Published in final edited form as:

Biomaterials. 2014 August ; 35(25): 6964–6971. doi:10.1016/j.biomaterials.2014.04.089.

Imaging of hepatocellular carcinoma patient-derived xenografts using ^{89}Zr -labeled anti-glypican-3 monoclonal antibody

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Abstract

Imaging probes for early detection of hepatocellular carcinoma (HCC) are highly desired to overcome current diagnostic limitations which lead to poor prognosis. The membrane protein glypican-3 (GPC3) is a potential molecular target for early HCC detection as it is over-expressed in >50% of HCCs, and is associated with early hepatocarcinogenesis. We synthesized the positron emission tomography (PET) probe ^{89}Zr -DFO-1G12 by bioconjugating and radiolabeling the anti-GPC3 monoclonal antibody (clone 1G12) with ^{89}Zr , and evaluated its tumor-targeting capacity. *In vitro*, ^{89}Zr -DFO-1G12 was specifically taken up into GPC3-positive HCC cells only, but not in the GPC3-negative prostate cancer cell line (PC3). *In vivo*, ^{89}Zr -DFO-1G12 specifically accumulated in subcutaneous GPC3-positive HCC xenografts only, but not in PC3 xenografts. Importantly, ^{89}Zr -DFO-1G12 delineated orthotopic HCC xenografts from surrounding normal liver, with tumor/liver (T/L) ratios of 6.65 ± 1.33 for HepG2, and 4.29 ± 0.52 for Hep3B xenografts. It also delineated orthotopic xenografts derived from three GPC3-positive HCC patient specimens, with T/L ratios of 4.21 ± 0.64 , 2.78 ± 0.26 , and 2.31 ± 0.38 at 168 h p.i. Thus, ^{89}Zr -DFO-1G12 is a highly translatable probe for the specific and high contrast imaging of GPC3-positive HCCs, which may aid early detection of HCC to allow timely intervention.

Keywords

Glypican-3; Hepatocellular carcinoma; Immuno-PET; ^{89}Zr ; Molecular imaging

1. Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer deaths worldwide [1–3]. Early detection of HCC is crucial and may significantly improve its outcome, since its typically late diagnosis is associated with limited treatment options and lower chances of survival [4–6]. Current diagnostic imaging of HCC based on ultrasonography, computed tomography, and magnetic resonance imaging [7] is limited as they are often incapable of detecting HCC lesions <2 cm, and of differentiating between HCC lesions and other benign liver lesions, leading to false-positive diagnoses [8]. Thus, it is imperative to develop new molecular imaging techniques which can improve the sensitivity and specificity of HCC detection.

Molecular imaging of cancer can potentially improve early diagnosis and clinical management of cancer patients [9,10]. Positron emission tomography (PET) using tumor-targeting radio-labeled-molecules has gained wide acceptance in oncology, allowing improved diagnosis and clinical management of cancer patients [9]. A variety of molecules, including monoclonal antibodies (mAbs), antibody fragments, small proteins, peptides, and small molecules can be used as tumor-targeting molecules with different levels of tumor accessibility and specificity [11–13]. The widespread availability of highly specific antibodies has led to rapid advances in antibody-based probe development for PET imaging [9,10,14]. Generally, intact antibody molecules have relatively slow pharmacokinetics, which require multiple days to reach their optimal biodistribution within the body [15,16]; therefore, PET radioisotopes with a long half-life such as ^{89}Zr (78.4 h) or ^{124}I (100.3 h) are particularly suitable for intact antibodies. However, since the liver is largely responsible for antibody clearance, the applicability of immuno-PET for HCC imaging remains unclear, with the major hurdle being high normal liver uptake and resulting poor tumor-to-liver ratio.

The successful early detection of HCC lesions will require the combined selection of highly specific targets and effective approaches to decrease non-specific liver uptake of the imaging probe. Although other HCC associated biomarkers such as epidermal growth factor receptor (EGFR) has been used as an imaging target for HCC imaging, these approaches were typically associated with high liver background and unfavorable tumor-to-liver ratios [17,18]. In HCC, the heparin sulfate proteoglycan glypican-3 (GPC3) is a rational molecular target for HCC diagnostic imaging because it is: (i). a cell-membrane receptor that is readily accessible for antibody-mediated targeting and binding [19]; (ii). expressed in more than 50% of HCC patients [20–22]; (iii). capable of distinguishing malignant HCCs from normal liver, and pre-neoplastic and benign liver lesions [8,22–24]; (iv). expressed at higher levels in small HCCs than in cirrhosis and other types of small focal lesions, suggesting that the transition from premalignant lesions to small HCC is usually associated with elevated GPC3 [20,22,24–26]. Thus, detection of HCC based on GPC3 expression may aid early diagnosis.

