Clinical Translation of a $^{68}$Ga-labeled Integrin $\alpha v\beta 6$-targeting Cyclic Radiotracer for PET Imaging of Pancreatic Cancer

Xun Feng$^1$, Yanpu Wang$^1$, Dehua Lu$^1$, Xiaoxia Xu$^2$, Xin Zhou$^2$, Huiyuan Zhang$^2$, Ting Zhang$^1$, Hua Zhu$^2$, Zhi Yang$^2$, Fan Wang$^1$, Nan Li$^2$,*, Zhaofei Liu$^1$,*

1 Medical Isotopes Research Center and Department of Radiation Medicine, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China

2 Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Nuclear Medicine, Peking University Cancer Hospital & Institute, Beijing 100142, China

Running Title: Clinical Translation of $^{68}$Ga-cycratide

Word count: 4999

For correspondence contact: Zhaofei Liu, Medical Isotopes Research Center, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China. E-mail: liuzf@bjmu.edu.cn; or Nan Li, Department of Nuclear Medicine, Peking University Cancer Hospital & Institute, Beijing 100142, China. Email: rainbow6283@sina.com

First author: Xun Feng

Master student in the Department of Radiation Medicine, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China. Email: fengxun@bjmu.edu.cn
ABSTRACT

The overexpression of integrin αvβ6 in pancreatic cancer makes it a promising target for noninvasive positron emission tomography (PET) imaging. However, currently, most integrin αvβ6-targeting radiotracers are based on linear peptides, which are quickly degraded in the serum by proteinases. Herein, we aimed to develop and assess a ⁶⁸Ga-labeled integrin αvβ6-targeting cyclic peptide (⁶⁸Ga-cycratide) for PET imaging of pancreatic cancer. **Methods:** ⁶⁸Ga-cycratide was prepared, and its PET imaging profile was compared with that of the linear peptide (⁶⁸Ga-linear-pep) in an integrin αvβ6-positive BxPC-3 human pancreatic cancer mouse model. Five healthy volunteers (two women and three men) underwent whole-body PET/CT imaging after injection of ⁶⁸Ga-cycratide, and biodistribution and dosimetry calculations were determined. PET/CT imaging of two patients was performed to investigate the potential role of ⁶⁸Ga-cycratide in pancreatic cancer diagnosis and treatment monitoring. **Results:** ⁶⁸Ga-cycratide exhibited significantly higher tumor uptake than did ⁶⁸Ga-linear-pep in BxPC-3 tumor-bearing mice, owing—at least in part—to markedly improved *in vivo* stability. ⁶⁸Ga-cycratide could sensitively detect the pancreatic cancer lesions in an orthotopic mouse model and was well tolerated in all healthy volunteers. Preliminary PET/CT imaging in patients with pancreatic cancer demonstrated that ⁶⁸Ga-cycratide was comparable to ¹⁸F-fludeoxyglucose for diagnostic imaging and post-surgery tumor relapse monitoring. **Conclusion:** ⁶⁸Ga-cycratide is an integrin αvβ6-specific PET radiotracer with favorable pharmacokinetics and dosimetry profile. ⁶⁸Ga-cycratide is expected to provide an effective noninvasive PET strategy for pancreatic cancer lesion detection and therapy response monitoring.
Keywords: Pancreatic cancer; integrin αvβ6; $^{68}$Ga radiotracer; PET/CT; cycratide
INTRODUCTION

Pancreatic cancer is a highly deadly malignancy, characterized by strong invasion and high resistance to treatment (1). Most patients are diagnosed at the late stages of this disease, resulting in a 5-year survival rate of only 4%-8% (2). The current anatomical imaging modalities for pancreatic cancer care mainly rely on CT, magnetic resonance imaging, and endoscopic ultrasound. Endoscopic ultrasound combined with fine needle aspiration has a diagnostic sensitivity that ranges between 80% and 95% (3). However, this approach is a relatively invasive procedure and is dependent upon experienced operators. Abdominal CT is most widely used to detect suspicious pancreatic cancer, but it has limited sensitivity in the detection of small tumors <2 cm (4). Magnetic resonance imaging has sensitivities and specificities in detecting and staging pancreatic cancers that are comparable with CT, but may be more accurate in characterizing small subcentimeter lesions and metastases (5).

Compared with anatomical imaging modalities, molecular imaging techniques such as positron emission tomography (PET) have advantages in providing functional and molecular information, which allows for early decision-making in patients with pancreatic cancer (4,6). Currently, $^{18}$F-fludeoxyglucose (FDG) PET is the most commonly used technique in the clinic for initial assessment of disease progression and monitoring of treatment response. However, the use of $^{18}$F-FDG has limitations, such as a lack of target-related specificity (7,8) and broad differential diagnosis including other conditions, such as inflammation (9). Therefore, there remains a need to develop new PET radiotracers with high specificity for targeting malignant lesions and for differentiating malignant lesions from nonmalignant cysts.

Integrin $\alpha_v\beta_6$, a member of the integrin family, is closely related to the malignant
behavior of a variety of tumors. In pancreatic cancer, the positive expression ratio of integrin $\alpha_v\beta_6$ is typically higher than 95% (10,11), making it a potential target for theranostics of pancreatic cancer. In the past several years, many peptide-based radiotracers have been developed for noninvasive PET (12-17) or SPECT (18-20) imaging of integrin $\alpha_v\beta_6$ in vivo. Most of the currently investigated integrin $\alpha_v\beta_6$-targeting peptides contain the “RGDLXXL” or “DLXXL” sequence (wherein “X” represents any undetermined amino acids), the major binding motif for integrin $\alpha_v\beta_6$ (21). Compared to antibodies, peptides possess advantages including low cost, easy synthesis, and low immunological reaction, which offers great potential for clinical use in nuclear medicine imaging. Recently, the $^{18}$F-labeled A20FMDV2 peptide containing the “RGDLQVL” sequence was tested in human subjects. The safety, biodistribution, and radiation dosimetry of the radiotracer (17), as well as its use in preliminary PET imaging in patients with malignant tumors (13), were assessed.

The preparation procedures of $^{18}$F-labeled peptides are typically time-consuming and tedious, and facilities such as on-site cyclotrons are needed. By contrast, $^{68}$Ga-labeled peptides can be prepared by kit formulation for rapid preparation of radiotracers for clinical use. Recently, integrin $\alpha_v\beta_6$-targeting peptides were radiolabeled with $^{68}$Ga; the resulting radiotracers were tested in preclinical mouse models (16) and clinical studies for PET imaging of non-small cell lung cancer (22-24). These pioneering studies demonstrated the potential of $^{68}$Ga-labeled peptides targeting integrin $\alpha_v\beta_6$ for potential clinical use in cancer detection and treatment monitoring.

