Application and Evaluation of $[^{99mTc}]$-Labeled Peptide Nucleic Acid Targeting MicroRNA-155 in Breast Cancer Imaging

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Abstract
It has been reported that dysregulation of microRNA-155 expression and function is associated with tumorigenesis, growth, tumor subtypes, invasion, and poor survival rates. Peptide nucleic acid (PNA), an artificially synthesized nucleic acid mimic, has been applied for molecular diagnosis. In this study, a PNA sequence that undergoes complementary binding to miR-155 was labeled with $^{99mTc}$ to evaluate whether the tracer could visualize the expression of miR-155 in breast cancer. Both antisense PNA (anti-PNA, fully complementary bound to human mature miR-155, referred to as “anti-PNA-155”) and mismatched PNA (referred to as “mis-PNA”) single strands containing 23-mer were synthesized. The relative expression of miR-155 in MCF-7 cells and tumors was higher than that in MDA-MB-231 cells and tumors. Single-photon emission computed tomography (SPECT) scan showed that radioactivity mainly accumulated in kidney. MCF-7 tumors, but not MDA-MB-231 tumors, were clearly visualized after $[^{99mTc}]$anti-PNA-155 injection. MCF-7 tumors were less visible when coinjected with 100-fold excess of anti-PNA-155 or injected with $[^{99mTc}]$mis-PNA, which suggested specific binding. Biodistribution study results were consistent with SPECT imaging. We successfully demonstrated that $[^{99mTc}]$anti-PNA-155 could visualize miR-155 expression in vivo, suggesting it may be a promising probe applied in breast cancer.

Keywords
breast cancer, miR-155, peptide nucleic acid (PNA), $^{99mTc}$, molecular imaging

Introduction
MicroRNAs (miRNAs) are a newly discovered class of endogenous and small noncoding single-stranded RNAs of 20 to 24 nucleotides. They can target specific messenger RNAs (mRNAs) and degrade them, regulating gene expression at the posttranscriptional level. They have been found to participate extensively in many physiological and pathological processes such as cell proliferation and growth, differentiation, apoptosis, and oncogenesis. Over the past decade, numerous studies have shown that altered expression of microRNA-155 (miRNA-155, miR-155) is related to cancer and other diseases, reflecting staging, progress, and treatment outcomes. Particularly, miR-155 is reported to aberrantly express in breast cancer, lung cancer, and B-cell lymphoma. It is reported that miR-155 may act as an oncogene or a tumor suppressor depending on its expression level. Acquiring the expression level of miR-155 whenever necessary may help investigate cancer progression, which is an appealing strategy for deciding treatment plans.

Numerous studies have supported that miRNAs obtained from tissues and body fluid play a promising role in cancer detection. It is vital to develop noninvasive detection techniques for human cancer and other diseases in living subjects.

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The visualization of miR-155 expression in vivo can help to tailor treatment schemes because its overexpression is related to genome instability and resistance to therapy. An in vivo study by Van Roosbroeck et al. showed that application of anti-miR-155 treatment could make miR-155-overexpressing tumors sensitive to cisplatin chemotherapy in an orthotopic lung cancer model, leading to an obvious decrease in tumor size. Therefore, it is necessary to develop a molecular probe targeting miR-155, to allow visualization and semi-quantitation of the expression of miR-155 in vivo with positron emission tomography (PET) or single-photon emission computed tomography (SPECT).

The third-generation antisense oligonucleotides (ASOs) such as peptide nucleic acid (PNA), locked nucleic acid, and phosphoramide morpholino were developed. Comparing to normal oligonucleotides, PNAs hybridize more specifically and strongly to RNA and DNA. In addition, PNAs resist both nucleases and proteases. They not only don’t interact with cellular proteins which often normally bind with negatively charged macromolecules but also don’t induce RNase degradation of bound miRNA or mRNA. Therefore, ASOs and their analogues have been widely studied in molecular imaging and gene therapy with promising application.

