Synthesis and Evaluation of Technetium-99m-labeled pH (low) Insertion Peptide Variant 7 for Early Diagnosis of MDA-MB-231 Triple-Negative Breast Cancer by Targeting the Tumor Microenvironment

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Research

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Abstract

Objective To prepare technetium-99m (^{99m}Tc)-labeled pH (low) insertion peptide variant 7 (pHLIP(Var7)) and carry out imaging of tumor-bearing nude mice in vivo, to study its value in the early diagnosis of triple-negative breast cancer (TNBC).

Methods The pHLIP(Var7) sequence was synthesized by solid-phase polypeptide synthesis. Four amino acids, Gly-(D)-Ala-Gly-Gly, were attached to the N-terminus of pHLIP(Var7) to form a strong chelating group containing an N4 structure. The polypeptide was directly labeled with ^{99m}Tc to determine the in vitro binding propensity of ^{99m}Tc-pHLIP(Var7) to MDA-MB-231 cells, to measure the in vivo biodistribution of ^{99m}Tc-pHLIP(Var7) in tumor-bearing nude mice, and to perform small-animal single-photon-emission computed tomography (SPECT)/computed tomography (CT) of these mice.

Results The radiochemical yield and radiochemical purity of ^{99m}Tc-pHLIP(Var7) were 99.49%±0.17% and 99.63±0.44%, respectively. The radiochemical purity was still more than 96% after 24 h in serum. The binding fraction of ^{99m}Tc-pHLIP(Var7) and MDA-MB-231 cells continuously increased in an acidic environment, being significantly higher than their cell binding fraction ($P < 0.01$) when pH = 7.4 and the cell binding fraction ($P < 0.01$) of ^{99m}Tc-kVar7 at different pH values (pH = 6.0, 6.5, 7.0 and 7.4) at each time point ($P < 0.01$). The biodistribution assay showed that the distribution of ^{99m}Tc-pHLIP(Var7) in tumors at each time point was significantly higher than that of ^{99m}Tc-kVar7 ($P < 0.01$). ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7 were also significantly distributed in the liver. SPECT/CT imaging was basically consistent with the biodistribution results. The tumor was clearly imaged at each time point after injection of ^{99m}Tc-pHLIP(Var7) but could not be imaged after injection of ^{99m}Tc-kVar7. The liver was clearly imaged at each time point in both groups.

Conclusion ^{99m}Tc-pHLIP(Var7) has high radiochemical yield and stability, and it concentrates highly in tumor tissues. The presence of a very strong radioactive background in the abdomen of tumor-bearing nude mice does not preclude the early diagnosis of TNBC.

Introduction

The tumor microenvironment (TME) of almost all solid tumors is acidic, with pH values as low as 6.0 [1, 2]. The acidic TME is stable and not affected by tumor clonal selection, making it a promising tumor detection target [2]. The pH (low) insertion peptide (pHLIP) family can target the acidic TME, spontaneously form an α-helix structure, and insert it into the tumor cell membranes in an acidic extracellular environment [3]. With an iodine-125-labeled pH (Low) insertion peptide variant 7 (PHLIP (VAr7)), single-photon-emission computed tomography (SPECT)/computed tomography (CT) of MDA-MB-231 triple-negative breast cancer (TNBC) mice has confirmed that pHLIP(Var7) can target MDA-MB-231 TNBC [4, 5]. However, the iodine-labeled compound is easily deiodinated, and its half-life is not suitable for imaging.

Technetium-99m (^{99m}Tc), which is better for SPECT imaging, was applied for labeling and imaging in this study. We used a novel ^{99m}Tc labeling method: Four amino acids that formed an N4 structure, Gly-d-Ala-Gly-Gly, were used as a chelating group and linked to the N-terminus of pHLIP(Var7) in polypeptide synthesis, thus realizing direct ^{99m}Tc labeling. The method simplifies the labeling process and yields a higher radiochemical yield and radiochemical purity than its predecessors [6].

Materials And Methods

1. Materials

1.1 Main instruments

The main instruments applied in this study were a Chirascan plus ACD spectropolarimeter (Applied Photophysics, United Kingdom), a CRC-55tR radiopharmaceutical dose calibrator (Capintec Inc., USA), a Wizard 1480 gamma counter (PerkinElmer Instruments Inc., USA), a Scan-RAM Radio-HPLC/TLC scanner (LabLogic, UK), and a U-SPECT +/CT small-animal SPECT/CT imaging system (MILabs, Netherlands).

1.2 Main reagents

The main reagents applied in this study were ^{99m}Tc-pertechnetate (Shanghai Xinke Pharmaceutical Co., Ltd., China), stannous chloride (Sinopharm Chemical Reagent Co., Ltd., China), isoflurane (Shenzhen RWD Life Technology Co., Ltd., China), and hydrochloric acid (Sinopharm Chemical Reagent Co., Ltd., China).

