Clinical significance of GLUT-1 expression in patients with esophageal cancer treated with concurrent chemoradiotherapy

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Abstract. This study aimed to investigate whether glucose transporter-1 (GLUT-1) expression in a pretreatment esophageal cancer biopsy was predictive of clinical outcomes in patients with esophageal cancer undergoing concurrent chemoradiotherapy (CRT). A total of 25 patients with esophageal cancer treated with concurrent CRT were reviewed. Radiotherapy was administered up to total doses of 40-66.6 Gy (median 66.6 Gy) with a single fraction of 1.8-2 Gy. Regarding chemotherapy, cisplatin (80 mg/m^2 on day 1) and 5-fluorouracil (800 mg/m^2 on days 2-6) were used concurrently with radiotherapy, every 3-4 weeks for a total of 1-2 courses. Tissue samples from esophageal carcinoma were obtained from the 25 patients by biopsy prior to concurrent CRT, and a semiquantitative analysis of GLUT-1 expression was performed using immunohistochemical staining. High GLUT-1 expression was observed in 7 of 25 (28%) patients, and GLUT-1 expression was significantly correlated with clinical T stage (p=0.0454), clinical N stage (p=0.0324) and initial response to CRT (p=0.0185). Patients with a high GLUT-1 expression had significantly poorer local control (LC) (5-year LC 28.6%) than those with a low expression (5-year LC 73.4%, p<005). Multivariate analysis revealed that GLUT-1 and the number of chemotherapy courses were independent prognostic factors for LC. Patients with a high GLUT-1 expression had significantly lower recurrence-free survival (RFS) compared to those with a low GLUT-1 expression (p=0.0405). Multivariate analysis revealed that GLUT-1, the number of chemotherapy courses and clinical M stage were independent prognostic factors for RFS. GLUT-1 expression was significantly correlated with clinical T stage, clinical N stage and initial response to concurrent CRT, and was predictive of LC and RFS for patients with esophageal cancer treated with concurrent CRT.

Introduction

Esophageal cancer is one of the most difficult malignancies to cure, and the prognosis for these patients is poor (1-3). Although surgery is the main curative treatment for these tumors, chemoradiotherapy (CRT) was identified as a viable option for the treatment of esophageal carcinoma. The Radiation Therapy Oncology Group (RTOG) 8501 trial showed that CRT is superior to radiotherapy alone as primary treatment (4). However, a number of studies demonstrated that the patterns of failure observed following definitive CRT showed that locoregional failure is frequent, with approximately 50% of patients experiencing local failure (4,5). The identification of predictive markers for the response to CRT may improve patient selection and allow for response modifications such as more intensive treatments for poor responders.

Glucose-transporter-1 (GLUT-1) is a membrane receptor that mediates the passive export of glucose across plasma membranes and its expression increases in hypoxic conditions due to reduced oxidative phosphorylation and hypoxia inducible factor-1 (HIF-1) induction (6). GLUT-1 facilitates the metabolic adaptation of cells to hypoxia and is essential for survival and proliferation of glycolytic metabolism (7,8). Therefore, GLUT-1 expression may be a suitable marker of hypoxia and glucose metabolism, measured simply and cost-effectively as part of the routine histological assessment of tumors (9,10). GLUT-1 has been immunohistochemically detected in a variety of malignant tissues, including tumors of the breast, thyroid, head and neck, bladder and lung (11-15). In all cases, the tumor expression was increased as compared to that of the corresponding normal tissues. The over-expression of GLUT-1 in tumors was reported to be a marker for poor prognosis in colorectal, ovarian and non-small cell lung cancers (16-18). In esophageal cancer, a number of studies showed that GLUT-1 over-expression was associated with tumor aggressiveness and poor prognosis in patients with esophageal cancer treated with surgical resection (19,20). However, little information is available on the clinical significance of GLUT-1 expression in patients with esophageal cancer treated with concurrent CRT.