We therefore hypothesized that an immuno-PET probe based on ^{89}Zr -radiolabeled, anti-GPC3 monoclonal antibody (mAb) may offer the potential to accurately identify GPC3-positive HCC cells. We tested this probe for its specificity for detecting HCC cells *in vitro* and *in vivo*, and further determined its suitability for clinical translation using orthotopic HCC patient-derived xenografts.

2. Materials and methods

2.1. Bioconjugation and radiolabeling

The anti-human GPC3 mAb (Clone 1G12, BioMosaics Inc., Burlington, VT) or non-targeting mouse IgG (Jackson ImmunoResearch, Inc.) were conjugated with desferrioxamine (DFO) and radiolabeled with ^{89}Zr (University of Wisconsin, Madison, WI) as previously described [27,28]. The radiolabeled products, ^{89}Zr -DFO-1G12 or ^{89}Zr -DFO-IgG respectively, were eluted by phosphate-buffered saline (PBS, pH 7.4) and passed through a 0.22- μm Millipore filter into a sterile vial for *in vitro* and animal experiments. The labeling yields were ~20%, and the specific activities were ~10 $\mu\text{Ci}/\mu\text{g}$.

2.2. Establishing subcutaneous and orthotopic xenografts from HCC cell lines and HCC patient specimens

Animal studies were carried out in compliance with Federal and local institutional rules for the conduct of animal experiments. To generate subcutaneous xenografts, $\sim 6\text{--}10 \times 10^6$ HCC cells were suspended in 100 μL of Dulbecco's Phosphate Buffered Saline (DPBS) (Invitrogen Life Technologies, Carlsbad, CA) and injected subcutaneously near the left (HepG2) or right (PLC/PRF/5, PC3) forelimb of 4–6 weeks old, adult male athymic nude mice (Charles River Laboratories, Inc., Cambridge, MA). Imaging was done when tumors have reached ~1.0 cm in largest diameter. Orthotopic xenografts from HCC cell lines were established as previously described [29], with weekly monitoring of tumor growth by bioluminescence imaging after intraperitoneal injection of D-luciferin (Xenogen IVIS® system).

Orthotopic mouse xenograft models based on primary human HCC tumor cells were established in 4 week old, male NOD.Cg-Prkdc^{scid}Il2rg^{tm1Wjl}/SzJ (Nod-SCID-Gamma, NSG) mice. Initial pairs of male and female NSG mice were obtained from the Jackson Laboratory (Bar Harbor, MA), and bred according to approved institutional protocols. Tissue specimens were obtained from three HCC patients undergoing surgical resection of their tumors at Stanford Hospital, with informed consent as approved by the Institutional Review Board at Stanford University. Tumors were cut into 1 mm^3 pieces and subcutaneously inserted into the shoulder of adult NSG mouse to initiate tumor growth. After 6–8 weeks, palpable subcutaneous xenografts were harvested and digested by collagenase into single cells for labeling with lentivirus containing luciferase gene for 3 h, and then subcutaneously injected back to another group of NSG mice. When the primary human xenografts with luciferase expression have grown, they were harvested and cut into 2 mm^3 pieces and surgically implanted onto the left lobe of the liver of another group of NSG mice. Growth of the primary orthotopic HCC xenografts was monitored with bioluminescence imaging.

2.3. Small animal PET, PET/CT, and image analysis

Subcutaneous HCC xenografts ($n = 4$ each for each group) were imaged using a micro-PET R4 rodent-model scanner (Siemens Medical Solutions USA, Inc., Knoxville, TN). Mice were injected intravenously with ^{89}Zr -DFO-1G12 or ^{89}Zr -DFO-IgG (~10 μCi , 0.37 MBq, ~1

µg) *via* the tail vein under isoflurane anesthesia. Starting 24 h post-injection (p.i.), static scans (5-min) were acquired every 24 h, till 168 h p.i.