2.Synthesis and characterization of peptides

pHLIP(Var7) and the reference sequence kVar7 [7] were synthesized by Shanghai Science Peptide Biological Technology Co., Ltd. (Shanghai, China) using solid-phase polypeptide synthesis. To facilitate ^{99m}Tc labeling, four amino acids, Gly-d-Ala-Gly-Gly, were attached to the N-terminal of pHLIP(Var7) and kVar7 to form a strong chelating group containing an N4 structure. To eliminate any spatial obstruction, gamma-aminobutyric acid (GABA) was introduced as a spacer between the N4 structure and pHLIP(Var7)/kVar7. The modified pHLIP(Var7) and kVar7 sequences were as follows:

pHLIP(Var7): GaGG-GABA- ACEEQNPWARYLEWLFPTETLLLEL-NH_2,

kVar7: GaGG-GABA- ACEEQNPWARYLKWLFPTKTLLLKL-NH_2.

pHLIP(Var7) and kVar7 were purified by high-performance liquid chromatography (HPLC). The LC-2010 chromatograph (Shimadzu Corporation, Japan) was equipped with a Kromasil 100-5C18 chromatographic column (AkzoNobel, Sweden) (5 μm,4.6×150 mm) eluted with a gradient from 25 to 85% solvent A (0.1% trifluoroacetic in 100% acetonitrile) and 75 to 15% solvent B (0.1% trifluoroacetic in 100% water) at 1.0 mL/min, with monitoring at 220 nm. The identities of pHLIP(Var7) and kVar7 were

confirmed by mass-spectrometric analysis using Agilent 6125B mass spectrometer (Agilent Technologies, Inc., USA).

3. Circular dichroism (CD)

A total of 7 μmol/L PHLIP (Var7) and 2 mmol/L1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC, diameter ≤50 nm) were dissolved in 10 mmol/L phosphate buffer (PB) when pH=8 and set aside at room temperature for at least 10 h. The pH value of the solution was adjusted to 4.0 with 0.1 mol/L HCl.

CD spectra of pHLIP(Var7) were recorded at pH = 8.0 and pH = 4.0 in a 1-mm-path-length quartz cuvette using a spectropolarimeter. The experimental settings were as follows: bandwidth, 1 nm, wavelength range, 190-260 nm, wavelength step size, 1 nm, time-per-point, 0.5 s, temperature, 25 °C.

4. Preparation of ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7

A total of 1 mg of the polypeptide powder was dissolved in 1 mL of 0.02 M phosphate-buffered saline (PBS). Ten microliters of the prepared solution was added to 90 μL PBS, 20 μL 1 mg/mL stannous chloride (in 0.01 M HCl), 20 μL sodium phosphate solution (8.2 mg/mL), and 74 MBq fresh ^{99m}Tc-pertechnetate. The mixture was then put in a 90 °C water bath for 15 min. The product was purified by a Sephadex G25 column (1.0 × 25 cm) and eluted with PBS (0.1 M, pH 7.4). The radiochemical yield and specific activity of ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7 were calculated.

The radiochemical purity of ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7 was determined by thin-layer chromatography (TLC). A thin polyamide layer was used as the stationary phase and acetonitrile as the developing solvent. A proper amount of the labeled product was sucked through a capillary pipette and spotted at the origin of the filter paper. The polyamide thin layer was air-dried and vertically placed in a test tube with developing solvent to be unfolded. The unfolded polyamide thin layer was taken out, dried, and placed on the TLC scanner for testing.

5. Stability in vitro of ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7

The markers were placed in PBS at room temperature (25°C) and fresh human serum at 37°C, and samples were taken to test the radiopurity 1, 4, 6, and 24 h after labeling.

5. Cell culture and xenograft model

MDA-MB-231 human breast cancer cells (National Collection of Authenticated Cell Cultures) were cultured in an incubator at 37°C and 5% CO_2 with high glucose in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum and 1% double antibodies (penicillin-streptomycin mixture).

Animal experiments were approved by ethics committee of Qingdao University, and the management of animals followed ethical standards. Four-to-5-week-old female BALB/c nude mice (Shanghai SLAC Laboratory Animal Co., Ltd, China) were used to build an MDA-MB-231 tumor model, and 0.1 mL of a cell

suspension (50 μL PBS + 50 μL Matrigel) containing 1×10^6 MDA-MB-231 cells was subcutaneously inoculated into the right axilla of each mouse.

6. Cell binding of ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7 to MDA-MB-231

a. When MDA-MB-231 cells grew to more than 90% confluence, they were digested. The cell concentration was adjusted to approximately 1.0×10^5 cells/mL, and 1 mL of the cell solution was added to each well of a 12-well plate and cultured overnight in a 37°C cell incubator.

b. The medium was discarded and washed with PBS two times. Then 2 mL fresh medium was added into each well. Each well was adjusted to four pH values (pH=7.4, 7.0, 6.5, and 6.0) after adding 37 kBq of ^{99m}Tc-pHLIP(Var7) or ^{99m}Tc-kVar7 (20-30 μL) to each well.

c. After culturing for 30, 60, 90, 120, or 180 min, the supernatant was aspirated into a supernatant tube, and each well was washed with PBS with the corresponding pH (pH=7.4, 7.0, 6.5, or 6.0) two times, and the cleaning solution was also added into the supernatant tube. The cells were digested fully with 0.25% trypsin, and the cell suspension was sucked into the cell tube and washed twice with PBS. The cleaning solution was also added to the cell tube. A γ counter was applied to measure the radioactivity count of the cell tube (B) and the radioactivity count of the supernatant tube (F). Cell binding fraction of the marker=B/(B+F)×100%.

