Clinical implication of glucose transport and metabolism evaluated by ^{18}F-FDG PET in hepatocellular carcinoma

BISHNUHARI PAUDYAL[1], PRAMILA PAUDYAL[1,2], NOBORU ORIUCHI[1], YOSHITO TSUSHIMA[1], TAKASHI NAKAJIMA[2] and KEIGO ENDO[1]

Departments of [1]Diagnostic Radiology and Nuclear Medicine, and [2]Tumour Pathology, Gunma University Graduate School of Medicine, Maebashi, Gunma 371-8511, Japan

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Abstract. Hepatocellular carcinoma (HCC) has variable ^{18}F-fluoro-2-deoxy-D-glucose (^{18}F-FDG) uptake and the relationship between ^{18}F-FDG uptake with the expression of glucose transporters (Gluts) and hexokinase II (HK-II) has not been extensively examined. Present study explored the role of ^{18}F-FDG positron emission tomography (PET) as a clinical significance and the association with Gluts and HK-II in patients with HCC. Whole body ^{18}F-FDG PET, immunohistochemistry and Western blot analysis of Glut-1 to Glut-5 and HK-II were performed in 31 patients (24 male and 7 female, range 48-75 years) with HCC. Significant correlation was found between ^{18}F-FDG uptake and overall expression of Glut-2 (ϱ=0.55, p=0.002) and HK-II (ϱ=0.37, p=0.04). Expression of HK-II was correlated with Glut-2 (ϱ=0.57, p=0.0009) but not with other Gluts, which indicated that Glut-2 is a major glucose transporter. The prognosis of patients with SUV ≥2 and positive Glut-2 were significantly worse than that with SUV <2 and negative Glut-2 (p=0.005 and p=0.03), respectively. Multivariate analysis showed that SUV and lymph node metastasis were independent prognostic factors. The present study indicated that combined evaluation of ^{18}F-FDG uptake and expression of Glut-2 might have an important role for management of patients with HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers of the world and causes about one million deaths annually (1). HCC is a primary liver tumour, which accounts for ~90% of all primary liver malignancy. The common risk factors for the development of HCC are chronic liver diseases caused by hepatitis B and hepatitis C viral infection (2).

Positron emission tomography (PET) using ^{18}F-fluoro-2-deoxy-D-glucose (^{18}F-FDG) is a reliable diagnostic imaging tool for various kinds of malignancy, allowing for functional assessment of tumour (3-6). Increased glucose uptake is found in malignant tumours because of increased levels of both glucose transporters (Gluts) and intracellular enzymes such as hexokinase and phosphofructokinase that promote glycolysis. The cellular concentration of ^{18}F-FDG in tumour represents the glycolytic activity of viable tumour cells, however, the detection of primary HCC was less successful because of variable ^{18}F-FDG uptake (7).

Hexokinase (HK) is the first enzyme of glycolytic pathway which converts hexose to hexose-6-phosphate (8). Among the four types of mammalian hexokinase (HK-II to V), HK-II is predominantly overexpressed and promotes gene amplification in HCC (9). The facilitative transport of glucose is mediated by the different isoforms of Gluts that are expressed in cancer cells. They exhibit distinct kinetic and regulatory properties that reflect their specific functional roles (10).

Glut-1 is believed to have ubiquitous distribution in most cell types, however, previous studies reported that 87 out of 154 malignant human neoplasms did not show detectable Glut-1 indicating that other Gluts may mediate glucose uptake in these tumours (11-13). Glut-2 is a high Michaelis constants (*K*m) isoform which is expressed in normal liver and different cancers (10,13-15). Glut-3 is a low *K*m isoform responsible for glucose uptake into neurons and detected in gastrointestinal and brain tumours (13). Breast cancer, gastric cancer and lung cancer showed expression of Glut-4. Glut-5 was expressed in lung and breast cancer. The expression of Gluts is variable in different cancers (10-15).