Orthotopic HCC xenografts were imaged using the Inveon PET/CT scanner (Siemens Medical Solutions, USA). ⁸⁹Zr-DFO-1G12 (0.37 MBq, 10 µCi, ~1 µg), was injected intravenously *via* the tail vein, and CT images acquired (632 slices at 206 µm) for photon attenuation correction and image co-registration with PET imaging data. A static 5-min PET scan was then performed, and PET images were reconstructed using the Ordered Subsets Expectation Maximization (OSEM) 2D algorithm (159 slices with 0.796 mm resolution). Static scans were performed every 24 h, till 168 h p.i. Region of interest (ROI) analysis was performed using the Inveon Research Workspace software. The maximum percent of injected dose per gram of tissue (%ID/g) upon normalization to injected dose was determined every 24 h.

After the final PET or PET/CT scan, animals were sacrificed, and tumors and organs of interest were excised, weighed, and their radioactivity was measured using a Cobra II auto-γ-counter B5002 (Packard, Virginia Beach, VA). Results are expressed as %ID/g.

2.4. Statistical analysis

Quantitative data were expressed as mean ± standard deviation (SD). Means were compared using one-way ANOVA and the student *t*-test. *p* Values less than 0.05 were considered statistically significant.

Other methods used in this paper are available as Supplementary Materials and Methods.

3. Results

3.1. Affinity and specificity of anti-GPC3-mAb in vitro

We first demonstrated that the mouse anti-GPC3 mAb (clone 1G12) has high binding affinity (mean K_D value = 0.41 ± 0.05 nM; Fig. 1A) to recombinant human GPC3 protein using an ELISA-based procedure [30]. Using the same mAb for Western blotting and immunofluorescence, we observed varying levels of GPC3 protein expression in a panel of HCC cell lines (HepG2, Hep3B, Huh 7, PLC/ PRF/5 and SNU449) and the prostate cancer cell line (PC3), with highest levels in HepG2 cells, and undetectable levels in SNU449 and PC3 cells (Fig. 1B). Immunofluorescence further confirmed the specificity of anti-GPC3 mAb, showing highest fluorescence intensity in HepG2 cells, moderate fluorescence intensity in Hep3B cells, and no signal in PC3 cells (Fig. 1C). Based on these results, we selected HepG2 and Hep3B cells to represent GPC3-high and GPC3-moderate HCC models for further *in vitro* and *in vivo* studies. The tumorigenic PC3 cells were used as GPC3-negative, non-HCC model.

3.2. In vitro cellular uptake of ⁸⁹Zr-DFO-1G12

We synthesized the PET probe, ⁸⁹Zr-DFO-1G12, and assessed its cellular uptake into a panel of human HCC cell lines (HepG2, Hep3B, SNU499) and a non-HCC cell line (PC3). We observed that overall cellular uptake of ⁸⁹Zr-DFO-1G12 corresponded with the level of GPC3 expression, with highest uptake in HepG2 cells, which was significantly higher than

in all other cell lines at every time point ($p < 0.005$; Fig. 2A). Moderate cellular uptake of ^{89}Zr -DFO-1G12 was observed in Hep3B cells, whereas negligible uptake was observed in SNU449 and PC3 cells. Immunoreactivity assessment of ^{89}Zr -DFO-1G12 demonstrated significantly higher binding percentage in HepG2 cells ($68.47 \pm 5.48\%$) at 40 h incubation compared to other cell lines ($p < 0.05$). Hep3B cells showed moderate level of specific binding ($44.68 \pm 2.43\%$), whereas PC3 cells showed minimal uptake ($11.59 \pm 2.36\%$), indicating non-specific binding (Fig. 2B).

Furthermore, we assessed the ability of ^{89}Zr -DFO-1G12 to be internalized within GPC3-positive HepG2 cells. About $8.64 \pm 0.58\%$ of the added radioactivity was detected in the internalized fraction 2 h post-incubation, which slowly increased to $27.42 \pm 1.38\%$ after 40 h (Fig. 2C), indicating that ^{89}Zr -DFO-1G12 can be internalized within HepG2 cells.

