Expression of glucose transporters and hexokinase II in cholangiocellular carcinoma compared using [^{18}F]-2-fluro-2-deoxy-D-glucose positron emission tomography

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Cholangiocellular carcinoma (CCC) has been reported to have a high glucose uptake; however, the mechanism of glucose entry into these cells is still unclear. We investigated the relationship between [^{18}F]-2-fluro-2-deoxy-D-glucose (^{18}F-FDG) uptake and the expression of facilitative glucose transporters (Glut) and hexokinase (HK) II, as well as the association between the expression of different histological types of CCC. The expression of Glut (1–5) and HK II was studied using immunohistochemistry of 26 patients with CCC who underwent whole-body ^{18}F-FDG positron emission tomography before surgery or biopsy. CCC expressed immunohistochemically detectable Glut 1 in 81%, Glut 2 in 54%, Glut 3 in 19%, and HK II in 77% of the total cases. Glut 1, Glut 2, Glut 3, and HK II were more often detected in moderately differentiated and poorly differentiated than in well-differentiated CCC. A significant correlation was observed between ^{18}F-FDG uptake and the staining scores of Glut 1 and HK II ($P = 0.02$, $\rho = 0.45$ and $P = 0.001$, $\rho = 0.59$). The staining scores of Glut 1 and HK II were also significantly correlated ($P = 0.002$, $\rho = 0.3$). Multivariate regression analysis revealed that lymph-node metastasis was independently associated with ^{18}F-FDG uptake. Our study showed a significant association between the expression of Glut 1 and HK II with ^{18}F-FDG uptake, indicating that Glut 1 is a major glucose transporter expressed in CCC and that HK II contributes to the increased metabolism of glucose, especially in moderately and poorly differentiated CCC. (*Cancer Sci* 2008; 99: 260–266)

Positron emission tomography (PET) using the glucose analog [^{18}F]-2-fluro-2-deoxy-D-glucose (FDG) is a rapidly developing functional-imaging modality that has shown great promise in the fields of primary tumor detection, planning and monitoring therapy, and the detection of metastasis and recurrence. It has become an established, essential imaging tool in oncology.[1–5]

High metabolism and increased rates of glucose utilization are major changes in malignant tumors that are associated with the increased expression of glucose transporters (Glut) in malignant cells.[6] The cellular mechanism underlying the increased ^{18}F-FDG accumulation in malignant tumors is associated with a higher rate of phosphorylation and diminished rate of dephosphorylation of intracellular phosphorylated glucose, a higher rate of glucose transport across the cell membrane, and higher activity of hexokinase (HK).[7] Several studies were focused on the expression of Glut and HK II activity to define the role of these in the regulation of ^{18}F-FDG uptake in various types of cancers, such as head and neck, esophageal, lung, breast, pancreatic, and uterine cancer.[8–14] Until now, only a few studies have reported the importance of ^{18}F-FDG PET in cholangiocellular carcinoma (CCC). ^{18}F-FDG PET has been reported to identify primary tumor sites accurately with a sensitivity of 90% and above.[15,16]

There are a few reports on the immunohistochemical study of Glut 1 and HK II expression in CCC,[17,18] but the association of ^{18}F-FDG uptake with Glut 1, HK II, and other Glut has not been clarified. Torizuka *et al*. mentioned that the phosphorylation step appears to be rate determining in the uptake of ^{18}F-FDG in primary breast cancer but not in primary lung cancer, suggesting that there may be differences in glucose transportation and metabolism among cancers derived from different tissues.[19] Previous studies have reported that the expression of Glut is tumor specific.[20,21] The present study using different Glut and HK II might lead to a better understanding of the clinical value of ^{18}F-FDG PET, which reflects the increased glucose utilization in CCC. Among the four subtypes of mammalian hexokinase, HK II was selected because it is a major subtype that is expressed predominantly in various tumors and cell lines.[22] In the present study we therefore tried to investigate the association between ^{18}F-FDG uptake and the expression of Glut 1, Glut 2, Glut 3, Glut 4, Glut 5, and HK II in CCC with various histological differentiations.

Material and Methods

Patients. The present study was carried out on 26 patients (14 male and 12 female, age range from 33 to 85 years, median age 69 years) who underwent ^{18}F-FDG PET imaging and a clinical follow up between April 2000 and March 2006 in our hospital. ^{18}F-FDG PET obtained under quiet respiration, and computed tomography studies were carried out under deep respiration on all patients before treatment. The average time interval between ^{18}F-FDG PET and surgery or biopsy was 2.3 weeks (range 1–4 weeks). The characteristics of patients and histological diagnosis are shown in Table 1. Tumor stage and disease grade were classified according to the 6th edition of the tumor, lymph node and metastasis classification of the International Union Against Cancer.[23] None of the patients had insulin-dependent diabetes. The blood-sugar level in all patients was less than 110 mg/dL at the time of ^{18}F-FDG PET administration. The Institutional Review Board of our institute approved the study protocols, and informed consent was obtained from all patients participating in the study.