Although many studies have focused on the expression of Gluts to define their role in the regulation of ^{18}F-FDG uptake in various malignancies, few studies have been done on HCC where ^{18}F-FDG PET was found to be effective for assessing histological grading, tumour viability and recurrence after therapy (6,7,16). Increased ^{18}F-FDG uptake relates to the more aggressive biologic properties and poor survival than those with low ^{18}F-FDG uptake in HCC (17). Although the

Correspondence to: Dr Paudyal Bishnuhari, Department of Diagnostic Radiology and Nuclear Medicine, Gunma University Graduate School of Medicine, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan
E-mail: paudyal@med.gunma-u.ac.jp

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prognostic value of ^{18}F-FDG PET in predicting the outcome of HCC has been reported (18,19), no study has been done to assess expression of different Gluts and HK-II in association with ^{18}F-FDG uptake as a prognostic biomarker in HCC. The aims of this study were to explore the association of ^{18}F-FDG uptake with the expression of Gluts and HK-II and to evaluate their role in clinical implication in patients with HCC.

Patients and methods

Patients. This study was performed on 31 patients (24 male and 7 female, range 48-75 years) with HCC who were examined between March 1999 and March 2007 in our university hospital. ^{18}F-FDG PET, computed tomography (CT) and magnetic resonance imaging (MRI) were performed on all patients before surgery. The time interval between ^{18}F-FDG PET and treatment was 1-4 weeks (mean 2.2 weeks). Surgical specimens were collected for the histological diagnosis, immunohistochemistry and Western blotting. The haematoxylin and eosin staining confirmed the diagnosis of HCC and specimens having most viable malignant components were chosen for immunohistochemical staining. The pathologic findings were classified according to the 6th edition of the Tumour, Lymph Node and Metastasis (TNM) Classification of the International Union Against Cancer (20). This study was conducted in accordance with the Helsinki Declaration and approved by the Institutional Review Board of our university and informed consent was obtained from all patients participating in this study.

^{18}F-FDG PET study. Patients fasted for at least six hours; then blood samples were collected for serum glucose measurement. Glucose level in all patients was <120 mg/dl at the time of the ^{18}F-FDG injection. ^{18}F-FDG was synthesized by the method of Hamacher *et al* (21). Data acquisition was performed 60 min after the injection of 5-6 MBq/kg body weight of ^{18}F-FDG by the simultaneous emission-transmission method with a rotating external source ^{68}Ge for absorption correction. PET study was performed using a SET2400W PET scanner (Shimadzu Corp., Japan) with a 59.5-cm transaxial field of view and a 20-cm axial field of view. Four to five bed positions from the head to the thigh were imaged for 8 min per position. The scanner produced 63 image planes, spaced 3.125 mm apart. Transaxial spatial resolution was 4.2 mm full width half-maximum (FWHM) at the center of the field of view and axial resolution was 5.0 mm FWHM. The attenuation-corrected transaxial images were reconstructed by the ordered subsets expectation maximization (OSEM) algorithm into a 128x128 matrix with pixel dimensions of 4.0 mm in a plane and 3.125 mm axially. Finally, every three consecutive slices of 9.8-mm thick transaxial and coronal images were generated for visual interpretation and quantitative analysis, using the standardized uptake value (SUV) as described previously by us (14).

Image analysis. ^{18}F-FDG PET images were interpreted by two experienced nuclear physicians in conjunction with the CT or MRI. They evaluated the uptake of the tracer in the lesions as compared with the surrounding normal structures. In order to evaluate the ^{18}F-FDG uptake in the tumour, either 4x4 pixel square regions of interest (ROIs) were placed on the tumour or the ROI were manually defined, depending on the shape of the tumour, including the area of the highest activity, but not covering the entire tumour. Positive lesions were identified if the uptake of ^{18}F-FDG in the tumour was higher than that in the background of the liver.

Antibodies. Polyclonal rabbit anti-glucose transporter antibodies reactive with the carboxy-terminus of Glut-1 (Dako, Carpinteria, CA, USA), Glut-2 (Chemicon International, Temecula, CA, USA), Glut-3 (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), Glut-4 (Santa Cruz Biotechnology) and Glut-5 (Chemicon International) were diluted at 1:1000, 1:1000, 1:500, 1:200, 1:100, respectively. HK-II expression was studied with a polyclonal rabbit anti-HK-II antibody reactive with C-terminal sequence of rat HK-II diluted 1:3000 (Chemicon International).

