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SPECT/CT imaging of HER2 expression in colon cancer-bearing nude mice using ^{125}I-Herceptin

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ABSTRACT

An accurate assessment of HER2 status in patients with colorectal cancer is very important, because only the patients overexpressing HER2 can benefit most from the anti-HER2 targeted therapy. In this study, we investigated the feasibility of detecting HER2 expression in colon cancer by SPECT imaging using ^{125}I-Herceptin, which showed high labeling rate, good *in vitro* stability and high binding specificity for HER2. HER2-positive mouse colon adenocarcinoma cell line (MC 38) was chosen as the colon caner cell model, and used for the establishment of colon cancer-bearing nude mice model. SPECT/CT imaging suggested that the tumors can be visualized at 12 h after the injection of ^{125}I-Herceptin, and the uptake of tracer in tumors reached the peak at 24 h after injection, and can be attenuated significantly by pretreatment with an excess of nonlabeled Herceptin. These results indicates that ^{125}I-Herceptin can be considered as an effective SPECT probe for the non-invasive detection of HER2 expression in colon cancer.

1. Introduction

Human epidermal growth factor receptor type 2 (hEGFR2, commonly referred to as HER2) is a receptor tyrosine kinase that is mainly involved in the regulation of cell survival, growth and differentiation [1]. It is noteworthy that HER2 overexpression has been found in multiple types of cancers, including breast cancer, gastric cancer, ovarian cancer and colorectal cancer, and is associated with poor therapeutic efficacy and prognosis [1–3]. The so-called anti-HER2 therapy, which disrupts the related molecular processes by targeting HER2 with antibodies or small molecular inhibitor, has been proven to be effective in HER2-positive cancers, but not ideal in HER2-negative cancers [4,5]. Therefore, an accurate assessment of HER2 status *in vivo* is very important, because only the "right patients" can benefit most from the HER2-taregted therapy. Routinely, HER2 expression can be evaluated in primary tumor tissue by IHC, ELISA and western blotting for HER2 protein, or PCR and FISH for HER2 gene amplification [6,7]. But these methods are invasive, and sometimes can not represent the metastases. Fortunately, non-invasive molecular imaging of HER2 using radiolabeled tracer can overcome these flaws and offer whole body and dynamic information about HER2 status, providing an ideal method for HER2 detection [8].

Colorectal cancer (CRC) is one of the most common malignant cancers, and the reported HER2-positivity rate in CRC is 1.6%–47.7% [9,10]. HER2 as a therapeutic target for CRC has been identified, and HER2-targeted therapeutic strategy has the potential to adjust and further optimize the treatment paradigm for the CRC patients according to the HER2 status [3]. As analyzed above, it is very necessary to evaluate the HER2 status in CRC before the targeted therapy application. Radionuclide ^{125}I has been widely used to radiolabel monoclonal antibody (mAb) in the field of SPECT molecular imaging, because ^{125}I is not only readily available and low cost, but also can be coupled to mAb by a relative simple mothod. In this study, Herceptin (Trastuzumab), the first FDA-approved humanized IgG1 mAb for the treatment of early and advanced breast cancer overexpressing HER2, was radiolabeled with ^{125}I, and the feasibility of the synthesized ^{125}I-Herceptin as a SPECT imaging probe for the *in vivo* HER2 detection in colon cancer-bearing nude mice model was tested.

2. Materials and methods

2.1. Materials

Herceptin was purchased from Shanghai Roche Pharmaceuticals

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Co., Ltd. and used without purification. Iodogen was purchased from Sigma-Aldrich Chemical Co. (Steinheim am Albuch, Germany). Na[^{125}I] solution was purchased from Shanghai Xinke Pharmaceutical Company. The mouse colon adenocarcinoma cell line (MC 38) was obtained from Shanghai Zishi Biotechnology Co., Ltd. RPMI 1640 medium and foetal bovine serum (FBS) was purchased from Gibco Life Technologies (New York, USA). Mouse anti-HER2 monoclonal antibody was purchased from Bioss Antibodies Biosynthesis Biotechnology Co., Ltd (Beijing, China), and the secondary antibody FITC-conjugated goat anti-mouse IgG (H + L) was purchased from Beyotime Institute of Biotechnology (Jiangsu, China). Other general agents were commercially available.

2.2. Preparation of ^{125}I-Herceptin

Herceptin was directly radiolabeled with ^{125}I using the Iodogen method. In brief, 18.5 MBq ^{125}I and 50 μg Herceptin in 55 μL water were added into an Eppendorf tube containing 40 μg of Iodogen. After incubating for 10 min at room temperature, the reaction was stopped by separating the supernatant liquid (^{125}I-Herceptin solution) and Iodogen.

2.3. The labeling rate and in vitro stability of ^{125}I-Herceptin

^{125}I-Herceptin solution (about 0.5 μL) was spotted on the origin point of Xinhua No.1 chromatography filter paper with saline as the mobile phase. The thin layer chromatography (TLC) migration was analyzed using a mini-scan radio TLC scanner with a flow-count detector (Eckert & Ziegler Radiopharma, Inc. Germany), and the quantification of radioactive bands was performed to calculate the labeling rate of ^{125}I-Herceptin using the system software.

