"Relationship between 18-F-fluoro-deoxy-D-glucose uptake and expression of glucose transporter 1 and pyruvate kinase M2 in intrahepatic cholangiocarcinoma."

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ABSTRACT

BACKGROUND: Cholangiocellular carcinoma is characterized by elevated glucose consumption, resulting in an increased uptake of 18F-2-fluoro-2-deoxy-d-glucose (18F-FDG). This study investigates the relationship between 18F-FDG uptake and tumour glucose metabolism. METHODS: This was a retrospective analysis of 19 patients with cholangiocellular carcinoma. Immunohistochemistry for glucose transporter 1 and pyruvate kinase type M2 were performed. Overall tumour glucose metabolism was evaluated by measuring 18F-FDG uptake and the protein expression levels of glucose transporter 1 and pyruvate kinase type M2. RESULTS: 18F-FDG uptake had a strong positive correlation with histological differentiation. Both tumour status (p=0.044) and tumour size (p=0.011) were correlated with primary tumour 18F-FDG uptake. Glucose transporter 1 expression correlated with histological differentiation (p=0.017), while pyruvate kinase type M2 expression tended to correlate with lymph node metastasis (p=0.051). Glucose transporter 1 expression was strongly related to the standard uptake value (p=0.001), but that of pyruvate kinase type M2 was not (p=0.461). CONCLUSIONS: Glucose transporter 1 expression exhibits a strong correlation with 18F-FDG uptake in cholangiocellular carcinoma tissue, while pyruvate kinase type M2 expression was not associated with fluoro-2-deoxy-d-glucose uptake. In addition to its glycolytic function, pyruvate kinase type M2 has a variety of roles and its expression may enhance tumour cell invasion and promote the lymph node metastasis of intrahepatic cholangiocarcinoma.

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Liver, Pancreas and Biliary Tract

Relationship between 18-F-fluoro-deoxy-\(d\)-glucose uptake and expression of glucose transporter 1 and pyruvate kinase M2 in intrahepatic cholangiocarcinoma

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**Abstract**

**Background:** Cholangiocellular carcinoma is characterized by elevated glucose consumption, resulting in an increased uptake of 18F-2-fluoro-2-deoxy-\(d\)-glucose (18F-FDG). This study investigates the relationship between 18F-FDG uptake and tumour glucose metabolism.

**Methods:** This was a retrospective analysis of 19 patients with cholangiocellular carcinoma. Immunohistochemistry for glucose transporter 1 and pyruvate kinase type M2 were performed. Overall tumour glucose metabolism was evaluated by measuring 18F-FDG uptake and the protein expression levels of glucose transporter 1 and pyruvate kinase type M2.

**Results:** 18F-FDG uptake had a strong positive correlation with histological differentiation. Both tumour status \((p = 0.044)\) and tumour size \((p = 0.011)\) were correlated with primary tumour 18F-FDG uptake. Glucose transporter 1 expression correlated with histological differentiation \((p = 0.017)\), while pyruvate kinase type M2 expression tended to correlate with lymph node metastasis \((p = 0.051)\). Glucose transporter 1 expression was strongly related to the standard uptake value \((p = 0.001)\), but that of pyruvate kinase type M2 was not \((p = 0.461)\).

**Conclusions:** Glucose transporter 1 expression exhibits a strong correlation with 18F-FDG uptake in cholangiocellular carcinoma tissue, while pyruvate kinase type M2 expression was not associated with fluoro-2-deoxy-\(d\)-glucose uptake. In addition to its glycolytic function, pyruvate kinase type M2 has a variety of roles and its expression may enhance tumour cell invasion and promote the lymph node metastasis of intrahepatic cholangiocarcinoma.

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1. Introduction

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary hepatic malignancy worldwide. ICC originates from the neoplastic transformation of cholangiocytes into intrahepatic tumours and has a poor prognosis with restricted treatment alternatives. Surgical resection is the only definitive treatment strategy for cholangiocellular carcinoma (CCC); however, local recurrence and metastasis are very common, and the 5-year survival rate is extremely low [1–4]. Previous studies attempted to identify useful prognostic factors, with some authors claiming that lymph node involvement is indicative of outcome [5–7]. Therefore, accurate staging including lymph node metastasis is essential for appropriate patient management [8].