Antisense imaging has been widely and intensively studied as an important method for noninvasively imaging oncogene expression in vivo. Oligonucleotide analogues labeled with radionuclides, fluorophores, or contrast agents as markers for antisense imaging of oncogenic mRNA are expected to have great potential for this. Tian et al. designed and synthesized a [99mTc]chelator-PNA-peptide probe to hybridize with cyclin D1 (CCND1, encoding cyclin D1 protein) mRNA and showed that high levels of CCND1 expression could be detected in vivo. Paudyal et al. showed that 64Cu-DOTA-PNA-peptide targeting human epidermal growth factor receptor 2 mRNA expression could reflect therapeutic efficacy or resistance sooner than computed tomography (CT) or magnetic resonance imaging (MRI). MicroRNAs, another widely studied target for antisense imaging, have been reported to play a key role in pathogenesis of various diseases such as cancer.

In this study, we developed a [99mTc]-labeled antisense PNA probe targeting miR-155 and evaluated its application in the visualization of miR-155 expression using human breast cancer xenograft models.

**Materials and Methods**

**Design and Synthesis of PNA Precursor**

The antisense PNA was designed to complement the human mature sequence hsa-miR-155-5p (5’-UAAUGCUAAUCGUGAUAGGGGUU-3’, MIR155HG, MIMAT0000646) on the basis of the miRBase database. The designed PNA sequence showed no gaps or mismatches when performed sequence alignment by Basic Local Alignment Search Tool among human genomic plus transcript (Human G+T) and mouse genomic plus transcript (Mouse G+T). The antisense PNA (anti-PNA-155) (5’-ACCCCTATACGGATTAGGATATA-3’), targeting miR-155 with highest specificity and affinity, and the mismatched PNA (mis-PNA) (5’-ACCCAATCGTCAATTCCATATA-3’), which was chosen to interact with none of the human genes, were synthesized and purified.

For 99mTc labeling, a short peptide consisting of 4 amino acids (Gly-(D)-Ala-Gly-Gly) which can form a N4 structure was added at the S’ end as a linker for the coupling of 99mTc by the ligand exchange method. For the purpose of decreasing steric hindrance, a 4-aminobutyric acid was introduced. Both antisense and mismatched PNA precursors were commissioned by Shanghai Science Peptide Biological Technology Co, Ltd (Shanghai, China) for synthesis and purification.

**Radiolabeling**

Radiolabeling was performed referring to the method of Zhao et al. In brief, 10 μL NaOH (0.15 M) was added to the PNA solution (1.7 nmol, 20 μL) and mixed. Subsequently, 200 μL (148-185 MBq) fresh [99mTc]-pertechnetate and 10 μL SnCl2·2H2O (1 mg/mL, 0.01 M HCl) were added immediately in sequence. The mixture was then reacted for 45 minutes at 37°C. At the end of reaction, phosphate-buffered saline (PBS, pH 7.0, 1 ×) was added for dilution, and the final pH was approximately 7.0. Afterward, the reaction mixture was subjected to high-performance liquid chromatography (HPLC) for analysis using a 4.6 × 250 mm Ultimate XB-C18 column (Welch Materials, Inc, Shanghai, China). High-performance liquid chromatography was performed on LC-20AT (Shimadzu Corporation, Tokyo, Japan) equipped with a SPD-20A UV/VIS detector and a flow count radiation detector (Bioscan, Washington, DC). The column was eluted at a flow rate of 1 mL/min at 25°C with a gradient of 5% to 90% MeCN/H2O with 0.1% trifluoroacetic acid for 20 minutes monitoring at 260 nm.

