

Original Article

Radiolabeling and evaluation of ^{64}Cu -DOTA-F56 peptide targeting vascular endothelial growth factor receptor 1 in the molecular imaging of gastric cancer

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Received September 27, 2015; Accepted October 6, 2015; Epub October 15, 2015; Published November 1, 2015

Abstract: Noninvasive imaging of vascular endothelial growth factor receptor 1 (VEGFR1) remains a great challenge in early diagnosis of gastric cancer. Here we reported the synthesis, radiolabeling, and evaluation of a novel ^{64}Cu -radiolabeled peptide for noninvasive positron emission tomography (PET) imaging of VEGFR1 positive gastric cancer. The binding of modified peptide WHSDMEWWYLLG (termed as F56) to VEGFR-1 expressed in gastric cancer cell BCG823 has been confirmed by immune-fluorescence overlap. DOTA-F56 was designed and prepared by solid-phase synthesis and folded *in vitro*. ^{64}Cu -DOTA-F56 was synthesized in high radiochemical yield and high specific activity (S.A. up to 255.6 GBq/mmol). It has excellent *in vitro* stability. Micro-PET imaging of ^{64}Cu -DOTA-F56 identifies tumor in BCG823 tumor-bearing mice, while that of ^{18}F -FDG does not. Immunohistochemical analysis of excised BCG823 xenograft showed colocalization between the PET images and the staining of VEGFR1. These results demonstrated that ^{64}Cu -DOTA-F56 peptide has potential as a noninvasive imaging agent in VEGFR1 positive tumors.

Keywords: Vascular endothelial growth factor receptor 1, copper-64, gastric cancer, molecular imaging, F56 peptide

Introduction

Cancer is still one of the most threaten to human health. It was estimated that there are 14.1 million new cancer cases and 8.2 million cancer deaths occurred in 2012 worldwide [1]. Among all cancer types, 951,600 new gastric cancer cases and 723,100 deaths are estimated to have occurred and with highest incidence rates in Eastern Asia, Central and Eastern Europe, and South America. Due to the limited clinical symptoms, early detection of gastric cancer relies on opportunistic screening [2]. About 90% of gastric cancer patients were initially diagnosed in advanced stage, with 5-year survival rate less than 20%, even in developed Asian countries [2, 3].

Early detection and treatment are critical to reduce mortality from gastric cancer. Although researches on biotherapy, immunotherapy and

gene therapy provide multi-dimensional treatments [4-6], the clinical results remain limited. In comparison, gastric cancer in early stage has excellent outcome from therapy, with five-year survival rate higher than 90%.

Noninvasive molecular imaging, especially PET, is becoming an effective method for diagnosis and staging of cancer, and monitoring of the efficiency of individual therapy [7]. [^{18}F] Fluorodeoxyglucose (^{18}F -FDG) PET has been gradually accepted by clinicians, especially for recurrent gastric cancer after surgical resection [8, 9]. However, ^{18}F -FDG has limited use due to its low sensitivity, with only about 50% detection rate in early gastric cancer [10].

There is still an urgent need of developing more sensitive and specific PET tracers for early detection of gastric cancer. Studies had revealed that elevated levels of VEGF-A, and its

receptor, VEGFR1, were found in gastric cancer patients [11]. In addition, VEGFR1 overexpression in peripheral blood was associated with advanced clinical stage of the cancer [12]. VEGF and VEGFR1 provide legible targets for molecular imaging [13-15]. Up to now, several PET tracers targeting VEGFRs have been designed, and most of them mimic the natural/mutated VEGF isoforms [16-18], and others are antibodies with high renal uptake and high normal organs background [19-21]. However, these bulky antibodies are generally difficult and costly to prepare and may induce immune reaction *in vivo*. A dimeric ^{64}Cu -DOTA-GU40C4 peptoid was used to perform noninvasive PET detection of VEGFR2 [22]. In addition, the clinical imaging using ^{123}I -VEGF165 in patients with gastrointestinal tumors and obtained 58% overall scan sensitivity [23, 24]. Due to limited supply of ^{123}I nuclide and high background in imaging, no further study was reported.

We previously discovered a novel VEGFR1-specific peptide, named as F56, through phage display screening with extracellular Ig-like domain I-IV of VEGFR1 fused with glutathione-S-transferase (GST) as target [25]. *In vitro* and *in vivo* studies show that F56 displaced VEGF binding to its receptor VEGFR1 and significantly inhibited tumor growth. The conjugates between F56 and multifunctional nanoparticles were developed to specifically target VEGFR1 in the molecular imaging of VEGFR1-expressing tumor xenograft in mice [26].

In this study, we aim to develop a novel F56-based PET tracer for the imaging of VEGFR-1 positive tumor. ^{64}Cu ($T_{1/2} = 12.7$ h, $\beta^+ = 0.653$ MeV; $\beta^- = 0.578$ MeV) is an excellent nuclide which has characteristics for PET imaging and targeted radiotherapy of cancer. It can also be produced in large quantities and with high specific activity [27, 28]. Here, we report the results of identifying ^{64}Cu -DOTA-F56 peptide as a PET probe, including a variety of chemical and biological analysis, animal Micro-PET imaging, and immunohistochemistry studies.