As cancer cell growth is heavily dependent on glucose metabolism, the underlying pathways could have a considerable effect on the prognosis of patients with CCC. Glycolytic metabolism lies at the centre of cancer biology, thus, understanding the relationship between the 18F-fluorodeoxyglucose (FDG) uptake and glucose transporter 1 (Glut-1) and pyruvate kinase M2 (PKM2) expression is important. Positron emission tomography (PET) with 18F-FDG provides metabolic information and has been widely used for cancer diagnosis, staging, and detection of recurrence. 18F-FDG is transported into tumour cells by glucose transporter proteins.
present on the cell membrane; intracellularly, it is phosphorylated by hexokinase to FDG-6-phosphate, a highly polar molecule that cannot diffuse out of the cell. FDG-6-phosphate within the cancer tissue can then be visualized by PET. Measuring the increased 18F-FDG uptake on PET scans has been used in diagnostic imaging of patients with CCC. Cancer cell growth is heavily dependent on glucose metabolism as its major energy source and, as a result, the 18F-FDG uptake pattern on PET may be an indicator of tumour growth and prognosis [9–12]. Investigations of liver tumours have shown that FDG-PET is useful for tumour characterisation as well as assessing therapeutic responses and outcomes [13,14]. However, 18F-FDG uptake patterns are variable in patients with ICC.

18F-FDG uptake by tumour cells largely depends on the presence of facilitated glucose transporters, and glucose metabolism is regulated by the glucose uptake induced by these transporters. Among the mechanisms that contribute to the glycolytic phenotype, glucose transporter 1 (Glut-1) overexpression has been reported for a large variety of tumours [15–17]. As an example, Glut-1 expression is significantly correlated with 18F-FDG uptake in patients with CCC [18]. In patients with colorectal or gastric carcinoma, Glut-1 overexpression was associated with tumour aggressiveness and poor survival [19].

Most cancer cells primarily metabolise glucose by glycolysis even in the presence of ample oxygen, whereas most normal cells completely catabolise glucose via oxidative phosphorylation. In other words, tumour cells convert large amounts of glucose to lactate, even in the presence of oxygen. This unique aspect of tumour metabolism is called aerobic glycolysis or the Warburg effect [20,21]. Recent studies have shown that this effect is regulated by the key glycolytic enzyme, PKM2 [22]. In addition to its glycolytic functions, PKM2 can be translocated into the nucleus, where it activates the transcription of various genes by interacting with, and phosphorylating, specific nuclear proteins [23]. Yang et al. reported that the endothelial growth factor receptor (EGFR) induces PKM2 nuclear translocation and stimulates PKM2’s involvement in gene transcription regulation [24]. This suggests that the protein kinase activity of PKM2 may play a pivotal role in controlling cell proliferation.

In the present study, we sought to identify factors associated with patient outcome after surgical management of ICC. We also examined the association between 18F-FDG uptake and the expression of Glut-1 and PKM2 with a focus on cancer cell glucose metabolism.

2. Materials and methods

2.1. Patients

We performed a retrospective study of ICC patients who underwent curative surgery from April 2002 to December 2012 at the Department of General Surgical Science, Graduate School of Medicine, Gunma University, Japan. Patients who did not undergo preoperative 18F-FDG imaging were excluded. ICC was defined as adenocarcinoma arising from the second order or more distal branches of intrahepatic bile ducts, and all ICCs were pathologically confirmed. ICCs were further classified into two groups based on their possible cell of origin: mucin-producing with CC features (muc-ICC), and focal hepatocytic differentiation with ductular areas (mixed-ICC) [25]. Before liver resection, all patients underwent liver function tests and cancer antigen 19-9 (CA 19-9) assays. Preoperative evaluation was carried out with ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), and FDG-PET. In addition, some patients underwent endoscopic retrograde cholangio-pancreatography (ERCP) and/or percutaneous transhepatic biliary drainage (PTCD) for diagnosis and/or biliary decompression. ICC was classified into three categories according to the macroscopic typing proposed by the Liver Cancer Study Group of Japan [26]: mass-forming (MF), periductal-infiltrating (PI), or intraductal-growth (IG). When more than one type was found, the predominant type was recorded.