**In Vitro Stability and Partition Coefficient of Probes**

Aliquots of [99mTc]anti-PNA-155 and [99mTc]mis-PNA were incubated in saline and fresh human serum at 37°C for 6 hours. Saline samples were subjected to analysis after incubation for 6 hours. Serum samples were mixed with an equal volume of anhydrous acetonitrile and then the mixture was centrifuged (13 000 g, 10 minutes) to precipitate plasma protein. The supernatant was then aspirated for HPLC analysis. The radiochemical purity of the samples was measured by HPLC under the above conditions.

Both 1.0 mL PBS (pH 7.4) and 1.0 mL 1-octanol were mixed in a 4-mL centrifuge tube. Then, [99mTc]anti-PNA-155 (370 kBq) and [99mTc]mis-PNA (370 kBq) were respectively added to the mixed solution. The mixture was then continuously vortexed for 4 minutes and centrifuged (4000 rpm, 5 minutes). Radioactivity counts of a 50 μL sample from each phase was measured by an automatic gamma counter (PerkinElmer WIZARD2 2470, Spokane, Washington). The partition
Reverse primer U6-2-R CGAATTTGCGTGTCATCCT
Reverse transcription primer RT-U6-2 CGAATTTGCGTGTCATCCT
Reverse primer RP2 CAGTGCAGGGTCCGAGGTAT
Forward primer hsa-miR-155-5p
Reverse transcription primer RT-hsa-miR-155-5p-F

Relative threshold cycle values were utilized to further analyze diluted and analyzed by radio-HPLC. The supernatant and cell lysates were collected respectively at the end of incubation at each time point and their radioactivity was measured with a γ-counter.

The following blocking experiment was carried out using MCF-7 cells. One group of cells was incubated only with $[^{99m}Tc]anti$-PNA-155 (37 KBq, 50 μL) for 6 hours at 37°C. The other group was incubated with a 100-fold excess of non-labeled anti-PNA-155 30 minutes in advance and then incubated with $[^{99m}Tc]anti$-PNA-155 (37 KBq, 50 μL) for 6 hours at 37°C.

MCF-7 and MDA-MB-231 cells were subjected to cell internalization and efflux assays. For cell internalization study, $[^{99m}Tc]anti$-PNA-155 (37 KBq, 50 μL) was added to each well and incubated at 37°C for 1, 2, 4, and 6 hours. At the end of incubation, the supernatant was removed and collected and acetone/Hank’s balanced salt solution (HBSS) (pH 4.0) was added and incubated at 37°C for 10 minutes. The supernatant and cells were collected and counted. For cell efflux study, $[^{99m}Tc]anti$-PNA-155 (37 KBq, 50 μL) was added and incubated at 37°C for 4 hours. Cell uptake at 4 hours was considered the zero point for efflux study. The supernatant of each well was collected after incubation for 4 hours. Then fetal bovine serum (FBS)-free DMEM medium was added and incubated for 1, 2, 4, and 6 hours. The remaining steps were the same as above. The cell uptake ratio was calculated as follows: cell uptake % = counts of cells/(counts of supernatant + counts of cells) × 100%.

**In Vitro Cell Study**

MCF-7 and MDA-MB-231 cells were collected at approximately 85% confluence. Then, cells were counted by a cytometer (Cellometer Mini; Nexcelom Bioscience LLC, Lawrence, Massachusetts). Cells were seeded in 24-well plates (3 duplicate wells per each group) at the amount of 2 × 10⁵ cells per well and cultured overnight. $[^{99m}Tc]anti$-PNA-155 (37 KBq, 50 μL) was added to each well and incubated at 37°C for 1, 2, 4, and 6 hours. For the control study, MCF-7 was used for uptake of $[^{99m}Tc]mis$-PNA. The supernatant and cell lysates were collected respectively at the end of incubation at each time point and their radioactivity was measured with a γ-counter.

