Glucose Transporter 1 (GLUT1) Expression is Associated with Intestinal Type of Gastric Carcinoma

Increased expression of glucose transporter1 (GLUT1) has been reported in many human cancers. We hypothesized that the degree of GLUT1 might provide a useful biological information in gastric adenocarcinoma. RT-PCR and immuno-staining were used to analyze GLUT1 expression in gastric cancer. RT-PCR showed GLUT1 expression was not largely detected in normal gastric tissue but was detected in cancerous gastric tissue of counterpart. By immunohisto-chemistry, GLUT1 protein was absent in normal gastric epithelium and intestinal metaplasia. 11 of 65 patients with gastric adenocarcinoma had specific GLUT1 immunostaining in a plasma membrane pattern with varied intensities. GLUT1 protein did not show any significant correlation with tumor stage and nodal metastasis ($p>0.05$ by Mann-Whitney test). However, the positive immunostaining for GLUT1 is associated with intestinal differentiation ($p=0.003$). Our results suggest that GLUT1 protein is associated with intestinal type of gastric cancer.

Key Words : Monosaccharide Transport Proteins; Stomach Neoplasms; Prognosis

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INTRODUCTION

Enhancement of glucose utilization, especially increase of glycolytic (anaerobic) metabolism is a widespread characteristic of malignant cells (1). The resulting large increase in glucose requirement implies a need for a corresponding glucose transport across the plasma membrane. Understanding of the distribution and function of these glucose transporters in carcinoma may lead to the development of new diagnostic and prognostic markers.

Two classes of glucose transporter have been described in mammalian cells (2): the Na^+-glucose cotransporters and facilitative glucose transporters. Facilitative glucose transport across the plasma membrane is mediated by a family of homologous protein, GLUT1-GLUT5 and GLUT7, that differ in tissue specific expression, transport kinetics, substrate specificity and in response to metabolic and hormonal regulation. GLUT6 is a pseudogene, which is not translated (2). Among them, glucose transporter1 (GLUT1), which in most normal tissue is restricted to erythrocytes and blood-tissue barriers (3), appears to be expressed aberrantly in many cancers. Increased expression of GLUT1 has also been demonstrated in gastro-intestinal carcinoma (4, 5), breast carcinoma (6), squamous cell carcinoma of the head and neck (7), renal cell carcinoma (8) and hepatoma (9). It was also observed that transformation of cultured cells with ras or src onco-genes rapidly induced GLUT1 mRNA (10). Increased GLUT1 expression, which means an increased utilization of energy, may correlate with aggressive behavior in many human cancers. Although GLUT1 has been observed in gastric carcinoma, few studies has been reported pathologic correlations in gastric carcinoma.

In this study we detected GLUT1 expression in gastric adenocarcinoma and investigated whether GLUT1 protein is associated with pathological parameters of gastric adenocarcinoma.

MATERIAL AND METHOD

Clinical material

Sixty-five patients (age range 26-79; average 56 years) with primary adenocarcinoma of the stomach were studied. Tumor tissues were taken either from subtotal or total gastrectomized specimens at Hanyang University Hospital. The tumors were assessed by depth of invasion, Lauren's histologic classification (11) and lymph nodal metastasis.

Reverse transcription polymerase chain reaction

Total cellular RNA was extracted by Chomczynski and

Sacchi method (12). Reverse transcription was performed using the total RNA extracted in a reverse transcription mixture containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 10 mM DTT, 3 mM MgCl₂, 0.5 mM dNTPs, 200 units M-MLV reverse transcriptase (Life Technologies, Gaithersburg, MD, U.S.A.), 2 units/μL RNase inhibitor (TaKaRa, Shiga, Japan) and 2.5 μM oligo dT (Life Technologies, Gaithersburg, MD, U.S.A.) in a total volume of 20 μL at 37℃ for 60 min. One μL of the first strand of cDNA was used as a template for PCR reaction with specific primers for GLUT1. The oligonucleotides used as primers were: sense 5'-TGTCCTATCTGAGCATCG-TG-3' and antisense 5'-CTCCTCGGGTGTCTTATCA-C-3'. All of the primers were purchased from Life Technologies. Each reaction mixture consisted of 1 μL of cDNA, 0.5 μM of each primer, 1 μL of 10×Ex *Taq* buffer, 0.8 μL of dNTP mix, 0.5 units of Ex *Taq* polymerase, and nuclease free water to a final volume of 10 μL. The 10×Ex *Taq* buffer, dNTP mix and Ex *Taq* polymerase were obtained from Perkin-Elmer cetus (Emeryville, CA, U.S.A.). The reaction mixture was subjected to 35 cycles of denaturation (94℃, 1 min), annealing (55℃, 1 min) and extension (55℃, 1 min). After PCR amplification, the reaction mixture was run on 1% agarose gel in Tris-HCl/boric acid/EDTA (TBE) buffer. The gel was then stained with ethidium bromide.

