Expression of Glucose Transporters in Human Pancreatic Tumors Compared with Increased FDG Accumulation in PET Study

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Although overexpression of GLUT-1 glucose transporter has already been reported in human cancers, the mechanism of glucose entry into pancreatic cancers remains unknown. To evaluate the relationship between GLUT glucose transporters and FDG uptake, FDG-PET was performed in 34 preoperative patients (mean age, 60.9 yr) with suspected pancreatic tumors, including 28 malignant and 6 benign tumors. **Methods:** FDG uptake at 50 min after injection of 185 MBq of [^{18}F]FDG with >5 hr of fasting was semiquantitatively analyzed as standardized uptake values (SUVs). The GLUT expression was studied by immunohistochemistry of paraffin sections from these tumors after operation using anti-GLUT-1, -2, -3, -4 and -5 antibodies to obtain immunohistochemical grading ("strong," "weak" and "negative") by three experienced physicians. **Results:** Of 26 malignant tumors proved by histological examination, 23 (88%) tumors were positive for the expression of GLUT-1 glucose transporter, and 17 (61%) showed strong expression. On the other hand, 13 (46%), 0 (0%), 9 (36%) and 13 (46%) malignant tumors were positive for the expression of GLUT-2, -3, -4 and -5 glucose transporters, respectively. Three of six benign tumors showed strong GLUT-1 expression. Concerning GLUT-2, -3, -4 and -5, only one benign tumor showed positive GLUT-5 expression. Thus, GLUT-1 showed relatively high sensitivity but low specificity (50%) for detecting malignant tumors, whereas GLUT-2, -3, -4 and -5 had lower sensitivities but higher specificities. Correlations between SUVs and grading of GLUT immunoreactivity were significant in GLUT-1 (strong, 4.49 ± 2.95; weak, 3.42 ± 1.21; negative, 2.52 ± 0.84) ($p < 0.05$) but not in the remaining four GLUT transporters. **Conclusion:** These data indicate that GLUT-1 has a significant role in the malignant glucose metabolism and may contribute to the increased uptake of FDG in PET imaging in patients with pancreatic tumor.

Key Words: pancreas tumor; GLUT expression; FDG-PET; immunohistochemistry

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PET with [^{18}F]FDG has shown promise in oncological imaging. An increase in FDG uptake has been demonstrated in a variety of malignant tumors (*1–8*).

For pancreatic tumors, we have reported the clinical values of FDG-PET for the detection and differentiation of pancreatic carcinoma (*9,10*).

The FDG accumulation is presumed to be due to enhanced exogenous glucose utilization in the tumor area (*11*). This theory is based on the observation that malignant tumors are characterized by increased glucose metabolism compared with healthy cells (*12*). Recent studies have demonstrated that facilitative glucose transport across the plasma membrane is mediated by a family of structurally related proteins, known as facilitated diffusion glucose transporters (designated GLUTs) (*13*). Brown et al. (*14*) reported significant positive correlation between the expression of GLUT-1 and FDG accumulation in viable cancer cells in the syngeneic rat breast cancer. In human studies as well, there is evidence of increased expression of glucose transporters in several malignancies (*15–20*). But as far as immunohistochemical study of human pancreatic cancers is concerned, only a few cases have been reported (*20*). In addition, no correlative clinical study has been reported between FDG accumulation and immunohistochemical expression of glucose transporters in the resected human tumor tissues.

In this study, we examined the immunohistochemical expression of five subtypes of glucose transporters (GLUT-1–5) in the resected pancreatic tumor tissue specimen to analyze the correlation between the FDG uptake value and the intensity of GLUT expression.

MATERIALS AND METHODS

Subjects

The study group was comprised of 34 patients with suspected pancreatic tumors (18 men and 16 women; mean age, 60.9 yr; age range, 19–79 yr) who were examined between June 1992 and June 1995 and who had an operation. Pancreatic tumors were suspected on the basis of clinical findings, laboratory data, ultrasound sonography and CT results. Patients had preoperative imaging with FDG-PET 1–3 wk before operation. They all had surgery, and paraffin sections were obtained from their resected tumors.

In 34 patients, a histological diagnosis was made after surgery. The results were as follows: ductal adenocarcinoma, n = 20; mucinous cystadenocarcinoma, n = 4; ampullary carcinoma, n = 2; islet cell tumor, n = 2; mass-forming pancreatitis, n = 2; primary sclerosing cholecystitis, n = 1; mucinous cystadenoma, n = 2; and solid and cystic tumor, n = 1.

Before being enrolled in this study, each patient gave written informed consent, as required by the Kyoto University Human Study Committee.

PET Imaging

Imaging Technique. Fluorine-18 was produced by a ^{20}Ne (d,α) ^{18}F nuclear reaction, and [^{18}F]FDG was synthesized with the acetyl hydrofluorite method (*21*). PET was performed with a whole-body PET camera that has eight rings, which provides 15 tomographic sections at 7-mm intervals. The intrinsic resolution was 4.6 mm FWHM at the center, and the axial resolution was 7 mm FWHM. The effective resolution after reconstruction was approximately 10 mm.

The patients fasted for at least 5 hr before the FDG injection. We

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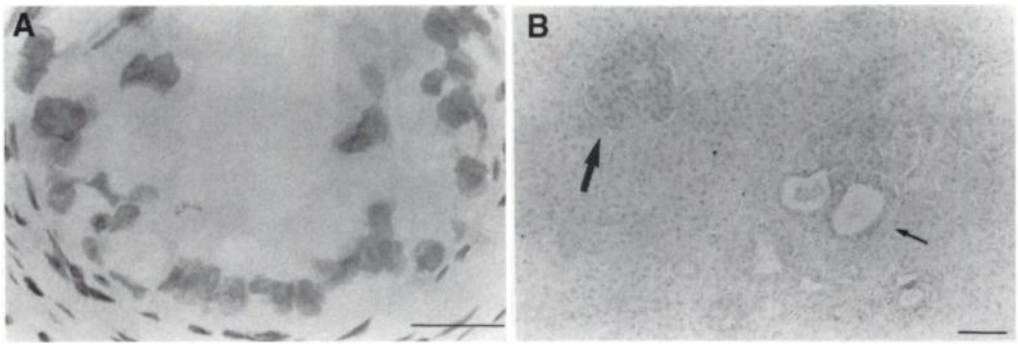