3.3. Imaging subcutaneous HCC xenografts using ^{89}Zr -DFO-1G12

We used ^{89}Zr -DFO-1G12 for PET imaging of subcutaneous xenografts generated using HepG2, Hep3B, and PC3 cells. Specificity of ^{89}Zr -DFO-1G12 for GPC3-expressing xenografts was demonstrated from the decay-corrected coronal and transaxial small-animal PET images in the tumor-bearing mice after injection of ^{89}Zr -DFO-1G12 (Fig. 3A). ^{89}Zr -DFO-1G12 clearly delineated GPC3-expressing, HCC xenografts regardless of their endogenous level of GPC3. Immunohistochemistry of GPC3 protein expression in HepG2, Hep3B, and PC3 xenografts confirmed that the *in vitro* GPC3 expression patterns of the respective cell lines were maintained *in vivo* (Fig. 3B). Both HepG2 and Hep3B xenografts showed increasing tumor uptake over time reaching the highest levels at 168 h p.i. (Fig. 3A and C), whereas non-specific liver signals in both xenograft models decreased over time (Fig. 3A and D). Quantification analysis revealed significantly higher radioactivity uptakes in HepG2 and Hep3B xenografts compared to PC3 xenografts ($p < 0.05$), starting 48 h p.i. (Fig. 3C). Minimal radioactivity was observed in PC3 xenografts, which did not increase over time, confirming specificity of ^{89}Zr -DFO-1G12 for GPC3-expressing xenografts only. The tumor-to-liver ratios in HepG2 and Hep3B xenograft models increased steadily over time, from 2.01 ± 0.19 at 48 h p.i. to 4.08 ± 0.54 at 168 h p.i. for HepG2, and from 1.40 ± 0.26 at 48 h p.i. to 2.47 ± 0.12 at 168 h p.i. for Hep3B (Fig. 3E), whereas the tumor-to-liver ratios in PC3 xenograft models did not. Similarly, biodistribution analysis showed significantly higher radioactivity uptake in HepG2 and Hep3B xenografts compared to PC3 xenografts at 168 h p.i. ($p < 0.005$) (Supplementary Table 1). The tumor-to-liver ratios at 168 h p.i. reached 4.10 ± 0.17 in HepG2 xenografts, and 3.18 ± 0.43 in Hep3B xenografts, which are significantly higher than that in PC3 xenografts (1.10 ± 0.27) ($p < 0.005$).

The non-targeting negative control ^{89}Zr -DFO-IgG showed negligible radioactivity accumulation in the HepG2 xenografts, which confirms the non-specificity of the normal mouse IgG to GPC3. Subsequent biodistribution analysis of ^{89}Zr -DFO-IgG at 168 h p.i. showed that the uptake in HepG2 xenografts and normal liver is $0.65 \pm 0.23\% \text{ID/g}$ and $1.47 \pm 0.35\% \text{ID/g}$ respectively (tumor-to-liver ratio of 0.43 ± 0.07), which revealed no significant difference between the tumor and normal tissues (Supplementary Fig. S1 and Supplementary Table 1).

3.4. Imaging orthotopic HCC xenografts using ^{89}Zr -DFO-1G12

The *in vivo* performance of ^{89}Zr -DFO-1G12 was next evaluated in clinically more relevant models of HCC, including orthotopic xenografts derived from HCC cell lines, and from primary HCC patient tumors. In the first set of orthotopic xenografts derived from HepG2 and Hep3B cells, ^{89}Zr -DFO-1G12 accumulated in both HCC xenografts with prolonged retention, whereas the normal liver signals decreased over time, allowing distinct delineation of the tumor from normal liver tissues as time elapsed (Fig. 4A). At 168 h p.i., the tumor xenografts were clearly defined with negligible signal from the surrounding normal liver. Negligible signals were also observed from normal liver of tumor-free mice injected with the same dose of ^{89}Zr -DFO-1G12.

Quantification analysis showed highest uptake of ^{89}Zr -DFO-1G12 into HepG2 xenografts, which increased over time. Uptake of ^{89}Zr -DFO-1G12 into Hep3B xenografts was relatively lower, and slightly decreased over time, which may be in part due to the lower levels of GPC3 expression in these tumors (Fig. 4B). Liver uptakes in both xenograft models were similarly low and decreased over time (Fig. 4C), resulting in high tumor-to-liver ratios (6.88 ± 0.95 for HepG2, and 5.03 ± 0.79 for Hep3B) (Fig. 4D) at 168 h p.i. Consistently, biodistribution analysis showed high tumor-to-liver ratios, with 6.65 ± 2.33 for HepG2, and 4.29 ± 0.52 for Hep3B (Supplementary Table 2).

