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Clinical Significance of Glucose Transporter 1 (GLUT1) Expression in Human Breast Carcinoma

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Glucose uptake and glycolytic metabolism are enhanced in cancer cells compared to normal cells and tissues. Increased expression of glucose transporter 1 (GLUT1) has been reported in human malignant cells. The aim of this study is to determine the expression of the facilitative glucose transporter protein GLUT1 in human breast carcinomas and a possible correlation between GLUT1 expression and clinical outcome including disease-free or overall survival. One hundred consecutive formalin-fixed, paraffin-embedded sections of invasive breast carcinomas were evaluated by means of immunohistochemical staining of GLUT1. Forty-seven (47%) of 100 breast carcinomas showed positive staining for GLUT1. Expression of GLUT1 correlated significantly with nuclear grade ($P<0.001$), estrogen receptor status ($P=0.002$), and progesterone receptor status ($P=0.001$). The mean disease-free survival periods of GLUT1-positive and -negative patients were 47±2.4 months and 54.3±1.3 months, respectively ($P=0.017$). The mean overall survival periods of GLUT1-positive and -negative patients were 48.7±2.2 and 56.1±1.3 months, respectively ($P=0.043$). In the multivariate analysis, disease-free survival correlated significantly with GLUT1, tumor size, and lymph node involvement ($P=0.043$, $P=0.014$, and $P=0.045$, respectively). In analysis of overall survival, however, lymph node involvement, tumor size, and nuclear grade were statistically significant ($P=0.024$, $P=0.023$, and $P=0.003$, respectively). Our data suggest that absence of GLUT1 expression significantly increases disease-free survival. These findings demonstrate that GLUT1 expression in breast carcinoma can be a marker of aggressive biological behavior and identifies a worse prognosis in breast carcinoma patients.

Key words: Breast carcinoma — GLUT1 — Immunohistochemistry — Prognosis

It has been recognized for many years that cancer cells exhibit enhanced rates of glycolysis compared with those of normal cells.[1)] Several studies suggest that glucose enters cells via a family of seven different facilitative glucose transporter (GLUT) isoforms (GLUT1–GLUT7), and among them, GLUT1 is known as a basic, high-affinity glucose transporter.[2)] GLUT1 is widely distributed in both fetal and adult tissues and its expression appears to be altered in human breast carcinoma.[3)] Grover-McKay *et al.* demonstrated that cell-surface GLUT1 expression was associated with the invasive ability of MCF-7, MDA-MB-435, and MDA-MB-231 human breast cancer cell lines.[4)] Elevated levels of GLUT1 mRNA and protein have been reported in carcinomas of the colorectum,[5–8)] thyroid,[9)] lung,[10, 11)] stomach,[12)] head and neck,[13)] urinary bladder,[14, 15)] kidney,[16)] and endometrium.[17)] The expression of GLUT1 would thus appear to be a potential marker for malignant transformation, and the degree of GLUT1 expression might correlate with biologic behavior, including clinical outcome.

The aim of this study is to determine whether the facilitative glucose transporter protein GLUT1 expression is increased in human breast carcinomas, and whether it is correlated with known prognostic markers such as axillary lymph node metastasis, tumor size, nuclear grade, age, estrogen receptor (ER), progesterone receptor (PR), C-erbB-2, and p53, and with the clinical outcome, including disease-free or overall survival.

MATERIALS AND METHODS

Patients and methods A total of 100 cases of invasive ductal carcinoma, excluding extensive intraductal component (EIC)-positive cancers and special types such as papillary, medullary, or tubular carcinoma of the breast, were analyzed in this study. All the patients underwent modified radical mastectomy or breast-conserving surgery with axillary lymph node dissection at Sungkyunkwan University Samsung Cheil Hospital in Seoul, Korea from January

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1996 to July 1997. These patients were followed up for a mean period of 57.4 months, ranging from 49 to 67 months.

Adjuvant systemic therapy such as anticancer chemotherapy or tamoxifen was prescribed according to the risk factors of the patients. The mean age was 48.3 years (range, 23–74 years). The mean tumor size was 2.4 cm (range, 1–5 cm). ER status, PR status, C-erbB-2, and p53 were available in all patients and were analyzed by immunohistochemical staining. All the histological slides were reviewed and reclassified into nuclear grades using 'Reversed Black Nuclear Grade.'[18)]

Immunohistochemistry staining for GLUT1 expression Sections of formalin-fixed, paraffin-embedded breast cancer specimens cut at 5 μm were prepared on polysine-coated slides.

