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Glut1 expression in T1 and T2 stage colorectal carcinomas: its relationship to clinicopathological features

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Abstract

Glucose uptake is mediated by glucose transporter (Glut) proteins, which exhibit altered expression in a variety of malignant neoplasms. Glut1 expression is thought to be a potential marker for malignant transformation. The aim of the present study was to investigate the expression of Glut1 protein in colorectal adenomas, T1 and T2 stage carcinomas. Immunohistochemical detection of Glut1 protein was examined in 141 formalin-fixed and paraffin-embedded colorectal tumour specimens (57 adenomas, 84 carcinomas). The degree of Glut1 immunostaining of a specimen was graded according to the proportion of Glut1-positive cells in it; absent (positive cells are 0%), weakly positive (less than 10%), moderately positive (10–50%), and strongly positive (more than 50%). Glut1 expression was present in 18% of the adenomas with low-grade dysplasia, and in 63% of the adenomas with high-grade dysplasia. The positivity in such lesions was usually weak, but was moderate in 8% of the adenomas with high grade dysplasia. For the carcinomas, there were significant correlations between Glut1-positivity and depth of invasion (T1 45% versus T2 74%, $P < 0.01$), histological differentiation (well 49% versus moderately to poorly 74%, $P < 0.05$) and morphological type (polypoid 42% versus depressed 73%, $P < 0.05$), if the cut-off value was set at 10% of cells. In conclusion, we clarified the relationship between Glut1 expression and clinicopathological features in T1 and T2 stage colorectal carcinomas, and our results suggested a high malignant potential of the depressed-type carcinoma.

Keywords: Glut1 expression; Immunostaining; Colorectal carcinoma; Depressed-type colorectal cancer

1. Introduction

Previous studies have shown an enhanced glycolytic metabolism in malignant neoplasms [1]. Increased glucose uptake is one of the major metabolic changes found in malignant tissue [2]. This uptake is mediated by glucose transporter (Glut) proteins, which are membrane proteins responsible for the transport of glucose across cellular membranes. These human glucose transporters have a distinct tissue distribution and contribute to the disposal of glucose under various conditions [3]. A family of seven glucose transporters have been cloned. Among these, *Glut*1 is expressed in erythrocytes, the blood–brain barrier, the perineurium of peripheral nerves and the placenta [3–6]. Several studies have demonstrated increased expression of *Glut*1 mRNA and protein in a variety of cancer tissues, indicating that Glut1 may play an important role in glucose uptake by these cancers [7–14]. In addition, these studies suggested that the expression of Glut1 could be useful as a marker for malignant transformation.

Previous reports have noted that Glut1 overexpression in colorectal carcinomas is correlated with lymph node metastases and poor prognosis [7,8]. Moreover, with recent advances in endoscopic instruments and techniques, depressed-type colorectal cancers are increasingly being diagnosed. Kudo and colleagues detected many depressed-type cancers of an aggressive nature [15,16]. However, the biological characteristics of these cancers remain obscure. There have been a few reports concerning the expression of Glut1 protein in

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early-stage colorectal cancer, including the depressed-type. It is also unclear at what stage alteration of Glut1 protein expression occurs during the development of colorectal cancer. Thus, we examined the expression of Glut1 proteins by immunohistochemistry in colorectal adenomas and early-stage carcinomas including the depressed-type, and evaluated the relationship between its expression and clinicopathological features.

2. Patients and methods

2.1. Subjects

The study material consisted of colorectal neoplastic lesions resected either endoscopically or surgically at the Kobe University Hospital from 1993 to 1998. Late stage cancers of the T3 and T4 stage in the TNM classification system [17] were not evaluated. Severely damaged specimens and those unsuitable for histochemical analysis were also excluded. There were 141 qualified lesions from 135 patients, none of whom had a familial history of polyposis. The adenoma cases and carcinoma cases were analysed separately.

2.1.1. Adenoma cases

There were 57 adenomatous lesions. The lesions were divided into two groups; there were 17 (30%) cases of adenoma with low-grade dysplasia (low-grade adenoma) and 40 (70%) cases with high-grade dysplasia (high-grade adenoma).