**Metabolism**

Four normal BALB/c nude mice (8 weeks old, female) were used to evaluate the metabolic stability of $[^{99m}Tc]anti$-PNA-155 and $[^{99m}Tc]mis$-PNA. Each mouse was injected via tail vein with the radiotracers (55.5-74 MBq). A sufficient amount of urine sample was collected at 2 hours postinjection and was mixed with double volume of acetonitrile. The mixtures were centrifuged at 13 000 rpm for 10 minutes. The supernatant was diluted and analyzed by radio-HPLC.

**Cell Culture**

The human breast cancer cell lines MCF-7 and MDA-MB-231 were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). They were both cultivated in Dulbecco’s modified Eagle high-glucose medium (DMEM; Gibco, Carlsbad, California) at 37°C in a humidified incubator with 5% CO₂. The medium was supplemented with 10% fetal bovine serum (Gibco), 100 μg/mL streptomycin, and 100 U/mL penicillin (Solarbio, Shanghai, China).

**Quantitative Real-Time Polymerase Chain Reaction**

Total RNA was extracted from MCF-7 and MDA-MB-231 cells and tumor tissues using NucliSens easyMAG extraction systems following the manufacturer’s instructions (bioMerieux, Inc, Durham North Carolina). Extracts were stored at −80°C for no longer than 1 week until use. All the primers are summarized in Table 1. Subsequently, an miRNA fluorescent quantitative polymerase chain reaction detection kit (BaiAoLaiBo, Haidian, Beijing, China) was introduced to test miR-155 in cells and tumor tissues. U6 was applied as an internal control to normalize the expression levels of miR-155. Relative threshold cycle values were utilized to further analyze the results of quantitative real-time polymerase chain reaction (qRT-PCR), converting to fold changes.

| Gene Name | Primer Name  | Primer Sequences (5′-3′) |
|-----------|--------------|--------------------------|
| Reverse transcription primer | RT-hsa-miR-155-5p | GTCGTATCCAGTGACGGGTCCGAGGTATTCGCACTGGATACGACacccct |
| Forward primer | hsa-miR-155-5p-F | GGCCTGGTAAACTGCTGACGACAG |
| Reverse primer | RP2 | CAGTCACCCGTCGAGGTAT |
| Reverse transcription primer | RT-U6-2 | CGAATTTCGGTGTCATCCT |
| Forward primer | U6-2-F | CTGCTTTCCGGCAGACATA |
| Reverse primer | U6-2-R | CGAATTTCGGTGTCATCCT |

**Animal Models**

All animal experiments were carried out according to the Institutional Animal Care and Use Committee of Tongji Medical College of Huazhong University of Science and Technology. Female BALB/c nude mice (5-6 weeks old) were purchased from Beijing HFK Bioscience Co Ltd (Beijing, China) and were kept in a specific pathogen-free barrier system room. For breast cancer xenograft models, female BALB/c nude mice were inoculated subcutaneously in the right shoulder with MCF-7 and MDA-MB-231 (1.0 × 10⁷, 125 μL), respectively. When tumor size reached 0.8 to 1.0 cm, the xenografted mice were subjected to following studies.
SPECT Imaging

The MCF-7 and MDA-MB-231 xenograft mice were injected via tail vein with $^{99m}$Tc-anti-PNA-155 (11.1-13 MBq, 125 μL). SPECT scan was performed using a human SPECT (Symbia T6; Siemens, Erlangen, Germany) equipped with a dedicated pinhole collimator at 1, 2, 4, and 6 hours postinjection with an acquisition time of 15 minutes. Mice were anesthetized intraperitoneally with 1% sodium pentobarbital aqueous solution (0.1 mL/20 g mouse) and placed on the table in the prone position. For a control study, another group of MCF-7 xenograft mice ($n=3$) were injected via tail vein with $^{99m}$Tc-mis-PNA (11.1-13 MBq, 125 μL) and underwent SPECT scanning under the same conditions. Blocking experiments were performed in another group of MCF-7 tumor models ($n=3$). The 100-fold excess of nonlabeled anti-PNA-155 was coinjected with $^{99m}$Tc-anti-PNA-155 (11.1-13 MBq, 125 μL).

