PET Imaging of β-Glucuronidase Activity by an Activity-Based $^{124}$I-Trapping Probe for the Personalized Glucuronide Prodrug Targeted Therapy

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Abstract

Beta-glucuronidase (βG) is a potential biomarker for cancer diagnosis and prodrug therapy. The ability to image βG activity in patients would assist in personalized glucuronide prodrug cancer therapy. However, whole-body imaging of βG activity for medical usage is not yet available. Here, we developed a radioactive βG activity–based trapping probe for positron emission tomography (PET). We generated a $^{124}$I-tyramine–conjugated difluoromethylphenol beta-glucuronide probe (TrapG) to form $^{124}$I-TrapG that could be selectively activated by βG for subsequent attachment of $^{124}$I-tyramine to nucleophilic moieties near βG-expressing sites. We estimated the specificity of a fluorescent FITC-TrapG, the cytotoxicity of tyramine-TrapG, and the serum half-life of $^{124}$I-TrapG. βG targeting of $^{124}$I-TrapG in vivo was examined by micro-PET. The biodistribution of $^{131}$I-TrapG was investigated in different organs. Finally, we imaged the endogenous βG activity and assessed its correlation with therapeutic efficacy of 9-aminocamptothecin glucuronide (9ACG) prodrug in native tumors. FITC-TrapG showed specific trapping at βG-expressing CT26 (CT26/mβG) cells but not in CT26 cells. The native TrapG probe possessed low cytotoxicity. $^{124}$I-TrapG preferentially accumulated in CT26/mβG but not CT26 cells. Meanwhile, micro-PET and whole-body autoradiography results demonstrated that $^{124}$I-TrapG signals in CT26/mβG tumors were 141.4-fold greater than in CT26 tumors. Importantly, Colo205 xenografts in nude mice that express elevated endogenous βG can be monitored by using infrared glucuronide trapping probes (NIR-TrapG) and suppressed by 9ACG prodrug treatment. $^{124}$I-TrapG exhibited low cytotoxicity allowing long-term monitoring of βG activity in vivo to aid in the optimization of prodrug targeted therapy. Mol Cancer Ther; 13(12): 2852–63. ©2014 AACR.
PET Imaging of βG Activity for Prodrug Therapy

lines (18). Elevated levels of βG present in the tumor environment are believed to be due to tumor overexpression (14) and release from necrotic tumor tissues (19) or tumor-infiltrating immune cells (15). Because βG has been considered as a tumor marker (14, 15, 20), it would be very useful to image βG activity in vivo to personalize glucuronide prodrug treatment and greatly improve exogenous or endogenous βG-based targeted therapy.

We have previously demonstrated that a fluorescein di-β-d-glucuronic acid probe (FDGlcU) can be applied for the assessment of βG activity in vivo. However, the fluorescent product FDGlcU rapidly leaked from the βG activity in subcutaneous tumors but not deeper tumors (2). To overcome these problems, we also developed fluorescent glucuronide trapping probes by conjugating a fluorescein isothiocyanate (FITC; λex = 495 nm, λem = 519 nm) and the near infrared dye IR-820 isothiocyanate (NIR; λex = 710 nm, λem = 820 nm) with the difluoromethylphenol beta-glucuronide trapping moiety to form FITC-TrapG and NIR-TrapG probes, respectively. Only the highly signal penetrating NIR-TrapG with near infrared spectrum properties can be used in long-term tracking (for about 72 hours) of βG activity in deep liver tissues; the FITC-TrapG cannot be. NIR-TrapG is a good probe to monitor the βG activity in small animals (21) but the near infrared fluorescent probes are still not feasible for human use as the large thickness of the human body causes a decrease in fluorescence emission and optical imaging systems for medical usage are not available. Development of a positron emission tomography (PET) glucuronide probe to image βG activity will provide a novel method to allow optimization of the protocols for βG-based personalized cancer therapy. Indeed, we previously described a 124I-phenolphthalein-glucuronide probe (124I-PTH-G) to image βG activity in vivo by micro-PET (3). The conversion of 124I-PTH-G by βG caused the in situ precipitation of 124I-PTH due to its hydrophobic property. However, this probe only allows in situ imaging (about 3 hours) and may induce carcino genesis based on a feed study of phenolphthalein in B6C3F1 mice and F344/N rats (22).