Our group previously developed a $^{99m}$Tc-labeled linear peptide containing the “RGDLATL” sequence for integrin $\alpha_v\beta_6$-targeted SPECT imaging of pancreatic cancer
and corresponding metastasis to the liver in mouse models (20). This integrin αvβ6-targeting peptide can also be modified for effective tumor-specific delivery of nanostructures and therapeutic agents (11,25,26). However, the linear peptide can be quickly metabolized in vivo after injection (20), which limits its further clinical translation. Cyclization has been proposed as a powerful strategy to improve in vivo stability of peptides (27,28). As “RGDLATL” is the key sequence for integrin αvβ6 binding, we recently synthesized an “RGDLATL”-containing cyclic peptide by adding two cysteine residues at the N- and C-terminals, respectively, to form a disulfide bridge. However, its integrin αvβ6-binding affinity was markedly reduced compared to the linear peptide (29). Therefore, in this study, we aimed to develop a new ⁶⁸Ga-labeled cyclic peptide based on the integrin αvβ6-targeting “RGDLATL” sequence. We evaluated the resulting radiotracer for its integrin αvβ6-specific tumor detection ability in animal models, assessed its biodistribution and dosimetry in healthy volunteers, and conducted clinical translational PET imaging in patients with pancreatic cancer.

MATERIALS AND METHODS

Synthesis of DOTA-conjugated Peptides and ⁶⁸Ga Radiolabeling

The integrin αvβ6-targeting linear peptide RGDLATLKC (denoted as linear-pep) and the cyclic peptide cyclo[-RGDLATL-] (denoted as cycratide) were custom made by ChinaPeptides. (Shanghai, China). Linear-pep or cycratide was conjugated with DOTA-NHS-ester (Macrocyclics, Dallas, TX) and then radiolabeled with ⁶⁸Ga as described in the Supplemental Materials.
**Cell Culture and Animal Models**

Integrin αvβ6-positive (20) BxPC-3 human pancreatic cancer cells were purchased from American Type Culture Collection (Manassas, VA). Cells were grown in RPMI-1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum at 37°C in a humidified atmosphere containing 5% CO2.

All animal experiments were performed in accordance with the Guidelines of the Peking University Animal Care and Use Committee. The subcutaneous and orthotopic tumor models were established as described in the Supplemental Materials.

**Small-animal PET Imaging**

Each subcutaneous BxPC-3 tumor-bearing nude mouse was injected with 5.55 MBq (~0.19 μg) of 68Ga-cycratide or 68Ga-linear-pep with or without blocking doses of cold peptides via the tail vein (n = 4 mice per group). Small-animal PET imaging was then performed as described in the Supplemental Materials.

For PET imaging experiments with the orthotopic BxPC-3 tumor model, mice were injected with 5.55 MBq of 68Ga-cycratide. At 0.5 h postinjection, 10-min static PET scans were acquired. One day later, the same mice were coinjected an excess dose of cold cycratide (50 mg/kg mouse body weight) with 5.55 MBq 68Ga-cycratide, and then PET imaging was performed at 0.5 h postinjection. After PET scanning, mice were euthanized, and the orthotopic tumor lesions were surgically excised. Half of each tumor was embedded in paraffin and then subjected to hematoxylin and eosin staining. The remaining half was frozen in OCT medium and cut into 5-μm-thick slices for immunofluorescence staining of integrin αvβ6.
Human Subjects

The Institutional Review Board of Peking University Cancer Hospital & Institute approved this study (#2018KT54), and all subjects signed a written informed consent. Five healthy volunteers (two women and three men; age 28–67 years; mean age ± standard deviation [SD]: 46.2 ± 15.2 years) and two patients with suspected pancreatic cancer (one woman and one man) were enrolled in this study.

PET/CT Procedures and Dosimetry in Healthy Volunteers

The five volunteers were deemed healthy based on history, physical examination, electrocardiogram, urinalysis, and routine blood testing. No fasting or other special preparations were requested on the day of $^{68}$Ga-cycratide PET/CT imaging. Any unusual or adverse clinical symptoms were recorded on the day of PET imaging and during the 2-week follow-up period. All five volunteers underwent a low-dose CT scan (120 kV, 35 mA, slice thickness: 0.6 mm, pitch: 0.8, and matrix: 512×512) and then dynamic PET acquisitions using a Siemens Biograph mCT Flow 64 scanner (Erlangen, Germany) at multiple time points from 0 min to 1 h after $^{68}$Ga-cycratide injection (3.7 MBq/~0.12 μg per kg of body weight). The whole body of each subject (from the top of skull to the middle of the femur) was covered by 6 bed positions, and the acquisition time was 40 s/bed position for the 5 and 15 min time points and 90 s/bed position for the 25, 35, 45, and 60 min time points. One additional 10-min static PET image was also acquired at 2 h postinjection. A Siemens workstation (MultiModality Workplace) was used for data processing, and the OLINDA/EXM 2.0 software (version 2.0; Hermes Medical Solutions
AB) was used for the dosimetry calculation. Detailed procedures for PET/CT data analysis and dosimetry calculation are described in the Supplemental Materials.

**PET/CT Imaging of Patients**

A total of two patients underwent $^{68}$Ga-cycratide PET/CT in a Siemens Biograph mCT Flow 64 scanner (Erlangen, Germany), and underwent $^{18}$F-FDG PET/CT for comparison within one week. A low-dose CT scan was used with the same parameters as described above. For $^{18}$F-FDG PET/CT, patients fasted for at least 6 h before intravenous injection of $^{18}$F-FDG at a dosage of 5.55 MBq/kg of body weight. $^{18}$F-FDG/CT PET scans were performed 1 h postinjection.

One patient (55 years old, female) with suspected pancreatic cancer underwent whole-body PET/CT acquisitions at 1 h after $^{68}$Ga-cycratide injection (3.7 MBq/~0.12 µg per kg of body weight), and the patient underwent surgical resection within 11 days after PET/CT imaging. Another patient (54 years old, male) with confirmed pancreatic cancer underwent 7-month post-surgery PET/CT scanning at 1 h after $^{68}$Ga-cycratide injection (3.7 MBq/~0.12 µg per kg of body weight). Abdomen contrast-enhanced CT of this patient was performed within 2 weeks of $^{68}$Ga-cycratide scanning, and was repeated for follow-up 3 months later. The patient fasted for at least 6 h, and dual-phase contrast-enhanced CT scans (120 kV, 300 mA, tube rotation time of 1 s, 0.625-mm thickness with 0.625-mm gap, 36-cm field of view) were performed in a GE Lightspeed VCT (GE Healthcare, Waukesha, WI) after intravenous administration of 100 mL of Iohexol (300 mg I/mL) at a rate of 2.5–3.5 mL/sec. The protocol during the acquisition period was at 30 s for the arterial phase and 90 s for the portal venous phase.
Statistical Analysis

The data were analyzed by GraphPad Prism 6.0 software (GraphPad, San Diego, CA). Unpaired Student's t test were used for statistical analysis. \( P \) values <0.05 were considered statistically significant.