FIGURE 1. Examples of negative expression of GLUT glucose transporters in sections of human pancreatic tumors and normal pancreas, immunostained with anti-GLUT-5 (A) and anti-GLUT-2 (B) and counterstained with Mayer's hematoxylin. (A) Duct-like structures of ductal adenocarcinomas without immunostaining. Magnification = ×400; Bar = 50 μm. (B) Almost all of the acinar cells were not stained. Only islets of Langerhans (large arrow) and the epithelial cells lining the pancreatic ducts (small arrow) were positively stained. Magnification = ×50; Bar = 200 μm.

certified and marked the exact position of pancreatic tumors by the ultrasound sonography. At the time of imaging, the patient was positioned on the PET camera bed by this marking and transmission scanning for attenuation correction was performed for 20 min. Immediately after the transmission scan, approximately 150–250 MBq (4.1–6.8 mCi) of FDG was administered intravenously, and static scanning was performed exactly 60 min later for 15 min. Plasma glucose levels were measured at the time of FDG injection (*9*).

Image Analysis. Initially, PET images were compared with the corresponding CT images, which permitted accurate identification of the tumor by anatomic landmarks (for example, the upper and lower part of the kidney, the shape of the liver and the gallbladder bed), and blinded PET interpretation was performed qualitatively by three experienced nuclear medicine physicians. FDG accumulation was also analyzed quantitatively by calculating the standardized uptake value (SUV) in the regions of interest (ROIs) placed over the tumor, the normal pancreas and the normal liver (*22*) as follows:

$$\text{SUV} = \frac{\text{PET count} \times \text{calibration factor (mCi/g)}}{\text{injection dose (mCi)/body weight (g)}}.$$

The ROI placed over the tumor was 10 × 10 mm (independent of tumor size) and was selected in areas of tumor that showed the highest FDG activity. The ROIs placed over the normal pancreas and the normal liver were 10 × 10 mm and 25 × 25 mm, respectively (*9*).

Immunohistochemistry

Antibodies to GLUT Glucose Transporters. The polyclonal rabbit antiglucose transporter antibody reactive with GLUT-1 (rat) (brain/erythrocyte type) was raised against a synthetic peptide (13-mer), based on the deduced amino acid sequence of the carboxy terminus of the rat brain glucose transporter (CGLFHPLGADSQV). It immunoreacts with a 50,000-Da glucose transporter species in rat brain and human erythrocytes and cross-reacts with the glucose transporter of human hepatocarcinoma cells (HEP G2). It was diluted 1:1000 with 0.05 *M* Tris-HCl buffer, pH 7.5, containing 1% bovine serum albumin.

The polyclonal rabbit antiglucose transporter antibodies reactive with GLUT-2, -3, -4 and -5 were raised against synthetic peptides consisting of the carboxy termini of human GLUT-2, human GLUT-3, rat adipocytes insulin regulated glucose transporter and human GLUT-5, respectively. Anti-GLUT-2 and -4 antibodies were diluted 1:1000, and anti-GLUT-3 and -5 antibodies were diluted to 5 μg/ml, as is shown above.

Immunohistochemical Procedure. Paraffin sections from each tumor, processed for routine pathology, were deparaffinized with xylene and ethanol and unmasked by target unmasking fluid. Then the sections were washed with PBS for 15 min (three times for 5 min each time) and blocked for 30 min at 25°C with 10% normal bovine serum in PBS. Then the sections were incubated with each of the anti-GLUT glucose transporter antibodies as primary antibody for 1 hr at 25°C. Parallel sections were incubated with healthy rabbit immunoglobulin G (at 2 and 20 μg/ml) as negative controls. Then the sections were washed with PBS for 15 min (three times for 5 min each time). In the following steps, each section was stained by the labeled streptavidin-biotin method. For linking, the sections were incubated with the second antibody for 10 min at 25°C and washed with PBS for 15 min. Then they were incubated with streptavidin peroxidase and washed with PBS for 15 min. 3,3′-Diaminobenzidine tetrahydrochloride was used as a substitute substrate–chromogen solution at 25°C for 10 min, diluted to 1 mg/ml with 0.05 *M* Tris-HCl buffer, pH 7.5. Then the sections were rinsed gently with distilled water and washed in flowing water for 15 min. In the last step, the sections were lightly counterstained with Mayer's hematoxylin, diluted 1:10, and then dehydrated, dealcoholized and coverslipped with mounting medium.

Other chemicals not mentioned above were of reagent grade or of the highest purity available.

For positive controls, normal tissues from the appropriate pancreatic and duodenal areas were available in some cases. We also used DAKO control slides as positive controls.

All slides were examined by light microscopy.

Immunohistochemical Grading. Immunohistochemical grading was performed by three independent, experienced physicians without knowledge of the SUVs in each case.

In the positive cases, differences in the intensities, the number and the patterns of the staining were observed in the same tumor. So we categorized the grading of GLUT immunoreactivity as follows: "negative," "weak" and "strong".

In the cases of neoplastic tumors, we observed only the staining of the tumor cells; the staining of fibroblasts and other interstitial cells between the tumor cells was not counted.

If there were no staining or if there were only some weak staining, as weak as the background staining, in all the tumor cells and mass-forming areas, we categorized the staining as negative (Fig. 1A).

Three types of strong staining were observed: we called them "cytoplasmic granular," "homogeneous" and "reticulated" types. In some of these positive cases (cytoplasmic granular type), we could observe cytoplasmic granules in the tumor cells, both weakly (Fig. 2A) and strongly (Fig. 3A). In other cases (homogeneous type), the cytoplasm of the tumor cells were stained homogeneously in brown, both weakly (Fig. 2B) and strongly (Fig. 3B). In yet other cases (reticulated type), the membranes of the tumor cells were strongly stained, and it seemed like a brown reticulated structure. This type of staining was only observed in the strongly stained cases, categorized as strong (Fig. 3B, C).