Contraindication for surgery was determined as follows: (1) distant metastasis, (2) peritoneal dissemination, (3) multiple paraaortic lymph node metastases, (4) extensive vascular involvement and/or multiple intrahepatic metastases, or (5) severe liver cirrhosis. Suspected regional lymph node metastasis on CT and/or MRI was not a contraindication for surgery. Curative resection was defined as total excision of the entire tumour including the primary tumour and associated lymph node. Staging was performed according to the pTNM classification of the International Union Against Cancer [27].

Standard demographic and clinicopathological data were collected, including age, gender, and primary tumour characteristics. Also, tumour-related data were collected, including tumour location, size, number, macroscopic type, pathological tumour staging, lymph node metastases, tumour differentiation, and surgical margin status. Data on treatment-related variables, such as type of surgery, resection of extrahepatic bile duct, operation time, and blood loss, were also recorded.

2.2. FDG-PET study

The detailed methods for PET scanning in our institution have been previously described [28]. Briefly, the PET study was carried out using a Discovery STE (GE Healthcare, Waukesha, WI, USA) and Biograph 16 (Siemens Medical Solutions, Malvern, PA, USA) scanners, with a 700-mm field of view (FOV) and slice thickness of 3.27 mm. Prior to FDG-PET scanning, patients fasted for at least 6 h, and 200–250 megabecquerel (MBq) 18F-FDG were injected intravenously. Whole-body PET-CT images were obtained 60 min after 18F-FDG administration. The emission scan started from the skull base and continued to the upper thigh in 2-dimensional mode 50–60 min after the injection. The PET scans were compared with the corresponding CT images for accurate tumour localization. The coronal, sagittal, and axial images of the patients were qualitatively evaluated to determine whether tumour 18F-FDG uptake was higher than that in the surrounding non-cancerous hepatic tissue. The standardized uptake value (SUV) was defined as the concentration of radioactivity in the tissue or lesion (MBq/g) × patient body weight (g)/injected dose (MBq).

2.3. Immunohistochemistry

The resected surgical specimens were fixed with 10% formaldehyde, embedded in paraffin blocks, cut into 4-μm-thick sections, and mounted on glass slides. The staining procedure was performed using standard streptavidin-biotin peroxidase complex (S-ABC) methods. All sections were incubated at 60 °C for 60 min, deparaffinized in xylene, rehydrated, and incubated with fresh 0.3% hydrogen peroxide in 100% methanol for 30 min at room temperature to block endogenous peroxidase activity. After rehydration through a graded series of the ethanol treatments, antigen retrieval was carried out in 10mM citrate buffer (pH 6.4) at 98–100 °C for 20 min, and sections were passively cooled to 30 °C. After rinsing the section in 0.1 M phosphate-buffered saline (PBS, pH 7.4), non-specific binding sites were blocked by incubation with 10% normal rabbit or goat serum for 30 min. The sections were incubated with a rabbit anti-PKM2 polyclonal antibody (Signalway Antibody, College Park, MD, USA) at a 1:200 dilution, and Glut-1 (AB15309; Abcam, Cambridge, UK) at a 1:400 dilution in PBS containing 1%
bovine serum albumin overnight at 4 °C, and then at room temperature for 30 min. The sections were washed in PBS, incubated with biotinylated anti-rabbit IgG, A, and M solution (Nichirei Co., Tokyo, Japan) for 30 min at room temperature, and finally incubated in a streptavidin–biotin peroxidase complex solution (Nichirei Co.) for 30 min. The chromogen 3, 3′-diaminobenzidine tetrahydrochloride was applied as a 0.02% solution containing 0.005% hydrogen peroxide in a 50 mM ammonium acetate–citrate acid buffer (pH 6.0). The sections were lightly counterstained in Mayer’s haematoxylin and mounted.