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Table I. GLUT-1 expression and clinicopathological characteristics in 25 patients with esophageal cancer treated with concurrent chemoradiotherapy.

Variable	No. of patients	GLUT-1 expression		P-value
		Negative	Positive	
Gender				
Male	24	18	6	0.1017
Female	1	0	1	
Age (years)				
<60	8	4	4	0.1663
≥60	14	11	3	
Clinical T stage (UICC 2002)				
T1-3	15	13	2	0.0454
T4	10	5	5	
Clinical N stage (UICC 2002)				
N0	8	8	0	0.0324
N1	17	10	7	
Clinical M stage (UICC 2002)				
M0	20	16	4	0.0747
M1	5	2	3	
KPS (%)				
100-70	23	17	6	0.4701
≤60	2	1	1	
Tumor site				
Ce or Ut	12	8	4	0.5682
Mt or Lt	13	10	3	
Total radiation dose				
<60 Gy	3	2	1	0.8264
≥60 Gy	22	16	6	
No. of chemotherapy courses				
1	7	4	3	0.3021
2	18	14	4	

UICC, International Union Against Cancer; KPS, Karnofsky performance status; Ce, cervical esophagus; Ut, upper thoracic; Mt, middle thoracic; Lt, lower thoracic.

In the current study, GLUT-1 expression was retrospectively semiquantitated. Moreover, whether or not these levels were associated with clinicopathological characteristics and the clinical outcomes in patients with esophageal cancer treated with concurrent CRT was investigated.

Materials and methods

Patients and sample collection. Between 1997 and 2002, 37 patients with esophageal cancer were treated with concurrent CRT at the University of the Ryukyus Hospital, Japan. The study comprised primary esophageal carcinoma specimens from pretreatment biopsies obtained from 25 patients. The disease characteristics of the 25 patients, including tumor stage and tumor sites, were not significantly different compared to those of the 12 patients from which carcinoma specimens were not obtained. The histopathological diagnosis of the 25 patients was squamous cell carcinoma. No patients received chemotherapy or radiotherapy prior to the biopsy. The carcinoma specimens were obtained from the tumor edge, thereby avoiding the necrotic center. The specimens were immediately fixed in 10% buffered formalin.

The patient characteristics of the 25 patients are shown in Table I. Of the 25 patients, 1 was female and 24 were male, and the ages ranged from 45-78 years (median 62). The study was performed according to the guidelines approved by the institutional review board of our institution, and written informed consent was obtained from the 25 patients.

Concurrent chemoradiotherapy. External beam radiotherapy (EBRT) was administered with megavoltage equipment of photon energies ≥4 MeV. The total doses of EBRT ranged

from 40-66.6 Gy with a single fraction of 1.8-2 Gy 5 days per week. The median total dose of the 25 patients was 66.6 Gy, and 22 of 25 patients (88.0%) were treated with a total dose of ≥60 Gy. The treatment field of EBRT consisted of a localized field in 4 of 25 patients (16%), and the primary tumor plus regional lymph nodes in the remaining 21 (84%). In the majority of patients, computed tomography (CT)-based treatment planning and conformal radiotherapy were used. Initially, anterior-posterior opposed fields were employed at doses of 32.4-40 Gy, and a booster dose of 14-34.2 Gy was administered, using bilateral oblique or multiple fields. The clinical target volume for the primary tumor was defined as the gross tumor volume plus 3 cm craniocaudally. The planning target volumes for the primary tumor and the metastatic lymph nodes were determined with 1 to 1.5 cm margins to compensate for set-up variations and internal organ motion. Lung heterogeneity corrections were not used.

Chemotherapy was administered concurrently with radiotherapy. One course of chemotherapy consisted of cisplatin (80 mg/m^2 on day 1) and 5-fluorouracil (800 mg/m^2 on days 2-6), with 3- to 4-week intervals for a total of 1-2 courses. The patients received antiemetics with granisetron and metoclopramide prior to chemotherapy administration.