Materials and methods

Materials

Phosphate-buffered saline (PBS), sodium tartrate (NaAc), *N,N*-dimethylformamide (DMF), hydroxybenzotriazole (HOBt) and 0.5 M diiso-

propylcarbodiimide (DIC), trifluoro-acetic acid (TFA), ethylenediaminetetraacetic acid (EDTA), and human serum albumin (HSA), were purchased from Sigma-Aldrich (St. Louis, MO, USA). ^{64}Cu (7.4 MBq/ μL) was provided by China Isotope and Radiation Company (Beijing, China). FITC-F56 peptide (sequence: WHSDM-EWWYLLG) was provided by China Peptides Co., Ltd (Shanghai, China).

Radiochemical purity was examined with radioactive thin-layer chromatography scanner (Bioscan, IAR-2000, Washington DC, USA), and HPLC (Agilent, USA), using YMC-Pack ODS-A C 18 column (4.6 mm $\times$ 250 mm, 5 μm) and the following gradient program: 0-10 min solvent B from 20% to 65%. The flow rate was 1.0 ml/min. Solvent A was 0.01% TFA water and solvent B was 0.01% TFA CH_3CN . The radioactivity was measured using an automated gamma scintillation counter (Perkin Elmer, 1470-002, PerkinElmer, Waltham, MA).

P53 mutant human gastric adenocarcinoma cell line BCG823 was provided by the Peking University Cancer Hospital (Beijing, China). This cell line was selected as a tumor model due to its adherent, poorly differentiated properties [29, 30]. Nude mice (nu/nu, female, 5-6 weeks old) were from Vital River (Beijing, China). Dulbecco's Modified Eagle's Medium (DMEM) was from Life Technologies (Grand Island, NY, USA). Fetal bovine serum was from Hyclone (Logan, UT, USA). Approximately 1×10^6 BCG823 cells suspended in 100 μl of PBS were implanted subcutaneously into the flanks of nude mice for Micro-PET imaging when tumors reached 0.1 cm^3 . The mice were kept under specific pathogen free conditions and were handled and maintained according to Institutional Animal Care and Use Committee guidelines.

To stain with F56 peptide, we generated a monoclonal antibody (manuscript in preparation) specific for a fragment of F56. An antibody (TA303515) against VEGFR1 was purchased from Origene Company (Rockville, MD, USA).

Chemistry and radiochemistry

Fluorescence visualization of N-terminal modified FITC-F56 peptide: BGC823 cells were incubated with FITC-conjugated F56 peptide (10 $\mu\text{g}/\text{ml}$) for 30 min. The cells were then fixed

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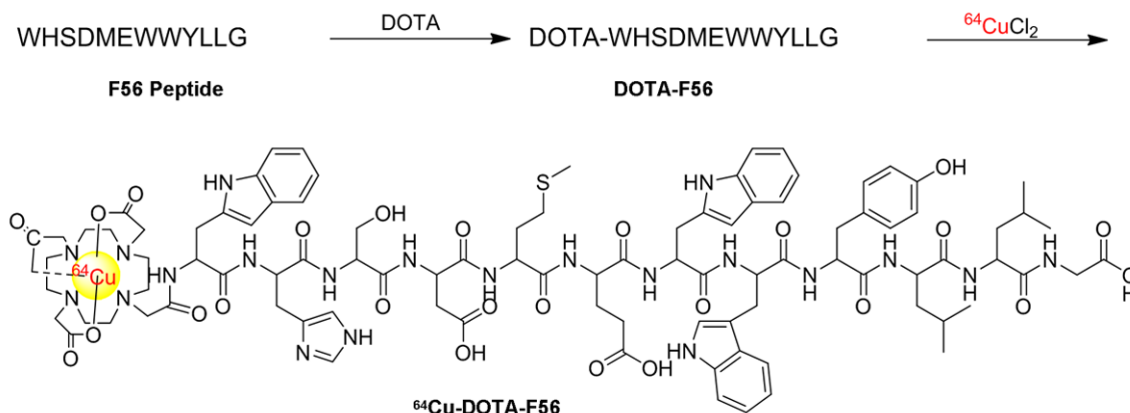


Figure 1. The Synthetic strategy and radiolabeling of ${}^{64}\text{Cu-DOTA-F56}$ peptide.

with 4% paraformaldehyde at room temperature for 30 min, and permeabilized with 0.1% Triton X-100 in PBS at room temperature for 10 min. The dye 4',6-diamidino-2-phenylindole (DAPI, 5 ng/ml) was added to stain nuclei for 10 min and washed three times with PBS. Samples were observed under a TCS-SP2 confocal microscopy (Leica Microsystems, Heidelberg, Germany).

