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Research Article

Development of radiolabeled peptides to target p185HER2/neu receptors of cancer

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Summary

The p185HER2/nue receptors which regulate the growth of cancer cells and are overexpressed in 20–30% of the cancers can be the markers for cancer diagnosis. This work describes the DTPA conjugation, Indium(III) complexes characterization, Tc-99m labeling, and Biacore test of the peptides such as [Cys^6-Cys^{12}]H-GAGGYCDGFYACYMDV-$CONH_2$ (anti-HER2/neu-peptidomics, (Ala^2)AHNP), [Cys^6-Cys^{12}]H-G(Abu)GGYCDGFYACYMDV-$CONH_2$ ((Abu^2)AHNP), and H-dYCALTYYDYECdFAY-$CONH_2$ (EP6, EGFR selective peptide) binding affinity to the p185HER2/neu proteins. The results show the evidence of EP6 binding to the immobilized p185HER2/neu proteins. The labeled EP6, [^{99m}Tc]DTPA-EP6, had displayed greater association percentage to the lysate of T6-17 cells expressing p185HER2/neu proteins compared to the labeled AHNPs. The uptake (in terms of ID%/g) of [^{99m}Tc]DTPA-EP6 in animal xenografted tumor was seven folds greater than that of the [^{99m}Tc](Ala^2)AHNP. The nature of the dual-receptors (EGFR and HER2/neu) binding of EP6 is a merit for the peptide to target cancer.

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Key Words: peptides; EP6; AHNP; technetium-99m; p185HER2/neu proteins

Introduction

The c-erbB1 and c-erbB2 encoded EGFR and p185HER2/neu proteins belong to the tyrosine kinase family. These proteins are important receptors for both malignant and non-malignant cell proliferation.[1–3] The p185HER2/neu proteins are overexpressed in 20–30% cancers of the breast,[4] prostate,[5] ovary,[6] and pancreas.[7] The overexpressed receptors promote the growth and

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metastasis of cancer cells.[8] Molecules such as IMC-C225 (anti-EGFR antibody),[9] Herceptin® (humanized anti-HER2/neu antibody),[10] and ZD1839,[11] can specifically target the receptors on cancer cells to interrupt the transduction of growth signals,[12] prohibit metastasis,[13] and induce apoptosis of the cancer cells.[14] Because of their receptor-binding specificity, IMC-C225,[15] Mab 528,[16] and Herceptin could be used as imaging/therapeutic agents for tumors when radiolabeled.[17,18] The radiolabeled antibodies, despite the high percentage of injected dose accumulated in tumors after a few days circulation, exhibited slow clearance from blood and therefore caused high background to interfere with tumor imaging and, generate excessive toxicity to normal organs in therapy. Smaller molecules with inherent receptor-binding affinity would overcome these problems because clearance of the molecules from circulation is faster compared to the monoclonal antibody. In recent years, two labeled peptides, [^{111}In]DTPA-octreotide and [^{99m}Tc]depreotide,[19,20] have been successfully developed and used in the clinic for somatostatin receptor imaging. In addition, several radiolabeled peptides, such as [^{99m}Tc]neurotensin (NT) for pancreatic tumor imaging,[21] ^{99m}Tc-labeled bombesin analogues for tumor imaging,[22] and [^{64}Cu]RGD for $\alpha_V\beta_3$ integrin imaging,[23] have shown promising results.

The peptide EP6 is a peptide of high affinity to EGFR, while the two anti-HER2/neu-peptides (AHNPs) are peptidomimetics derived from anti-p185HER2/neu antibody.[24,25] Here we report the radiolabeling of the EP6 and the two AHNPs and test the binding affinity of the peptides and the radiolabels to p185HER2/neu proteins.

Results and discussion

The peptides of EP6, (Abu^2)AHNP, (Ala^2)AHNP, and CD4 (as control) were cyclized before use. Cyclized peptides were rigid in structure and exhibited reproducible receptor binding affinity. The sequences of the peptides are shown in Figure 1. The conjugations of DTPA to the peptides were carried out in an established manner with some modifications.[26] The yields of the conjugation ranged from 35–68% after RP HPLC purification. The molecular weights of the DTPA conjugates were determined by MALDI-TOF mass spectrometry and the results are summarized in Table 1. The results showed that one DTPA was conjugated to one peptide. Non-radioactive indium(III) complexes of the peptides were prepared as references for the radioactive counterparts. The chelation of indium(III) ion by a DTPA-peptide was carried out at room temperature and was complete in 1 h. After RP HPLC purification, the yield of the products ranged from 20–35%. The molecular weights (Table 1) of indium(III) chelates revealed that one indium(III) ion was chelated by one DTPA-peptide.