^{18}F-FDG PET study. The PET study was carried out using a SET2400W PET scanner (Shimadzu Corporation, Kyoto, Japan) with a 59.5-cm transaxial field of view and 20-cm axial field of view. The scanner produced 63 image planes, spaced 3.125 mm apart. Transaxial spatial resolution was 4.2 mm full-width

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Table 1. Clinical details, [^{18}F]-2-fluro-2-deoxy-D-glucose uptake, and results of immunohistochemical analysis of glucose transporters (Glut) and hexokinase (HK) II in 26 patients with cholangiocellular carcinoma

Patient no.	Age (years)	Sex	Histology	TNM classification	SUV	Glut 1			Glut 2			Glut 3			HK II		
						Int.	%	Score	Int.	%	Score	Int.	%	Score	Int.	%	Score
1	61	M	WD	T2N0M0 II	2.51	W	50	1	N	0	0	N	0	0	W	80	2
2	62	M	WD	T3N0M0 IIa	2.72	N	0	0	W	0	0	N	0	0	S	50	3
3	65	M	WD	T3N0M0 III	2.84	N	0	0	N	60	2	N	0	0	S	30	3
4	61	F	WD	T3N0M0 III	0.50	N	0	0	N	0	0	N	0	0	S	50	3
5	53	F	MD	T4N1M1IVb	4.65	W	50	1	N	0	0	N	0	0	S	100	4
6	78	F	MD	T3N0M0 IIIa	4.50	S	50	3	N	0	0	N	0	0	W	100	2
7	69	F	MD	T2N0M0 II	3.47	W	100	2	N	0	0	N	0	0	S	30	3
8	33	M	MD	T4N1M0 IV	5.12	W	50	1	W	60	2	N	0	0	N	0	0
9	73	F	MD	T4N0M0 IVa	3.64	S	50	3	S	60	4	W	30	1	N	0	0
10	67	M	MD	T4N0M0 IV	4.80	S	50	3	N	0	0	W	60	2	S	60	4
11	70	M	MD	T4N1M1IVb	4.50	S	50	3	N	0	0	N	0	0	S	50	3
12	65	F	MD	T4N0M0 IV	4.50	S	80	4	N	0	0	N	0	0	W	100	2
13	75	F	MD	T3N1M0 IV	8.38	S	50	3	S	50	3	N	0	0	W	50	1
14	79	F	MD	T3N0M0 IIIa	3.50	S	80	4	W	70	2	N	0	0	N	0	0
15	70	M	MD	T2N0M0 II	3.90	S	50	3	S	20	3	N	0	0	N	0	0
16	52	F	PD	T4N1M0 IVa	6.60	W	100	2	W	70	2	N	0	0	S	60	4
17	55	M	PD	T3N0M0 IIa	4.29	S	50	3	N	0	0	W	40	1	W	50	1
18	64	F	PD	T2N0M0 Ib	3.31	N	0	0	N	0	0	N	0	0	S	80	4
19	85	F	PD	T4N1 M IVa	6.25	W	50	1	W	100	2	N	0	0	S	40	4
20	72	F	PD	T3N1M0 IV	2.93	N	0	0	W	30	1	N	0	0	S	100	4
21	70	M	PD	T3N1M1IV	8.01	W	100	2	W	50	1	N	0	0	N	0	0
22	71	M	PD	T3N1M1IVb	4.35	S	50	3	W	50	1	W	30	1	W	50	1
23	71	M	PD	T3N0M0 III	3.60	S	30	3	S	30	3	W	60	2	W	80	2
24	68	M	PD	T4N1M0 IV	13.34	S	100	4	S	60	4	N	0	0	W	100	2
25	69	M	PD	T4N1M0 IVa	2.94	W	100	2	W	30	1	N	0	0	S	50	3
26	60	M	PD	T4N1M1 IVb	3.20	S	40	3	N	0	0	N	0	0	N	0	0

F, female; Int, intensity; M, male; MD, moderately differentiated; N, negative; PD, poorly differentiated; S, strong; SUV, standardized uptake value; W, weak; WD, well differentiated.

half-maximum (FWHM) at the center of the field of view, and axial resolution was 5.0 mm FWHM. ^{18}F-FDG was synthesized using the method pioneered by Hamacher *et al*.[24]

Patients fasted for at least 6 h before ^{18}F-FDG PET scanning. Data acquisition was initiated at 60 min after the injection of 5–6 MBq/kg ^{18}F-FDG by simultaneous emission–transmission with a rotating external source for absorption correction. Four to five bed positions from the head to the thigh were imaged for 8 min per position.