If there were some stained area of membrane and cytoplasm of the tumor cells, this positive type of staining was categorized as

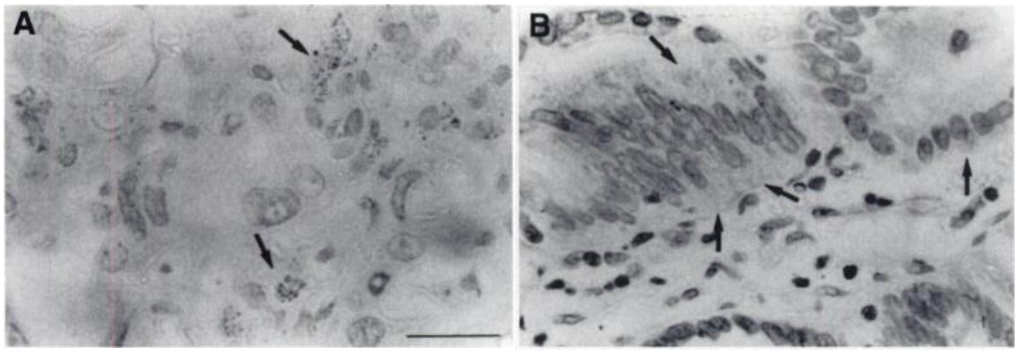

FIGURE 2. Examples of weak expression of GLUT glucose transporters in sections of human pancreatic tumors and normal pancreas, immunostained with anti-GLUT-4 (A) and anti-GLUT-2 (B), and counterstained with Mayer's hematoxylin. (A) Cytoplasmic granular type. Cytoplasmic granules in the tumor cells were observed dispersively stained in the section of ductal adenocarcinoma. (B) Homogeneous type. Cytoplasm of the tumor cells were stained homogeneously (arrows) in the section of ductal adenocarcinoma. Magnification = ×400; Bar = 50 μm.

weak or strong. There was no need for all the tumor cells to be stained.

If a case had some part of strong staining in its tumor cells, the grade of GLUT immunoreactivity of the case was categorized as strong.

In cases of inflammatory and nonneoplastic tumors, we observed the staining of their fibroblasts and other interstitial cells, inflammatory cells and islet cells. If there was stronger staining than the staining of the counterparts by and between the tumor cells in tumor cases, we categorized them as weak or strong. If they were stained less or the same as the counterpart in tumor cases, we categorized them as negative (Fig. 1B).

Statistical Analysis

The data presented in this paper are expressed as means ± s.d. The statistical analysis in SUVs between malignant and benign tumors was performed by ANOVA, followed by Student's t-test for unpaired data. The nonparametric statistical analysis between SUVs and the immunohistochemical results was performed by ANOVA, followed by the Kruskal–Wallis test and Mann–Whitney U-test. Probability values of less than 0.05 indicated a statistically significant difference.

RESULTS

Table 1 summarizes the results of FDG-PET imaging and the immunohistochemical findings of the 34 patients studied.

PET Imaging Diagnosis

The histological examination showed that 28 of 34 tumors were malignant and 6 were benign. FDG-PET accurately identified an increase in FDG uptake in 26 of the 28 malignant tumors and no increase in FDG uptake in five of the six benign cases (Table 1).

In the quantitative analysis of FDG-PET uptake, the SUVs of malignant tumors ranged from 1.76 to 15.12 with a mean value of 4.22 ± 2.55; these values were significantly higher than those of benign lesions (range, 1.20–3.68; mean, 2.34 ± 1.17) ($p < 0.05$) (Fig. 4). When the SUV of 2.03 was set as a threshold, all but one malignant lesion showed an increase in SUV above the threshold, whereas all but two benign tumors were below the threshold.

Immunohistochemical Findings in Tumors

Negative control sections showed no staining with healthy rabbit immunoglobulin G (at 2 and 20 μg/ml).

The results of the grading by the three physicians were concordant in more than 90% cases. There were no cases of three different grades assigned by the three physicians. The interobserver variance in our grading was 16/170 (9.4%).

In the cases of neoplastic tumors, all three staining patterns, cytoplasmic granular, homogeneous and reticulated types, were observed in the GLUT-1 immunohistochemical findings. But homogeneous and reticulated patterns were far more often observed than cytoplasmic granular patterns in GLUT-1. In GLUT-1 expression, the three patterns were often seen in a same tumor, separately or side-by-side. In the GLUT-2 immunohistochemical findings, the homogeneous type was mainly observed. The cytoplasmic granular pattern was rare, and the reticulated pattern was not seen. In GLUT-3, none of the three staining patterns was observed. In GLUT-4, only the cytoplasmic granular type was observed. In GLUT-5, cytoplasmic granular and homogeneous types were observed.

In the GLUT-1 immunohistochemical findings, there were some differences between histological types. In the cases of ductal adenocarcinoma, all three staining patterns, cytoplasmic granular, homogeneous and reticulated types, were observed. In the cases of cystadenoma and cystadenocarcinoma, all three staining patterns were observed, but homogeneous and reticulated types were mainly observed. In the cases of islet cell tumor and solid and cystic tumor, the homogeneous type was

