7. Torizuka T, Tamaki N, Inokuma T, et al. Value of fluorine-18-FDG PET to monitor hepatocellular carcinoma after interventional therapy. *J Nucl Med* 1994;35:1965-1969.
8. Wahl RL, Cody RL, Hutchins GD, Mudgett EE. Primary and metastatic breast carcinoma: initial clinical evaluation with PET with the radiolabeled glucose analog 2-[^{18}F]-fluoro-2-deoxy-D-glucose. *Radiology* 1991;179:765-770.
9. Inokuma T, Tamaki N, Torizuka T, et al. Evaluation of pancreatic tumors with positron emission tomography and ^{18}F-fluorodeoxyglucose: comparison with CT and US. *Radiology* 1995;195:345-352.
10. Inokuma T, Tamaki N, Torizuka T, et al. Value of fluorine-18-fluorodeoxyglucose and thallium-201 in the detection of pancreatic cancer. *J Nucl Med* 1995;36:229-235.
11. Yonekura Y, Benua RS, Brill AB, et al. Increased accumulation of 2-deoxy-2-[^{18}F]Fluoro-D-glucose in liver metastases from colon carcinoma. *J Nucl Med* 1982; 23:1133-1137.
12. Warburg O. The metabolism of tumors. *Constable* 1930:254-270.
13. Mueckler M, Caruso C, Baldwin SA, et al. Sequence and structure of a human glucose transporter. *Science* 1985;229:941-945.
14. Brown RS, Leung JY, Fisher SJ, Frey KA, Ethier SP, Wahl RL. Intratumoral distribution of tritiated-FDG in breast carcinoma: correlation between Glut-1 expression and FDG uptake. *J Nucl Med* 1996;37:1042-1047.
15. Boden G, Murer E, Mozzoli M. Glucose transporter proteins in human insulinoma [published erratum appears in *Ann Intern Med* 1994;121:470]. *Ann Intern Med* 1994;121:109-112.
16. Brown RS, Wahl RL. Overexpression of Glut-1 glucose transporter in human breast cancer. An immunohistochemical study. *Cancer* 1993;72:2979-2985.
17. Mellanen P, Minn H, Grenman R, Harkonen P. Expression of glucose transporters in head-and-neck tumors. *Int J Cancer* 1994;56:622-629.
18. Nishioka T, Oda Y, Seino Y, et al. Distribution of the glucose transporters in human brain tumors. *Cancer Res* 1992;52:3972-3979.
19. Su TS, Tsai TF, Chi CW, Han SH, Chou CK. Elevation of facilitated glucose-transporter messenger RNA in human hepatocellular carcinoma. *Hepatology* 1990;11: 118-122.
20. Yamamoto T, Seino Y, Fukumoto H, et al. Overexpression of facilitative glucose transporter genes in human cancer. *Biochem Biophys Res Commun* 1990;170:223-230.
21. Tamaki N, Yonekura Y, Yamashita K, et al. Relation of left ventricular perfusion and wall motion with metabolic activity in persistent defects on thallium-201 tomography healed myocardial infarction. *Am J Cardiol* 1988;62:202-208.
22. Woodard HQ, Bigler RE, Freed B. Expression of tissue isotope distribution. *J Nucl Med* 1975;16:958-959.
23. Merrall NW, Plevin R, Gould GW. Growth factors, mitogens, oncogenes and the regulation of glucose transport. *Cell Signal* 1993;5:667-675.
24. Mally MI, Otonkoski T, Lopez AD, Hayek A. Developmental gene expression in the human fetal pancreas. *Pediatr Res* 1994;36:537-544.
25. Torizuka T, Tamaki N, Inokuma T, et al. In vivo assessment of glucose metabolism in hepatocellular carcinoma with FDG-PET. *J Nucl Med* 1995;36:1811-1817.
26. Graham MM, Spence AM, Muzi M, Abbott GL. Deoxyglucose kinetics in a rat brain tumor. *J Cereb Blood Flow Metab* 1989;9:315-322.
27. Birnbaum MJ, Haspel HC, Rosen OM. Transformation of rat fibroblasts by FSV rapidly increases glucose transporter gene transcription. *Science* 1987;235:1495-1498.
28. Dermietzel R, Krause D, Kremer M, Wang C, Stevenson B. Pattern of glucose transporter (Glut 1) expression in embryonic brains is related to maturation of blood–brain barrier tightness. *Dev Dyn* 1992;193:152-163.
29. Higashi K, Clavo AC, Wahl RL. Does FDG uptake measure proliferative activity of human cancer cells? In vitro comparison with DNA flow cytometry and tritiated thymidine uptake. *J Nucl Med* 1993;34:414-419.
30. James DE. Targeting of the insulin-regulatable glucose transporter (Glut-4). *Biochem Soc Trans* 1994;22:668-670.
31. Birnbaum MJ. The insulin-sensitive glucose transporter. *Int Rev Cytol* 1992;137A: 239-297.
32. Wahl RL. Targeting glucose transporters for tumor imaging: "sweet" idea, "sour" results. *J Nucl Med* 1996;37:1038-1041.
33. Brown RS, Leung JY, Fisher SJ, Frey KA, Ethier SP, Wahl RL. Are inflammatory cells important? *J Nucl Med* 1995;36:1854-1861.
34. Hermanek P, Sobin LH. TNM classification of malignant tumors, 4th ed., Revised. New York, NY: Springer-Verlag; 1987.

