Radiosynthesis and Biological Evaluation of N-[18F]Labeled Glutamic Acid as a Tumor Metabolic Imaging Tracer

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Abstract

We have previously reported that N-(2-[18F]fluoropropionyl)-l-methionine ([18F]FPMET) selectively accumulates in tumors. However, due to the poor pharmacokinetics of [18F]FPMET in vivo, the potential clinical translation of this observation is hampered. In this study, we rationally designed and synthesized [18F] or [11C]labeled N-position L-glutamic acid analogues for tumor imaging. N-(2-[18F]fluoropropionyl)-l-glutamic acid ([18F]FPGLU) was synthesized with a 30 ± 10% (n = 10, decay-corrected) overall radiochemical yield and a specific activity of 40 ± 25 GBq/μmol (n = 10) after 130 min of radiosynthesis. In vitro cell experiments showed that [18F]FPGLU was primarily transported through the Xc⁺ system and was not incorporated into protein. [18F]FPGLU was stable in urine, tumor tissues, and blood. We were able to use [18F]FPGLU in PET imaging and obtained high tumor to background ratios when visualizing tumors tissues in animal models.

Introduction

Positron emission tomography (PET) provides noninvasive images of physiologic function and is now regularly used in the diagnosis and staging of cancer [1]. Targeting of tumor specific metabolic pathways represents a useful strategy for early tumor detection and characterization. At present, a glucose analog, [18F]fluorodeoxy-D-glucose ([18F]FDG) as a widely used PET tumor tracer, is based on increased glucose utilization in tumor cells [2]. However, [18F]FDG has several disadvantages and limitations. [18F]FDG has a high uptake into the brain and nonmalignant, inflammatory cellular elements [3]. In addition, a noticeable portion of tumors with a low metabolic rate, such as prostate [4,5], bladder, renal cancer, and other [18F]FDG non-avid cancers [6], are [18F]FDG PET-negative and cannot be detected by [18F]FDG PET. To be able to overcome these disadvantages, work has been conducted to identify new tracers for tumor imaging that has greater sensitivity and better specificity for detecting and characterizing tumors. At present, a glucose analog, [18F]FDG-negative tumors may use a different metabolic pathway called-gluconolysis [9–12]. Hence, in the adapted intermediary metabolism of tumors, it is possible that key roles are played by glutamine and glutamate. Recently, some [18F] labeled glutamate and glutamine derivatives have been reported [2,13–16]. 4-[18F]fluoroglutamic acid (BAY 85-8050, 1; see Figure 1) [13] and [18F]fluoroglutamine (4-[18F]FGN, 2; see Figure 1) [2,14] have demonstrated high uptake in tumor cells that undergo high growth and proliferation, but both of them become defluorinated in animals models, resulting in suboptimal images. Another derivative of glutamic acid, 4S-L-4-[18F]fluoropropionyl)-l-glutamate (BAY 94-9392, 3; see Figure 1) can be useful for detecting system Xc⁻ (SLC7A11) activity in vivo, which is a potential biomarker for tumor oxidative stress. Animal models and use in humans have confirmed these observations [13,16].

Our previous studies proposed the hypothesis that radiolabeled N-position amino acid analogues could function as potential PET tracers, which was confirmed by our early studies of N-(2-[18F]fluoropropionyl)-l-methionine ([18F]FPMET, 4; see Figure 1) as a typical tool for tumor imaging [17]. Although intriguing uptake tumor was seen, its potential use in a clinical setting was hampered by poor stability and a high background signal in vivo.
In the present study, we design and radiosynthesize N-(2-\([\text{\textsuperscript{18}F}]\)fluoropropionyl)-L-glutamic acid (\([\text{\textsuperscript{18}F}]\)FPGLU) and N-(\([\text{\textsuperscript{11}C}]\)methyl)-L-glutamic acid (\([\text{\textsuperscript{11}C}]\)MGLU). Furthermore, we examine the biological evaluation of \([\text{\textsuperscript{18}F}]\)FPGLU as a stable PET tracer for tumor imaging.