In the second set of orthotopic xenografts derived from primary HCC patient tumors (HCC-1, -2, and -3), ^{89}Zr -DFO-1G12 distinctly demarcated the xenografts from the normal liver starting at 48 h p.i. (Fig. 5A; Supplementary Video 1). Radioactivity accumulated over time in all three xenografts, whereas the liver signals decreased over time, allowing clear demarcation of the tumor from the liver at 168 h p.i. Elevated GPC3 protein expressions in all three HCC patient tumors were confirmed by Western blotting of the original tumors, and by immunohistochemistry on corresponding xenografts in mice (Fig. 5B).

Supplementary video related to this article can be found at <http://dx.doi.org/10.1016/j.biomaterials.2014.04.089>.

Quantification analysis showed increasing ^{89}Zr -DFO-1G12 uptake for the first 72 h, followed by a slight decrease over time for all three primary orthotopic xenografts (Fig. 5C). Liver radioactivity uptakes in all three xenograft models were consistently lower than xenograft uptakes, and decreased steadily over time (Fig. 5D), resulting in high tumor-to-liver ratios (4.21 ± 0.64 , 2.78 ± 0.26 , and 2.31 ± 0.38 at 72 h, respectively) (Fig. 5E). These were supported by biodistribution analysis (Table 1). Our data suggest that ^{89}Zr -DFO-1G12 can clearly differentiate HCC lesions from the normal liver in orthotopic HCC xenografts, including those derived from HCC patients.

4. Discussion

We successfully synthesized the ^{89}Zr -DFO-1G12 immuno-PET probe for imaging of HCC based on GPC3 expression, and demonstrated its ability to specifically identify GPC3-expressing HCC cells *in vitro* and *in vivo*. Importantly, ^{89}Zr -DFO-1G12 delineated GPC3-

expressing orthotopic HCC patient-derived xenografts from surrounding normal liver tissue, suggesting its potential for clinical translation.

Despite recent advances in positron emission tomography imaging using tumor-seeking radiolabeled-molecules (immuno-PET), the applicability using immuno-PET for HCC imaging still remains questionable. As the liver is largely responsible for exogenous molecules clearance, high normal liver uptake and resulting poor tumor-to-liver ratio are typical, and limits clinical application. None of the most commonly used PET probes for HCC imaging, including ^{18}F -FDG and ^{11}C -labeled acetate, can provide ideal tumor-targeting specificity: ^{18}F -FDG misses up to 30%–50% of HCC lesions in the liver [31]; whereas ^{11}C -labeled acetate represents a non-specific probe for HCC imaging [32]. Thus, identifying an HCC-specific molecular target is paramount in the development of an effective immuno-PET probe, together with careful selection of the targeting molecule and the positron-emitting radionuclide [33].

GPC3 is an excellent molecular target in HCC, due to its preferential expression in HCC cells only, even in the early stages of malignant transformation. Recently, at least two other groups have attempted to target GPC3 for diagnostic imaging using either multifunctional nanoparticles or MRI-specific superparamagnetic iron oxide anti-GPC3, but their studies are limited to *in vitro* evaluation only and lack critical *in vivo* performance evaluation [34,35]. In our study, we chose to use an intact monoclonal antibody (1G12) as the targeting molecule because of its demonstrated high affinity and specificity for recognizing GPC3. Given that an intact antibody has relatively slow pharmacokinetics, we rationalized that the use of a long half-life radioisotope, ^{89}Zr , might help to achieve clinically favorable tumor-to-liver ratios as its long decay half-life (3.3 day; 78.4 h) matches the biological half-life of intact mAbs, and thus, allows imaging at late time points (up to seven days p.i.) for obtaining maximum information [36]. Additionally, the ability of ^{89}Zr to be residualized and retained within the target cell after internalization and intracellular degradation of the tracer results in enhanced uptake in the tumor when an internalized antibody is used [36].

Using a panel of HCC and non-HCC cell lines expressing varying levels of GPC3, we demonstrated that ^{89}Zr -DFO-1G12 could be specifically taken up by GPC3-expressing cells only; and confirmed that ^{89}Zr -DFO-1G12 can be internalized into the HepG2 cells *in vitro*. Furthermore, ^{89}Zr -DFO-1G12 demonstrated promising *in vivo* performance in achieving high tumor-to-liver (T/L) ratios due to enhanced tumor accumulation and reduced non-specific liver accumulation, in both subcutaneous and orthotopic HCC xenograft models. Tumor uptake and corresponding tumor-to-liver ratios in the xenografts correlate with the endogenous expression levels of GPC3 in respective HCC cell lines, demonstrating specificity of the probe for GPC3. In particular, ^{89}Zr -DFO-1G12 distinctly delineated orthotopic HCC xenografts (derived from HCC cell lines and from primary HCC specimens) from the normal liver when imaged seven days p.i., providing high imaging contrast of the xenografts. ^{89}Zr -DFO-1G12 detected all GPC3-positive orthotopic HCC xenografts regardless of GPC3 expression levels, implying specificity for GPC3-expressing HCCs and highlighting its clinical value in the diagnosis of all GPC3-expressing HCCs.