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-

trolling cytoreductive therapy of a multitude of human malignant tumors with PET (2,3).

Elevation of glucose transport and overexpression of glucose transporter 1 (Glut-1) and key regulatory glycolytic enzymes are well established in cells that are stimulated by mitogens or growth factors or are transformed by various viruses or activated oncogenes (4,5). In src-, ras- or fps-transformed fibroblasts, activated signal transduction mechanisms were associated with substantially increased cellular deoxyglucose uptake and elevated Glut-1 mRNA and protein levels (6). The *Glut-1* gene was activated within 30 min after oncogenic transformation (7). Indirect evidence suggested that the *Glut-1* gene was activated in response to oncogenes by biochemical intracellular signaling pathways similar or identical to those of growth factors (8). Ras-mediated and phospholipase C-dependent pathways and the mitogen-activated protein kinase cascade may be involved in activated signal transduction, leading finally to the activation of the *Glut-1* gene in transformed cells (7). Mitogen-activated protein kinase has been suggested as a convergence point, integrating mitogenic and metabolic signals (9). The molecular mechanisms of intracellular signaling, ultimately activating the *Glut-1* gene in tumor cells, however, are far from being completely understood at present.

Oxygen and nutrient deprivation are alternative stimuli, well known to substantially increase the rate of glycolysis in cultured cells and human tumor xenografts (10). Whether increased glucose consumption in solid human malignancies in vivo is secondary to oxygen and nutrient deprivation or due to constitutive overexpression of key regulatory proteins and enzymes of glucose metabolism is presently unknown. Therefore, we examined tumoral glucose uptake and expression of Glut-1 and Glut-4 in 12 patients with pancreatic carcinoma (PC) and 15 patients with mass-forming pancreatitis (MFP), serving as controls. Tissue glucose uptake in PC or MFP was measured with FDG and PET. Expression of glucose transporters was studied in operatively removed tissue samples by Nothern analysis and reverse transcriptase-polymerase chain reaction (RT-PCR).

MATERIALS AND METHODS

Patients

Twelve patients with PC and 15 patients with MFP were included into the study. Two patients with MFP were diabetic, and one of these was hyperglycemic during the PET study (Table 1). Patients were fasted and all medications were discontinued for at least 12 hr before the PET study. Patients were scheduled for partial pancreatectomy due to clinical requirements for palliation or relief of bile obstruction. All patients gave informed consent into the study, which was approved by the ethical committee of the medical faculty of the University of Ulm.

PET scanning was done within 1–2 wk before surgery. Before PET scanning, blood was taken from a peripheral vein, and serum glucose concentration was measured with a commercial enzymatic test.

