of the stomach, colon, pancreas, as well as in brain tumors [3, 12, 16, 17]. Immunohistochemical studies revealed overexpression of GLUT-1 protein in a variety of carcinomas including breast cancer [5, 6, 13, 15, 18, 19].

Breast carcinomas display a considerable variation in the expression of facilitative glucose transporters expression, also GLUT-1. Although GLUT-1 has been reported to be upregulated in ductal and lobular carcinomas of the breast, the extent of its expression, distribution pattern and its possible association with clinicopathologic parameters have not been fully elucidated [1]. The correlation of GLUT-1 expression with ER-α and ER-β expression pattern has also not been studied in previous studies. The objective of the present study was to correlate the expression of GLUT-1 with clinicopathologic features of the tumor and to investigate, whether intensity of GLUT-1 expression correlates with ER-α and ER-β expression status.

Material and methods

Tumor samples were obtained from patients who underwent mastectomy or breast conserving surgery in Regional Cancer Centre and Hospital of the Ministry of Internal Affairs and Administration in Białystok. Informed consent was obtained from every patient prior to the investigation. This study was conducted in 69 consecutive patients with primary breast carcinoma; 57 cases of ductal infiltrating and 12 of lobular infiltrating. The mean age was 53.8 years (range 31–74 years). Patients had not received any preoperative chemo- or hormonal therapy. Information regarding the clinical and pathologic characteristics of the patient population was obtained from the medical records.

Histological grading was performed according to the BLOOM and RICHARDSON [2].

GLUT-1, ER-α and ER-β immunostaining. Two representative blocks of each tumor were used for routine and immunohistochemistry staining. All samples were representative of the primary tumor.

GLUT-1, ER-α and ER-β were detected by immunohistochemistry using the labeled streptavidin biotin (LSAB) procedure [11]. Sections were deparaffinized in xylene, and cells were rehydrated in decreasing ethanol solutions. Endogenous peroxidase was neutralized with 2% hydrogen peroxide for 3 minutes. The slides were subjected to heat-induced antigen retrieval using a microwave. They then were washed and incubated for 30 minutes at 22 °C with:

– a monoclonal rabbit anti-human ER-α antibody (D-12, Santa Cruz Biotechnology, USA) at dilution 1:200. The antigen used for immunization is a peptide corresponding to amino acids 2-185 mapping at the amino terminus of ER-α of human origin.

– a polyclonal rabbit anti-human ER-β antibody (H-150, Santa Cruz Biotechnology, USA) at dilution 1:200. The antigen used for immunization is a peptide corresponding to amino acids 1-150 mapping at the amino terminus of ER-β of human origin.

– a polyclonal rabbit anti-human GLUT-1 (Dako, Denmark) at dilution 1:100. Antibody was raised against a 12-amnioacid synthetic peptide corresponding to carboxyl terminus of human GLUT-1. In Western blotting, anti-GLUT-1 recognizes the 55-kilodalton GLUT-1 protein in immunoblots of solubilized human erythrocytes [19].

The samples were then washed off, and biothenylated secondary antibody was applied to the slides for 25 minutes in a humidity chamber. The slides were again washed and incubated with streptavidin peroxidase for additional 25 minutes and submerged in DAB bath for 5 minutes. Tissues were counterstained with hematoxylin. All studies were examined by light microscopy.

Tumors were considered positive for GLUT-1 only when strong membrane associated immunoreactivity was observed (Fig. 1A). For ER-α nuclear (Fig. 1B) and for ER-β cytoplasmatic immunostaining was examined (Fig. 1C). Appropriate immunohistochemical controls were done. Positive controls included breast carcinomas, which were known for positive immunostaining for ER-α, ER-β and GLUT-1. Moreover, as a positive control we used ER-α-, ER-β-positive MCF-7 cell line. Negative controls included omission of primary and secondary antibodies. Specific staining abolished in negative controls.

For the purpose of below described quantification system cases in which 100% of the cells were ER-α, ER-β and GLUT-1 negative by immunocytochemistry were considered ER-α, ER-β and GLUT-1 negative.

Quantification of GLUT-1, ER-α and ER-β was performed using immunoreactive score (IRS) of REMELLE and STEGNER [14]. Briefly, the tumors were categorized by the percentage of tumor cells with positive staining: 0%–20% (I), 21%–50% (II), 51%–80% (III), and 81%–100% (IV). The intensity of staining was categorized as weak staining (I), medium staining (II), and intense staining (III). The immunoreactive score was calculated by multiplication of the groups, resulting in a scale ranging from 1 to 12. To reduce variability in the staining procedure, all available tumors were stained on the same day. The slides were examined by two skilled observers who were blinded as to the clinicopathological features of the patients, and the final agreements was reached by consensus using a 2-head microscope when the evaluation differed.