7. In vivo biodistribution

When the tumor diameter reached 0.8-1.0 cm, 24 tumor-bearing nude mice were selected for in vivo biodistribution experiment. Each nude mouse was injected with approximately 1480 kBq/200 μL ^{99m}Tc-pHLIP(Var7) or ^{99m}Tc-kVar7 through the tail vein. The mice were sacrificed humanely 1 h, 2 h, 4 h, and 6 h after injection, and their tissues and organs, such as the brain, heart, lung, liver, kidney, stomach, small intestine, blood, muscle, bone, and tumor, were weighted and measured for radioactive count. The results are expressed as the percentage of injected dose per gram of tissue (%ID/g).

8. Small-animal SPECT/CT imaging

When the tumor diameter reached 0.8-1.0 cm, six tumor-bearing nude mice were selected for imaging. Each nude mouse was injected with approximately 11.1 MBq/200 μL ^{99m}Tc-pHLIP(Var7) or ^{99m}Tc-kVar7 through the tail vein. The tumor-bearing mice were anesthetized with 2% isoflurane 1 h, 2 h, 4 h, and 6 h after injection and were fixed on the imaging bed of a small-animal SPECT/CT imaging system in the prone position. A pinhole collimator was applied to image them. The main parameters were matrix size 256×256, magnification power 1.45, and acquisition time at each time point 10 min.

9. Statistical analysis

SPSS 24.0.0.0 (IBM) statistical software was applied for data processing. Quantitative data are expressed as mean (M) ± standard deviation (SD). Variables were compared by one-way analysis of

variance. $P < 0.05$ was deemed significant.

Results

1. Peptide synthesis

PHLIP (var7) and kvar7 were successfully synthesized by solid-phase polypeptide synthesis (see Electronic Supplementary Material for details). HPLC analysis of pHLIP(Var7) showed the formation of several compounds consisting of a major product (98.12%, retention time of 12.85 min) accompanied by two smaller peaks at retention times of 12.75 and 13.24 min, respectively. HPLC analysis of kVar7 showed the formation of several compounds consisting of a major product (98.03%, retention time of 11.82 min) accompanied by two smaller peaks at retention times of 11.47 and 12.33 min.

The MS analysis of pHLIP(Var7) showed two mass peaks with m/z values of 1131.70 ([M+3H]3H+) and 1697.13 ([M+2H]2H+). The MS analysis of kVar7 showed two mass peaks with m/z values of 1130.80 ([M+3H]3H+) and 1695.70 ([M+2H]2H+). The measured molecular weights (3392.10, 3389.40) of pHLIP(Var7) and kVar7 were consistent with the theoretical molecular weights (3391.80, 3388.98).

2. CD spectroscopy

The secondary structure of pHLIP(Var7) was determined by CD at pH 8.0 and 4.0. Figure 1 shows that pHLIP(Var7) exhibited a typical pH-dependent transition from a nonstructural conformation to an α-helical structure when the pH decreased from 8.0 (blue line) to 4.0 (red line) in the presence of POPC.

3. The preparation of ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7

^{99m}Tc-pHLIP(Var7) had a radiochemical yield of 99.49%±0.17% and radiochemical purity of 99.63 ± 0.44%. ^{99m}Tc-kVar7 had a radiochemical yield of 99.46%± 0.14% and radiochemical purity of 99.63±0.35%. The specific activities of ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7 were both 7.36 ± 0.01 MBq/μg.

4. Stability in vitro of ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7

The radiochemical purity of both ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7 was >99% in PBS at room temperature at 24 h and >96% in fresh human serum at 37°C at 24 h (Figure 2).

5. The binding fractions of ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7 to MDA-MB-231 Cells

At pH = 6.0, 6.5 and 7.0, the binding fractions of ^{99m}Tc-pHLIP(Var7) and MDA-MB-231 cells steadily increased, and reached the highest at 180 min, which were 3.33 ± 0.12%, 3.02 ± 0.04% and 2.87 ± 0.03% respectively. At pH= 7.4, the binding fractions of ^{99m}Tc-pHLIP(Var7) and MDA-MB-231 cells remained low at each time point (30, 60, 90, 120, 180 min). The binding fractions of ^{99m}Tc-kVar7 and MDA-MB-231

cells remained low at each time point (30, 60, 90, 120, 180 min) at all tested pH values (6.0, 6.5, 7.0 and 7.4) (Figure 3).

The cell-binding fractions of ^{99m}Tc-pHLIP(Var7) at pH=6.0, 6.5, and 7.0 at various time points (30, 60, 90, 120, 180 min) were significantly higher than its cell binding fractions at pH = 7.4 ($P < 0.01$) and significantly higher than the cell-binding fractions of ^{99m}Tc-kVar7 at pH=6.0, 6.5, 7.0, and 7.4 ($P < 0.01$).

6. Biodistribution

The distribution of ^{99m}Tc-pHLIP(Var7) in tumors at 1, 2, 4, and 6 h was 3.63±0.92, 6.11±0.66, 5.33±0.40, and 3.72±0.94%ID/g, respectively, all higher than that of ^{99m}Tc-kVar7 in tumors ($P<0.01$). Outside of tumors, the distribution of ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7 had high levels of distribution in the liver and blood (Figure 4A) (n=3).

The tumor/non-tumor (T/NT) ratio increased over time in the ^{99m}Tc-pHLIP(Var7) group, while no significant increasing trend was seen in the T/NT ratio of the ^{99m}Tc-kVar7 group. The T/NT ratio in the ^{99m}Tc-pHLIP(Var7) group was higher at each time point than in the ^{99m}Tc-kVar7 group ($P<0.05$) (Figure 4B) (n=3).

