

## Significance of Glucose Transporter Type 1 (GLUT-1) Expression in the Therapeutic Strategy for Pancreatic Ductal Adenocarcinoma

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### ABSTRACT

**Background.** This study aimed to examine the prognostic relevance of glucose transporter type 1 (GLUT-1), which is a key regulator of the glucose metabolism. In particular, the study aimed to examine the association between GLUT-1 expression and the therapeutic effect of chemoradiotherapy (CRT) in pancreatic ductal adenocarcinoma (PDAC).

**Methods.** Patients with PDAC were enrolled in the study. Patients with distant metastases and those who received only chemotherapy as treatment were excluded from the study. Specimens for immunohistochemical evaluations were obtained through surgical resection and endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) of the primary tumor before any treatment.

**Results.** This study included 197 patients. Of these 197 patients, 100 underwent upfront surgery, and 97 received neoadjuvant CRT (NACRT), which was performed mainly for patients with locally advanced tumors. Of the 97 patients who received NACRT, 21 later underwent surgical resection. For the patients who underwent upfront surgery, low GLUT-1 expression was an independent factor for a better prognosis. For the patients who underwent NACRT,

low GLUT-1 expression was significantly associated with greater tumor size reduction, a higher resection rate, and a better prognosis. Additionally, GLUT-1 expression was significantly increased after NACRT treatment.

**Conclusions.** Among the patients with PDAC, those with low GLUT-1 expression in the primary tumor had a better prognosis than those with high GLUT-1 expression. Moreover, the patients with low GLUT-1 expression displayed a better therapeutic response to NACRT.

Pancreatic ductal adenocarcinoma (PDAC), one of the most lethal malignancies, is associated with a very poor prognosis and a 5-year survival rate of only 6%.<sup>1</sup> Surgical resection is the only potentially curative therapy for PDAC. However, most patients have advanced tumors at diagnosis<sup>2</sup> and experience local or distant metastases even after resection.<sup>3</sup>

To improve the curative resection rate and prognosis, neoadjuvant chemoradiotherapy (NACRT) has been adopted in cases of borderline resectable and unresectable diseases without distant metastasis.<sup>4–6</sup> Tumor radiosensitivity is thought to be a critical factor in the use of NACRT.

A change in the energy metabolism is a hallmark of tumor cells and a key contributor to tumor progression.<sup>7,8</sup> Malignant cells take advantage of the physiologic preferential use of glycolysis over oxidative phosphorylation, resulting in higher rates of glucose uptake and lactate production. This is known as the Warburg effect.<sup>9</sup>

Tumor radioresistance may result from the interaction of multiple tumor-intrinsic and tumor-extrinsic factors. The metabolic shift to glycolysis is apparently involved in

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radioresistance.<sup>10,11</sup> The transmembrane glucose transporter type 1 (GLUT-1) mediates the first rate-limiting step in glucose transport across the plasma membrane.<sup>12</sup> Hypoxia-inducible factor 1 (HIF-1) upregulates GLUT-1,<sup>10</sup> whereas mutant p53 promotes translocation of GLUT-1 to the plasma membrane.<sup>13</sup> Both factors stimulate the Warburg effect. Hypoxia, which drives tumor progression, is an important characteristic of the microenvironment of malignant tumors, including PDAC.<sup>14</sup> Moreover, mutations in *TP53* are frequently observed in PDAC.<sup>15,16</sup>

Overexpression of GLUT-1 has been shown to correlate with poor prognosis in several malignancies.<sup>17–19</sup> Reported findings show GLUT-1 expression to be significantly higher in PDAC lesions than in adjacent non-cancerous tissue.<sup>20</sup> Expression of GLUT-1 progressively increases from low- to high-grade dysplastic lesions in both pancreatic intraepithelial neoplasms and intraductal papillary mucinous neoplasms.<sup>21</sup> A recent meta-analysis has shown that high GLUT-1 expression is associated with shorter overall survival (OS) for patients with PDAC.<sup>22</sup> However, the association between GLUT-1 expression and radioresistance in PDAC was not examined.