Immunohistochemical staining was performed using the streptoavidin-biotin immunoperoxidase method, according to the supplier's protocol (DAKO, LSAB kit, Carpinteria, CA). In brief, paraffin-embedded sections were deparaffinized in xylene and rehydrated with graded ethanol. After quenching of endogenous peroxidase activity in 0.3% hydrogen peroxide for 30 min and treatment with blocking reagent for 30 min, primary polyclonal rabbit anti-human GLUT1 (DAKO) was applied to the sections at a dilution of 1:200 and the sections were incubated in a moist chamber for 2 h at room temperature. The excess complex was washed out, and the localization of antibodies was visualized by incubating the section for 10 min with 3,3′-diaminobenzidine tetrahydrochloride (Research Genetics, Huntsville, AL). Positive control for GLUT1 was red blood cells present in each section. In the negative control, normal serum was substituted for the primary antibody.

GLUT1 immunostaining was quantified by grading the proportion of cells that were GLUT-positive. The grading system was as follows: absence of immunoreactive cells =negative; less than 10% of immunoreactive cells=1+; 10% to 50% of immunoreactive tumor cells=2+; and greater than 50% of immunoreactive cells=3+ (Fig. 1). For statistical analyses, each tissue section was classified as GLUT1-positive or -negative.

Immunohistochemical staining for ER, PR, C-erbB-2, and p53 Tissue preparation was done conventionally in the same way as for GLUT1, except for antigen retrieval.

The sections were autoclaved in 10 m*M* citrate buffer at 121°C for 20 min for antigen retrieval. Immunohistochemical staining was performed using monoclonal mouse anti-

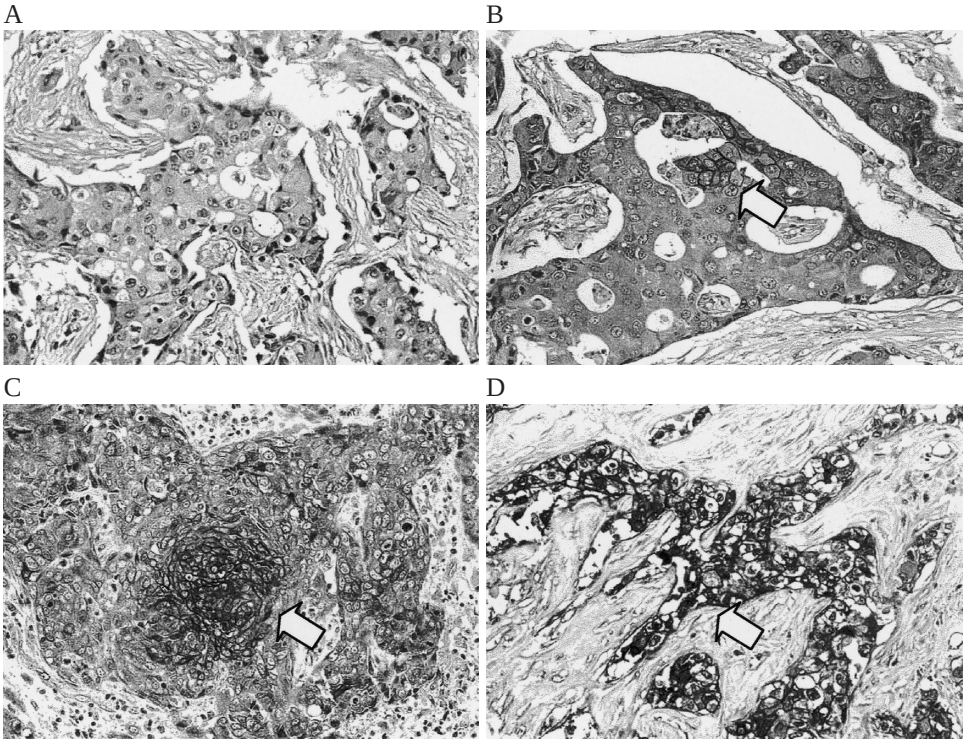

Fig. 1. Immunohistochemical staining for GLUT1 protein in invasive ductal carcinoma of breast (×200). A, absence of immunoreactive cells=negative; B, less than 10% of immunoreactive cells=1+; C, 10% to 50% of immunoreactive tumor cells=2+; and D, greater than 50% of immunoreactive cells=3+. Arrows indicate GLUT1 positive area.