Western blot analysis. Western blotting was performed as described previously by Shibata *et al*, with slight modification (22). The normal liver tissues and HCC were homogenized with phosphate buffered saline containing protease inhibitor cocktail (Sigma, St Louis, MO). Normal liver tissue was obtained from surgical excisions of a patient with liver metastasis of colon cancer. Protein concentration of 1:50 diluted sample was measured with Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, USA). Samples (10 μg) were separated on 10% SDS-polyacrylamide gel and then transferred into polyvinylidene difluoride membranes at 150 mA for 1 h. After blocking with 5% skim-milk, the membranes were incubated with the primary antibodies (Glut-1 to Glut-5 and HK-II) for 2 h at room temperature. The bound antibodies were detected with horseradish peroxidase-labeled rabbit anti-goat IgG (Jackson Immuno Research Laboratories, West Grove, PA). Following the extensive washes with Tris-buffered saline with Tween-20, the membranes were visualized using the enhanced chemiluminescence detection system (ECL kit; Amesrsham International, Buckinghamshire, UK).

Immunohistochemical staining for Gluts and HK-II. Immunohistochemical staining of the sections for Glut-1 to Glut-5 and HK-II were performed as we described previously (14). Specimens were deparaffinized, rehydrated and incubated with 10% hydrogen peroxide for 10 min to block the endogenous peroxidase activity. After antigen retrieval method using a microwave oven in a 0.01 mol/l citrate buffer (pH 6.0) at 98˚C for 15 min, blocking treatment was performed by normal goat serum for 30 min. The sections were incubated with the primary polyclonal antibody as described above, with 1% bovine serum albumin at 4˚C overnight. Signals were detected using an En Vision Kit (Dako) and diaminobenzidine tetrahydrochloride. Negative controls were prepared by substituting normal mouse serum for each primary antibody.

Quantitative evaluation of immunohistochemical staining. The evaluation of immunohistochemical staining was performed as we described previously (14). In brief, the intensity, cellular pattern of staining and number of positive cells were

recorded for every specimen. The percentages of positively stained cells were rated using a semiquantitative scale as 0-10, 11-50 and 51-100%. The intensity of the staining was graded as negative, weak and strong. The staining result was scored from 0 to 4 according to the intensity and percentage of positively stained cells. All stained sections were reviewed under a double-headed light microscope by two experienced pathologists who had no prior knowledge of the clinical outcome.

Statistical analysis. Data were expressed as mean ± standard deviation. Spearman's rank correlations were analyzed between ^{18}F-FDG uptake and expression of Gluts and HK-II. Difference of the variables was evaluated by the Kruskal-Wallis test and Mann-Whitney's non-parametric test. The probability of survival rate was analyzed using the Kaplan-Meier method and the log-rank test was used for comparison. The survival time was defined from the date of diagnosis until the death or last follow-up. The Cox's proportional hazard model for the risk ratio was used to assess survival in univariate and multivariate analysis. Probability values of $p<0.05$ were considered statistically significant. All statistical calculations were performed using the StatView version 5.0 (SAS Institute, NC, USA).

Results

^{18}F-FDG uptake in relation to clinicopathology. ^{18}F-FDG uptake and clinicopathological variables were analyzed by univariate analysis as summarized in Table I. ^{18}F-FDG uptake was significantly higher in poorly differentiated HCC (n=7, 3.83±2.01) and moderately differentiated (n=15, 2.15±1.68) than well differentiated HCC (n=9, 0.89±0.62). A significant correlation was observed between ^{18}F-FDG uptake and tumour size (p=0.004), histological differentiation (p=0.024), T status (p=0.022), lymph node metastasis (p=0.005), distant metastasis (p=0.009) and tumour grade (p=0.013), whereas it was not significant with age, gender or tumour location.