RESULTS

Chemistry and Radiochemistry

The identity of DOTA-cycratide (Fig. 1A) and DOTA-linear-pep (Supplemental Fig. 1A) were confirmed by mass spectroscopy. The decay-corrected labeling yield for \( ^{68}\text{Ga}\)-cycratide and \( ^{68}\text{Ga}\)-linear-pep ranged from 95% to 98%. After purification, the radiochemical purity of both radiotracers was >99%, with the specific activity of 37–55.5 MBq/nmol. Both \( ^{68}\text{Ga}\)-cycratide and \( ^{68}\text{Ga}\)-linear-pep showed favorable in vitro stability, with a radiochemical purity higher than 85% after 4 hours (Supplemental Fig. 1B).

In Vitro Cell-binding Studies

The IC\(_{50}\) values of cycratide (16.30 ± 2.36 nM), DOTA-cycratide (20.17 ± 1.68 nM), and linear-pep (19.58 ± 2.49 nM) were comparable (Fig. 1B). The binding values of \( ^{68}\text{Ga}\)-cycratide to BxPC-3 cells (AD% per 10\(^6\) cells) were significantly inhibited by the addition of excess doses of cycratide (11.06 ± 2.51 vs. 1.90 ± 0.41; n = 4, \( P <0.01 \)) or linear-pep (11.06 ± 2.51 vs. 1.96 ± 0.53; n = 4, \( P <0.01 \); Fig. 1C).

In Vivo Metabolic Stability

The linear peptide radiotracer \( ^{68}\text{Ga}\)-linear-pep was rapidly metabolized after injection
with almost no intact peptide present in the blood and urine at 0.5 h postinjection (Supplemental Fig. 1C). In contrast, the cyclic radiotracer $^{68}$Ga-cycratide showed favorable metabolic stability \textit{in vivo}, with no evident metabolites in the blood and urine at 0.5 h postinjection (Fig. 1D).

\textbf{Small-animal PET Imaging and Biodistribution}

The BxPC-3 tumors could be clearly visualized with good tumor-to-background contrast for $^{68}$Ga-cycratide at 0.5 h and 1 h, but rapidly cleared, with low tumor uptake at 2 h (Fig. 2A). The region of interest-derived tumor uptake of $^{68}$Ga-cycratide was significantly higher than that of $^{68}$Ga-linear-pep at 0.5 h (3.82 ± 1.44 vs. 1.40 ± 0.38 %ID/g; n = 4, \(P < 0.01\)) and 1 h postinjection (2.47 ± 1.18 vs. 0.90 ± 0.16 %ID/g; n = 4, \(P < 0.01\)). The tumor-to-muscle ratio of $^{68}$Ga-cycratide was also significantly higher than that of $^{68}$Ga-linear-pep at 0.5 h (4.77 ± 1.62 vs. 2.27 ± 0.63; n = 4, \(P < 0.05\)) and 1 h postinjection (4.19 ± 1.29 vs. 2.27 ± 0.53; n = 4, \(P < 0.05\); Fig. 2B).

To validate the PET imaging results, biodistribution studies were performed. As shown in Fig. 2C, the tumor uptake of $^{68}$Ga-cycratide was significantly higher than that of $^{68}$Ga-linear-pep (2.15 ± 0.46 vs. 0.94 ± 0.58 %ID/g; n = 4, \(P < 0.05\)) at 0.5 h postinjection. The tumor uptake of $^{68}$Ga-cycratide was reduced significantly from 2.15 ± 0.46 to 1.10 ± 0.18 %ID/g at 0.5 h postinjection (n = 4, \(P < 0.01\)) after blocking with cold cycratide.

Orthotopic BxPC-3 tumor lesions in the pancreas were clearly detected by $^{68}$Ga-cycratide, with high contrast (Fig. 3A). A 74% reduction in the calculated %ID/g uptake of $^{68}$Ga-cycratide was observed in the pancreas after blocking (Fig. 3A). After PET imaging, the presence of tumor lesions in the pancreas was verified by hematoxylin and eosin
staining (Fig. 3B), and immunofluorescence staining further confirm the positive expression of integrin \( \alpha \beta 6 \) in the tumor lesions (Fig. 3C).

**Biodistribution and Dosimetry of \( ^{68} \)Ga-cycratide in Healthy Volunteers**

No adverse events or obvious changes in signs or the results of clinical laboratory test were found by any of the volunteers during the PET imaging procedure and within 2 weeks after injection of \( ^{68} \)Ga-cycratide. Kidney and bladder showed the highest radioactivity accumulation, whereas the heart, liver, and spleen had relatively low activity (Fig. 4). The highest SUV values were observed in the kidney at all time points examined. The uptake of \( ^{68} \)Ga-cycratide in other organs, such as the lung, intestine, bone marrow, brain, and muscle, was relatively low (Supplemental Table 1).

The estimated radiation-absorbed doses derived from the PET/CT images for each organ of the five volunteers are listed in Supplemental Table 2. The whole-body effective dose was 5.49E-02 ± 4.69E-02 mSv/MBq. The highest absorption doses were observed in the urinary bladder wall (5.59E-01 ± 6.40E-01 mGy/MBq), as \( ^{68} \)Ga-cycratide was excreted predominantly via the renal route. The adjacent organs next to the urinary bladder walls and the kidneys, such as the ovaries, and the uterus, also showed high absorption doses.

**PET Imaging of \( ^{68} \)Ga-cycratide in Patients with Pancreatic Cancer**

In a 55-year-old female patient, uptake of \( ^{68} \)Ga-cycratide was evident in the region of the pancreatic mass, with a SUVmax of 4.86, and \(^{18}\)F-FDG also showed high radioactivity accumulation in that region, with a SUVmax of 6.90 (Fig. 5A). This patient was later confirmed to suffer moderately differentiated pancreatic ductal adenocarcinoma (stage:
pT3N1) after surgery and pathological examination. Immunohistochemical examination confirmed the expression of integrin αvβ6 in the tumor lesion (Fig. 5B).

Another patient (54 years old, male) had been previously diagnosed with pancreatic cancer. This patient had received surgery 7 months before and had undergone 7 periods of chemotherapy (gemcitabine plus tegafur, gimeracil, and oteracil potassium capsules) after surgery. Retroperitoneal soft tissue mass were observed on enhanced CT examination (Fig. 5C). $^{68}$Ga-cycratide showed a low uptake with a SUVmax of 1.6 and SUVmean of 0.9, and $^{18}$F-FDG exhibited a SUVmax of 2.2 and SUVmean of 1.8 in the pancreas. After 3 months, enhanced CT was performed again, and the mass was markedly reduced (Fig. 5C), suggesting an inflammatory reaction rather than a tumor relapse.

**DISCUSSION**

Herein, we prepared a novel $^{68}$Ga-radiolabeled cyclic peptide targeting integrin αvβ6, tested it in a preclinical orthotopic model, investigated its biodistribution and dosimetry profile in healthy volunteers, and performed preliminary clinical PET imaging in patients with pancreatic cancer. We found that $^{68}$Ga-cycratide is an integrin αvβ6-specific PET radiotracer that can be easily prepared, has a favorable pharmacokinetics and dosimetry profile.