Immunohistochemical analysis

Tissue sections were prepared from formalin fixed, paraffin-embedded archival specimens of gastric carcinoma obtained at surgery. Normal tissues in this study included grossly and microscopically unremarkable tissues removed during operations for malignant tumors involving the same organs. Anti-GLUT1 antibody was kindly provided by Dr. Wan Lee (State University of New York, Buffalo, NY, U.S.A.) (13). The avidin biotin complex method was used for immnunohistochemical staining. All the staining procedures were carried out at room temperature. Five-micrometer sections were cut from paraffin-embedded tissue blocks and hydrated. After exposure to a mixture of hydrogen peroxide and methanol, which is for the inhibition of endogenous peroxide activity, the sections were treated with normal goat serum to block nonspecific protein binding. The sections were incubated for 2 hr in a 1:400 dilution of GLUT1 antibody. Then, the biotinylated goat anti-rabbit IgG was applied to the sections, and the sections were incubated for 30 min. They were then further incubated for 30 min with avidin-biotin complex (DACO, Carpinteria, CA, U.S.A.). The final complex formed was visualised by exposure of the sections to 0.5 mg/mL 3,3-diaminobenzidine (DAB) and 0.005% hydrogen peroxide. After counterstaining

with hematoxylin, the sections were dehydrated and mounted. We used the erythrocytes in the tissue sections for the positive control of GLUT1 expression. Staining was considered to be positive only when strong membrane associated immunoreactivity was observed. Negative control sections were obtained by incubating with phosphated buffered saline instead of the same concentration of GLUT1 antibody. The sections were consistently negative.

Statistical analysis

Contingency tables were used to assess stage, nodal metastasis and Lauren's classification significance for GLUT1 expressions. Statistical analysis was performed using the SPSS 7.5 software package (SPSS Inc., Chicago, IL, U.S.A.). The proportions were compared using Mann-Whitney test to determine the significance of these associations.

RESULTS

We analyzed GLUT1 expression in gastric adenocarcinoma using RT-PCR and immunostaining. RT-PCR analysis showed that GLUT1 was not largely expressed in 3 normal gastric tissues but was expressed in cancerous tissue (Fig. 1). All the cases studied by RT-PCR are moderately differentiated tubular adenocarcinoma in histologic examination.

Immunostaining of GLUT1 was localized on cell membrane. There was nonspecific granular cytoplasmic staining, mainly localized in Golgi area and also occasionally present in the adjacent normal mucosal and intestinal metaplastic epithelium. RBCs in tissue sections were always positive and served as an internal positive control (Fig. 2). Positive staining for GLUT1 was detected in 11 of 65 tumors (16.9%), which could be readily divided into groups showing weak and strong (Fig. 3). Strong staining was observed in 2 tumors (18.2%) and weak
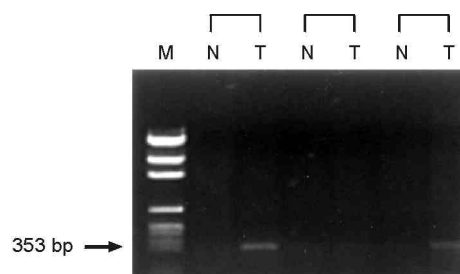