The attenuation-corrected transaxial images were reconstructed from an ordered subsets expectation maximization algorithm into a 128×128 matrix with pixel dimensions on a plane of 4.0 mm and at 3.125 mm axially. Finally, every three consecutive slices were added to generate a transaxial image 9.8-mm thick for visual interpretation and quantitative analysis, using the standardized uptake value (SUV). Coronal images 9.8-mm thick were also reconstructed from attenuation-corrected transaxial images. All PET images were analyzed semiquantitatively using the SUV, which was calculated as follows:

SUV = radioactivity in the tissue or lesion (MBq/g)/injected dose (MBq)/patient's bodyweight (g)

In order to evaluate the ^{18}F-FDG uptake in the tumor, 4×4 pixel square regions of interest were placed on the tumor, including the area of the highest activity, but not necessarily covering the entire tumor. Positive lesions were identified if the uptake of ^{18}F-FDG in the tumor was higher than that in the background of the liver. Thus, in our study, ^{18}F-FDG PET images were evaluated qualitatively by two experienced nuclear physicians in conjunction with computed tomography, and the results were compared with clinical findings because of the poor anatomical localization and the poor resolution of the PET.

Immunohistochemical staining for Glut and HK II. Samples from the surgical resection of 24 patients and two cases of biopsy specimens having enough viable malignant components were chosen for immunohistochemical staining. All specimens were fixed with 10% formalin and embedded in a paraffin block. Immunohistochemical staining of the section for Glut and HK II was carried out according to the standard procedure.[25] Sections 2-μm thick were incubated in an oven for 1 h and then deparaffinized with xylene and rehydrated, and endogenous peroxidase activity was blocked for 10 min in 10% hydrogen peroxide. Antigen retrieval methods were carried out for all sections before immunostaining in 0.01-mol/L citrate buffer (pH 6.0) using a microwave oven at 98°C for 15 min. After cooling the sections were then washed with phosphate-buffered saline (PBS) and reacted with a blocking treatment using normal goat serum for 30 min. The sections were then incubated with the primary polyclonal antibody at a dilution of 1:1000 for Glut 1 (Dako, Carpinteria, CA, USA) and Glut 2 (Chemicon International, Temecula, CA, USA), 1:500 for Glut 3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), 1:200 for Glut 4 (Santa Cruz Biotechnology), 1:100 for Glut 5 (Chemicon International), and 1:3000 for HK II (Chemicon International) in 1% bovine serum albumin at 4°C overnight. After washing with PBS, signals were detected using an En Vision Kit (Dako) and diaminobenzidine tetrahydrochloride. Finally, specimens were counterstained with hematoxylin–eosin (HE), dehydrated, and mounted. Parallel sections were incubated with normal mouse serum to substitute for the primary antibody and used as negative controls. Human red blood cells, normal liver tissues, testes, skeletal muscle, small intestine, and rat skeletal muscle served as positive controls for Glut 1, Glut 2, Glut 3, Glut 4, Glut 5, and HK II, respectively. No staining was observed in a parallel section incubated with normal mouse serum.

Table 2. Evaluation of immunohistochemistry

Stained cells (%)	Intensity	
	Weak	Strong
0–10	0	2
11–50	1	3
51–100	2	4

Evaluation of stained sections. Immunohistochemical staining for Glut and HK II was evaluated simultaneously by two experienced pathologists who did not have any knowledge of the clinical outcome, using a double-headed light microscope in one sit-up, and any discrepancy was resolved by a third observer. The intensity, cellular pattern of staining, and number of positive cells were recorded for every specimen and the presence of positive stained cells was interpreted as indicative of expression. The percentages of positively stained cells were rated using a semiquantitative scale as 0–10%, 11–50%, or 51–100%. The intensity of the staining was graded as negative, weak, or strong, as described previously.[9] The staining result was scored from 0 to 4 according to the intensity and percentage of positively stained cells as shown in Table 2, which was a modification of the scoring system described previously.[26]

Data analysis. Data was expressed as mean ± SD. Spearman's rank correlation was computed between ^{18}F-FDG uptake and expression of Glut or HK II. Differences between the variables were evaluated using the Kruskal–Wallis test and Mann–Whitney's non-parametric test and it was adjusted for multiple comparisons by Holm's method. Multivariate analysis was carried out to compare ^{18}F-FDG uptake and clinicopathological parameters. Probability values less than 0.05 were considered statistically significant.

