Cancer Detection Using a PET Tracer, \( ^{11} \text{C-Go} \text{lycylsarcosine}, \) Targeted to \( H^+ / \text{Peptide Transporter} \)

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H+/peptide transporter, PEPT1, is functionally expressed in some human cancer cell lines and can be a candidate molecular target for detection of cancers in vivo using PET. The aim of the present study was to establish a novel tumor-imaging technology using a PET tracer targeted to H+/peptide transporter(s). We also compared the tracer with \( ^{18} \text{F-FDG} \), focusing on the specificity of their accumulation between tumor and inflammatory tissues. Methods: A dipeptide PET tracer, \( ^{11} \text{C-glycylsarcosine} \) (\( ^{11} \text{C-Gly-Sar} \)), was injected intravenously into athymic mice transplanted with human pancreatic, prostate, and gastric cancer cells. The distribution patterns of \( ^{11} \text{C-Gly-Sar} \) and \( ^{18} \text{F-FDG} \) in the tumor-bearing mice, and in mice with inflammatory tissue, were assessed by imaging with a positron planar imaging system (PPIS). Tissue distributions of tracer radioactivity were also measured. The expression levels of PEPT1 and PEPT2 (PEPTs) proteins in tumor xenografts and inflammatory tissue were examined by immunohistochemical analysis. Results: All 3 tumor xenografts were well visualized with the PPIS after injection of \( ^{11} \text{C-Gly-Sar} \). Expression of PEPTs in those xenografts was confirmed by immunohistochemical analysis. Tumor-to-blood concentration ratios of \( ^{11} \text{C-Gly-Sar} \) increased in a time-dependent manner and were much higher than unity. Most of the radioactivity found in the tumor tissue was recovered as the intact tracer. These results indicated that \( ^{11} \text{C-Gly-Sar} \) was taken up by the PEPTs in tumor xenografts. It is noteworthy that \( ^{11} \text{C-Gly-Sar} \) was minimally present in inflammatory tissues that expressed no PEPT1 or PEPT2 protein, whereas \( ^{18} \text{F-FDG} \) was highly accumulated, with the values of selectivity index being \( >25.1 \) and \( 0.72 \) for \( ^{11} \text{C-Gly-Sar} \) and \( ^{18} \text{F-FDG} \), respectively. The mRNAs of PEPT1 and PEPT2 were expressed in 27.6% and 93.1%, respectively, of the cancer cell lines examined in the present study. Conclusion: The present study indicates that \( ^{11} \text{C-Gly-Sar} \) is a promising tumor-imaging agent and is superior to \( ^{18} \text{F-FDG} \) for distinguishing between tumors and inflammatory tissue. Because PEPTs were ubiquitously expressed in various types of tumor cells examined, \( ^{11} \text{C-Gly-Sar} \) could be useful for the detection of many types of cancers.

Key Words: H+/peptide transporter; PEPT1; PEPT2; peptide transport activity; dipeptide PET tracer; \( ^{11} \text{C-glycylsarcosine}; \) cancer detection

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The H+/peptide transporters (PEPTs) PEPT1 (\( \text{SLC15A1} \)) and PEPT2 (\( \text{SLC15A2} \)) are members of the proton-coupled peptide transporter SLC15A family. In healthy humans, expression of PEPT1 is restricted to the small intestinal epithelial cells, the brush-border membrane of epithelial cells in the kidney proximal tubule S1 segment, and the apical membrane of bile duct epithelial cells (7). PEPT1 has enormous potential as an oral drug delivery target, because it also mediates the intestinal absorption of peptide-mimetic and nonpeptide substrates, in addition to numerous peptides derived from dietary proteins (2,3). A higher-affinity isomorph, PEPT2, is localized in the brush-border membrane of S2 and S3 segment cells of the proximal tubule, lung, mammary gland, and choroid plexus, as well as in glial cells in the central nervous system (4). PEPT2 also transports various peptides and peptide-mimetics (5). One of the peptide-mimetics, 5-aminolevulinic acid, which has been used as a photosensitizer and also to guide the surgical resection of glioblastoma multiforme (6), is a substrate of PEPT1 (7) and PEPT2 (8). Antitumor agent ubenimex ((\(-\))-\( N^-\)-(2S,3S)-3-amino-2-hydroxy-4-phenylbutyryl)-L-leucine), which induces apoptosis in glioma (9), is also a substrate of PEPTs (10). In our previous study, repeated oral Ubenimex administration dramatically suppressed the growth of HeLa cells expressing human PEPT1 inoculated into mice (11). These observations suggest that PEPTs could be responsible for peptide transport activity in cancer cells.
We have previously reported that a peptide transport activity exists in the human fibrosarcoma cell line HT1080 but does not exist in the normal fibroblast cell line IMR-90 cells (12). Gonzalez et al. reported that human pancreatic carcinoma cell lines AsPC-1 and Capan-2 also possess peptide transport activity and express PEPT1 (13). Subsequently, our group (11) and Knüttner et al. (14) reported that human gastric cancer cell line MKN45, human osteosarcoma cell line MG-63, human bladder cancer cell line T24, and human hepatocarcinoma cell line SK-ChA-1 also exhibit peptide transport activity. These findings suggest that the PEPTs may be a promising target for cancer imaging using PET.