2.1.2. Carcinoma cases

There were 84 carcinoma specimens; 49 (58%) T1 stage (invading the submucosal layer) tumours and 35 (42%) T2 stage (invading the muscularis propria) tumours. The degree of histological differentiation was well-differentiated in 57 (68%) lesions and moderately to poorly differentiated in 27 (32%) lesions. Among the surgically resected cases, nodal metastases were negative in 59 (70%), and positive in 11 cases (13%). Early-stage colorectal carcinomas can be morphologically classified into three types; polypoid, flat-elevated and depressed-types [18]. The polypoid-type was defined as tumours in which the height of the neoplastic mucosa was more than twice the thickness of the adjacent non-neoplastic mucosa. The flat-elevated type was defined as tumours with a neoplastic mucosa not greater than twice the thickness of the adjacent non-neoplastic mucosa. The depressed-type was defined as tumours in which the height of the neoplastic mucosa was less than that of the adjacent non-neoplastic mucosa. Out of 84 carcinoma cases, there were 31 (37%) polypoid lesions and 30 (36%) depressed lesions. 23 cases (27%) consisted of flat-elevated lesions, and unclassified types in which the intramucosal lesions were absent.

2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue sections were immunostained with anti-Glut1 antibody MYM (DAKO, Carpinteria, CA, USA), a rabbit polyclonal antibody raised against a 12-amino acid synthetic peptide corresponding to the carboxyl terminus of human Glut1.

Tissue sections were deparaffinised in xylene and rehydrated in a graded ethanol series. The sections were washed with phosphate-buffered saline (PBS, pH 7.6), and microwaved in 10 mM citrate buffer (pH 6.0) for 15 min. Deparaffinised sections were incubated with 0.3% hydrogen peroxide for 10 min to block intrinsic peroxidase, and then washed in PBS. The sections were then incubated with bovine serum albumin for 10 min at room temperature to reduce the non-specific immunoreactivity, and washed in PBS. Treated tissue sections were incubated with MYM antibody diluted 1:100 in Tris–HCl buffer containing carrier protein and 15 mM sodium azide, at room temperature for 30 min. After rinsing with PBS, the sections were incubated with peroxidase labelled anti-rabbit immunoglobulin (DAKO Envision System, DAKO) for 30 min. Sections were washed with PBS and coloured with 3,3′-diaminobenzidine (DAB). Finally, the sections were counterstained lightly with haematoxylin, dehydrated and mounted. Adjacent sections incubated with healthy rabbit immunoglobulin G were used as negative controls. Red blood cells present in each section served as positive controls for Glut1.

The degree of Glut1 immunostaining of a specimen was graded according to the proportion of Glut1-positive cells in it; absent (positive cells are 0%), weakly positive (positive cells are less than 10%), moderately positive (positive cells are 10–50%) and strongly positive (positive cells are more than 50%). In the literature, a cut-off value of 50% was analysed, but in this study, the significance of lower cut-off values in early-stage colorectal carcinomas and adenomas was also evaluated.

2.3. Statistical analysis

Differences were analysed for statistical significance using the Chi-square test. A P-value of less than 0.05 was considered significant.

3. Results

3.1. Glut1 expression in colorectal adenomas

In adenomas with low-grade dysplasia, Glut1 expression was absent in 14 cases (82%) and weakly positive in 3 cases (18%). In adenomas with high-grade dysplasia, Glut1 positivity was absent in 15 cases (38%), weak