7. Micro-SPECT/CT imaging

SPECT/CT imaging was performed 1, 2, 4, and 6 h after the injection of ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7 (Figure 5) (n=3). The tumor was clearly imaged at each time point after injection of ^{99m}Tc-pHLIP(Var7), but not after injection of ^{99m}Tc-kVar7. The liver was clearly imaged in both groups at each time point.

Discussion

TNBC can easily metastasize and has a poor prognosis. Early diagnosis of TNBC could effectively improve the prognosis. In daily medical practice, the imaging diagnosis of breast cancer is done mainly by breast X-ray examination, magnetic resonance imaging, and ultrasound [8]. Since TNBC is more likely to occur in young women [9], the relatively dense breast tissue could interfere with the detection of tumor lesions by conventional imaging. In this study, ^{99m}Tc, which has a low cost and suitable half-life and radiation energy for imaging, was used to label pHLIP(Var7) for SPECT imaging for the first time [4, 5, 10, 11]. SPECT imaging is characterized by high sensitivity and low cost, so it is widely used. Combined with this molecular probe, it could be used for functional imaging of TNBC and was expected to be used for early diagnosis and screening of TNBC.

At present, indirect labeling is mainly used for ^{99m}Tc labeling, which is relatively complex, having an insufficient radiochemical yield and poor stability in vitro. In this study, by referring to the labeling technology mentioned by Zhao et al. [6], four amino acids (Gly-d-Ara-Gly-Gly) were attached to one end of

the polypeptide as a chelating group for direct ^{99m}Tc labeling. The labeling method was simple and easy. The radiochemical yield and radiochemical purity of that radioactive molecular probe in this experiment were both greater than 99%, indicating that the labeling method can achieve high radiochemical yield and radiochemical purity, and the radioactive probe can be used for in vivo imaging without purification. The 24-h radiochemical purity of the molecular probe in fresh human serum at 37°C in vitro was more than 96%, suggesting that the radioactive probe has high resistance to proteases in blood and high stability. The application of small peptides as bifunctional coupling agents has the following advantages: these can be directly synthesized by solid-phase polypeptide synthesis, and they can form stable compounds with ^{99m}Tc [12].

^{99m}Tc-pHLIP(Var7) showed a high binding fraction to MDA-MB-231 cells in the acidic environment, significantly higher than the binding fraction of the control peptide ^{99m}Tc-kVar7 with MDA-MB-231 cells, suggesting that the N-terminal labeling of pHLIP(Var7) by ^{99m}Tc did not change the ability of pHLIP(Var7) to insert itself into tumor cell membranes in acidic environments. The insertion of pHLIP family peptides into the lipid bilayer of cell membranes is triggered by low pH. In acidic environments, the negatively charged amino acid residues (Asp or Glu) contained in pHLIP are protonated, enhancing the hydrophobicity of the polypeptide, and an α-helix forms for insertion into the cell membrane [3, 13]. kVR7, a pH-insensitive peptide obtained by replacing the protonated Glu residue in the insertion region of pHLIP(Var7) with a positively charged Lys residue, does not target acidic tissues [7]. Therefore, kVR7 was used as the control peptide in this study.

Biodistribution showed that ^{99m}Tc-pHLIP(Var7) and ^{99m}Tc-kVar7 had slow blood clearance, suggesting they might have had strong binding to plasma proteins and/or red blood cells. Complexes formed by binding are not conducive to probe elimination and have an adverse effect on image quality. In future studies, we will consider changing the characteristics of probe molecules (such as molecular weight, amino acid sequence, and charge number) to reduce their binding to plasma proteins and/or red blood cells. The high distribution of the probes in the kidney suggested that the probes might be excreted through the urinary system. One study found [14] that the pHLIP peptide family is mainly excreted through the urinary system because the kidney is the main excretion route of low-molecular-weight proteins, and normal urine is weakly acidic and so facilitates excretion of the pHLIP peptide family. That study [14] showed that the addition of weakly alkaline bicarbonate buffer to the drinking water of mice reduced the accumulation of pHLIP family peptides in their urinary system. Urine may be appropriately acidified to improve the ability of the probe to be excreted in urine for the purpose of reducing the radiological background of the image.

The distribution of both probes in the liver was high. The liver uptake of fluorescence-labeled pHLIP(Var7) was much lower than that of ^{99m}Tc-pHLIP(Var7) [15], probably because ^{99m}Tc-labeled pHLIP(Var7) bound strongly to intrahepatic tissue proteins and ^{99m}Tc labeling changed the original elimination characteristics of pHLIP(Var7), so it was partially excreted in bile. Biodistribution data further showed that although there was a very strong radioactive background in the abdomen of mice, the distribution of

probes in tumor tissues was significantly higher than that in muscle, bone, brain, and other tissues. Based on the biodistribution characteristics of the probes, it could be predicted that tumors in organs other than the abdomen should be easily identifiable in SPECT images, which was valuable for the early diagnosis of TNBC.

