Nuclear Medicine and Biology 42 (2015) 256–262

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

journal homepage: www.elsevier.com/locate/nucmedbio

Evaluation of ^{99m}Tc-HYNIC-TMTP1 as a tumor-homing imaging agent targeting metastasis with SPECT

Fei Li [a], Teng Cheng [a], Qingjian Dong [b], Rui Wei [a], Zhenzhong Zhang [a], Danfeng Luo [a], Xiangyi Ma [a], Shixuan Wang [a], Qinglei Gao [a], Ding Ma [a], Xiaohua Zhu [b,*], Ling Xi [a,**]

[a] Cancer Biology Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Avenue, Wuhan, Hubei 430030, People's Republic of China
[b] Department of Nuclear Medicine,Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jiefang Avenue, Wuhan, Hubei 430030, People's Republic of China

ARTICLE INFO

Article history:
Received 13 July 2014
Received in revised form 20 October 2014
Accepted 3 November 2014

Keywords:
TMTP1
^{99m}Tc-HYNIC-TMTP1
Ovarian cancer
SPECT imaging
Metastasis
Tumor imaging

ABSTRACT

Introduction: TMTP1 (NVVRQ) is a novel tumor-homing peptide, which specifically targets tumor metastases, even at the early stage of occult metastasis foci. Fusing TMTP1 to therapeutic peptides or proteins can increase its anti-cancer efficacy both in vivo and in vitro. Here, we labeled TMTP1 with ^{99m}Tc to evaluate its targeting properties in an ovarian cancer xenograft tumor mouse model and a gastric cancer xenograft mouse model.
Methods: The invasion ability of SKOV3 and highly metastatic SKOV3.ip cell lines were performed by the Transwell Invasion Assays, and then Rhodamine-TMTP1 was used to detect its affinity to these two cells. Using the co-ligand ethylenediamine-*N*, *N*′-diacetic acid (EDDA) and the bifunctional chelator 6-hydrazinonicotinic acid (HYNIC), the TMTP1 peptide was labeled with ^{99m}Tc. A cell-binding assay was performed by incubating cancer cells with ^{99m}Tc-HYNIC-TMTP1 with or without an excess dose of cold HYNIC-TMTP1. To evaluate the probe in vivo, nude mice bearing SKOV3, SKOV3.ip and MNK-45 tumor cells were established and subjected to SPECT imaging after injection with ^{99m}Tc-HYNIC-TMTP1. Ex vivo γ-counting of dissected tissues from the mice was used to evaluate its biodistribution.
Results: ^{99m}Tc-HYNIC-TMTP1 was successfully synthesized. The radiotracer also exhibited high hydrophilicity and excellent stability in vitro and in vivo. It has strong affinity to highly metastatic cancer cell lines but not to poorly metastatic cell lines. After mice were injected with ^{99m}Tc-HYNIC-TMTP1, non-invasive SPECT imaging detected SKOV3.ip and MNK-45 xenograft tumors but not SKOV3 xenograft tumors. This result can be inhibited by excess HYNIC-TMTP1. The uptake of ^{99m}Tc-HYNIC-TMTP1 in SKOV3.ip xenograft tumors was 0.182 ± 0.017% ID/g at 2 h p.i. with high renal uptake (74.32 ± 15.05% ID/g at 2 h p.i.).
Conclusion: ^{99m}Tc-HYNIC-TMTP1 biodistribution and SPECT imaging demonstrated its ability to target highly metastatic tumors. Therefore, metastasis can be non-invasively investigated by SPECT imaging using ^{99m}Tc-HYNIC-TMTP1. Meanwhile, this radiotracer has some shortages in the low % ID/g of tumors and high accumulation in the kidney.

1. Introduction

Metastasis is a significant factor for selecting treatment programs and estimating prognosis [1,2]. The majority of ovarian cancers are diagnosed at a late stage, and fifty percent of patients will die within 5 years of diagnosis [3]. Peritoneal metastases are often the first presentation of ovarian malignancy. Evaluating the extent of disease critically determines tumor resectability and can also predict outcome. Thus, it is critical to diagnose ovarian cancer at an early stage and peritoneal metastases. Contrast-enhanced CT has routinely been used in the staging of ovarian cancer. However, owing to poor contrast between peritoneal implants and adjacent normal structures, the sensitivity of this technique is decreased to 7–25% when maximal diameter is <1 cm [4,5]. Hence, the site of malignant tumor may be not detected by conventional imaging techniques. PET/CT hybrid imaging may increase detectable rate of ovarian cancer, but increased ^{18} F-FDG uptake is associated with malignancy as well as physiological or inflammatory hypermetabolic activity. So it is necessary to find a useful diagnostic tool for detecting ovarian cancer [5–7].