**Materials and Methods**

**General**

All chemicals obtained commercially were used without further purification unless otherwise indicated. \([\text{\textsuperscript{18}F}]\)FDG was prepared as previously reported [18]. Sep-Pak light QMA, Plus C18, and Oasis HLB cartridges were obtained from Waters Corporation (Milford, MA, USA). Sep-Pak light QMA cartridges were preconditioned with 5 mL NaHCO\(_3\) aqueous (8.4%) and 10 mL water before use. Plus C18 and Oasis HLB cartridges were preconditioned with 10 mL ethanol and water before use. HPLC separation was carried out at the PET-MF-2V-IT-I synthesizer module (PET Co. Ltd., Beijing, China) built-in HPLC system with a semi-preparative reverse-phase C18 column (10 × 250 mm) equipped with a UV detector (Alltech 201, USA) and a radioactivity detector (PET Co. Ltd., China). The mobile phase was 55% solvent A (0.1% TFA in water): 45% solvent B (0.1% TFA in MeCN) with the flow rate of 4 mL/min. Analytical HPLC was performed using an Agilent 1200 Series HPLC system equipped with a ZORBAX Eclipse XDB-C18 analytic column (4.6 × 150 mm, 5 μm) using the flow rate of 1 mL/min. The gradient program started from 98% solvent A (0.1% TFA in water): 2% solvent B (0.1% TFA in MeCN) ramped to 90% solvent A: 10% solvent B at 8 min and ramped to 20% solvent A: 80% solvent B at 20 min. The elution profile was detected with an ultraviolet detector (Agilent interface 35900E, Agilent Technologies, USA) at 210 nm and a B-FC-3200 high energy PMT Detector (Bioscan, Inc, Washington DC, USA). Radioactivity was measured by a calibrated ion chamber (Capintec CRC-15R) or a gamma counter (γ-counter) (GC-1200, USTC Chuangxin Co. Ltd. Zonkia Branch, China). No-carrier-added \([\text{\textsuperscript{18}F}]\)fluoride was measured by a calibrated ion chamber (Capintec CRC-15R) or a γ-counter (GC-1200, USTC Chuangxin Co. Ltd. Zonkia Branch, China). No-carrier-added \([\text{\textsuperscript{18}F}]\)fluoride was trapped in a loop ring counter (γ-counter) (GC-1200, USTC Chuangxin Co. Ltd. Zonkia Branch, China). No-carrier-added \([\text{\textsuperscript{18}F}]\)fluoride was added to a solution of diethyl L-glutamate hydrochloride (9, 100 μg) in DMSO (200 μL) and DIPEA (20 μL). The reaction mixture was heated for 5 min at 40°C and was quenched by adding of 5% acetic acid (600 μL) and was diluted with water (10 mL). The dilution was passed through a Plus C18 cartridge, and then the cartridge was washed with water (10 mL). The ester 10 was eluted with ether (5 mL) from the Plus C18 cartridge. The ether was evaporated under nitrogen flow at room temperature. The residue was added NaOH aqueous (1 M, 50 μL). The ester 11 was formulated in 0.9% saline and passed through a 0.22 μm Millipore filter for studies.

**Synthesis of N-(2-fluoropropionyl)-L-glutamic Acid and N-methyl-L-glutamic Acid**

The synthesis of N-(2-fluoropropionyl)-L-glutamic acid (FPGLU) was performed by a similar method as previously described [17]. Radiochemistry procedures for the FPGLU are included in File S1. The synthesis of N-methyl-L-glutamic acid (MGLU) was prepared based on previous procedures [21].

**Radiosynthesis of \([\text{\textsuperscript{18}F}]\)FPGLU**

\([\text{\textsuperscript{18}F}]\)FNP (8) was prepared and dried based on previously procedures [22]. Anhydrous \([\text{\textsuperscript{18}F}]\)FNP was added a solution of diethyl L-glutamate hydrochloride (9, 100 μg) in DMSO (200 μL) and DIPEA (20 μL). The reaction mixture was heated for 5 min at 40°C and was quenched by adding of 5% acetic acid (600 μL) and was diluted with water (10 mL). The dilution was passed through a Plus C18 cartridge, and then the cartridge was washed with water (10 mL). The ester 10 was eluted with ether (5 mL) from the Plus C18 cartridge. The ether was evaporated under nitrogen flow at room temperature. The residue was added NaOH aqueous (1 M, 50 μL). The ester 11 was formulated in 0.9% saline and passed through a 0.22 μm Millipore filter for studies.