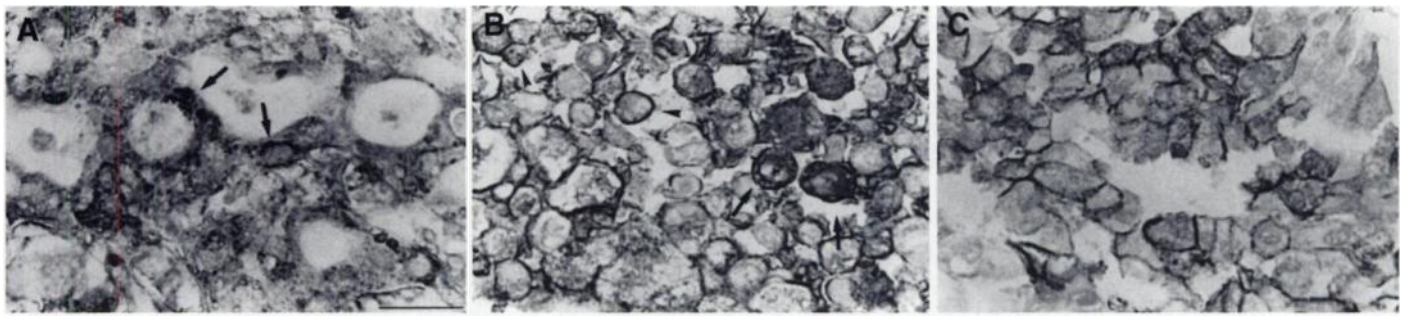

FIGURE 3. Examples of strong expression of GLUT glucose transporters in sections of human pancreatic tumors and normal pancreas, immunostained with anti-GLUT-1 and counterstained with Mayer's hematoxylin. Magnification = ×400; Bar = 50 μm. (A) Cytoplasmic granular type. Cytoplasmic granules (arrows) in the tumor cells were observed massively stained in the section of ductal adenocarcinoma. (B) Homogeneous type. Cytoplasm of the tumor cells were stained homogeneously strongly in the sections of ductal adenocarcinoma (arrows). We also observed the reticulated structure (arrowheads). (C) Reticulated type. Membrane of the tumor cells were stained strongly positive in the sections of ductal adenocarcinoma. It appeared as a brown reticulated structure.

TABLE 1
Results of FDG-PET Imaging and Immunoreactivity of Glucose Transporters in 34 Pancreatic Lesions

Patient no.	Age (yr)	Sex	Tumor characteristics: Histological diagnosis	Size (mm)	Stage*	FDG-PET diagnosis	SUV	Grade of immunoreactivity: GLUT-1	GLUT-2	GLUT-3	GLUT-4	GLUT-5
Malignant lesions (n = 28)												
1	65	M	Ductal adenocarcinoma	28	T2N1M0	Malignant	2.52	N	N	N	Weak	N
2	63	M	Ductal adenocarcinoma	30	T2N0M0	Malignant	2.80	N	N	N	N	N
3	72	M	Ductal adenocarcinoma	60	T3N1M1	Malignant	2.87	N	N	N	Weak	N
4	62	M	Cystadenocarcinoma	10	T1aN0M0	False-negative	2.04	Weak	Weak	N	N	Weak
5	57	F	Islet cell tumor	45		Malignant	2.44	Weak	N	N	N	N
6	77	M	Cystadenocarcinoma	30	T1bN0M0	Malignant	2.50	Weak	Weak	N	Weak	Weak
7	71	F	Ductal adenocarcinoma	18	T1aN0M0	Malignant	2.74	Weak	Weak	N	N	Strong
8	55	M	Ductal adenocarcinoma	40	T3N1M0	Malignant	3.62	Weak	Strong	N	Weak	Strong
9	59	F	Ductal adenocarcinoma	50	T2N1M1	Malignant	3.88	Weak	N	N	N	N
10	64	F	Ductal adenocarcinoma	80	T3N1M1	Malignant	4.67	Weak	N	N	N	N
11	55	F	Ductal adenocarcinoma	30	T1bN1M0	Malignant	5.49	Weak	Weak	N	N	Strong
12	77	M	Cystadenocarcinoma	28	T1bN0M0	False-negative	1.76	Strong	Strong	N	N	Strong
13	69	F	Ampullary carcinoma	15	T2N0M0	Malignant	2.78	Strong	Weak	N	N	Weak
14	45	M	Cystadenocarcinoma	70	T2N0M0	Malignant	2.88	Strong	N	N	Strong	N
15	67	F	Ductal adenocarcinoma	20	T1bN1M0	Malignant	3.09	Strong	N	N	N	N
16	68	F	Ductal adenocarcinoma	50	T3N1M0	Malignant	3.17	Strong	Strong	N	Strong	Strong
17	79	M	Ductal adenocarcinoma	30	T3N0M0	Malignant	3.28	Strong	Strong	N	Weak	Strong
18	76	F	Ductal adenocarcinoma	70	T3N1M1	Malignant	3.49	Strong	N	N	N	N
19	51	M	Ductal adenocarcinoma	25	T2N0M0	Malignant	3.55	Strong	Weak	N	N	N
20	52	M	Ductal adenocarcinoma	40	T3N1M0	Malignant	3.71	Strong	Weak	N	Strong	Weak
21	58	M	Ductal adenocarcinoma	50	T3N1M1	Malignant	4.19	Strong	N	N	N	Weak
22	67	F	Ductal adenocarcinoma	80	T3N1M1	Malignant	4.84	Strong	Weak	N	N	Strong
23	69	M	Ductal adenocarcinoma	80	T3N1M1	Malignant	5.71	Strong	N	N	N	N
24	66	M	Ductal adenocarcinoma	70	T3N1M1	Malignant	5.93	Strong	N	N	N	N
25	66	M	Ductal adenocarcinoma	70	T3N1M0	Malignant	5.98	Strong	N	N	N	N
26	55	F	Ductal adenocarcinoma	55	T3N1M1	Malignant	6.27	Strong	N	N	N	N
27	46	M	Ductal adenocarcinoma	70	T3N1M1	Malignant	6.90	Strong	N	N	N	N
28	61	F	Islet cell tumor	60		Malignant	15.12	Strong	Weak	N	Strong	Weak
Benign lesions (n = 6)												
29	64	M	Chronic pancreatitis	15		Benign	1.20	N	N	N	N	N
30	61	M	Chronic pancreatitis	50		Benign	2.03	N	N	N	N	N
31	44	F	Primary sclerosing cholangitis	45		False-positive	3.68	N	N	N	N	N
32	46	F	Cystadenoma	40		Benign	1.31	Strong	N	N	N	N
33	65	F	Cystadenoma	30		Benign	1.94	Strong	N	N	N	N
34	19	F	Solid and cystic tumor	35		False-positive	3.9	Strong	N	N	N	Weak

M = male; F = female; N = negative.
*Tumor stage according to Reference *34*.

mainly observed, and cytoplasmic granular and reticulated types were rare.

