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Gold nanoparticle-based 2′-O-methyl modified DNA probes for breast cancerous theranostics

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ABSTRACT

MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulated diverse cellular processes including differentiation, proliferation, apoptosis, metabolism and signal transduction pathways. An increasing number of data suggested that miRNA-21 could be identified as diagnostic and therapeutic biomarker for breast cancer. Meanwhile, inhibiting the function of miRNA-21, resulting in cells growth inhibition and apoptotic cells death. To realize miRNA-21detection and inhibition to diagnostic and therapeutic breast cancer cells, we developed gold nanoparticle-based 2′-O-methyl modified DNA probes (AuNP-2′-OMe-DNA probes) for diagnostic and therapeutic breast cancer. Gold nanoparticles were functionalized with chemically modified miRNA-21 inhibitor to suppress the function of miRNA-21 for the therapeutic breast cancer, at the same time, fluorophore-labeled DNA molecules were hybridized with antimiRNA-21 for diagnostic breast cancer. The results showed that the 2′-O-methyl modified DNA can improve stability, increase binding affinity to target strands and enhance the therapeutic effects. The experimental results also demonstrated that antimiR-21 were efficiently introduced into the cells and knocked down miRNA-21 to inhibit its function, leading to growth inhibition and apoptotic cells death. We prospected that chemically modified miRNA-21 inhibitor based on gold nanoparticles would be as a promising diagnostic and therapeutic platform for breast cancer clinically.

1. Introduction

Breast cancer is leading cause of cancer death in women worldwide [1]. Recently, many kinds of therapies have been used for treatment of breast cancer, in which the most successful treatments have been chemotherapy [2,3] and radiotherapy [4–6]. However, these therapies are often accompanied by side effects [7,8]. To alleviate these side effects, many new therapies [9–11] have been tried for therapeutic breast cancer, of which miRNA-mediated gene silence is an evolving therapeutic strategy [12–16].

MiRNAs are a class of small non-coding RNAs that modulate protein expression by binding to complementary or partially complementary target mRNAs and thereby targeting the mRNAs for degradation or translational inhibition [17–19]. MiRNA is a promising endogenous stimulus, as well as a potential therapeutic target because it shows considerable differential expression between the malignant tissues and their normal counterparts [20]. Especially, emerging evidence demonstrates an important role of miRNAs in regulating diverse cellular processes including differentiation, proliferation, apoptosis, metabolism and signal transduction pathways [21–23]. Individual miRNAs engage numerous mRNAs targets, often encoding multiple components of complex intracellular networks. Thus, the manipulation of miRNAs expression or function can have profound impact on cellular phenotypes [24]. Hence, inhibiting the function of miRNAs by antisense oligonucleotide (that is, antimiRs) is a potential therapeutic strategy, which may yield patient benefits unobtainable by other therapeutic approaches [25–27]. In recent years, there has been increasing efforts in exploiting antimiRs. For example, Mo et al. transfected antisense oligonucleotide of miRNA-21(antimiR-21) in breast cancer cell and revealed that miRNA-21 is overexpressed in breast tumour tissues and antimiR-21 inhibits both cell growth in vitro and tumour growth in vivo [28]. Ju et al. proposed molecular beacon (MB) conjugated to multifunctional SnO_2 nanoparticles with a disulfide linkage using folic acid for cell-specific delivery for imaging and inhibiting intracellular miRNAs-21 [29]. Recently, a novel antimiRs therapeutic platform that targets the acidic microenvironment of tumours has been described,

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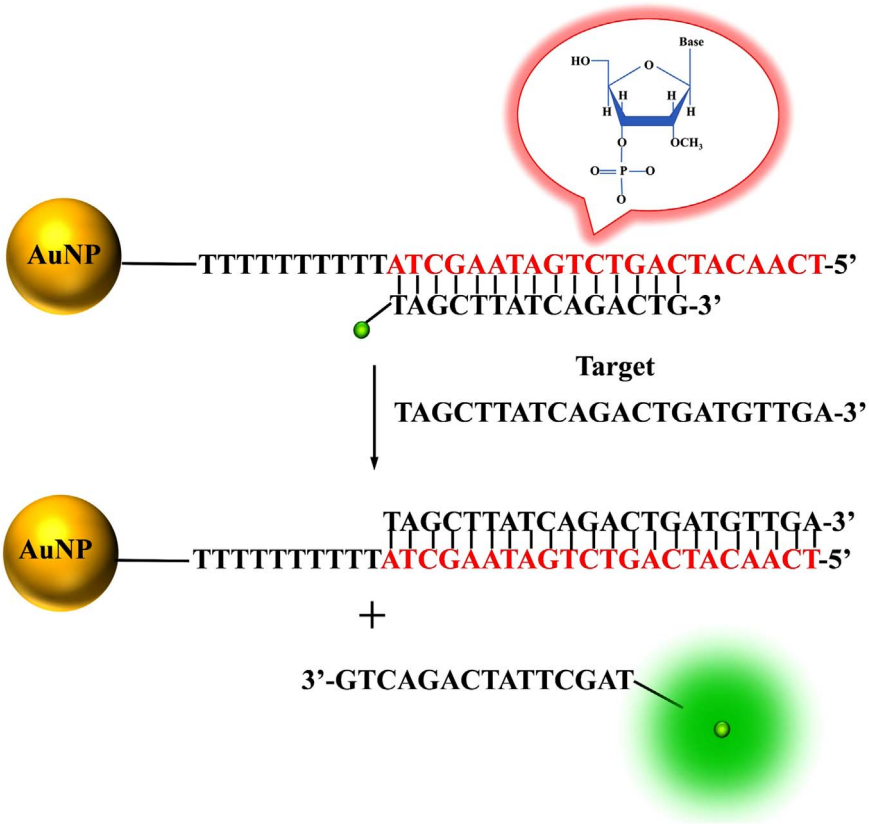