**Radiosynthesis of \([\text{\textsuperscript{11}C}]\)MGLU**

\([\text{\textsuperscript{11}C}]\)CO\(_2\) was produced by \([\text{\textsuperscript{14}N}]\) gas \([\text{\textsuperscript{11}C}]\)C nuclear reactions using a Cyclone 10/5 cyclotron (IBA) and was delivered to the radiochemical laboratory. \([\text{\textsuperscript{11}C}]\)CO\(_2\) was trapped in a loop ring cooled with liquid nitrogen. \([\text{\textsuperscript{11}C}]\)CH\(_3\)I was prepared from reduction of \([\text{\textsuperscript{11}C}]\)CO\(_2\) with LiAlH\(_4\). Hydrolysis of the intermediated formed organometallic complex, and subsequent iodination of \([\text{\textsuperscript{11}C}]\)methanol with hydrogen iodide, \([\text{\textsuperscript{11}C}]\)CH\(_3\)I translated to \([\text{\textsuperscript{11}C}]\)CH\(_3\)OTf with AgOTf column [23]. \([\text{\textsuperscript{11}C}]\)CH\(_3\)OTf was passed into a solution of diethyl L-glutamate hydrochloride (9, 2 mg) in acetone (1 mL) and DIPEA (40 μL). The reaction was reacted for 5 min and diluted with water (10 mL). The compound ester 12 was purified by plus C18 and eluted with ether (5 mL) from the Plus C18 cartridge. The ether was evaporated under nitrogen flow at room temperature. The residue was added NaOH aqueous (1 M, 100 μL) and reacted at room temperature for 10 min. The

**Cell Culture and Animal Models**

The S-180 Cell line and mice were obtained from the Laboratory Animal Center of Sun Yat-Sen University (Guangzhou, China). LTEP-a-2 and SPC-A-1 (SPCA-1) human lung adenocarcinoma cell lines were obtained from Shanghai Institute of Cellular Biology of Chinese Academy of Sciences (Shanghai, China). SPCA-1 cell line was originally isolated from the surgical specimens of a Chinese man with advanced lung adenocarcinoma by Shanghai Chest Hospital and Cellular Institute of Chinese Academy of Science in 1980 [19,20]. The cells were cultured in RPMI 1640 medium with a physiologic glucose concentration (1.0 g/L) containing 5% fetal calf serum at 37°C in a humidified atmosphere of 5% CO\(_2\) and 95% air. Two days before the experiments in vitro, human lung adenocarcinoma SPCA-1 cell lines were trypanized and 2 × 10\(^5\) cells per well were seeded into 12-well plates. Mice were housed 5 animals per cage under standard laboratory conditions at 25°C and 50% humidity. Tumor cells (1–2 × 10\(^5\)) were injected subcutaneously and allowed to grow for 1 to 3 weeks. At the time of the experiments, the tumor reached 6–12 mm (diameter), the mice were 5–8 wk old, and weighed 18–28 g. All work studies were approved by the Institutional Animal Care and Utilization Committee (IACUU) of the First Affiliated Hospital, Sun Yat-Sen University (approval no. 2012.001). All efforts were made to minimize animal suffering, to reduce the number of animals used and to utilize alternatives to in vivo techniques, if available.
solution was neutralized with HCl (1 M, 100 µL). The product 13 \(^{13}\text{C}\)MGLU was formulated in 0.9% saline and passed through a 0.22 µm Millipore filter for studies.

PET Studies

PET imaging of \([^{18}\text{F}]\)FPGLU (11), \([^{18}\text{F}]\)FPGLU ester (10), or \([^{13}\text{C}]\)MGLU (13) with S180 fibrosarcoma-bearing model mice (2 ~ 3 mice per group) are included in File S1.

Octanol-Water Partition Coefficient Study (logP)

Octanol-water partition coefficient for \([^{18}\text{F}]\)FPGLU was determined by measuring the distribution of radiolabeled compound in n-octanol and phosphate-buffered saline (pH = 7.4). A 20 µL sample of \([^{18}\text{F}]\)FPGLU (740 KBq, 20 µCi) in saline was added to a vial containing 5 mL each of n-octanol and phosphate-buffered saline. After being stirred in a vortex mixer for 1 min, the vial was centrifuged for 4 min to ensure complete separation of layers. Three hundred milliliters of each layer were measured using a γ-counter. logP value was calculated using the following formula: log10 P = log10 (counts in 0.3 mL of octanol/counts in 0.3 mL of water) [24].

Competitive Inhibition Studies and Protein Incorporation

The methods of competitive inhibition and protein incorporation were similar as reported previously [17,25,26]. Detailed procedures for studying \([^{18}\text{F}]\)FPGLU using human lung adenocarcinoma SPCA-1 cells are included in File S1.

**In Vivo Biodistribution of \(^{18}\text{F}\)FPGLU**

For single-isotope \(^{18}\text{F}\) biodistribution studies, Kunming mice (body weight range, 18-22 g) were anesthetized with 5% chloral hydrate solution (6 mL/kg) before injection of radiotracer. They were injected with 0.74-1.48 MBq (20-40 µCi) of \([^{18}\text{F}]\)FPGLU in 100-200 µL of saline through the tail vein. Radioactivity in the syringe before and after administration was measured in a calibrated ion chamber. The animals were killed by cervical dislocation at various times after injection, blood was obtained through the eyeball, and the organs of interest (blood, brain, heart, lung, liver, kidneys, pancreas, stomach, and intestine) were rapidly dissected and weighed, and \(^{18}\text{F}\) radioactivity was counted with a γ-counter. All measurements were background-subtracted and decay-corrected to the time of injection, then averaged together.