PET-scanning was performed as described previously (11). Briefly, a transmission scan was acquired with a ^{68}Ge/^{68}Ga ring source before 185–448 MBq of FDG was injected intravenously. Emission scans of the upper and middle abdomen were recorded in two bed positions (10.1 cm/position) for 10 min starting 45 min postinjection. Scans were reconstructed by an iterative reconstruction algorithm. Standard uptake values (SUVs) were obtained from regions of interest placed inside the pancreatic mass. Standard uptake value was defined as the radioactivity concentration inside the region of interest divided by injected activity per body weight

(3). All patients had CT scanning with contrast infusion during their preoperation work-up. Maximal diameter of the pancreatic mass, determined from CT scans, was taken as an index of the size of the pancreatic mass.

Tissues and mRNA Extraction

Extraction and purification of mRNA was performed on samples of about 30–50 mg (wet weight) using guanidinium thiocyanate/oligo(dT)-cellulose chromatography.

Northern Analysis

The isolated RNA (20 μg each) was separated on a 3% formaldehyde agarose gel, blotted, transferred to a nylon membrane and fixed under ultraviolet light. Hybridization was performed with a 2.47-kb Glut-1 cDNA fragment in a solution containing 50% formamide, 5× standard saline citrate, 5× Denhardt's solution and 1% sodium dodecyl sulfate. To ensure the uniform quality of RNA isolation and analysis, hybridization of 7S rRNA was also performed with the corresponding cDNA probe as a standard control.

Oligonucleotides

Primers 1 and 2 for Glut-1 amplification were 20-mers with binding sites to the amino acid sequences at positions 258–265 and 456–462, respectively, and amplified a PCR product of 614 bp. Primers 1 and 2 for Glut-4 amplification (20-mers) bound to amino acid sequences at positions 297–303 and 491–497, respectively, producing a 602-bp PCR product.

RNA Reverse Transcription

cDNA synthesis was performed with Moloney murine leukemia virus reverse transcriptase using a first-strand cDNA synthesis kit.

Polymerase Chain Reaction Amplification

Polymerase chain reaction was performed at a final concentration of 1× PCR buffer (50 mM KCl and 20 mM Tris-HCl, pH 8.4), 0.75 mM MgCl$_2$, 0.01 μM each dNTP, 0.5 μM each 5' and 3' primers and 1.25 units of Taq DNA polymerase in a final volume of 50 μl. Simultaneously with the specific cDNA of glucose transporters from tissues, a neutral DNA fragment with binding sites for the specific Glut-1 and Glut-4 primers was coamplified as internal standard in a defined amount (1 pg). This neutral DNA fragment (442 bp) was synthesized using a PCR construction kit (12).

The PCR mixture was amplified within 30 cycles (denaturation at 95°C for 30 sec; annealing at 55°C for 1.5 min; extension at 72°C for 2 min). After 30 cycles, tubes were kept at 72°C for 10 min and then stored on ice.

Qualitative Analysis

For qualitative analysis, each PCR mixture was subjected to electrophoresis on 6% agarose gels in 1× TRIS − acetate/EDTA buffer (80 V for 3 hr).

Semiquantitative Analysis

To determine the ratio of Glut-1 to Glut-4 expression, we amplified the specific cDNA in a radioactive PCR using [^{32}P]dCTP. After electrophoretic separation of the PCR products for Glut-1 and Glut-4, the two bands were excised from the gel, and the radioactivity was quantified by a triple determination (13).

Quantitative Analysis

Quantification of the amplified PCR products was performed by high-performance liquid chromatography analysis using a standard calibration curve. We used a TSK, produced by TOSOH in Japan, DEAE (diethylaminoethyl) and NPR (nonporous resin) ion-exchange column and an aqueous gradient mixture of 0.02 M

TABLE 1
Patient Characteristics and Results of Glut-1 and Glut-4 Expression Analyses