In the present study, we developed a 124I-tyramine-difluoromethylphenol-glucuronide probe (124I-TrapG) for imaging βG activity by micro-PET. 124I-TrapG can be converted by βG to form the quinine methide derivative of 124I-Trap to quickly react with any nearby nucleophile moiety such as membrane proteins (23) (Fig. 1B). The specificity of the probe was tested in vitro by incubating a FITC-TrapG probe with either CT26/mβG (CT26 cancer cells engineered to express membrane-anchored βG) or parental CT26 cells. The specificity and cytotoxicity of TrapG were also examined by incubating the probe with CT26/mβG or CT26 cells. For in vivo imaging studies, the 124I-TrapG was injected into mice bearing both CT26/mβG and parental CT26 tumors to assess intratumoral βG activity by micro-PET. We also examined the biodistribution of 124I-TrapG in various tissues. To estimate whether TrapG probes can be used to detect endogenous βG activity in vivo, immunodeficient mice bearing Colo205 (human βG high) and SW620 (human βG low) tumors were injected with NIR-TrapG for optical imaging. Finally, to evaluate whether the endogenous βG can specifically activate glucuronide prodrugs, mice bearing Colo205 or SW620 tumors were injected with 9-aminoacanthothemin glucuronide (9AGC) for βG-mediated prodrug therapy. 9AGC is a water-soluble substrate of βG that displays antineoplastic activity after being hydrolyzed by βG to release a topoisomerase I inhibitor, 9AC. Our results indicate that 124I-TrapG can systemically image the location and the expression of βG in vivo, promoting βG usage as a reporter gene for future clinical therapy protocols.

Materials and Methods

Reagents, cells, and mice

The 9AGC was synthesized as described (24). D-saccharic acid 1,4-lactone monohydrate (SAL) was purchased from Sigma-Aldrich. We previously generated CT26/mβG cells (2) in 2007. CL1-5 cells (25) were obtained from Dr. Cheng-Wen Wu’s laboratory (Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan) in 2003. CT26, SW480, Hela, HCC36, SW620, and Colo205 cells were obtained from ATCC (American Type Culture Collection) in 2002. All these cell lines were maintained in Dulbecco’s minimal essential medium (DMEM; Sigma-Aldrich) supplemented with 10% heat-inactivated bovine calf serum, 100 U/mL penicillin and 100 µg/mL streptomycin at 37°C in an atmosphere of 5% CO2. The cell lines were not authenticated by our laboratory. All cell lines were propagated for less than 6 months after resuscitation. Six- to 8-week-old female BALB/cByJNarl and BALB/cAnN.Cg-Foxn1nu/CrlNarl (BALB/c nude) mice were purchased from the National Laboratory Animal Center, Taipei, Taiwan. Mice were pretreated with 0.2% Lugol solution (Sigma-Aldrich) in their drinking water for 2 days before injection of radioiodinated probes, as previously described (3) to reduce thyroid uptake. All animal experiments were conducted in specific pathogen-free conditions and in accordance with guidelines approved by the Animal Care and Use Committee of the National Yang-Ming University.

Radioiodination of 131I or 124I-TrapG

Compound 5 shown in Supplementary Methods (Tyramine-TrpG) was labeled with iodine 124 (124I) or 131I NaI solution (Nuclear Science and Technology Development Center, National Tsing Hua University, Taiwan) using chloramine-T methods as previously described (3). The specific activity of 131I-TrapG and 124I-TrapG were about 500 MBq/mol with > 95% purity as determined by radio-thin-layer chromatography. The radiochemical yields of 131I-TrapG and 124I-TrapG were 65% and 70%, respectively.
Analysis of cell surface trapping of FITC-TrapG probe

CT26 and CT26/miG cells were stained with 0.5 μmol/L FITC-TrapG probes in serum-free PBS (pH = 6.5) in the presence or absence of SAL (0.5 mg/mL) for 30 minutes at 37°C. Unreactive probes were removed by washing with cold PBS twice. The surface fluorescence of 10^4 viable cells was measured on a BD LSR II Flow Cytometer (Becton Dickinson) and analyzed with FlowJo (TreeStar). Similarly, cells (3 × 10^5 cells) were incubated with 0.69 μmol/L FITC-TrapG in pH 6.5 PBS for 45 minutes at room temperature. The cells were boiled in reducing SDS buffer, electrophoresed on a SDS-PAGE, and transferred to PVDF membranes. Membranes were sequentially stained with mouse anti-FITC antibody followed by HRP-conjugated goat anti-mouse antibody (Jackson ImmunoResearch).
Laboratories). Bands were visualized by ECL detection (Thermo Scientific).