Small tumor-homing peptides are another approach for delivering radioactivity to tumors. Linked to an appropriate radionuclide, tumor-targeting peptides are increasingly considered a promising strategy for molecular imaging and therapy [8,9]. For instance, the radiolabelled RGD peptides, Octreotide (TOC) and NGR are currently under development and undergoing clinical trials [10,11].

Using a bacterial peptide display system, we previously identified a novel peptide, TMTP1(NVVRQ), that can bind specifically to highly metastatic tumor cells but not to poorly metastatic and normal cells

* Correspondence to: X. Zhu, 1095 Jiefang Avenue, Wuhan, Hubei 430030, China. Tel.: +86 2783663244; fax: +86 2783663446.
** Correspondence to: L. Xi, 1095 Jiefang Avenue, Wuhan, Hubei Province, 430030, China. Tel.: +86 27 83662681; fax: +86 27 83662779.
E-mail addresses: evazhu@vip.sina.com (X. Zhu), lxi@tjh.tjmu.edu.cn (L. Xi).

http://dx.doi.org/10.1016/j.nucmedbio.2014.11.001
0969-8051/

[12]. TMTP1 also strongly and specifically targets metastatic foci in tumor-bearing mice. Recently, researchers developed some derivatives of TMTP1, including DT390-triTMTP1, TMTP1-GG-$(KLAKLAK)_2$ and TMTP1-TAT-NBD, with a remarkable ability to target highly metastatic tumors and with the most powerful toxicity against cancer[13–16].

TMTP1 could be a promising candidate peptide-based probe for targeted imaging of metastases. However, radiolabelled TMTP1 derivatives have not been investigated using single-photon emission computed tomography (SPECT) or PET imaging. Therefore, in this study, we labeled TMTP1 with 99mTechnetium and evaluated its potential to target highly metastatic ovarian tumors in vitro and in vivo. Meanwhile, we chose highly metastatic MNK-45 cell as a positive control in terms of previous research [12–14,17].

2. Materials and methods

2.1. Materials and equipment

Tricine (*N*-[Tris(hydroxymethyl)methyl]glycine), EDDA (ethylenediamine-*N*,*N*′-diacetic acid) and $SnCl_2$ were purchased from Sigma/Aldrich (St. Louis, MO, USA). Rhodamine-TMTP1 and HYNIC-TMTP1 (HYNIC-E-G-Cyclo(CGNVVRQGC)) were synthesized by GL Biochem (Shanghai) Ltd. $Na^{99m}TcO_4$ was produced according to standard procedures using the 99Mo/99mTc generator in the nuclear medicine department of Tongji Hospital.

Instant thin-layer chromatography silica-gel paper strips (iTLC-SG) and RP-HPLC were used to obtain radiochemical purity. Sep-pak c18 cartridges (Waters Company, America) were prepared for purifying ^{99m}Tc-HYNIC-TMTP1. Radioactivity was measured by a Radio-immune Gamma Counter (Anhui USTC Zonkia Scientific Instruments, Hefei, Anhui, China), and planar imaging studies were performed on a SPECT MPR (GE, USA) with the ^{99m}Tc Thyroid Scan procedure.

2.2. Radiochemistry

In a rubber-sealed vial, 8 nmol of HYNIC-TMTP1 was incubated with 1 mL of EDDA/tricine solution (112 mM tricine, 57 mM EDDA in PBS buffer, pH 6–7), 1 mL of $^{99m}TcO_4$ solution (230 MBq) and 20 μL of tin (II) solution (10 mg of $SnCl_2$ in 10 mL of 0.1 N HCl) for 15 min at 100 °C. Reactions were then cooled down at room temperature [18]. The ratio among free 99mTc, colloid and the radiolabeled peptides was performed using thin layer chromatography-silica gel plates and RP-HPLC. For TLC analysis, three different mobile phases were used: acetone was used to determine the amount of free $^{99m}TcO_4$ ($R_f = 1$); 0.1 M sodium citrate (pH 5) was used to determine 99mTc-co-ligand and $^{99m}TcO_4$ ($R_f = 1$); and methanol: 1 M ammonium acetate (1:1 v/v) was used for 99mTc-colloid ($R_f = 0$). The Rf values of the radiolabeled peptide in each system were 0.0, 0.0 and 0.7–1.0, respectively [19]. HPLC analyses were performed with a Shimadzu instrument (Shimadzu, Kyoto, Japan) running with both radioactivity and UV-photo-diode array in-line detectors and an Agilent C18 column. A gradient using 0.1% TFA/water as solvent A and 0.1% TFA/acetonitrile as solvent B was use at a flow rate of 1 ml/min. The gradient began at 100% solvent A for 3 min, was changed to 50% solvent A over 10 min and was maintained thus for 10 min; it was then changed to 30% solvent A over 3 min and finally returned to 100% solvent A over 4 min. In this system, the retention time for free 99mTc, 99mTc-coligand and ^{99m}Tc-HYNIC-TMTP1 were 1.6, 2.2 and 13.0–13.6 min, respectively. [20]. A reverse-phase Sep-Pak C-18 cartridge was preconditioned with 5 mL of ethanol followed by 5 mL of 0.9% NaCl solution and 5 mL of air. An aliquot of 2 mL of the labeled peptide was loaded on the preconditioned column followed by 5 mL of 0.9% NaCl solution to elute free $^{99m}TcO_4$. The radiolabeled peptide was eluted with 0.2 mL of 70% ethanol. The second and third drops were collected (80% of total radioactivity), and the first drop and the remaining solution were discarded.