Patient	Age (yr)	Weight (kg)	Glucose (mg/dl)	DM	Height (cm)	"Tumor" size (cm)	BMI (kg/m²)	BSA (m²)	SUV	Glut-1 mRNA (nmol/μg)	Glut-4 Northern blot	Glut-1 Northern blot	PCR Glut-1	PCR Glut-4	Glut-1 to Glut-4 (count/count)
MFP															
B.J.	53	78	96		170	nd	27.0	1.89	1.14	nd	nd	−	−	+	0.57
C.Z.	35	72	80		180	nd	22.2	1.91	0.78	0	−	nd	nd	nd	nd
C.H.	56	75	82		178	9	23.7	1.93	1.60	0.035	−	−	−	+	0.45
F.E.	40	53	75		170	11	18.3	1.61	1.02	0	−	nd	nd	nd	nd
H.M.	51	65	74		170	nd	22.5	1.75	0.97	0	−	nd	nd	nd	nd
H.K.	53	62	73	DM	172	8	21.0	1.73	0.95	0	−	nd	nd	nd	nd
J.P.	25	75	74		180	nd	23.1	1.94	0.83	0.094	−	nd	nd	nd	nd
K.W.	37	50	78		171	nd	17.1	1.58	1.55	0	−	nd	nd	nd	nd
K.H.	38	62	93		178	nd	19.6	1.78	0.42	0	−	nd	nd	nd	nd
L.B.	48	76	290	DM	175	nd	24.8	1.91	1.34	nd	nd	−	−	+	nd
L.H.	31	67	76		185	5	19.6	1.89	0.76	0.186	−	−	+	+	0.38
N.A.	73	72	53		160	nd	28.1	1.75	1.39	0	−	nd	nd	nd	nd
P.M.	36	55	60		172	8	18.6	1.65	1.69	0.02	+	−	−	+	0.29
T.U.	53	55	73		165	nd	20.2	1.60	1.96	1.55	−	nd	nd	nd	nd
Z.K.	55	62	88		186	9	17.9	1.83	2.31	0	−	nd	nd	nd	nd
Mean	45.7	65.3	91.0		174	8.33	21.6	1.78	1.25	0.15					0.42
s.d.	12.5	9.2	56.1		7	1.97	3.3	0.13	0.51	0.43					0.12
n	15	15	15		15	6	15	15	15	13					4
PC															
B.K.	66	69	75		163	4	26.0	1.74	3.95	nd	−	+	+	nd	5.81
B.R.	59	70	81		170	3.5	24.2	1.81	1.80	2.31	+	−	+	+	nd
B.U.	57	61	80		168	4	21.6	1.69	3.63	0.21	+	nd	nd	−	nd
B.H.	63	62	73		168	nd	22.0	1.70	2.62	0.0446	+	nd	nd	−	nd
D.K.	59	57	105		168	10	20.2	1.64	2.31	9.43	−	+	+	+	4.67
K.E.	54	68	84		160	nd	26.6	1.71	1.42	0.102	−	nd	nd	−	nd
L.E.	64	78	109		170	nd	27.0	1.89	1.72	nd	nd	+	+	nd	7.7
R.A.	70	58	70		185	nd	16.9	1.78	2.74	0.225	+	nd	nd	−	nd
R.L.	56	58	74		162	nd	22.1	1.61	3.10	nd	−	nd	nd	−	nd
W.P.	30	55	59		168	4	19.5	1.62	5.67	0.904	−	nd	nd	−	nd
W.A.	74	58	92		156	nd	23.8	1.57	2.48	0.475	2+	nd	nd	−	nd
W.R.	55	69	88		165	2.5	25.3	1.76	4.30	nd	nd	+	+	+	6.48
Mean	58.8	63.6	82.5		167	4.67	22.9	1.71	2.98	1.71					6.17
s.d.	10.9	7.1	14.4		7	2.68	3.1	0.09	1.23	3.21					1.27
n	12	12	12		12	6	12	12	12	8					4
p < 0.05	s*	ns*	ns*		s*	s†	ns*	ns*	s*	ns†					s*

*t-test.
†Chi square test of Brandt and Snedecor.
DM = diabetes mellitus; BMI = body mass index; BSA = body surface area; nd = not determined; s = significant; ns = not significant.

Tris-HCl (pH 9.0) (A) and 0.02 M Tris-HCl + 1.0 M NaCl (B), with a linear gradient of 45%–60% B within 15 min and a flow of 1.5 ml/min. Detection of the peaks was achieved photometrically at 260 nm.

Sequence Analysis

Identity of the PCR products was proven by nonradioactive sequence analysis.

Statistical analysis was performed using Student's t-test and the chi square test of Brandt and Snedecor to test for differences between groups (14). Statistical significance was assumed for $p < 0.05$ or 0.01, when indicated.