Statistical analysis. The exact Fisher test was used to test correlation between the expression of ER-α, ER-β and GLUT-1. Test of independence χ^2 as well as exact Fisher test were used to study the correlation between the expression of ER-α, ER-β and GLUT-1 and patients age, histological type, tumor size, tumor grade and lymph node status. Regression analysis was used to determine the relationships between immunoreactive scores (IRS) of GLUT-1 (ScoreG) vs ER-α (ScoreA) and GLUT-1 vs ER-β (ScoreB). All statistical tests were performed at 5% level of significance.

Statistical package SAS STAT 6.12 and Prism 3.0. were used for statistical analysis.

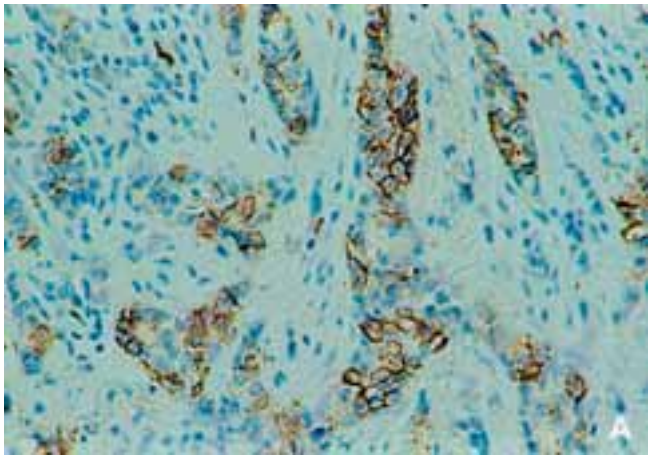
Results

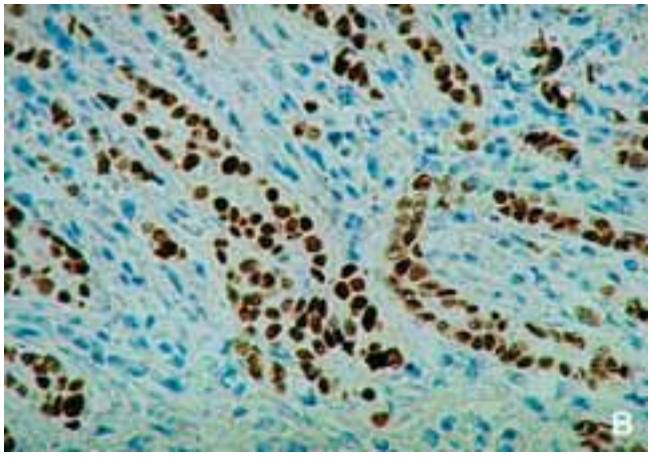
A total of 69 paraffin-embedded breast carcinomas tissue sections were examined using GLUT-1, ER-α and ER-β immunohistochemical staining (Fig. 1A, B, C).

Fifty-three cases (76.8%) were ER-α positive, whereas 64 (92.7%) were ER-β positive. Thirty-seven (53.6%), of total 69, were GLUT-1 positive. Table 1 shows that among all GLUT-1-positive tumors 24 cases (45.3%) were ER-α-positive and 35 (54.7%) were ER-β-positive. Thirteen of 16 cases (81.3%) of ER-α-negative tumors were GLUT-1-positive (Tab. 1).

The Fisher exact test showed statistically significant correlation between the expression of ER-α and GLUT-1. No other statistically significant correlation was found between other combinations of expression of GLUT-1, ER-α and ER-β (Tab. 1).

The analysis of immunoreactive score values (IRS) between GLUT-1 and ER-α and GLUT-1 and ER-β revealed no statistically significant results. Correlation ratio values were 0.02 and 0.08, respectively for IRS of score G (GLUT-1) vs score A (ER-α), and score G vs score B (ER-β). It practically means that no correlation exists between the above variables. Coefficient A values (slope of regression function) in linear regressive equation (Y=AX+B) are also different from zero. We can therefore assume that there is no linear dependence between the values of analyzed variables.