The SPECT/CT imaging results of tumor-bearing nude mice were basically consistent with the in vivo biodistribution trend. The tumors of ^{99m}Tc-pHLIP(Var7) mice were imaged clearly at various time points after injection, while the tumors with the control probe ^{99m}Tc-kVar7 was not imaged after injection, suggesting that ^{99m}Tc-pHLIP (Var7) was electrically neutral in the protonation of the negatively charged Glu residue in the acidic TME, and the polypeptide changed from a disordered structure to an α-helix structure and inserted itself into the tumor cell membrane. ^{99m}Tc-kVar7 remained positively charged in the acidic TME and did not convert to an α-helix to insert itself into tumor cell membranes. Although the intense signal of the liver in the tumor-bearing nude mice interfered with the detection of abdominal metastases, it did not hinder the imaging of primary TNBC lesions by ^{99m}Tc-pHLIP(Var7), so this could be used for the early diagnosis of TNBC and screening for breast cancer in high-risk groups.

Conclusion

^{99m}Tc-pHLIP(Var7) has high radiochemical yield, high stability, and high concentration in TNBC tissues. Although ^{99m}Tc-pHLIP(Var7) has very strong radioactive concentration in the abdomen, this does not prevent the imaging of primary TNBC. ^{99m}Tc-pHLIP(Var7) is expected to become a valuable radioactive molecular probe for the early diagnosis and screening of TNBC.

Abbreviations

CD: Circular dichroism, CT: Computed tomography, HPLC: high-performance liquid chromatography, pHLIP: pH (low) insertion peptide, pHLIP (var7): pH (low) insertion peptide variant 7, POPC: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine, SPECT: Single-photon emission computed tomography, TME: Tumor microenvironment, TNBC: Triple-negative breast cancer

Declarations

Ethics approval and consent to participate

Animal studies were approved by the ethics committee of Qingdao University, and the management of animals corresponded to animal ethical standards.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that they have no conflict of interest.

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Authors' Contributions

CYH, SY, PXF, SXX and ZWJ performed the experiments and wrote the paper. YMM designed and supervised the research. All authors read and approved the final manuscript.

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Not applicable

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Figures

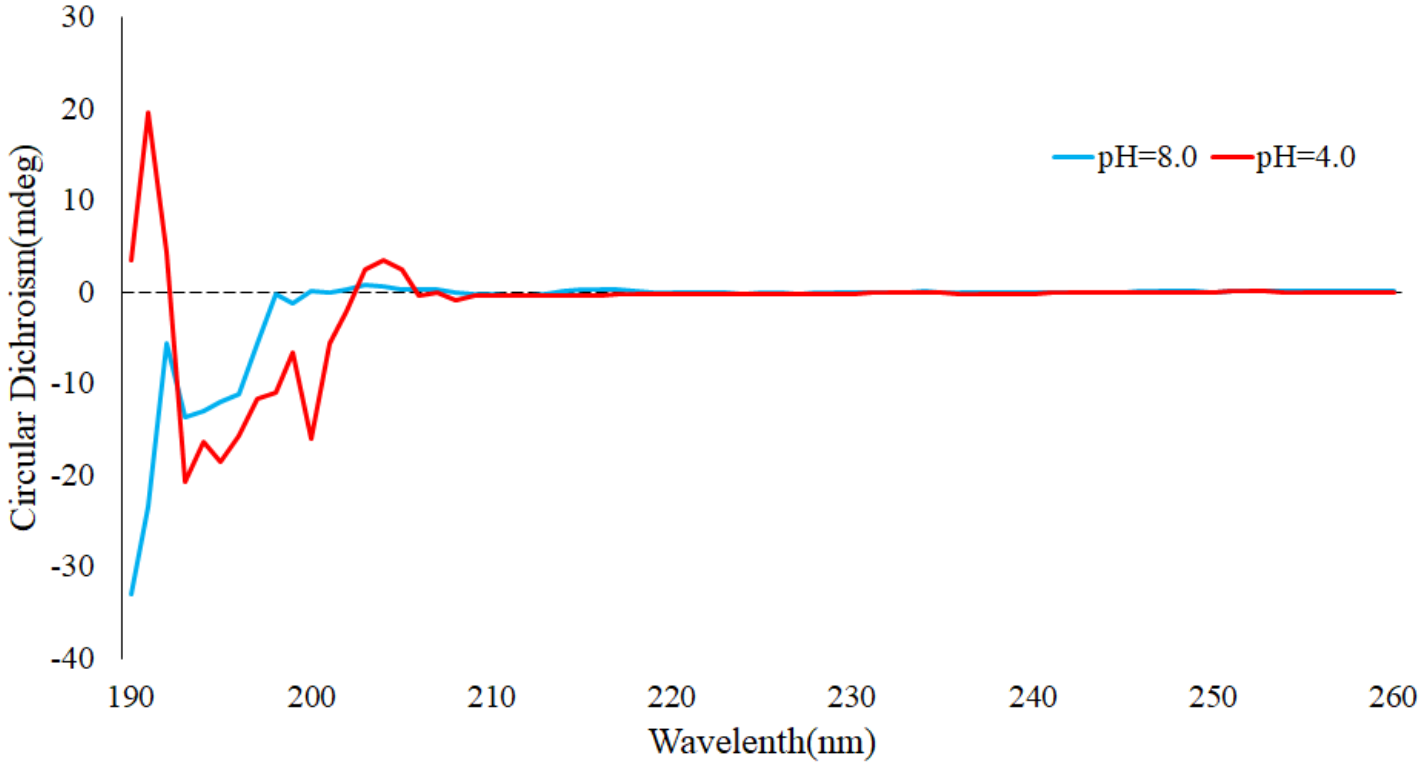

Figure 1

Circular dichroism of pHLIP(var7) at pH 4.0 (red line) and pH 8.0 (blue line).