RESULTS

Ten of 12 patients with PC had adenocarcinoma, one patient suffered from cystadenocarcinoma and one had a rare mixed papillary cystic PC. In three patients with PC, significant concomitant chronic pancreatitis was noted. Compared to the group of patients with MFP, there were more women with PC (7 of 12 compared to 2 of 15); they were older (59 yr compared to 46 yr; $p < 0.05$) and shorter (167 cm compared to 174 cm;

$p < 0.05$); they had comparable body weights (64 kg compared to 65 kg; not significant) and body mass indices (22.9 compared to 21.6; not significant); and they had a lower body surface area (1.71 m² compared to 1.78 m²) and a smaller pancreatic mass (4.7 cm compared to 8.3 cm; $p < 0.05$) in six patients with PC or MFP, respectively (Table 1). Serum glucose was 82.5 mg/100 ml in patients with PC and 91.0 mg/100 ml in patients with MFP (not significant). PET scans showed a focally increased FDG uptake in all patients with PC, whereas a borderline increased FDG uptake was noted in only one patient with MFP. Standard uptake value was 2.98 ± 1.23 (mean ± s.d.) in patients with carcinoma and 1.25 ± 0.51 (mean ± s.d.) in MFP ($p < 0.05$). Northern analysis showed intense Glut-1 expression in four of five patients with PC. In PC, Glut-1 and Glut-4 transcripts were found in five of five and three of 10 patients, whereas in MFP, Glut-1 was detected in one of five and Glut-4 was detected in five of five patients, respectively. The Glut-1-to-Glut-4 transcript count ratio was 6.17 ± 1.27 in patients with cancer and 0.42 ± 0.12 in patients with MFP (Table 1). Mean Glut-1 concentration in eight patients with cancer was

1.71 (nmol of Glut-1 mRNA/μg mRNA) (range, 0.0446–9.43) and 0.15 (p < 0.05) (range, 0–1.55) in 13 patients with MFP.

DISCUSSION

The results of this study demonstrate a concomitant overexpression of Glut-1 and enhanced glucose utilization in PC. In contrast, glucose uptake in MFP and expression of Glut-1, as compared to Glut-4, were very low. Virtually identical concentrations of serum glucose indicated a comparable metabolic state in both patient groups.

Considering that both MFP and PC produce "tumors" with highly increased content of connective tissue and compromised tissue perfusion (3) and that MFP was nearly twice as large as PC in this study, selective Glut-1 overexpression and enhanced glucose utilization in PC render stimulation of glycolysis by limited oxygen and nutrient supply unlikely. Rather, the findings suggest a specific stimulation of glucose utilization in cancer tissue. This interpretation is supported by observations of Haspel et al. (15), who reported that nutrient deprivation augmented glucose transport and Glut-1 protein but not Glut-1 mRNA in 3T3-C2 murine fibroblasts. There are no reports on the effect of nutrient deprivation on Glut-1 expression in cancer cells of human pancreas in vivo at present. However, preliminary results of an immunofluorescence study using a polyclonal rabbit anti-Glut-1 serum in PC tissue of some of our patients indicated selective staining of Glut-1 protein in cancer cells but not in tumor stroma or normal ductal epithelia (16).

Compared to Glut-1, Glut-4 was only weakly expressed in PC, resulting in an about 10-fold higher Glut-1-to-Glut-4 ratio in PC than in MFP. Other authors reported Glut-4 mRNA below the detection limit of Northern analysis in cancer of the pancreas (17). Higher sensitivity of RT-PCR may explain detection of a Glut-4 transcript in the cancer tissue of three of 10 patients in this study.

Our results support and extend findings of Yamamoto et al. (17) and Schek et al. (18), who described overexpression of Glut-1 glycolytic enzymes in a few cases of human PCs. Recent immunofluorescence and molecular genetic studies of several groups described increased expression of Glut-1 and Glut-3 in malignant human tumors of the brain, head and neck and breast (19–21). However, functional relevance of Glut-1 overexpression in malignant tumors described in these studies remained speculative.