2.3. In vitro stability

^{99m}Tc-HYNIC-TMTP1 was dissolved in 1 mL of saline or cysteine solution (1 mg/mL), incubated at room temperature and analyzed by iTLC-SG at 0, 1, 3, 6, and 18 h post-incubation.

The study of metabolic stability was performed in human serum. Human serum from healthy donors was incubated at 37 °C with ^{99m}Tc-HYNIC-TMTP1 for different time periods (1, 3, 6, and 18 h). After incubation, a sample of 250 μL was precipitated with 750 μL of acetonitrile/ethanol ($V_{acetonitrile}/V_{ethanol} = 1:1$) and centrifuged (3 min at 3000 rpm). The supernatants were passed through a filter and analyzed by RP-HPLC and iTLC-SG. The results were plotted as the radiochemical purity (RCP) at different time points [21,22]. The metabolic stability was also performed in BALB/c mice, the blood samples of three mice were withdrawn immediately from the retroorbital sinus at 4 h post-injection, and the following steps were fulfilled as above described.

2.4. Log D values

^{99m}Tc-labeled HYNIC-TMTP1 (0.5 mL) in phosphate-buffered saline (PBS) was added to 0.5 mL of octanol in an Eppendorf vial. The tube was vigorously vortexed over a period of 15 min and centrifuged at 5000 g for 3 min. A 100 μL aliquot of the aqueous and octanol layers was collected and counted in a Radio-immune Gamma Counter, and log P values were calculated [22].

2.5. Cell culture

The MNK-45 cell line was cultured in RPMI 1640 medium supplemented with 10% fetal serum at 37 °C in a humidified 5% CO_2 atmosphere. The SKOV3.ip cell line was established from ascites that developed in a nude mouse given an i.p. injection of SKOV3 cells. Paired highly metastatic human serous ovarian cancer cells (SKOV3.ip) and poorly metastatic human serous ovarian cells (SKOV3) were cultured in DMEM medium supplemented with 10% fetal serum [23,24].

2.6. Invasion assay

The invasion assay was performed using BD Biocoat matrigel invasion chambers following the manufacturer's instructions. In brief, 5×10^4 cells were placed on the upper chambers in 100 μL of serum-free medium. The lower chambers were filled with conditioned NIH-3 T3 medium. After 48 h, the matrigel membranes were stained with crystal violet and the migratory cells were counted under a microscope. This experiment was repeated in triplicate independently.

2.7. Immunofluorescence staining and fluorescence microscopy

A total of 2×10^4 tumor cells were seeded onto glass cover slips and cultivated for 24 h. The culture medium was replaced with 1 mL of fresh RPMI-1640 medium supplemented with 10% fetal calf serum. Then, 1 μM rhodamine-conjugated peptide was added to the cell culture and cultivated for 120 min in a 37 °C atmosphere containing 5% CO_2. The cells were washed thrice with phosphate-buffered saline (pH 7.2) and fixed with 4% paraformaldehyde for 7 minutes. The nuclei were then stained with DAPI. The tumor cells were examined by fluorescence microscopy (Nikon, Tokyo, Japan) and by laser scanning confocal microscopy (Olympus, Tokyo, Japan) [12,16].

2.8. Cell binding assay at different incubation times

Three tumor cells were grown in culture until a sufficient number of cells were available. The cells were seeded into 24-well plates at a density of 1.25×10^5 cells per well and incubated overnight at 37 °C to allow adherence. After briefly washing with PBS, tumor cells were