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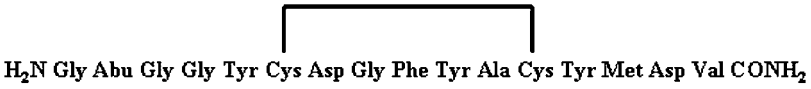
1. EP6

2. (Ala^2)AHNP

3. (Abu^2)AHNP



4. CD4

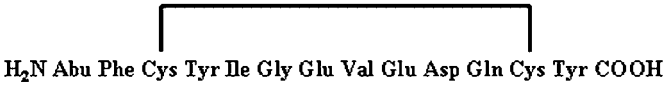


Figure 1. Amino acid sequences of the cyclized EP6, (Abu^2)AHNP, (Ala^2)AHNP, and CD4

Table 1. Molecular weights of the DTPA conjugates/indium(III) complexes of EP6 and AHNPs as determined by MALDI-TOF MS

Compounds		Mass	
Name	Formula	Calculated	Found
DTPA-EP6	$C_{99}H_{126}N_{18}O_{33}S_2$	2158.8176	2159.801 [M + H$_+$]
DTPA-(Ala$_2$)AHNP	$C_{88}H_{118}N_{20}O_{32}S_3$	2062.7383	2063.72 [M + H$_+$]
DTPA-(Abu$_2$)AHNP	$C_{89}H_{120}N_{20}O_{32}S_3$	2076.7540	2077.714 [M + H$_+$]
DTPA-CD4	$C_{83}H_{115}N_{17}O_{32}S_2$	1925.7336	1926.6598 [M + H$_+$]
[In]DTPA-EP6	$C_{99}H_{123}InN_{18}O_{33}S_2$	2270.6980	2271.583 [M + H$_+$]
[In]DTPA-(Ala$_2$)AHNP	$C_{88}H_{115}InN_{20}O_{32}S_3$	2174.6187	2175.748 [M + H$_+$]
[In]DTPA-(Abu$_2$)AHNP	$C_{89}H_{117}InN_{20}O_{32}S_3$	2188.7305	2189.865 [M + H$_+$]

The indium(III) complexes were water soluble and stable in water or PBS for months without significant change. Lipophilicity of the compounds was evaluated by the retention time of RP HPLC. The retention time of the DTPA-EP6 (25.7 min) was longer than those of DTPA-(Abu^2)AHNP (24.2 min) and DTPA-(Ala^2)AHNP (24.2 min) suggesting that the lipophilicity

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of the former molecule was greatest of the three. Upon chelating an indium(III) ion, the retention time of the [In]DTPA-EP6 increased (26.1 min) suggesting the increase of lipophilicity.

The radiolabeling of the DTPA conjugates started with ^{99m}Tc because of its wide availability. The ^{99m}Tc labeling of the DTPA-peptides was carried out in the presence of $SnCl_2$ as a reducing agent. 4 μg (21 nmol) of $SnCl_2$ was sufficient to reduce $Na^{99m}TcO_4$ up to 3 mCi (equivalent to 5.8 pmol). The labeling efficiency was analyzed by RP HPLC, where the labeled products appeared on the chromatogram with a retention time similar to that of the indium(III) complexes (chromatograms not shown). Paper chromatography (Whatman No. 1 paper with acetone and saline as mobile phases, respectively) assay of the labeling solution revealed that over 98% of the pertechnetate had been reduced and bound to the peptides. However, only ~40% of the radioactivity was recovered from the RP HPLC which might imply that a significant amount of the ^{99m}Tc was non-specifically bound to the peptides. Non-specifically bound ^{99m}Tc was unstable on the peptides and would be released in circulation causing difficulties in interpreting the results of *in vitro* and *in vivo* tests. For this reason, the ^{99m}Tc labels were always purified by size-exclusion (SE) HPLC (Figure 2) before use. The purified radiolabeled peptides were stable in solution for over 12 h without significant change.

