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A novel Indium-111-labeled gonadotropin-releasing hormone peptide for human prostate cancer imaging

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Abstract

The purpose of this study was to evaluate the tumor targeting and imaging properties of a novel ¹¹¹In-labeled gonadotropin-releasing hormone (GnRH) peptide {1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-Ahx-(D-Lys⁶-GnRH1)} for human prostate cancer. The biodistribution and tumor imaging properties of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) were determined in DU145 human prostate cancer-xenografted nude mice. ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) exhibited rapid tumor uptake (1.27 ± 0.40 %ID/g at 0.5 h post-injection) coupled with fast whole-body clearance through the urinary system. The DU145 human prostate cancer-xenografted tumor lesions were clearly visualized by single photon emission computed tomography (SPECT)/CT at 0.5 h post-injection of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1). The successful imaging of DU145 human prostate cancer-xenografted tumor lesions using ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) highlighted its potential as a novel imaging probe for human prostate cancer imaging.

Keywords

Gonadotropin-releasing hormone receptor; receptor-targeting peptide; prostate cancer; imaging

Prostate cancer was the most commonly diagnosed cancer in males (217,730 new cases) and the second leading cause of cancer-related death among men (32,050 fatalities) in the United States in 2010.¹ Upon diagnosis by current detection regimens, about 30% of patients have metastases.^{2,3} Approximately 50% of patients eventually develop metastases.⁴ Survival times for patients with metastases are generally 2~3 years from the time when the metastases are diagnosed.⁵ [5]. Unfortunately, current prostate cancer treatments (radical prostatectomy, chemotherapy, immunotherapy, hormonal therapy and radiation therapy) are far from satisfactory and no curative treatment exists for metastatic prostate cancer. Early diagnosis of prostate cancer is critical for appropriate treatment decisions and may provide the patients the best opportunities for cures or prolonged survivals.

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Currently, prostate-specific antigen (PSA) test is the first-line clinical screening tool for prostate cancer. However, PSA test is lack of sensitivity and specificity since PSA is an organ-specific biomarker rather than a cancer-specific biomarker. Approximately 43% of patients with organ-confined prostate cancer do not have elevated PSA levels,⁶ indicating the high percentage false-negative rate of the PSA test. On the other hand, benign prostatic hyperplasia (BPH), which is extremely common in men, commonly results in elevated PSA levels.⁷ Hence, PSA test has 60-80% false-positive findings based on prostate biopsies.⁸

The clinical single photon emission computed tomography (SPECT) scan using ¹¹¹In-capromab Pendetide (ProstaScint® Scan, Cyt-356) targeting the prostate-specific membrane antigen (PSMA) provides more accurate localization and staging of a new or recurrent prostate cancer than the PSA test.⁹⁻¹² However, relatively low sensitivity (62%) and overall accuracy (68%) of the ProstaScint® scan limits its widespread application. At present, 2-[¹⁸F]fluoro-2-deoxy-D-glucose ([¹⁸F]FDG) is the most commonly used positron emission tomography (PET) imaging agent for the detection of various tumors including prostate cancer.¹³⁻¹⁶ However, the delineation of prostate cancer by PET with [¹⁸F]FDG is generally unsatisfactory due to the low uptake of [¹⁸F]FDG in prostate cancer.^{15,16} Relatively low sensitivity (60-70%) of [¹⁸F]FDG PET is due to the fact that the glucose utilization is not significantly higher in prostate cancer cells than that in normal cells. The limited clinical application of ProstaScint® and [¹⁸F]FDG underscores the urgent need for novel cancer-specific imaging probes for prostate cancer detection.