It is noteworthy that many PET tracers used for cancer detection are thought to be substrates of specific transporters. For example, 18F-FDG is a substrate of GLUT1 (SLC2AJ1) (15,16). 3'-Deoxy-3'-18F-fluorothymidine (18F-FLT), a thymidine analog, is a substrate of ENT1 (SLC29A1) (17). Tyrosine derivatives, such as O-(2-18F-fluoroethyl)-L-tyrosine and O-11C-methyl-1-tyrosine (18,19), may be substrates of LAT1 (SLC7A5) coupled with 4F2hc (SLC3A2) (20). These examples suggest that strategies using novel PET tracers targeting transporters expressed in tumors have potential value for cancer detection.

We hypothesized that PEPTs would be a suitable target for cancer detection using a peptide-based PET probe. Such a peptide-based PET probe may also be useful to distinguish tumors from inflammatory tissues, because, to our knowledge, there is no report of peptide transport activity in inflammatory tissues. Therefore, we examined the feasibility of using the peptide transport activity in cancer cells for imaging the tumors in tumor-bearing mice. Because the tissue localizations of PEPTs in mice are similar to those in humans (21–24), mice were expected to be a suitable model to evaluate peptide tracer characteristics. Recently, 11C-glycylsarcosine (11C-Gly-Sar) was reported as a PET tracer that can evaluate PEPT function (25). We describe here the use of 11C-Gly-Sar as a PET tracer targeted to the PEPTs for cancer detection in mice.

**MATERIALS AND METHODS**

**Animals and Cells**

Male athymic mice (CAnN.Cg-Foxn1nu/Crlj) were obtained from Charles River Laboratories Japan, Inc. CRL-1682 AsPC-1 cells (American Type Culture Collection [ATCC]) were cultured in RPMI+GlutaMAX medium (Invitrogen Japan K.K.) containing 10% fetal bovine serum. Other cell lines were obtained from the Cell Engineering Division of RIKEN BioResource Center.

**Transplantation**

The cells for xenotransplantation were grown to 95% confluence and then trypsinized (TrypLE Express; Invitrogen), and the single-cell suspension was centrifuged at 90g for 4°C for 5 min. The cell pellet was suspended directly with ice-cold PBS(−) (Invitrogen), which was followed by addition of the same volume of ice-cold liquid Matrigel (Becton-Dickinson). Adult mice (5 wk old) were injected subcutaneously with 5 × 106 AsPC-1 cells or 1 × 106 PC-3 and MKN45 cells and maintained for 2 wk after xenotransplantation; the mice were then used for experiments at 7 wk of age (body weight, 22.6 ± 1.5 g). Mice were maintained and handled in accordance with the recommendations of the National Institutes of Health, and animal experiments were performed in compliance with the requirements of the Animal Ethics Committee of The Medical and Pharmacologic Research Center Foundation.

**Chemicals**

3H-Glycylsarcosine (18.5 GBq/mmol, Moravek Biochemicals Inc.) was obtained from Daiichi Pure Chemicals Co., Ltd. Oil of turpentine was purchased from Wako Pure Chemical Industry. Novo-Heparin was purchased from Mochida Pharmaceutical Co., Ltd. All other reagents were of the highest grade available.

**Synthesis of Labeled Compound**

The synthesis of 11C-Gly-Sar reported by Nabulusi et al. (25) was modified slightly. The synthetic route for 11C-Gly-Sar is indicated in Figure 1. The crude intermediate 11C-1-methyl-2,5-piperazinedione was synthesized from glycyglycine ethyl ester hydrochloride using 11C-methyl triflate according to the reported method (25). The reaction mixture was purified with a high-performance liquid chromatography (HPLC) system and eluted with 25 mM NH4OAc (flow rate, 4.0 mL/min; column: DAISOPAK, SP-120-5-ODS-BP 10 × 250 mm; Diao Chemical Co., Ltd.) to give a fraction containing 11C-1-methyl-2,5-piperazinedione, which was collected in a reaction vessel containing 5N NaOH (120 µL). The mixture was heated to 140°C for 10 min and then cooled to ambient temperature. To this solution, 0.2 M Na2HPO4 (3 mL) was added and the mixture was passed through a sterile 0.22-µm filter (Millex GV; Millipore Corp.) to give 11C-Gly-Sar as an injectable solution. The total synthesis time was 41 min from the end of bombardment. The decay-corrected radiochemical yield was 9.3%, the radiochemical purity was >97%, and the specific activity was 52.5 GBq/µmol.