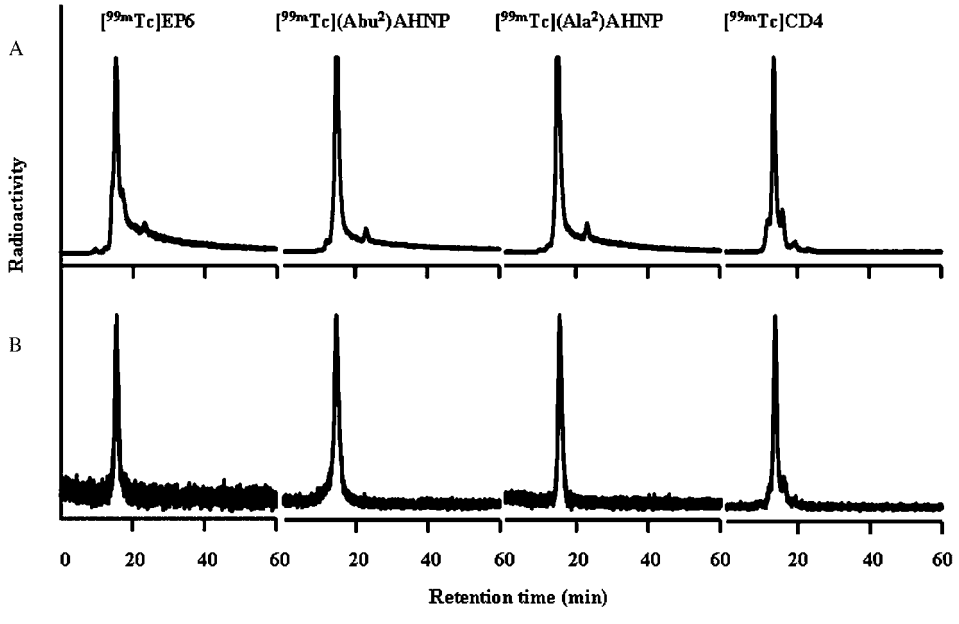

Figure 2. Size-exclusion HPLC analyses of ^{99m}Tc labeled DTPA-EP6, DTPA-(Abu^2)AHNP, DTPA-(Ala^2)AHNP, and DTPA-CD4 are shown in panel A (unpurified) and panel B (purified)

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Cellular accumulation of a radiolabel usually contains a non-specific portion, which is related more or less to the lipophilicity of the molecule. The lipophilicity of the ^{99m}Tc labeled peptides was evaluated by their partition ratios (PRs) between oil and phosphate buffer. The PRs of the four radiolabels of [^{99m}Tc]DTPA-EP6, [^{99m}Tc]DTPA-(Abu^2)AHNP, [^{99m}Tc]DTPA-(Ala^2)AHNP, and [^{99m}Tc]DTPA-CD4 were shown in Table 2. The data indicated that the PR of the labeled EP6 was 2.3–3.4 fold greater over the AHNPs, indicating that the labeled EP6 was the least hydrophilic.

The binding affinity of the cyclised peptides, the DTPA conjugates, and indium(III) complexes of the peptides to the immobilized p185HER2/neu proteins was measured by surface-plasmon-resonance technique (Biacore). EP6 had exhibited significant dose dependent responses on the diagram (Figure 3) indicating binding to the p185HER2/neu proteins. By contrast, the

Table 2. Results of the partition ratios (*PR*s) of the ^{99m}Tc labeled peptides between *n*-octanol and pH 7.4 phosphate buffer

Radiolabels	PR (n = 3)
[^{99m}Tc]DTPA-EP6	0.0017 ± 0.0002
[^{99m}Tc]DTPA-(Ala^2)AHNP	0.00075 ± 0.00005
[^{99m}Tc]DTPA-(Abu^2)AHNP	0.00050 ± 0.00005
[^{99m}Tc]DTPA-CD4	0.00064 ± 0.00004