Over-expression of gonadotropin-releasing hormone (GnRH) receptors on prostate cancer cells and specimens, dramatic low level expression on healthy prostate cells and no expression on most normal tissue cells¹⁷⁻²⁵ highlights the potential of GnRH receptor as a distinct molecular target for developing novel prostate cancer-specific imaging probes. Native GnRH peptide is a peptide with 10 amino acids (pGlu¹-His²-Trp³-Ser⁴-Tyr⁵-Gly⁶-Leu⁷-Arg⁸-Pro⁹-Gly¹⁰-NH₂). Both pGlu¹-His²-Trp³ and Arg⁸-Pro⁹-Gly¹⁰-NH₂ motifs are crucial for GnRH receptor binding.²⁶ A backbone metal cyclization between the N-terminus and C-terminus of the GnRH peptides dramatically decreased their GnRH receptor binding affinities,²⁷ confirming that both N-terminus and C-terminus need to be reserved for strong GnRH receptor binding. The replacement of Gly⁶ with a D-amino acid enhances the binding affinity and reduces the metabolic clearance of the peptide.²⁸ [28].

We have been interested in developing radiolabeled GnRH peptides to target the GnRH receptors for cancer detection.²⁹ Specifically, we coupled the radiometal chelator DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) to both epsilon and alpha amino group of D-Lys⁶ in D-Lys⁶-GnRH via an aminohexanoic acid (Ahx) linker to generate novel DOTA-Ahx-(D-Lys⁶-GnRH1) and DOTA-Ahx-(D-Lys⁶-GnRH2),²⁹ respectively. The introduction of the Ahx hydrocarbon linker between the DOTA and D-Lys⁶-GnRH enhanced the lipophilicity of the moiety attached to D-Lys⁶-GnRH, which was favorable for GnRH receptor binding.³⁰ Interestingly, DOTA-Ahx-(D-Lys⁶-GnRH1) displayed 36.1 nM GnRH receptor binding affinity, whereas DOTA-Ahx-(D-Lys⁶-GnRH2) exhibited 10.6 mM GnRH receptor binding affinity.²⁹ These receptor binding results clearly demonstrated that the epsilon amino group was more suitable for DOTA conjugation. Moreover, the successful imaging of MDA-MB-231 human breast cancer-xenografted tumor lesions (GnRH receptor-positive) using ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) suggested its potential as a novel imaging probe for human breast cancer imaging.²⁹ In attempt to extend the application on this novel peptide for human prostate cancer imaging, we determined the tumor targeting and imaging properties of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) in DU145 human prostate cancer-xenografted nude mice in this study.

DOTA-Ahx-(D-Lys⁶-GnRH1) was synthesized according to our published procedure.²⁹ DOTA-Ahx-(D-Lys⁶-GnRH1) was readily synthesized and purified by RP-HPLC. The peptide displayed greater than 90% purity after the HPLC purification. The identity of the peptide was confirmed by electrospray ionization mass spectrometry. ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) (Fig. 1) was readily prepared with greater than 95% radiolabeling yield in a 0.5 M NH₄OAc buffer at pH 4.5. Figure 2 illustrates the radioactive HPLC profile of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) and UV profile of DOTA-Ahx-(D-Lys⁶-GnRH1). ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) was completely separated from the excess DOTA-Ahx-(D-Lys⁶-GnRH1) by RP-HPLC. The retention times of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) and DOTA-Ahx-(D-Lys⁶-GnRH1) were 12.0 and 8.3 min, respectively.

The specific GnRH receptor binding of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) was determined using human GnRH receptor preparations obtained from Millipore, Inc (Billerica, MA). Approximately 86% of the binding of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) was competed off by 1 μM of DOTA-Ahx-(D-Lys⁶-GnRH1) peptide (Fig. 2C). The GnRH receptor expressions on DU145 human prostate cancer-xenografted tumor slices were confirmed by immunohistochemistry staining. The staining results are presented in Figure 3. The GnRH receptor expressions were positively stained in DU145 human prostate cancer-xenografted tumors. Thus, we determined the tumor targeting and pharmacokinetic properties of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) in DU145 human prostate cancer-xenografted nude mice.