(human Ig) antibodies directed against ER (DAKO), PR (DAKO), and p53 (DAKO). Immunohistochemistry for C-erbB-2 was done with polyclonal rabbit anti-(human Ig) antibody. The primary antibodies for ER, PR, C-erbB-2, and p53 proteins were retrieved for 1 h at 1:100, 1:50, 1:150, and 1:100, respectively.

For ER, PR, and p53, nuclear immunostaining was examined. The cases showing more than 5% of nuclear immunostaining were classified as positive. For C-erbB-2, membranous immunostaining was counted. The cases stained diffusely and strongly along the cytoplasmic membrane qualified as positive.

Statistical analysis The expression of GLUT1 was compared with clinico-pathological features and known prognostic parameters including age, tumor size, lymph node metastasis, ER, PR, C-erbB-2, p53, and nuclear grade. All statistical tests were performed using the SPSS 10.0 data analysis program. Data were analyzed using the χ^2 test and Fisher's exact test to compare the expression of GLUT1 protein with age, tumor size, lymph node metastasis, ER, PR, C-erbB-2, p53, and nuclear grade. Disease-free and overall survival data were analyzed by using the Kaplan-Meier method and were assessed by log-rank test to compare differences in survival between the patients whose tumor expressed positive GLUT1 staining and those whose tumor did not express GLUT1 protein. By using the proportional hazards model of Cox, multivariate analyses were performed on GLUT1 protein expression, age, lymph node metastasis, tumor size, and nuclear grade in the patients with breast carcinomas. $P<0.05$ was regarded as statistically significant.

RESULTS

A total of 100 paraffin-embedded breast carcinoma tissue sections containing normal tissues were examined using GLUT1 immunohistochemical staining (Fig. 1). Normal breast tissue or hyperplastic ducts did not show GLUT1 protein expression (data not shown). Forty-seven (47%) of 100 breast carcinomas showed positive GLUT1 expression with heterogeneous intensity: 1+, 29 cases (61.7%); 2+, 9 cases (19.1%); and 3+, 9 cases (19.1%) (Table I). GLUT1 staining was usually recognized in the cell membrane of cancer cells. In positively stained lesions, the staining was strongly positive in the center of the necrotic areas and infiltrative areas of the carcinoma.

Table I. Glucose Transporter GLUT1 Expression in Breast Carcinoma Tissues

Expression of GLUT1	Number of patients (%)
Positive	47
1+	29 (61.7)
2+	9 (19.1)
3+	9 (19.1)
Negative	53
Total	100

Table II shows the correlations between GLUT1 expression and several clinico-pathological prognostic parameters. The incidence of GLUT1 expression correlated significantly with nuclear grade ($P<0.001$), ER ($P=0.002$), and PR ($P=0.001$). The incidence of GLUT1 expression in lesions with higher nuclear grade, ER-negative (61.1%), and PR-negative (63.3%) was higher than in those with lower nuclear grade, ER-positive (30.4%), and PR-positive (31.4%). No significant difference was found in age, tumor size, axillary lymph node metastasis, C-erbB-2, or p53 overexpression.

When assessed by log-rank test, the disease-free survival rate for patients with positive GLUT1 expression was significantly lower than that for patients without GLUT1 expression ($P=0.017$). The mean disease-free survival periods of GLUT1-positive and -negative patients

Table II. Expression of GLUT1 and Clinico-pathological Prognostic Parameters

Clinico-pathological parameters	No. of cases	No. of GLUT1 positive (%)	*P*-value
All	100	47 (47)	
Age (yr.), $n=100$			0.35
<50	56	24 (42.9)	
≥50	44	23 (52.3)	
Tumor size, $n=99$			>0.1
<2 cm	37	12 (32.4)	
≥2 cm	62	35 (56.5)	
Axillary lymph node metastasis, $n=100$			0.86
Positive	47	23 (48.9)	
Negative	53	24 (45.3)	
Nuclear grade, $n=100$			<0.001
1	7	0 (0)	
2	38	12 (31.6)	
3	55	35 (63.6)	
ER, $n=100$			0.002
Positive	46	14 (30.4)	
Negative	54	33 (61.1)	
PR, $n=100$			0.001
Positive	51	16 (31.4)	
Negative	49	31 (63.3)	
C-erbB-2, $n=100$			0.34
Positive	61	31 (50.8)	
Negative	39	16 (41)	
p53, $n=100$			0.27
Positive	41	22 (53.7)	
Negative	59	25 (42.4)	