Immunohistochemical staining for glucose-transporter-1 and evaluation of staining. Formalin-fixed, paraffin-embedded tumor sections were dewaxed in xylene and dehydrated using a series of ethanol solutions of increasing dilution. Staining for GLUT-1 was then perfromed using the Envision Dual link. This protocol uses a 3,3'-diaminobenzidine substrate system that enabled visualization of the GLUT-1 protein as a brown stain. For GLUT-1 staining, a 1/100 (10 mg/ml protein) concentration of affinity-pure rabbit antihuman GLUT-1 (Chemicon Europe) was utilized. An incubation time was performed overnight at 4˚C for the primary antibody steps, whereas an incubation time of 30 min at room temperature was selected for each secondary antibody. The negative controls were prepared by omitting the primary antibodies. Following staining, sections were rinsed with water, counterstained with Gill's hematoxylin and coverslipped using an aqueous mountant.

Two independent pathologists blinded to the clinicopathological data performed the scoring of the immunohistochemical staining. The percentage of positive tumor cells was semiquantitatively determined by assessing the whole biopsy specimen, and the mean percentage of positive tumor cells was calculated by the two pathologists. In the current study, each sample was characterized as either 1, low (0-30%); or 2, high (31-100%).

Statistical analysis. The median follow-up of 9 surviving patients was 57.8 months (range 2.8-107.7). In the current study, the initial response of the primary tumor was evaluated using the criteria of the Japanese Society for Esophageal Disease, which were based on findings from esophagograms and esophagoscopies (21). In brief, complete response (CR) was defined as the complete disappearance of tumor and no appearance of new lesions at least 4 weeks after treatment. Partial response (PR) was defined as a >50% reduction in the product of the perpendicular diameters of the tumor and no

appearance of new lesions at least 4 weeks after treatment. Progressive disease (PD) was defined as a >25% increase in the product of the perpendicular diameters of the tumor or any new tumor. Any further conditions were defined as no change (NC). Disease recurrence was defined as recurrence or progression of the initial disease or the occurrence of new disease following CRT detected by a CT scan and/or esophagoscopy, which were performed every 3-4 months for 2 years and then twice a year. Overall survival (OS), recurrence-free survival (RFS) and local control (LC) rates were calculated actuarially according to the Kaplan-Meier method (22), and were measured from the first day of CRT. Differences between the groups were estimated using the Chi-square and generalized Wilcoxon tests (23). Multivariate analysis was performed using the Cox regression model (24). P=0.05 was considered to be statistically significant. Statistical analysis was performed with the SPSS software package (version 6.1; SPSS, Inc., Chicago, IL, USA).
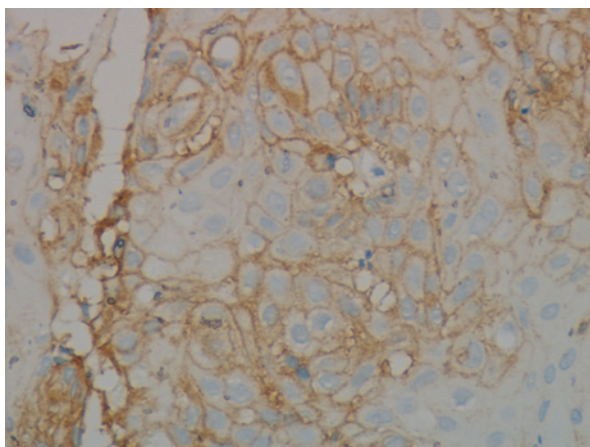
Results

Representative examples of high or low GLUT-1 expression by immunohistochemical analysis are shown in Fig. 1. In the current study, a high GLUT-1 expression was observed in 7 of 25 (28%) patients, and the patients were divided into low and high GLUT-1 expression groups. Table I shows the clinical data and GLUT-1 expression in the tumor biopsies of the 25 patients. The proportion of patients with T4 tumors was significantly higher (p=0.0454) in the high GLUT-1 expression group than that of patients with low GLUT-1 expression. Moreover, the proportion of patients with N1 tumor was significantly higher (p=0.0324) in the high GLUT-1 expression group than that of patients with low GLUT-1 expression. Other characteristics, such as age and gender, did not correlate with the GLUT-1 expression.

