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Radiolabeled antibodies in prostate cancer: A case study showing the effect of host immunity on antibody bio-distribution

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ABSTRACT

Objectives: Human tumors xenografted in immunodeficient mice are crucial models in nuclear medicine to evaluate the effectiveness of candidate diagnostic and therapeutic compounds. However, little attention has been focused on the biological profile of the host model and its potential effects on the bio-distribution and tumor targeting of the tracer compound under study. We specifically investigated the dissimilarity in bio-distribution of ^{111}In-DTPA-5A10, which targets free prostate specific antigen (fPSA), in two animal models.
Methods: In vivo bio-distribution studies of ^{111}In-DTPA-5A10 were performed in immunodeficient BALB/c-nu or NMRI-nu mice with subcutaneous (s.c.) LNCaP tumors. Targeting-specificity of the tracer was assessed by quantifying the uptake in (a) mice with s.c. xenografts of PSA-negative DU145 cells as well as (b) BALB/c-nu or NMRI-nu mice co-injected with an excess of non-labeled 5A10. Finally, the effect of neonatal Fc-receptor (FcRn) inhibition on the bio-distribution of the conjugate was studied by saturating FcRn-binding capacity with nonspecific IgG1.
Results: The inherent biological attributes of the mouse model substantially influenced the bio-distribution and pharmacokinetics of ^{111}In-DTPA-5A10. With LNCaP xenografts in BALB/c-nu mice (with intact B and NK cells but with deficient T cells) versus NMRI-nu mice (with intact B cells, increased NK cells and absent T cells), we observed a significantly higher hepatic accumulation (26 ± 3.9 versus 3.5 ± 0.4%IA/g respectively), and concomitantly lower tumor uptake (25 ± 11 versus 52 ± 10%IA/g respectively) in BALB/c-nu mice. Inhibiting FcRn by administration of nonspecific IgG1 just prior to ^{111}In-DTPA-5A10 did not change tumor accumulation significantly.
Conclusions: We demonstrated that the choice of immunodeficient mouse model importantly influence the bio-distribution of ^{111}In-DTPA-5A10. This study further highlighted important considerations in the evaluation of pre-clinical tracers, with respect to gaining information on their performance in the translational setting. Investigators utilizing xenograft models need to assess not only radiolabeling strategies, but also the host immunological status.

1. Introduction

There is an urgent need for molecular imaging methods to sensitively and accurately detect and monitor aggressive forms of prostate cancer. Initially proposed over half a century ago, radiolabeled antibodies successfully target tumor-specific molecules and provide an attractive platform for non-invasive *in vivo* imaging in nuclear medicine. Radiolabeled antibodies, with high affinity for antigens that are either disease-specific or overexpressed in disease states, accumulate at disease sites. This activity can be measured by planar or tomographic imaging [1]. Continual refinements in instrumentation, antibody production and radiochemistry over the intervening decades have made antibody imaging one of the most promising tools for staging and monitoring disseminated prostate cancer (PCa).

While many novel radiotracers have been developed for the screening and diagnosis of prostate cancer, translation into clinical practice has proven challenging. Candidate molecules often accrue through the *in vitro* screening of species-specific proteins that are up-regulated in

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human cancer cell lines. Subsequently, tumor-specific accumulation of a tracer in subcutaneous and homogeneous antigen-positive xenografts in immune deficient mice is regarded as preclinical evidence of success. The definition of tumor-specific uptake is often based on criteria which focus on the significantly lower uptake in tumors deprived of the targeted protein, or tumors in which the radiolabeled tracer has been successfully blocked by an excess of unlabeled compound.

Studies of antibody elimination in rodents have shown that clearance is depending on immunodeficiency profile and endogenous levels of antibodies [2,3]. Surprisingly little attention has been paid to the potential impact of the immunological profile of the host model on tumor uptake of the tracer, and the possible effects this may have on the critical outcomes of bio-distribution and tumor targeting.

Recently, members of our research group reported on the development of a Zirconium-89 labeled PET tracer, ^{89}Zr-DFO-5A10, which specifically targets a unique epitope only available on free (uncomplexed) forms of prostate specific antigen, fPSA [4]. PSA is an androgen-regulated kallikrein-related serine protease that is exclusively expressed in human prostatic epithelial tissue, including malignantly deranged forms [5]. PSA is best known for its use as a blood biomarker for prostate cancer [6] in humans and naturally deficient in mice [7]. Approximately, only one millionth of the PSA present in prostate epithelium is normally released into the blood [8,9]. Upon reaching the perivascular space, a predominant proportion of PSA is covalently and irreversibly bound to abundant extra-cellular proteinase inhibitors and converted to a non-catalytic complexed form (complexed PSA, cPSA) [10]. By employing a probe that specifically recognizes a unique epitope overlapping the catalytic cleft of PSA that is not available on complexed PSA [11,12]. Thus, the complexed forms of PSA in blood are excluded from being targeted.

Here, we utilized an ^{111}In-labeled 5A10 mouse antibody to investigate the potential influence of mouse models on the kinetics and bio-distribution of tracers. Human prostate cancer cell lines (LNCaP, DU145) were subcutaneously inoculated into two commonly-employed immunodeficient mouse strains: (a) NMRI-nu mice, characterized as athymic with a very low T cell population that responds poorly to T cell-dependent antigen presentation; (b) BALB/c-nu mice, also regarded as athymic but with no T cell population. Both strains have intact B cells with antibody response confined to IgM class. Both strains have natural killer (NK) cells intact. However, the level of NK cells in NMRI-nu mice is particularly increased in comparison to normal NMRI mice.

