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Multifunctional aptamer-based nanoparticles for targeted drug delivery to circumvent cancer resistance

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ABSTRACT

By its unique advantages over traditional medicine, nanomedicine has offered new strategies for cancer treatment. In particular, the development of drug delivery strategies has focused on nanoscale particles to improve bioavailability. However, many of these nanoparticles are unable to overcome tumor resistance to chemotherapeutic agents. Recently, new opportunities for drug delivery have been provided by oligonucleotides that can self-assemble into three-dimensional nanostructures. In this work, we have designed and developed functional DNA nanostructures to deliver the chemotherapy drug doxorubicin (Dox) to resistant cancer cells. These nanostructures have two components. The first component is a DNA aptamer, which forms a dimeric G-quadruplex nanostructure to target cancer cells by binding with nucleolin. The second component is double-stranded DNA (dsDNA), which is rich in -GC- base pairs that can be applied for Dox delivery. We demonstrated that Dox was able to efficiently intercalate into dsDNA and this intercalation did not affect the aptamer's three-dimensional structure. In addition, the Aptamer-dsDNA (ApS) nanoparticle showed good stability and protected the dsDNA from degradation in bovine serum. More importantly, the ApS&Dox nanoparticle efficiently reversed the resistance of human breast cancer cells to Dox. The mechanism circumventing doxorubicin resistance by ApS&Dox nanoparticles may be predominantly by cell cycle arrest in S phase, effectively increased cell uptake and decreased cell efflux of doxorubicin. Furthermore, the ApS&Dox nanoparticles could effectively inhibit tumor growth, while less cardiotoxicity was observed. Overall, this functional DNA nanostructure provides new insights into the design of nanocarriers to overcome multidrug resistance through targeted drug delivery.

1. Introduction

Aptamers are small, simple, single-stranded DNA or RNA oligonucleotides that assume a specific three-dimensional (3D) structure and bind to target molecules with high specificity and affinity [1–4]. Recently, the biomedical field has given increasing attention to aptamers as a result of their unique advantages, such as low molecular weight, lack of immunogenicity, and high specificity [5–8]. Nucleolin, a multifunctional protein involved in RNA transcription, DNA replication, rRNA processing, and Bcl-2 stabilization, is highly expressed in continuously proliferating cells, such as cancer cells, and it has been reported to shuttle between the nucleus, the cytoplasm, and the cell surface [9,10]. In this study, we chose an aptamer which is able to target the nucleolin molecule with specificity equal to that of antibody and, at the same time, has therapeutic effect on the tumor region [11–13]. This aptamer, named AS1411, which consists of 26-base-pair, guanine-rich oligonucleotides, is the first aptamer administrated at the clinical level. As reported by many papers previously, this aptamer could form a G-quartet-containing structure that remained remarkable stability in serum-containing medium [14]. The G-quadruplex (four-stranded) structure of AS1411 can be determined using CD spectrum, which exhibits a positive peak at 262 nm and a negative peak at 238 nm [1]. The most significant properties of AS1411 aptamer are that, it is not only able to target nucleolin molecule and specifically accumulate in the cancer cells, but also has the

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therapeutic effect on the tumor region. AS1411 has shown growth-inhibitory properties against a wide range of cancer cells such as prostate (DU145), breast (MDA-MB-231 and MCF-7), and lung (A549) cancer cells [7,15–17].

It is well known that multidrug resistance (MDR) has been one of the major impediments to successful chemotherapy [18]. Doxorubicin (Dox) is an effective drug widely used as a chemotherapeutic agent for clinical treatment of cancers [19–21]. Closely related to the natural product daunomycin, it contains flat aromatic rings and works like all anthracyclines by intercalating into the genomic DNA, namely preferentially binding to double-stranded 5′-GC-3′ or 3′-GC-5′ base pairs through noncovalent intercalation, and then inhibits DNA replication [3,22,23]. Cancer cells that are resistant to doxorubicin remain a significant obstacle to drug treatment. Overexpression of P-glycoprotein (P-gp) in resistant cells induces increased drug efflux and decreased intracellular drug accumulation [24]. Recently, several attempts to circumvent doxorubicin resistance using a nanoparticle-based delivery system have been reported [21,25–27]. Nanoparticles could enter cells by an endocytosis pathway, which may be independent of the P-gp pathway. Nanoparticles have a wide range of potential biomedical and biotechnological applications. In this study, we chose the aptamer molecule for its targeting capability and therapeutic effect on cancer cells. Our goal was to functionalize aptamers as a drug nanocarrier to deliver Dox into breast cancer cells, thus circumventing chemotherapeutic resistance. This functional Aptamer-dsDNA (ApS) nanocarrier has two DNA components. The aptamer part forms a dimeric G-quadruplex nanostructure after annealing, and this can target the cancer cells. The targeting capability of the functionalized aptamer is based on the overexpression of nucleolin in breast cancer cells and its subsequent endocytosis [28]. The other part of the nanocarrier is a double-stranded DNA (dsDNA) sequence, which consists of 5′-GC-3′ or 3′-GC-5′ base pairs, to deliver the Dox payload. As a novel carrier, the multifunctional ApS nanostructure could potentially exploit the therapeutic efficacy of Dox and the binding characteristics of aptamers (Fig. 1). According to the functionalized nucleic acid nanocarrier, a simple, but effective, targeted doxorubicin delivery system was developed. Compared with some common nanosystems, such as inorganic and polymeric nanocarriers, our developed DNA nanoparticles are more bio-friendly, as all the components are biodegradable.