Intravascular red cells, which stained strongly in all tissue sections, served as internal controls. Glut-1 immunoreactivity was cytoplasmically localized in cancer cells, and the staining intensity and number of positive cells were scored for each specimen. The percentage of Glut-1-positive cells was rated using a semiquantitative scale as 0–10% or 11–100%, considered as low or high expression, respectively (Fig. 1).

The proportion of tumour cells was classified as follows based on staining intensity: 0–25% (low expression) or >25% (high expression) of PKM2-positive tumour cells (Fig. 2). The epithelial membrane antigen (EMA, MUC1) was used to classify Muc-ICC and Mixed-ICC according to what reported in a previous publication [25].

### 2.4. Statistical methods

Statistical computations were performed with the JMP software (SAS Institute, Cary, NC, USA). Continuous variables are expressed as medians and were compared using the Wilcoxon test, whereas categorical variables were compared using the Fisher’s exact or chi-square test. The Kaplan–Meier method was used to analyse overall survival, and the log-rank test was used to assess differences in survival. Statistical significance was defined as p < 0.05. For disease-specific survival, only deaths attributable to recurrent cancer were considered as events. Patients who died from secondary causes without recurrence were censored.

![low expression of Glut-1](image1)

![high expression of Glut-1](image2)

**Fig. 1.** Immunohistochemistry for glucose transporter 1 in cholangiocellular carcinoma. Glucose transporter 1 expression in representative areas of tumours in the negative and the positive. Sections were immunolabeled using a glucose transporter 1 antibody and an avidin-biotin technique. Magnification ×400.

![low expression of PKM2](image3)

![high expression of PKM2](image4)

**Fig. 2.** Immunohistochemistry for pyruvate kinase M2. Low and high pyruvate kinase M2 expression in representative areas of tumour tissue. Tumours with more than 25% pyruvate kinase M2-positive cells were considered positive, and those with less than 25% pyruvate kinase M2-positive cells were considered negative. Glut-1, glucose transporter 1.
Table 1
Patient demographics, clinicopathologic characteristics and surgical results.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of patients (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;70</td>
<td>7 (36.8)/12 (63.2)</td>
<td>0.354</td>
</tr>
<tr>
<td>≥70</td>
<td>10 (57.9)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>12 (63.2)/7 (36.8)</td>
<td>0.759</td>
</tr>
<tr>
<td>Location</td>
<td>9 (47.4)/10 (52.6)</td>
<td>0.069</td>
</tr>
<tr>
<td>Right/left</td>
<td>10 (52.6)/9 (47.4)</td>
<td>0.921</td>
</tr>
<tr>
<td>Macroscopic tumour growth type</td>
<td>3 (15.8)/11 (57.9)/5 (26.3)</td>
<td>0.147</td>
</tr>
<tr>
<td>Histological classification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muc-ICC/Mixed-ICC</td>
<td>12 (63.2)/7 (36.8)</td>
<td>0.241</td>
</tr>
<tr>
<td>Maximum diameter (mm)</td>
<td>11 (57.9)/8 (42.1)</td>
<td>0.135</td>
</tr>
<tr>
<td>T1, T2/T3/T4</td>
<td>8 (42.1)/11 (57.9)</td>
<td>0.036</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>13 (68.4)/6 (31.6)</td>
<td>0.453</td>
</tr>
<tr>
<td>Stage</td>
<td>12 (63.2)/7 (36.8)</td>
<td>0.299</td>
</tr>
<tr>
<td>I, II, III, IV</td>
<td>8 (42.1)/11 (57.9)</td>
<td>0.301</td>
</tr>
<tr>
<td>Lobectomy</td>
<td>10 (52.6)/9 (47.4)</td>
<td>0.007</td>
</tr>
<tr>
<td>Resection of extrahepatic bile duct</td>
<td>14 (73.7)/5 (26.3)</td>
<td>0.114</td>
</tr>
<tr>
<td>Radicility</td>
<td>3 (15.8)/16 (84.2)</td>
<td>0.017</td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>6 (31.6)/13 (68.4)</td>
<td>0.907</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>8 (42.1)/11 (57.9)</td>
<td>0.090</td>
</tr>
<tr>
<td>CA19-9 (U/ml)</td>
<td>11 (57.9)/8 (42.1)</td>
<td></td>
</tr>
<tr>
<td>Glut-1 expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PKM2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CA19-9: carbohydrate antigen 19-9; IG: intraductal growth type; MF: mass-forming type; PI: periductal infiltrating type; Muc-ICC: mucin producing ICC; Mixed-ICC: ICC with mixed features; ICC: intrahepatic cholangiocarcinoma; SUV: standardized uptake value