The current study examined the association between GLUT-1 expression and prognosis for patients with PDAC who underwent upfront surgery. In addition, we explored the significance of GLUT-1 expression for patients who underwent NACRT using specimens obtained through endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) biopsy.

## MATERIALS AND METHODS

### *Patient and Tumor Sampling*

Patients who underwent upfront surgery or NACRT for PDAC between January 2000 and December 2015 at the Department of Digestive Surgery, Breast and Thyroid Surgery, Kagoshima University were enrolled in the study. Since 2006, NACRT has been performed mainly for patients with a diagnosis of locally advanced tumors. Patients who had distant metastases or received chemotherapy were excluded from the study. This study was approved by the institutional ethics review board of our hospital, and written informed consent for data analysis and publication was obtained from all patients.

Specimens for immunohistochemical evaluation of GLUT-1 expression were obtained by surgical resection and EUS-FNA of the primary tumor, and EUS-FNA was performed before any treatment. All resected specimens were examined histologically according to the seventh tumor-node-metastasis (TNM) classification system.<sup>23</sup> Imaging examinations, including computed tomography (CT) and magnetic resonance imaging (MRI), were

performed every 4 months for the first 2 years, then every 6 months during the following 3 years. The CRT regimens included hyperfractionated accelerated radiotherapy administered with S-1 (TS-1; Taiho Pharmaceutical Co, Ltd, Tokyo, Japan) at a dose of 80 mg/m<sup>2</sup> for the first 21 days.<sup>24</sup> A total of 50–58 Gy was administered in 40 fractions during 4 weeks. Then, 1 month after CRT completion, S-1 was administered for 2 weeks, followed by a 2-week rest period. Either CT or MRI was performed 1 month after CRT and every 3 months afterward. Tumor response after NACRT was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST).<sup>25</sup>

### *Immunohistochemistry of GLUT-1*

Paraffin blocks were sliced into 3- $\mu$ m-thick sections. After deparaffinization and rehydration, endogenous peroxidase activity was blocked by immersing the slides in an absolute methanol solution containing 0.3% hydrogen peroxide for 30 min. The sections then were treated with 1% bovine serum albumin for 30 min to block nonspecific reactions. Heat-induced antigen retrieval was performed by autoclave pretreatment (120 °C for 5 min) in citrate buffer solution (pH 6.0). The sections then were incubated overnight at 4 °C with rabbit polyclonal anti-GLUT-1 antibody (ab15309; Abcam, Cambridge, UK) at a dilution of 1:400.

After incubation with the appropriate secondary antibodies, staining was performed using an avidin–biotin complex detection kit (Vector Laboratory, Burlingame, CA, USA) and a diaminobenzidine substrate system according to the manufacturer's instructions. The slides were counterstained with hematoxylin before mounting. Positive controls for GLUT-1 expression consisted of red blood cells in each section. Negative controls were obtained by omitting the primary antibody.

### *Evaluation of GLUT-1 Expression*

All tissue sections were simultaneously assessed by two investigators (H.K. and K.M.) who were blinded to the patient's clinicopathologic details. Levels of GLUT-1 expression were evaluated by measuring the intensity and frequency of tumor cell membrane staining. The expression of GLUT-1 was considered high when more than 30% of the tumor cells were intensely stained and low when 30% or less of the tumor cells were intensely stained. For evaluation of GLUT-1 expression, we used only EUS-FNA specimens that contained more than 500 tumor cells.

### *Statistical Analysis*

Associations between different categorical variables were assessed using the Chi square test or Fisher's exact

test. The Wilcoxon signed-rank test was used to compare continuous variables before and after NACRT for the same patients. Survival curves were plotted using the Kaplan–Meier method and analyzed using the log-rank test. The unstratified Cox proportional hazard model was used to estimate the hazard ratio (HR) and its corresponding 95% confidence interval (CI).

Overall survival was calculated as the time between initial treatment and death by any cause. Recurrence-free survival (RFS) was calculated as the time between surgical resection and initial recurrence. Progression-free survival (PFS) was calculated as the time between initial treatment and tumor progression for patients who underwent NACRT.