The results of this study indicate that measuring tumoral glucose utilization with FDG and PET could provide a sensitive diagnostic tool for differentiating PC from MFP. Indeed, two recent studies with larger patient groups showed high sensitivity and specificity (85%–95%) of this biochemical imaging approach in determining dignity of pancreatic mass lesions (11,22).

It is generally believed that tumoral FDG-uptake is primarily related to hexokinase activity in cancer tissue (23), although convincing evidence for this concept has not been presented as yet in human cancer tissue in vivo. Results of Schek et al. (18) and others (24–27) indicated that Glut-1 is overexpressed in pancreatic cancer tissue and other human malignancies, together with hexokinase and other glycolytic enzymes. The exact functional relation between activation of glucose inward flux regulating genes and their function, i.e., glucose consumption or FDG-uptake, remains to be determined.

In this study, we found no relationship between SUV as a measure of FDG uptake and tissue content of Glut-1, measured by quantitative RT-PCR (data not shown). Random tissue sampling during operation and the relatively small amounts of tissue available for expression analysis, together with heteroge-

neous distribution of tumor cells in tumor tissue and limitations inherent in quantitative RT-PCR, precluded a comprehensive and quantitative analysis of whole tumoral Glut-1 content necessary for a valid comparison with SUV of FDG in whole tumor tissue. In addition, Glut-1 mRNA and Glut-1 protein may be regulated through synthesis rate, stabilization and degradation (4,5). Thus, changes in the steady-state levels of tissue mRNA would probably not simply lead to equal changes in the amounts of functional protein, located inside the cell membrane and hence, glucose utilization.

The molecular mechanisms underlying overexpression of Glut-1 in malignant tumors are not well understood at present. Besides a large number of extracellular signal molecules, such as hormones, growth factors, cytokines, drugs and mitogens (4,5,9), activated oncogenes ras, scr and fps may play a pivotal role in stimulating Glut-1 transcription, translation and Glut-1 protein function (4–9). Interestingly, K-ras, which is overexpressed in PC by 60%–90% (28), was found in tissue of six of nine patients with PC in this study (data not shown).

In Fujinami sarcoma virus-transformed rat fibroblasts, Birnbaum et al. (7) have shown that activation of the Glut-1 gene and glucose transport is a very early event in malignant transformation (7). If this holds true also for human tumors, increased glucose metabolism might be used as metabolic fingerprint for early diagnosis of human malignancies through expression analysis of glycolytic enzymes, Glut-1 and Glut-3 in tissue sections (24) or in vivo with PET and FDG. New PET scanners can detect structures as small as 1 mm in diameter with a target-to-nontarget radioactivity concentration gradient of 10–20. Tumor cell uptake of FDG may well build up such a concentration gradient. Interestingly, two recent reports have demonstrated malignant infiltration in lymph nodes as small as 3–5 mm in diameter with FDG and PET (29,30).

It has been observed that glucose transport and Glut-1 mRNA are elevated in growing cells compared to contact-inhibited cells but not to the extent caused by malignant transformation (7). Little is known of a potential biological role of augmented glucose utilization, exceeding increased energy requirements of cellular proliferation in malignancies. In interleukin 3-dependent hemopoietic progenitor cells, Kan et al. (31) presented evidence that growth factor- or oncogene-mediated increases in glucose uptake may represent an important mechanism in the suppression of apoptosis. If this also holds true for PC, overexpression of Glut-1 might be a fruitful target for therapeutic strategies aimed at suppression of tumoral glycolysis. We therefore think that pathophysiology of increased glucose utilization in PC deserves further careful investigation.