3. Results

Patient demographics and surgical results are shown in Table 1. The 19 patients who underwent surgical resection with curative intent were 12 males and 7 females with a median age of 70 years (range 57–80 years). The CA19-9 levels were elevated in 9 patients (47%). With regard to the macroscopic findings of the excised tumour, 11 (57.9%), 5 (26.3%), and 3 (15.8%) were MF, PI, and IG type, respectively. The median tumour size was 4.8 cm (range 2.8–8.5 cm). Histological examination revealed poorly differentiated carcinoma in 6 cases (31.6%), well-differentiated or moderately differentiated carcinoma in 13 cases (68.4%). Based on the cell-of-origin classification, 12 ICCs were diagnosed as Muc-ICCs (63.1%), and 7 were Mixed-ICCs (36.8%). Among the 19 cases, 7 had affected lymph nodes (36.8%). More than half of the patients (57.9%) were diagnosed with advanced stage III or IV cancer. With regard to preoperative FDG-PET scanning, the detection rate for ICC was 89.4% (17/19), and the mean tumour SUV was 6.0 (range 1.5–14.5). A lobectomy was performed in 14 cases (73.7%), and curative resection was achieved in 7 patients (26.3%). Extrahepatic bile duct resection was performed in 3 patients (15.8%). Major lobectomy significantly improved the 3-year overall survival (51.4% vs. 0%; p = 0.007). When R0 resections were compared with R1/R2 resections, curative resection also significantly improved the 3-year overall survival (83.3% vs. 18.3%, p = 0.017; data not shown). The patients with muc-ICC tended to have poorer prognoses compared to those with mixed-ICC.

3.1. Relationship between clinicopathological data and SUV in ICC

The two criteria for correct detection by PET/CT are positive FDG uptake and correct anatomical location of the tumour. ICC was detected in 17/19 patients by PET/CT and 15/19 patients by CT. As shown in Table 2, there was no significant relationship between tumour SUV and macroscopic type, histological classification, CA19-9 level, lymph node metastasis, tumour grade, or curability. However, primary tumour SUV was inversely correlated with histological differentiation (p = 0.004). Moreover, both tumour status (p = 0.026) and size (p = 0.011) were also correlated with primary tumour FDG uptake.

3.2. Correlations between Glut-1 expression and clinicopathological factors

We examined Glut-1 expression in representative negative and positive tumour areas. Sections were immunolabeled with a glucose 1 antibody using the avidin-biotin technique. Glut-1 immunolabling was positive in 13 (68.4%) of the 19 primary lesions. Table 3 shows the comparative analysis between Glut-1 immunohistochemical findings and clinicopathological characteristics. Glut-1 expression was correlated with histological differentiation (p = 0.017), but there were no significant relationships between Glut-1 expression and other clinicopathological characteristics including maximum diameter, lymph node metastasis, and stage. However, 83% (10/13) of muc-ICCs had positive Glut-1 expression, suggesting a possible association between muc-ICC and Glut-1 expression.

3.3. Correlations between PKM2 expression and clinicopathological factors

The PKM2 protein was detected in 11 cases (57.9%). As shown in Fig. 2, PKM2 was mainly localized in the cytoplasm of primary
Table 3
Correlations between glucose transporter 1 expression, pyruvate kinase type m2 expression and clinicopathological factors.