The multivariate Cox proportional hazards model was used to estimate adjusted HR and to determine independent factors associated with survival using significant factors from the univariate analysis as covariates. All  $p$  values lower than 0.05 were considered to indicate statistical significance. Statistical evaluation was performed using SigmaPlot version 12.5 for Windows (HULINKS Inc., Tokyo, Japan).

## RESULTS

### *Patient Characteristics*

Among the 197 patients involved in this study, 100 received upfront surgery and 97 received NACRT, 21 of whom also were eligible for surgery. The patients who underwent NACRT had more advanced T factor (Table S1). Of the 100 patients who underwent upfront surgery, 65 received postoperative adjuvant therapy, with 14 receiving 5-fluorouracil, 27 receiving gemcitabine, and 24 receiving S-1. The study excluded 78 patients who underwent NACRT without EUS-FNA ( $n = 70$ ) or without EUS-FNA specimens feasible for evaluation of GLUT-1 expression ( $n = 8$ ). The median follow-up period was 20.5 months (mean, 29.4 months).

### *GLUT-1 Expression and Prognosis for Patients Who Underwent Upfront Surgery*

We evaluated GLUT-1 expression using immunohistochemistry of resected specimens from patients who underwent upfront surgery. Acinar and ductal cells of the adjacent noncancerous tissue showed no GLUT-1 staining. The findings showed GLUT-1 expressed primarily in tumor cell membranes (Fig. 1a–d). Expression of GLUT-1 was classified as low in 40 patients (40%) and high in 60 patients (60%).

Table 1 shows the analyses of OS for patients who underwent upfront surgery. Univariate analysis found male gender, tumor larger than 30 mm, lymph node metastasis, moderate or poor differentiation, serum carbohydrate antigen 19-9 level higher than 37 U/mL, R1 resection, no adjuvant therapy, and high expression of GLUT-1 to be significant factors for a poor prognosis. Multivariate analysis indicated that all these factors, except for adjuvant therapy, remain as independent prognostic factors.

Figure S1 shows OS curves according to the GLUT-1 expression. The median survival time (MST) was 19 months (95% CI 14.2–23.8 months) for the patients with high GLUT-1 expression and 35.1 months (95% CI 18.5–51.7 months) for the patients with high low GLUT-1 expression ( $p = 0.008$  log-rank; adjusted HR, 0.328; 95% CI 0.184–0.585). Furthermore, high GLUT-1 expression was an independent factor of shorter RFS (Table S2).