REFERENCES

1. Warburg O. The metabolism of tumors. New York: Richard R. Smith Inc.; 1931:129–169.
2. Strauss LG, Conti PS. The applications of PET in clinical oncology. J Nucl Med 1991;32:623–648.
3. Wahl RL. Positron emission tomography: applications in oncology. In: Murray ICP, Ell PJ, eds. Nuclear medicine in clinical diagnosis and treatment, Vol. 2. Edinburgh: Churchill Livingston; 1994:801–820.
4. Mueckler M. Facilitative glucose transporters. Eur J Biochem 1994;219:713–725.
5. Baldwin SA. Mammalian passive glucose transporters: members of an ubiquitous family of active and passive transport proteins. Biochem Biophys Acta 1993;1154:17–49.
6. Flier JS, Mueckler MM, Usher P, Lodish HF. Elevated levels of glucose transport and transporter messenger RNA are induced by ras or src oncogenes. Science 1987;235:1492–1495.
7. Birnbaum MJ, Haspel HC, Rosen OM. Transformation of rat fibroblasts by FSV rapidly increases glucose transporter gene transcription. Science 1987;235:1495–1498.
8. Williams SA, Birnbaum MJ. The rat facilitated glucose transporter gene. Transformation and serum-stimulated transcription initiate from identical sites. J Biol Chem 1988;263:19513–19518.
9. Merrall NW, Plevin R, Gould GW. Growth factors, mitogens, oncogenes and the regulation of glucose transport. Cell Signalling 1993;6:667–675.
10. Vaupel P, Kallinowski F, Okunieff P. Blood flow, oxygen and nutrient supply, and

metabolic microenvironment of human tumors: a review. *Cancer Res* 1989;49:6449–6465.

11. Stollfuss JC, Glatting G, Friess H, Kocher F, Beger HG, Reske SN. 2-(Fluorine-18)-Fluoro-2-deoxy-D-glucose PET in detection of pancreatic cancer: value of quantitative image interpretation. *Radiology* 1995;195:339–344.

12. Siebert PD, Larrick JW. PCR mimics: competitive DNA fragments for use as internal standards in quantitative PCS. *BioTechniques* 1993;14:244–249.

13. Sivitz WI, Lee EC. Assessment of glucose transporter gene expression using the polymerase chain reaction. *Endocrinology* 1991;128:2387–2394.

14. Sachs L. *Angewandte Statistik: anwendung statistischer methoden.* Berlin: Springer Verlag, 1992; 580–585.

15. Haspel HC, Wilk EW, Birnbaum MJ, Cushman SW, Rosen OM. Glucose deprivation and hexose transporter polypeptides of murine fibroblasts. *J Biol Chem* 1986;261:6778–6789.

16. Komrumpf D, Schulz HJ. Überexpression des Glut-1 glukosetransporters im menschlichem pankreaskarzinom. Eine immunhistologische studie [Abstract]. *Nucl Med* 1995; 34:A127.

17. Yamamoto T, Seino Y, Fukumoto H, et al. Overexpression of facilitative glucose transporter genes in human cancer. *Biochem Biophys Res Commun* 1990;170:223–230.

18. Schek N, Hall BL, Finn OJ. Increased glyceraldehyde-3-phosphate dehydrogenase gene expression in human pancreatic adenocarcinoma. *Cancer Res* 1988;48:6354–6359.

19. Boado RJ, Black KL, Pardridge WM. Gene expression of Glut-3 and Glut-1 glucose transporters in human brain tumors. *Mol Brain Res* 1994;27:51–57.

20. Mellanen P, Minn H, Grénman R, Härkönen P. Expression of glucose transporters in head-and-neck tumors. *Int J Cancer* 1994;56:622–629.

21. Brown RS, Wahl RL. Overexpression of Glut-1 glucose transporter in human breast cancer. *Cancer* 1993;72:2979–2985.

22. Bares R, Klever P, Hauptmann S, et al. F-18-fluorodeoxyglucose PET in vivo evaluation of pancreatic glucose metabolism for detection of pancreatic cancer. *Radiology* 1994;192:79–86.

23. Wahl RL. Targeting glucose transporters for tumor imaging: "sweet" idea, "sour" result. *J Nucl Med* 1996;37:1038–1041.

24. Ahn, YS, Rempel A, Zerban H, Bannasch P. Overexpression of glucose transporter isoform Glut-1 and hexokinase I in rat renal oncocytic tubules and oncocytomas. *Virchows Arch* 1994;425:63–68.

25. Shinohara Y, Yamamoto K, Kogure K, Ichihara J, Terada H. Steady state transcript levels of the type II hexokinase and type 1 glucose transporter in human tumor cell lines. *Cancer Lett* 1994;82:27–32.

26. Rempel A, Mathupala SP, Griffin CA, Hawkins AL, Pedersen PL. Glucose metabolism in cancer cells: amplification of the gene encoding type II hexokinase. *Cancer Res* 1996;56:2468–2471.