Immunohistochemical staining of Gluts and HK-II in HCC. Fig. 1 shows a representative case of HCC with ^{18}F-FDG PET, corresponded CT and immunohistochemical staining of Glut-1, Glut-2 and HK-II. Twenty-two out of 31 cases (71%) showed positive (weak and strong) expression of Glut-2 (staining score 1.74±1.46, percentage of positive cells 39.36±30.21%). Glut-2 was expressed mainly in plasma membrane and some in cytoplasm and the intensity of staining was heterogeneous. Strong staining was detected around the necrotic and hypovascular area (data not shown). Twenty-five cases (81%) showed positive expression of HK-II (staining score 2.03±1.38, percentage of positive cells 48.07±27.38%). Expression of HK-II was diffuse and granular type in cytoplasm. However, some positive cells showed focal staining suggesting mitochondrial localization. HK-II also showed strong staining around the necrotic area of cancer cells. The weak membranous staining for Glut-1 was detected in five cases (16%), (staining score 0.26±0.63, percentage of positive cells 9.03±21.35%). Immunohistochemical staining did not show any reactivity of Glut-3, Glut-4 nor Glut-5. No detectable staining was observed in the negative control.

Table I. Association of ^{18}F-FDG uptake and clinicopathological variables in patients with HCC.

Variables	Total	SUV (mean ± SD)	p-value
SUV (range 0.5-7.0)	31	2.01±1.80	
Age (range 48-75 years)			
<62	14	2.37±2.47	
≥62	17	1.70±0.97	0.95
Gender			
Male	24	2.12±1.97	
Female	7	1.60±1.04	0.85
Tumour location			
Lt. lobe	11	(1.77±1.61)	
Rt. lobe	20	(2.13±1.92)	0.48
Tumour size			
<3 cm	16	2.1±0.58	
≥3 cm	15	4.63±2.61	0.004
Histology			
Well differentiated	9	0.89±0.62	
Moderately differentiated	15	2.15±1.68	
Poorly differentiated	7	3.83±2.01	0.024
T status			
T1	3	0.63±0	
T2	7	1.5±0.99	
T3	14	1.91±1.75	
T4	7	3.62±2.05	0.022
Lymph node metastasis			
Absent	24	1.52±1.51	
Present	7	3.39±1.95	0.005
Distant metastasis			
Absent	25	1.58±1.46	
Present	6	3.78±2.14	0.009
Tumour grade			
I	5	0.95±0.66	
II	6	2.25±2.53	
III	15	1.67±0.90	
IV	5	3.66±2.41	0.013

Moderately and poorly differentiated HCC showed higher expression of Glut-2, HK-II and Glut-1 compared to well differentiated HCC however, the difference did not reach the statistical significance (Table II). Low expression of Gluts and HK-II in the well differentiated HCC was similar to the normal hepatocytes. Glut-2 was positive in 55 % of the patients