### *GLUT-1 Expression Using EUS-FNA Specimens and the Effect of NACRT*

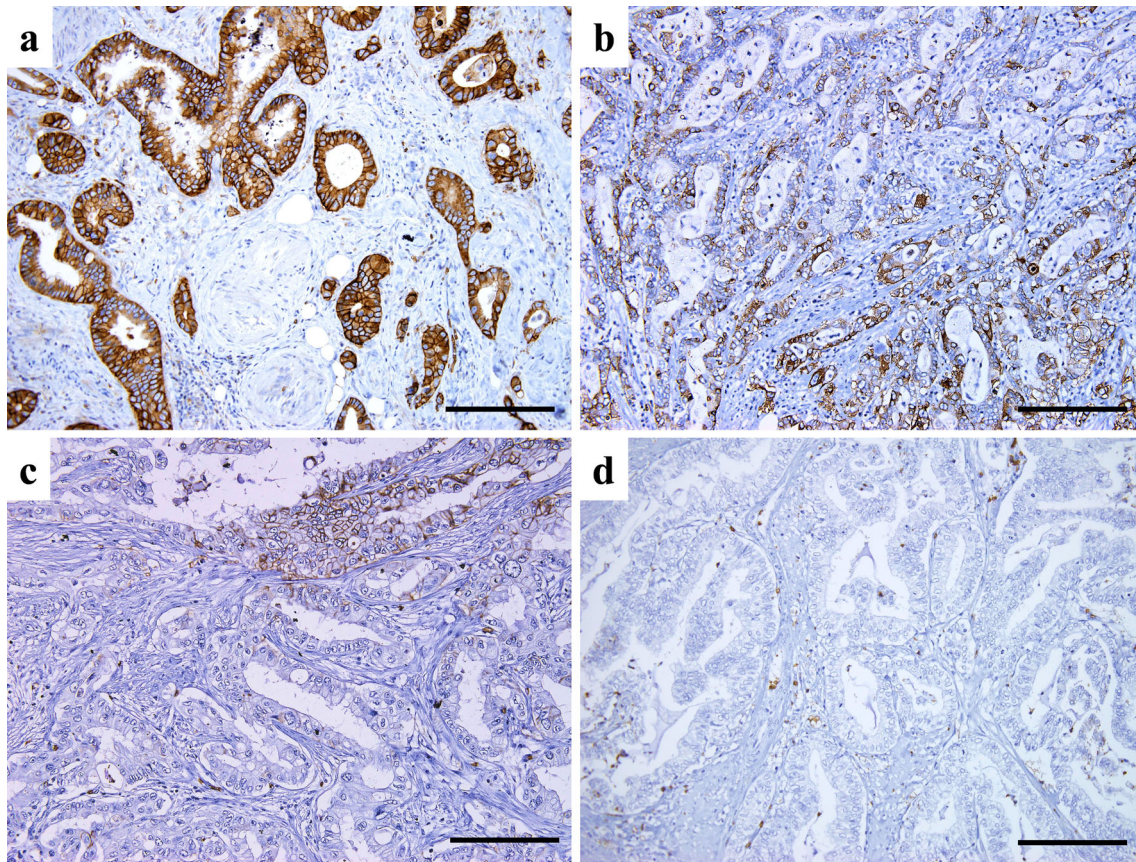
We evaluated GLUT-1 expression in specimens obtained through EUS-FNA according to the same criteria used for surgically resected specimens (Fig. 2). Tumor size and serum CA 19-9 were significantly reduced after NACRT (Table S3).

The findings showed 54 patients with CR/PR and 43 patients with SD/PD. One patient showed CR. We then examined the association between clinical factors and the effect of NACRT (Table 2). Low GLUT-1 expression was significantly associated with the rate of CR/PR.

Figure 3a shows the OS of patients who underwent NACRT. The MST was 17 months (95% CI 13.0–21.0 months) for the patients with high GLUT-1 expression and 26.6 months (95% CI 16.4–36.8 months) for the patients with low GLUT-1 expression ( $p = 0.008$ , log-rank; adjusted HR, 0.640; 95% CI 0.380–1.078; Table S4).

Figure 3b shows the resection rates after NACRT according to GLUT-1 expression. Of the 42 patients with low GLUT-1 expression, 14 underwent surgical resection after NACRT. By contrast, only 7 of the 55 patients with high GLUT-1 expression underwent surgical resection. The resection rate for low GLUT-1-expressing tumors (33.3%) was significantly higher than for high GLUT-1-expressing tumors (12.7%).

We examined the prognostic impact of GLUT-1 expression using EUS-FNA specimens according to surgical resection after NACRT (Fig. S2). For the patients who underwent surgical resection after NACRT, high GLUT-1 expression was a significant factor for a shorter RFS ( $p = 0.043$ , log-rank; HR, 0.260; 95% CI 0.063–1.076). For those who did not undergo surgical



**FIG. 1** Representative images of immunohistochemical glucose transporter type 1 (GLUT-1) staining in resected specimens (magnification,  $\times 200$ ; bar, 100  $\mu\text{m}$ ). **a** Diffuse positive staining of GLUT-1.

**b** 50% positive staining of GLUT-1. **c** 10% positive staining of GLUT-1. **d** No staining of GLUT-1

resection after NACRT, the high GLUT-1 expression group tended to have a shorter PFS than the low GLUT-1 expression group ( $p = 0.057$ , log-rank; HR, 0.629; 95% CI 0.383–1.035).

#### *Comparison of GLUT-1 Expression Between EUS-FNA Specimens and Surgically Resected Specimens*

Expression of GLUT-1 was evaluated in surgically resected and EUS-FNA specimens from 20 patients who underwent upfront surgery. The findings of GLUT-1 expression in the surgically resected specimens were concordant with those in the EUS-FNA specimens from 18 patients (90%). We then evaluated GLUT-1 expression using surgically resected and EUS-FNA specimens from 20 patients who underwent surgical resection after NACRT (excluding one patient with a pathologic complete response). Among the 20 patients, 8 (40%) experienced a low-to-high change in GLUT-1 expression due to NACRT. However, none of the patients experienced a high-to-low change in GLUT-1 expression. Nonetheless, prognosis did

not differ significantly according to changes in GLUT-1 expression (Fig. S3).

