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# Comparing the intracellular fate of components within a noncovalent streptavidin nanoparticle with covalent conjugation☆

Yuxia Liu[a], Dengfeng Cheng[a], Xinrong Liu[a], Guozheng Liu[a], Shuping Dou[a], Nan Xiao[b], Ling Chen[a], Mary Rusckowski[a], Donald J. Hnatowich[a,*]

[a]*Department of Radiology, University of Massachusetts Medical School, Worcester, MA 01655, USA*
[b]*Department of Medicine, University of Massachusetts Medical School, Worcester, MA 01655, USA*

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**Abstract**

**Introduction:** Auger radiotherapy requires adequate tumor delivery and high nuclear accumulation and retention. We hypothesize that the noncovalent nature of a streptavidin/biotin three-component nanoparticle possessing these qualities may be required for dissociation of the radiolabeled oligomer and its accumulation into the cell nucleus.
**Methods:** As a test of our hypothesis, the intracellular fate of an antisense oligomer when incubated as the nanoparticle and when incubated while covalently conjugated to the antibody was compared. The three-component noncovalent nanoparticle consisted of streptavidin linking three biotinylated components: a Cy3-labeled anti-RIα antisense phosphorodiamidate morpholino (MORF) oligomer, a tat transfecting peptide and the anti-Her2 herceptin antibody. The covalent constructs included an anti-RIα antisense DNA conjugated to a radiolabeled herceptin and a fluorescent DNA conjugated to native herceptin. Fluorescence microscopy in SK-BR-3 (Her2+) cells was used to evaluate the fate of the fluorescent Cy5.5-DNA and Cy3-MORF, while the subcellular accumulation of the $^{111}$In-labeled herceptin and herceptin-DNA in both SK-BR-3 and MDA-MB-231 (Her2) cells was determined by isolating and counting the nuclear fractions.
**Results:** Previously, we demonstrated that when incubated as the three-component nanoparticle consisting of herceptin and streptavidin and $^{99m}$Tc-labeled antisense MORF, only the MORF accumulated in the nucleus of Her2+ cells. In this investigation, clear evidence was observed of nuclear accumulation of the antisense oligomer within the noncovalent nanoparticle as before, but when incubated as the covalent construct, by both fluorescence microscopy and nuclear counting, no evidence of nuclear accumulation was observed.
**Conclusion:** The weaker noncovalent biotin–streptavidin bond may be essential for adequate delivery of the radiolabeled antisense oligomer to the nucleus of tumor cells.
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*Keywords:* Nanoparticle; Antisense oligomer; Herceptin; Streptavidin

## 1. Introduction

This laboratory has been developing methods of improving tumor accumulation following intravenous administration of radiolabeled antisense DNA and other oligomers [1–5]. Lately, the emphasis has included methods of targeting the nucleus within tumor cells with Auger-emitting radionuclides such as indium-111 ($^{111}$In) and iodine-125 ($^{125}$I) that are particularly effective for radiotherapy if carried to the nucleus [6,7]. In a number of studies, we have demonstrated that a streptavidin nanoparticle consisting of a biotinylated antitumor antibody [8] (for improved tumor accumulation), a biotinylated transfecting peptide such as tat [9–11] (potentially to improve cell membrane transport without entrapment) and a biotinylated radiolabeled antisense MORF or DNA oligomer [6,12] (to carry the therapeutic radionuclide to the nucleus to be retained therein by binding to its target mRNA) appears to be a viable solution [13,14]. We have shown that the streptavidin does not appear to influence the properties of the three components. In particular, we have shown rapid and

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* Corresponding author. Department of Radiology, Division of Nuclear Medicine, University of Massachusetts Medical School, Worcester, MA 01655, USA. Tel.: +1 508 856 3791; fax: +1 508 856 6363.
*E-mail address:* donald.hnatowich@umassmed.edu (D.J. Hnatowich).

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essentially quantitative accumulation of radiolabeled antisense oligomers within the nucleus when incubated as the nanoparticle and, as a result, we have reported successful radiotherapy of cancer cells in culture using the nanoparticle with $^{111}$In- or $^{125}$I-labeled antisense oligomers [6,7].

The streptavidin was employed as a linker of these components because of the simplicity of linking biotinylated agents via this protein [15,16] that stands in contrast to covalent conjugations [17]. More importantly, we hypothesized that a covalent bond linking the radiolabeled antisense oligomer to the antibody may not dissociate sufficiently in cells to permit the required migration of the oligomer to the nucleus.

We report herein on a study designed to support our hypothesis that migration of an antisense oligomer to the nucleus of cancer cells requires the dissociation of the three-component nanoparticle by showing that an antisense oligomer does not accumulate in the nucleus following incubation in cell culture when covalently conjugated to the antibody.