The successful imaging of HCC lesions based on GPC3 expression is potentially valuable for the early detection of HCC, and may also allow more accurate prognostication of HCC patients (GPC3-positive HCC patients have been reported to have significantly lower 5-year survival rate [26]), and eventually, lead to improved clinical management and overall patient survival rate. The translational potential of this probe could be further validated using a humanized anti-GPC3 antibody, or antibody fragments (such as a minibody or a diabody), radiolabeled with ^{89}Zr or another more appropriate radionuclide. These radiolabeled humanized antibody moieties should be systematically evaluated for their GPC3-binding capacity and human serum stability, and preclinical potential as PET imaging probes. Besides diagnostic imaging of GPC3-positive HCC lesions, these probes can also be adapted for targeted delivery of therapeutic agents, such as radionuclides (e.g. ^{90}Y) or small anti-tumor molecules to GPC3-positive HCC.

5. Conclusions

We have demonstrated that immuno-PET imaging of HCC based on GPC3 is a feasible and highly translatable approach. The specificity provided by the anti-GPC3 antibody, coupled with the high tumor-to-liver ratios provided by the ^{89}Zr -mAb, makes ^{89}Zr -DFO-1G12 the most specific immuno-PET probe for HCC to date that can achieve high resolution imaging of GPC3-expressing HCCs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the T.S. Kwok Liver Research Foundation, the C.J. Huang Foundation (to the Asian Liver Center, Stanford University), and the Stanford Cancer Center (DCRA). The authors thank Dr. Tim Doyle from the Stanford Center for Innovation in *in vivo* Imaging for his expert knowledge and technical advice for PET/CT imaging.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.biomaterials.2014.04.089>.

Fig. 1. Anti-GPC3 mAb binds to recombinant human GPC3 and specifically identifies GPC3-expressing HCC cells. (A) Binding of anti-GPC3 mAb (clone 1G12) to recombinant human GPC3 protein was assessed using an affinity binding assay. Fluorescence counts corresponding to each serial dilution of the anti-GPC3 mAb were measured ($n = 3$). The mean K_D value was determined to be 0.41 ± 0.05 nM. (B) GPC3 protein expression level was measured in various human HCC cell lines (HepG2, Hep3B, Huh 3, PLC/PRF/5 and SNU449) and non-HCC PC3 cell line by Western blot (B) and immunofluorescence staining (C). For immunofluorescence, anti-GPC3 mAb (clone 1G12) was used as the primary antibody, and AlexaFluor 660 goat anti-mouse IgG used as the secondary antibody. Overlay images of GPC3 staining (green fluorescence signals) and cell nuclei DAPI staining (blue fluorescence signals) are shown.

Fig. 2. Specific uptake and cellular internalization of ^{89}Zr -DFO-1G12 mAb. (A) Cellular uptake of ^{89}Zr -DFO-1G12 in HepG2, Hep3B, SNU449, and PC3 cells over time at 37 °C. $**p < 0.001$. (B) Immunoreactivity assay measuring cell uptake at concentrations ranging from 0.2 to 10.0 nM of ^{89}Zr -DFO-1G12. (C) Cell-associated (internalized) radioactivity as a function of time after incubation of HepG2 cells with ^{89}Zr -DFO-1G12. All data are presented as means $\pm$ SD ($n = 4$).

Fig. 3. PET imaging of ^{89}Zr -DFO-1G12 in subcutaneous xenografts. (A) Representative decay corrected coronal (top) and transaxial (bottom) PET images in HepG2, Hep3B, and PC3-tumor bearing mice at different time points after tail vein injection of ^{89}Zr -DFO-1G12. Arrows indicate the location of the tumors. (B) GPC3 expression in the HepG2, Hep3B, and PC3 xenograft sections are shown by immunohistochemistry. Time-activity curves of tumor uptake (C), liver uptake (D), and tumor-to-liver ratios (E) derived from multiple-time point small-animal PET images after tail injection of ^{89}Zr -DFO-1G12. Data are presented as mean $\pm$ SD %ID/g ($n = 4$). $**p < 0.001$. $***p < 0.0001$.