Scheme 1. Schematic illustrating the working mechanism of the AuNP-2′-OMe-DNA probes, where the antimiR-21 strand is modified with 2′-O-methyl (red).

which deliver a peptide nucleic acid (PNA) modified antimiR-155 by pHLIP (low pH-induced transmembrane structure) to inhibit miR-155 in a mouse model of lymphoma and delay tumour growth [30]. Conde et al. used gold-nanobeacon as a theranostic probe for gene specific silencing [31,32]. MiRNA therapeutics may be superior over other therapies (e.g., single proteins or small molecules) as a miRNA can potentially regulate complex biological processes [33]. miRNA-mediated gene silence had advantages of stability and targeting delivery without potential side effects [34]. However, an ideal miRNA inhibitor platform would display the following properties: high affinity to target genes; low toxicity; high specificity; resistance to exonucleases; relatively low cost for synthesis and the ability to enter cells without use of transfection agents [35].

To satisfy the above criteria, herein, we developed gold nanoparticle-based 2′-O-methyl modified DNA probes (AuNP-2′-OMe-DNA probes) for miRNA-21 detection and inhibition in breast cancer cells. To make sure the better detection and therapeutic effect, antimiR-21 strands are fully modified by 2′-O-methyl to increase binding affinity with tumour gene and improve stability and resistance to exonucleases to prolonged inhibition of miRNAs [36]. As shown in Scheme 1, Au NPs are functionalized with miRNA-21 inhibitors that are hybridized with fluorophore-labeled DNA molecules named “flares”, miRNA-21 inhibitors are 2′-O-methyl modified antisense oligonucleotide that are complementary to the mature miRNA-21 to suppress its function, resulting in cells growth inhibition and apoptotic cells death [27]. On the contrary, abnormal expression miRNA-21 in breast cancer cells may function as an oncogene by blocking expression of critical apoptosis-related genes to promote the growth of cancer cells [27]. In the absence of targets, the close proximity of the fluorophore to the AuNP surface leads to quenching of the fluorescence. However, when a target miRNA-21 binds to the antisense oligonucleotide, the concomitant displacement of the flare can be detected as a corresponding increase in fluorescence and the function of miRNA-21 can be inhibited to realize diagnostic and therapeutic breast cancer cells. (Scheme 1).

2. Experimental section

2.1. Materials and instruments

Trisodium citrate was obtained from Sinopharm Chemical Reagent Co., Ltd. (China). Chloroauric acid ($HAuCl_4{\cdot}4HO_2$) was obtained from Shanghai Chemical Reagent Company (Shanghai, China). 3-(4,5-Dimethylthiazol-2-yl)-2-diphenyltetrazolium bromide (MTT) and fetal bovine serum (FBS) were purchased from Sinopharm Chemical Reagent Co., Ltd. (China). Deoxyribonuclease I (DNase I) was purchased from Sangon Biotechnology Co., Ltd (Shanghai, China). Loading buffer was purchased from TaKaRa Bio Inc. (Dalian, China). SYBR Gold was purchased from Invitrogen (U.S.A.). All other reagents were analytically grade. All aqueous solutions were prepared using ultrapure water (≥ 18 MΩ, Milli-Q, Millipore). All oligonucleotides were synthesized and HPLC purified by Sangon Biotechnology Co., Ltd (Shanghai, China) These sequences are listed as following.

miRNA-21 target: 5′-TAGCTTATCAGACTGATGTTGA-3′
one-base mismatched miRNA-21 target: 5′-TAGCATATCAGACTGATGTTGA-3′
antimiR-21 sequence: 5′-TCAACATCAGTCTGATAAGCTAT_{10}-SH-3′
(underline section: 2′-O-methyl modified)
flares: 5′-FAM TAGCTTATCAGACTG-3′
miRNA-141 target: 5′-TAACACTGTCTGGTAAAGATGG-3′
let-7d target: 5′-AGAGGTAGTAGGTTGCATAGTT-3′
miRNA-429 target: 5′-TAATACTGTCTGGTAAAACCGT-3′
miRNA-200b target: 5′-TAATACTGCCTGGTAATGATGAC-3′

The transmission electron microscopic (TEM) images were obtained on a JEM-2100 transmission electron microscope (JEOL Ltd., Japan).