Results

Pathological results in relation to ^{18}F-FDG uptake. Out of 26 patients, four patients (16%) had well-differentiated CCC, 11 patients (42%) had moderately differentiated CCC, and 11 patients (42%) had poorly differentiated CCC. All tumors except that of one patient showed increased ^{18}F-FDG uptake compared with the surrounding non-tumor tissues. The patients with well, moderately, and poorly differentiated CCC had ^{18}F-FDG uptakes ranging from 0.5 to 2.84 (2.14 ± 1.10), from 3.47 to 8.38 (4.63 ± 1.36), and from 2.93 to 13.34 (5.35 ± 3.15), respectively. A significant difference was observed between the ^{18}F-FDG uptakes of well-differentiated and poorly differentiated CCC (2.14 ± 1.10 vs 5.35 ± 3.15, $P = 0.004$), which also differed from that of moderately differentiated tumors (4.63 ± 1.36, $P = 0.009$). No significant difference was noted between poorly differentiated and moderately differentiated tumors, as shown in Figure 1.

Table 3 shows the results of univariate analysis of clinicopathological variables and ^{18}F-FDG uptake. A statistically significant correlation was observed between ^{18}F-FDG uptake and histological differentiation ($P = 0.006$), lymph-node metastasis ($P = 0.0013$), and tumor grade ($P = 0.034$). Multivariate regression analysis between ^{18}F-FDG uptake and different clinicopathological variables such as histological differentiation, tumor status, lymph-node metastasis, distant metastasis, and tumor grade demonstrated that only lymph-node metastasis was independently associated with ^{18}F-FDG uptake ($P = 0.02$), as shown in Table 4.

Expression of Glut and HK II in CCC. The results of the immunohistochemical findings as well as the ^{18}F-FDG PET imaging are summarized in Table 1. Patterns of Glut and HK II expression were revealed in both the membrane and cytoplasm.

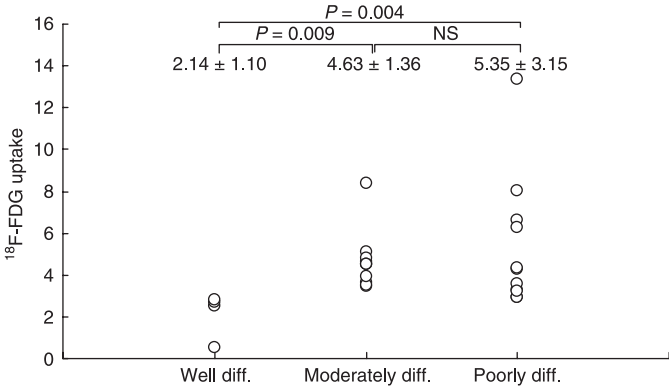

Fig. 1. Comparison between [^{18}F]-2-fluro-2-deoxy-D-glucose (FDG) uptake and different histological types of cholangiocellular carcinoma (CCC). A significant difference was seen between well-differentiated and moderately differentiated CCC, and also between well-differentiated and poorly differentiated CCC. However, no significant difference (NS) was noted between poorly differentiated CCC and moderately differentiated CCC.

Table 3. Univariate analysis of clinicopathological factors and [^{18}F]-2-fluro-2-deoxy-D-glucose uptake in cholangiocellular carcinoma

Factor	*n*	Standardized uptake value (mean ± SD)	*P*-value
Histological differentiation			
Well differentiated	4	2.14 ± 1.10	
Modrately differentiated	11	4.63 ± 1.36	
Poorly differentiated	11	5.35 ± 3.15	0.006*
Tumor status			
T2	4	3.30 ± 0.58	
T3	11	4.15 ± 2.29	
T4	11	5.41 ± 2.86	0.07*
Lymph-node metastasis			
Absent	14	3.43 ± 1.09	
Present	12	5.86 ± 2.98	0.0013**
Distant metastasis			
Absent	20	4.37 ± 2.66	
Present	6	5.16 ± 1.70	0.17**
Tumor grade			
I	1	3.31	
II	5	3.38 ± 0.76	
III	5	2.99 ± 1.51	
IV	15	5.55 ± 2.73	0.034*
Tumor grade			
Low (I and II)	6	3.37 ± 0.68	
High (II and IV)	20	4.91 ± 2.71	0.05**

P-values were evaluated using the *Kruskal–Wallis and **Mann–Whitney *U*-tests.

Positive staining was observed mainly in cancer cells around the necrotic area and the intensity of expression was heterogeneous within a tumor. Figure 2d–f shows the immunohistochemical expression of Glut 1, Glut 2, and HK II. Glut 1 immunostaining was positive in 21 out of 26 cases (81%). The percentage of stained cells was 51.2 ± 33.0% and an average staining score of 2.08 ± 1.35. The expression of Glut 1 was observed mainly along the membrane of the cancer cells but some positive granules were found in the cytoplasm and also in cancer cells around the necrotic area.