The biodistribution results of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) are shown in Table 1. ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) exhibited rapid tumor uptake. The tumor uptake was 1.27 ± 0.40 and 0.55 ± 0.23 %ID/g at 0.5 and 2 h post-injection. The tumor uptake decreased to 0.38 ± 0.16 and 0.31 ± 0.09 %ID/g at 4 and 24 h post-injection. ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) displayed rapid whole-body clearance, with approximately 91% of the injected radioactivity cleared through the urinary system by 2 h post-injection. The kidneys were the normal organs with the highest uptakes after 2 h post-injection. The renal uptake was 10.93 ± 1.53 , 6.41 ± 0.34 , 7.29 ± 0.90 and 2.72 ± 0.64 % ID/g at 0.5, 2, 4 and 24 h post-injection. The tumor imaging property of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) was examined in DU145 human prostate cancer-xenografted nude mice. Representative three-dimensional, coronal and transversal SPECT/CT images are presented in Figure 4. The DU145 xenografted tumors were clearly visualized by SPECT/CT using ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) as an imaging probe in at 0.5 h post-injection, highlighting the potential use of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) for human prostate cancer imaging.

The kidneys were the normal organs with the highest radioactivity uptakes in the biodistribution results and SPECT/CT images. Thus, the urinary metabolites of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) were analyzed by RP-HPLC at 2 h post-injection. Figure 5 shows the radioactive HPLC profile of the urine sample. The urine analysis revealed that only 9.8% of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) remained intact, whereas 90.2% of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) was metabolized into two compounds with higher polarity. Although the identities of the metabolites need to be confirmed in future studies, it was likely that both ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) and its metabolites contributed to the renal uptake. Lysine co-injection could be potentially utilized to decrease the renal uptake of ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) since it was successfully used to reduce the renal uptakes of ¹¹¹In-labeled alpha-melanocyte stimulating hormone (α-MSH) peptides by 70%.³¹ Besides the strategy to decrease renal uptake, it is equally important to increase the tumor uptake in future studies. It was reported that the DOTA-conjugated bombesin peptides with the linkers ranging from 5-carbon (Ava) to 8-carbon (Aoc) exhibited 0.6-1.7 nM receptor binding affinities. Either shorter or longer hydrocarbon linkers dramatically reduce the receptor binding affinity by 100-fold,³² indicating the profound effect of hydrocarbon linker on the receptor binding affinity. DOTA-Ahx-(D-Lys⁶-GnRH1) displayed 36.1 nM

GnRH receptor binding affinity in this study. Substituting the Ahx linker with other hydrocarbon linkers could be a potential way to improve the receptor binding affinity.

The experimental details are presented in References and notes.³³⁻³⁶

In conclusion, the successful imaging of DU145 human prostate cancer-xenografted tumor lesions using ¹¹¹In-DOTA-Ahx-(D-Lys⁶-GnRH1) highlighted its potential as a novel imaging probe for human prostate cancer imaging.