Fig. 4. PET/CT imaging of ^{89}Zr -DFO-1G12 in orthotopic HCC xenografts derived from human cell lines. (A) Representative decay corrected sagittal PET/CT images of mice bearing orthotopic xenografts of HepG2 and Hep3B cells are shown. As controls, images from normal mice at every time point (24 h, 48 h, 72 h, 120 h, and 168 h) are also shown. Scale bars indicate signal density for CT, and %ID/g for PET. Time-activity curves of tumor uptake (B), liver uptake (C), and tumor-to-liver ratios (D) derived from multiple-time point small-animal PET images after tail injection of ^{89}Zr -DFO-1G12. Data are presented as mean $\pm$ SD %ID/g ($n = 4$).

Fig. 5. PET/CT imaging of ^{89}Zr -DFO-1G12 in orthotopic HCC xenografts derived from human patient specimens. (A) Representative decay corrected sagittal PET/CT images of orthotopic HCC xenografts derived from three human HCC patients (HCC-1, -2, and -3) are shown at various time points. Scale bars indicate signal density for CT, and %ID/g for PET. (B) Western blot of these three pairs of HCC tumors (T) and their adjacent non-tumor tissues (NT) for GPC3 and GAPDH (as internal control). Immunohistochemistry staining for GPC3 using xenografts harvested from mice are shown to validate GPC3 expression in animal models. Time-activity curves of tumor uptake (C), liver uptake (D), and tumor-to-liver ratios (E) derived from multiple-time point small-animal PET images after tail injection of ^{89}Zr -DFO-1G12 are shown. Data are presented as mean $\pm$ SD %ID/g ($n = 4$).

Table 1 Biodistribution of ^{89}Zr -DFO-1G12 in orthotopic HCC patient-derived xenografts at 168 h p. i.

^{89}Zr -DFO-1G12	HCC-1	HCC-2	HCC-3
Tissues (%ID/g)			
Tumor	13.21 $\pm$ 1.62	8.50 $\pm$ 1.60	6.64 $\pm$ 0.80
Blood	5.37 $\pm$ 0.35	1.17 $\pm$ 0.81	4.63 $\pm$ 0.61
Heart	1.32 $\pm$ 0.16	0.49 $\pm$ 0.23	1.59 $\pm$ 0.02
Lungs	2.84 $\pm$ 0.36	0.90 $\pm$ 0.36	2.86 $\pm$ 0.34
Liver	2.91 $\pm$ 0.45	2.37 $\pm$ 0.14	2.47 $\pm$ 0.41
Spleen	4.95 $\pm$ 1.01	2.48 $\pm$ 1.11	4.62 $\pm$ 0.69
Pancreas	0.78 $\pm$ 0.07	0.30 $\pm$ 0.05	0.79 $\pm$ 0.20
Stomach	0.83 $\pm$ 0.12	0.42 $\pm$ 0.11	0.72 $\pm$ 0.05
Brain	0.15 $\pm$ 0.01	0.05 $\pm$ 0.03	0.15 $\pm$ 0.03
Intestine	0.66 $\pm$ 0.09	0.33 $\pm$ 0.12	0.50 $\pm$ 0.05
Kidneys	1.67 $\pm$ 0.22	2.62 $\pm$ 0.88	1.62 $\pm$ 0.18
Skin	1.56 $\pm$ 0.06	0.74 $\pm$ 0.19	1.49 $\pm$ 0.07
Muscle	0.51 $\pm$ 0.07	0.28 $\pm$ 0.09	0.48 $\pm$ 0.07
Bone	2.15 $\pm$ 0.35	1.53 $\pm$ 0.13	2.27 $\pm$ 0.51
Uptake ratio of tumor/normal tissue			
Tumor/liver	4.60 $\pm$ 1.05	3.58 $\pm$ 0.61	2.75 $\pm$ 0.62
Tumor/muscle	26.06 $\pm$ 6.71	32.35 $\pm$ 10.11	13.88 $\pm$ 2.25

Data are given as mean $\pm$ SD of percentage administered activity (injected dose) per gram of tissue (%ID/g).