Synthesis of DOTA-F56 peptide: The DOTA-F56 peptide (amino acid sequence shown in **Figure 1**) was synthesized on a CS Bio CS336 instrument (Menlo park, CA) by Fmoc-based solid-phase peptide method. Briefly, Rink amide resin was swollen in DMF for 30 min. Fmoc groups were removed with 20% piperidine in DMF. Aliquots of amino acids (1 mM) were activated in a solution containing 1 mM of HOBT and 0.5 M DIC in DMF. During the synthesis, side-chain deprotection and resin cleavage were achieved by addition of a mixture of TFA/trimethylsilane/ethanedithiol/water (v/v, 94:2.5:2.5:1) for 2 h at room temperature. The crude product was precipitated with cold anhydrous diethyl ether and purified by a preparative RP-HPLC using a Varian Prostar instrument and Vydac C18 columns. Peptide purity was analyzed by analytical-scale RP-HPLC using a Vydac C18 column. DOTA-F56 peptide was further characterized by MADL-TOF-MS.

${}^{64}\text{Cu}$ Radiolabeling of DOTA-F56: The radiolabeling of DOTA-F56 by ${}^{64}\text{Cu}$ was performed as follows: each amount of DOTA-F56 (1-20 μg) was incubated with 222 MBq ${}^{64}\text{CuCl}_2$ in 0.1 M sodium acetate buffer (pH 5.5) for 30 min at 60°C (**Figure 1**). The reaction was monitored by

Radio-HPLC and terminated with the addition of EDTA. ${}^{64}\text{Cu-DOTA-F56}$ was purified using a sep-pak C-18 column and eluted with 1.0 ml 80% ethanol. After the evaporation of ethanol by vacuum, the final product ${}^{64}\text{Cu-DOTA-F56}$ in water was passed through a 0.22 μm filter before further cell and animal experiments. The radiolabeling yield and radiochemical purity were determined by Radio-HPLC, > 99% respectively. The specific activity of ${}^{64}\text{Cu-DOTA-F56}$ was > 45 GBq/mmol (or 0.6 mCi/ μg).

In vitro evaluation of ${}^{64}\text{Cu-DOTA-F56}$: Partition coefficient of ${}^{64}\text{Cu-DOTA-F56}$: The partition coefficient of ${}^{64}\text{Cu-DOTA-F56}$ complex was determined by measuring the distribution of radioactivity in equal volume of 1-octanol and 0.01 M PBS. Briefly, a 10 μl aqueous solution of ${}^{64}\text{Cu-DOTA-F56}$ in PBS was added to a vial containing 1.00 ml of 1-octanol and 0.99 ml of PBS. After 8 min vortexes, the vial was centrifuged for 5 min to ensure complete separation of layers. Then, 10 μl of each layer was pipetted into separate test tubes, to calculate $\log P = \log (\text{counts in octanol}/\text{counts in water})$.

In vitro stability test: The stability of the ${}^{64}\text{Cu-DOTA-F56}$ peptide was examined by measuring its radiochemical purity by performing Radio-HPLC at different time intervals after the incubation. Specifically, ${}^{64}\text{Cu-DOTA-F56}$ (3.7 MBq) was added to a test tube containing 1.0 ml of 0.01 M PBS (pH 7.4), 0.1 M NaOAc (pH 5.5), or 5% HSA, respectively. The mixtures were incubated at 37°C with shaking in a thermo mixer. For PBS and NaOAc system, the radiochemical purity was measured directly at 30 min, 1 h, 3 h, and 18 h by Radio-HPLC. For