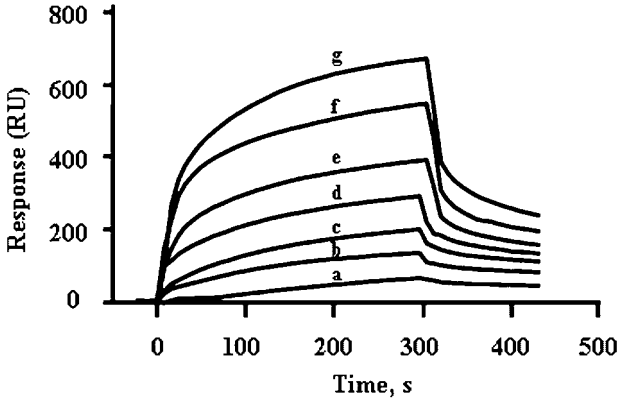

Figure 3. Biacore sensorgrams show binding of the cyclized EP6 to immobilized p185HER2/neu proteins in a dose-dependent manner. The concentrations of the EP6 injected were 8.6 µM (a), 25 µM (b), 43 µM (c), 86 µM (d), 125 µM (e) 200 µM (f), and 250 µM (g). The association of the peptide to the protein was exhibited within the first 300 s from when the peptide was injected, whereas the dissociation was seen in the following 240 s when buffer was injected

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control peptide, CD4, showed no binding to the immobilized p185HER2/neu proteins. When linking with a DTPA, EP6 lost its affinity for p185HER2/neu proteins. This might be due to the interference of the 4 free carboxylic acid groups of the DTPA. However, the protein binding affinity was recovered when the DTPA conjugate of EP6 had chelated an indium(III) ion (diagram not shown). The results suggested that the character of the dual binding affinity to ERFR and p185HER2/neu proteins of EP6 and EP6 indium(III) complex could be favorable for these molecules to target cancers. It was assumed that the p185HER2/neu proteins binding affinity of [^{99m}Tc]DTPA-EP6 was similar to that of [In]DTPA-EP6 because of the structural similarity of the two molecules.

In addition to the Biacore test, the binding affinity of the ^{99m}Tc labeled EP6 was evaluated by using cell lysate of T6-17 cells. The T6-17 cells are transgenetic ones which express p185HER2/neu proteins, while the control NR6 cells do not. Upon mixing the radiolabeled peptides with lysate of the cells and injection into SE HPLC, the signal from the bound labeled peptide would be shifted to 9.5 min (retention time of p185HER2/neu proteins) on the chromatogram, while the unbound remained at 15.5 min (retention time of the labeled EP6). [^{99m}Tc]DTPA-EP6 and [^{99m}Tc]DTPA-(Abu^2)AHNP were more greatly shifted by the T6-17 lysate when compared to the shift observed in the presence of the NR6 cell lysate, suggesting the evidence for p185HER2/neu proteins binding selectivity.

Tumor selectivity of the radiolabels was tested by nude mice with xenografted T6-17 tumor. The uptakes of [^{99m}Tc]DTPA-EP6 and [^{99m}Tc]DTPA-(Ala^2)AHNP in the major organs of the mice were shown in Table 3. The data exhibited that the dosage of the two labels accumulated in

Table 3. Biodistribution results of ^{99m}Tc labeled DTPA-EP6 and DTPA-(Ala^2)AHNP at 3 h after i.v. injection in nude mice bearing transplanted T6-17 tumor

Organs	[^{99m}Tc]DTPA-EP6		[^{99m}Tc]DTPA-(Ala^2)AHNP	
	%ID/g	Standard deviation	%ID/g	Standard deviation
Liver	6.8	3.2	1.7	0.55
Heart	0.78	0.39	0.18	0.038
Kidney	14	3.2	8.6	3.0
Lung	2.4	1.4	0.39	0.12
Spleen	2.1	1.0	0.92	0.17
Small intestine	3.4	3.6	0.30	0.06
Larger intestine	7.3	5.1	0.46	0.29
Muscle	0.51	0.28	0.083	0.032
Tumor	1.7	0.93	0.24	0.050
Blood	3.0	1.7	0.59	0.15
Tumor/muscle	3.30	0.10	3.0	0.64
Tumor/blood	0.55	0.13	0.43	0.15

Values are mean and standard deviation, n= 3.