**FIGURE 1. Synthetic route for 11C-Gly-Sar. Synthesis of 11C-Gly-Sar as reported by Nabulusi et al. (25) was modified slightly. Asterisk denotes position of 11C labeling. DMSO = dimethyl sulfoxide.**
Tissue Distribution Assay
The radiolabeled compound (0.7–29.8 MBq) dissolved in 150 µL of physiologic saline (Otsuka Pharmaceutical Factory, Inc.) was injected into each mouse through the tail vein. The animals were killed by decapitation at 5, 15, 30, and 60 min after injection; samples of blood, brain, heart, lung, liver, pancreas, kidney, bone, muscle, and tumor were rapidly removed and weighed, and the radioactivity was measured with a γ-counter (Wizard3, Wallac). Tracer uptake by various organs was quantified as dimensionless standardized uptake values (SUVs) using the formula: SUV = (Bq per gram of tissue/Bq injected per gram of body weight). For metabolite analysis of Gly-Sar, athymic mice bearing AsPC-1 cells were injected intravenously with 150 µL of 3H-Gly-Sar (185 kBq/mouse) through the tail vein. The mice were immediately decapitated 60 min after receiving the intravenous dose of 3H-Gly-Sar, and tissue samples were collected. The heparinized blood (Novo-Heparin, 5 U/mL of blood) was centrifuged to separate the plasma and weighed, and the tumor tissues were homogenized in an Ultra-Turrax T8 homogenizer (IKA-Werke GmbH & Co. KG) on ice. Acetonitrile was then added to plasma or tumor samples (sample/acetonitrile, 1:1, w/w), which was followed by centrifugation. Aliquots of 100 µL of supernatant were analyzed by reverse-phase HPLC, using a Shimadzu LC-6A pump, CTO-2A column oven, and SCL-6A system controller (Shimadzu Corp.). 3H-Gly-Sar was separated on a YMC-Pack CN S-5 column (5 µm, 4.6 x 150 mm; YMC Co., Ltd.). Isocratic elution was performed by mixing acetonitrile and water containing 5 mM sodium dodecyl sulfate and 50 mM sodium dihydrogen phosphate adjusted to pH 2.6 with phosphate (5:95), at a constant flow rate of 1.0 mL/min. The retention time of 3H-Gly-Sar was 4.6 min. Radioactivity of the collected 3H-Gly-Sar fraction was measured with an LSC-5200 liquid scintillation counter (Aloka Co., Ltd.).

Whole-Body Imaging of Tumor-Bearing Mice
Kinetics and biodistribution patterns of each radiolabeled compound were determined with a positron planar imaging system (PPIS) (IPS-1000-6XII, Hamamatsu Photonics K.K.) (19). Mice anesthetized with sevoflurane (Maruishi Pharmaceutical Co., Ltd.) were positioned with the spine on an acrylic plate and placed on anesthetized with sevoflurane (Maruishi Pharmaceutical Co., Ltd.) (PPIS) (IPS-1000-6XII, Hamamatsu Photonics K.K.) (19). Mice were killed by decapitation at 5, 15, 30, and 60 min after injection (19). The selectivity index was defined as (tumor uptake − muscle uptake)/(inflammation uptake − muscle uptake)—that is, tumor-to-inflammation ratio corrected for background radioactivity.

Immunohistochemical Study of Expression of PEPT1 and PEPT2 in Tumor Xenografts and in Inflammatory Tissue
To examine PEPT1 and PEPT2 protein expression in xenografts of tumor-bearing mice, postmortem tumor, inflammatory, and normal tissues were harvested, embedded in OTC compound (Tissue-Tek; Sakura Finechemical Co., Ltd.), and fixed with paraformaldehyde. They were then frozen and cut into 10-µm sections with a Tissue-Tek cryostat. After incubation with a PEPT1- or PEPT2-specific polyclonal antibody (Santa Cruz Biotechnology, Inc.) at a dilution of 1:100 or 1:400, respectively, PEPT1 or PEPT2 immunoreactivity was visualized by reaction with EnVision (DaKoCytomation) or biotinylated antigen IgG (Vector Laboratories), respectively. The specimens were examined with a Biozero microscope (Keyence Corp.). The objective lens was a Plan Apo 20 × 0.75 (Nikon Corp.).