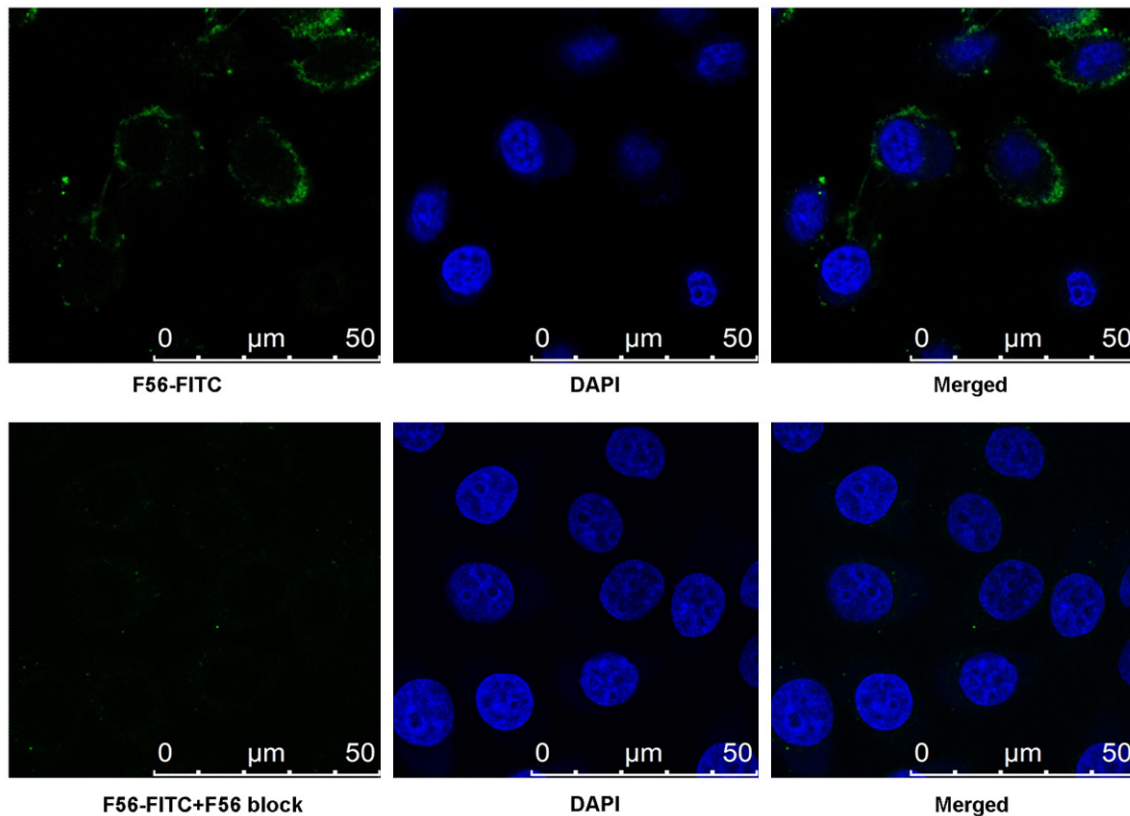


Figure 2. Fluorescence visualization of FITC-F56 binds to BGC823 cells. BGC823 cells were incubated with 10 μ g/ml FITC-F56 peptide for 30 min, fixed, stained with 5 ng/ml DAPI (blue), and observed under a confocal microscopy. Scale bar = 50 μ m.

5% HSA system, 200 μ l of solution was mixed with 200 μ l of acetonitrile to precipitate the protein, and the supernatant was analyzed by Radio-HPLC.

Cell uptake and micro-PET imaging studies

Briefly, BGC823 cells were seeded at 2.0×10^5 per well onto 12-well plates and allowed to attach overnight. Cells were washed twice with serum-free medium and incubated with radio-tracer (2 μ Ci per well, 74 kBq) in 400 μ l of serum-free medium at 37°C. After desired time points (10 min, 30 min, 60 min and 120 min), cells were washed three times with cold PBS and lysed in 200 μ l of 0.2 M NaOH. Radioactivity of the cells was counted using a PerkinElmer 1470 automatic γ -counter. Cell uptake data were expressed as the percentage of the applied radioactivity per total radioactive.

PET imaging on mice bearing BGC823 xenograft was performed on a micro-PET scanner (Siemens Medical Solutions USA, Inc.). The mice bearing BGC823 tumor were injected with

37 MBq of ^{64}Cu -DOTA-F56 (1.5 μ g), ^{18}F -FDG, or $^{64}\text{CuCl}_2$, respectively. At indicated time after injection, the mice were anesthetized with 1.5% isoflurane and placed in the prone position, near the center of the field of view of micro-PET. The 10-min static scans were obtained, and the images were reconstructed by a two-dimensional ordered subsets expectation maximum (OSEM) algorithm. No back-ground correction was performed.

Hematoxylin eosin staining (HE) and immunohistochemistry of BGC823 xenografts

For HE staining, the BGC823 xenografts were dissect from mice and air-dried at 40°C before freezing and storage. Then the frozen tissue slices were washed by PBS, and stained with hematoxylin and eosin to show different structures of the tissue.

For immunohistochemistry analysis, paraffin-embedded sections were dewaxed using standard histological procedures. In order to block internal peroxidase, the frozen tissue sections

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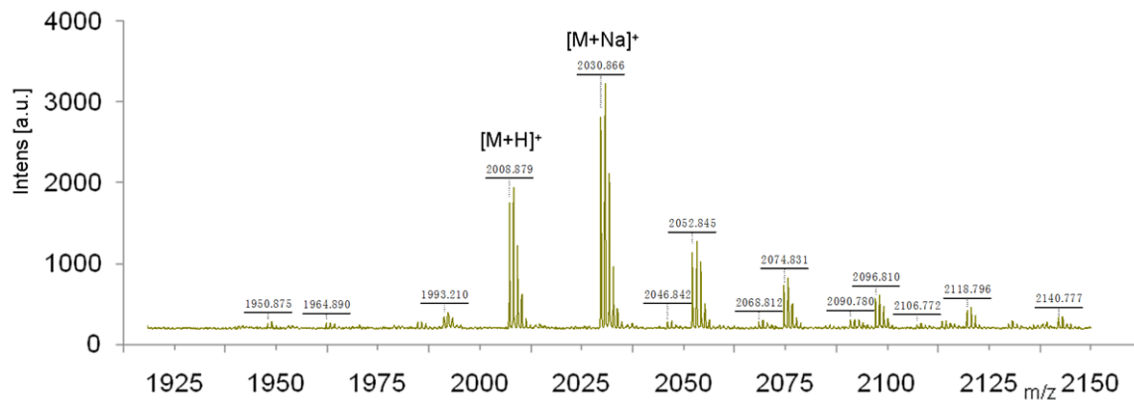


Figure 3. MALDI-TOF mass spectra of DOTA-F56 peptide. The purified DOTA-F56 was characterized by MALDI-TOF-MS and the measured molecular weight was consistent with the expected molecular weight.