Table II shows GLUT-1 expression and the initial response in 25 patients. A total of 12 of 13 patients (92.3%) exhibited a CR response in the low GLUT-1 expression group, while 6 of 12 patients (50.0%) exhibited a CR response in the high GLUT-1 expression group. Significant differences were noted between the low and high GLUT-1 expression groups regarding the initial response to concurrent CRT (p=0.0185).

At the time of this study, 16 patients (91.0%) had disease recurrences (local, 6 patients; regional lymph nodes, 2; distant metastasis, including bone or lung, 4; and multiple sites, 4 patients). Of the 16 patients with multiple recurrences, 2 patients had simultaneous local recurrence. Local recurrence occurred in 8 patients (32.0%) in total. The 5-year actuarial LC rate in the 25 patients was 61.9%. Fig. 2 shows the LC curves according to the GLUT-1 expression. Patients with high GLUT-1 expression had a significantly poorer LC (5-year LC; 28.6%) than those with low GLUT-1 expression (5-year LC; 73.4%; p<005). The univariate analysis showed that GLUT-1 expression, the number of chemotherapy courses, total radiation dose and clinical M stage had a significant impact on LC (Table III), while the multivariate analysis showed that GLUT-1 expression and the number of chemotherapy courses were independent prognostic factors for LC (Table IV).
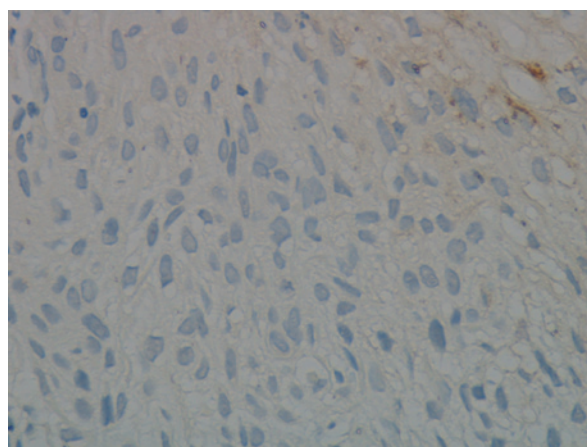
A

B

Figure 1. Representative example of GLUT-1 expression in esophageal carcinoma. Immunohistochemical staining with anti-GLUT-1 antibody in esophageal carcinoma cells. (A) High and (B) low GLUT-1 expression.

Table II. GLUT-1 expression and initial response in 25 patients with esophageal cancer treated with concurrent chemoradiotherapy.

Initial response	No. of patients	GLUT-1 expression		P-value
		Low (%)	High (%)	
CR	13	12 (92.3)	1 (7.7)	0.0185
PR or NC	12	6 (50.0)	6 (50.0)	

GLUT-1, glucose transporter 1; CR, complete response; PR, partial response; NC, no change.
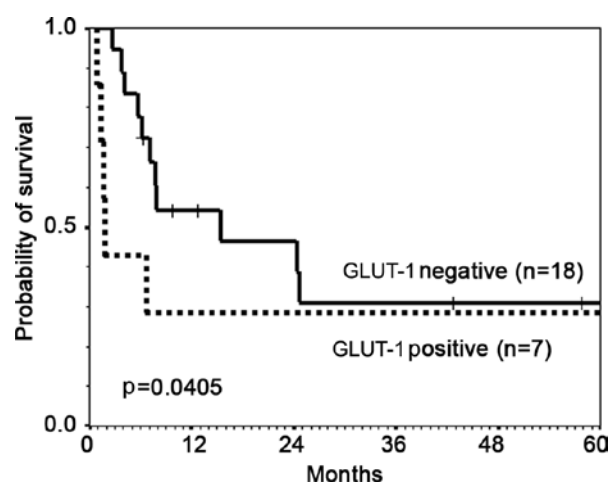