**Cytotoxicity of the TrapG probe**
Graded concentrations of Tyramine-TrapG or the glucurononide prodrug (p-hydroxyaniline mustard glucuronide, BHAMG; ref. 7) were added to the CT26 cells (1 × 10^4 cells per well) with or without recombinant E. coli β-glucuronidase (eβG; 2 μg/well) at 37°C for 24 hours. 37 kBq 3H-thymidine was added to the cells during the last 12-hour period of culture. Results are expressed as percentage of 3H-thymidine incorporation as compared with untreated cells by the following formula:

\[
\% \text{ of Control} = \frac{\text{cpm sample} - \text{cpm background}}{\text{cpm control} - \text{cpm background}} \\
\]

**The specificity of 124I-TrapG**
Graded concentrations of 124I-TrapG in PBS were added to the CT26 and CT26/mβG cells (8 × 10^3 cells per well) at room temperature for 1 hour. Likewise, the cells were incubated with 0.74 MBq of 124I-TrapG for various times, following washing with PBS (supplemented with 0.495 mmol/L MgCl2 and 0.9 mmol/L CaCl2, pH = 3.0) to remove nonbound probe. The radioactivity of the cells was measured in a 1470 Wizard gamma counter (Wallac).

**Liver function evaluation**
Groups of BALB/c mice (n = 6) were i.v. injected with PBS or 40 mg/kg TrapG probes. Blood was collected in serum separation 7 days postinjection. Liver injury was assessed by measuring serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (TBIL) levels using a Fuji Dri-Chem 3500 biochemistry analyzer (Fujifilm).

**Serum half-life of 124I-TrapG probe**
BALB/c mice (n = 3) were i.v. injected with 1.85-MBq 124I-TrapG, and blood samples were collected at different time points. The blood samples were weighed and assayed for radioactivity in a gamma counter. The radioactivity in the samples was expressed as the percentage of injected dose per gram of tissue. The elimination half-life (t1/2) of 124I-TrapG was calculated by a two-phase exponential decay equation with use of software (Graphpad Software).

**Imaging of βG activity in vivo**
BALB/c mice (n = 3) bearing 200 mm^3 CT26/mβG (right hind leg) and control CT26 (left hind leg) tumors were i.v. injected with 3.7 MBq of 124I-TrapG. The whole-body micro-PET imaging of pentobarbital-anesthetized mice was performed at 1, 8, and 20 hours postinjection using a micro-PET R4 scanner (Concorde Microsystems). To test the specificity of 124I-TrapG in vivo, the mice were i.p. injected with SAL inhibitors (1 g per kilogram of body weight) 1 hour before 124I-TrapG injection. Fully 3-dimensional list-mode data were collected using an energy window of 350 to 750 keV and a time window of 6 nanoseconds. Image pixel size was 0.85 mm transaxially with a 1.21 mm slice thickness. BALB/c nude mice (n = 3) bearing 100 mm^3 SW480, SW620, and Colo205 tumors or CL1-5, Hela, and HCC36 tumors were also i.v. injected with 100 μg of NIR-TrapG (21). Whole-body optical images were acquired on an IVIS Spectrum imaging system (Caliper Life Sciences) at 24 hours after probe injection at an acquisition time of 10 seconds.

**In vivo antitumor activity**
Groups of 5 BALB/c nude mice bearing 100 mm^3 subcutaneous SW620 and Colo205 tumors in their right flank were i.p. injected with doses of 50 mg/kg 9ACG or PBS on days 1, 3, and 6. Tumor volumes were measured every 2 to 3 days. Tumor sizes were calculated according to the formula: length × width × height × 0.5. The relative tumor volume was determined as: % = 100% (tumor size)/initial tumor size).