The introduction of NOTA-Al$^{18}$F chelating strategy has markedly simplified the preparation of $^{18}$F-labeled peptides, with potential kit-formulation for clinical application (30). We prepared the $^{18}$F-labeled NOTA-cycratide with high purity by using Al$^{18}$F (Supplemental Fig. 2), and observed a comparable tumor cell uptake pattern of $^{18}$F-cycratide *in vitro* with that of $^{68}$Ga-cycratide (Supplemental Fig. 3). However, compared
with $^{68}$Ga-cycratide, evident radioactivity uptake in the spine was observed 2 h after injection of $^{18}$F-cycratide (Supplemental Fig. 4). The biodistribution showed that the uptake of $^{18}$F-cycratide in the bone increased with time from 0.5 h to 2 h (Supplemental Fig. 5). Compared with $^{68}$Ga-cycratide, a significantly higher uptake in the kidney, bone, and muscle at 1, and 2 h postinjection, and a significantly lower tumor-to-muscle ratio at 0.5, 1, and 2 h postinjection was observed for $^{18}$F-cycratide ($P<0.05$; $n=4$; Supplemental Fig. 6). Therefore, the superior in vitro characterization and in vivo behaviors of $^{68}$Ga-cycratide as compared to the $^{18}$F-labeled counterpart (Supplemental Table 3), as well as its easy availability, facilitate its use in clinical settings.

We therefore tested the in vivo behavior of $^{68}$Ga-cycratide in healthy volunteers. Dynamic scan results showed that $^{68}$Ga-cycratide was cleared via renal and bladder routes, and background in the blood and surrounding abdominal organs was quite low, which would allow for sensitive detection of pancreatic tumor lesions. Notably, background uptake in the abdomen and intestine were much lower than that recently reported of a $^{68}$Ga-labeled integrin $\alpha v \beta 6$-targeting radiotracer in humans (22,23), which may have resulted at least in part from the improved in vivo stability of $^{68}$Ga-cycratide. The dosimetry of $^{68}$Ga-cycratide was comparable to other $^{68}$Ga-labeled peptides (31,32), and no adverse events, or abnormal vital signs or clinical laboratory tests were observed after injection, confirming that $^{68}$Ga-cycratide is safe and well tolerated in healthy volunteers.

$^{68}$Ga-cycratide and $^{18}$F-FDG imaging both confirmed the advantages of $^{18}$F-FDG and $^{68}$Ga-cycratide over enhanced CT for monitoring post-surgery relapse (Fig. 5C). In a patient with suspected pancreatic cancer, both $^{68}$Ga-cycratide and $^{18}$F-FDG were able to identify the lesions (Fig. 5A). Although the SUV of $^{68}$Ga-cycratide was slightly lower than that of
$^{18}$F-FDG, immunohistochemical analysis confirmed integrin-specific targeting of $^{68}$Ga-cycratide (Fig. 5B). Despite the fact that $^{18}$F-FDG is more sensitive than CT, $^{18}$F-FDG suffers from false-positive uptake in the presence of focal mass-forming pancreatitis ($4,33$). Altmann et al. recently demonstrated that $^{68}$Ga-labeling integrin $\alpha \beta_6$-targeting peptides are more specific for lung cancer differentiation with inflammation compared to $^{18}$F-FDG (22), and integrin $\alpha \beta_6$-targeted PET imaging can sensitively detect a variety of metastasis sites including the lungs, liver, bone, and brain (13). Indeed, in our animal studies, side-by-side comparison of the PET images of $^{68}$Ga-cycratide and $^{18}$F-FDG demonstrated that integrin $\alpha \beta_6$-specific imaging using $^{68}$Ga-cycratide holds potential advantages for the differentiation of tumors compared to $^{18}$F-FDG (Supplemental Fig. 7). As integrin $\alpha \beta_6$ and transforming growth factor-$\beta$ affect the tumor suppressor pathway to promote pancreatic cancer progression (34), further investigation into the correlation of $^{68}$Ga-cycratide uptake with integrin $\alpha \beta_6$ expression, as well as tumor progression, is warranted to further demonstrate the role of $^{68}$Ga-cycratide in noninvasive pancreatic cancer PET staging.

This study has several limitations. First, tumor uptake of $^{68}$Ga-cycratide was relatively low (approximately 2 %ID/g; Fig. 2C). The integrin $\alpha \beta_6$-binding affinity of $^{68}$Ga-cycratide could be further improved to increase the tumor uptake and retention, which may result in higher SUV in humans. We have tested the multimerization strategy by construction of a dimeric cycratide; however, the receptor-binding affinity as well as the tumor uptake did not show evident improvement (data not shown), consistent with recent observations by Notni et al. (16). Therefore, optimization strategies other than multimerization may be needed in the future studies. Further, we only tested PET imaging
of $^{68}$Ga-cycratide in two patients with pancreatic cancer. This is a very small sample size, and further large scale prospective clinical studies comparing $^{68}$Ga-cycratide and $^{18}$F-FDG, as well as correlation studies regarding the SUV of $^{68}$Ga-cycratide with that of integrin $\alpha \beta 6$ expression levels, are necessary to evaluate the full potential of $^{68}$Ga-cycratide in pancreatic cancer detection, staging, and prognosis monitoring.

CONCLUSION

The cyclic radiotracer $^{68}$Ga-cycratide showed high targeting specificity for integrin $\alpha \beta 6$ in vitro and in pancreatic cancer mouse models. Pilot clinical studies demonstrated that this radiotracer is safe and can specifically detect pancreatic cancer and monitoring therapy. Larger-scale clinical trials of $^{68}$Ga-cycratide and comparisons to $^{18}$F-FDG are warranted for further characterization of the utility of $^{68}$Ga-cycratide for detection and/or treatment monitoring in patients with pancreatic cancer.

ACKNOWLEDGMENTS

This work was supported by National Key R&D Program of China (2018YFE0205300 and 2018YFC1313300), National Natural Science Foundation of China (81671747, 81873907 and 81920108020), Beijing Nova Program Interdisciplinary Cooperation Project (Z181100006218136), Beijing Natural Science Foundation (L172007 and JQ19026), and the Clinical Medicine Plus X-Young Scholars Project of Peking University (PKU2019LCXQ023).

Conflict of Interest: No potential conflicts of interest relevant to this article exist.
KEY POINTS

QUESTION: Can the integrin αvβ6-targeting cyclic peptide radiotracer $^{68}$Ga-cycratide be used for PET imaging of pancreatic cancer in the clinic?

PERTINENT FINDINGS: $^{68}$Ga-cycratide can be rapidly prepared with high radiochemical purity and has favorable *in vivo* pharmacokinetics. It is safe with a favorable dosimetry profile. PET imaging using $^{68}$Ga-cycratide can detect integrin αvβ6-positive lesions in patients with pancreatic cancer.