Figure 3. Disease-free survival curves according to the GLUT-1 expression in esophageal carcinoma patients treated with concurrent chemoradiotherapy.
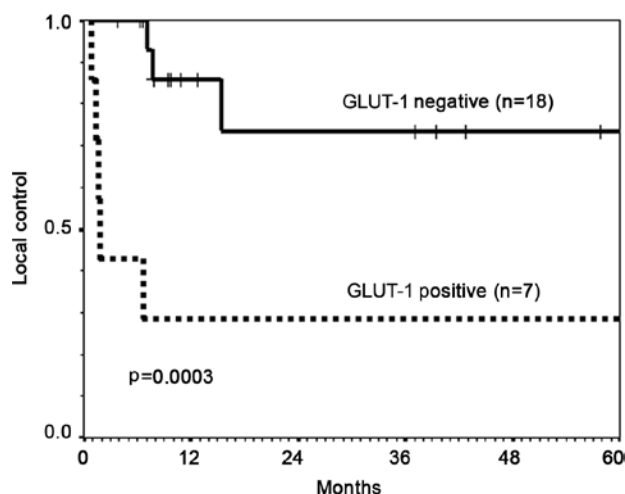

Figure 2. Local control curves according to the GLUT-1 expression in esophageal carcinoma patients treated with concurrent chemoradiotherapy.

Of the 25 patients, 16 (64.0%) succumbed to the disease during this study. Of the 16 patients, 13 succumbed to esophageal carcinoma and the remaining 3 patients succumbed without any sign of clinical recurrence (1, radiation pneumonitis; 1, pneumonia; and 1, unknown causes). The 5-year actuarial OS and RFS rate for the 25 patients was 27.3 and 31.3%, respectively. Fig. 3 shows the RFS curves according to GLUT-1 expression. Patients with a high GLUT-1 expression had a significantly lower RFS compared to those with a low GLUT-1 expression (p=0.0405). The univariate analysis showed that GLUT-1 expression, the number of chemotherapy courses and clinical M stage had a significant impact on OS, while the multivariate analysis, the three factors proved to be significant prognostic factors for RFS (Tables V and VI).

Late complications of NCI-CTC Grade 4-5 were observed in 2 patients (4.0%). One patient suffered grade 4 pericardial effusion requiring pericardial puncture 58 months after the completion of CRT. The remaining patient suffered grade 5 radiation pneumonitis and succumbed to radiation pneumonitis 4 months after CRT. The 2 patients were treated with a total dose of 66.6 Gy radiotherapy and 2 courses of chemotherapies.

Table III. Univariate analysis of various potential prognostic factors for LC in patients with esophageal cancer treated with concurrent chemoradiotherapy.

	No. of patients	Univariate analysis	
		LC, 5-year rate (%)	P-value
Gender			
Male	24	59.7	0.5065
Female	1	100	
Age (years)			
<60	8	62.5	0.3847
≥60 or more	14	59.6	
Clinical T stage (UICC 2002)			
T1-3	15	63.3	0.2851
T4	10	60.0	
Clinical N stage (UICC 2002)			
N0	8	60.0	0.4436
N1	17	66.7	
Clinical M stage (UICC 2002)			
M0	20	68.1	0.0067
M1	5	40.0	
KPS (%)			
100-70	23	62.3	0.1776
≤60	2	50.0	
Tumor site			
Ce or Ut	12	71.4	0.8294
Mt or Lt	13	59.4	
Total radiation dose			
<60 Gy	3	33.3	0.0058
≥60 Gy	22	66.1	
No. of chemotherapy courses			
1	7	19.5	0.0012
2	18	78.7	
GLUT-1 expression			
Low	18	73.4	0.0003
High	7	28.5	

LC, local control; UICC, International Union Against Cancer; KPS, Karnofsky performance status; Ce, cervical; Ut, upper thoracic; Mt, middle thoracic; Lt, lower thoracic.