Our results highlight the importance of mouse models, in particular the immunological competence (or lack thereof), in the interpretation of pre-clinical targeted imaging experiments in general.

2. Materials and methods

All reagents were purchased from Thermo Scientific, unless otherwise stated.

2.1. Conjugation and radiolabeling

5A10, the murine IgG1, (provided by the University of Turku, Finland) was conjugated with the chelator CHX-A"-DTPA (Macrocyclics Inc.) as follows: A solution of 5A10 in PBS was adjusted to pH 9.2 using 0.07 M sodium borate buffer (Sigma Aldrich), and thereafter concentrated on an Amicon Ultra-2 centrifugal filter (2 mL, 100 K; Millipore). The chelator compound CHX-A"-DTPA was then added to the protein solution in a molar ratio of 3:1 (chelator to antibody), and incubated with gentle shaking at 40 °C. The reaction was terminated after 4 h. The next step was separating CHX-A"-DTPA-5A10, hereafter referred to as DTPA-5A10, from the free chelator by size-exclusion chromatography on an NAP-5 column (GE Healthcare) equilibrated with 20 mL 0.2 M ammonium acetate buffer, pH 5.5. Finally, conjugated 5A10 was eluted with 1 mL ammonium acetate buffer, and aliquot samples were stored at −20 °C.

For radiolabeling, typically 300 μg DTPA-5A10 (1 μg/μL in 0.2 M ammonium acetate buffer, pH 5.5) was mixed with a predetermined amount (~15 MBq) of $^{111}InCl_3$ (Mallinkrodt Medical). After incubating the mixture at room temperature for 1.5–2 h, the labeling was terminated and the radiotracer purified on an NAP-5 column, equilibrated with PBS. Labeling efficiency and kinetics were monitored with instant thin layer chromatography (Biodex), eluted with 0.2 M citric acid (Sigma Aldrich). In this system, the radiolabeled conjugate remains at the origin line, while free ^{111}In migrates with the front of the solvent. The radioactive distribution was determined with a Cyclone Storage Phosphor System using the Optiquant quantification software (both from Perkin Elmer).

2.2. In vitro stability studies

To assess the stability of the radio-immunoconjugate ^{111}In-DTPA-5A10, the compound was incubated in triplicate at 37 °C in murine serum from NMRI-nu (Taconic) and BALB/c-nu mice (Charles River), respectively. 10 μL of DTPA-5A10 was mixed with 100 μL of each strain's mouse serum. Approximately 20 μL of each sample was collected from the two DTPA-5A10 mixtures at 2, 3 and 9 days of incubation and analyzed by SDS-PAGE on NuPAGE 4–12% Bis-Tris gel (Invitrogen) in MES buffer (200 V constant, ~50 min). ^{111}In-DTPA and free ^{111}In diluted in PBS were run in parallel with the incubated sample as controls. The distribution of the samples along the gel was evaluated using Cyclone Storage Phosphor System (Perkin Elmer).

2.3. Cell lines

PSA-expressing LNCaP and control DU145 (PSA-negative) cell lines were purchased from the American Type Culture Collection. Cells were cultured in RPMI 1640 medium enriched with 10% fetal bovine serum, to which penicillin 100 IU/mL and 100 μg/mL streptomycin were added. The cells were maintained at 37 °C in a humidified incubator at 5% CO_2 and were detached with trypsin-EDTA solution.

2.4. Animal models

All animal experiments were conducted in compliance with the national legislation on laboratory animals' protection, and with the approval of the Ethics Committee for Animal Research (Lund University, Sweden). The experimental hosts were 6–8 week old male immunodeficient mice of BALB/c-nu and NMRI-nu strains, with the average weights of 25 g and 30 g, respectively. All mice were inoculated in the right flank by subcutaneous injection of 5–8 × 10^6 tumor cells in a 200 μL cell suspension of 1:1 mixture of medium with Matrigel (BD Biosciences). We did not note any difference in levels of free, complexed and total PSA in these models (data not shown).

2.5. Bio-distribution studies

Bio-distribution studies were conducted to evaluate the uptake of ^{111}In-DTPA-5A10 in human prostate cancer xenograft models, as well as in non-tumor bearing mice. All mice received either 20 μg or 50 μg of ^{111}In-DTPA-5A10 [0.4–0.6 MBq (11–16 μCi)] in approximately 100 μL PBS through tail-vein injection. The animals (n = 3–4 per group) were euthanized by intraperitoneal (i.p.) injection of Ketalar–Rompun solution (20 μL of solution per gram of body weight; Ketalar, 10 mg/ml; Rompun 1 mg/ml; from Pfizer and Bayer Animal Health, respectively) at 4, 24, 48, 72 and 168 hours post-injection. Blood was immediately harvested by cardiac puncture. Twelve tissue types (including tumor) were removed from each animal and placed in plastic 20 mL vials (Zinsser Analytic). After weighing, their ^{111}In radioactivity was assayed using a NaI(Tl) well counter (1480 Wizard, Wallac). The mass of tracer injected into each animal was measured and used to determine the count rate by comparison with a standard syringe