Fig. 1. Expression of GLUT1 in gastric adenocarcinoma. The expression of GLUT1 in cancer gastric tissue (T) and normal counterpart (N) was determined using RT-PCR. The lane marked M is 1 kb ladder marker.
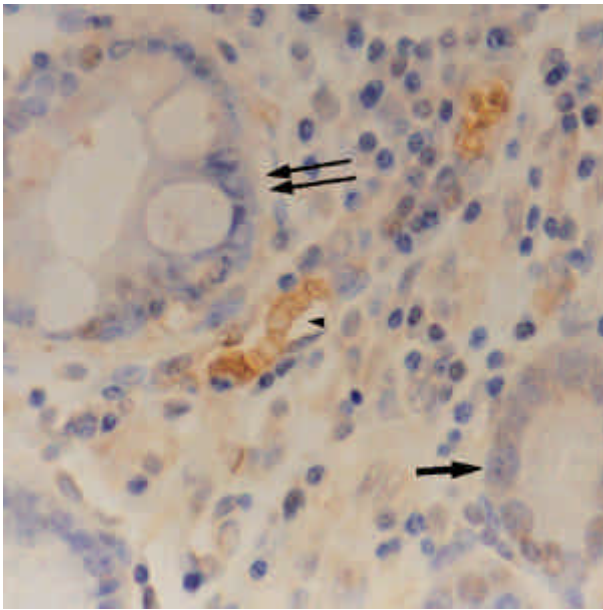
Fig. 2. Immunohistochemical finding of GLUT1 in normal and intestinal metaplastic gastric epithelium. No specific membranous staining is present in normal (arrow) and metaplastic epithelium (double arrow). RBCs show consistently strong positivity (arrow head) (×400).

Fig. 4. Immunohistochemical finding of GLUT1 in diffuse type of gastric adenocarcinoma. The tumor cells show positive membranous staining (arrow) (×400).
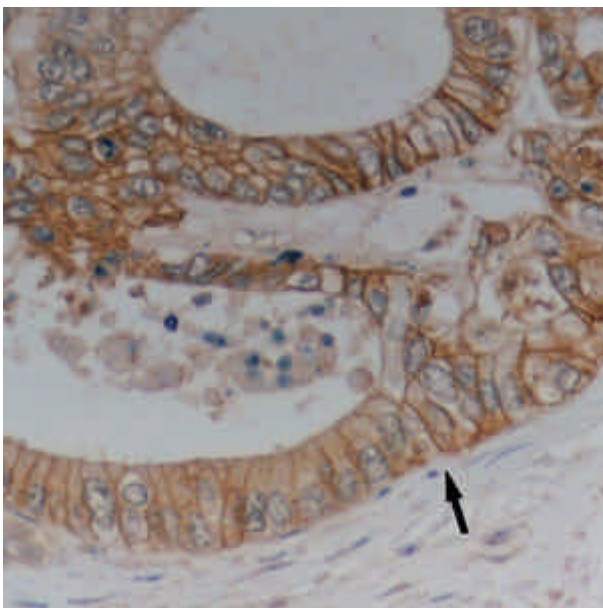

Fig. 3. Immunohistochemical staining for GLUT1 in intestinal type of gastric adenocarcinoma. Many tumor cells are positive for GLUT1 with intense, membranous staining (arrow) (×400).

staining was observed in 9 tumors (81.8%). Among the positive staining, diffuse or signet ring cell type of carcinoma showed weak positivity in only 2 of 38 cases (5.3%) (Fig. 4). Intestinal types of carcinoma were stained positive in 9 of 27 cases (33.3%). Analysis of GLUT1 staining in relation to the pathological charac-
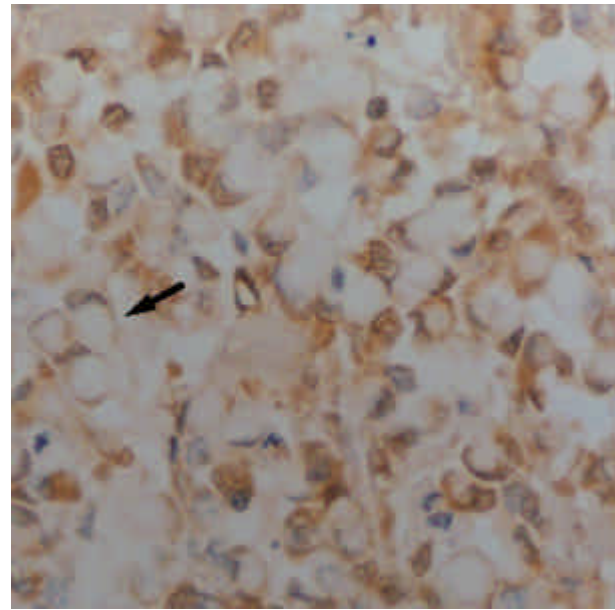
teristics of the patient population is shown in Table 1. Positive staining for GLUT1 was not associated with depth of invasion and lymph node metastasis. Our results suggests that GLUT1 expression in gastric adenocarcinoma is associated with intestinal type of gastric carcinoma ($p=0.003$).