Discussion

The current study showed that GLUT-1 expression is significantly correlated with clinical T and N stages in patients with esophageal carcinoma. The percentage of patients with T4 tumor was significantly higher in the high GLUT-1 expression group than that of patients with low GLUT-1 expression. Moreover, the percentage of patients with N1 tumor was significantly higher in the high GLUT-1 expression group than that of patients with a low GLUT-1 expression. A number of studies found an association between GLUT-1 expression and tumor stage (19,20). Tohma et al found that pathological T3-4 esopha-

geal cancers showed higher percentages of GLUT-1 strong positive cells than T1-2 cancers, and lesions exhibiting lymph node metastasis showed higher percentages of GLUT-1 strong positive cells than lesions without lymph node metastasis (19). Kato et al showed that significant correlations between GLUT-1 expression and tumor stage were observed in patients with esophageal squamous cell carcinoma (20). The results of these authors, together with our results, proved that GLUT-1 expression is correlated with tumor aggressiveness in patients with esophageal carcinoma. GLUT-1 may be linked to invasiveness by supporting glycosis, which enhances tumor cell viability and provides energy for cell division and tumor growth (25).

Table IV. Multivariate analysis of various potential prognostic factors for LC in patients with esophageal cancer treated with concurrent chemoradiotherapy.

		Multivariate analysis	
		RR (95% CI)	P-value
GLUT-1	(Low vs. high)	0.047 (0.005-0.434)	0.0070
Chemotherapy course	(1 course vs. 2 courses)	29.598 (2.872-304.993)	0.0040
Clinical M stage	(M0 vs. M1)	0.260 (0.001-45.361)	0.6090
Total radiation dose	(<60 Gy vs. ≥60 Gy)	0.683 (0.000-3409.927)	0.9300

LC, local control; RR, relative ratio; CI, confidence intervals.

Table V. Univariate analysis of various potential prognostic factors for RFS in patients with esophageal cancer treated with concurrent chemoradiotherapy.

		Univariate analysis	
	No. of patients	RFS, 5-year rate (%)	P-value
Gender			
Male	24	27.9	0.3125
Female	1	100	
Age (years)			
<60	8	25.0	0.3782
≥60	14	34.2	
Clinical T stage (UICC 2002)			
T1-3	15	25.9	0.5743
T4	10	40.0	
Clinical N stage (UICC 2002)			
N0	8	60.0	0.0566
N1	17	22.1	
Clinical M stage (UICC 2002)			
M0	20	39.1	<0.0001
M1	5	0.0	
KPS (%)			
100-70	23	31.6	0.4884
≤60	2	50.0	
Tumor site			
Ce or Ut	12	35.9	0.5099
Mt or Lt	13	25.9	
Total radiation dose			
<60 Gy	3	33.3	0.0539
≥60 Gy	22	32.4	
No. of chemotherapy courses			
1	7	0.0	0.0004
2	18	43.8	
GLUT-1 expression			
Low	18	30.1	0.0405
High	7	28.5	

RFS, recurrence-free survival; UICC, International Union Against Cancer; KPS, Karnofsky performance status; Ce, cervical; Ut, upper thoracic; Mt, middle thoracic; Lt, lower thoracic.

Table VI. Multivariate analysis of various potential prognostic factors for DFS in patients with esophageal cancer treated with concurrent chemoradiotherapy.

		Multivariate analysis	
		RR (95% CI)	P-value
GLUT-1	(Low vs. high)	0.113 (0.019-0.668)	0.0160
Chemotherapy course	(1 course vs. 2 courses)	6.614 (1.473-25.797)	0.0130
Clinical M stage	(M0 vs. M1)	0.065 (0.008-0.513)	0.0090
Total radiation dose	(<60 Gy vs. ≥60 Gy)	2.580 (0.332-20.005)	0.3650
Clinical N stage	(N0 vs. N1)	4.474 (0.958-20.892)	0.0570

DFS, disease-free survival; RR, relative ratio; CI, confidence intervals.