IMPLICATIONS FOR PATIENT CARE: $^{68}$Ga-cycratide is clinically translatable for PET imaging of pancreatic cancer and may also be extended to enable treatment response monitoring and prognosis staging.
REFERENCES


19. Zhu X, Li J, Hong Y, et al. 99mTe-labeled cystine knot peptide targeting integrin...


29. Liu H, Gao L, Yu X, et al. Small-animal SPECT/CT imaging of cancer xenografts and


Fig. 1. (A) Chemical structure of DOTA-cycratide. (B) Inhibition of $^{64}$Cu-cycratide binding to integrin $\alpha v \beta 6$ on BxPC-3 cells by cycratide, DOTA-cycratide, and linear-pep. Data are shown as mean ± SD, n = 4. (C) Binding of $^{68}$Ga-cycratide to BxPC-3 with or without the blocking of cold cycratide or linear-pep. %AD/10$^6$ cells = percentage of total added dose per million cells. Data are shown as mean ± SD, n = 4. **, $P < 0.01$. (D) Metabolic stability of $^{68}$Ga-cycratide in the blood and urine of BALB/c mice (data are representative of three independent experiments).
Fig. 2. (A) Small-animal PET images obtained at 0.5, 1, and 2 h after injection of $^{68}$Ga-cycratide or $^{68}$Ga-linear-pep in BxPC-3 tumor-bearing mice without or with blocking doses of cold cycratide or linear-pep, respectively. Tumors are indicated by red arrows. (B) Quantification of the tumor uptake and tumor-to-muscle ratio of $^{68}$Ga-cycratide and $^{68}$Ga-linear-pep shown in panels A. Data are shown as mean ± SD, n = 4. (C) Biodistribution of $^{68}$Ga-cycratide or $^{68}$Ga-DOTA-linear-pep in BxPC-3 tumor-bearing mice without or with a blocking dose of cold cycratide. Data are shown as mean ± SD, n = 4. *, $P < 0.05$; **, $P < 0.01$. 
Fig. 3. (A) PET imaging of the orthotopic pancreatic cancer lesions in nude mice at 0.5 h after injection of $^{68}$Ga-cycratide without or with a blocking dose of cold cycratide. Tumors are indicated by white arrows. (B) Hematoxylin and eosin (H&E) staining of tumor tissues harvested from the orthotopic tumor model. (C) Left, immunofluorescence staining of integrin αvβ6 from tumor tissues harvested from the orthotopic tumor model. Right, negative control with secondary antibody only.
**Fig. 4.** Multiple-time-point whole-body maximum-intensity-projection PET images of a male healthy volunteer after injection of $^{68}$Ga-cycratide.
Fig. 5. (A) PET/CT images of a female patient with suspected pancreatic cancer obtained at 1 h after intravenous administration of $^{68}$Ga-cycratide or $^{18}$F-FDG. The tumors are indicated by arrows. (B) Immunohistochemical (IHC) staining for integrin $\alpha_\nu\beta_6$ in the tumor sample from the same patient as in panels A. (C) Contrast-enhanced CT (CECT) image and PET/CT images of a male patient with pancreatic cancer 7 months after surgery at 1 h after administration of $^{68}$Ga-cycratide or $^{18}$F-FDG, as well as a CECT image of the same patient 3 months later (10 months after surgery). Occupancy lesions in CT are indicated by arrows.
Supplemental Materials for

Clinical Translation of a 68Ga-labeled Integrin αvβ6-targeting Cyclic Radiotracer for PET Imaging of Pancreatic Cancer

Xun Feng1, Yanpu Wang1, Dehua Lu1, Xiaoxia Xu2, Xin Zhou2, Huiyuan Zhang2, Ting Zhang1, Hua Zhu2, Zhi Yang2, Fan Wang1, Nan Li2*, Zhaofei Liu1*

1 Medical Isotopes Research Center and Department of Radiation Medicine, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China
2 Key Laboratory of Carcinogenesis and Translational Research (Ministry of Education/Beijing), Department of Nuclear Medicine, Peking University Cancer Hospital & Institute, Beijing 100142, China

For correspondence contact: Zhaofei Liu, Medical Isotopes Research Center, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China. E-mail: liuzf@bjmu.edu.cn; or Nan Li, Department of Nuclear Medicine, Peking University Cancer Hospital & Institute, Beijing 100142, China. Email: rainbow6283@sina.com
SUPPLEMENTAL MATERIALS AND METHODS

Synthesis of DOTA-conjugated Peptides

Five $\mu\text{mol}$ cycratide or linear-pep were mixed with 15 $\mu\text{mol}$ DOTA-NHS-ester in 300 $\mu\text{L}$ $N,N$-dimethylformamide. $N,N$-Diisopropylethyamine was then added to the mixture so as to adjust the pH to 8.0–9.0. After stirring at room temperature for 6 h, the DOTA-conjugated peptides were isolated by high-performance liquid chromatography (HPLC). DOTA-cycratide was obtained in 60% yield, and its purity was determined by HPLC to be >95%. Matrix-assisted laser desorption/ionization time-of-light analysis revealed an m/z of 1241.8 for $[\text{MH}]^+$ ($C_{53}H_{92}N_{16}O_{18}$; calculated molecular weight 1240.68). DOTA-linear-pep was obtained in 50% yield, the purity of which was determined by HPLC to be >95%. Matrix-assisted laser desorption/ionization time-of-light analysis revealed an m/z of 1361.58 for $[\text{MH}]^+$ ($C_{56}H_{100}N_{18}O_{19}S$; calculated molecular weight 1360.71).

$^{68}$Ga Radiolabeling

Ten nmol of DOTA-cycratide or DOTA-linear-pep were dissolved in 300 $\mu\text{L}$ NaOAc buffer, and then reacted with 370–555 MBq $^{68}$GaCl$_3$ for 10 min at 99°C. The reaction mixtures were then purified with Sep-Pak C18 cartridges (Waters, Milford, MA) and passed through 0.22-µm Millipore filters into sterile multidose vials for further use. The radiochemical purity of the final products, $^{68}$Ga-cycratide and $^{68}$Ga-linear-pep, was determined by analytical HPLC.

Cell Binding Studies

In vitro integrin $\alpha\beta$6-binding affinities of cycratide, DOTA-cycratide, and linear-pep
were determined on BxPC-3 cells by cell binding assay, as described previously (1). Because of the short half-life of $^{68}$Ga, we used $^{64}$Cu-labeled DOTA-cycratide ($^{64}$Cu-cycratide) as an alternative radioligand for $^{68}$Ga-cycratide in the in vitro cell binding assay. $^{64}$Cu-cycratide was prepared using a previously reported method (2). The best-fit 50% inhibitory concentration (IC$_{50}$) values were calculated by fitting the data with nonlinear regression using Prism software (version 6.0; GraphPad Software, San Diego, CA).