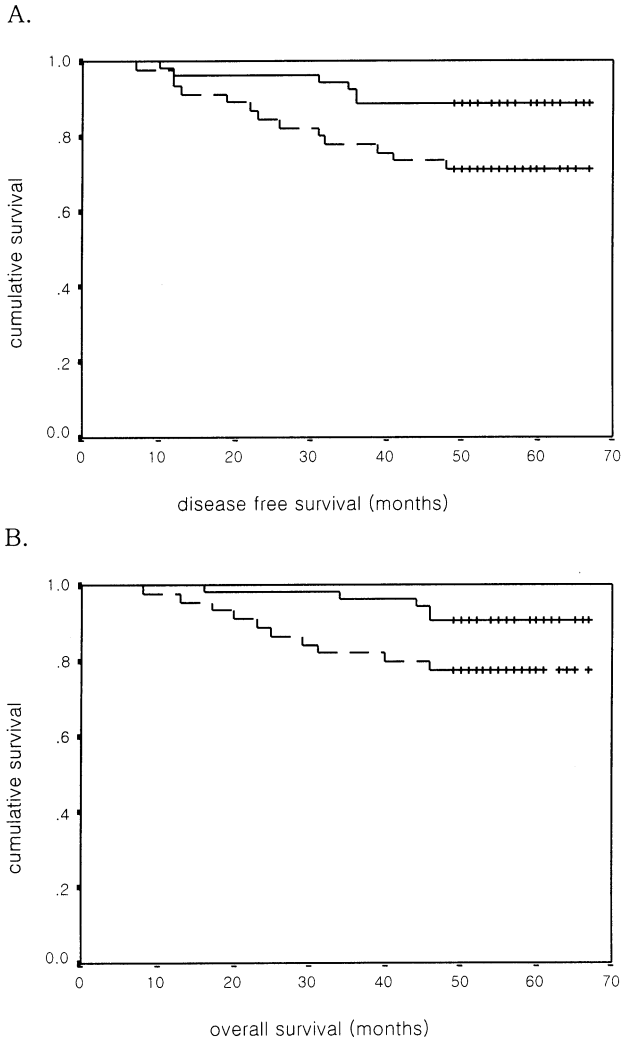

Fig. 2. Kaplan-Meier survival curves for disease-free and overall survival of patients stratified with positive or negative GLUT1 expression by immunohistochemical staining in breast carcinomas. A, P=0.017. —— GLUT1(–), ----- GLUT1 (+); B, P=0.043. —— GLUT1 (–), ----- GLUT1 (+).

were 47±2.4 months and 54.3±1.3 months, respectively (Fig. 2A).

The overall survival rate for patients whose lesions showed GLUT1 expression was significantly lower than that for patients without GLUT1 expression (P=0.043). The mean overall survival periods of GLUT1-positive and -negative patients were 48.7±2.2 and 56.1±1.3 months, respectively (Fig. 2B).

The disease-free and overall survival rate were significantly different in relation to lymph node involvement and tumor size. The statistical significance of disease-free survival stratified with lymph node involvement was P=0.038, and that with tumor size was P=0.039, while the statistical significance of overall survival stratified with lymph node involvement was P=0.047, and that with tumor size was P=0.045.

Table III. Correlations of Various Factors with Disease-free and Overall Survival, as Determined by Cox Proportional Hazard Model

	Disease-free survival (P-value)	Overall survival (P-value)
GLUT1[a)] (positive or negative)	0.043	NS[b)] (0.089)
Age (50> or 50≤)	NS (0.879)	NS (0.738)
Lymph node metastasis (positive or negative)	0.045	0.024
Tumor size (2 cm> or 2 cm≤)	0.014	0.023
Nuclear grade[c)] (1, 2, or 3)	NS (0.055)	0.003

a) GLUT1: glucose transporter 1.
b) NS: not specific.
c) Nuclear grade: Reversed Black Nuclear Grade.[18)]

As shown in Table III, in the multivariate analysis using the Cox proportional hazards model, disease-free survival correlated significantly with GLUT1, tumor size, and lymph node involvement (P=0.043, P=0.014, and P=0.045, respectively). In the analysis for overall survival, however, lymph node involvement, tumor size, and nuclear grade were statistically significant (P=0.024, P=0.023, and P=0.003, respectively). There was a trend for negative GLUT1 expression to favor overall survival. Overall survival periods for the patients with negative GLUT1 expression were longer than for the patients with positive GLUT1 expression, but the difference was not statistically significant (P=0.089).