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the tumor was 3–3.3 fold greater than that found in the normal tissue. Among the organs studied, kidneys showed highest radioactivity uptake in terms of %ID/g due to the hydrophilic nature of the peptides. [^{99m}Tc]DTPA-EP6 displayed seven fold greater accumulation in the tumor compared to the [^{99m}Tc]DTPA-(Ala^2)AHNP. The results suggested that the labeled EP6 was superior for tumor targeting due to its character of dual receptors binding. However, there could be a certain portion of the labeled EP6 accumulated non-specifically in the tumor, because the uptakes of the labeled EP6 in other tissues and organs (where EGFR and p185HER2/neu proteins were not supposed to be over expressed) were also greater than that of the labeled AHNP. For example, by comparison with [^{99m}Tc]DTPA-(Ala^2)AHNP, [^{99m}Tc]DTPA-EP6 displayed five fold higher accumulation level in blood, 3.9 fold higher in liver, 4.3 fold higher in heart, 6.2 fold higher in lungs, 6.1 fold higher in normal tissue. Since those tissues were not expected to overexpress EGFR or p185HER2/neu proteins, the uptake of the radioactivity in those tissues or organs could include non-specific portion. In this circumstance, molecular size and lipophilicity of a labeled peptide may play an important role in determining the level of non-specific cellular uptakes. A peptide of greater lipophilicity might exhibit a higher level of cellular uptakes. Non-specific tumor accumulation of the peptides might be due to the hindrance of the labeled peptides binding to their target molecules, and/or the smaller density of the p185HER2/neu receptors expressing on the tumor cells.[27-28] However, this remains to be confirmed. The clearance rates of the ^{99m}Tc and ^{111}In labeled DTPA-(Abu^2)AHNP in animal blood was fast (half-life less than 30 min, data not shown) as expected.

Experimental

The two AHNP peptides, [Cys^6-Cys^{12}]H-GAGGYCDGFYACYMDV-$CONH_2$ ((Ala^2)AHNP) and [Cys^6-Cys^{12}]H-GAbuGGYCDGFYACYMDV-$CONH_2$ ((Abu^2)AHNP, where Abu stands for γ-(aminobutyric acid) were designed to mimic the sequences and structure of the complementary determining regions (CDRs) of the anti-p185HER2/neu antibodies (1FVD and 7.16.4) as described previously.[24-25] The two AHNPs, EP6 ([Cys^2-Cys^{11}] H-dYCALTYYDYECdFAY-$CONH_2$), and control peptide CD4 ([Cys^3-Cys^{12}]H-AbuFCYIGEVEDQCY-OH) were synthesized by the Protein Chemistry Laboratory, University of Pennsylvania (Philadelphia, PA). They were cyclized, purified, and structurally confirmed by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS, MicroMass TofSpec, Micromass Inc., Beverly, MA). The sequences of the peptides are shown in Figure 1.

$Na^{99m}TcO_4$ was obtained from ^{99}Mo/^{99m}Tc generators produced by DuPont Pharma (Billerica, MA). Diethylenetriaminepentaacetic acid

DOI: 10.1002/jlcr

anhydride (DTPA), indium(III) chloride, *tert*-amyl alcohol, 2,2,2 tribromoethanol (Avertin), 5,5′-dithio-bis(2-nitrobenzoic acid (DTNB), and all other chemicals were purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI). Dulbecco's Modified Eagle's Medium (DMEM) and L-glutamine were produced by BioWhittaker (Walkersville, MD). Penicillin–streptomycin was obtained from GibcoBRL (Grand Island, NY 14072), and fetal bovine serum (FBS) was bought from HyClone (Logan, UT).

Waters HPLC Alliance system (Waters Corporation, Milford, MA) consisting of a Waters 626 pump, Waters 600S controller, Waters 996 photodiode array detector, Waters 717 autosampler and Waters Millennium®32 Chromatography Manager Software (system 1) was used for peptide purification and analysis with an ODS C18 (10×250 mm) column (Beckman Instruments, Inc., Fullerton, CA). Analysis and purification of the radiolabeled products was carried out on another Waters HPLC system (system 2) consisting of two Waters 501 pumps, Waters U6K injector, Waters Lambdamax model 481 LC spectrophotometer, and FC-3200 radiometric detector (Bioscan, Inc., Washington, DC). Zorbax SB300 C18 reversed-phase column (4.6×250 mm, 5 μm) was purchased from Agilent Technology (Palo Alto, CA), while size-exclusion (SE) Superdex peptide 10/30 column was bought from Pharmacia Biotech AB (Uppsala, Sweden). Binding affinity of the peptides, DTPA conjugates, and non-radioactive indium(III) chelates of the peptides was recorded on a Biacore 3000 (Biacore AG, Uppsala, Sweden). Radioactivity in the samples was measured by Cobra II gamma counter (Packard, Downers Grove, IL).

Cyclization and diethylenetriaminepentaacetic acid (DTPA) conjugation of the peptides.