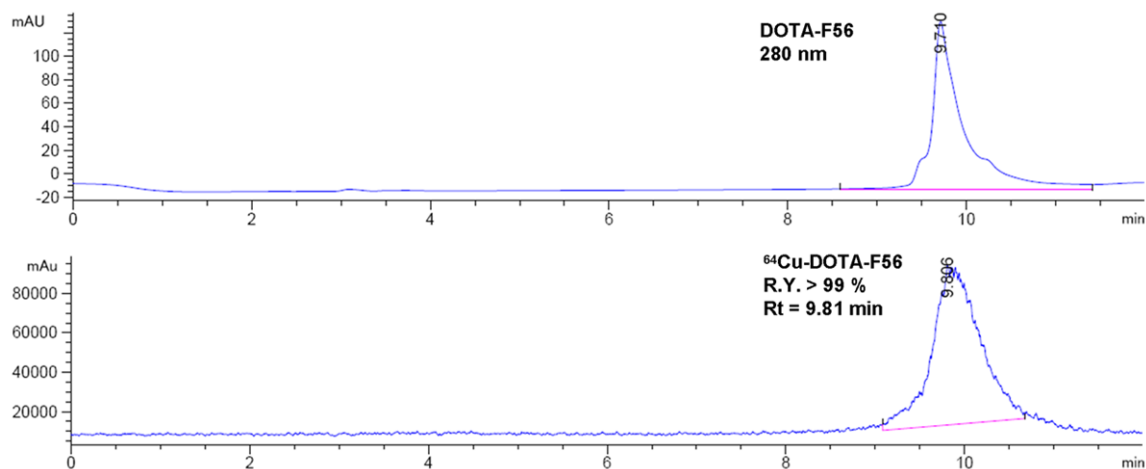


Figure 4. HPLC chromatographs of DOTA-F56 and ^{64}Cu -DOTA-F56.

were dipped in 3% H_2O_2 solution at room temperature for 5 min. Next, sections were placed into 0.01% pronase at 40°C for 40 min for antigen retrieval, and cooled to room temperature. After washing by PBS, sections were washed by the DAKO blocking solution to block non-specific binding for 5 min. The sections were incubated in primary antibodies overnight at 4°C in a humidified chamber and washed by PBS. Then they are detected using the EnVisionTM Kit (DAKO, Carpinteria, CA). Yellow or brown granules in cytoplasm or karyon were considered as positive staining.

Statistical analysis

The statistical computations were performed using the Prism software program. In all experiments, P -value < 0.05 was identified as statistically significant by a student's t -test.

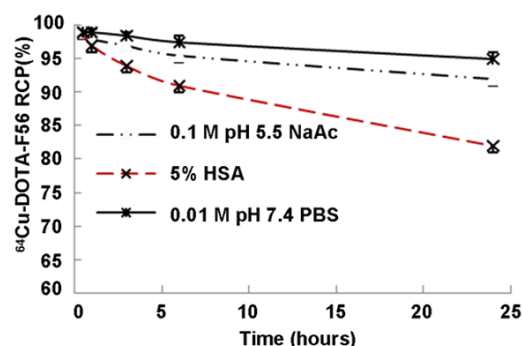
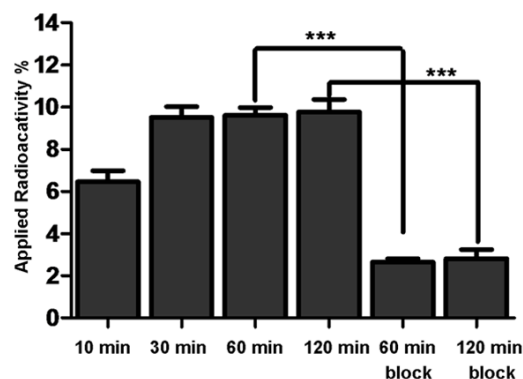
Results

Binding of FITC-F56 to BGC823 cells

F56 peptide is an antagonist of VEGF in its binding with VEGFR1. To confirm the binding of N-terminal modified F56 peptide to VEGFR1 positive BGC823 cells *in vitro*, FITC-F56 was incubated with BGC823 cells. The positive staining of the conjugate was detected at the peripheral of the cells (**Figure 2**). Moreover, the fluorescent signal from the surface of cells significantly was reduced by incubation of the cells with excess unconjugated F56 peptide (100 times than FITC-F56), demonstrating the binding specificity of FITC-F56. The *in vitro* binding of the conjugate led us to believe that other N-terminal modified F56 peptides would remain their binding affinity for VEGFR1, and they have potential for further *in vivo* tumor imaging as described below.