#### **DISCUSSION**

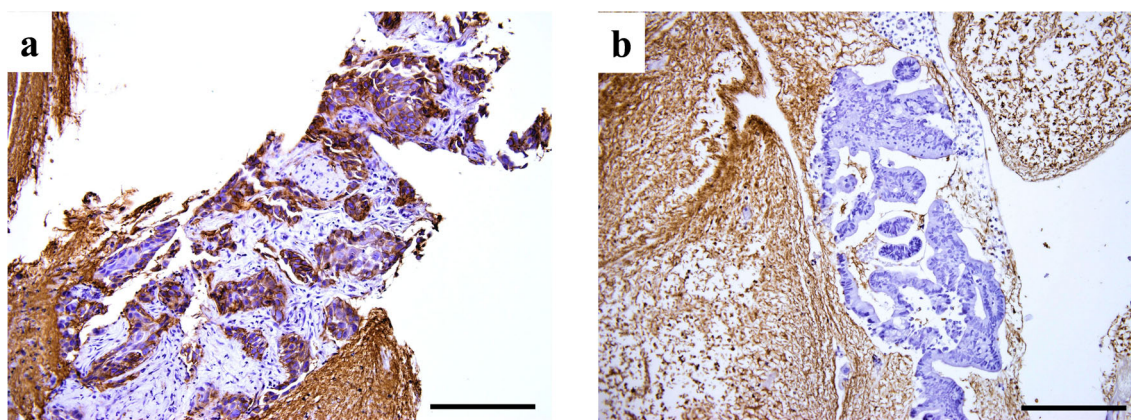
Reports show that CRT improves the resection rate and prognosis for patients with locally advanced PDAC. However, its therapeutic effect on the primary tumor varies considerably across individuals.<sup>4-6</sup> Predicting the therapeutic effect of CRT is pivotal in selecting the optimal therapy for locally advanced PDAC. The metabolic shift to glycolysis, a hallmark of tumor cells, seems to be an important factor in radioresistance.<sup>10,11</sup> For this process, GLUT-1 is the key molecule.<sup>12</sup> Chikamoto et al.<sup>26</sup> demonstrated that GLUT-1 expression is associated with the maximum standardized uptake value of <sup>18</sup>F-fluorodeoxyglucose positron emission tomography and the prognosis for patients with pancreatic cancer.

In the current study, GLUT-1 expression was an independent prognostic factor for the patients with PDAC who underwent upfront surgery: Those with low GLUT-1 expression showed a significantly better prognosis.

**TABLE 1** Association between glucose transporter type 1 (GLUT-1) expression and overall survival in patients with pancreatic cancer who underwent upfront surgery ( $n = 100$ )

Factor ( $n$ )	Events	Univariate analysis		Multivariate analysis	
		MST (months)	$p$ value	Adjusted HR (95% CI)	$p$ value
Age (years)	< 75 ( $n = 70$ )	56	22.5	0.496	
	$\geq 75$ ( $n = 30$ )	21	22.5		
Gender	Male ( $n = 60$ )	52	18.5	0.406 (0.235–0.700)	0.001
	Female ( $n = 40$ )	25	37.5		
Tumor position	Head ( $n = 69$ )	53	24.5	0.972	
	Body-tail ( $n = 31$ )	24	27.0		
Tumor size (mm)	$\leq 30$ ( $n = 58$ )	38	38.5	0.470 (0.273–0.809)	0.006
	$> 30$ ( $n = 42$ )	39	18.0		
T factor	1/2 ( $n = 6$ )	5	28.0	0.460	
	3 ( $n = 94$ )	72	22.5		
N factor	0 ( $n = 35$ )	24	42.0	0.396 (0.218–0.721)	0.002
	1 ( $n = 65$ )	53	19.0		
Histology	WD ( $n = 48$ )	32	35.0	0.508 (0.298–0.867)	0.013
	MD/PD ( $n = 52$ )	45	19.0		
CA 19-9 (U/mL)	$\leq 37$ ( $n = 41$ )	27	35.0	0.520 (0.308–0.879)	0.015
	$> 37$ ( $n = 59$ )	50	18.0		
R0 resection	No ( $n = 23$ )	21	15.0	0.379 (0.216–0.663)	< 0.001
	Yes ( $n = 77$ )	56	28.0		
GLUT-1 expression	Low ( $n = 40$ )	29	35.1	0.328 (0.184–0.584)	< 0.001
	High ( $n = 60$ )	48	19.0		
Postoperative adjuvant therapy	No ( $n = 35$ )	29	18.0	0.614 (0.369–1.022)	0.061
	Yes ( $n = 65$ )	48	30.1		