DISCUSSION

It has become increasingly apparent that activation of *GLUT* gene expression for enhanced uptake and metabolism of glucose is a molecular feature of the malignant phenotype in a variety of cancers. The apparent overexpression of GLUTs suggests an important role for these transporters in tumor biology. Increased GLUT1 expression has been demonstrated in colorectal carcinoma (14), lung carcinoma (14), breast carcinoma (6), gastric carcinoma (4, 5), squamous cell carcinoma of head and neck (7), renal cell carcinoma (8), and hepatoma (9). It has been also reported that GLUT1 expression correlates with neoplastic progression, poor prognosis and tumor aggressiveness in many cancers (5, 6, 14, 15). For gastric carcinoma, Yamamoto et al. (4) showed that the level of GLUT1 mRNA in tumor was higher than normal gastric tissue. Noguchi et al. (5) reported that GLUT1 mRNA was not detectable in normal gastric mucosa but was detected in 95% of gastric carcinoma sample and GLUT1 protein was detected in 19% of tumors by im-

Table 1. Comparison of GLUT1 expression, Lauren's classification, depth of invasion and lymph node metastasis in 65 patients with gastric adenocarcinoma

Pathologic factor	GLUT1−(%)	GLUT1+(%)	Significance
Laurens' classification			p=0.003
Intestinal type	18 (66.7)	9 (33.3)	
Diffuse type	36 (94.7)	2 (5.3)	
Depth of invasion			NS
Mucosa/Submucosa	8 (100.0)	0 (0.0)	
Muscularis propria	4 (80.0)	1 (20.0)	
Subserosa/Serosa	42 (80.8)	10 (19.2)	
Lymph node metastasis			NS
Negative	7 (77.3)	2 (22.2)	
Positive	47 (83.9)	9 (16.1)	
Total	54 (83.1)	11 (16.9)	

NS, not significant ($p>0.05$)

munostaining. They also indicated that the GLUT1 protein was positively correlated with the tumor invasion into the gastric wall, lymphatics or blood vessel and with lymph node metastasis. Based on our results, GLUT1 protein was present in 16.9% of gastric carcinoma and did not show any significant correlations with tumor stage and nodal metastasis. GLUT1 protein, however, is positively associated with intestinal type of gastric carcinoma ($p<0.05$). We therefore suggest the positive correlation between GLUT1 expression and the intestinal type of gastric carcinoma. We did not detect GLUT1 protein in all samples that were positive for GLUT1 mRNA in RT-PCR. It may be inherent limitation of immunohistochemistry or the presence of a given mRNA in certain tissues does not always correlate with expression of the corresponding protein.

Stomach cancer is histologically classified into the well-differentiated or intestinal type and the poorly differentiated or diffuse type (11). Interestingly, the scenario of multiple gene changes in stomach cancer differs depending on the two histologic types, indicating that the two types of stomach cancer may have different genetic pathways (16). Werner et al. (17) have shown that GLUT1 mRNA levels in rat heart and liver increases at the embryonic stage, but they markedly decreases at the postnatal stage. Since GLUT1 have been demonstrated to be closely related with tissue development, the over-expression of GLUT1 in the present study might contribute to differentiation of intestinal type in gastric cancer. Although the precise mechanism still remains to be determined, the present results suggest that the induction of GLUT1 gene expression, which is closely related with tissue development, may force to increase glucose uptake by transformed cells.

Although no significant correlation is present between GLUT1 expression and studied prognostic factors, no GLUT1 staining was detectable in 8 cases of early gastric carcinomas. The mortality of patients with these tumors was much lower, even curable than those whose advanced gastric carcinomas (11 of 57, 19.3%). These data may suggest that tumors with no GLUT1 staining may possible favor prognosis. With this possibility, GLUT1 does not exclude a potential role as prognostic factor. To investigate this possibility, further studies based on large number of cases with complete follow-up data will be needed.

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