Glut 2 showed positive staining in 14 out of 26 cases (54%). The percentage of stained cells was 28.5 ± 30.8% and the

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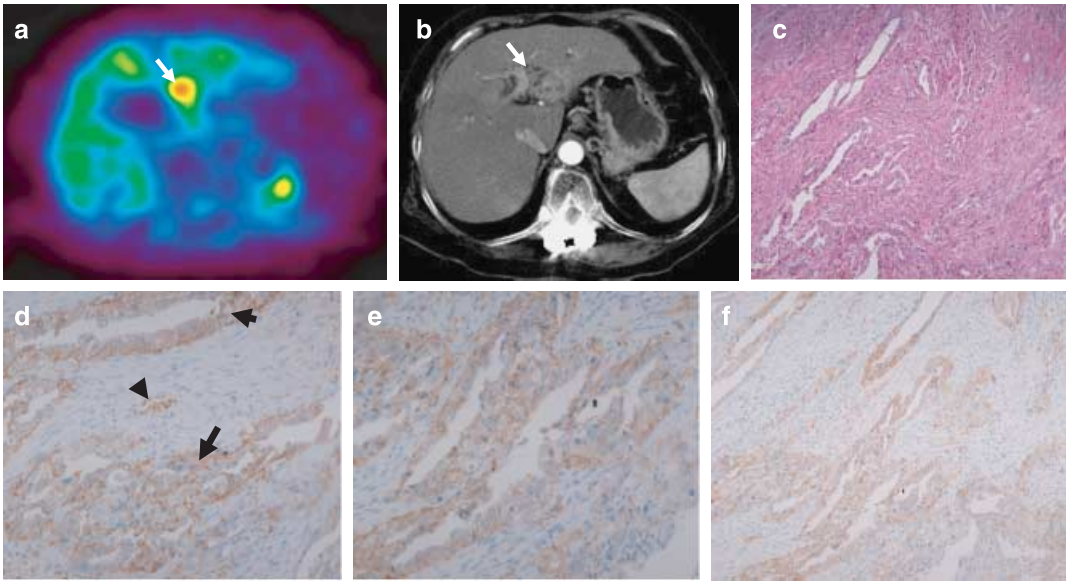

Fig. 2. [^{18}F]-2-fluro-2-deoxy-D-glucose (FDG) positron emission tomography, corresponding computed tomography (CT), and expression of glucose transporters (Glut) in cholangiocellular carcinoma (CCC). (a) High ^{18}F-FDG uptake (standardized uptake value = 3.64) was noted in the tumor (arrow). (b) CT showed a heterogeneously enhanced mass (arrow) with dilated intrahepatic bile ducts, which is consistent with CCC. (c) Hematoxylin–eosin staining confirmed the histological diagnosis of CCC. (d) Immunohistochemical staining showed strong Glut 1 expression in the cell membranes (short arrow) and a few positive granules in the cytoplasm (long arrow). Red blood cells (arrowhead) showed strong staining and served as an internal positive control. (e) Immunohistochemical staining showed strong expression of Glut 2. (f) Immunohistochemical staining of hexokinase (HK) II showed strong expression of HK II (original magnification ×400).

Table 4. Multivariate regression analysis of clinicopathological variables and [^{18}F]-2-fluro-2-deoxy-D-glucose uptake in cholangiocellular carcinoma

Variable	Estimate coefficient	Standard error	*P*-value
Histological difference	0.82	0.63	0.21
Tumor status	–0.57	1.03	0.59
Lymph-node metastasis	3.18	1.35	0.02
Distant metastasis	–1.94	1.22	0.13
Tumor grade	0.41	0.88	0.65

Observation, 26; adjusted R^2, 0.288; *F*-statistic, 3.017 on 5 and 20 degree of freedom; *P*-value, 0.03.

staining score was 1.2 ± 1.36. The expression of Glut 2 was observed mainly in the cytoplasm with a faint and diffuse pattern compared with Glut 1 expression. Glut 3 showed positive staining in five out of 26 cases (19%). The percentage of stained cells was 8.5 ± 18.3% and the average staining score was 0.27 ± 0.59. The expression of Glut 3 in the cytoplasmic area was weaker in intensity than that of Glut 1 and Glut 2. None of the tumors showed Glut 4 or Glut 5 expression. HK II showed positive staining in 20 out of 26 cases (77%). The percentage of stained cells was 50.38 ± 34.69% and the average staining score was 2.12 ± 1.48. Expression was observed mainly along the cytoplasm of the cancer cells but some positive granules were found in the membrane. No association was observed among the expression of different Glut with any parameters.