**Small-Animal PET Imaging**

Small-Animal PET-CT imaging studies with tumor-bearing mice were carried out using the Inveon small-animal PET/ computed tomography (CT) scanner (Siemens). 3.7–7.4 MBq of \([^{18}\text{F}]\)FPGLU were injected intravenously in conscious animals via the tail vein. Ten minutes later, the mice were anesthetized with 5% chloral hydrate solution (6 mL/kg) and were then placed on a heating pad to maintain body temperature throughout the procedure. Animals were visually monitored for breathing and any other signs of distress throughout the entire imaging period. Ten-minute static PET images were acquired at four time points (30, 60, 90, and 120 min) postinjection. For a comparative study, mice were kept fasting for 4 h and were anesthetized with 5% chloral hydrate solution (6 mL/kg) and imaged with \([^{18}\text{F}]\)FDG (3.7 MBq) at 60 min after intravenous injection. Imaging started with a low-dose CT scan, immediately followed by a PET scan. The CT scan was used for attenuation correction and localization of the lesion site. The images were reconstructed by two-dimensional ordered-subsets expectation maximum (OSEM). For each small-animal PET scan, ROIs were drawn over the tumor and major organs on decay-corrected whole-body coronal images using Inveon Research Workplace 4.1 software. The radioactivity concentration (accumulation) within a tumor or an organ was obtained from mean pixel values within the multiple ROI volume, which were converted to MBq/mL by using a conversion factor. Assuming a tissue density of 1 g/mL, the ROIs were converted to MBq/g and then divided by the administered activity to obtain an imaging ROI-derived %ID/g.

**In Vivo and in Vivo Stability**

In **in vitro** experiment, a sample of \([^{18}\text{F}]\)FPGLU (0.74 MBq, 20 µL) dissolved in normal saline was added to 200 µL of mouse serum and incubated at 57°C [27]. An aliquot of the serum sample was filtered through a 0.22 µm Millipore filter and was injected into a radio-HPLC column to analyze the stability of \([^{18}\text{F}]\)FPGLU in mouse serum within 2 h. In **in vivo** stability experiment, Kunming mice bearing S180 fibrosarcoma were anesthetized with 5% chloral hydrate solution (6 mL/kg) and were injected with a dose of approximately 30 MBq (810 µCi) of \([^{18}\text{F}]\)FPGLU in 200 µL of normal saline via the tail vein [8]. The mice were sacrificed at 0.5 h and 1 h after injection. The amount of unchanged tracer in tissue samples was determined by radio-HPLC. Tumors were separately homogenized in phosphate-buffered saline (PBS) and centrifugation (18000 g, 6 min). Supernatant was filtered through a 0.22 µm Millipore filter and analyzed by radio-HPLC. Blood was obtained by extirpating eyeballs, centrifuging (18000 g, 6 min). The plasma was filtered through a 0.22 µm Millipore filter and analyzed by radio-HPLC. The urine was collected from the bladder and was injected into an HPLC column to analyze the metabolic fate of \([^{18}\text{F}]\)FPGLU in vivo.

**Statistical Analysis**

Data were expressed as mean ± SD. Statistical analysis was performed with SPSS software, version 13.0 (SPSS Inc.), for Windows (Microsoft). Continuous variables were analyzed using the Student’s t-test. A P value of less than 0.05 was considered to indicate statistical significance.

**Results**

**Radiochemistry**

Nonradioactive FPGLU and MGLU were synthesized for use as a reference standard for characterizing radioactive \([^{18}\text{F}]\)FPGLU and \([^{13}\text{C}]\)MGLU by HPLC. \([^{18}\text{F}]\)FPGLU (11) was synthesized as shown in Figure 2. The prosthetic group \([^{18}\text{F}]\)NFP (8) was synthesized in an automated process from ethyl 2-bromopropionate (9) as the precursor via a three-step, one-pot procedure on the modified PET-MF-2V-IT-I synthesizer [22]. Compound 9 was coupled with dried \([^{18}\text{F}]\)NFP and purified with a C18 cartridge. The compound 10 was further hydrolyzed with sodium hydroxide and neutralized with hydrochloric acid to afford 11 \([^{18}\text{F}]\)FPGLU. The decay-corrected radiochemistry yield of \([^{18}\text{F}]\)FPGLU was 90±5% (n = 10) from \([^{18}\text{F}]\)NFP for 30 min. The total decay-corrected radiochemical yield of \([^{18}\text{F}]\)FPGLU was 30±10% (n = 10) from \(^{18}\text{F}\) for 130 min, with a specific activity of 40±25 GBq/µmol (n = 10). As can be seen from Figure 3, \([^{18}\text{F}]\)FPGLU had a radiochemical purity of more than 95% and the tracer was initially evaluated as an epimeric mixture.