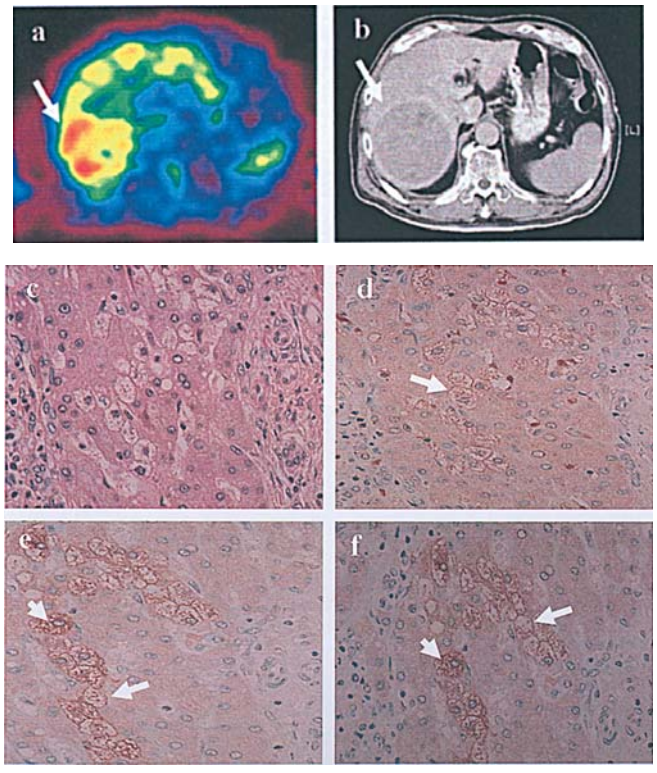

Figure 1. ^{18}F-FDG PET, corresponded CT and immunohistochemical analysis for Gluts and HK-II in HCC. (a) High ^{18}F-FDG uptake (SUV=2.79) is noted in the right hepatic lobe (arrow). (b) Enhanced CT of the upper abdomen demonstrated a large tumour in the right hepatic lobe with a capsular structure (arrow), which is consistent with HCC. (c) Hematoxylin and eosin staining confirmed the histological diagnosis of moderately differentiated HCC. (d) Immunohistochemical staining of Glut-1 shows weak staining along the cell membrane (arrow). (e) Immunohistochemical staining of Glut-2 shows strong expression along the cell membrane (long arrow) and some granular staining pattern in the cytoplasm (short arrow). (f) Immuno-histochemical staining shows strong expression of HK-II in the cytoplasm (short arrow) and also along the cell membranes (long arrow) (Original magnification x400).

Table III. Results of correlation analysis between ^{18}F-FDG uptake and expression of Glut-1, Glut-2 and HK-II in 31 patients with HCC.

Variables	(Mean ± SD)	(ϱ)	p-value
Glut-1			
Staining score	0.26±0.63	0.22	0.22
% of staining cells	9.03±21.35	0.21	0.23
Intensity	1.16±0.37		0.26
Glut-2			
Staining score	1.74±1.46	0.55	0.002
% of staining cells	39.36±30.21	0.39	0.03
Intensity	2.03±0.80		0.001
HK-II			
Staining score	2.03±1.38	0.37	0.04
% of staining cells	48.07±27.38	0.35	0.05
Intensity	2.16±0.73		0.29

with well differentiated HCC (staining score 1.33±1.50), 73% with moderately differentiated HCC (staining score 1.87±1.40) and 86% with poorly differentiated HCC (staining score 2.00±1.63). HK-II was positive in 67% of the patients with well differentiated HCC (staining score 1.44±1.42), 86% with moderately differentiated HCC (staining score 2.20±1.27) and 86% with poorly differentiated HCC (staining score 2.71±1.38) whereas Glut-1 was positive in only 11% of the patients with well differentiated (staining score 0.11±0.33), 20% with moderately differentiated HCC (staining score 0.33±0.72) and 14% with poorly differentiated HCC (staining score 0.29±0.76).

Association of ^{18}F-FDG uptake, Glut-2 and HK-II expression. ^{18}F-FDG uptake and expression of Glut-1, Glut-2 and HK-II were analyzed. ^{18}F-FDG uptake was significantly correlated with the expression of Glut-2 and HK-II as shown in Table III.

Further, we examined the correlation between the staining score, percentage of stained cells and intensity of Glut-2 and HK-II expression in patients with HCC. Staining score of Glut-2 vs. HK-II (ϱ=0.57, p=0.0009), percentage of stained cells of Glut-2 vs. HK-II (ϱ=0.53, p=0.003), and intensity of Glut-2 vs. HK-II (p=0.006) were found to be statistically significant.

Table II. Expression of Glut-1, Glut-2 and HK-II according to histological differentiation of HCC.

	Well differentiated				Moderately differentiated				Poorly differentiated			
Antibody	Negative N (%)	Weak N (%)	Strong N (%)	Score Mean ± SD	Negative N (%)	Weak N (%)	Strong N (%)	Score Mean ± SD	Negative N (%)	Weak N (%)	Strong N (%)	Score Mean ± SD
Glut-1	8 (89)	1 (11)	0	0.11±0.33	12 (80)	3 (20)	0	0.33±0.72	6 (86)	1 (14)	0	0.29±0.76
Glut-2	4 (45)	3 (33)	2 (22)	1.33±1.50	4 (27)	6 (40)	5 (33)	1.87±1.40	1 (14)	3 (43)	3 (43)	2.00±1.63
HK-II	3 (33)	4 (45)	2 (22)	1.44±1.42	2 (14)	8 (53)	5 (33)	2.20±1.27	1 (14)	1 (14)	5 (72)	2.71±1.38