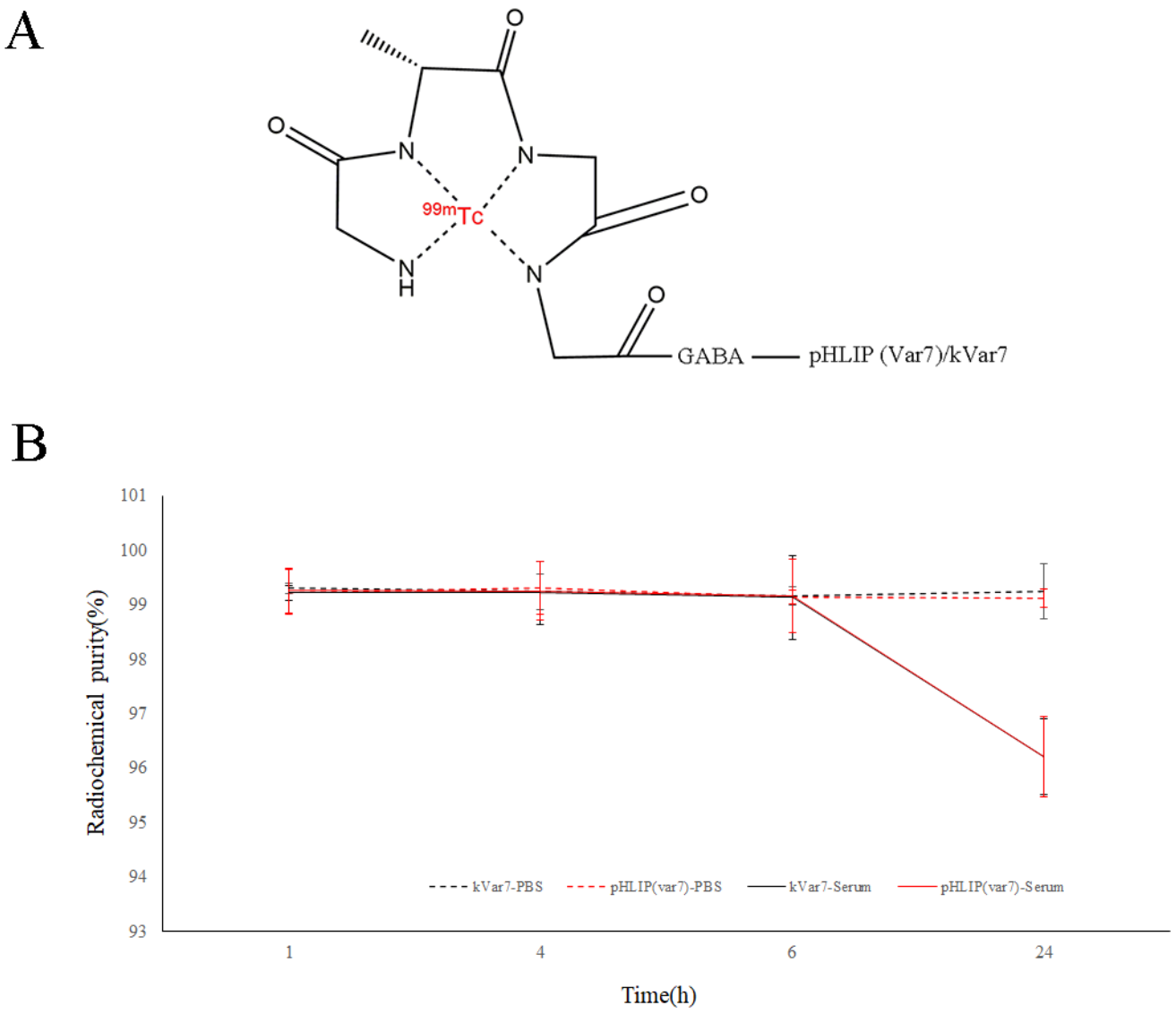

Figure 2

(A) N4 structure of 99mTc-pHLIP(Var7)/kVar7. (B) Stability of 99mTc-pHLIP(Var7) and 99mTc-kVar7 in PBS at room temperature and in fresh human serum at 37°C.