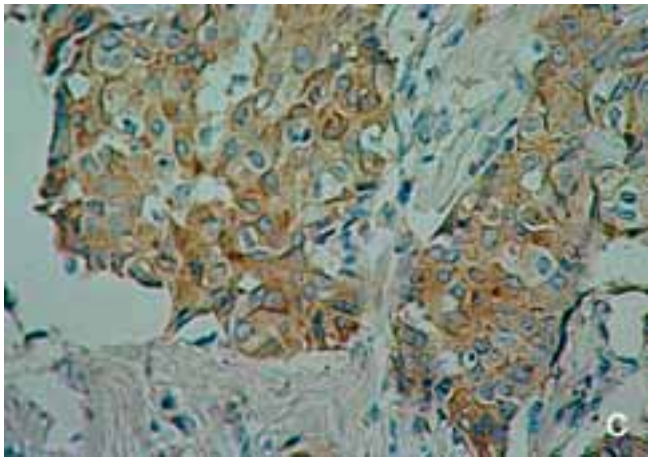


Figure 1. Typical membranous GLUT-1 expression in breast cancer (A). Immunostaining for ER-α was restricted to the nucleus of breast tumor cells (B), whereas ER-β staining was only cytoplasmic (C). The same ER-α, ER-β immunostaining pattern we observed in MCF-7 cell line.

Table 1. Relationship between ER-α and ER-β and GLUT-1 expression

	GLUT-1-positive tumors n=37	p value
ER-α status		
ER-α positive	24/53 (45.3)[a,b]	0.011
ER-α negative	13/16 (81.3)	
ER-β status		
ER-β positive	35/64 (54.7)	NS
ER-β negative	2/5 (40)	
ER-α and ER-β status		
ER-α positive/ ER-β positive	22/48 (45.8)	NS
ER-α positive/ ER-β negative	2/5 (40)	
ER-α negative/ ER-β positive	13/16 (81.3)	
ER-α negative/ ER-β negative	0	

[a]Numbers in parentheses represent percentage. [b]p<0.05 when compared, by Fisher exact test, between ER-α-positive and ER-α-negative tumors.

Clinicopathologic characteristics of our patient population according to ER-α, ER-β and GLUT-1 expression are shown in Table 2.

Statistical analysis revealed no significant associations between GLUT-1, ER-α and ER-β expression and patient age, tumor size, histological type, grade and lymph node status (Tab. 3).

Discussion

Our results provide evidence that invasive human breast cancer expresses immunohistochemically detectable GLUT-1 in 37 out of 69 cases and its expression is statistically correlated with ER-α status. Our findings are not strikingly different from the results reported by others. In a very recent study by BROWN et al [4] it is shown that 61% of breast cancers are GLUT-1 positive, which is similar to our results where 53.6% of tumors immunostained positively for GLUT-1. The percentage of GLUT-1 positive breast cancer cells (30%) found in study by AVRIL et al [1] was, however, lower and the authors observed a wide inhomogeneity of positive staining among tumors.

The discrepancy between findings may be due to both: different immunocytochemical procedures used in the above cited studies and different qualification of positive cells. For the purpose of our study we employed immunocytoreactive score be REMMELLE and STEGNER [14] where cases in which 100% of the cells were negative by immunocytochemistry were considered negative. In other above cited studies, the discrimination was based on cut-off 10% or sometimes 20% of immunopositive cells – from which

Table 2. Relationship between GLUT-1, ER-α and ER-β expression and clinicopathological characteristics of breast cancers

	All tumors ER-α-positive	All tumors ER-α-negative	All tumors ER-β-positive	All tumors ER-β-negative	All tumors GLUT-1-positive	All tumors GLUT-1-negative
Age						
<50	19(27.5)[a]	4(5.8)	21(30.4)	2(2.9)	14(20.3)	9(13.1)
≥50	34(49.3)	12(17.4)	43(62.3)	3(4.4)	23(33.3)	23(33.3)
Tumor size						
pT1	38(55.1)	8(11.6)	43(62.3)	3(4.4)	25(36.2)	21(30.4)
pT2	15(21.7)	8 (11.6)	21(30.4)	2(2.9)	12(17.4)	11(16)
Histology						
invasive ductal	42(60.9)	15(21.7)	54(78.3)	3(4.3)	32(46.4)	25(36.3)
invasive lobular	11(15.9)	1(1.5)	10(14.5)	2(2.9)	5(7.2)	7(10.1)
Histological grade						
G1	–	–	–	–	–	–
G2	30(53.6)	7(12.5)	35(62.5)	2(3.6)	19(33.9)	18(32.1)
G3	12(21.4)	7(12.5)	18(32.1)	1(1.8)	12(21.4)	7(12.5)
Lymph node metastasis						
negative (0)	27(39.1)	10(14.5)	34(49.3)	3(4.3)	20(29)	17(24.6)
positive (1)	26(37.7)	6(8.7)	30(43.5)	2(2.9)	17(24.6)	15(21.8)

[a]Numbers in parentheses represent percentage data.