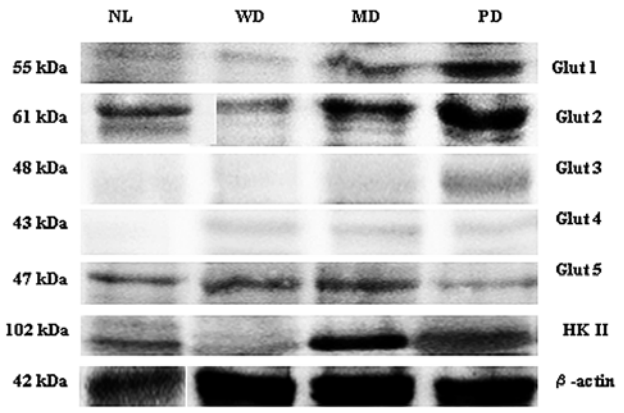

Figure 2. Western blot analysis for Gluts and HK-II in different histological types of HCC. Well differentiated (WD), moderately differentiated (MD), poorly differentiated (PD) HCC and a normal liver (NL) were analyzed. Duplicate samples were probed with polyclonal antibody to ß-actin to verify the presence of equal amount of total protein loaded in each line. Data are representative of three independent experiments performed with each specimen.

Detection of Gluts and HK-II by Western blot analysis. Western blot analysis of Gluts and HK-II protein was performed on HCC and a normal liver tissue for a control as shown in Fig. 2. The analysis confirmed the results of immunohistochemical staining. Moderately or poorly differentiated HCC had a higher level of Glut-2, HK-II and Glut-1 expression than well differentiated HCC. Interestingly, Western blot analysis revealed the expression of Glut-5, although immunohistochemical study failed to show positive staining.

^{18}F-FDG uptake, expression of Glut-2 and HK-II and survival of patients. The average survival of 31 patients with HCC was 3.37±2.26 years, median 3.08 years, range 0.25-7.92 years. The survival rate of patients with HCC was significantly higher (p=0.005) in the low SUV group as compared with the higher SUV group (Fig. 3a). Patient with Glut-2 negative HCC showed higher survival rate (p=0.03) as compared with Glut-2 positive HCC (Fig. 3b). However, the survival rate of HK-II negative patients was not significantly higher than those with HK-II positive patients (Fig. 3c). We further analyzed associations between the examined factors and survival using the Cox's proportional hazard model for the risk ratio. Univariate analysis found that SUV, lymph node metastasis, distant metastasis and tumour grade were significantly associated with poor prognosis, whereas, expression of Glut-2, HK-II and T status failed to predict the prognosis. Multivariate analysis found that SUV and lymph node metastasis were significant and independent unfavorable prognostic factors in HCC (Table IV).

Discussion

The present study found a significant correlation between ^{18}F-FDG uptake and the expression of Glut-2 and HK-II. HK-II was significantly correlated with Glut-2 but not with other Gluts indicating that Glut-2 is a major transporter in HCC.