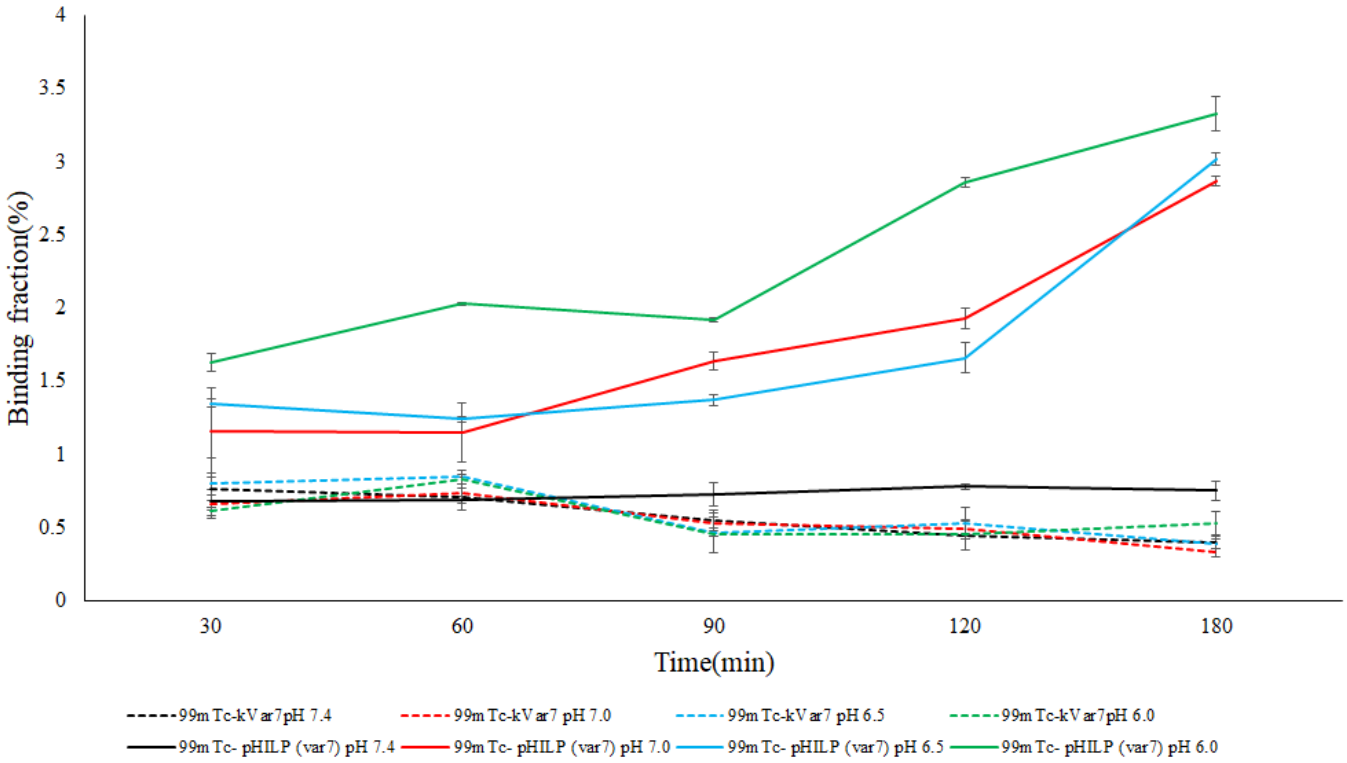

Figure 3

Binding fraction of 99mTc-pHLIP(Var7) and 99mTc-kVar7 to MDA-MB-231 cells at different times and at different pH values