Table 3. Correlation of various clinicopathologic parameters with expression status of ER-α, ER-β and GLUT-1

	All tumors p-values ER-α	All tumors p-values ER-β[a]	All tumors p-values GLUT-1
Age (50> or 50≤)	0.42	1.0	0.393
Tumor size (pT1 or pT2)	0.107	1.0	0.864
Histological type of tumor (ductal or lobular)	0.18	0.206	0.361
Histological grade (1, 2, or 3)	0.142	1.0	0.4
Lymph node status (positive or negative)	0.649	1.0	0.555

[a]p-value of Fisher exact test; The remaining p values – test of independence χ^2

level the tumors are considered positive. Intensity of staining was also excluded from determination procedure. In order to study the correlation between the expression level, method that includes both numbers of positive cells as well as intensity of staining, expressed as immunoreactive score, seems to be more appropriate.

The explanation of the absence of immunohistochemically detected GLUT-1 in 32 out of 69 cases is not difficult. One of the reasons might be owing to the inability of immunocytochemistry to detect low levels of GLUT-1. The other plausible explanation is that in those cases glucose uptake is mediated by other than GLUT-1 glucose transporters, such as GLUT-5 which was found to be specifically expressed in breast cancer [20].

In our study we found no significant correlation between GLUT-1 expression and tumor size, tumor grade and lymph node status. Recent study by KANG et al [9] performed on 100 cases of ductal invasive breast cancer showed that the positive rate of GLUT-1 expression was affected by nuclear grade and ER but not by patient age, tumor size and lymph node status. In study by YOUNES et al [18] it has been found that a positive correlation exists between GLUT-1 expression and nuclear grade but not between ER status and other clinicopathologic features.

Different results obtained in the studies by KANG et al [9], YOUNES et al [18] and AVRIL et al [1] as to the correlation of GLUT-1 with ER status, may be owing to different methodologies used in their studies and lack of discrimination between ER-α and ER-β. Although GLUT-1 expression was significantly correlated with immunocytochemically detected ER in the study by KANG et al [9], the study by YOUNES et al [18] failed to show this correlation. The assay the authors used was based on dextran charcoal system with sucrose gradient centrifugation method on frozen breast cancer tissue [18]. We believe that this method could yield slightly but efficiently different results as compared to immunocytochemistry.

Since statistically significant correlation was found between ER-α and GLUT-1 status, which was not observed with ER-β, we therefore attempted to check, whether the

expression levels, expressed as immunocytoreactive score, of either of ERs correlates GLUT-1.

Although immunoreactive score of the estrogen receptor did not correlate with the intensity of GLUT-1 staining in the study of AVRIL et al [1], they did not discriminate between ER-α and ER-β [1]. In our study we were not able to show, by the use of regression analysis, the relationship between the expression level of GLUT-1 vs. ER-α and GLUT-1 vs. ER-β. We showed, however, by the use of Fisher exact test, that regardless of the expression level (IRS) – the expression status (considering negative versus positive staining) of GLUT-1 is statistically correlated with ER-α (p=0.011). Of 37 GLUT-1 positive cases 24 were ER-α-positive and 13 ER-α-negative. The ER-α-negative group of tumors consists of 16 cases out of total 69 and most of them (13 of 16 i.e. 81.3%) were found to be GLUT-1-positive.

Our recent results [10] showed that specific nitric oxide donor Glucose-2-SNAP, which possibly binds with GLUT receptors [7, 8], exerts cytostatic effect towards estrogen negative breast cancer cells MDA-MB-231. We can therefore hypothetically consider the use of gluco-S-nitrosothiols in the treatment of breast cancer, especially in ER negative tumors which would otherwise be excluded from hormonal therapy.

Our findings are also consistent with the widely accepted theory, which states that the malignancy of tumors correlates with the metabolic rate of tumors and loss of hormone-dependence. It is feasible to assume that in cases in which the expression of estrogen receptor α is lost, breast cancer cells start to over-express GLUT-1 which could facilitate their survival and possibly enhance progressiveness.

In summary, our data demonstrate that GLUT-1 is relatively widely expressed in primary breast cancer. The pattern of GLUT-1 staining shows membrane localization in both ductal and lobular invasive carcinomas. A significant correlation between GLUT-1 and ER-α, found in our study, might possibly point to a role of estrogens in the regulation of GLUT-1 expression in breast cancer tissue.

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