MST median survival time, HR hazard ratio, CI confidence interval, WD well-differentiated, MD moderately differentiated, PD poorly differentiated, CA 19-9 carbohydrate antigen 19-9

**FIG. 2** Representative images of immunohistochemical glucose transporter type 1 (GLUT-1) staining in endoscopic ultrasound-guided fine-needle aspiration biopsy specimens (magnification,  $\times 200$ ; bar, 100  $\mu\text{m}$ ). **a** Diffuse positive staining of GLUT-1. **b** No staining of GLUT-1

Furthermore, evaluation of GLUT-1 expression using EUS-FNA specimens before treatment was significantly associated with the therapeutic effect of NACRT. Low GLUT-1 expression was significantly associated with greater

reduction in tumor size, higher resection rates, and a better prognosis.

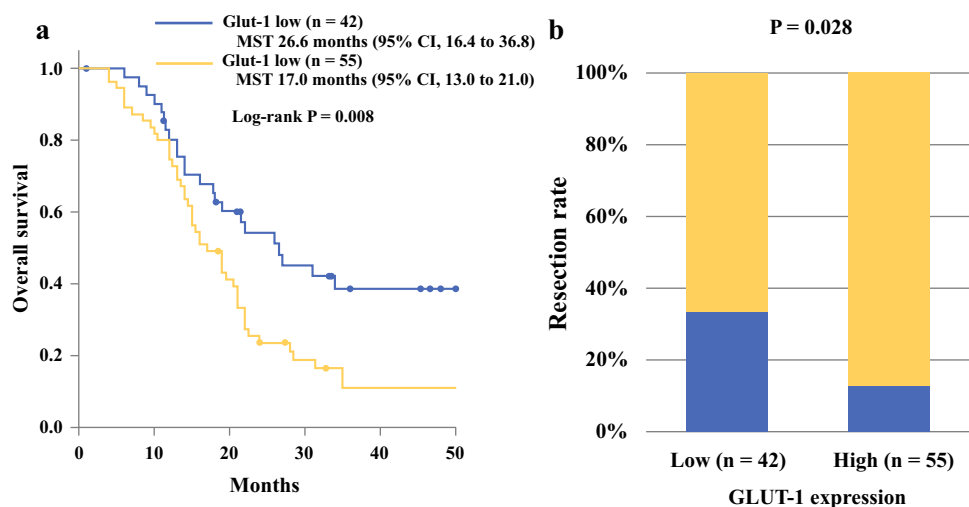
The effects of radiotherapy, due partly to radiation-induced radical and oxidative stress, can be reduced in tumor cells via the upregulation of their endogenous antioxidant

**TABLE 2** Association between clinical factors and the effect of neoadjuvant chemoradiation therapy (NACRT) ( $n = 97$ )

Factor ( $n$ )	Tumor size reduction rate after NACRT		$p$ value
	CR/PR ( $n = 54$ )	SD/PD ( $n = 43$ )	
Age (years)	< 75 ( $n = 69$ )	37	0.681
	$\geq 75$ ( $n = 28$ )	17	
Gender	Male ( $n = 53$ )	31	0.683
	Female ( $n = 44$ )	23	
Tumor position	Head ( $n = 68$ )	36	0.545
	Body-tail ( $n = 29$ )	18	
Tumor size (mm)	$\leq 30$ ( $n = 44$ )	20	0.101
	$> 30$ ( $n = 53$ )	34	
CA 19-9 (U/mL)	$\leq 37$ ( $n = 29$ )	17	0.874
	$> 37$ ( $n = 68$ )	37	
GLUT-1 expression	Low ( $n = 42$ )	16	0.005
	High ( $n = 55$ )	38	