**Biodistribution Studies**

For biodistribution experiments, grouping was the same as with SPECT imaging. Either $^{99m}$Tc-anti-PNA-155 (1.85 MBq/mouse) or $^{99m}$Tc-mis-PNA (1.85 MBq/mouse) was injected via tail vein into each group of tumor xenografted mice ($n=3$ in each group). The mice were euthanized 6 hours postinjection. Organs and tissues of interest were removed quickly and washed. All samples were weighed, and radioactivity was measured. The biodistribution result was expressed as a percentage of the injected dose per gram of tissue (% ID/g). All radioactivity measurements were corrected for decay.

**Statistical Analysis**

Data are expressed as mean ± standard deviation. Statistical analysis was performed using Student’s t test using commercial software (GraphPad Prism 8; GraphPad Software, La Jolla, California). $P<.05$ was considered a statistically significant difference.

**Results**

**Chemical and Radiochemical Characterization of Probes**

The molecular weights of anti-PNA-155 and mis-PNA were 6485.6 and 6468.1 g/mol determined by Matrix Assisted Laser Desorption Ionization-Mass Spectrometry (MALDI-MS) (Supplementary Figure S1). MALDI-MS (Shimadzu Biotech Axima Assurance, Shimadzu Biotech, Manchester, UK): $m/z$ calcd for (N-term)G-dA-G-G-(g-aminobutyric acid)-acc cct atc aac gac att aat tcc atg (C-term) [M + 1], Found 6485.6; (N-term)G-dA-G-G-(g-aminobutyric acid)-acc caa tcc taat tct ata ta (C-term) [M + 1], Found 6468.1. The labeling process for $^{99m}$Tc-anti-PNA-155 and $^{99m}$Tc-mis-PNA was simple with a molar activity of 97.94 ± 8.89 GBq/μmol ($n=5$). The radioactivity yield and purity were 95.24 ± 2.69% and 93.62 ± 3.85%, respectively ($n=5$), as measured by analytic HPLC. The absorption peaks of anti-PNA-155 and mis-PNA at 260 nm were in line with their
radiation peaks (Figure 1A and B). As shown in Figure 1C and D, the proportion of intact probes still exceeded 90\% after incubation for 6 hours at 37°C in saline and serum, indicating good stability in vitro. The Log $P$ of $[^{99mTc}]$anti-PNA-155 and $[^{99mTc}]$mis-PNA were $-1.47 \pm 0.02$ and $-2.00 \pm 0.02$, respectively, indicating that the two probes are relatively water soluble and hydrophilic.

**Metabolic Properties**

Metabolism study of $[^{99mTc}]$anti-PNA-155 and $[^{99mTc}]$mis-PNA was conducted using normal BALB/c nude mice. Since both the probes are mainly excreted from renal, urine sample was collected and analyzed. As shown in Figure 2, the major radioactivity was still the intact $[^{99mTc}]$-tracers, while they showed a certain degree of metabolism (around 15\%) in urine sample at 2 hours postinjection.

**Characterization of miR-155 Expression in Cells and Tumor Tissues**

A qRT-PCR assay was performed to characterize the expression of $\text{miR-155}$ by MCF-7 and MDA-MB-231 cells and tumor tissues. As can be seen from Figure 3, the relative expression level of $\text{miR-155}$ by MCF-7 cells and tumor tissues was significantly higher than that of MDA-MB-231 ($P < .001$).