In GLUT-2, -3, -4 and -5, there were no significant differences between histological types.

In cases of inflammatory and nonneoplastic tumors, the intensities of the staining of fibroblasts and other interstitial cells, inflammatory cells and the islet cells were all the same as those in the neoplastic tumor cases. All three immunohistochemical findings were negative.

Table 2 shows the results of 28 malignant and six benign pancreas tumors with each GLUT histochemical grade.

GLUT-1 immunohistochemical findings showed the following. Of 28 malignant tumors proven by histological examination, 25 (89%) tumors were positive for expression of GLUT-1 glucose transporter. Only three cases (11%) of ductal adenocarcinomas did not show the GLUT-1 immunoreactivity. Of 28 malignant tumors, eight (29%) showed weak-grade GLUT-1 glucose transporter immunoreactivity, and 17 (61%) showed strong immunoreactivity. Of six benign tumors proven by histological examination, three (50%) tumors showed strong

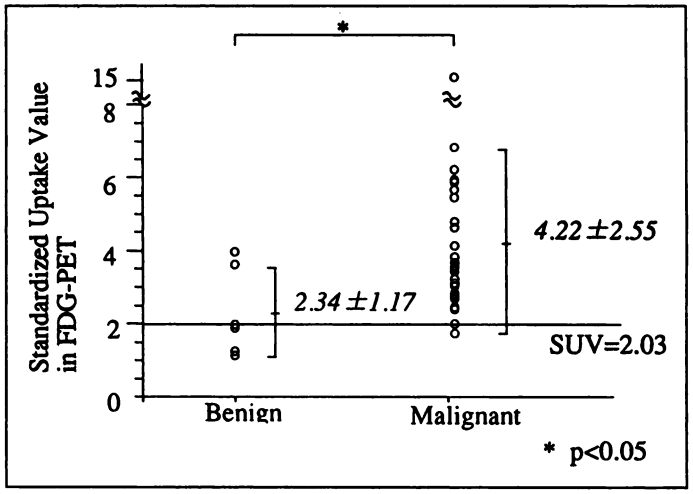

FIGURE 4. Comparison of SUVs of malignant and benign lesions of the pancreas. When an SUV of 2.03 was set as a threshold, all malignant lesions but one showed an increase in SUV above the threshold, and all benign but two showed a decrease.

TABLE 2
Results of Immunohistochemical Grading of Glucose Transporters in 28 Malignant and Six Benign Pancreatic Tumors

Glucose transporter	Malignant tumors (n = 28)			Benign tumors (n = 6)		
	Grade			Grade		
	Negative	Weak	Strong	Negative	Weak	Strong
GLUT-1	3 (11%)	8 (29%)	17 (61%)	3 (50%)	0 (0%)	3 (50%)
GLUT-2	15 (54%)	9 (32%)	4 (14%)	6 (100%)	0 (0%)	0 (0%)
GLUT-3	28 (100%)	0 (0%)	0 (0%)	6 (100%)	0 (0%)	0 (0%)
GLUT-4	19 (68%)	5 (18%)	4 (14%)	6 (100%)	0 (0%)	0 (0%)
GLUT-5	15 (54%)	6 (21%)	7 (25%)	5 (83%)	1 (17%)	0 (0%)
Total no. of sections	80 (57%)	28 (20%)	32 (23%)	26 (87%)	1 (3%)	3 (10%)

expression of GLUT-1 glucose transporter (all of them were neoplastic tumors). All three inflammatory pseudotumors were negative for GLUT-1 expression. The expression of GLUT-1 protein was strong and had high sensitivity but was revealed to have low specificity.

In the remaining four GLUT immunohistochemical studies, 13 (46%), 0 (0%), 9 (32%) and 13 (46%) malignant tumors were positive for the expression of GLUT-2, -3, -4 and -5, respectively (Table 2). Furthermore, of the strong-grade tumors, only four (14%), zero (0%), four (14%) and seven (25%) tumors showed strong positive expression of GLUT-2, -3, -4 and -5, respectively. The expression of these four transporters had less intensity than the expression of GLUT-1. But of six benign tumors proved by histological examination, only one tumor was positive for the expression of the GLUT-5 glucose transporter. In all the remaining cases, the expression was negative. Compared with GLUT-1 expression, the expressions of GLUT-2, -3, -4 and -5 had lower sensitivity but higher specificity.

Immunohistochemical Findings in Normal Pancreatic Tissues and Positive Controls

In GLUT-1, normal acinar cells of pancreas were not stained in all cases. Only some acinar cells near the malignant tumor surface, which were severely compressed and not considered to be normal and healthy, were stained positive in some cases. Cytoplasm of the healthy epithelial cells lining the pancreatic ducts and the healthy cells of the islets of Langerhans were positive in many cases. The columnar epithelia of the duodenal mucosa were also stained positive in many cases. Positive staining of endothelial cells of blood vessels and perineurium of nerve fibers was observed in some cases. Fibroblasts and other interstitial cells in the duodenal submucosa and pancreas also stained positive in many cases. Fat tissue of the pancreas also stained positive in some cases. All the structures mentioned above were stained more weakly than the strongly GLUT-1-stained tumor cells. Erythrocytes were strongly stained in all cases.

Almost all the structures of the pancreas were positive with GLUT-2, -4 and -5, but many of them stained more weakly than with GLUT-1.

In GLUT-2, perineurium of nerve fibers was not stained, but nerve fibers themselves were positively stained. The islet cells of Langerhans were positively stained.

In GLUT-2 and -5, fat tissue, fibroblasts and other interstitial cells in the duodenal submucosa and pancreas were stained strongly in many cases. Erythrocytes were positively stained in some cases.

In GLUT-3, almost all the structures of pancreas stained negatively.