Moderately and poorly differentiated tumors showed a higher expression of Glut 1, Glut 2, Glut 3, and HK II compared with well-differentiated tumors, as shown in Table 5. Glut 1 was positive in one out of the four patients (25%) with well-differentiated CCC, with an average staining score of 0.25 ± 0.5. For moderately differentiated CCC, Glut 1 was positive in all 11 patients (100%), with an average staining score of 2.73 ± 1.01. For poorly differentiated CCC, 9 out of the 11 patients (82%) were positive for Glut 1, with an average staining score of 2.09 ± 1.3. Glut 2 was positive in one out of four patients (25%) with well-differentiated CCC, with an average staining score of 0.5 ± 1. Five of the 11 patients (45%) with moderately differentiated CCC were positive for Glut 2, with an average staining score of 1.27 ± 1.56. Eight out of 11 patients (73%) with poorly differentiated CCC were positive for Glut 2, with an average staining score of 1.36 ± 1.29. Glut 3 was positive in none of the patients with well-differentiated CCC. Of the patients with moderately differentiated CCC, two out of 11 (18%) were positive for Glut 3, with an average staining score of 0.27 ± 0.65. For poorly differentiated CCC, three out of 11 patients (27%) were positive for Glut 3, with an average staining score of 0.36 ± 0.67. HK II was positive in one out of four patients (25%) with well-differentiated CCC, with an average staining score of 0.75 ± 1.5, in 11 out of 11 patients (100%) with moderately differentiated CCC, with an average staining score of 2.64 ± 1.12, and in 8 out of 11 patients (73%) with poorly differentiated CCC, with an average staining score of 2.09 ± 1.64.

^{18}F-FDG uptake in relation to intensity of Glut and HK II. A statistically significant association was observed between ^{18}F-FDG uptake and intensity, the staining score, and the percentage of Glut 1 and HK II stained cells, whereas Glut 2 and Glut 3 were found to be not significant. A positive correlation was observed between ^{18}F-FDG uptake and staining scores of Glut 1 and HK II ($P = 0.02$, $\rho = 0.45$, and $P = 0.001$, $\rho = 0.59$, respectively) and between ^{18}F-FDG uptake and the percentage of Glut 1 and HK II stained cells ($P = 0.006$, $\rho = 0.56$, and $P = 0.001$, $\rho = 0.63$, respectively). A significant difference was noted between ^{18}F-FDG uptake and the intensity of Glut 1 (negative vs weak, $P = 0.05$, and negative vs strong, $P = 0.01$) and HK II (negative vs weak, $P = 0.011$, and negative vs strong, $P = 0.012$), as shown in Figure 3. No significant difference was observed between FDG uptake and the intensity of Glut 2 and Glut 3. We observed

Table 5. Results of immunohistochemical expression of glucose transporters (Glut) and hexokinase (HK) II according to different histological type of 26 patients with cholangiocellular carcinoma

Histology	*n*	Glut 1				Glut 2				Glut 3				HK II			
		Neg.	Weak	Strong	Score	Neg.	Weak	Strong	Score	Neg.	Weak	Score		Neg.	Weak	Strong	Score
		n (%)	*n* (%)	*n* (%)	(mean ± SD)	*n* (%)	*n* (%)	(mean ± SD)	*n* (%)	*n* (%)	*n* (%)	(mean ± SD)		*n* (%)	*n* (%)	*n* (%)	(mean ± SD)
WD	4	3 (75)	1 (25)	0 (0)	0.25 ± 0.5	3 (75)	1 (25)	0 (0)	0.5 ± 1	4 (100)	0 (0)	0 ± 0		3 (75)	0	1 (25)	0.75 ± 1.5
MD	11	0 (0)	3 (27)	8 (73)	2.73 ± 1.01	6 (55)	2 (18)	3 (27)	1.27 ± 1.56	9 (82)	2 (18)	0.27 ± 0.65		0	5 (45)	6 (55)	2.64 ± 1.12
PD	11	2 (18)	4 (36)	5 (46)	2.09 ± 1.3	3 (27)	6 (55)	2 (18)	1.36 ± 1.29	8 (73)	3 (27)	0.36 ± 0.67		3 (27)	3 (27)	5 (46)	2.09 ± 1.64
Total	26	5 (19)	8 (31)	13 (50)	2.08 ± 1.35	12 (46)	9 (35)	5 (19)	1.2 ± 1.36	21 (81)	5 (19)	0.27 ± 0.59		6 (23)	8 (31)	12 (46)	2.12 ± 1.48

MD, moderately differentiated; Neg, negative; PD, poorly differentiated; WD, well differentiated.

a weak correlation between the staining scores of Glut 1 and HK II, as shown in Figure 4 ($P = 0.002$, $\rho = 0.3$).

The expression of Glut 1, Glut 2, and HK II was higher in advanced tumor grades compared to the lower tumor grade of CCC. Glut 1 was positive in four out of six (67%) patients with lower tumor grade (I and II) and in 17 out of 20 (85%) patients with higher tumor grade (III and IV). Glut 2 was positive in two out of six (33%) patients with lower tumor grade and in 12 out of 20 (60%) patients with higher tumor grade. HK II was positive in three out of six (50%) patients with lower tumor grade and in 17 out of 20 (85%) patients with higher tumor grade (data not shown).