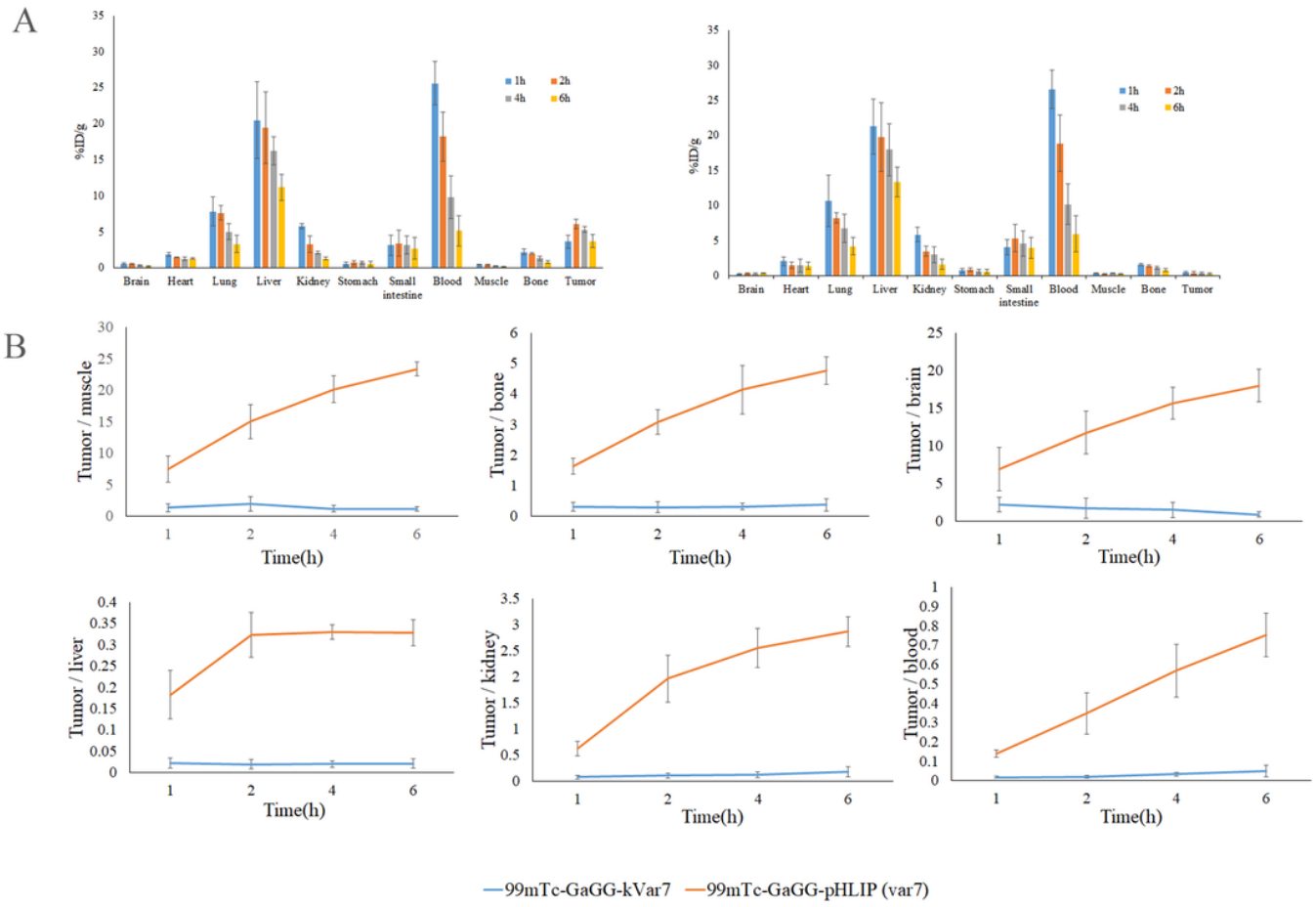

Figure 4

Biodistribution and T/NT ratio of 99mTc-pHLIP(Var7) and 99mTc-kVar7 in mice with MDA-MB-231 tumors (n=3).

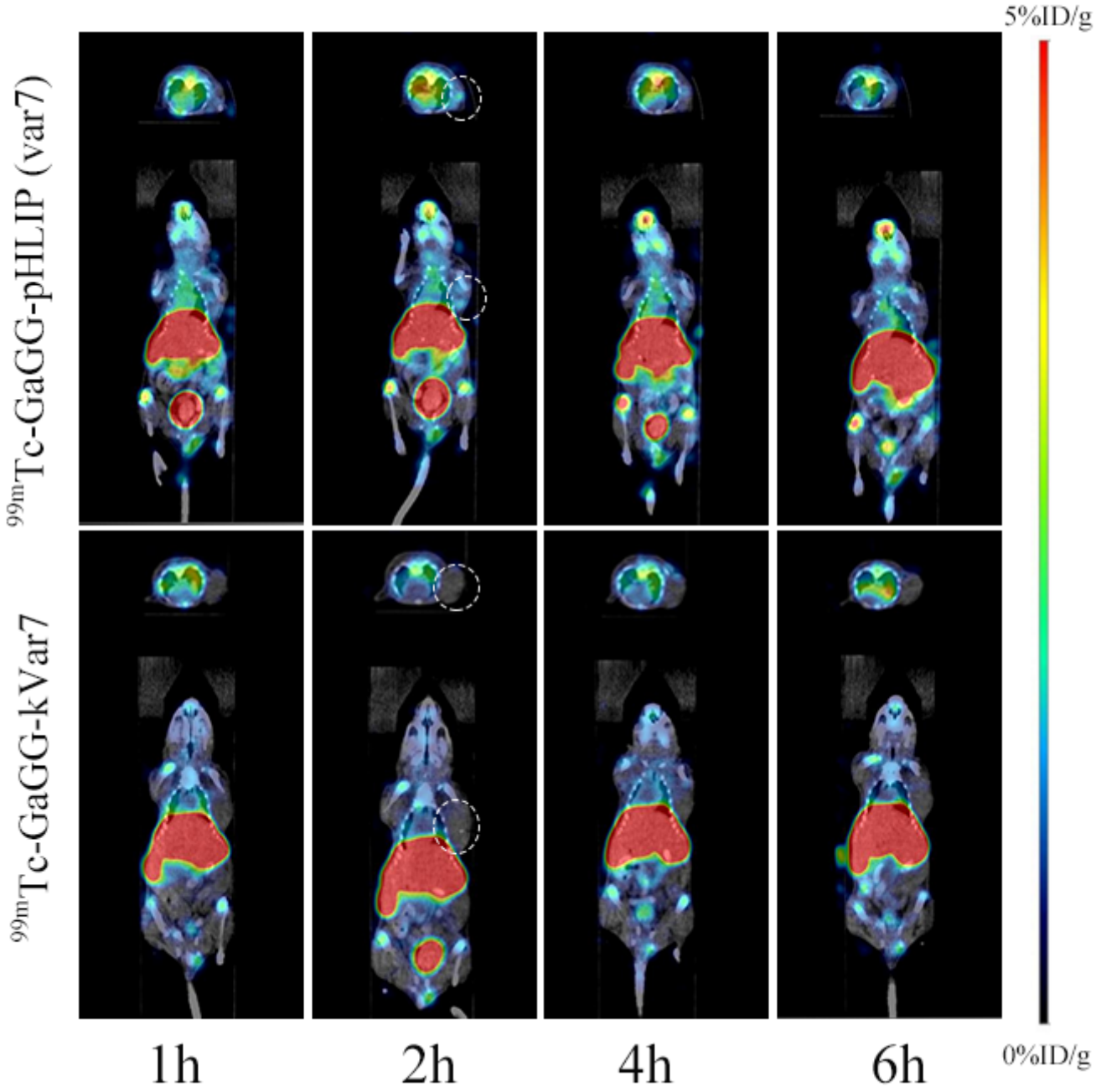

Figure 5

SPECT/CT imaging of 99mTc-pHLIP(Var7) and 99mTc-kVar7 in the MDA-MB-231 tumor model (n=3).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- VAR7.pdf
- kVar7.pdf