CR complete response, PR partial response, SD stable disease, PD progressive disease, CA19-9 carbohydrate antigen 19-9, GLUT-1 glucose transporter type 1

**FIG. 3** Kaplan–Meier survival curves of overall survival (OS) from the initial treatment. **a** OS of all patients who underwent neoadjuvant chemoradiotherapy (NACRT). Patients with low glucose transporter type 1 (GLUT-1) expression showing better OS than those with high GLUT-1 expression. **b** Resection rate after NACRT according to GLUT-1 expression. The resection rate for low GLUT-1-expressing tumors was significantly higher than for high GLUT-1-expressing tumors ( $p = 0.028$ )



capacity through the accumulation of pyruvate and lactate caused by increased glycolysis in tumor cells.<sup>10</sup> Expression and localization of GLUT-1 are regulated by HIF-1 and mutant p53.<sup>10,13</sup> The current study demonstrated that GLUT-1 expression evaluated in EUS-FNA specimens is associated with the effectiveness of NACRT. The role of EUS-FNA in the diagnosis of pancreatic masses is increasing.<sup>27</sup> Furthermore, recent studies have demonstrated that immunohistochemical evaluations using EUS-FNA specimens can predict distant metastasis and prognosis for patients with PDAC.<sup>28,29</sup> Yamada et al.<sup>30</sup> used EUS-FNA specimens to demonstrate that human equilibrative nucleoside transporter expression can predict responses to gemcitabine-based CRT.

Immunohistochemical evaluation using EUS-FNA specimens is thought to be affected by intratumoral heterogeneity due to the small amount of tissue obtained from this procedure. It remains unclear how many tumor cells are required for adequate immunohistochemical evaluation. The current study used only EUS-FNA specimens that included more than 500 tumor cells. The concordance of the evaluation between EUS-FNA and surgically resected specimens of patients who underwent upfront surgery was 90%. For evaluation in EUS-FNA specimens, GLUT-1 appears to be a suitable candidate given its absence in the noncancerous pancreatic ducts and acinar cells. Furthermore, erythrocytes, which usually are included in EUS-FNA specimens, can be used as positive controls.

Eight (40%) of the patients who underwent surgical resection after NACRT showed a low-to-high change in GLUT-1 expression due to NACRT. However, none of the patients experienced a high-to-low change in GLUT-1 expression. One possible reason should be that subclones of high GLUT-1-expressing tumor cells had survived and proliferated during NACRT.

The current study showed no significant difference in prognosis according to changes in GLUT-1 expression. However, analysis of a larger sample is required to show the true prognostic impact of changes in GLUT-1 expression. A recent study demonstrated that inhibition of GLUT-1 expression by antisense oligodeoxynucleotides improved radiosensitivity in laryngeal carcinoma.<sup>11</sup> Therefore, GLUT-1 expression may be not only an important factor associated with the effect of NACRT but also a potent target for improving the therapeutic effect of NACRT in PDAC.

The current study had several limitations. First, given its retrospective nature, it had no defined criteria for resection after NACRT. Second, the number of patients who underwent surgical resection after NACRT was relatively small. A prospective study including a larger sample is required for further confirmation. Despite these limitations, the current study demonstrated that GLUT-1 expression was an independent prognostic factor for patients with PDAC who underwent upfront surgery. Furthermore, our findings suggest that the evaluation of GLUT-1 expression in EUS-FNA specimens before treatment can help to predict the effects of NACRT.

In conclusion, our findings demonstrated that among patients with PDAC, those with low GLUT-1 expression in the primary tumor have a better prognosis than those with high GLUT-1 expression. Moreover, patients with low GLUT-1 expression before treatment show better therapeutic responses to NACRT.

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