Cellular Uptake Study
For the cellular uptake study, cancer cells were seeded at a density of 5.0 × 10^4 (AsPC-1) or 2.0 × 10^4 (PC-1 and MKN45) cells per well on 96-well plates (Nalge Nunc International K.K.) and grown for 2 d. The passage numbers were in the ranges of 4–7 for AsPC-1, 9–12 for PC-3, and 8–11 for MKN45 cells. Uptake of 2 µM 3H-Gly-Sar by the cultured cells was examined at 37°C, pH 6.0 or 7.4. The procedures of the uptake experiments were as described previously (12). The radioactivity was determined using an LSC-6100 liquid scintillation counter (Aloka Co., Ltd.). Uptake of 3H-Gly-Sar was described as uptake clearance (µL/min/mg protein), defined as the value obtained by dividing the uptake rate (nmol/min/mg protein) by the 3H-Gly-Sar concentration in the uptake medium (2 µM).

Quantitative Real-Time Polymerase Chain Reaction (RT-qPCR) Analysis
Quantification of the messenger RNAs (mRNAs) coding for human PEPT1 and PEPT2 in cancer cell lines was performed by using the LightCycler system (Roche Diagnostics) with the LightCycler FastStart DNA MasterPLUS HybProbe (Roche Diagnostics) according to the manufacturer’s protocol. The PEPT1 and PEPT2 primers were designed and synthesized by TaKaRa Bio Inc. Hybridization probe sets (Fluorescein Probe and LCRed640 Probe) were designed and synthesized by Nihon Gene Research Laboratories Inc. The absolute concentration of the external standard was measured with a NanoDrop ND-1000 (NanoDrop Technologies).

Statistical Analysis
Results are presented as mean ± SD. Differences of SUVs in the various tissues and in tissue-to-blood ratios were compared using ANOVA, which was followed by the Dunnett or Scheffé post hoc tests (KaleidaGraph; Synergy Software). Comparisons between conditions were performed using an unpaired, 2-tailed Student t test. A P value < 0.05 was considered significant.

RESULTS

Whole-Body PPIS Imaging of 11C-Gly-Sar in Tumor-Bearing Mice
To evaluate 11C-Gly-Sar as a tumor-detecting agent, whole-body PPIS imaging (static collection, 10 min) with 11C-Gly-Sar in athymic mice bearing pancreatic cancer AsPC-1 was performed. Data are displayed as the images accumulated every 10 min, up to 60 min after injection (Fig. 2A). 11C-Gly-Sar showed clear delineation of the tumor in the right hind limb compared with the contralateral normal tissue (left hind limb). Remarkable accumulation of 11C-Gly-Sar in the tumor was apparent from an early time point after injection. Prostate cancer PC-3 (Fig. 2B) and gastric cancer MKN45 (Fig. 2C) were also well visualized by 11C-Gly-Sar injection. The kidneys were...
found to be the primary sites of uptake of $^{11}$C-Gly-Sar (Fig. 2), as reported previously (25) after intravenous administration. $^{14}$C-Gly-Sar was reported to be mainly excreted in urine (8), and $^{11}$C-Gly-Sar was rapidly accumulated in the bladder (Fig. 2). HPLC with radiochemical detection demonstrated that 72.9% ± 8.7% (mean ± SD, n = 5) of the radioactivity recovered in the AsPC-1 xenograft at 60 min after an intravenous bolus dose of $^{3}$H-Gly-Sar was that of intact Gly-Sar.

**Biodistribution of $^{11}$C-Gly-Sar in Tumor-Bearing Mice**

Figure 3 shows the biodistribution of $^{11}$C-Gly-Sar in athymic mice bearing AsPC-1 (Fig. 3A), PC-3 (Fig. 3B), and MKN45 (Fig. 3C). Significant accumulation of $^{11}$C-Gly-Sar in the tumor was detected from an early time point after injection, and tumor uptakes of $^{11}$C-Gly-Sar were comparable during 5–60 min after injection (Fig. 3). The SUVs of $^{11}$C-Gly-Sar in AsPC-1, PC-3, and MKN45 xenografts at 60 min after intravenous dosing were 0.70 ± 0.11, 0.53 ± 0.05, and 0.42 ± 0.03 (mean ± SD, n = 5), respectively. The SUVs of $^{11}$C-Gly-Sar in muscle ranged from 0.11 to 0.22 at 60 min. The tracer uptake values obtained from various tissues at 60 min after injection in AsPC-1 xenograft-bearing mice were initially tested using overall repeated-measures ANOVA, which was found to be significant (F$_{9,39}$ = 76.1, P < 0.0001). The post hoc test revealed that tracer uptake in the tumor tissue was significantly higher than that in blood (P = 0.0011), brain (P = 0.0005), muscle (P = 0.0020), and heart (P = 0.012) but was lower than that in pancreas (P < 0.0001) or kidney (P < 0.0001). No significant differences in the tracer uptake were found between tumor tissue and bone (P = 0.19), lung (P = 1.0), or liver (P = 1.0) (Fig. 3A). Similar results were obtained in PC-3- and MKN45-bearing mice (Figs. 2B and 2C). The T/B and T/M ratios, diagnostically important parameters, were elevated until 30 min after intravenous injection, in contrast to the M/B ratio (Figs. 2A–2C, insets). The T/B ratio of $^{11}$C-Gly-Sar was significantly higher than the M/B ratio (P < 0.0001) from 5 to 60 min after intravenous injection.