DISCUSSION

Malignant cells show enhanced glucose uptake and utilization of glycolysis when compared to normal cells. It has been suggested that this process is mediated, at least in part, by members of the facilitative glucose transporter proteins (GLUT) family, of which GLUT1 is believed to be the most widely distributed and to have no tissue specificity.[19)] The expression and activity of facilitative glucose transporters are regulated by various types of oncogene and growth factors.[20–22)]

We evaluated GLUT1 protein overexpression by immunohistochemical staining in 100 tissue sections of invasive breast carcinomas containing normal mammary tissue. None of the normal breast tissue or hyperplastic ducts showed overexpression of this GLUT1 protein in our

study (data not shown). The expression of GLUT1 protein in normal breast tissue is controversial. As in our study, Younes *et al.*[23] failed to detect it in normal breast tissue. In contrast, both Zamora-Leon *et al.*[3] and Brown and Wahl[24] suggested the presence of GLUT1 in normal breast tissue. In a recent study, Alo *et al.*[25] reported that GLUT1 analyzed using purified monoclonal mouse antibody specific to GLUT1 was expressed in 36% of typical/atypical hyperplastic adjacent tissues and in 31% of adjacent normal tissues. However, specimens from 10 patients surgically treated for fibrocystic disease and who did not have cancer expressed no GLUT1 immunostaining. A possible explanation for this disagreement may be that GLUT1 is expressed during the late phase of the menstrual cycle when breast tissue is undergoing rapid growth, and the various study methodologies have different sensitivity.[2] In the present study, 47 (47%) of 100 breast carcinomas expressed GLUT1 protein, as evaluated by immunohistochemical staining.

There has been controversy regarding the expression of GLUT1 in breast carcinoma tissue, and this may reflect the variations in the procedures used to detect GLUT1 protein by each group. Younes *et al.*[23] and Binder *et al.*[26] reported that GLUT1 was expressed in 49 (42%) out of 118 and 17 (57%) out of 30 human breast carcinomas, respectively.

Fifty-three breast carcinomas did not show any positive staining pattern for GLUT1 protein in our study. There are two possible reasons for this. One is that glucose uptake in some breast carcinomas is mediated by glucose transporters other than GLUT1. Zamora-Leon *et al.*[3] demonstrated that the breast cancer cell lines MCF-7 and MDA-468 express the glucose transporters GLUT1 and GLUT2 and also found that the breast cancer cell lines transport fructose and express the fructose transporter GLUT5. The other possible explanation is that low levels of GLUT1 are not detected by immunohistochcemical staining. Even the presence of a specific mRNA in certain tissues does not always correlate with the expression of the corresponding protein. The cause of negative staining may be that GLUT1 was expressed in these tissues at a level too low to be detected by immunohistochemistry.

In this study, the positive rate of GLUT1 expression was affected by nuclear grade, ER, or PR. No significant correlation, however, was found with age, tumor size, axillary lymph node involvement, C-erbB-2, or p53 overexpression.

Younes *et al.*[23] found a positive correlation between GLUT1 expression and nuclear grade. In contrast, GLUT1 immunostaining did not correlate with ER status, tumor size, or lymph node involvement. This contradictory result regarding ER status could be explained by the different techniques used. They assayed ER content using the dextran charcoal assay (DCCA) with sucrose gradient centrifugation method on frozen breast cancer tissues.

Although some studies suggest that GLUT1 expression correlates with poor prognosis in carcinomas of colorectum,[6, 8] lung,[10] and urinary bladder,[15] it was not established whether there is any correlation between GLUT1 expression and the prognosis of breast carcinoma. Our data clearly indicate an influence of GLUT1 expression on disease-free and overall survival. Furthermore, we found that the correlation of GLUT1 and the prognosis of breast carcinoma was independent of lymph node status and tumor size.

Multivariate analysis also demonstrated that positive staining of GLUT1 protein correlated with disease-free survival. Although patients with positive GLUT1 expression had poorer overall survival in multivariate analysis, this was not statistically significant.

Our data demonstrate that GLUT1 expression correlates significantly with negative ER, negative PR, and higher nuclear grade, and may play a useful role as a predictor for poor prognosis in breast carcinoma patients. Breast carcinomas with positive GLUT1 expression exhibited more aggressive behavior and more malignant potential than those with negative GLUT1 expression.

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