<table>
<thead>
<tr>
<th>Characteristics (%)</th>
<th>Glut 1 expression</th>
<th>PKM2 expression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative (n = 6)</td>
<td>Positive (n = 13)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;70 (36.8)</td>
<td>2/4</td>
<td>5/8</td>
</tr>
<tr>
<td>≥70 (63.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (63.2)/Female (36.8)</td>
<td>5/1</td>
<td>7/6</td>
</tr>
<tr>
<td>CA19-9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;37 (52.6)/≥37 (47.4)</td>
<td>4/2</td>
<td>6/7</td>
</tr>
<tr>
<td>Histological classification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muc-ICC (63.2)/Mixed-ICC (47.4)</td>
<td>2/4</td>
<td>10/3</td>
</tr>
<tr>
<td>Maximum diameter</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40 (57.9)/≥40 (42.1)</td>
<td>5/1</td>
<td>6/7</td>
</tr>
<tr>
<td>Histologic grading</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well or moderate (68.4)/poor (31.6)</td>
<td>6/0</td>
<td>7/6</td>
</tr>
<tr>
<td>T stage (TNM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1, T2 (42.1)/T3, T4 (57.9)</td>
<td>4/2</td>
<td>4/9</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent (63.2)/Present (36.8)</td>
<td>5/1</td>
<td>7/6</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I, II (42.1)/III, IV (57.9)</td>
<td>2/4</td>
<td>6/7</td>
</tr>
<tr>
<td>SUV max</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;4 (52.6)/≥4 (47.4)</td>
<td>6/0</td>
<td>4/9</td>
</tr>
</tbody>
</table>

Glut-1, glucose transporter 1, PKM2, pyruvate kinase type M2, CA19-9, carbohydrate antigen 19-9, Muc-ICC, mucin producing ICC, Mixed-ICC, ICC with mixed features, ICC, intrahepatic cholangiocarcinoma, SUV, standardized uptake value.

Fig. 3. (a) Relationship between [18F]-2-fluoro-2-deoxy-D-glucose uptake and glucose transporter 1 expression in cholangiocellular carcinoma. A statistically significant difference in uptake was noted between glucose transporter 1-negative and -positive cells. PKM2, pyruvate kinase type M2. (b) Relationship between [18F]-2-fluoro-2-deoxy-D-glucose uptake and pyruvate kinase M2 expression in cholangiocellular carcinoma. No significant difference was observed between pyruvate kinase M2-negative and -positive cells. FDG, [18F]-2-fluoro-2-deoxy-D-glucose, SUV, standardized uptake value.

cancer cells. We examined whether PKM2 protein upregulation was linked to the clinical characteristics of ICC (Table 3). There was no correlation between PKM2 expression and maximum diameter, histological grading, T stage, or stage. However, PKM2 expression tended to correlate with lymph node metastasis (p = 0.051). Overall, 85.8% of patients with lymph node metastases had PKM2-positive tumours.

3.4. 18F-FDG uptake in relation to Glut-1 and PKM2 expression

In surgically resected masses, FDG uptake within the primary masses was compared with Glut-1 and PKM2 immunolabelling. We observed that Glut-1-positive tumours had higher SUVs than Glut-1-negative tumours (Fig. 3a). However, PKM2 expression was not correlated with SUV (Fig. 3b), and no significant correlation was observed between Glut-1 and PKM2 expression (p = 0.637, data not shown).

4. Discussion

The present study showed that FDG uptake is associated with tumour expression of Glut-1 but not PKM2. However, PKM2 tended to correlate with lymph node metastasis in patients with ICC. Collectively, these results suggest that PKM2 plays a pivotal role in balancing glucose metabolism and cellular growth.