Acknowledgments

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References and notes

1. Jemal A, Siegel R, Xu J, Ward E. CA Cancer J Clin. 2010; 60:277. [PubMed: 20610543]
2. Pienta KJ, Naik H, Lehr JE. Urology. 1996; 48:164. [PubMed: 8693645]
3. Naik H, Lehr JE, Pienta KJ. Urology. 1996; 48:508. [PubMed: 8804514]
4. Yogoda A, Petrylak D. Cancer. 1993; 71:1098. [PubMed: 7679039]
5. Crawford ED, Blumenstein BA, Goodman PJ, Davis MA, Eisenberger MA, McLeod DG, Spaulding JT, Benson R, Dorr FA. Cancer. 1990; 66(suppl. 5):1039. [PubMed: 2118417]
6. Oesterling JE. J Urol. 1991; 145:907. [PubMed: 1707989]
7. Hudson MA, Bahnson RR, Catalona WT. J Urol. 1989; 142:1011. [PubMed: 2477559]
8. Stephan C, Cammann H, Meyer HA, Lein M, Jung K. Cancer lett. 2007; 249:18. [PubMed: 17292541]
9. Kahn D, Williams RD, Seldin DW, Libertino JA, Hirschhorn M, Dreicer R, Weiner GJ, Bushnell D, Gulfo J. J Urol. 1994; 152:1490. [PubMed: 7523704]
10. Kahn D, Williams RD, Manyak MJ, Haseman MK, Seldin DW, Libertino JA, Maguire RT. J Urol. 1998; 159:2041. [PubMed: 9598514]
11. Kahn D, Williams RD, Haseman MK, Reed NL, Miller SJ, Gerstbrein J. J Clin Oncol. 1998; 16:284. [PubMed: 9440754]
12. Hinkle GH, Burgers JK, Neal CE, Texter JH, Kahn D, Williams RD, Maguire R, Rogers B, Olsen JO, Badalament RA. Cancer. 1998; 83:739. [PubMed: 9708939]
13. Chang CH, Wu HC, Tsai JJ, Shen YY, Changlai SP, Kao A. Urol Int. 2003; 70:311. [PubMed: 12740497]
14. Heicappell R, Muller-Mattheis V, Reinhardt M, Vosberg H, Gerharz CD, Muller-Gartner H, Ackermann R. Eur Urol. 1999; 36:582. [PubMed: 10559612]
15. Shreve PD, Grossmann HB, Gross MD, Wahl RL. Radiology. 1996; 199:751. [PubMed: 8638000]
16. Effert PJ, Bares R, Handt S, Wolff JM, Bull U, Jakse G. J Urol. 1996; 155:994. [PubMed: 8583625]
17. Limonta P, Dondi D, Moretti RM, Maggi R, Motta M. J Clin Endocrinol Metab. 1992; 75:207. [PubMed: 1320049]
18. Limonta P, Dondi D, Moretti RM, Fermo D, Garattini E, Motta M. J Clin Endocrinol Metab. 1993; 76:797. [PubMed: 8445038]
19. Dondi D, Limonta P, Moretti RM, Montagnani MM, Garattini E, Motta M. Cancer Res. 1994; 54:4091. [PubMed: 8033142]
20. Limonta P, Moretti RM, Montagnani MM, Dondi D, Parenti M, Motta M. Endocrinology. 1999; 140:5250. [PubMed: 10537155]
21. Straub B, Muller M, Krause H, Schrader M, Goessl C, Heicappell R, Miller K. Clin Cancer Res. 2001; 7:2340. [PubMed: 11489810]