**Whole-body autoradiography in mice**
Mice (n = 3) were i.v. injected with 1.85 MBq of 124I-TrapG 3 hours before the mice were sacrificed by means of chloroform inhalation. Animals were dipped into isopentane at liquid nitrogen temperatures and embedded on a cryostat holder (7 × 5 cm) in 4% carboxymethylcellulose. The frozen carcass was sliced into 30-μm whole-body sections then attached on microscopic slides. The whole-body sections were applied to an imaging plate (BAS cassette 2040; Fujifilm). After 36 hours of exposure, the phosphor images were acquired with a FLA5000 reader (Fujifilm).

**Biodistribution of the radioiodinated TrapG probe**
BALB/c mice (n = 4) bearing established CT26 and CT26/mβG tumors (200 mm^3) on the right and left shoulders, respectively, were i.v. injected with 1.85 MBq of 124I-TrapG. The mice were sacrificed at 1, 8, 20, and 40 hours postinjection, and the radioactivity in selected tissues was measured in a multichannel gamma counter. The biodistribution of the probe was expressed as percentage of injected dose per gram tissue. The tumors were harvest at 20 hours after 124I-TrapG injection then embedded in tissue embedding medium (Tissue-Tek O.C.T.) at −80°C, and sectioned into 5-μm slices. Consecutive sections were either directly placed onto a phosphor imaging plate for autoradiography and detected by Typhoon 9410 phosphorimager (GE Healthcare Life Sciences) or stained with the β-glucuronidase Reporter Gene Staining Kit (Sigma-Aldrich) as previously described (21).

**Reverse transcription polymerase chain reaction**
SW620 and Colo205 tumor tissue samples from tumor-bearing nude mice were snap frozen in liquid nitrogen before grinding the samples into fine powder. Total RNA
was extracted by using the NucleoSpin RNA Isolation Kit (MACHEREY-NAGEL) according to the manufacturer’s protocol. Five micrograms of total RNA was reverse transcribed by using the SuperScript III RT-PCR system with oligo(dT) primers (Invitrogen). Two microliters of cDNA sample was amplified by PCR and visualized on an agarose gel. The primers 5’-TCACCAAGAAGCAGCCCTTC-3’ and 5’-CTAGCTGGAAATGTTCGCTGC-3’ for mouse βG; 5’-CTGGCGCTGCCAGTTCTTCAA-3’ and 5’-GGTGAAACCCCTGCAA-3’ for human βG; 5’-GACCCAGTCCATGCCCATCACT-3’ and 5’-TCCACCACCTGTTGCTGTAG-3’ for human GAPDH genes were used in PCR.

Results

Synthesis of 131I-TrapG and 124I-TrapG

The synthesis of 131I-TrapG and 124I-TrapG are shown in Fig. 1A. Methyl 1-O-(2-difloromethyl-4-amino)-2,3,4-tri-O-acetyl-β-D-glucopyranuronate (1) was prepared as described previously (21). To prepare of Tyramine-TrapG, tyramine was coupled with compound 1 via an amide linkage to obtain compound 3. The acetyl groups in 3 were removed by treatment with sodium methoxide and the methyl ester was changed to carboxylic acid via methyl trimethylsilanoate in 1 N hydrochloric acid to give compound 5, Tyramine-TrapG. To prepare 131I-TrapG and 124I-TrapG, Tyramine-TrapG was labeled with iodine 124 (124I) or 131I (131I) solution and purified on a Sep-Pak PLus C18 cartridge. Details of synthesis are described in Supplementary Methods.

The specificity of the TrapG probe in βG-expressing cells

To examine whether the activated TrapG probes could be specifically trapped on βG-expressing cells, parental CT26 and CT26/5βG cells (CT26 cells engineered to express membrane-tethered βG on their surface, 93 kDa) were stained with 0.5 μmol/L FITC-TrapG probes in the presence or absence of SAL inhibitors. After washing with PBS, FITC that remained attached to cells was detected on a flow cytometer. As shown in Fig. 2A, CT26/5βG cells were specifically bound to FITC-TrapG probes in the absence of SAL inhibitors as compared with the CT26 cells. Meanwhile, the addition of SAL inhibitors completely suppressed the fluorescence retained by CT26/5βG, indicating that the activation of FITC-TrapG depended on βG activity (Fig. 2A). To verify that TrapG could be covalently cross-linked to the cell surface after reaction with βG, FITC-TrapG was added to CT26 or CT26/5βG cells and analyzed by anti-FITC Western blot. Compared with the control cells, only CT26/5βG cell samples showed bands that were detected by anti-FITC antibodies, consistent with covalently cross-linking of the TrapG to the cell surface proteins as noncovalently bound FITC would dissociate from proteins boiled in reducing SDS-PAGE electrophoresis buffer (Fig. 2B). We conclude that activated FITC-Trap can covalently label bystander nucleophiles on βG-expressing cells.