**In Vitro Cell Studies**

As shown in Figure 4A, the uptake of $[^{99mTc}]$anti-PNA-155 by MCF7 cells increased gradually over time, and reached maximum at 6 hours, which was significantly higher than the uptake by MDA-MB-231 cells ($P < .001$). For control experiments, Figure 4B shows that the uptake of $[^{99mTc}]$anti-PNA-155 was obviously higher than that of $[^{99mTc}]$mis-PNA in MCF7 cells ($P < .001$). Cell-blocking studies were performed to assess the binding specificity of $[^{99mTc}]$anti-PNA-155 in vitro. The uptake of $[^{99mTc}]$anti-PNA-155 by another group of MCF7 cells decreased significantly when pretreated with excessive unlabeled anti-PNA-155 ($P < .001$) (Figure 4C). The cell internalization ratio of MCF-7 was much higher than MDA-MB-231 ($P < .001$; Figure 4D) and was similar to the cell uptake ratio of MCF-7. As shown in Figure 4E, the retention ratio of $[^{99mTc}]$anti-PNA-155 in MCF-7 cells was obviously higher than that in MDA-MB-231 cells ($P < .001$).
SPECT Imaging

SPECT scans were conducted to evaluate whether $[^{99m}Tc]$anti-PNA-155 could specifically visualize breast tumors with relative high expression levels of miR-155. As shown in Figure 5, radioactivity mainly accumulated in the abdomen and pelvis, which demonstrated that $[^{99m}Tc]$anti-PNA-155 and $[^{99m}Tc]$mis-PNA are metabolized mainly via kidney and liver. The contour of MCF-7 tumor was clearly visualized by $[^{99m}Tc]$anti-PNA-155 at 1, 2, 4, and 6 hours postinjection, while MDA-MB-231 tumor was not visible during the observation period (Figure 5A and B). For further determining the specificity of $[^{99m}Tc]$anti-PNA-155, blocking studies were conducted in another group of MCF-7 xenograft mice. The blocking studies showed that MCF-7 tumors were less visible and tumor uptake markedly decreased when blocked by excessive unlabeled anti-PNA-155 (Figure 5C). As control, MCF-7 tumors were less visualized by $[^{99m}Tc]$mis-PNA (Figure 5D).

Biodistribution Studies

Biodistribution studies were conducted in the xenografted mice to further evaluate pharmacokinetic characteristics as well as targeting affinity and specificity for miR-155 in vivo of $[^{99m}Tc]$anti-PNA-155. The results of biodistribution are shown in Figure 6 and Supplementary Table S1. The highest radioactivity in the kidneys of MCF-7 ($5.66 \pm 0.99 \%$ID/g) and MDA-MB-231 ($4.43 \pm 0.21 \%$ID/g) indicated that renal clearance is the main metabolic pathway of $[^{99m}Tc]$anti-PNA-155, which is consistent with imaging findings. $[^{99m}Tc]$mis-PNA ($6.31 \pm 0.86 \%$ID/g in kidney) uptake was similar. The mean tumor uptake of $[^{99m}Tc]$anti-PNA-155 by MCF-7 xenograft mice ($0.51 \pm 0.05 \%$ID/g) was significantly higher than that by MDA-MB-231 ($0.22 \pm 0.02 \%$ID/g, $P = .0019$), which demonstrated the excellent ability to differentiate between tumors with relatively high and low expression level of onco-miR-155. The mean tumor uptake of $[^{99m}Tc]$anti-PNA-155 was higher than that of $[^{99m}Tc]$mis-PNA ($0.38 \pm 0.02 \%$ID/g, $P = .034$) in MCF-7 xenograft mice. In addition, when blocked...
with anti-PNA-155, the tumor uptake was significantly reduced (0.34 ± 0.04 %ID/g, $P = 0.024$).

**Discussion**

Numerous studies have reported that dysregulation of miRNA expression level plays important roles in the progression of diseases including cardiovascular disorders, neurological disease, diabetes, and especially cancer. Upregulation of the expression of miR-155, an oncogenic miRNA, is related to the development of breast cancer, which suggests its use as a potential tumor marker for breast cancer. Based on their desirable properties, including that they are resistant to enzymatic degradation, hybridize more strongly and specifically to RNA, are stable over a wide range of pH, and display the highest melting temperature (Tm) for duplexes, PNAs are promising for development as antisense imaging agents. In this study, we applied an antisense PNA probe specifically targeting miR-155 to visualize its expression, thus providing some diagnostic and therapeutic information at the oncogene level for breast cancer.