Table 1. The actual specific activity of ^{64}Cu -DOTA-F56 under each condition

DOTA-F56 (amount)	20 μg	10 μg	5 μg	1 μg	0.5 μg
R.Y. (100%)	> 98%	> 98%	96.2%	36.9%	28.4%
S.A. (GBq/mmol)	22.50	45.00	86.58	166.05	255.60

**Figure 5.** *In vitro* stability of ^{64}Cu -DOTA-F56. ^{64}Cu -DOTA-F56 was mixed with 0.01 M PBS (pH 7.4), 0.1 M NaOAc (pH 5.5), or 5% HSA, respectively. After incubation at 37 °C with shaking in a thermo mixer, the radiochemical purity was measured by Radio-HPLC.**Figure 6.** *In vitro* BCG-823 cell uptake assay. BCG823 cells were seeded in 12-well plate and incubated overnight. After incubation with radiotracer at 37 °C for desired time points (10 min, 30 min, 60 min and 120 min), cells were washed and lysed. Radioactivity of the cells was counted using a PerkinElmer 1470 automatic γ -counter. Cell uptake data were expressed as the percentage of the applied radioactivity per total radioactive. ***P < 0.001 student's t-test.

Chemistry and radiochemistry

The conjugate DOTA-F56 was synthesized through conventional solid phase peptide synthesis and purified by semi-preparative HPLC. The purified peptide was generally obtained in about 20% yield. The retention time on analyti-

cal HPLC for DOTA-F56 was found with 98.6% chemical purity under 280 nm wavelength.

The purified DOTA-F56 was characterized by MALDI-TOF-MS and ESI-MS. The measured molecular weight was consistent with the expected molecular weight (M.W.) as calculated for $\text{C}_{95}\text{H}_{126}\text{N}_{21}\text{O}_{26}\text{S}^+ [\text{M}+\text{H}]^+ = 2008.8898$, found at 2008.879, calculated for $\text{C}_{95}\text{H}_{125}\text{N}_{21}\text{NaO}_{26}\text{S}^+ [\text{M}+\text{Na}]^+ = 2030.8718$, found at 2030.866. The main peak and relative isotope peaks were shown in **Figure 3**.

The radio labeled conjugate, ^{64}Cu -DOTA-F56, was synthesized by the reaction of DOTA-F56 peptide with $^{64}\text{Cu}^{2+}$ at 60 °C after 30 min incubation. The labeling yield with $^{64}\text{Cu}^{2+}$ was generally over 98%. After purification by C18 sep-pak column, the radio-chemical purity of ^{64}Cu -DOTA-F56 was over 99% with the retention time at 9.81 min, which was slightly different from that of DOTA-F56 at 9.71 min (**Figure 4**).

To increase the specific activity of ^{64}Cu -DOTA-F56, the amount of DOTA-F56 used for the radiolabeling reaction was gradually reduced from 20 μg , 10 μg , 5 μg , 1 μg , to 0.5 μg , respectively, and the radioactivity of $^{64}\text{CuCl}_2$ (222 MBq) remained the same under the same radiolabeling conditions. The radiolabeling yield decreased from 98% to 28%, and the Specific Activity (S.A.) increased from 22.50 GBq/mmol to 255.60 GBq/mmol, when the amount of DOTA-F56 was reduced from 20 μg to 0.5 μg (**Table 1**).

In vitro stability and LogP of ^{64}Cu -DOTA-F56

There is more than 80% intact ^{64}Cu -DOTA-F56 after 24 h of incubation in 3 conditions, 0.01 M PBS at pH 7.4, 0.1 M NaOAc at pH 5.5, and 5.0% HSA (**Figure 5**). ^{64}Cu -DOTA-F56 peptide showed excellent *in vitro* stability in PBS and NaOAc at 37 °C up to 24 h, and more than 90% of conjugates retained their original structures. It had excellent resistance to proteolysis and transchelation in 5.0% HSA with > 96% of the probe intact after a 3 h incubation and with > 80% after 24 h incubation, as monitored by Radio-HPLC with the Retention time of 9.81 min.

The log *P* value of ^{64}Cu -DOTA-F56 was determined to be 2.81 ± 0.12 , indicating high lipophilicity of the radiolabeled peptide, from the

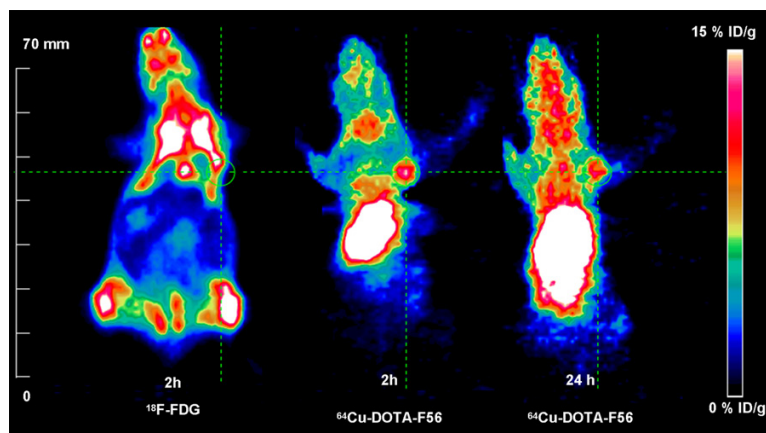


Figure 7. Micro-PET imaging of a nu/nu mouse bearing BCG823 tumor cells. Images were obtained at 2 h PET imaging of ^{18}F -FDG and 2 h, 24 h after injection of ^{64}Cu -DOTA-F56. Static Micro-PET images are presented in 3D and the green line circles the tumors.

octanol-water partition coefficient measurements.