27. Weber G. Enzymology of cancer cells (second of two parts). *N Engl J Med* 1977;296:541–551.

28. Tabata T, Fujimori T, Maeda S, Yamamoto M, Saitoh Y. The role of Ras mutation in pancreatic cancer, precancerous lesions and chronic pancreatitis. *Int J Pancreatol* 1993;14:237–244.

29. Braams JW, Pruim J, Freling NJM, et al. Detection of lymph node metastases of squamous-cell cancer of the head and neck with FDG-PET and MRI. *J Nucl Med* 1995;36:211–216.

30. Laubenbacher C, Saumweber D, Wagner-Manslau C, et al. Comparison of fluorine-18-fluorodeoxyglucose PET, MRI and endoscopy for staging head and neck squamous-cell carcinomas. *J Nucl Med* 1995;36:1747–1457.

31. Kan O, Baldwin SA, Whetton AD. Apoptosis is regulated by the rate of glucose transport in an interleukin 3-dependent cell line. *J Exp Med* 1994;180:917–923.

Fractional Retention of Technetium-99m-Sestamibi as an Index of P-Glycoprotein Expression in Untreated Breast Cancer Patients

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The multidrug-resistant phenotype is characterized by the reduced intracellular retention of several structurally and functionally unrelated cytotoxic compounds due to the energy-dependent pump activity of P-glycoprotein (Pgp). Because ^{99m}Tc-sestamibi is a suitable transport substrate of Pgp, we tested whether the time-dependent fractional retention of this tracer could be used as an index of Pgp expression in untreated breast carcinomas. **Methods:** Twenty-seven patients with histologically confirmed breast carcinoma were intravenously injected with 740 MBq (20 mCi) of ^{99m}Tc-sestamibi, and static planar images of the breast were obtained at 10, 60 and 240 min. The fractional retention of ^{99m}Tc-sestamibi was then calculated as the ratios between 60 and 10 min (R60/10) and between 240 and 10 min (R240/10) of decay-corrected counts/pixel registered in the region of interest drawn around the tumor. Surgically excised tumors were then obtained from each patient, and Pgp levels were determined using ^{125}I-labeled MRK16 monoclonal antibody and in vitro quantitative autoradiography. **Results:** The fractional retention of ^{99m}Tc-sestamibi at 60 and 240 min was significantly higher in tumors with low Pgp levels (Group I, n = 18) as compared to that measured in tumors with high Pgp expression (Group II, n = 9) (p < 0.001). In particular, R60/10 values were 0.86 and 0.59 in breast carcinomas of Groups I and II, respectively, whereas the values of R240/10 were 0.56 and 0.25 in low- and high-Pgp-expressing tumors, respectively. **Conclusion:** The determination of fractional retention of ^{99m}Tc-sestamibi may be used as

a simple functional test for Pgp expression in untreated breast cancer. A preliminary estimate of the sensitivity and the specificity of the test indicates its potential use in clinical practice to identify patients with a high probability of developing multidrug resistance.

Key Words: technetium-99m-sestamibi; multidrug resistance; P-glycoprotein; breast carcinoma

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The development of the multidrug-resistant phenotype, which is characterized by the ability of tumor cells to survive exposure to different cytotoxic compounds (such as anthracyclines, *Vinca* alkaloids and actinomycin D) (*1*), is a major problem during cancer treatment. One of the mechanisms responsible of such resistance is the reduced intracellular retention of chemotherapeutic agents due to the energy-dependent pump activity of P-glycoprotein (Pgp), a M_r 170,000 transmembrane protein encoded by the *MDR1* gene (*2,3*). Experimental evidence of the association of the multidrug-resistant phenotype with increased levels of Pgp in many cultured tumor cell lines has been well documented (*1–3*). Elevated levels of Pgp also have been found in certain normal tissues (*4,5*), as well as in both treated and untreated human malignant tumors, including renal, colonic, adrenal and hepatocellular carcinoma (*4,6*). Untreated breast carcinomas have been reported to express relatively low levels of *MDR1* mRNA or Pgp as compared to other malignant solid tumors (*7*). However, these low levels of Pgp may reflect the