22. Halmos G, Arencibia JM, Schally AV, Davis R, Bostwick DG. J Urol. 2000; 163:623. [PubMed: 10647698]
23. Tieva A, Stattin P, Wikstrom P, Bergh A, Damber JE. Prostate. 2001; 47:276. [PubMed: 11398175]
24. Fekete M, Zalatnai A, Comaru-Schally AM, Schally AV. Pancreas. 1989; 4:521. [PubMed: 2573055]
25. Grundker C, Volker P, Griesinger F, Ramaswamy A, Nagy A, Schally AV, Emons G. Am J Obstet Gynecol. 2002; 187:528. [PubMed: 12237622]
26. Millar RP. Animal Reproduction Sci. 2005; 88:5.
27. Barda Y, Cohen N, Lev V, Ben-Aroya N, Koch Y, Mishani E, Fridkin M, Gilon C. Nucl Med Biol. 2004; 31:921. [PubMed: 15464394]
28. Beckers T, Bernd M, Kutscher B, Kuhne R, Hoffman S, Reissmann T. Biochem Biophys Res Commun. 2001; 289:653. [PubMed: 11726197]
29. Guo H, Lu J, Hathaway H, Royce ME, Prossnitz ER, Miao Y. Bioconjug Chem. 2011 in press.
30. Schottelius M, Berger S, Poethko T, Schwaiger M, Wester HJ. Bioconjug Chem. 2008; 19:1256. [PubMed: 18510351]
31. Guo H, Yang J, Gallazzi F, Prossnitz ER, Sklar LA, Miao Y. Bioconjug Chem. 2009; 20:2162. [PubMed: 19817405]
32. Hoffman TJ, Gali H, Smith CJ, Sieckman GL, Hayes DL, Owen NK, Volkert WA. J Nucl Med. 2003; 44:823. [PubMed: 12732685]
33. **Specific GnRH receptor binding of ^{111}In -DOTA-Ahx-(d-Lys⁶-GnRH1):** DOTA-Ahx-(d-Lys⁶-GnRH1) was synthesized and radiolabeled with ^{111}In according to our published procedure.²⁹ The specific GnRH receptor binding of ^{111}In -DOTA-Ahx-(d-Lys⁶-GnRH1) was determined using Millipore ChemoScreen™ human GnRH membrane preparations (Millipore, Inc., Billerica, MA). Briefly, 50 μL of human GnRH membrane preparations were incubated at 25°C for 3 h with approximately 60,000 cpm of HPLC-purified ^{111}In -DOTA-Ahx-(d-Lys⁶-GnRH1) in 50 μL of binding medium {50 mM N-(2-hydroxyethyl)-piperazine-N'-(2-ethanesulfonic acid), 5 mM MgCl_2 , 1 mM CaCl_2 , pH 7.4, 0.2% bovine serum albumin (BSA)} with or without 1 μM of DOTA-Ahx-(d-Lys⁶-GnRH1) peptide blockade. After the incubation, each membrane preparation was mixed with 800 μL of ice-cold washing buffer first, and then filtered through a GF/C filter (Waterman, Clifton, NJ) pre-soaked in 1% polyethylenimine. Each filter was rinsed with 1 mL of ice-cold washing buffer for three times and counted in a Wallac 1480 automated gamma counter (PerkinElmer, Waltham, MA). Statistical analysis was performed using the Student's t-test for unpaired data to determine the significance of differences between the GnRH receptor binding of ^{111}In -DOTA-Ahx-(d-Lys⁶-GnRH1) with or without 1 μM of DOTA-Ahx-(d-Lys⁶-GnRH1) blockade. Difference at the 95% confidence level ($p < 0.05$) was considered significant.
34. **Immunohistochemistry staining of DU145 human prostate cancer-xenografted tumor:** The immunohistochemistry staining was performed on DU145 human prostate cancer-xenografted tumors to demonstrate the GnRH receptor expression. DU145 human prostate cancer cells were obtained from American Type Culture Collection (Manassas, VA). GnRHR antibody (N-20, sc-8682) and the goat ABC staining system (sc-2023) were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA) for immunohistochemistry (IHC) staining of DU145 human prostate cancer-xenografted tumor. The DU145 human prostate cancer-xenografted tumors were generated through flank subcutaneous inoculations of DU145 cells (1×10^7 cells/mouse) in male athymic nude mice. The tumor weights reached approximately 0.3 g at 18 days post cell inoculation. The immunoperoxidase staining of the xenografted DU145 tumor slices (4- μm thickness) were performed according to the protocol of goat ABC staining system. Briefly, the tumor slices were treated with 3% H_2O_2 for 15 min followed by a 20 min-treatment with the blocking serum at 25°C. Then, the tumor slices were incubated with primary goat anti-human GnRH antibody (1:40) for 1.75 h at 25°C. Thereafter, the tumor slices were incubated with biotinylated secondary antibody for 30 min and followed by a 30 min-incubation with AB enzyme reagent. The tumor slices were incubated with the peroxidase substrate for 5 min followed by a dehydration process using ethanol and xylene. After the immunoperoxidase staining, the tumor slices were washed with de-ionized water and counterstained with Gill's formulation #2 hematoxylin. One to two drops of DPX permanent mounting medium were immediately added to

the tumor slices after the counterstaining. Then, the tumor slices were covered with glass coverslips and observed by the light microscopy. As controls, the xenografted DU145 tumor slices (4- μ m thickness) were incubated (without primary goat anti-human GnRH antibody) with secondary biotinylated antibody, AB enzyme reagent and peroxidase substrate, respectively.