In GLUT-1, normal brain and testis sections stained strongly positive, and normal kidney, small and large intestine, adrenal gland and heart sections stained positively. The DAKO control slides and sections from carcinoid tumor, malignant fibrous histiocytoma, rhabdomyosarcoma, malignant melanoma, undifferentiated carcinoma, mesothelioma, astrocytoma, prostatic carcinoma, pancreatic carcinoma, gastric carcinoma, breast carcinoma, colonic carcinoma, renal cell carcinoma, hepatocellular carcinoma and lung adenocarcinoma all stained strongly positive.

In GLUT-2, normal liver, kidney, small and large intestine and testis sections stained strongly positive, and normal pancreas and adrenal gland sections stained positively. Of the multitumor block DAKO control slides, some sections, such as astrocytoma, colonic carcinoma, hepatocellular carcinoma and lung adenocarcinoma, stained positive.

In GLUT-3, none of the checkerboard normal multitissue block DAKO control slides were stained. And of the multitumor block DAKO control slides, some sections, such as astrocytoma, ovarian carcinoma, colonic carcinoma and hepatocellular carcinoma, stained weakly positive.

In GLUT-4, normal brain and testis sections were stained strongly positive, and normal kidney, small and large intestine, adrenal gland and heart sections were stained positively. Of the multitumor block DAKO control slides, some sections, such as rhabdomyosarcoma, astrocytoma, breast carcinoma, colonic carcinoma, hepatocellular carcinoma and lung adenocarcinoma, stained strongly positive.

In GLUT-5, normal brain and testis sections stained strongly positive, and normal kidney, small and large intestine, adrenal gland and heart sections stained positively. Of the multitumor block DAKO control slides, some sections, such as rhabdomyosarcoma, astrocytoma, breast carcinoma, colonic carcinoma, hepatocellular carcinoma and lung adenocarcinoma, stained strongly positive.

Correlation Between SUVs and GLUT-1–5 Expression

Figure 5 shows the comparative results of SUVs in FDG-PET and GLUT-1 immunoreactivity in the histochemistry of the 34 pancreas tumors. There were six cases of negative-grade GLUT-1 immunoreactivity, and the mean of their SUVs was 2.52 ± 0.84. There were eight cases of weak grade, and the mean of their SUVs was 3.42 ± 1.21. There were 20 cases of strong grade, and the mean of their SUVs was 4.49 ± 2.95. The SUVs increased as the grade of immunoreactivity increased from negative to strong. SUVs were significantly different among the three groups, negative, weak and strong ($p < 0.05$). Figure 6 shows each comparative result of SUVs in FDG-PET and GLUT-2–5 immunoreactivity in the histochemistry of the 34 pancreas tumors studied. There were no significant correlation between SUVs and GLUT-2–5 immunoreactivity.

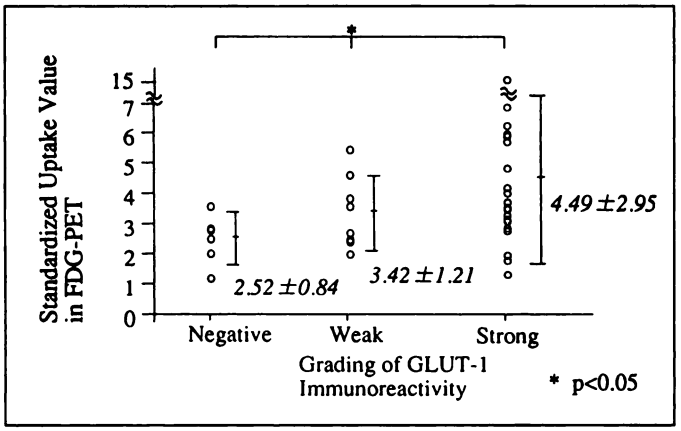

FIGURE 5. Comparison of SUVs of all the malignant and benign lesions of the pancreas according to the grading of GLUT-1 immunoreactivity. The average of the SUVs increased as the grade became stronger.

DISCUSSION

Our data showed the close correlation between the immunoreactivity of GLUT-1 and SUVs in FDG-PET. On the contrary, GLUT-2–5 immunoreactivity did not correlate with SUVs in FDG-PET. These findings suggest that increased expression of GLUT-1 transporter molecules in human pancreatic tumors may contribute to a higher rate of entry of the FDG into the tumor cells compared with normal pancreatic tissues. Furthermore, there were close relationships between the malignant potential of human tumors, SUVs in FDG-PET imagings and the increased expression of GLUT-1 glucose transporter. Recently, Brown et al. (*14*) reported a significant positive correlation between the expression of GLUT-1 and FDG accumulation in viable cancer cells in the syngeneic rat breast cancer. Our clinical data were almost compatible with theirs.

GLUT-1 is omnipresent and is thought to be the most primitive type of glucose transporter. GLUT-1 is expressed in most tissue types, all cell lines, transformed cells and tumor cells, and is thought to be responsible for "housekeeping" levels of glucose transport (*23*). Furthermore, GLUT-1 was the predominant isoform expressed in most of the human fetal pancreas tissues (*24*). These reports also support our results that most of the pancreas malignant tumors expressed GLUT-1 protein strongly.

It has long been recognized that cancer cells have increased rates of glucose metabolism compared with benign cells (*12*). A variety of mechanisms have been proposed for the accelerated glucose use seen in growing tumors and in transformed and malignant cells, such as increased concentrations of hexokinase (*25*), decreased rates of glucose-6-phosphatase-mediated dephosphorylation (*26*) and enhanced rates of glucose uptake. Activation of glucose transporters and elevated glucose consumption are considered to be early and prominent features of oncogene-mediated malignant transformation (*23*).

But high uptake of FDG does not seem to be specific for malignancy. Several studies have shown not only acceleration of glucose uptake and of glucose transporter induction during transformation (*27*) but also normal development and growth conditions (*28*). A tumor is counted as a cancer only if it is malignant, that is, only if its cells have the ability to invade surrounding tissue. And recently, invasiveness were thought to be correlated not with the increased glucose consumption but with the other cancerous characters, such as migration and adhesion.