Discussion

The results of our study indicate that of Glut 1–5, Glut 1 is the primary glucose transporter in CCC. Other glucose transporters might have a minor role in CCC as found by their low expression, assessed by immunohistochemical analysis. The PET study revealed that ^{18}F-FDG uptake is correlated with the expression of Glut 1 and HK II but not with other Glut, indicating that Glut 1 and HK II provide glucose as a source of energy in rapidly growing tumor cells of CCC.

A recent study by Lee *et al*. showed that seven out of seven cases with CCC were Glut 1 positive but HK II was positive in only one case.[25] The reason behind the negative expression of HK II was not stated in their study. In contrast, our study showed a significant association between the amount of ^{18}F-FDG uptake and the staining score, percentage of cells stained, and the intensity of Glut 1 and HK II, indicating that Glut 1 and HK II contribute to increased transport of ^{18}F-FDG into the tumor cells compared with other transporters, and increased retention of ^{18}F-FDG-6-phosphate in CCC. Previous studies showed that 13 out of 16 (81.3%)[17] and 21 out of 42 (50%) cases were Glut 1 positive in CCC,[18] but these studies investigated neither the expression of HK II nor the association between Glut 1 expression and ^{18}F-FDG uptake. The discrepancy in expression of Glut 1 and HK II in these studies might be due to the use of different antibodies (monoclonal vs polyclonal), detection methods, and patient populations.

A previous study by Younes *et al*. mentioned that 87 out of 154 malignant human neoplasms did not show detectable Glut 1, indicating that other Glut may mediate glucose uptake in these tumors.[20] The expression of Glut 1 in only a portion of cancer cells suggests that glucose uptake in these tumors may be mediated by Glut other than Glut 1. This was consistent with the findings of Yamamoto *et al*. where an overexpression of mRNA for more than one type of Glut was found in some carcinomas of the digestive system.[21] Our results showed that CCC expresses Glut 1 in 81%, Glut 2 in 54%, Glut 3 in 19%, and HK II in 77% of patients, but no detectable expression of Glut 4 or Glut 5 was noted. The expression of Glut 1 to Glut 3 and HK II was more often detected in moderately and poorly differentiated CCC than in well-differentiated CCC. The expression was also higher in advanced tumor stages compared with low tumor stages of CCC.

A previous study by Kunkel *et al*. reported Glut 1 expression as a negative biomarker of prognosis in patients with oral squamous cell carcinoma.[27] High Glut 1 expression was associated with an increased risk of death, and low SUV on ^{18}F-FDG PET scans was associated with longer survival. In our study a significant association was observed between ^{18}F-FDG uptake and different clinicopathological variables, such as histological differentiation, lymph-node metastasis, and tumor grade, which resembles the results of previous studies on other malignancies.[28,29] Using multivariate regression analysis among different clinicopathological variables, only lymph-node metastasis was independently associated with ^{18}F-FDG uptake. However, in the

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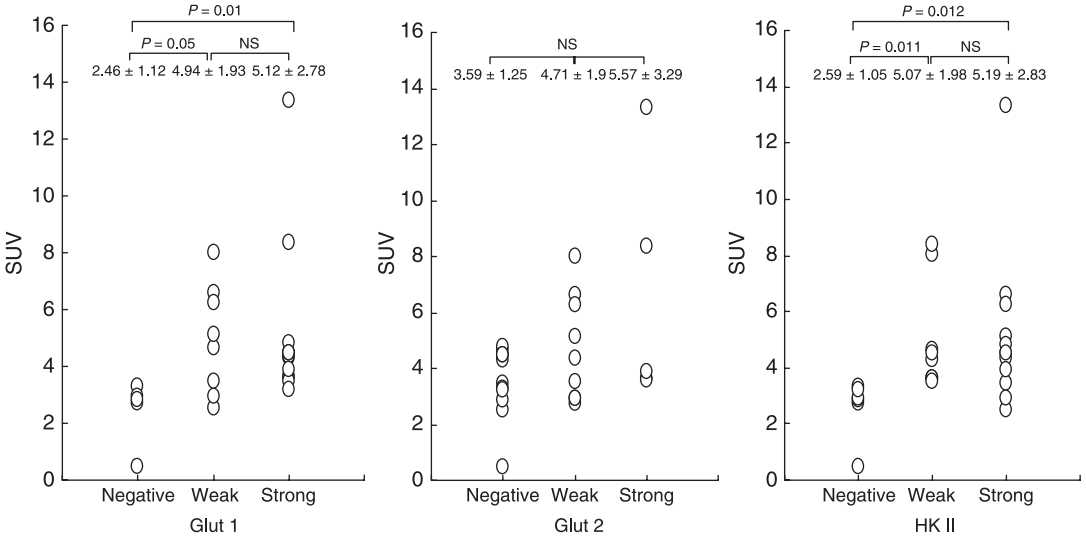