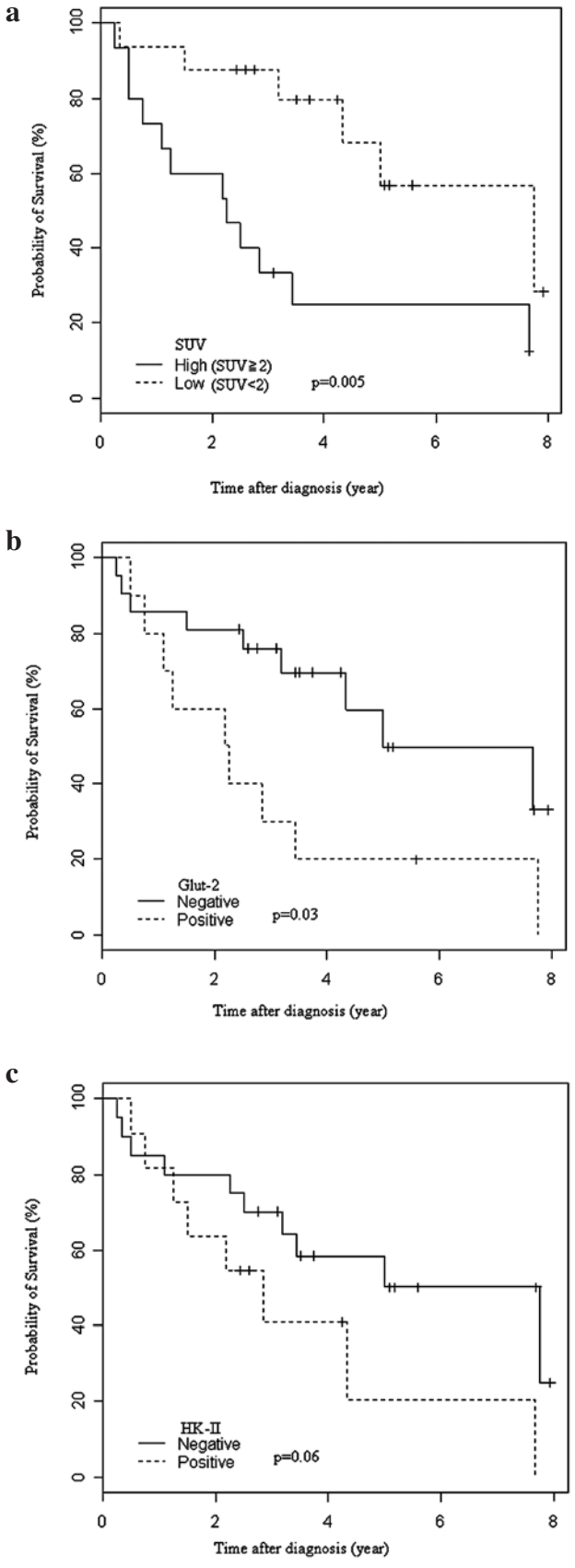

Figure 3. The overall survival rate of patients with HCC according to ^{18}F-FDG uptake, expression of Glut-2 and HK-II. (a) Survival rate was significantly higher in low SUV group than in the high SUV group (p=0.005). (b) Survival rate was significantly higher in Glut-2 negative than in the Glut-2 positive (p=0.03). (c) HK-II negative patients seems to show better survival than those with positive HK-II, but no significant difference was obtained (p=0.06).

Table IV. Prognostic factors of patients with HCC in Cox proportional hazards model.

Factors	Unfavorable status	Univariate			Multivariate		
		Hazard ratio	95% CI	p-value	Hazard ratio	95% CI	p-value
SUV	High (≥2)	1.39	1.11-1.73	0.004	1.49	1.03-2.15	0.03
HK-II	Positive	1.75	0.49-6.22	0.39	2.15	0.31-14.53	0.43
Glut-2	Positive	1.8	0.58-5.55	0.31	0.78	0.14-4.19	0.77
T status	High (T3/T4)	1.53	0.53-4.44	0.43	0.32	0.05-1.95	0.22
Lymph node metastasis	Present	1.95	1.05-3.63	0.03	0.05	0.00-0.96	0.04
Distant metastasis	Present	3.47	1.27-9.53	0.02	5.72	0.59-54.71	0.13
Tumour grade	High (III/IV)	2.08	1.09-3.98	0.03	5.69	0.90-34.92	0.06

CI, confidence interval; SUV, standarized uptake value.

Although several studies showed that Glut-1 was not expressed in HCC (23,24,27), the present study detected immunohistochemical reactivity of Glut-1 in 16% and Glut-2 in 71% of HCC. Present results support the findings of the recent study by Godoy *et al* (10) who reported the weak positive reactivity of Glut-1 and a strong expression of Glut-2 and no expression of Glut-3 and Glut-4 in human HCC. However, weak positive staining of Glut-5 was detected in their study. Our immunohistochemical study showed no staining but the Western blot analysis showed positive expression of Glut-5. Previous studies revealed that Glut-2 and Glut-5 are involved in fructose uptake, a molecule that may be involved in metabolic activity (10). Western blotting is more specific than that of immunohistochemistry for detecting small amounts of protein in fresh samples of the tumour despite small pitfall of contamination. This result gives rise to the hypothesis that fructose is transported for energy metabolism and may be used as an alternative metabolic substrate in HCC.