\([^{13}\text{C}]\)MGLU was synthesized as shown in Figure 4. Compound 9 was reacted with \([^{13}\text{C}]\)CH₃OTf and purified with a C18 cartridge. The ester compound 12 was hydrolyzed and neutralized to afford the desired compound 13 \([^{13}\text{C}]\)MGLU. The decay-corrected radiochemistry yield of \([^{13}\text{C}]\)MGLU was 20±5% (n = 5).
from $[^{11}\text{C}]\text{CO}_2$ for 60 min, with a specific activity of 100±30 GBq/μmol (n = 5).

**PET Studies**

The whole-body $[^{18}\text{F}]\text{FPGLU}$, $[^{18}\text{F}]\text{FPGLU}$ ester, and $[^{11}\text{C}]\text{MGLU}$ PET/CT imaging of S180 fibrosarcoma-bearing mice was performed. Comparative imaging studies demonstrated the superior performance of $[^{18}\text{F}]\text{FPGLU}$ over $[^{18}\text{F}]\text{FPGLU}$ ester or $[^{11}\text{C}]\text{MGLU}$ for imaging S180 fibrosarcoma, with excellent tumor-to-background contrast (Figure 5).

**Octanol-Water Partition Coefficient Study (logP)**

The lipophilicity logP value of $[^{18}\text{F}]\text{FPGLU}$ at pH 7.4 is $-1.54±0.06$. As expected, the tracer was hydrophilic, as indicated by the logP of this derivative of an amino acid.

**Competitive Inhibition Studies**

To investigate the transport mechanisms involved in the uptake of $[^{18}\text{F}]\text{FPGLU}$, we conducted a series of competitive inhibition studies in SPCA-1 cells using specific inhibitors for system A, ASC, L, $X_C^-$, and $X_AG^-$, which are the major amino acid transport systems in mammalian cells and are potentially responsible for the uptake of glutamate and its analogs [14,28,29]. Figure 6 showed the results from these inhibition experiments when competing amounts of inhibitors were included and when Na$^+$ was either present or absent. The data showed that the system A inhibitor $N$-methyl-2-amino-isobutyric acid (MeAIB) had no inhibitory effect on the uptake of $[^{18}\text{F}]\text{FPGLU}$. In presence of Na$^+$, the uptake of $[^{18}\text{F}]\text{FPGLU}$ was inhibited by 23% by the system L inhibitor 2-amino-2-norbornane-carboxylic acid (BCH). The system ASC inhibitors, L-serine (Ser) and L-glutamine (Gln), inhibited uptake...
of \([^{18}\text{F}]\text{FPGLU}\) by 23% and 19%, respectively. The system \(X_\text{C}^-\) and \(X_\text{AG}\) inhibitor L-glutamic acid (Glu) inhibited uptake of \([^{18}\text{F}]\text{FPGLU}\) by 52%, and the system \(X_\text{AG}\) inhibitor D-aspartic acid (Asp) inhibited uptake of \([^{18}\text{F}]\text{FPGLU}\) by 54%. Replacement of \(\text{NaCl}\) by choline chloride reduced uptake of \([^{18}\text{F}]\text{FPGLU}\) by 53%. In the absence of \(\text{Na}^+\), BCH reduced uptake of \([^{18}\text{F}]\text{FPGLU}\) by 62%. Asp reduced uptake of \([^{18}\text{F}]\text{FPGLU}\) by 67%, and other inhibitors (MeAlB, serine, Gln, and Glu) did not markedly reduce the uptake of \([^{18}\text{F}]\text{FPGLU}\). These results indicated that \(\text{Na}^+\)-dependent \(X_\text{AG}\) and \(X_\text{AG}\) were involved in the transport of \([^{18}\text{F}]\text{FPGLU}\), with \(X_\text{AG}\) possibly playing a more dominant role.