Toxicity, specificity, and half-life of the TrapG probe

To estimate the cytotoxicity of TrapG to cells, graded concentrations of tyramine-TrapG and BHAMG (an anticancer glucuronide prodrug as positive control) were added to CT26 cells with or without the addition of 2 μg eβG. The incorporation of 3H-thymidine into cellular DNA was measured as an index of cell viability. Figure 3A shows that 200 μmol/L tyramine-TrapG and BHAMG were not cytotoxic to CT26 cells in absence of βG. In the presence of βG, tyramine-TrapG displayed some toxicity at concentrations greater than about 100 μmol/L, whereas BHAMG was much more cytotoxic with an IC50 value of about 5 μmol/L. These results indicate that native tyramine-TrapG exhibited low cytotoxicity to cells with or without βG activation.

To examine the specificity of 124I-TrapG, CT26/5βG or CT26 cells were incubated with graded concentrations...
of 124I-TrapG in the presence or absence of the βG inhibitor SAL. In addition, the cells were also incubated with 124I-TrapG for different time periods. After washing cells with acidic PBS buffer, the radioactivity retained on the cells was measured on a gamma counter. Figures 3A and B show that addition of SAL completely blocked the radioactivity retained by cells expressing membrane-tethered βG, showing that the activation of 124I-TrapG depended on βG activity. In addition, the radioactivity (cpm) retained by CT26/mβG cells after washing with acidic PBS was 1.6 to 10.3-fold higher than CT26 cells and was both dose and time dependent (Fig. 3B and 3C). These results indicate that 124I-TrapG could be selectively activated by βG, resulting in covalent retention of 124I-TrapG at βG-expressing cells.

To investigate the toxicity of TrapG probes in vivo, mice were i.v. injected with tyramine-TrapG (40 mg/kg) on June 22, 2017. © 2014 American Association for Cancer Research.
tumors but not CT26 tumors (Fig. 4B). The region of interest ratios of CT26/mβG to CT26 tumors were 3.3, 21, and 141.4 at 1, 8, and 20 hours, respectively, suggesting that 124I-TrapG was preferentially hydrolyzed into 124I-Trap by βG-mediated activation. The radioactivity accumulated in CT26/mβG tumors could last up to 20 hours, indicating that long-term imaging of βG activity can be achieved by using the βG-activated trapping 124I-TrapG probe. To confirm whether conversion of 124I-TrapG is βG dependent, mice bearing CT26/mβG and CT26 tumors were i.p. injected with the βG inhibitor SAL before probe injection for PET imaging. Figure 4B shows that SAL completely obstructed the generation and accumulation of radioactivity in CT26/mβG tumors, suggesting that the conversion of 124I-TrapG to 124I-Trap was blocked by the suppression of βG activity. A previous study showed that intraperitoneal administration of SAL in mice can prevent
CPT-11–induced mucosa damage by inhibiting bacterial βG (26). Although the pretreatment of SAL hampered the accumulation of cell-associated radioactivity in CT26/mβG tumors (Fig. 4B), strong abdominal signals were still observed. This result indicates that conversion of the fecal excreted 124I-TrapG by intestinal βGi not the major source of the abdominal signals. To estimate the colocalization of βG activity and specific 124I-TrapG retention, CT26 and CT26/mβG tumors were frozen, and sectioned 20 hours after the injection of 124I-TrapG in tumor-bearing mice. The adjacent tumor sections were either directly exposed to phosphor imaging for autoradiography or stained with X-GlcA substrate to visualize βG activity. Figure 4C shows that autoradiography and βG activity matched in adjacent CT26/mβG tumor sections.