## 2. Materials and methods

### 2.1. Chemicals and cell lines

The 18-mer uniform phosphorothioate DNA (5′-GCG TGC CTC CTC ACT GGC-3′) antisense to the RIα mRNA was obtained with a thiol group on the 3′ end via a C3 linker and with a Cy5.5 fluorophor on the opposite end (Alpha DNA, Quebec, Canada). The antisense MORF, a DNA analogue with the same sequence, was obtained with a biotin group on the 3′ equivalent end via a 6-aminohexanoic acid linker and with a primary amine on the opposite end (GeneTools, Philomath, OR, USA). The herceptin was obtained from Genentech (South San Francisco, CA, USA) as the clinical product. The tat (biotin-G-R-K-K-R-R-Q-R-R-R) was purchased as the native L isomer with the biotin attached to the amine end via a 6-aminohexanoic acid linker and high-performance liquid chromatography (HPLC) purified (21st Century Biochemicals, Marlboro, MA, USA). Streptavidin was obtained from Sigma (St. Louis, MO, USA). The *N*-[a-maleimidoacetoxy]succinimide ester (AMAS) was purchased from Pierce (Rockford, IL, USA). The cyclic diethylenetriamine pentaacetic acid (cDTPA) anhydride was purchased from Sigma. The TBE gels were obtained from Bio-Rad Laboratories (Hercules, CA, USA). The $^{99m}$Tc-pertechnetate was eluted from a $^{99}$Mo-$^{99m}$Tc generator (Bristol-Myers Squibb Medical Imaging, North Billerica, MA, USA). The $^{111}InCl_3$ was from Perkin Elmer Life Science (Waltham, MA, USA). All other chemicals were reagent grade and were used without purification.

The SK-BR-3 cells were cultured and maintained in the McCoy's 5A medium with 10% fetal bovine serum (FBS). The MDA-MB-231 cells were cultured in Leibovitz L15 medium supplemented with 10% FBS. Cells were maintained at 37°C in the presence of 5% $CO_2$.

### 2.2. Preparation of herceptin-DNA-Cy5.5

The clinical-grade herceptin was purified from histidine by dialyzing against 0.1 M $NaHCO_3$ solution (pH 8.5) overnight. After dialysis, AMAS (10 µl, 10.6 µg/µl) in dimethyl sulfoxide (DMSO) was added to the mixture of DNA-Cy5.5 (20 µl, 12.0 µg/µl) and herceptin (186 µl, 33.9 µg/µl), both in 0.1 M phosphate-buffered saline (PBS) (pH 7.2). The final molar ratio of DNA/AMAS/herceptin was 1:10:1. The reaction mixture was then incubated at room temperature for 2 h before purification on a 0.7×20-cm Sephadex G200 column with 0.1 M PBS (pH 7.2). Fractions off the G200 column were analyzed for herceptin-DNA-Cy5.5 by size-exclusion HPLC with in-line UV detection and also by native polyacrylamide gel electrophoresis. To each sample, 3 µl of non-denatured loading buffer was added and the gel was run at low voltage (80 V) on a vertical gel electrophoresis system. The gel was then stained with Coomassie Blue-R250 solution in 10% acetic acid and 50% methanol for 2 h and destained in 10% acetic acid with 50% methanol for 2 h at room temperature. The Cy5.5 fluorescence of the gel was also measured. The yields of conjugates were roughly estimated according to the results of HPLC and gel electrophoresis.

### 2.3. Preparation of $^{111}$In-DTPA-herceptin

Cyclic DTPA anhydride (8 µl, 21 µg/µl) in DMSO was added dropwise to 100 µl of purified herceptin (15 µg/µl) to a final cDTPA/herceptin molar ratio of 50:1. The reaction mixture was then incubated at room temperature for 1 h before purification on a 0.7×20-cm P4 column (Bio-Rad) with 0.1 M PBS (pH 7.2 eluant). The fractions corresponding to the product were collected and the protein concentration of these was determined by UV spectrometry. The radiolabeling with $^{111}$In was similar to that described previously [18]. The conjugated and purified DTPA-herceptin (10 µl, 4.7 µg/µl) was labeled by incubation with 10 µCi $^{111}$In acetate for 1 h at room temperature and analyzed by size-exclusion HPLC on a 1×30-cm Superose-12 column using 20% acetonitrile in 0.1 M Tris-HCl (pH 8.0) at a flow rate of 0.6 ml/min with in-line radioactivity detection. The conjugate yield was calculated based on the HPLC results.

### 2.4. Preparation of $^{111}$In-DTPA-herceptin-DNA

Briefly, AMAS (2 µl, 5 µg/µl) in DMSO was added to a mixture of DNA (2 µl, 11.2 µg/µl) and DTPA-herceptin (120 µl, 4.7 µg/µl) in 0.1 M PBS (pH 7.2). The final molar ratio of DNA/AMAS/DTPA-herceptin was 1:10:1. The reaction mixture was then incubated at room temperature for 2 h before the product was purified on the G-200 column and concentrated by ultrafiltration (Centricon YM-10, Millipore, Massachusetts, USA). The concentrated product (10 µl, 3.6 µg/µl) was labeled by incubation with 10 µCi of $^{111}$In acetate for 1 h at room temperature and analyzed as above for

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