Fig. 3. Relationship between [^{18}F]-2-fluro-2-deoxy-D-glucose (FDG) uptake and intensity (negative, weak, or strong) of glucose transporter (Glut) 1, Glut 2, and hexokinase (HK) II expression in cholangiocellular carcinoma (CCC). A statistically significant difference in ^{18}F-FDG uptake was noted between the negatively and weakly stained cells, and between the negatively and strongly stained cells for Glut 1 and HK II. However, no significant difference (NS) was observed between ^{18}F-FDG uptake and the expression of Glut 2. SUV, standardized uptake value.

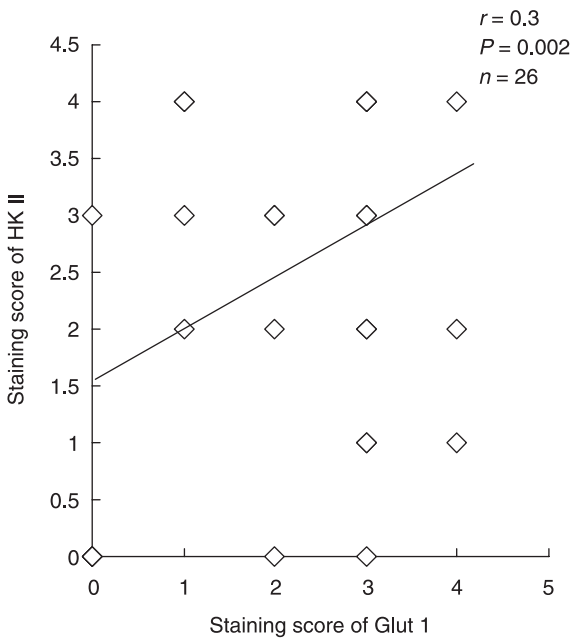

Fig. 4. Relationship between intensity score of glucose transporter (Glut) 1 and hexokinase (HK) II expression in cholangiocellular carcinoma. A weak correlation was noted between Glut 1 and HK II.

clinical setting, it is difficult to evaluate histological characteristics such as malignant potential and the expression of glucose transporters in every patient. Thus, ^{18}F-FDG PET may have clinical value as a non-invasive procedure to postulate the expression of Glut 1, HK II, malignant potential, histological differentiation, and lymph-node metastasis of CCC.

Our findings on Glut expression with high and low ^{18}F-FDG uptake in CCC reflect the metabolic heterogeneity of tumors. Increased ^{18}F-FDG uptake indicates viability and hypoxia of the tumor, whereas decreased uptake indicates necrosis in an animal model.[30] Rapidly growing tumors have deficient vascular systems characterized by the formation of necrosis, which reflects the cell death caused by hypoxia, and hypoxia increases the ^{18}F-FDG uptake *in vitro*.[30,31]

The present results on the expression of Glut in membranous and cytoplasmic areas correspond to the stimulation due to hypoxia or ischemia, which induces the translocation of existing glucose transporters from cytoplasmic vesicles to the plasma membrane and eventually increases the synthesis of Glut mRNA. Protein expression is a consequence of both oxygen and glucose starvation. Thus, it is logical that these areas of Glut staining, particularly the areas of intense staining seen round necrotic foci, are deprived of both oxygen and nutrients. It is well established that a reduction in oxidative phosphorylation, which might be a consequence of the increased proliferation of cancer cells, enhances expression of Glut.[32]

There was only one patient in our study (case 4) with a very low ^{18}F-FDG uptake who had an infiltrating extrahepatic CCC. Extrahepatic CCC often present as infiltrating tumors that have only loosely connected cell nests embedded in conspicuous fibrous stroma on histology.[15] They usually do not show sufficient levels of ^{18}F-FDG uptake, because of a lower population of tumor cells and relatively higher amount of fibrous tissue.

There are several areas of expansion in the present study. Some patients showed negative expression of all Glut, including Glut 1. Glucose transporters other than Glut 1 to Glut 5 could be postulated to be expressed in these patients who show an uptake of ^{18}F-FDG. An earlier study reported that in principle, a higher level of glucose transporters does not guarantee increased FDG uptake in cancer cells.[22] Assessment of glucose-6-phosphatase activity would be needed to more clearly understand the clinical implication of ^{18}F-FDG PET for the evaluation of histological malignancy and the prognosis of patients with CCC.

Conclusion

The present study demonstrates a significant association between ^{18}F-FDG uptake and the expression of Glut 1 and HK II, suggesting that Glut 1 is the major transporter contributing to the higher rate of entry of glucose into the tumor cells, and that HK II contributes to the increased retention of glucose in CCC.

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