**Biodistribution of the 131I-TrapG in mice**

To investigate the biodistribution of 131I-TrapG in vivo, BALB/c mice (n = 4) bearing established CT26/mβG and CT26 tumors were i.v. injected with 131I-TrapG and then examined by measuring radioactivity of the probe in organs at 1, 8, 20, and 40 hours after probe injection. Consistent with the micro-PET analysis, mean radioactivity of CT26/mβG tumors was significantly higher than the CT26 tumors (Fig. 5A). The accumulation of radioactivity in CT26/mβG tumors were 2.5-, 4.9-, 7.1-, and 9.60fold higher than the CT26 tumor at 1, 8, 20, and 40 hours, respectively. Notably, 15% and 15.7% of the total 131I-TrapG were found in the gallbladder and urine, respectively, at 1 hour after injection (Fig. 5A). At 40 hours, 9.3% and 1.3% of the total 131I-TrapG were found in the gallbladder and urine, respectively. This result suggests that 131I-TrapG undergoes both renal and biliary excretion. 131I-TrapG was eliminated rapidly by renal excretion but metabolized slowly by biliary excretion. To confirm this result, whole-mouse sections were detected by autoradiography at 40 hours after probe injection. Figure 5B shows that higher radioactivity accumulated in CT26/mβG tumors as compared with control CT26 tumors. However, we also found nontargeted 124I-TrapG in the urine, gallbladder, liver, and intestines, which may be caused by the biliary excretion of 124I-TrapG into the intestinal tract.

**Imaging of βG activity in endogenous βG-overexpressing tumors for targeted 9ACG prodrug therapy**

To further investigate whether TrapG probes can be used to image different levels of endogenous βG activity in tumors, mice bearing SW480, HCC36, CL1-5, SW620, Hela, or Colo205 tumors were i.v. injected with NIR-TrapG and imaged on an IVIS optical imaging system at 24 hours after injection. As shown in Fig. 6A and Supplementary Fig. S1, Colo205, CL1-5, and Hela (human βG high) tumors displayed more fluorescence intensity than SW620, SW480, and HCC36 (human βG low) tumors. These results suggest that TrapG probes can specifically image the different levels of βG activity in tumors. To investigate whether NIR-TrapG tumor imaging and 9ACG cancer therapy were contributed by increased βG in the tumor microenvironment, the mouse βG expression levels in human SW620 and Colo205 tumors were determined by reverse transcription PCR (RT-PCR). As shown in Fig. 6B, Colo205 tumors displayed elevated mouse βG as compared with the SW620 tumors, indicating that the conversion of TrapG probes and 9ACG prodrugs relies on extracellular βG in the tumor microenvironment. To
PET imaging. The in vitro and in vivo results demonstrated that the $^{124}$I-TrapG probe can be activated by $\beta$G to radioactively image $\beta$G-expressing cells or tumors. In the cytotoxicity assay, native tyramine-TrapG displayed modest cytotoxicity in the presence of $\beta$G. We also found that the therapeutic efficacy of $\beta$ACG prodrug is correlated to the $\beta$G levels in tumors that are determined by TrapG imaging. These results indicate that $^{124}$I-TrapG may be a useful probe for the detection of $\beta$G activity by PET technology to optimize $\beta$G-based targeted therapies in the clinic.

Although $^{124}$I-TrapG can be specifically converted by $\beta$G and thereby trapped in $\beta$G-expressing tumors, we also observed strong abdominal signals in mice. Two major causes may result in this background signals. First, liver cells can absorb xenobiotic glucuronides from the circulation by organic anion-transporting polypeptides and transport them to the intestines via biliary excretion. To diminish this effect, several methods have been used, including depletion of intestinal microbes by antibiotics (30, 31) and inhibition of bacterial $\beta$G by chemical inhibitors (32, 33). Therefore, we treated mice with antibiotics or a $\beta$G inhibitor before $^{124}$I-TrapG injection. However, the abdominal signals were not reduced by either elimination of intestinal bacteria (data not show) or inhibition of bacterial $\beta$G activity (Fig. 4B). However, biliary excretion may be reduced by repeated injections of acetaminophen to induce Mrp3 expression and provoke the clearance of glucuronide conjugates through the urinary pathway (34).