The intramolecular cyclization of the peptides (0.1 mg/ml or 0.5–0.7 mM, pH 8–8.5) was carried out at 4°C as described previously.[24] The cyclization process was monitored by testing the concentration of the free thiol groups of the peptide by the DTNB method.[24] Procedures of the test were: (a) 400 μl of the reaction solution was mixed with 100 μl 0.1M pH 8 phosphate buffer and 5 μl 10 mM pH 8 of DTNB; (b) the mixture was incubated at 4°C in the dark for 30 min; (c) absorption at 412 nm of the mixture was measured with the reaction solution as reference; (d) cyclization was considered to be completed when the absorptions at 412 nm of the mixture and the reaction solution become identical. After the completion of the cyclization, the solvents were removed by lyophilization. The purity of the cyclized peptides was tested by HPLC (ODS C18 column, 0.01 M pH 5.2 NH_4OAc/MeOH as mobile phase with gradient of 40% MeOH to 90% MeOH over 25 min) before further use. The purity of the cyclized peptides were typically over 90%. 2.0 mg (1.1–1.2 μmol) of a cyclized peptide was dissolved in 1.5 ml 0.13 M pH 8.6

DOI: 10.1002/jlcr

$NaHCO_3$. To the solution was added 3 mg (8.4 μmole) anhydride DTPA dissolved in 100 μl DMSO. The mixture was stirred at room temperature for 1 h and the DTPA conjugate was purified by HPLC (system 1) with 0.01 M pH 5.2 NH_4OAc/MeOH as mobile phase and a gradient of 40% MeOH to 90% MeOH over 25 min. After removing most of the solvent by rotary evaporation at 35°C followed by lyophilization, the molecular weights of the DTPA conjugates were determined by MALDI-TOF MS. The purity of the purified DTPA conjugates was over 95%, while the yields of them ranged from 36–68%.

Preparation of non-radioactive indium(III) complexes of DTPA conjugates

4 mg (~2 μmol) of a DTPA-conjugated peptide was dissolved in 2 ml 0.13 M pH 8.6 $NaHCO_3$. To the solution was added 0.44 mg (2 μmol) $InCl_3$ in 0.02 N HCl. After stirring at room temperature for 1 h, the indium(III) complex was purified by RP HPLC (system 1) with the same mobile phases and gradient mentioned above. After removing the solvent, the samples were lyophilized. The purity of the purified indium(III) complexes was over 95%, while the yields of them ranged from 25–35%. The molecular weights of the complexes were determined by MALDI-TOF MS.

Technetium-99m labeling of the DTPA conjugates

1 μg of a DTPA conjugate in 10 μL water was mixed with 100 μl 0.05 M pH 8.5 $NaHCO_3$, 1-3 mCi of $Na^{99m}TcO_4$ (10–100 μl), and 4–8 μg $SnCl_2$ in 15 μl 0.04 N HCl. After incubation at room temperature for 30 min, the labeled peptide was analyzed by RP HPLC (system 2) on a Zorbax C18 (4.6 × 250 mm) column with 0.01 M pH 5.2 NH_4OAc/MeOH (gradient 0% MeOH to 90% MeOH over 25 min) as mobile phase, while purification was carried out on a size-exclusion (SE) Superdex peptide 10/30 column with 0.01 M pH 7.39 phosphate buffer as mobile phase. The RP HPLC showed that [^{99m}Tc]EP6 had longer retention time (25.2 min) over [^{99m}Tc](Ala^2)AHNP (25.0 min) and [^{99m}Tc](Abu^2)AHNP (25.0 min). The SE HPLC retention time of those three radiolabels were 15.1 , 15.4 , and 16.1 min respectively. The test by Whatman No. 1 paper strips with saline and acetone as mobile phases respectively indicated that over 98% of [^{99m}TcO4]$^-$ had been reduced and less than 2% of colloid was formed. In practice, the labeled peptides were always purified by SE HPLC (yields were approximately 40%) before use.

Partition between n-octanol and phosphate buffer (pH 7.4)

In triplicate, 100 μl aliquots of a ^{99m}Tc labeled peptide were placed in microcentrifuge tubes, diluted by 400 μl 2 mM pH 7.4 phosphate buffer, mixed with 500 μl *n*-octanol, and vortexed at room temperature for 1 min. The tubes

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were centrifuged at 12000g for 4 min and 3×100 µl of the aqueous phase and 3×100 µl of the organic phase from each tube were transferred to plastic tubes separately for radioactivity counting. The partition ratios of the labeled peptides were calculated by dividing the counts of the organic phase by that of aqueous phase per unit volume.