In vitro cell uptake assay

Cell uptake of ^{64}Cu -DOTA-F56 was evaluated using BCG823 cells, and the results are shown in **Figure 6**. The cell uptake values of ^{64}Cu -DOTA-F56 at 10, 30, 60, and 120 min at 37°C were $6.5 \pm 1.1\%$, $10.40 \pm 1.3\%$, $9.6 \pm 0.8\%$, and $9.8 \pm 1.3\%$, respectively. While block groups values of $2.7 \pm 0.3\%$, $2.8 \pm 1.0\%$ at 60 and 120 min were observed, respectively. A 4-fold greater accumulation of the probe occurred in cells incubated at 37°C compared with those block group, indicating that cell surface binding could be significantly inhibited by the presence of a large excess of F56 ($P < 0.001$).

Micro-PET imaging study

The BCG823 tumor was clearly visible at 2 h after injection of ^{64}Cu -DOTA-F56, better contrast images were showed at 24 h (**Figure 7**, decay-corrected coronal micro-PET images). The uptake of ^{64}Cu -DOTA-F56 in liver and kidney was relatively high, but it slowly decreased from 2 h to 24 h. Some radioactivity uptake was also observed in the region of thyroid, due to dissociation of ^{64}Cu -DOTA-F56. For comparison, the tumor was barely visible on micro-PET imaging at 2 h after injection of ^{18}F -FDG (**Figure 7**, decay-corrected coronal micro-PET images). When 37 MBq of $^{64}\text{CuCl}_2$ was injected into the mice, micro-PET imaging indicated almost purely liver uptake, with no uptake in tumor.

Semi-quantification analysis of Micro-PET images showed that the region of interest (ROI) value at tumor site was 4.7% ID/g and 8.0% ID/g at 2 h and 24 h post injection of ^{64}Cu -DOTA-F56, while no specific binding was observed from ^{18}F -FDG or $^{64}\text{CuCl}_2$.

Immunohistochemistry study

Immunohistochemical stain studies were performed to demonstrate the binding specificity of F56 peptide with VEGFR-1 receptors. This was achieved through the comparison of the stainings of F56 peptide and VEGFR1 anti-

body. F56 peptide imaging showed no staining in regions of bleeding, light staining in regions of diffuse tumor cells, and heavy staining in regions of liquefaction, which are believed to be the VEGFR-1 highly expression area (**Figure 8A**, **8B**). The same pattern was observed by staining with VEGFR1 antibody (**Figure 8A**, **8C**).

Discussion

We have confirmed that the binding of FITC-F56 peptide with VEGFR1 is comparable with that of F56 peptide by fluorescence imaging in BCG823 cells. We deduce that the N-terminal modified F56 is tolerable for binding with VEGFR1 receptor. This is also confirmed by our study of ^{64}Cu -DOTA-F56 reported in this paper.

^{64}Cu -DOTA-F56 was synthesized in a high radiochemical yield ($> 98\%$) and radiochemical purity ($> 99\%$), and its specific activity ($22.5\text{--}255.6 \text{ GBq/mmol}$) is controllable through variation of the ratio between the 2 starting materials. The controllable SA may allow appropriate dose for gastric tumor therapy. ^{64}Cu -DOTA-F56 showed lower lipophilicity as compared with F56 peptide, which is extremely insoluble in water or other aqueous media. The lipophilicity of ^{64}Cu -DOTA-F56, as shown by $\log P$ of 2.81 ± 0.12 , is in the right range for its tissue permeability.

Micro-PET imaging of ^{64}Cu -DOTA-F56 showed excellent contrast images of the tumor having positive VEGFR1 positive expression in BCG823 tumor xenograft model were obtained at 2 h or