**Immunohistochemistry of PEPT1 or PEPT2 in Human Cancer Xenografts Implanted in Mice and Inflammatory Tissue**

The expression of PEPT1 or PEPT2 in cancer xenografts inoculated into athymic mice was examined by immunohistochemistry using specific antibodies. Strong positive signals with antihuman PEPT1 or antihuman PEPT2 antibody were observed in AsPC-1, PC-3, and MKN45 xenografts (Fig. 4), shown as brown arrowheads. In inflammatory tissues that were induced subcutaneously with oil of turpentine, specific signals for PEPT1 and PEPT2 were negligible (Fig. 4B). Positive signals for PEPT1 and PEPT2 were observed in the kidney (positive controls), whereas no signals were observed in the muscle (negative controls) (Fig. 4C).

**In Vitro Uptake of $^{3}$H-Gly-Sar by Cancer Cell Lines**

The uptake of 2 μM $^{3}$H-Gly-Sar in 10 min by cancer cells was measured in the absence or presence of unlabeled Gly-Sar (20 mM). The initial uptake clearances were 3.88 ± 0.20 or 0.94 ± 0.20 (AsPC-1), 1.39 ± 0.13 or 1.22 ± 0.11 (PC-3), and 1.64 ± 0.03 or 0.77 ± 0.08 (MKN45) μL/min/mg protein (mean ± SD, n = 4), respectively. $^{3}$H-Gly-Sar uptake observed in AsPC-1 cells was pH-dependent, with saturable uptake values (μL/min/mg protein) of 2.94 ± 0.32 and 1.62 ± 0.13 at pH 6.0 and 7.4, respectively.

**Accumulation of $^{18}$F-FDG and Lack of Accumulation of $^{11}$C-Gly-Sar in Inflammatory Tissues**

No specific uptake of $^{11}$C-Gly-Sar was found in inflammatory tissues, whereas $^{18}$F-FDG had a higher accumulation in inflammatory tissue than that in tumor tissue in mice inoculated with AsPC-1 cells (Fig. 5). The mice without injection of turpentine oil are shown as controls. The tissue distribution of $^{11}$C-Gly-Sar in inflammatory tissue was compared with that of $^{18}$F-FDG in athymic mouse bearing.
AsPC-1 (Fig. 5B). One-way factorial ANOVA (F2,15 = 54.2, P < 0.0001) and the Scheffé post hoc test revealed that the inflammation-to-blood ratio (I/B) and M/B of 11C-Gly-Sar were not significantly different (P = 0.98). The I/B ratio of 11C-Gly-Sar was significantly smaller than the T/B ratio (P < 0.0001). However, 18F-FDG accumulation was 6.8-fold higher in inflammatory tissue than in the non-inflamed contralateral muscle (P < 0.0001). The selectivity index (tumor-to-inflammatory tissue ratio corrected for uptake in healthy muscle) was >25.1 for 11C-Gly-Sar and 0.72 ± 0.13 for 18F-FDG, respectively. The SD for 11C-Gly-Sar could not be calculated because, in some animals, tracer uptake in inflammatory tissue was smaller than that in the contralateral healthy muscle. The difference in SUV between 11C-Gly-Sar and 18F-FDG in the tumor was not significant (P = 0.95). Furthermore, The T/B ratios of

FIGURE 3. Biodistribution of 11C-Gly-Sar as determined by radioactivity assay in tumor-bearing mice. Postmortem tissues were harvested at 5 (white bars), 15 (black hatched bars), 30 (white hatched bars), and 60 (black bars) min after intravenous injection of 11C-Gly-Sar in AsPC-1-bearing (A), PC-3-bearing (B), and MKN45-bearing (C) mice (n = 5). Uptakes are expressed as mean ± SD of SUV. (Insets) Tumor-to-blood (circles), tumor-to-muscle (squares), and muscle-to-blood (triangles) SUV ratios of 11C-Gly-Sar are plotted against the time period after injection.