**Protein Incorporation**

SPCA-1 cells were incubated with \([^{18}\text{F}]\text{FPGLU}\) for 30 min, then trichloroacetic acid was used to precipitate them, which showed there was less than 1% of the radioactivity in the acid precipitable fraction. Therefore, there was no incorporation of \([^{18}\text{F}]\text{FPGLU}\) into the protein, which was similar to many labeled non-protein-composition amino acids tracers (e.g., BAY 85-8050, \([^{18}\text{F}]\text{FPMET},\) 6-[\(^{18}\text{F}\)]fluoro-L-dihydroxyphenylalanine, and O-(2-[\(^{18}\text{F}\)]fluoroethyl)-L-tyrosine) [15,17,30,31].

**In Vivo Biodistribution of \([^{18}\text{F}]\text{FPGLU}\)**

The in vivo biodistribution data of \([^{18}\text{F}]\text{FPGLU}\) were summarized in Table 1. The rapid uptake of \([^{18}\text{F}]\text{FPGLU}\) was observed within the kidneys but it was quickly excreted through the urinary bladder, with 30.0 and 1.15%ID/g in the kidneys at 5 and 120 min, respectively. Blood clearance dropped fairly quickly with time, and showed low blood activity (1.04%ID/g) at 120 min after injection. \([^{18}\text{F}]\text{FPGLU}\) showed a moderate liver uptake with a relatively slow washout rate. Lung, spleen, intestine, heart, pancreas and stomach, showed relatively moderate uptake of radioactivity at 5 min, which decreased over the course of the 2 h study. There were negligible or very low levels (<1%ID/g) of uptake in other organs of interest, for example, the brain, bone, and muscle.

**Small-Animal PET Imaging**

Small-animal PET-CT imaging using \([^{18}\text{F}]\text{FPGLU}\) was performed on S180 fibrosarcoma, SPCA-1, and LTEP-a-2 human lung adenocarcinoma mouse models (\(n = 3\) per group) (Figures 7A and 7B). The tumors were clearly visible with high contrast to the contralateral background (muscle, bone, brain, neck, and other tissues) within each animal model. PET imaging provided the consistent distribution data obtained from the ex vivo biodistribution result of \([^{18}\text{F}]\text{FPGLU}\). During the early part of the experiment, prominent renal uptake was measured and accumulation in the bladder was observed, which suggests that the renal-bladder route was the main excretory system. As shown in Figures 7A and 7B, the tumor could be readily visualized with \([^{18}\text{F}]\text{FPGLU}\). Regions of interest (ROIs) from the whole organ on the coronal images were measured so that the accumulation of the radioactivity in the small-animal PET scans could be quantified. The uptake between \([^{18}\text{F}]\text{FPGLU}\) and \([^{18}\text{F}]\text{FDG}\) in the S180 fibrosarcoma and SPCA-1 human lung adenocarcinoma has no significant difference at 1 h (5.3±1.0%ID/g vs. 4.8±0.8%ID/g for S180 fibrosarcoma, \(n = 3\), \(P > 0.05\); 3.1±0.4%ID/g vs. 2.9±0.5%ID/g for SPCA-1 human lung adenocarcinoma, \(n = 3\), \(P > 0.05\)). In the LTEP-a-2 human lung adenocarcinoma, the uptake of \([^{18}\text{F}]\text{FPGLU}\) was significantly higher than that of \([^{18}\text{F}]\text{FDG}\) at 1 h (26.2±0.3%ID/g vs. 18.2±0.2%ID/g, \(n = 3\), \(P < 0.05\)) (Figure 7C).

**In Vitro and in Vivo Stability**

Radio-HPLC analysis showed that \([^{18}\text{F}]\text{FPGLU}\) in serum was stable. \([^{18}\text{F}]\text{FPGLU}\) in mouse serum at 37°C was more than 98% intact after 2 h. The stability of \([^{18}\text{F}]\text{FPGLU}\) in vivo and its metabolic fate was analyzed in urine, plasma, and tumor tissue (Figure 8). For the plasma and tumor tissue collected after 1 h, it was not possible to use HPLC to detect the radioactivity, as its level was too low. Therefore, data points for up to 0.5 h were the only ones that could be analyzed for the metabolites.