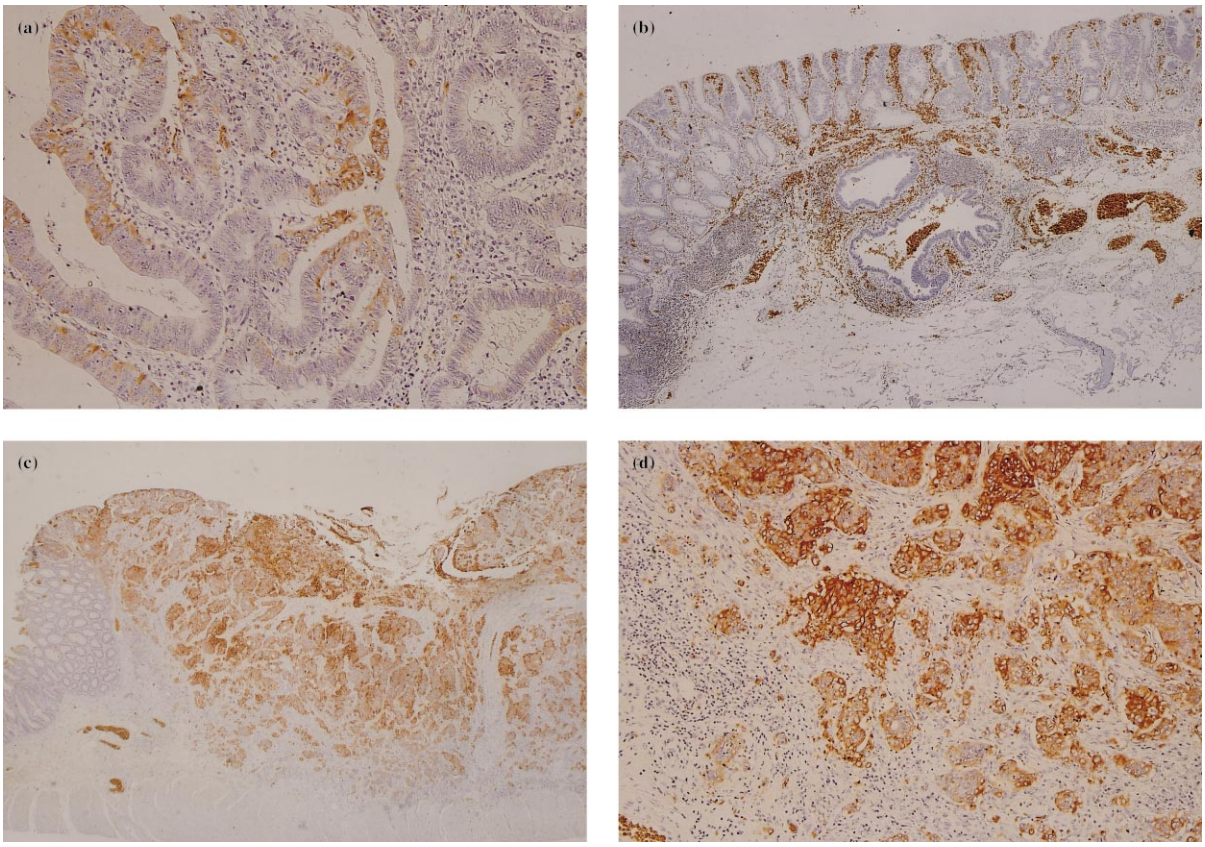

Fig. 1. Glut1 immunostaining in colorectal tumours. (a) Glut1-positive adenoma with high-grade dysplasia (haematoxylin counterstaining, original magnification×10). (b) Glut1-negative depressed-type T1 carcinoma. Erythrocytes were always positive and served as an internal positive control (haematoxylin counterstaining, original magnification ×4). (c) Glut1-positive depressed-type T2 carcinoma with Glut1-negative normal mucosa (haematoxylin counterstaining, original magnification ×2). (d) Higher magnification of the invasive area in the same lesion as shown in (c). Note membranous and partially cytoplasmic staining of carcinoma cells (haematoxylin counterstaining, original magnification ×10).

in 22 cases (55%) and moderate in 3 cases (8%) (Fig. 1a). The positive rate of the Glut1 expression was significantly different ($P < 0.01$) between the low-grade adenomas and high-grade adenomas (18% versus 63%).

3.2. Glut1 expression in colorectal carcinomas

The basic characteristics of the carcinoma cases are shown in Table 1.

3.3. Relationship between Glut1 expression and depth of invasion

In T1 carcinomas, Glut1 expression was absent in 19 cases (39%), weak in 8 cases (16%), moderate in 14 cases (29%) and strong in 8 cases (16%). In T2 lesions, Glut1 expression was absent in 1 case (3%), weak in 8 cases (23%), moderate in 18 cases (51%) and strong in 8 cases (23%) (Fig. 1b–d). Glut1 expression was significantly different ($P < 0.01$) between the T1 and T2 groups (positivity of 61% versus 97%). The rate of moderate and strong Glut1 expression was also significantly different ($P < 0.01$) between these two groups (45% versus 74%).

3.4. Relationship between Glut1 expression and histological differentiation

In well-differentiated adenocarcinomas, Glut1 expression was absent in 19 cases (33%), weak in 10 cases (18%), moderate in 19 cases (33%) and strong in 9 cases (16%). In moderately to poorly differentiated lesions, Glut1 expression was absent in 2 cases (7%), weak in 5 cases (19%), moderate in 13 cases (48%) and strong in 7 cases (26%). Glut1 expression was significantly different ($P < 0.05$) between the well differentiated and less differentiated group (positivity of 67% versus 93%). The rate of Glut1 moderate and strong expression together was also significantly different ($P < 0.05$) between these two groups (49% versus 74%).