Earlier studies reported the expression of Glut 2 in various cancers such as breast, lung, cholangiocellular carcinoma, however, only few cases in their studies had strong expression while the majority of the cases showed faint and weak staining for Glut-2 (14,15). Present study revealed that Glut 2 is expressed stronger in HCC than normal liver, which is consistent with the findings of previous immunohistochemical (10) and mRNA studies in liver cancer (13). In contrast some studies revealed that Glut 2 is expressed in rat hepatocytes and normal liver but not in HCC (25,26). The discrepancies in these results might be due to the species related differences in glucose transport system, variation in the expression pattern of glucose transporter in HCC, and tumour-specific expression of different transporters arising from different tissues.

Earlier studies by Lee *et al* (27), stated that Glut-1 is not expressed in HCC, because the growth of HCC is supposed to be glutamine dependent and fatty acids are its major source of energy. In contrast, another study reported that Glut-1 is expressed in hepatocarcinogenesis. They described that Glut-1 has high affinity to glucose efflux and low *K*m for glucose influx, which resulted in the expression of Glut-1 (26). It was also reported that regulatory mechanisms of Glut-2 are different from those of Glut-1 (28). The present study revealed that Glut-2 is a major transporter in HCC although it seems inappropriate in other malignant tumours.

We observed moderate association between ^{18}F-FDG uptake and staining scores of HK-II expression. Expression of HK-II was diffuse and granular type in cytoplasm and showed variations in staining intensity however, some positive cells showed focal staining. Previous studies demonstrated a significant correlation between SUVs and the expression of HK-II in cholangiocellular cancer, lung cancer and HCC (2,14,29), which is contradictory to a study that found no association of HK-II with ^{18}F-FDG uptake in breast cancer (8). The discrepancy is postulated to be owing to the variety of HK-II activities in tumours with different origins.

In our study, poorly and moderately differentiated HCC had significantly higher ^{18}F-FDG uptake than well differentiated HCC. Similarly, poorly and moderately differentiated HCC showed higher expression of Glut-1, Glut-2 and HK-II than well differentiated HCC, however no statistically significant association was noted. Well differentiated HCC exhibits similar FDG metabolism and similar expression of Gluts and HK-II to that of normal hepatocytes. It is supposed to be the consequence of a high glucose-6-phosphatase concentration. This finding is consistent with previous reports that concluded FDG-PET does not effectively detect well differentiated HCC (7,16), however it is useful in poorly and moderately differentiated HCC.

Our study showed significant associations between ^{18}F-FDG uptake and clinicopathological variables reflecting the aggressiveness of the disease. The prognosis of patients with high SUV was significantly worse than those with low SUV in patients with HCC which is consistent with previous studies (18,19). The patients with positive expression of Glut-2 showed worse prognosis than those with negative expression. These results suggest that ^{18}F-FDG uptake and Glut-2 expression may be predictors of survival in HCC. The survival rate of

HK-II negative patients was higher than those with HK-II positive patients however, the difference did not reach statistical significance (p=0.06). The fewer cases of HK-II negative patients (n=6) might have caused this statistical deviation.

Multivariate statistical analysis showed that only SUV and lymph node metastasis were independent prognostic factors. Therefore, in post-operative patients with HCC, who have high SUV, a careful follow-up of lymph node metastasis is very important to avoid metastasis and recurrence. Thus, ^{18}F-FDG PET has a valuable role in clinical implication for the patient management such as to detect the malignancy and predict the prognosis of patients with HCC.

The exact mechanisms of the differential ^{18}F-FDG uptake and the expression of glucose transporters and HK-II in different cancer are still unclear (11). Assessment of glucose-6-phosphatase activity, different glucose transporters and HK-II with a larger population is required for better understanding of the mechanism of expression of glucose transporters; prognosis of patients and the clinical implication of ^{18}F-FDG PET in patients with HCC.

In conclusion, the present study indicates that Glut-2 serves as a main glucose transporter in HCC. High ^{18}F-FDG uptake and expression of Glut-2 are associated with clinical aggressiveness and poor survival of HCC. Thus, the combined evaluation of ^{18}F-FDG uptake and the expression of Glut-2 might have an important role for the management of patients with HCC.

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