For the stability test, ^{125}I-Herceptin was mixed with physiological saline or RPMI-1640 medium containing 10% FBS, and the mixture was incubated at 37 °C. At different time points (0.5, 1, 4, 8, 12, and 24 h), aliquots of physiological saline or FBS solution were collected, and the radiochemical purity of ^{125}I-Herceptin, which represents the *in vitro* stability, was measured according to the above-stated method.

2.4. Cell culture and immunofluorescence staining

MC38 cells were cultured in RPMI-1640 medium containing 10% FBS at 37 °C in a humidified incubator with 5% CO_2. The expression of HER2 in MC38 cells was identified by immunofluorescence staining method. Brifely, MC38 cells were seeded and cultured in a 35 mm diameter culture dish containing a coverslip at the bottom overnight at 37 °C. After this, cells were washed with ice-cold PBS, fixed with formaldehyde, blocked with 1% BSA, incubated with anti-HER2 antibody and stained with secondary antibody FITC-conjugated goat anti-mouse IgG following the experimental instruction. At last, cellular nuclei were stained with DAPI, and immunofluorescence images were obtained using a fluorescence microscopy.

2.5. Cell binding assay

MC38 cells were seeded in a 24-well plate at a density of 1×10^5 cells/well, and a dilution series of ^{125}I-Herceptin (1–120 nM in PBS) was added to each well. Cells were incubated at 4 °C for 2 h, and then the free radioactivity was removed by washing cells three times with ice-cold PBS. The cells were harvested from each well using 0.1 M NaOH, and the cell-bound radioactivity was measured by a γ-counter. To determine the nonspecific binding, various concentrations of ^{125}I-Herceptin and the corresponding 1000 times of unlabeled Herceptin were added to each well and co-incubated under identical experimental conditions. These experiments were performed in triplicate. The specific bind (SB) was calculated by subtracting nonspecific binding (NSB) from total binding (TB). The saturation curves, in which the concentration of ^{125}I-Herceptin was set as the abscissa and the radioactive counts of TB, NSB or SB were set as the vertical axis, were fitted and the K_D value was calculated using GraphPad Prism 5.

2.6. Establishment of colon cancer-bearing nude mice model

The nude mice were obtained from the Laboratory Animal Center of the Second Military Medical University, and all animal procedures were in agreement with the guidelines of the Institutional Animal Care and Use Committee. Six to eight weeks nude mice with a body weight of 20 g were chosen for the establishment of colon cancer animal model. 100 μL of MC38 cells (2×10^7 cells/mL) in the logarithmic growth phase were prepared and subcutaneously injected in the right side near the armpit of each nude mouse, and the tumors were observed and used for next step experiment until their diameters reached about 10 mm.

2.7. SPECT/CT imaging study

Colon cancer-bearing nude mice were anesthetized and injected with ^{125}I-Herceptin (18.5 MBq/40 μg per mouse) through the tail vein. SPECT/CT imaging was performed at different time points (6, 12, 24, 48 h after the injection of tracer) using the clinical used SPECT/CT scanner (Symbia T16, Siemens, Germany) equipped with a pinhole collimator. The image acquisition parameter was set as follow: energy peak, 27 KeV; window width, ± 10%; matrix, 64 × 64; zoom, 1.23. After imaging finished, coregistration of SPECT and CT images were performed, and the ^{125}I-Herceptin uptake ratios of tumor to muscle at different times were measured by a ROI technique. According to the results of SPECT imaging, the uptake of ^{125}I-Herceptin tumor reached the maximum at 24 h after injection. So, in the blocking experiment, 4 h before the injection of ^{125}I-Herceptin, colon cancer-bearing nude mice were injected with an excess of unlabeled Herceptin (4 mg per mouse), and then SPECT/CT imaging was performed at 24 h after injection of the radiotracer by adopting the above-mentioned image acquisition condition. In order to assess the *in vivo* biodistribution of ^{125}I-Herceptin, the radioactivity in tumor and normal tissue (e.g., muscle, brain, bone, liver, kidney, stomach, gut, thyroid) were obtained from the mean values measured by ROI, and the ratios of T/NT were calculated.

2.8. Statistical analysis

Quantitative data are expressed as the means ± SD and analyzed by the *t*-test of two sample mean comparison. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Radiolabeling and characterization of ^{125}I-Herceptin

Radioiodination of Herceptin was performed using Iodogen method, and the synthesized ^{125}I-Herceptin has a very high labeling rate, which was about 99% (Fig. 1A), suggesting it can be used directly for the subsequent experiments without additional purification. The specific activity of ^{125}I-Herceptin was about 5.55 MBq/nmol. For the stability test, there was almost no deiodination of the mAb occurred in physiological saline or medium-containing 10% FBS at 37 °C within 4 h, and the dissociation was still lower than 20% even up to 24 h after incubation, indicating good *in vitro* stability of ^{125}I-Herceptin (Fig. 1B).

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