FIGURE 4. Immunohistochemistry of PEPT1 or PEPT2 in human cancer xenografts implanted in mice and in inflammatory tissue. Microscopic images were obtained of sections of human pancreatic cancer AsPC-1, prostate cancer PC-3, and gastric cancer MKN45 xenografts (A) and inflammatory tissue (B) at 3 d after injection of turpentine oil. Immunoreactivity of antihuman PEPT1 or antihuman PEPT2 antibodies was also examined in tissue sections of positive control (kidney) or negative control (muscle) (C). Tissue sections were counterstained with 4'-6-diamidino-2-phenylindole (DAPI) (blue) to show cell nuclei (bars = 50 μm).

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CANCER DETECTION BY 11C-GLYCYSARCOSINE • Mitsuoka et al. 619

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Table 1). The by PPIS with $^{11}$C-Gly-Sar and $^{18}$F-FDG injection, and accumu-

3 d before tracer injection (red arrowheads). Mice were imaged for cancer detection. We were able to detect human

geta. mRNAs coding for human PEPT1 and PEPT2 in

Cycler system with HybProbes specific for each mRNA

60 min after injection are presented in E. Mean

accumulation images were created from 0 to 20 and 41 to 60 min,

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TRANSCRIPT. Accumulation of $^{18}$F-FDG but absence of accumu-

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Widespine xenograft was clearly visualized by $^{11}$C-Gly-Sar in PC-3
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AsPC-1 cells, ovary serous adenocarcinoma JHOS-2, ovary

tissue sections, but not in muscle (Fig. 4), together with the

PET. On the other hand, there was an apparent discrepancy

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promising target for peptide transporters, targeting of peptide transporters was also able to distinguish between tumor and inflammatory tissue, in combination with $^{18}$F-FDG.

Whole-body PPIS imaging effectively visualized an

AsPC-1 tumor xenograft when $^{11}$C-Gly-Sar was injected

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Expression of mRNA of PEPT1 and PEPT2 in Various Human Cancer Cell Lines

Quantitative RT-PCR was performed by using the Light-

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58 human cancer cell lines were examined (Table 1). The expression levels of mRNA of PEPT1 were high in renal
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DISCUSSION

This study demonstrates that PEPTs are promising tar-

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$^{11}$C-Gly-Sar and $^{18}$F-FDG were not significantly different

(Fig. 5B, $P = 0.71$).

Subsequently, to corroborate the potential usefulness of

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58 human cancer cell lines were examined (Table 1). The expression levels of mRNA of PEPT1 were high in renal
cell carcinoma VMRC-RCW, pancreatic adenocarcinoma

AsPC-1 cells, ovary serous adenocarcinoma JHOS-2, ovary

clear cell carcinoma JHOS-8, and poorly differentiating
gastric adenocarcinoma MKN45 cells. The percentage of
cell lines containing PEPT1 mRNA over the quantification

limit was 27.6%. On the other hand, mRNA levels of PEPT2 were above the quantification limit in 54 of 58 cell

lines (93.1%). Notably, poorly differentiated esophageal

squamous cell carcinoma TE-9, tongue squamous cell

carcinoma HSC-3, primitive neuroectodermal tumor cell

derived from kidney (FU-RPNT-2), epidermoid carcinoma

A431, and ovary serous adenocarcinoma JHOS-2

exhibited high levels of PEPT2 mRNA.
xenograft (Fig. 4), we speculate that PEPTs might have been induced by the inoculation of the cells into the mice in vivo, leading to uptake of 11C-Gly-Sar and consequently resulting in the detection of the cancer by in vivo imaging. Further experiments are needed to examine the possible induction of these PEPTs in the PC-3 xenograft and its physiologic role in vivo. PEPT1 is a high-capacity, low-affinity transporter, in contrast to PEPT2, which is a low-capacity, high-affinity transporter (4). The contribution ratio of PEPT1 and PEPT2 to the uptake of 11C-Gly-Sar by tumors in vivo was not determined in this study and should also be evaluated.

The vicinity of tumor tissues in vivo is known to be more acidic than that of normal tissue (28), and this acidic environment may favor the function of PEPTs, as transport of peptides is activated by an inwardly directed proton gradient (29). This idea was confirmed in the present study, because transport into AsPC-1 cells was almost 2 times larger at pH 6.0 than at pH 7.4. The relatively small accumulation of 11C-Gly-Sar in inflammatory tissue (Fig. 5) suggests that 11C-Gly-Sar may be an effective probe to distinguish tumors from the inflammatory tissues in vivo in combination with 18F-FDG, at least for some cancers. Such tumor-specific imaging by 11C-Gly-Sar is consistent with the absence of PEPTs in inflammatory tissue (Fig. 4) and is in marked contrast to the case of 18F-FDG, which exhibited greater accumulation in inflammatory tissues than tumor xenograft (Fig. 5).