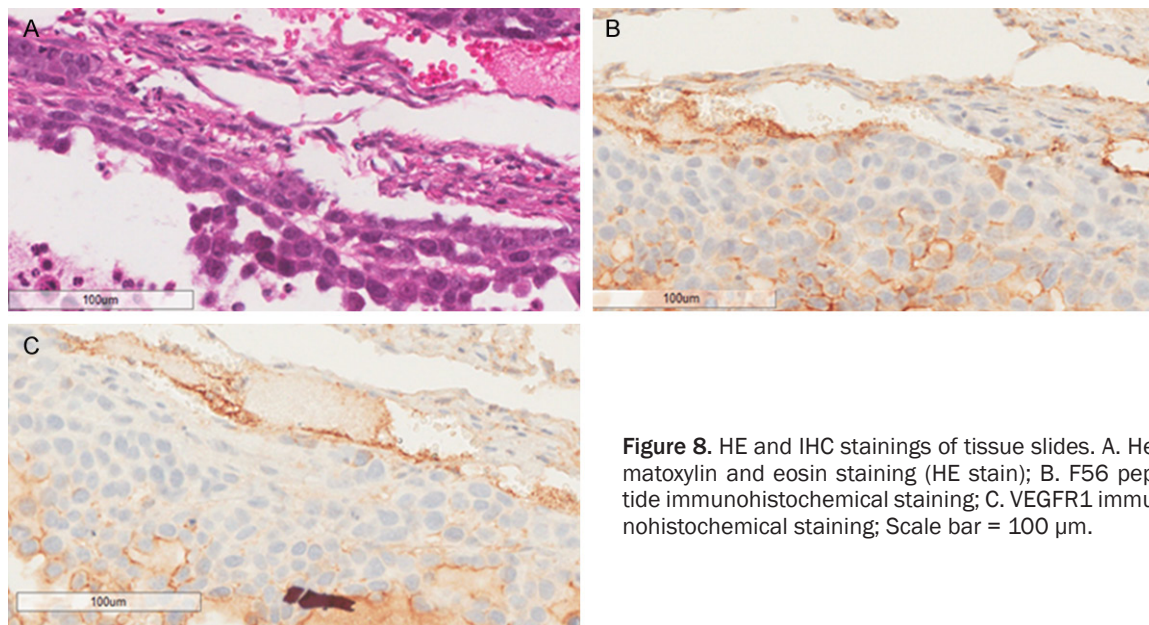


Figure 8. HE and IHC stainings of tissue slides. A. Hematoxylin and eosin staining (HE stain); B. F56 peptide immunohistochemical staining; C. VEGFR1 immunohistochemical staining; Scale bar = 100 μ m.

24 h after post the injection of ^{64}Cu -DOTA-F56. In contrast, no accumulation of ^{18}F -FDG was shown in tumor region by PET imaging. We also observed a prominent uptake of ^{64}Cu -DOTA-F56 in the tumor at 24 h after injection of ^{64}Cu -DOTA-F56.

The micro-PET imaging by released $^{64}\text{Cu}^{2+}$ was excluded by the Micro-PET studies of $^{64}\text{CuCl}_2$. No uptake of radioactivity was observed in tumor (data not shown). It is also known that human copper transporter 1 (hCTR1) is the primary protein responsible for importing copper. It has been proved to be over expressed in non-small cell lung cancer, liver cancer and malignant melanomas [31, 32]. However, no hCTR1 was expressed in malignant tissue of gastric carcinoma (0/29) [31]. No interference from free $^{64}\text{Cu}^{2+}$ was observed, supporting the specific binding of ^{64}Cu -DOTA-F56 in gastric cancer.

The receptor specificity of the probe was verified by the *in vitro* cell blocking experiment. Unlabeled F56 significantly reduced cell uptake both in fluorescence visualization and *in vitro* cell uptake test. In order to further verify the specificity of ^{64}Cu -DOTA-F56 in its binding affinity toward VEGFR1, we have studied immunohistochemical staining of F56 and VEGFR1 antibody. The staining pattern of the F56 peptide was similar to the distribution of VEGFR1 expression, allowing us to conclude that micro-

PET images also reflected the expression of VEGFR1 *in vivo*.

The designed ^{64}Cu -DOTA-F56 peptide could be useful for detection of early gastric cancer and other VEGFR1 positive tumors, and monitoring the efficacy of VEGFR1-based tumor treatment. To our knowledge, this is the first report of ^{64}Cu -radiolabeled peptide which can bind to VEGFR1 for PET imaging. In addition, due to the inherent therapeutic effect of F56 peptide and ^{64}Cu nuclide, ^{64}Cu -DOTA-F56 peptide could also be used for VEGFR1-based peptide receptor radionuclide therapy (PRRT). Further research of ^{64}Cu -DOTA-F56 as a therapeutic agent is still in progress.

Conclusion

We have successfully developed ^{64}Cu -DOTA-F56 peptide targeted VEGFR1 for noninvasive molecular imaging of gastric tumor. It was synthesized in both high radiolabeling yield and controllable specific activity, and it showed excellent stability. Micro-PET imaging of ^{64}Cu -DOTA-F56 showed clear images of tumors from 2 h to 24 h in nude mice bearing BCG823 cells, while no accumulation of ^{18}F -FDG or $^{64}\text{CuCl}_2$ was observed under the same conditions. The specificity of ^{64}Cu -DOTA-F56 for VEGFR1 *in vivo* was deduced from the close match between stainings of F56 and that of VEGFR1. Overall, ^{64}Cu -DOTA-F56 had high spe-

cific activity, high binding specificity, and gave excellent detection of gastric tumor as compared with ^{18}F -FDG. ^{64}Cu -DOTA-F56 peptide has potential for early detection of VEGFR1 positive gastric cancer, and potentially as a therapeutic agent.

Acknowledgements

This work was supported by the Major Project on Drug Research and Development for the 12th Five-Year Plan of China (2012ZX09103-301-013), the National 973 Program of China (2015CB553906), the National Natural Science Foundation of China (81172083, 813715-92, 81401467, 81301966, 81571705), and the Beijing Natural Science Foundation (7132040, 7154188).

Disclosure of conflict of interest

None.

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