ICC is associated with poor prognosis, and newly diagnosed patients are frequently found to have ICC that has progressed beyond surgery. The resectability of ICC remains low because of its early metastasis and advanced stage when diagnosed [29]. The postoperative 5-year survival rates have been reported to range from 20% to 30% [8]. Surgery for ICC often requires extended hepatic resection. We performed major hepatic resections in 73.4% (14/19) of the cases included in the present study. The patients who underwent major hepatectomy had a better prognosis than those who did not (p = 0.007), and lobectomy often increased the rate of radical resection (R0). We achieved radical resection in 31.6% of patients, with a median survival after R0 resection of 79 months compared to 18 months for R+ patients (p = 0.017). Alfredo et al. also reported that Curative resection of ICC is the only therapy that can achieve long-term survival [5]: they achieved curative resection (R0) in 83% of the patients and performed a major hepatic resection in 63%. On the other hand, it is difficult to make a prediction of the ICC patients with a better prognosis, because the unfavourable prognosis of ICC may be attributable to a lack of clear clinical signs and symptoms; therefore, it is important to identify predictive factors. Moreover,
as cancer cell growth is heavily dependent on glucose as a major energy substrate, it is important to examine glucose metabolism in patients with ICC.

A better understanding of the association between FDG uptake and clinicopathological features will provide more information. We found a significant correlation between several tumour characteristics and FDG uptake. Specifically, tumour differentiation was strongly correlated with FDG uptake. Adler et al. reported that breast cancer patients with poorly differentiated tumours have significantly higher FDG uptake than patients with well or moderately differentiated tumours [30]. In line with this, we also observed fewer differentiated tumours with higher FDG uptakes, suggesting that FDG uptake allows indirect assessment of the differentiation degree in patients with ICC. Moreover, FDG uptake was strongly correlated with tumour size (p = 0.011). A significant relationship between tumour size and SUV has been reported in many studies and various organs [31]. The FDG-PET SUV determined on the basis of Ki67 expression plays an essential role in assessing the proliferative status of breast cancer [12]. A high growth rate requires increased glucose intake, and a large tumour could exhibit increased FDG uptake. Unlike other cancer types, we found no correlation between the SUV and regional lymph node metastasis or stage for ICC. This may be due to the limited number of patients in this series. However, we did observe a significant correlation between SUV and lymph node metastasis with regard to mass-forming type (data not shown).

We found a wide range of Glut-1 expression in ICC surgical specimens. We divided the tumours into two groups based on low (<10%) or high (≥10%) numbers of Glut-1-positive cells in the tumour sample, and found a significant correlation between Glut-1 expression and histological grading. Moreover, Glut-1 expression was strongly correlated with the SUV of the primary tumour. In this study, positive Glut-1 expression was found in all patients with poorly differentiated carcinoma. Less differentiated tumours are surrounded by a hypoxic microenvironment, and hypoxia has the potential to inhibit tumour cell differentiation [32]. Moreover, low oxygen levels can influence the cellular phenotypes by altering the expression of specific genes and are generally thought to give cancer cells an advantage by promoting factors considered beneficial for tumour growth and survival [33]. Hypoxia inducible factor 1 (HIF-1) is overexpressed in low-oxygen environments and is the primary transcription factor mediating several physiological and biological changes, including Glut-1 overexpression. Alok et al. also reported that hypoxia upregulates Glut-1 expression in vitro [34]. Hypoxia increases Glut-1 expression, which would result in increased FDG uptake. In this study, Glut-1 expression was strongly correlated with the maximum SUV (p = 0.001). Glut-1 expression is significantly and positively correlated with SUV in colorectal cancer [31]. 18F-FDG uptake in malignant tumours largely depends on the presence of facilitated glucose transporters including Glut-1 and hexokinase type II (HKII) [35]; glucose phosphorylation by HKII, which is downstream of Glut-1, is also important. On the other hand, Alok and colleagues insisted that FDG uptake correlates better with the FDG-phosphorylating activity of mitochondrial preparations rather than the expression of Glut-1 or hexokinase I and II genes in vitro [34]. This suggests that there may be differences among cancers originating from different tissues with regard to glucose metabolism, such as glucose uptake, glucose transporter rates, and hexokinase activity in the glycolytic pathway.