3.5. Relationship between Glut1 expression and lymph node metastasis

In carcinomas without nodal metastasis, Glut1 expression was absent in 13 cases (22%), weak in 12 cases (20%), moderate in 21 cases (36%) and strong in 13 cases (22%). In lesions with nodal metastasis, Glut1 expression was absent in 1 case (9%), weak in 1 case

Table 1
Basic characteristics of T1 and T2 colorectal carcinomas

	Depth of invasion	
	T1 ($n=49$)	T2 ($n=35$)
Age (mean±S.D.) (years)	44–85 (63.4±9.5)	42–86 (64.8±10.0)
Sex (no. of patients)		
Male	33 (67)	20 (57)
Female	16 (33)	15 (43)
Tumour location (no. of patients)		
Proximal colon	13 (27)	6 (17)
Distal colon and rectum	36 (73)	29 (83)
Tumour size (mm) (mean±S.D.)	6–48 (17.7±9.5)	12–65 (31.9±13.0)
Histological differentiation (no. of patients)		
Well	33 (67)	24 (69)
Moderately to poorly	16 (33)	11 (31)
Lymph node metastasis (no. of patients)		
Positive	4 (11)	7 (20)
Negative	31 (89)	28 (80)
Morphological type (no. of patients)		
Polypoid	20 (41)	11 (31)
Depressed	20 (41)	10 (29)
Flat-elevated/unclassified	9 (18)	14 (40)

S.D., standard deviation.

(9%), moderate in 6 cases (55%) and strong in 3 cases (27%). The positive rate of Glut1 expression was higher in the metastatic group than non-metastatic group, but the difference was not statistically significant.

3.6. Relationship between Glut1 expression and morphological type

In order to clarify the characteristics of the depressed-type carcinomas, a comparison between the polypoid-type and the depressed-type was made. The flat-elevated-type lesions were excluded from the comparison. There were 31 polypoid carcinomas (22 well-differentiated adenocarcinomas, 9 moderately to poorly differentiated adenocarcinomas) and 30 depressed lesions (16 well differentiated adenocarcinomas, 14 moderately to poorly differentiated adenocarcinomas). The depth of the lesions (Table 1) and the histological differentiation in both groups were not significantly different. In the polypoid-type, Glut1 expression was absent in 11 cases (35%), weak in 7 cases (23%), moderate in 8 cases (26%) and strong in 5 cases (16%). In the depressed-type, Glut1 expression was absent in 4 cases (13%), weak in 4 cases (13%), moderate in 17 cases (57%) and strong in 5 cases (17%). Glut1 expression was significantly different ($P<0.05$) between the polypoid group and the depressed group (positivity of 65% versus 87%). The rate of Glut1 expression of more than a moderate degree was also significantly different ($P<0.05$) between these groups (42% versus 73%).

4. Discussion

Previous studies have indicated that malignant tissues show increased glucose uptake and utilisation. Several immunohistochemical studies have shown that a significant number of malignant tumours, including colorectal carcinomas, express Glut1 [7–14]. Higher levels of Glut1 expression in neoplastic tissue reflect an increased glycolytic metabolism. Younes and colleagues reported that a high level of Glut1 expression was significantly associated with the presence of lymph node metastases in colorectal carcinoma [7], while Haber and associates noted that assessment of the extent of Glut1 immunostaining in colorectal carcinoma could identify patients with a poorer prognosis [8]. These studies suggested that the expression of Glut1 could be a marker for malignant potential.

Younes and colleagues demonstrated that approximately 10% of the adenoma cases expressed Glut1, but all of the Glut1-positive cases showed only weak immunoreactivity of less than 10% of the cells. They also pointed out that the frequency of Glut1 expression in adenomas increased with size [7]. In our study, Glut1 expression was positive in 18% of low-grade adenomas, and in 63% of high-grade adenomas. The positivity in such lesions was usually weak (less than 10% of cells) but was moderate (10–50%) in 8% of the high-grade adenomas. Glut1 expression was significantly different ($P<0.01$) between the low-grade adenomas and high-grade adenomas. However, we found no difference in

the average diameter between Glut1-positive and negative adenomas (data not shown). The discrepancy between Younes' study and our results may be due to differences in the selection of sample lesions or due to differences in the sensitivity of the immunostaining system used.