The binding specificity of $^{68}$Ga-cycratide to integrin $\alpha$$\beta$6-positive BxPC-3 cells was determined as described previously (1). Briefly, $^{68}$Ga-cycratide (74 kBq) was added to cells cultured in 12-well plates with or without excess (5 nmol) of cold cycratide and linear-pep peptides. After incubation at 4°C for 2 h, cells were washed and collected. Cell-associated radioactivity was measured using a $\gamma$ counter (Packard, Meriden, CT). The result was expressed as the percentage of the total added dose (%AD) per million cells.

**Animal Models**

All animal experiments were performed in accordance with the Guidelines of the Peking University Animal Care and Use Committee. Female BALB/c nude mice (5 weeks of age) were obtained from the Department of Laboratory Animal Science of Peking University (Beijing, China). For the subcutaneous tumor model, $1 \times 10^7$ BxPC-3 cells were inoculated subcutaneously into the right flank of BALB/c nude mice. Tumor growth was measured with a caliper, and tumor volume was calculated using the formula: volume = (length $\times$ width$^2$) / 2. For the orthotopic tumor model, BALB/c nude mice were anesthetized, and small incisions were made in the abdomen of each mouse to expose the spleen. Next, $1 \times 10^7$ BxPC-3 cells in 50 $\mu$L of phosphate-buffered saline (PBS) mixed with Matrigel (BD
Biosciences, San Jose, CA) at a 1:1 ratio were injected into the pancreas, after which the incision was closed using sutures.

**Small-animal PET Imaging**

Each subcutaneous BxPC-3 tumor-bearing nude mouse was injected with 5.5 MBq (~0.19 μg) of $^{68}$Ga-cycratide or $^{68}$Ga-linear-pep via the tail vein (n = 4 mice per group). At 0.5, 1, and 2 h postinjection, 10-min static PET scanning was performed using a small-animal PET/CT scanner (Mediso, Budapest, Hungary). For the blocking study, BxPC-3 tumor-bearing nude mice were co-injected with 50 mg/kg mouse body weight of excess cycratide and 5.5 MBq $^{68}$Ga-cycratide, or 50 mg/kg mouse body weight of linear-pep and 5.5 MBq $^{68}$Ga-linear-pep. Ten-minute static PET scans were then acquired at 0.5 h postinjection (n = 4 per group). PET images were analyzed and the region of interest-derived percent injected dose per gram of tissue (%ID/g) values were calculated as described previously (3).

**Biodistribution Studies of $^{68}$Ga-cycratide and $^{68}$Ga-linear-pep**

Female BALB/c nude mice bearing subcutaneous BxPC-3 tumors were injected with 3.7 MBq $^{68}$Ga-cycratide or $^{68}$Ga-linear-pep to evaluate the distribution of the radiotracer in the main organs (n = 4 per group). Mice were euthanized at 0.5, 1, and 2 h postinjection, and blood, tumor, main organs, and tissues were harvested, weighed, and measured using a γ counter. For the blocking experiment, four tumor-bearing nude mice were administered 3.7 MBq $^{68}$Ga-cycratide along with an excess dose of cold cycratide (50 mg/kg). At 0.5 h postinjection, all four mice were euthanized to determine organ biodistribution as described
**In Vivo Metabolic Stability of $^{68}\text{Ga}$ -cycratide and $^{68}\text{Ga}$-linear-pep**

Female BALB/c normal mice ($n = 3$ per group) were injected with 37 MBq of $^{68}\text{Ga}$ -cycratide or $^{68}\text{Ga}$-linear-pep via the tail vein. At 0.5 h postinjection, mouse serum and urine samples were collected. After centrifugation, the supernatant was diluted with an aqueous solution of 50% acetonitrile, filtered through a 0.22-$\mu$m Millipore filter, and analyzed by radio-HPLC.

**PET/CT Data Analysis and Dosimetry Calculation in Healthy Volunteers**

A Siemens workstation (MultiModality Workplace) was used for data processing, and the PET images were reconstructed using ordered-subsets expectation maximization. Regions of interest of each normal organ were drawn manually by two experienced nuclear medicine physicians using 3-D ellipsoid isocontours on each image with the correction of the corresponding CT images. The radioactivity concentrations (Bq/mL) of each organ and the single-voxel standardized uptake value (SUV) in the volumes of interest were obtained through the Siemens workstation software.

The dosimetry calculation was performed using the OLINDA/EXM 2.0 software (version 2.0; Hermes Medical Solutions AB) as previously described (4). Briefly, the decay-uncorrected time–activity curve was generated according to the radioactivity concentrations (Bq/mL) of each organ. The time integrated activity coefficient of each organ was determined by fitting the data using a bi-phase exponential model. The void time of the bladder was set as 60 min, and the absorbed doses were calculated by entering the
time-integrated activity coefficient for all source organs into the software using the standard adult male (73.7 kg of body weight) and female (56.9 kg of body weight) phantoms.

**Immunofluorescence Staining and Immunohistochemistry**

The expression of integrin $\alpha \beta 6$ in tumor tissue was analyzed by immunofluorescence staining. Briefly, frozen tumor sections were fixed with ice-cold acetone for 10 minutes and then blocked with 1% bovine serum albumin (in PBS) for 1 h. Then, tissues were incubated with mouse anti-human integrin $\alpha \beta 6$ antibody (clone E7P6; Chemicon/Millipore, Billerica, MA) for 1 h at room temperature and then visualized with fluorescent dye-conjugated secondary antibodies using a Leica TCS-NT confocal microscope.

For immunohistochemistry, paraffin-embedded tumor tissues from patients were deparaffinized. After abolishing endogenous peroxidase activity using 0.3% hydrogen peroxide and performing antigen retrieval by microwave, tumor tissues were incubated with rabbit anti-human integrin $\alpha \beta 6$ antibody (bs-5791r; Bioss, Beijing, China) overnight at 4°C. Afterwards, tumor sections were incubated with a horseradish peroxidase-conjugated secondary antibody for 2 h, and then was visualized by incubation with the diaminobenzidine substrate.

**Preparation of $^{18}$F-labeled Cycratide**

For $^{18}$F radiolabeling, cycratide was first conjugated with NOTA-NHS ester (CheMatech, Dijon, France) using a previously described method (5). Briefly, 2 $\mu$mol of
cycratide was mixed with 6 μmol of NOTA-NHS in 0.1 N NaHCO$_3$ solution (pH 9.0). After stirring at room temperature for 4 h, the NOTA-conjugated cycratide (NOTA-cycratide) was purified by semipreparative HPLC and the product was confirmed by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry: $m/z$ 1,140.31 for [MH]$^+$ (C$_{49}$H$_{85}$N$_{15}$O$_{16}$, calculated molecular weight 1,139.63 Da).