The current study showed that GLUT-1 expression was significantly correlated with initial response to concurrent CRT, and patients with a high GLUT-1 expression had a significantly poorer LC (5-year LC, 28.6%) than those with a low expression (5-year LC, 73.4%; p<005). Multivariate analysis revealed that GLUT-1 and the number of chemotherapy courses were independent prognostic factors for LC. The relationship between GLUT-1 expression and the reduction in response to radiotherapy and chemotherapy is explained by the fact that GLUT-1 expression is a marker of cellular adaptive responses to hypoxia (26-28). The biological effect of radiotherapy was reported to have increased approximately 3-fold when irradiation was performed under well-oxygenated conditions compared to anoxic ones (29). Regarding rectal cancers, Brophy *et al* found that GLUT-1 expression was significantly associated with a reduced response to CRT (26). These authors showed that GLUT-1 expression appears to be a surrogate for tumor response to CRT in patients with esophageal carcinoma.

Regarding RFS, patients with a high GLUT-1 expression had significantly lower RFS compared to those with a low GLUT-1 expression (p=0.0405). The multivariate analysis showed that GLUT-1, the number of chemotherapy courses and clinical M stage were independent prognostic factors for RFS. Previous studies found that the GLUT-1 over-expression in esophageal carcinoma is a risk factor for mortality (19,20) The results of these authors, together with our results, suggest that GLUT-1 expression is predictive of clinical outcomes in patients with esophageal carcinoma treated with CRT.

It is well established that hypoxic conditions due to reduced oxidative phosphorylation, which may be a consequence of the increased proliferation observed in tumors, enhances GLUT-1 expression (6,30). GLUT-1 is considered to enhance glycolytic metabolism which has been correlated with tumor proliferative activity (8). Therefore, GLUT-1 expression appears to be a surrogate marker for not only hypoxia but also the metabolic activity of malignant tumors (31). Since the presence of hypoxia causes treatment resistance by radiotherapy and chemotherapy, and the high metabolic activity causes a higher proliferative activity in tumors, a high GLUT-1 expression may result in poor treatment outcome in patients with malignant tumors.

The increase in glucose transport noted in malignant tumors has also been detected using 18-fluorodeoxyglucose positron emission tomography (FDG-PET), which can quantify tumor glucose consumption (32,33). Regarding esophageal carcinoma, Taylor *et al* found that in patients undergoing surgical resection, increasing standardized uptake values (SUVmax) correlated with an increased GLUT-1 expression (34). GLUT-1 activity enhanced glycolytic metabolism which has been correlated with tumor proliferative activity (8). Westerterp *et al* showed that in patients with esophageal carcinoma, a significant correlation was found between FDG uptake and GLUT-1 expression (35). Kato *et al* found that GLUT-1 expression was related to FDG uptake and assessment of both FDG uptake and GLUT-1 expression may be useful for providing prognostic data in patients with esophageal carcinoma (36). However, GLUT-1 expression is obtained from superficial biopsy tissues and may not be representative of the tumor as a whole. Further studies are therefore required to investigate the correlations of GLUT-1 and FDG-PET in patients with esophageal carcinoma.

In conclusion, our results showed that GLUT-1 expression is significantly correlated with initial T and N stages, and response to concurrent CRT. Moreover, GLUT-1 is predictive of LC and DFS for patients with esophageal carcinoma treated with concurrent CRT. These findings suggest a role for GLUT-1 as a new prognostic biomarker for patients with esophageal cancer treated with CRT, and may allow for the selection of patients most likely to benefit from more intensive treatments. Furthermore, understanding the biological role of GLUT-1 may allow for response modification by targeting specific pathways. However, this study is a retrospective study with a relatively limited number of patients. Therefore further prospective studies are required to confirm our results.

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