Bovine serum albumin (BSA) binding

100 µl of the purified ^{99m}Tc labeled peptides (5–10 µCi, 9.6–19 fmol) was mixed with an equal volume of BSA solution in water with serial concentration (ranging from 0.005 to 50 mg/ml) and incubated at room temperature for 1 h before SE HPLC analysis. The percentage of the binding was evaluated by the shift of the radioactivity on the chromatogram.

Binding assay by Biosensor

The kinetic binding affinity of the cyclized peptides, DTPA conjugates, and non-radioactive indium(III) complexes to the immobilized p185HER2/neu proteins were performed on a Biacore 3000 in a similar manner and experimental conditions as described previously.[24] Recombinant purified p185HER2/neu proteins were immobilized on the surface of CM5 (carboxymethyl dextran surface) chip. Phosphate buffered saline containing 0.005% Tween 20 (PBST) was used as a running buffer. 100 µl of the peptides with various concentration were injected and run through the surface of the sensor chip for 300 s (as association phase) with a flow rate of 20 µl/min followed by a 240 s running period (as dissociation phase) of PBST. The binding of peptide-protein was measured by the surface-plasmon-resonance (real time) technique. The kinetic and equilibrium association/dissociation constants, k_a/k_d and K_A/K_D, were evaluated by BIA evaluation 3.0 software (Biacore International AB, Uppsala, Sweden). After each assay, the chip was regenerated by two injections of PBST and 0.2% SDS.

Cell lines

T6-17 cell line was transgenic cells expressing human p185HER2/neu receptors. They are derived from NIH3T3 (NR6) cells which were used as control. The expression of the receptors on these cells was confirmed by FACS from time to time in an established manner.[25] The two cell lines were cultured in Dulbeco's Modified Eagle's Medium (DMEM) with supplements of 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin in a 37°C incubator with 95% humidity and 5% CO_2. The cells were harvested in 0.05% trypsin/0.02% EDTA and re-suspended in fresh medium for transplanting to animals. The cell viability was evaluated by the trypan blue dye exclusion assay.[25]

DOI: 10.1002/jlcr

Cell lysis

T6-17 cells grown in a dish with 80% confluence (the cell number was approximately 22×10^6) were rinsed with cold PBS for three times after aspirating the culture medium. 1 ml freshly made PI/RIPA lysis buffer was added and shaken gently at 4°C for 1 h. The proteins were scraped and the lysate was transferred to a microcentrifuge tube and centrifuged at 5000*g* for 5 min at 4°C. The supernatant was isolated from the DNA and then stored in a −20°C freezer for later use.

Biodistribution assay of ^{99m}Tc labeled peptides

Approximately one million T6-17 cells in PBS were implanted in the right flank of each of the 6 nude mice. After the tumor grew to 1 cm in any dimension, three animals each received 3 μCi of the purified ^{99m}Tc labeled DTPA-(Ala^2)AHNP and another 3 received approximately 3–6 μCi ^{99m}Tc labeled DTPA-EP6 by i.v. injection. After 3 h, each animal was anesthetized by 0.2 ml of Avertin (a mixture of 39.5 ml saline and 0.5 ml avertin stock solution prepared by dissolving 25 g of avertin in 15.5 ml hot *tert*-amyl alcohol) and then sacrificed. Blood was sampled and organs were removed and weighed. Radioactivity in the samples was measured by a gamma well counter against a standard of each injectate.

Conclusion

The Biacore sensorgrams and T6-17 cell lysate test had shown evidence of binding selectivity of EP6 and [In]DTPA-EP6 to p185HER2/neu proteins. The character of the dual-receptors (EGFR and HER2/neu) binding of the EP6 is a merit for this peptide to target cancer cells. The biodistribution experiment had exhibited that the %ID/g of [^{99m}Tc]DTPA-EP6 in the xenografted T6-17 tumor was seven fold greater than that of the [^{99m}Tc]DTPA-(Ala^2)AHNP. In order to improve the specificity of the EP6 labels to the tumor expressing HER2/neu and EGFR, it would be advisable to use the ^{111}In labeled EP6 for the following reasons: (1) the yield of ^{111}In labeling is high and therefore purification procedure can be avoided and, (2) the [^{111}In]DTPA-EP6 is a more stable compound compared to [^{99m}Tc]DTPA-EP6.

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