NOTA-cycratide was then labeled with $^{18}$F via NOTA-Al$^{18}$F chelation using a previous described method with minor modifications (6). Briefly, No-carrier-added $^{18}$F$^-$ (0.74–1.48 GBq) was mixed with 24 nmol AlCl$_3$ in 100 μL sodium acetate buffer (0.1 M, pH 4.0) for 5 min at room temperature. Subsequently, 40 nmol NOTA-cycratide was added and the mixture was heated at 110°C for 15 min. After purification with Sep-Pak C18 cartridges (Waters, Milford, MA), the product was passed through 0.22-μm Millipore filters into sterile vials for further use. The radiochemical purity of $^{18}$F-cycratide was determined by analytical HPLC.

**Cell Uptake of $^{68}$Ga-cycratide and $^{18}$F-cycratide**

The integrin αvβ6-positive BxPC-3 cells were seeded into 12-well plates and incubated overnight at 37°C to allow adherence. After a brief wash with PBS, cells were incubated with $^{68}$Ga-cycratide or $^{18}$F-cycratide (37 kBq per well) at 37°C for 10, 20, 30, 60, 120, and 240 min. After washing six times with chilled PBS, cells were collected and cell-associated radioactivity was measured using a γ counter (Packard, Meriden, CT). The cell uptake was expressed as the percent added dose (% AD) after decay correction. Experiments were performed 3 times with four parallel samples.
Comparison of $^{18}$F-cycratide with $^{68}$Ga-cycratide for Small-animal PET Imaging

For direct comparison of the PET imaging of $^{18}$F-cycratide with $^{68}$Ga-cycratide, each BxPC-3 tumor-bearing nude mouse ($n = 4$) was injected with 5.55 MBq $^{18}$F-cycratide via the tail vein. At 0.5, 1, and 2 h postinjection, 10-min static PET scanning was performed using a small-animal PET/CT scanner (Siemens Medical Solutions). On the second day, the same mice were injected with 5.55 MBq $^{68}$Ga-cycratide via the tail vein, and 10-min static PET imaging was performed at 0.5, 1, and 2 h postinjection using the same protocol.

Biodistribution of $^{18}$F-cycratide

Female BALB/c nude mice bearing subcutaneous BxPC-3 tumors were injected with 1.85 MBq $^{18}$F-cycratide to evaluate the distribution of the radiotracer in the main organs ($n = 4$ per group). Mice were euthanized at 0.5, 1, and 2 h postinjection, and blood, tumor, and main organs/tissues were harvested, weighed, and measured using a $\gamma$ counter.

$^{18}$F-FDG and $^{68}$Ga-cycratide PET Imaging in a Dual Tumor and Inflammation Mouse Model

For the dual BxPC-3 and inflammation mouse model, 100 $\mu$L of turpentine was injected in the left thigh muscle of the BxPC-3 tumor-bearing nude mice at 24–48 h before the PET imaging experiments. Mice ($n = 3$) were injected with 3.7 MBq $^{18}$F-FDG via the tail vein, and 10-min static PET scans were acquired at 1 h postinjection. One day later, the same mice were injected with 5.55 MBq $^{68}$Ga-cycratide, and then PET imaging was performed at 0.5 h postinjection using a small-animal PET/CT scanner (Siemens Medical Solutions).
SUPPLEMENTAL REFERENCES


**Supplemental Table 1.** Biodistribution of $^{68}$Ga-cycratide in healthy volunteers (SUV, $n = 5$)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Heart</th>
<th>Liver</th>
<th>Spleen</th>
<th>Lung</th>
<th>Kidney</th>
<th>Intestine</th>
<th>Bone marrow</th>
<th>Brain</th>
<th>Muscle</th>
<th>Pancreas</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5.05 ± 1.06</td>
<td>2.52 ± 0.70</td>
<td>3.34 ± 0.94</td>
<td>1.24 ± 0.53</td>
<td>13.94 ± 4.39</td>
<td>0.91 ± 0.28</td>
<td>1.36 ± 0.43</td>
<td>0.49 ± 0.13</td>
<td>0.84 ± 0.07</td>
<td>3.18 ± 0.96</td>
</tr>
<tr>
<td>15</td>
<td>2.93 ± 0.69</td>
<td>1.58 ± 0.43</td>
<td>1.99 ± 0.52</td>
<td>0.80 ± 0.39</td>
<td>8.72 ± 3.03</td>
<td>0.72 ± 0.17</td>
<td>0.81 ± 0.22</td>
<td>0.14 ± 0.04</td>
<td>0.66 ± 0.08</td>
<td>1.99 ± 0.66</td>
</tr>
<tr>
<td>25</td>
<td>2.21 ± 0.44</td>
<td>1.22 ± 0.30</td>
<td>1.49 ± 0.33</td>
<td>0.58 ± 0.21</td>
<td>6.19 ± 1.59</td>
<td>0.60 ± 0.22</td>
<td>0.64 ± 0.24</td>
<td>0.09 ± 0.04</td>
<td>0.57 ± 0.12</td>
<td>1.41 ± 0.55</td>
</tr>
<tr>
<td>35</td>
<td>1.84 ± 0.32</td>
<td>1.01 ± 0.27</td>
<td>1.26 ± 0.25</td>
<td>0.52 ± 0.24</td>
<td>5.57 ± 1.69</td>
<td>0.55 ± 0.20</td>
<td>0.57 ± 0.20</td>
<td>0.06 ± 0.03</td>
<td>0.49 ± 0.09</td>
<td>1.15 ± 0.31</td>
</tr>
<tr>
<td>45</td>
<td>1.55 ± 0.22</td>
<td>0.86 ± 0.17</td>
<td>1.00 ± 0.14</td>
<td>0.41 ± 0.18</td>
<td>4.55 ± 1.44</td>
<td>0.44 ± 0.30</td>
<td>0.56 ± 0.26</td>
<td>0.06 ± 0.03</td>
<td>0.45 ± 0.14</td>
<td>1.06 ± 0.33</td>
</tr>
<tr>
<td>60</td>
<td>1.35 ± 0.19</td>
<td>0.74 ± 0.14</td>
<td>0.95 ± 0.16</td>
<td>0.37 ± 0.15</td>
<td>4.11 ± 1.20</td>
<td>0.44 ± 0.20</td>
<td>0.55 ± 0.18</td>
<td>0.05 ± 0.03</td>
<td>0.37 ± 0.08</td>
<td>0.82 ± 0.26</td>
</tr>
<tr>
<td>120</td>
<td>0.71 ± 0.05</td>
<td>0.40 ± 0.08</td>
<td>0.55 ± 0.15</td>
<td>0.18 ± 0.03</td>
<td>2.49 ± 1.45</td>
<td>0.18 ± 0.14</td>
<td>0.33 ± 0.13</td>
<td>0.02 ± 0.01</td>
<td>0.18 ± 0.03</td>
<td>0.41 ± 0.14</td>
</tr>
</tbody>
</table>
**Supplemental Table 2.** Radiation-absorbed dose estimates of $^{68}$Ga-cycratide in healthy volunteers (mGy/MBq; n = 5, [2 women, 3 men])