Our results showed that the expression of GLUT-1 protein was strong and had high sensitivity but low specificity. All the three benign neoplastic tumors, without characters of invasiveness, had strong immunoreactivity in our grading. Therefore, there was an enormous range of SUVs in strong cases (Fig. 5).

Of course, we had only three benign neoplastic and only three nonneoplastic tumor cases. But we had an impression in this study that there might actually be a closer relationship between the neoplastic cellular character and the increased glucose consumption than that between the malignant potential and the increased glucose consumption. It is true that some benign neoplastic tumor cells are supposed to have the increased glucose consumption and FDG uptake. We must discuss the problems of glucose consumption and GLUT expression separately at the cellular level and at the tissue level. Some other factors should have some influence on the total count of FDG uptake within a ROI at a clinical study level.

We considered the following four factors: cellularity, expression pattern of transporters, intratumoral heterogeneity of transporter and inflammatory changes.

First, we must discuss the problem of the cellularity. We did not evaluate the number of viable tumor cells, and we categorized the grade of GLUT immunoreactivity as strong if a case had any strong staining in its tumor cells. In the case of islet cell tumor (Patient 28), the case with highest SUV, there were huge numbers of tumor cells in the section, and almost all of the

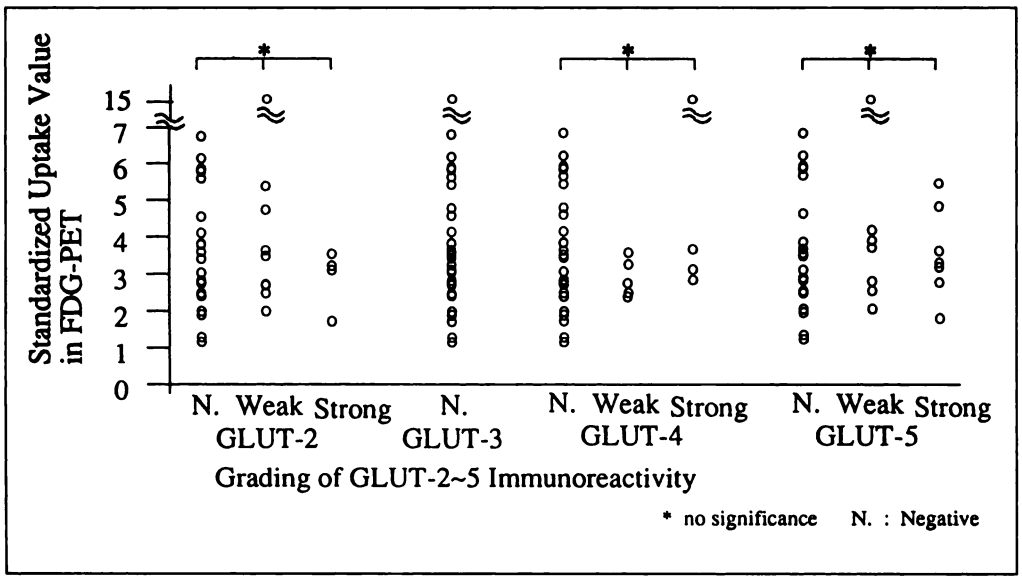

FIGURE 6. Comparison of SUVs of all the malignant and benign lesions of the pancreas according to the grading of GLUT-2–5 immunoreactivity. There were no significant correlations between SUVs and GLUT-2–5.

tumor cells stained strongly positive. On the other hand, in the case of cystadenocarcinoma (Patient 12), the case with lowest SUV in the strong grade, there were far fewer tumor cells in the section. Especially in the cases of cystic tumors, the number of viable tumor cells might have some effect on the discrepancies between FDG uptake and GLUT-1 immunoreactivities. And of course, even within the cystic tumors, cystadenoma cases had fewer tumor cells than cystadenocarcinoma cases, which had mural nodules. Recent reports showed that FDG uptake does not relate to the proliferative activity of cancer cells, but it strongly relates to the number of viable tumor cells (*29*). The differences between the FDG uptake of malignant tumors and that of benign neoplastic tumors might be due to the differences between the cellularities in the tumors, despite the similarly high expression of GLUT-1.

Furthermore, we observed that the cases of negative GLUT-1 (Patients 1, 2 and 3) seemed to have relative high cellularity. Both higher cellularity and higher GLUT-1 expression seemed to be needed for higher FDG accumulation. However, it is difficult to evaluate the cellularity of tumor cells at the whole tissue level, including cystic space, fat and fibrous tissue, as wide as ROIs in the actual FDG-PET study. Further clinical study with a sophisticated method is needed.

Second, there is the problem of the expression pattern of transporters. In our immunohistochemical results of neoplastic tumors, all three staining patterns were observed only in GLUT-1, whereas none of the remaining four GLUTs had expression of the reticulated pattern. Brown et al. (*14*) emphasized the importance of the high membranous expression of GLUT-1. It is well-known that GLUT-4 levels at the cell surface can rise five- to 40-fold over levels during prolonged fasting due to translocation to the membrane from vesicles and markedly increased glucose utilization (*30*). Some models for the cellular trafficking of glucose transporter proteins are considered to explain these facts (*31*). In these theories, the strong staining pattern of cytoplasmic granular in our study is supposed to be the expression of GLUT location in the trans-Golgi network and intracellular vesicles. The reticulated pattern is considered to be located in the cell membrane. We supposed this pattern probably corresponds to the high membranous expression of GLUT-1 in the report by Brown et al. (*14*). Homogeneous pattern is considered to be a mixed type. Recent studies showed that there are three potential mechanisms that could account for the observed increases in glucose transport: increase of the total level of glucose transporter proteins; increasing numbers of functional transporters at the cell surface; and increasing activity of individual glucose transporter proteins (*23*). In this study, we count only the total level of glucose transporter proteins, not the function or activity. But if the reticulated pattern had a closer relationship to the real function or activity in glucose transport than to the other two patterns, then the role of GLUT-1 in entry of the FDG into the tumor cells should be more significant than those of the remaining four GLUTs. Our immunohistochemical findings of staining patterns might be a further confirmation of the importance of the role of GLUT-1.