35. **Biodistribution and tumor imaging of ^{111}In -DOTA-Ahx-(d-Lys⁶-GnRH1):** All the animal studies were conducted in compliance with Institutional Animal Care and Use Committee approval. The pharmacokinetics of ^{111}In -DOTA-Ahx-(d-Lys⁶-GnRH1) was determined in DU145 human prostate cancer-xenografted male athymic nude mice (Harlan, Indianapolis, IN). The nude mice were subcutaneously inoculated with 1×10^7 DU145 cells on the right flank of each mouse to generate DU145 xenografted tumors. The tumor weights reached approximately 0.3 g at 18 days post cell inoculation. Each tumor-bearing mouse was injected with 0.037 MBq of ^{111}In -DOTA-Ahx-(d-Lys⁶-GnRH1) via the tail vein. Groups of 5 mice were sacrificed at 0.5, 2, 4 and 24 h post-injection, and tumors and organs of interest were harvested, weighed and counted. Blood values were taken as 6.5% of the whole-body weight. To determine the tumor imaging property of ^{111}In -DOTA-Ahx-(d-Lys⁶-GnRH1), approximately 33.3 MBq of ^{111}In -DOTA-Ahx-(d-Lys⁶-GnRH1) was injected into a DU145 human prostate cancer-xenografted nude mouse (18 days post the cell inoculation) via the tail vein. The mouse was sacrificed for small animal SPECT/CT (Nano-SPECT/CT[®], Bioscan) imaging at 0.5 h post-injection. The CT imaging was immediately followed by the whole-body SPECT imaging. The SPECT scans of 24 projections were acquired. Reconstructed SPECT and CT data were visualized and co-registered using InVivoScope (Bioscan, Washington DC).
36. **Urinary metabolites of ^{111}In -DOTA-Ahx-(d-Lys⁶-GnRH1):** One hundred microliters of HPLC purified ^{111}In -DOTA-Ahx-(d-Lys⁶-GnRH1) (2.4 MBq) was injected into a DU145 human prostate cancer-xenografted nude mouse through the tail vein. At 2 h post-injection, the mouse was sacrificed and the urine was collected for metabolites analysis. The urine was centrifuged at 16,000 g for 5 min prior to the HPLC analysis. The radioactive metabolites in the urine were analyzed by injecting aliquots of the urine into HPLC. A 20-minute gradient of 15-25% acetonitrile / 20 mM HCl was used for the urine analysis.

Figure 1.
Structure of ^{111}In -DOTA-Ahx-(D-Lys⁶-GnRH1).

Figure 2.

Radioactive HPLC profile of ^{111}In -DOTA-Ahx-(D-Lys⁶-GnRH1) (A) and UV HPLC profile of DOTA-Ahx-(D-Lys⁶-GnRH1) (B); Binding of ^{111}In -DOTA-Ahx-(D-Lys⁶-GnRH1) on human GnRH receptor membrane preparations with (■) or without (■) the presence of 1 μM of DOTA-Ahx-(D-Lys⁶-GnRH1). The percentage radioactivity bound was normalized by taking the binding without peptide blockade as 100%. * $P < 0.05$.

Figure 3. Immunohistochemistry staining of GnRH receptor expressions in DU145 human prostate cancer-xenografted tumor (A, $\times 400$). The DU145 xenografted tumor exhibited strong brown cytoplasmic staining. As a comparison, the DU145 xenografted tumor (B, $\times 400$) were stained without primary goat anti-human GnRH antibody.