Tumour cells convert large amounts of glucose to lactate, even in the presence of oxygen, as opposed to large amounts of ATP synthesis. This phenomenon of aerobic glycolysis has been termed the Warburg effect [20]. In the glycolytic process, pyruvate kinase (PK) catalyses the last reaction, which is the transfer of a high-energy phosphate group from phosphoenolpyruvate to ADP, yielding ATP and pyruvate. PKM2 is the predominant PK in proliferating cancer cells [36], and PKMS-expressing cells produce more lactate and consume less oxygen than cells expressing other PKs. Rapidly growing cells must take up glucose at a high rate and maintain a balance between energy production (e.g., ATP synthesis) and anaerobic processes such as protein, lipid, and nucleic acid synthesis. PKM2 plays a critical role in aerobic glycolysis and allows proliferating cells to regulate their anabolic and catabolic metabolism needs [37]. In this study, we could not demonstrate a correlation between PKM2 and Glut-1 expression; however, PKM2 has a nonglycolytic function in addition to its role in glycolysis. PKM2 is found in both the cytoplasm and nucleus, where it is associated with chromatin [36]. Yang et al. reported that PKM2 nuclear translocation, facilitated by EGFR activation, promotes β-catenin transactivation, leading to increased expression of cyclinD1 and c-Myc [24]. In our study, we identified translocation from the cytoplasm to the nucleus in the ICC cell line. In addition to the regulation in the glycolytic process, PKM2 is subject to complex regulation both by oncogenes and a tumour suppressor [23].

We found a tendency towards a correlation between PKM2 expression and lymph node metastasis, which is regarded as an important prognostic factor for patients with ICC [8]. Park showed that vascular endothelial growth factor-C (VEGF-C) expression in cancer cells correlated with lymph node metastasis in ICC [38], and another group demonstrated increased matrix metalloproteinase-9 expression in ICC with lymphatic metastasis [39]. On the other hand, several studies have described the nonglycolytic functions of PKM2, e.g., it directly interacts with HIF-1 and promotes transactivation of HIF-1 target genes [40]. The PKM2 translocated into the nucleus in response to the epidermal growth factor (EGF) associates with β-catenin, and this complex leads to increased expression of cyclin D1 and c-Myc [24]. Nuclear PKM2 also acts as a protein kinase that can phosphorylate signal transducer and activator of transcription 3 (STAT3) and thereby activate the transcription of cancer-relevant genes [41]. Collectively, the evidence suggests that high PKM2 expression might be a key factor contributing to tumour cell invasion and metastasis, including lymph node metastases. Kimura et al. showed that HIF-1α expression is associated with vascular endothelial growth in human oesophageal squamous cell carcinoma [42] and plays a role in lymphatic invasion and lymph node metastasis through the induction of VEGF-C in oesophageal cancer. These findings are consistent with our results, which suggest that PKM2 might promote lymph node metastasis of ICC through cancer-relevant proteins such as HIF-1, STAT3, and β-catenin.

The cell-of-origin-based ICCs classification has shown the clinicopathological difference between Muc-ICCs and Mixed-ICCs [25]. Briefly, Muc-ICCs showed a more aggressive behaviour than Mixed-ICCs. Likewise, our study showed that the median survival time of 18.2 months in patients with muc-ICCs was shorter than the 98.7 months in patients with mixed-ICCs. In addition, patients with muc-ICCs tended to have poorer prognoses compared with patients with mixed-ICCs. However, there were no significant differences between the two groups. This may be also due to the small number of cases included. Nevertheless, 83% of patients with muc-ICCs showed positive Glut-1 expression. Glut-1 expression is increased under hypoxic conditions, and hypoxia is related to the inhibition of tumour cell differentiation. The patients with muc-ICC included 5 cases of poorly differentiated adenocarcinoma. Unfortunately, our data do not demonstrate a direct link between Glut-1 expression and muc-ICC. Further studies are needed to show a more convincing association.

In this study, Glut-1, but not PKM2 expression, strongly correlated with FDG uptake. PKM2 plays a variety of roles in addition to its glycolytic function, and may enhance tumour cell invasion and promote lymph node metastasis of ICC. However, further studies are required to clarify the energy metabolism and 18F-FDG uptake
patterns in association with various oncogenic alterations that regulate glycolytic pathways, cancer proliferation, and tumourigenesis.

Conflict of interest
None declared.

References