Radio-HPLC analysis of the extracted activity indicated that more than 40% of the \([^{18}\text{F}]\text{FPGLU}\) remained intact in the plasma 0.5 h post-injection. In tumor tissue, more than 65% of the tracer remained intact 0.5 h post-injection. The percentage of unchanged \([^{18}\text{F}]\text{FPGLU}\) in urine was 90% 0.5 h post-injection, which was reduced to 78% 1 h post-injection. HPLC analysis also showed that the main radioactive metabolites in tissues were highly polar products as they were eluted very fast.
Discussion

Glutamate and its derivative play key roles in the adapted intermediary metabolism of tumors. Glutamate can be converted into and functions as a substitute for glutamine. Glutamate’s transamination product, α-ketoglutarate is directly channeled into the truncated tricarboxylic acid (TCA) cycle [13]. Its derivative N-acetyl-L-glutamate is the essential allosteric activator of the first urea cycle enzyme (carbamoyl phosphate synthetase I), which is a

Table 1. Biodistribution of [18F]FPGLU in Normal Micea.

| Organ        | 5 min    | 30 min   | 60 min   | 120 min   |
|--------------|----------|----------|----------|-----------|
| Brain        | 0.68±0.21| 0.83±0.10| 0.89±0.21| 0.75±0.08 |
| Heart        | 1.80±0.50| 1.68±0.07| 1.27±0.32| 0.98±0.25 |
| Lung         | 3.06±0.55| 2.00±0.43| 1.17±0.27| 0.92±0.07 |
| Liver        | 3.53±0.12| 3.27±0.80| 2.07±0.56| 1.35±0.17 |
| Spleen       | 1.79±0.48| 1.60±0.20| 0.99±0.18| 0.60±0.17 |
| Pancreas     | 1.60±0.18| 1.30±0.20| 0.77±0.13| 0.67±0.07 |
| Kidneys      | 3.0±6.3  | 18.9±4.0 | 3.25±0.42| 1.15±0.15 |
| Intestine    | 1.41±0.29| 1.56±0.19| 1.03±0.23| 0.96±0.04 |
| Muscle       | 0.85±0.17| 0.86±0.13| 0.74±0.19| 0.60±0.09 |
| Stomach      | 1.51±0.46| 1.78±0.62| 0.94±0.13| 0.87±0.19 |
| Bone         | 0.92±0.05| 0.85±0.09| 0.75±0.20| 0.53±0.06 |
| Blood        | 2.17±0.48| 1.91±0.21| 1.54±0.15| 1.04±0.30 |

aMeans ± SD (n = 4). Data are average % ID/g.

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key regulator of this crucial cycle for ammonia detoxification in animals [32]. We have previously proposed that [18F]labeled N-acyl amino acids could be potential tracers for tumor imaging, as exemplified by [18F]FPMET. To further develop a more desirable PET tracer for tumor imaging with enhanced tumor-targeting efficacy and improved in vivo pharmacokinetics, we designed N-position positron-emitting radionuclide-labeled L-glutamic acid as novel tumor imaging tracers.

PET images of S180 fibrosarcoma-bearing mice with [18F]FPGLU, [18F]FPGLU ester, or [11C]MGLU showed radioactivity selectively accumulated in tumor. Comparative PET imaging characteristics of [18F]FPGLU with [18F]FPGLU ester or [11C]MGLU showed that [18F]FPGLU was superior over [18F]FPGLU ester or [11C]MGLU for imaging S180 fibrosarcoma, with excellent tumor-to-background contrast.

Malignant tumor cells accumulate amino acids and their close analogs because increased levels of their transporters are produced by the tumor, which also has a greater rate of protein synthesis. Adaptations of amino acid transport systems in cancer cells can offer opportunities to provide novel diagnostic and therapeutic targets. The BAY 94-9392 targeting system X<sub>C</sub> is considered to be a potential tracer for tumor imaging [15,16]. In our experiments, [18F]FPGLU transport in SPCA-1 cells consisted of three processes, two saturable Na<sup>+</sup>-dependent activities and a non-saturable Na<sup>+</sup>-independent route. In the presence of Na<sup>+</sup>, [18F]FPGLU uptake was moderately inhibited by serine since the Na<sup>+</sup>-dependent system ASC contributed to [18F]FPGLU transport. In the presence and absence of Na<sup>+</sup>, [18F]FPGLU uptake was moderately inhibited by BCH since the Na<sup>+</sup>-independent system L also contributed to [18F]FPGLU transport. Glu and Asp potently inhibited the uptake of [18F]FPGLU in the presence of Na<sup>+</sup>. However, when Na<sup>+</sup> was absent, the uptake of [18F]FPGLU was not further reduced by Glu. The X<sub>AG</sub> system is a Na<sup>+</sup>-dependent transport system for anionic amino acids [33].