<table>
<thead>
<tr>
<th>Organ</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenals</td>
<td>1.57E-02</td>
<td>2.66E-03</td>
</tr>
<tr>
<td>Brain</td>
<td>2.70E-03</td>
<td>1.49E-03</td>
</tr>
<tr>
<td>Esophagus</td>
<td>8.80E-03</td>
<td>3.38E-03</td>
</tr>
<tr>
<td>Eyes</td>
<td>5.78E-03</td>
<td>4.58E-03</td>
</tr>
<tr>
<td>Gallbladder wall</td>
<td>1.10E-02</td>
<td>2.01E-03</td>
</tr>
<tr>
<td>Left colon</td>
<td>1.30E-02</td>
<td>1.73E-03</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1.75E-02</td>
<td>7.39E-03</td>
</tr>
<tr>
<td>Stomach wall</td>
<td>2.24E-02</td>
<td>1.87E-02</td>
</tr>
<tr>
<td>Right colon</td>
<td>1.02E-02</td>
<td>2.40E-03</td>
</tr>
<tr>
<td>Rectum</td>
<td>2.62E-02</td>
<td>1.87E-02</td>
</tr>
<tr>
<td>Heart wall</td>
<td>2.83E-02</td>
<td>7.99E-03</td>
</tr>
<tr>
<td>Kidneys</td>
<td>7.87E-02</td>
<td>4.98E-02</td>
</tr>
<tr>
<td>Liver</td>
<td>2.14E-02</td>
<td>1.27E-02</td>
</tr>
<tr>
<td>Lungs</td>
<td>2.61E-02</td>
<td>1.41E-02</td>
</tr>
<tr>
<td>Ovaries*</td>
<td>2.25E-01</td>
<td>7.78E-03</td>
</tr>
<tr>
<td>Pancreas</td>
<td>6.93E-03</td>
<td>1.25E-03</td>
</tr>
<tr>
<td>Prostate**</td>
<td>2.32E-02</td>
<td>1.97E-02</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>1.03E-02</td>
<td>2.37E-03</td>
</tr>
<tr>
<td>Red marrow</td>
<td>1.06E-02</td>
<td>4.37E-03</td>
</tr>
<tr>
<td>Osteogenic cells</td>
<td>1.52E-02</td>
<td>6.79E-03</td>
</tr>
<tr>
<td>Spleen</td>
<td>3.49E-02</td>
<td>2.82E-02</td>
</tr>
<tr>
<td>Testes**</td>
<td>1.34E-02</td>
<td>4.60E-03</td>
</tr>
<tr>
<td>Thymus</td>
<td>8.88E-03</td>
<td>3.16E-03</td>
</tr>
<tr>
<td>Thyroid</td>
<td>2.86E-02</td>
<td>2.25E-02</td>
</tr>
<tr>
<td>Urinary bladder wall</td>
<td>5.59E-01</td>
<td>6.40E-01</td>
</tr>
<tr>
<td>Uterus*</td>
<td>1.42E-01</td>
<td>4.95E-03</td>
</tr>
<tr>
<td>Total body</td>
<td>1.30E-02</td>
<td>1.98E-03</td>
</tr>
<tr>
<td>Effective dose (mSv/MBq)</td>
<td>5.49E-02</td>
<td>4.69E-02</td>
</tr>
</tbody>
</table>

**Note:** * n = 2; ** n = 3.
**Supplemental Table 3.** Characterizations of radiotracers reported in this study.

<table>
<thead>
<tr>
<th>Radiotracer</th>
<th>Labeling yield</th>
<th>Synthesis time</th>
<th>RCP</th>
<th>Metabolic stability*</th>
<th>Tumor uptake (0.5 h p.i.)</th>
<th>T/M ratio (0.5 h p.i.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{68}$Ga-linear-pep</td>
<td>&gt; 95%</td>
<td>~20 min</td>
<td>&gt; 99%</td>
<td>0%</td>
<td>0.94 ± 0.58 %ID/g</td>
<td>2.27 ± 0.71</td>
</tr>
<tr>
<td>$^{68}$Ga-cycratide</td>
<td>&gt; 95%</td>
<td>~20 min</td>
<td>&gt; 99%</td>
<td>&gt; 95%</td>
<td>2.15 ± 0.46 %ID/g</td>
<td>3.06 ± 0.28</td>
</tr>
<tr>
<td>$^{18}$F-cycratide</td>
<td>10~20%</td>
<td>~40 min</td>
<td>&gt; 99%</td>
<td>&gt; 95%</td>
<td>1.64 ± 0.41 %ID/g</td>
<td>1.98 ± 0.82</td>
</tr>
</tbody>
</table>

**Note:** RCP, radiochemical purity; p.i., postinjection; T/M ratio, tumor-to-muscle ratio; *, Metabolic stability in the blood at 0.5 h postinjection.
Supplemental Fig. 1. (A) Chemical structure of DOTA-linear-pep. (B) In vitro stability of $^{68}$Ga-cycratide and $^{68}$Ga-linear-pep in the fetal bovine serum (FBS) and PBS ($n = 3$, mean ± SD). RCP, radiochemical purity. (C) Metabolic stability of $^{68}$Ga-linear-pep in the blood and urine of BALB/c mice (data are representative of three independent experiments).
Supplemental Fig. 2. Chemical structure (A) and HPLC radiochromatogram (B) of synthesized $^{18}$F-cycratide.
Supplemental Fig. 3. Cell uptake assay of $^{68}$Ga-cycratide and $^{18}$F-cycratide in BxPC-3 tumor cells (n = 4, mean ± SD).
Supplemental Fig. 4. (A) Small-animal PET images obtained at 0.5, 1, and 2 h after injection of $^{18}$F-cycratide in the BxPC-3 tumor-bearing BALB/c nude mice (n = 4). (B) Small-animal PET images obtained at 0.5, 1, and 2 h after injection of $^{68}$Ga-cycratide in the same mice on the second day after $^{18}$F-cycratide PET imaging. Tumors are indicated by arrows.
Supplemental Fig. 5. Biodistribution of $^{18}$F-cycratide in BxPC-3 subcutaneous tumor-bearing BALB/c nude mice. Data are shown as mean ± SD, n = 4.
Supplemental Fig. 6. Comparison of the biodistribution of $^{18}$F-cycratide and $^{68}$Ga-cycratide in BxPC-3 subcutaneous tumor-bearing BALB/c nude mice. Data are shown as mean ± SD, n = 4. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$. 
Supplemental Figure 7. PET imaging of $^{18}$F-FDG and $^{68}$Ga-cycratide in the dual inflammation and BxPC-3 tumor-bearing BALB/c nude mouse model ($n = 3$). (A) A representative of photograph of the dual inflammation and BxPC-3 tumor-bearing BALB/c nude mice. (B) Representative small-animal PET images obtained at 1 h after injection of $^{18}$F-FDG and one day later at 0.5 h after injection of $^{68}$Ga-cycratide in the same mouse. Tumors and inflammations are indicated by red and white dashed-line circles, respectively.