We first hypothesized that Dox would intercalate into the GC sequences of the functionalized ApS nanocarrier and be efficiently delivered into drug-resistant MCF-7 breast cancer cells. This innovative ApS&Drug nanocomplex, which employs 1) target-specific G-quadruplex aptamer and 2) GC-rich dsDNA to deliver drug payload, is based on the precise self-assembly of DNA building blocks to form self-assembled nanostructures [22,29–32]. Most importantly, the resistance of breast cancer cells was effectively circumvented *in vitro* and *in vivo*.

2. Materials and methods

2.1. Materials

Doxorubicin was purchased from Zhejiang Hisun Pharmaceutical Co. Ltd (Zhejiang, China). Oligonucleotides shown in Table S1 were purchased from Beijing Sunbiotech Co. Ltd. (Beijing, China) and used without further purification. TE buffer is composed of 10 mM tris hydroxymethyl aminomethane hydrochloric acid (Tris-HCl), 1 mM ethylenediaminetetraacetic acid (EDTA), and 100 mM NaCl. The annealing buffer consists of 28 mM Tris-HCl, 200 mM KCl, and 4 mM $MgCl_2$.

2.2. dsDNA annealing and doxorubicin intercalation

We designed the following oligonucleotide sequences: S2, S4, S6, S6-1, S6-2, S6-3, S6-4, S8, S10, S20, ApS6, and ApS10 (Table S1). S6 was dissolved in annealing buffer (200 mM KCl, 4 mM $MgCl_2$, and 28 mM Tris-HCl) and TE buffer (10 mM Tris-HCl, 1 mM EDTA, and 100 mM NaCl), respectively, and then annealed at 100 °C for 5 min, followed by slow cooling to room temperature. The samples were run on a 1% agarose gel and stained with ethidium bromide.

The dsDNA oligonucleotides (S2, S4, S6, S6-1, S6-2, S6-3, S6-4, S8, S10, and S20) and the aptamer-dsDNA oligonucleotides (ApS6, and ApS10) were separately dissolved in annealing buffer, annealed at 100 °C for 5 min, and then slowly cooled to room temperature to form double-stranded DNA and/or G-quadruplex structure. Doxorubicin at a concentration of 3 μM was incubated with the dsDNA or ApS in an ice bath for 2 h. Fluorescence was monitored at an excitation wavelength of 485 nm and emission wavelength between 500 nm and 720 nm on a Tecan Infinite M200 continuous spectrum multi-function microplate reader.

2.3. Preparation of aptamer and ApS&Dox nanoparticles

G-quadruplex nanostructures of aptamer were prepared as reported previously [1]. In brief, the aptamer oligonucleotide was dissolved in annealing buffer as described above, annealed at 100 °C for 5 min, then slowly cooled to room temperature to form the structure of aptamer. For the preparation of ApS&Dox nanoparticles, the ApS6 or ApS10 oligonucleotides were annealed as described above to form nanostructures; then doxorubicin was incubated with the solution for 2 h in an ice bath and stored at 4 °C until needed.

2.4. Stability analysis of the functionalized aptamer

The aptamer, double-stranded DNA, and functionalized ApS&Dox nanoparticles were separately mixed with 10% FBS and incubated at 37 °C. After incubation for 2, 24, or 48 h, the mixtures were run on a 1% agarose gel and stained with ethidium bromide. The band intensity was quantitated by Image J.

2.5. Drug release from ApS&Dox nanoparticles

Release of doxorubicin from the ApS&Dox nanoparticles was determined by fluorescence spectroscopy. ApS10&Dox and ApS6&Dox were dissolved in PBS at pH 7.4 and pH 5.0 at 37 °C. After 0 h, 2 h, 5 h, 12 h, 24 h, 48 h, and 72 h, the sample was taken to test the fluorescence of doxorubicin.

2.6. Nanoparticle characterization

The sizes of aptamer, ApS6&D ox, and ApS10&Dox were characterized by transmission electronmicroscopy (TEM, Tecani-G2-20-S, TWIN, FEI, USA), atomic force microscopy (AFM, Nano-scopeIII SPM system, Veeco, USA), and dynamic light scattering (DLS, NanoZS ZEN3600, Malvern Instruments, Malvern, UK). Circular dichroism (CD) measurements of nanoparticles were performed on a J-810 Circular Dichroism Spectrometer (JASCO INC, Easton, MD, USA).

2.7. Cell culture

The human breast MCF-7 cancer cell lines sensitive (MCF-7S) and resistant (MCF-7R) to doxorubicin were cultured in Dulbecco's Modified Eagle's Medium (DMEM, HyClone) with high glucose and RPMI 1640 medium, and the human normal liver L02 cell line was