Figure 7. Small animal PET imaging and quantification. Decay-corrected whole-body PET images were acquired at different time points. (A) PET images of S180 fibrosarcoma-bearing mouse static scans at 0.5, 1, 1.5, and 2 h after the injection of [18F]FPGLU. The same S180 fibrosarcoma-bearing mouse static scan at 1 h after injection of [18F]FDG. (The red arrows indicate the tumor.) (B) PET images of SPCA-1 or LTEP-a-2 human lung adenocarcinoma-bearing nude mouse static scans at 1 h after the injection of [18F]FPGLU or [18F]FDG. (The red arrows indicate the tumor.) (C) A comparison of tumor uptake of [18F]FPGLU and [18F]FDG in S180 fibrosarcoma, LTEP-a-2, and SPCA-1 human lung adenocarcinoma at 1 h after injection. (n = 3 per group; bars represent means ± SD). doi:10.1371/journal.pone.0093262.g007
Therefore, \[^{18}\text{F}]\text{FPGLU}\) uptake was primarily transported through \(\text{Na}^+\)-dependent \(X_M\) transport. Since \(^{18}\text{F}]\text{FPGLU}\) was initially evaluated as an epimeric mixture, it was possible that the amino acid transport mechanism of this epimeric mixture was different from that of its single epimer, primarily due to its specificity for the various amino acid transport systems. A more detailed study on the biological transport properties of its single epimer \textit{in vivo} and \textit{in vitro} would require further study.

The biodistribution of \(^{18}\text{F}]\text{FPGLU}\) in mice showed that its radioactivity rapidly accumulated in kidneys and was quickly excreted through the urinary-bladder route. The radioactivity in other tissues was relatively low during the entire observation time. In comparison with \(^{18}\text{F}]\text{FPMET}\) \cite{17}, \(^{18}\text{F}]\text{FPGLU}\) had a lower uptake in the organs except in the kidneys at 30 and 60 min. It was suggested that \(^{18}\text{F}]\text{FPGLU}\) had a lower background to signal ratio than that of \(^{18}\text{F}]\text{FPMET}\) \textit{in vivo}. The \textit{in vivo} biodistribution results were confirmed by small-animal PET imaging. Small-animal PET studies using \(^{18}\text{F}]\text{FPGLU}\) exhibited the expected high uptake and retention in S180 fibrosarcoma, SPCA-1, and LTEP-a-2 human lung adenocarcinoma mice models. Future studies would be further observed the tumor uptake of \(^{18}\text{F}]\text{FPGLU}\) in other subcutaneous human tumor mouse models. \(^{18}\text{F}]\text{FPGLU}\) was stable in mouse serum at 37°C for 2 h. However, the tracer was metabolized \textit{in vivo}, which could be due to the lack of peptidases for peptide bond cleavage in the serum \cite{34}. The level in the tumor tissue of the intact \(^{18}\text{F}]\text{FPGLU}\) was greater than that in the plasma 0.5 h post-injection. Perhaps \(^{18}\text{F}]\text{FPGLU}\) was rapidly accumulated in specific tumors through amino acid transporters and therefore maintained long-time stability in the tumor. It has been reported that the stability of \(^{18}\text{F}]\text{FPMET}\) was 25% of the unchanged \(^{18}\text{F}]\text{FPMET}\) in plasma and 8% of the unchanged \(^{18}\text{F}]\text{FPMET}\) in urine 0.25 h post-injection \cite{17}. In these tissues studied, \(^{18}\text{F}]\text{FPGLU}\) was more stable than \(^{18}\text{F}]\text{FPMET}\).
Conclusion
In this study, we successfully designed and prepared the new tracer $[^{18}F]$ and $[^{11}C]$ labeled $N$-glutamic acid analogues, and examined their potential application in imaging analysis of solid tumors in vivo. $[^{18}F]$FPGLU showed good uptake and good tumor-to-background contrast in S180 fibrosarcoma, SPCA-1, and LTEP-a-2 human lung adenocarcinoma mouse models. $[^{18}F]$FPGLU was primarily transported through the Na+-dependent $XAG$ system. In addition, $[^{18}F]$FPGLU showed relatively favorable pharmacokinetics in vivo. This work has shown that it is possible to use $[^{18}F]$FPGLU as a tumor metabolic imaging tracer with PET.

Supporting Information

File S1
The synthesis of nonradioactive N-(2-fluoropropionyl)-l-glutamate (FPGLU); the detail procedures of PET imaging of $[^{18}F]$FPGLU, $[^{11}C]$FPGLU ester, or $[^{14}C]$MGLU with S180 fibrosarcoma-bearing mouse mice; the detail procedures of competitive inhibition and protein incorporation experiments; and the H&E staining of LTEP-a-2 and SPCA-1 human lung tumor.

(DOC)

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Author Contributions
Conceived and designed the experiments: KH GT LZ. Performed the experiments: KH KD SY HW XL BY TH. Contributed reagents/materials/analysis tools: KH KD GT LZ. Wrote the paper: KH GT.

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