$\beta$G is considered as a tumor marker (14, 15, 20) and a prodrug-activating enzyme for cancer prodrug therapies (35, 36). Connors and Whisson demonstrated that high $\beta$G levels present in plasmacytomas were correlated with higher drug sensitivity of the tumors (37). Indeed, we also found that Colo205 xenografts exhibited high levels of endogenous $\beta$G activity based on the imaging results using TrapG probes. Meanwhile, several studies have found that $\beta$G accumulates in the necrotic areas of human cancers (14, 18, 38, 39). In addition, glucuronide conjugates usually exhibit enhanced solubility and decreased cell permeability that greatly increases the utility and specificity of prodrugs or proprobes relying on selective hydrolysis by the $\beta$G enzyme. Many $\beta$G activity–based prodrugs have been developed for cancer-targeted therapies, including $\beta$ACG (24), BHAMG (40), daunorubicin-GA3 (DNR-GA3; ref. 35), and glucuronide-conjugated doxorubicin (DOX-GA3, HMR1826; ref. 35). Thus, the $\beta$G activity–based $^{124}$I-TrapG imaging system may provide a powerful tool for tracking tumor associated $\beta$G activity by PET imaging to improve $\beta$G-based personalized anticancer therapies.

Development of a functional PET probe to image the $\beta$G activity in patients would improve $\beta$G-based targeted therapy. PET imaging is a sensitive, noninvasive, and clinical used technology for detecting organ function.
and disease diagnosis. The high penetration of radioactive signals facilitates deep tissue/organ imaging (41, 42). Antunes and colleagues generated 1-O-(4-(2-13)fluoroethyl-carbamoyloxymethyl)-2-nitrophenyl)-O-ß-D-glucopyronuronate ([13]F)-FEAnGA for in vivo tracking βG activity by PET imaging (43). However, the optimal radioactive accumulation of [13]F)-FEAnGA was 2-fold higher in βG-tumor than in control tumors at 1 hour (4). We have also previously developed a 124I-labeled βG-based hydrophobic conversion probe ([124]I-PTH-G) that can specifically accumulate at βG-expressing tumors. However, the optimal imaging efficiency of 124I-PTH-G was only 3.6-fold greater in βG-expressing tumors than in control tumors at 1 hour (3). These studies indicate that both [13]F)-FEAnGA and 124I-PTH-G rapidly diffuse away from βG-expressing sites and limit their utility for clinical use. In the present study, we describe a new 124I-TrapG probe for monitoring of βG enzyme activity by micro-PET imaging. The optimal radioactive accumulation of 124I-TrapG was 141.4-fold higher in βG-expressing tumors than in control tumors at 20 hours after probe injection. These results indicate that PET imaging of 124I-TrapG is potentially useful for long-term monitoring of βG activity in vivo.

An imaging probe should display low toxicity, high specificity, and strong sensitivity. We previously demonstrated that a near infrared glucuronide trapping probe (NIR-TrapG) is an activity-based probe that allows specific and direct detection of βG activity in preclinical models (21). In addition, the glucuronide trapping probe did not inhibit βG enzyme activity, which can improve substrate activation, imaging intensity, and might not affect the conversion of βG-based prodrugs (43, 44). Here, we also verified that TrapG probe exhibits relatively low toxicity (IC50 > 200 μmol/L). In contrast, FIAU, the substrate for HSV-tk, is much more toxic (IC50 = 0.073 μmol/L; ref. 45). Moreover, the glucuronide trapping moiety (difluoromethylphenol) can be conjugated with various probes for multimaging systems, such as fluorescent dyes (FITC and IR-820) for optical imaging (21) and radioactive isotopes (124I) for micro-PET imaging. In the future, the glucuronide trapping probe could also be linked to clinical used diethylether reporter system in the laboratory and clinical medicine.

The βG activity-based PET probe, 124I-TrapG, is useful for noninvasive imaging of βG expression in the living body. The 124I-TrapG possesses several advantages, including (i) the low toxicity of TrapG should allow clinical imaging, (ii) high specificity and signal amplification due to the catalytic hydrolysis of probes by βG, (iii) possibility of generating a wide range of imaging probes by attachment of TrapG groups, and (iv) retention of probes upon βG conversion leading to long-term imaging in vivo. On the basis of these advantages, 124I-tyramine-TrapG may be paired with βG-based prodrugs to generate a personalized therapy system.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y.-C. Su, T.-C. Cheng, C.-H. Chuang, H.-E. Wang, T.-L. Cheng
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Y.-C. Su, T.-C. Cheng, J.-Y. Wang, H.-E. Wang, T.-L. Cheng
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): C.-H. Kao, K.-C. Chen, H.-E. Wang
Study supervision: H.-E. Wang, T.-L. Cheng

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