Third, Brown et al. (*14*) emphasized the importance of the heterogeneity of the intratumoral distribution of GLUT-1 expression and FDG uptake and the relationship between the heterogeneity and hypoxic condition. We must admit that the heterogeneity of the intratumoral distribution of GLUT expression was not considered in this study. If a case had strong staining in its tumor cells, the grade of GLUT immunoreactivity of the case was categorized as strong, independent of the distribution of GLUT-expressed cells. Because this was a clinical study and we had to evaluate many different types of tumors with several different histological characters, solid or cystic, hypervascular or hypovascular, it was impossible to evaluate the heterogeneity or hypoxic condition at the tissue level.

Last, inflammatory changes might have some effect on our results. In one case of primary sclerosing cholangitis, massive infiltration of inflammatory cells was observed with near-background-level GLUT-1 expression (one of our physicians diagnosed this case as positive). Some reports said that, although there may also be a component of uptake of FDG to nonmalignant inflammatory cells in the tumors, these contributions are modest in many tumor systems (*25,32,33*). These massive lymphocytes might be related to the false-positive PET result of this case.

Finally, we discuss the GLUT subtypes. Our results showed that GLUT-2–5 immunoreactivity did not correlate with SUVs in FDG-PET. Compared with GLUT-1 expression, the expressions of GLUT-2, -3, -4 and -5 had lower sensitivity but higher specificity. GLUT-2, -4 and -5 have specific characteristics and tissue and cellular distributions. GLUT-4 and -5 are thought to be absent from pancreas tissue. GLUT-2 are thought to be limited in the islet cells, not the pancreatic duct cells. Benign tumors, thought to have characteristics of the original organ, could not express such well-differentiated types of glucose transporters. The roles of GLUT-2, -4 and -5 in entry of the FDG into the benign tumor cells were thought to be less significant than that of GLUT-1. In malignant tumors, their roles in glucose transporting are questionable because of the pattern of expression already mentioned. The GLUT-3 glucose transporter, as well as GLUT-1, may be responsible for the basal glucose uptake, and there is evidence of increased expression of GLUT-3 glucose transporter in several human malignancies in some reports (*17,18,20*), especially in some of the pancreatic cancer cases (*20*). Our results were incompatible with these previous reports, but Yamamoto et al. (*20*) used the Northern blot method. The difference between the expression level in mRNA and protein might have some effect on our results.

CONCLUSION

Our findings suggest that there is a close relationship between SUVs in FDG-PET imagings and the increased expression of GLUT-1 glucose transporter but, at least in this study, the relationship is independent of the malignant potential of human tumors. GLUT-1 has a significant role in the glucose metabolism of pancreatic tumors and may contribute to the increased uptake of FDG in PET imaging.

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Overexpression of Glucose Transporter 1 and Increased FDG Uptake in Pancreatic Carcinoma

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Increased glycolysis is a characteristic metabolic feature of a malignant transformed phenotype. In cultured cells transformed by viruses or activated oncogenes, enhanced glycolytic metabolism is mediated by the overexpression of glucose transporter 1 (Glut-1) and key regulatory glycolytic enzymes. Whether increased glucose metabolism in solid human malignant tumors is related to the overexpression of key regulatory proteins of glucose metabolism is presently unknown. We thus studied the expression of Glut-1 and glucose uptake, assessed with 2-fluorodeoxyglucose (FDG) and PET in human pancreatic carcinoma (PC) and chronic mass-forming pancreatitis (MFP). **Methods:** Glucose uptake was measured in the fasting state with FDG and PET in 12 patients with PC and 15 patients with MFP. The standardized uptake value (SUV) of FDG was determined as a global quantitative measure of tissue glucose utilization in cancer tissue or MFP. The expression of Glut-1 and Glut-4 was analyzed from operatively removed cancer or MFP tissue by Northern analysis or semiquantitative reverse transcriptase-polymerase chain reaction. The count ratio of Glut-1 to Glut-4 transcripts was used as an indicator of selective Glut-1 up-regulation. **Results:** The SUVs of FDG in patients with cancer and MFP were 2.98 ± 1.23 and 1.25 ± 0.51 ($p < 0.01$), respectively. Northern analysis showed intense Glut-1 expression in four of five patients with cancer but not in any of the five patients with MFP that were tested. In PC, Glut-1 and Glut-4 transcripts were found in five of five and three of 10 patients, respectively, using reverse transcriptase-polymerase chain reaction, whereas in MFP, Glut-1 was detected in one of five and Glut-4 was detected in all five patients. The Glut-1-to-Glut-4 transcript ratios were 6.17 ± 1.27 in patients with cancer and 0.42 ± 0.12 in patients with MFP. The mean Glut-1 concentration in eight patients with cancer was 1.71 nmol of Glut-1 mRNA/μg of mRNA (range, 0.0446–9.43) and 0.15 (range, 0–1.55) ($p < 0.05$) in 13 patients with MFP. **Conclusion:** The concomitant enhancement of glucose utilization and selective overexpression of Glut-1 mRNA in pancreatic cancer but not in MFP suggested constitutive activation of *Glut-1* gene or decreased degradation of Glut-1 mRNA in human pancreatic cancer. These findings may imply a potential for the early detection of pancreatic cancer with FDG and PET and identify new targets for anticancer therapy.

Key Words: PET; Glut-1; pancreatic carcinoma; chronic mass-forming pancreatitis

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Increased rates of respiration, glucose uptake and glucose metabolism in malignant tumors have been documented since the early observations of Warburg (*1*) and are among the most characteristic biochemical markers of the transformed phenotype. With the availability of sensitive PET scanners and 2-fluorodeoxyglucose (FDG) for measuring regional tissue glucose metabolism, enhanced tumoral glucose consumption has gained considerable clinical interest, forming one important pathophysiological mechanism for detecting, staging and con-

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