Another advantage of 11C-Gly-Sar was the much smaller accumulation in the brain (Figs. 2 and 3). Accumulation of 11C-Gly-Sar in the brain, which does not express PEPTs, was only 5% of 18F-FDG uptake at 60 min (P < 0.00001). This result may imply that brain tumors could also be identified with peptide PET tracer. The marked accumulation of 11C-Gly-Sar in the PC-3 xenograft (Fig. 2) suggested that this tracer may be suitable for detection of prostate cancer. Because 11C- and 18F-labeled choline has been recently been used for prostate cancer imaging (27,30–32),

TABLE 1
mRNA Expression of PEPT1 and PEPT2 in Various Human Cancer Cell Lines

<table>
<thead>
<tr>
<th>Source tissue &amp; cell name</th>
<th>PEPT1</th>
<th>PEPT2</th>
<th>Source tissue &amp; cell name</th>
<th>PEPT1</th>
<th>PEPT2</th>
<th>Source tissue &amp; cell name</th>
<th>PEPT1</th>
<th>PEPT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>UQL</td>
<td></td>
<td>Bladder</td>
<td></td>
<td></td>
<td>Brain</td>
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<tr>
<td>A549</td>
<td>39 ± 10</td>
<td></td>
<td>T24</td>
<td>0.2 ± 0.0</td>
<td>2 ± 0</td>
<td>T98G</td>
<td>UQL</td>
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<td>PC-10</td>
<td>53 ± 2</td>
<td></td>
<td>Uterus</td>
<td></td>
<td></td>
<td>IMR-32</td>
<td>UQL</td>
<td>11 ± 5</td>
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<tr>
<td>Lu99B</td>
<td>23 ± 5</td>
<td></td>
<td>JHUEM-7</td>
<td>UQL</td>
<td>12 ± 4</td>
<td>SCCH-26</td>
<td>UQL</td>
<td>82 ± 31</td>
</tr>
<tr>
<td>T3 M-12</td>
<td>29 ± 27</td>
<td></td>
<td>JHUCS-3</td>
<td>1.5 ± 0.0</td>
<td>41 ± 15</td>
<td>SCCH-26</td>
<td>UQL</td>
<td>166 ± 0</td>
</tr>
<tr>
<td>EBC-1</td>
<td>76 ± 5</td>
<td></td>
<td>Cervix</td>
<td></td>
<td></td>
<td>Pleural effusion</td>
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<tr>
<td>LK-2</td>
<td>1.1 ± 0.2</td>
<td>19 ± 10</td>
<td>HeLa</td>
<td>UQL</td>
<td>29 ± 6</td>
<td>U-937</td>
<td>UQL</td>
<td>333 ± 99</td>
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<td>Kidney</td>
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<tr>
<td>ACHN</td>
<td>29 ± 2</td>
<td></td>
<td>JHOS-2</td>
<td>63.2 ± 8.7</td>
<td>1,887 ± 429</td>
<td>ACC-MESO-1</td>
<td>UQL</td>
<td>151 ± 53</td>
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<tr>
<td>VMRC-RCW</td>
<td>279.4 ± 72.7</td>
<td>35 ± 11</td>
<td>JHOS-4</td>
<td>0.5 ± 0.1</td>
<td>361 ± 86</td>
<td>Peripheral blood</td>
<td>UQL</td>
<td>188 ± 6</td>
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<td>FU-RPNT-2</td>
<td>3,118 ± 16</td>
<td>29 ± 33</td>
<td>JHOS-8</td>
<td>29.1 ± 3.3</td>
<td>UQL</td>
<td>PCM6</td>
<td>0.8 ± 0.4</td>
<td>188 ± 6</td>
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<tr>
<td>PK-45-H</td>
<td>UQL</td>
<td>UQL</td>
<td>KGN</td>
<td>UQL</td>
<td>97 ± 46</td>
<td>EoL-1</td>
<td>UQL</td>
<td>577 ± 129</td>
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<td>PK-1</td>
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<td>Connective tissue</td>
<td>UQL</td>
<td>8 ± 1</td>
<td>OIH-1</td>
<td>UQL</td>
<td>406 ± 7</td>
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<tr>
<td>AsPC-1</td>
<td>105.3 ± 51.3</td>
<td>93 ± 4</td>
<td>HT1080</td>
<td>UQL</td>
<td>81 ± 1</td>
<td>IUH-1</td>
<td>UQL</td>
<td>248 ± 55</td>
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<tr>
<td>Stomach</td>
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<tr>
<td>MKN45</td>
<td>40.7 ± 4.4</td>
<td>3 ± 2</td>
<td>A431</td>
<td>UQL</td>
<td>3,037 ± 131</td>
<td>TDP-1</td>
<td>UQL</td>
<td>278 ± 124</td>
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<tr>
<td>LMSU</td>
<td>UQL</td>
<td>UQL</td>
<td>Esophagus</td>
<td>UQL</td>
<td>116 ± 33</td>
<td>Jurkat</td>
<td>UQL</td>
<td>116 ± 33</td>
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<td>AZ521</td>
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<td>TE-15</td>
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<td>MOLT-4</td>
<td>UQL</td>
<td>59 ± 12</td>
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<td>KATO III</td>
<td>0.1 ± 0.0</td>
<td>10 ± 1</td>
<td>TE-5</td>
<td>UQL</td>
<td>162 ± 79</td>
<td>Bone marrow</td>
<td>UQL</td>
<td>1,392 ± 708</td>
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<td>Liver</td>
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<tr>
<td>HepG2</td>
<td>24 ± 3</td>
<td></td>
<td>Thyroid</td>
<td>UQL</td>
<td>182 ± 79</td>
<td>Bone marrow</td>
<td>UQL</td>
<td>1,392 ± 708</td>
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<td>Huh7-6</td>
<td>15 ± 4</td>
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<td>HCT/3</td>
<td>1.4 ± 0.3</td>
<td>76 ± 22</td>
<td>MG-63</td>
<td>UQL</td>
<td>22 ± 7</td>
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<tr>
<td>Bile duct</td>
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<tr>
<td>HuCCT1</td>
<td>7.7 ± 1.6</td>
<td>43 ± 12</td>
<td>CJM</td>
<td>UQL</td>
<td>608 ± 224</td>
<td>SK-Mel-28</td>
<td>UQL</td>
<td>2 ± 0</td>
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<td>T47-D</td>
<td>UQL</td>
<td>1 ± 0</td>
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<td>842 ± 376</td>
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<td>CRL1500</td>
<td>UQL</td>
<td>432 ± 2</td>
<td>Tongue</td>
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<tr>
<td>PC-3</td>
<td>0.1 ± 0.0</td>
<td>11 ± 2</td>
<td>HSC-3</td>
<td>0.5 ± 0.6</td>
<td>3,556 ± 1,534</td>
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</tbody>
</table>