The [$^{99m}$Tc]-labeled PNA antisense probe in our study was developed with a simple labeling process. It has a high radiochemical yield, high radiochemical purity, and good stability. The uptake by MCF-7 cells with their relatively higher expression of miR-155 was significantly higher than by MDA-MB-231 cells, indicating that [$^{99m}$Tc]anti-PNA-155 complementarily bound to miR-155 in cytoplasm, thus reducing the probe efflux.

For in vivo SPECT imaging, MCF-7 tumors with relatively high miR-155 expression were clearly visible, while MDA-MB-231 tumors with low miR-155 expression were not visible after injection of [$^{99m}$Tc]anti-PNA-155 at all scan time points, indicating superior specificity and targeting capability of the probe. For biodistribution, the organs with the highest uptake of probe were kidney and liver, which is consistent with the studies of Mardirossian et al and Sun et al. The uptake by MCF-7 tumor was obviously higher than MDA-MB-231 tumor post-injection of [$^{99m}$Tc]anti-PNA-155 at 6 hours. Consistent with the in vitro findings, the uptake by MCF-7 tumor of [$^{99m}$Tc]mis-PNA or of [$^{99m}$Tc]anti-PNA-155 when blocked by unlabeled anti-PNA-155 was low, again signifying that [$^{99m}$Tc]anti-PNA-155 specifically targets miR-155, and can visualize its expression in vivo.

It cannot be ignored that there are several shortcomings in this study. First, radioactivity in the blood was relatively high probably for the reasons that their hydrophobicity promotes PNA aggregation and seems to promote their relatively nonspecific adherence to both other macromolecules and larger surfaces. Second, high uptake by kidney and liver resulted in high imaging background, mainly because the probe is mainly excreted by these organ systems. Finally, tumor uptake was relatively low due to unfavorable delivery efficiency of the probe, which is not conducive to imaging and further treatment. Tian et al reported that tumor uptake was 0.20 ± 0.05 %ID/g postinjection of [$^{99m}$Tc]MYC antisense probe at 24 hours.
et al\textsuperscript{17} developed $[^{99m}\text{Tc}]$CCND1-PNA-IGF1 chimeras with tumor uptake of $0.20 \pm 0.06 \%\text{ID/g}$ (4 hours), $0.17 \pm 0.06 \%\text{ID/g}$ (12 hours), and $0.11 \pm 0.05 \%\text{ID/g}$ (24 hours), respectively. Owing to PNA being a noncharged nucleotide analogue, the delivery of unmodified PNAs to target cells is less efficient.\textsuperscript{33} Many strategies have been proposed to improve the delivery efficiency of PNAs. Chemical modifications were initially designed for conjugation to PNA to facilitate uptake such as cell-penetrating peptide R6-penetratin,\textsuperscript{34} 4 lysine residues,\textsuperscript{34} and octreotide.\textsuperscript{35} Application of delivery carriers, such as gold nanoparticles,\textsuperscript{36} cationic nanocomplexes,\textsuperscript{37} cationic shell crosslinked nanoparticles,\textsuperscript{38} liposomes,\textsuperscript{39} and exosomes,\textsuperscript{40} could improve the cell delivery efficiency of PNA and other nucleic acids.

## Conclusion

In this study, we successfully prepared an antisense PNA-mediated probe and showed that $[^{99m}\text{Tc}]$anti-PNA-155 could specifically and efficiently target oncogene miR-155, noninvasively visualizing miR-155 expression in vivo, thus providing helpful information at gene level for breast cancer. However, relatively higher radioactivity in blood, lower delivery efficiency, and relatively low tumor uptake need to be improved in the future.

## Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

Supplemental material for this article is available online.

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