As for Glut1 expression in carcinoma cases, Haber and colleagues [8] studied 112 cases and the positive rate was 90%. They concluded that the proportion of low Glut1 staining (less than 50% of cells) and high Glut1 staining (more than 50% of cells) did not correlate with Dukes' stage of the cancer. In our study, Glut1 expression was positive in 61% of T1 carcinomas, and 97% of T2 lesions, and the difference was statistically significant. The rate of Glut1 expression of more than a moderate degree (10–50%) was also significantly different ($P < 0.01$) between these two groups (45% versus 74%). However, if the cut-off value was set at 50%, Glut1 expression was not significantly different between the T1 and T2 lesions. The discrepancy between Haber's study and ours could have been caused by differences in the clinical characteristics of the subjects between the two studies. Haber's study included only 6 Dukes' A cases and all other cases were more advanced, while our study analysed only T1 and T2 stage cases. Our speculations are as follows; in early-stage carcinomas Glut1 positivity is low, but correlates with the depth of the lesion. In contrast, in the more advanced stages, the tumour cells already show high Glut1 expression, and no further increase of Glut1 expression occurs, even when the cancer invades more deeply. Thus, we believe that when one studies the Glut1 expression in early colorectal carcinomas, the cut-off level should be set at approximately 10%, and not at 50%.

Likewise, the correlation between Glut1 expression and histological differentiation was examined in our study using the 10% cut-off value. The rate of Glut1 expression of 10% or more was significantly different ($P < 0.05$) between well-differentiated adenocarcinomas and moderately to poorly differentiated lesions (49% versus 74%). If the cut-off value was set at 50%, there was no significant difference between these two groups. Previous reports have concluded that there is no correlation between Glut1 expression and histological differentiation [7,8]. The reasons for this discrepancy may be also be caused by differences in the tumour stages and the cut-off values between studies. Interestingly, Ito and colleagues reported on Glut1 immunostaining in lung adenocarcinomas and concluded that it was stronger in tumours with less differentiation [14]. This was similar to that of colorectal carcinomas in our study.

Previous studies have shown that there is a correlation between strong Glut1 expression in colorectal carcinomas and the frequency of lymph node metastases [7]. Our findings showed that the rate of Glut1 expression in carcinomas with nodal metastases was higher than that in those without, but the difference was not significant. Although the difference was not significant, due to the small sample size of lymph node metastases-positive carcinomas, the higher incidence of Glut1 expression in the lymph node metastases-positive carcinomas was thought to be compatible with previous findings.

Recently, flat- or depressed-type colorectal tumours have been increasingly detected not only in Japan, but also in other countries [19–23]. Kudo and colleagues detected many depressed cancers and demonstrated their invasive tendency, despite their small size. Furthermore, they concluded that the depressed-type carcinoma should be regarded as a *de novo* carcinoma [15,16]. This type of cancer was shown to progress rapidly and have a high malignant potential as reported in other clinicopathological studies [18]. The genetic characteristics of the depressed-type cancers have not yet been elucidated. Infrequent detection of the *K-ras* gene mutation is one of the major characteristics detected by molecular analysis of these flat- or depressed-type tumours [18,24,25]. There have been some reports about p53 immunostaining of these tumours, but the incidence of p53 immunoreactivity varies among several reports. Some authors have suggested that the *de novo* type shows a higher incidence of p53 immunoreactivity [20,22], while others have reported that the form of cancer has no effect on p53 status [26–30]. Apart from these reports, a previous study indicated a higher rate of loss of heterozygosity (LOH) at the D17S520 (17p) locus located close to the *TP53* gene in the *de novo* type [22]. However, there have been few studies of the molecular biological characteristics of this lesion. Our study is the first report of Glut1 expression in depressed-type early cancers. We showed that the rate of Glut1 expression of more than a moderate degree in the depressed-type was higher than in the polypoid-type (73% versus 42%, $P < 0.05$). The depth and histological differentiation in both groups were not significantly different. Thus, the difference in Glut1 expression between these two groups was considered significant independent of the depth or histological differentiation. This finding supported previous clinicopathological studies [15–18,30] which showed a high malignant potential of the depressed-type. Most studies of the flat- or depressed-type carcinoma and *de novo* carcinoma have originated in Japan. Although recently we have begun to see more reports from western countries [19–23], it remains necessary to accumulate more data, utilising not only clinicopathological studies but also molecular biological analyses.

In conclusion, our study suggested that Glut1 expression in colorectal T1 and T2 stage carcinomas could be related to the depth of invasion and histological differentiation, and that the depressed-type carcinoma may have a more malignant potential than the polypoid-type carcinoma.

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