Figure 4. Three-dimensional (A), coronal (B) and transversal (C) SPECT/CT images of DU145 human prostate cancer-xenografted tumor at 0.5 h post-injection of 33.3 MBq of ^{111}In -DOTA-Ahx-(D-Lys⁶-GnRH1). The prostate cancer lesions (T) were highlighted with arrows on the images.

Figure 5.

Radioactive HPLC profile of the urine sample of a DU145 human prostate cancer-xenografted nude mouse at 2 h post-injection of ^{111}In -DOTA-Ahx-(D-Lys⁶-GnRH1). The arrow indicated the retention time of the original ^{111}In -DOTA-Ahx-(D-Lys⁶-GnRH1) prior to the tail vein injection.

Table 1

Biodistribution of ^{111}In -DOTA-Ahx-(D-Lys⁶-GnRH1) in DU145 human prostate cancer-xenografted nude mice. The data were presented as percent injected dose/gram or as percent injected dose (Mean $\pm$ SD, n=5).

Tissue	0.5 h	2 h	4 h	24 h
Percent injected dose/gram (%ID/g)				
Tumor	1.27 $\pm$ 0.40	0.55 $\pm$ 0.23	0.38 $\pm$ 0.16	0.31 $\pm$ 0.09
Brain	0.14 $\pm$ 0.06	0.10 $\pm$ 0.05	0.06 $\pm$ 0.04	0.07 $\pm$ 0.02
Blood	1.25 $\pm$ 0.23	0.59 $\pm$ 0.26	0.46 $\pm$ 0.38	0.49 $\pm$ 0.23
Heart	0.62 $\pm$ 0.20	0.20 $\pm$ 0.07	0.24 $\pm$ 0.09	0.10 $\pm$ 0.04
Lung	2.10 $\pm$ 0.12	0.32 $\pm$ 0.05	0.31 $\pm$ 0.05	0.19 $\pm$ 0.05
Liver	0.97 $\pm$ 0.11	0.57 $\pm$ 0.10	0.48 $\pm$ 0.02	0.56 $\pm$ 0.09
Spleen	0.63 $\pm$ 0.21	0.46 $\pm$ 0.04	0.52 $\pm$ 0.15	0.28 $\pm$ 0.06
Stomach	0.46 $\pm$ 0.09	0.33 $\pm$ 0.33	0.17 $\pm$ 0.07	0.06 $\pm$ 0.03
Kidneys	10.93 $\pm$ 1.53	6.41 $\pm$ 0.34	7.29 $\pm$ 0.90	2.72 $\pm$ 0.64
Muscle	0.36 $\pm$ 0.23	0.14 $\pm$ 0.07	0.22 $\pm$ 0.05	0.25 $\pm$ 0.27
Pancreas	0.61 $\pm$ 0.11	0.14 $\pm$ 0.04	0.12 $\pm$ 0.03	0.07 $\pm$ 0.04
Bone	1.66 $\pm$ 0.11	0.59 $\pm$ 0.19	0.68 $\pm$ 0.94	0.55 $\pm$ 0.67
Skin	2.01 $\pm$ 0.50	0.26 $\pm$ 0.08	0.27 $\pm$ 0.09	0.24 $\pm$ 0.16
Uptake ratio of tumor/normal tissue				
Tumor/blood	1.02	0.93	0.83	0.63
Tumor/muscle	3.53	3.93	1.73	1.24
Percent injected dose (%ID)				
Intestines	1.03 $\pm$ 0.14	1.12 $\pm$ 0.81	0.51 $\pm$ 0.07	0.40 $\pm$ 0.09
Urine	77.12 $\pm$ 4.54	91.44 $\pm$ 1.78	92.47 $\pm$ 0.78	95.75 $\pm$ 0.34