UQL = under quantification limit (>0.05 copy/ng total RNA for PEPT1; >0.5 copy/ng total RNA for PEPT2). Expression levels are reported as copy/ng total RNA (mean ± SD; n = 4). Determinations were performed in duplicate on each sample by 2-step real-time quantitative PCR using complementary DNA obtained by reverse transcription from total RNA of human cancer cell lines.
it would be interesting to compare the potential usefulness of the peptide tracer and choline analogs. It may be difficult to detect renal or bladder tumors with peptide tracers because of the high accumulation of these tracers in the clearance organs (Fig. 2) (8,25). Inoue et al. (26) reported that PEPT1 was induced by 5-fluorouracil treatment, in contrast to a decrease of GLUT1 at the mRNA and protein levels. 11C-Gly-Sar may also be a promising tracer for assessment of the efficacy of chemotherapy with 5-fluorouracil, and this seems worthy of further study. Recently, Knüttet et al. (33) developed a high-affinity transported substrate for PEPT1, Bip-Pro, which is a stable dipeptide. Bip-Pro will be also a valuable candidate for cancer detection by positron labeling as well as 11C-Gly-Sar. As the tissue distributions of PEPTs in healthy mice are similar to those in healthy humans (21–24), 11C-Gly-Sar-PET seems to be an excellent candidate for cancer detection in the clinical situation.

CONCLUSION

Our results indicate that the peptide PET tracer 11C-Gly-Sar is a promising candidate for cancer detection. Unlike 18F-FDG, this tracer can distinguish tumors from inflammatory tissues.

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Cancer Detection Using a PET Tracer, $^{11}$C-Glycylsarcosine, Targeted to H$^+$/Peptide Transporter

Keisuke Mitsuoka, Sosuke Miyoshi, Yukio Kato, Yoshihiro Murakami, Rie Utsumi, Yoshiyuki Kubo, Akihiro Noda, Yukio Nakamura, Shintaro Nishimura and Akira Tsuji

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