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Aptamer–integrated DNA nanoassembly: A simple and sensitive DNA framework to detect cancer cells

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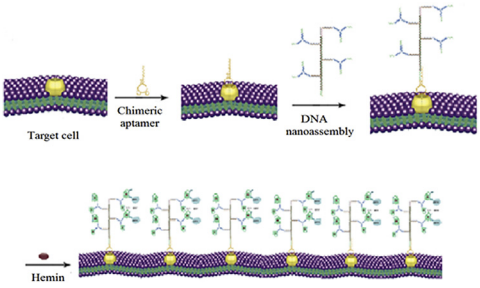
HIGHLIGHTS

- A label-free colorimetric nano-aptasensor was developed for cancer cell detection.
- Signal amplification is an interesting process to increase sensitivity.
- DNA Self-assembly is a remarkable process for organizing systems to create functional structures and amplifying signal.
- The colorimetric nanoaptasensor offered high sensitivity and selectivity toward target cancer cells.

GRAPHICAL ABSTRACT



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ABSTRACT

The development of powerful techniques to detect cancer cells at early stages plays a notable role in diagnosing and prognosing cancer patients and reducing mortality. This paper reports on a novel functional DNA nanoassembly capable of detecting cancer cells based on structural DNA nanotechnology. DNA nanoassemblies were constructed by the self-assembly of a DNA concatemer to a plenty of sticky-ended three-way junctions. While an aptamer moiety guided the nanoassembly to the target cancer cell, the peroxidase-mimicking DNAzymes embedded in the nanoassemblies were used as the sensing element to produce colorimetric signals. As proof-of-concept, as low as 175 cancer cells were detected by the assay, and color change was clearly distinguished by the naked eyes. The proposed system enjoys potential applications for point-of-care cancer diagnosis, with its excellent sensitivity and selectivity.

1. Introduction

Sensitive and specific detection of cancer cells serves a crucial role in the prevention, effective treatment, and examination of cancer metastasis [1,2]. Nowadays, the most common techniques in molecular biology for detecting cancer cells are immunophenotyping by flow cytometry or antibody microarray, identification of

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cancer-specific (epi)genetic mutations by PCRs and sequencing, and analysis of the transcriptome by DNA microarrays and RNA-seq technologies [3]. In particular, immunophenotyping technologies due to the analysis of cell-surface biomarkers without labor-intensive sample pretreatments have become more popular in recent years. However, employing different labeled antibodies, which require expensive equipment and lack an appropriate signal amplification strategy for improving the sensitivity, has limited their applications in diagnosis [3–7].

The emergence of nucleic acid nanotechnology, based on the programmability of Watson-Crick base-pairing, has made DNA as a widely used building block for the assembly of nanoscale addressable materials and dynamic molecular architectures [8,9]. Such biomimetic molecular devices in a wide range of size and function, are characterized by such features as sequence programmability, evident biocompatibility, chemical addressability, and exceptional biostability. These nanoassemblies can autonomously move or process information and combine with different functional elements such as aptamers – artificial nucleic acids with binding capability to particular targets. Aptamer moieties can guide the intended nanodevices to target cell surfaces and provide a distinguished platform for in situ construction with accurate spatial control of nanofactories on target living cell surfaces. Such a platform can be utilized for smart functions such as sensing, computation, signal amplification or manipulation of biological activities [9–15].

Signal amplification, the capability of increasing the signal intensity to an acceptable level, is a ubiquitous feature in biology and engineering [16–18]. Nanomaterial-based signal amplifications have attracted much attention due to their rapid analysis procedures and easy miniaturization [19–21]. In these strategies, the nanomaterials usually serve as catalysts to trigger the detectable signals in which the interaction of the reporter and the target lead to a multitude of reporter molecules (catalysis) or carriers/loaders for signal tags (multivalency) [21,22]. Among the nanomaterial building blocks, DNA – as a natural bridge between nanotechnology and biotechnology with exceptional properties – provides a generic material for nanoscale engineering and enzyme-free signal amplifications [23]. The improvements in this field have resulted in the design of simple and logical ultrasensitive sensing platforms through the construction of structural bulk-scale nanoassemblies, such as linear and branched nanostructures, DNA origamis, DNA hydrogels, and DNA multi-arms [8,24–26]. As a result, numerous aptamer-integrated DNA nanostructures have recently been proposed for cancer imaging and detection [13,27–29]. For example, Kelley and coworkers designed a self-assembled quantum dot DNA hydrogel for xenografted breast cancer tracing [30]. Using DNA hydrogel formation technology, Zuo and coworkers proposed a strategy in which porous DNA hydrogels serve as cloaking networks of circulating tumor cells for subsequent culture and analysis [31]. Douglas et al. designed an autonomous aptamer-guided DNA nanorobot capable of sensing and releasing of molecular payloads to tumor cells [32].

In order to convert the aptamer-target binding events into color signals, numerous sensitive non-apparatus detection approaches have been reported [33–35]. Colorimetric assays, particularly those exploiting G-quadruplex DNAzymes with peroxidase activity as signal-amplifying elements have been used in the detection of cancer cells, due to simplicity in design, robustness across diverse conditions, and cost-effectiveness [33,36,37]. Using this signal amplifier and other relatives like split G-quadruplex partzymes, Shi and coworkers developed a colorimetric aptasensor for the detection of human leukemic lymphoblast (CCRF-CEM cells) [36]. Furthermore, the Li group proposed a label-free aptamer-based strategy and a G-quadruplex sequence as the signal probe to detect the same cancer cells [37]. To improve the sensitivity of G-quadruplex DNAzyme-based sensors, various strategies such as rolling circle amplification (RCA) [38], polymerase chain reaction (PCR) [39], autonomous DNA machine [40] and strand displacement amplification (SDA) [41] have been employed. However, an ideal aptasensor which offers ultrasensitive colorimetric sensing platform for cancer cell detection is still in need of exploration.

In the present study, by using a bottom-up approach, we constructed a programmable aptamer-based DNA nanoassembly coupled with HRP-mimicking DNAzymes to detect target cancer cells by naked eyes. We used sgc8c aptamer as a model. This aptamer specifically binds to protein tyrosine kinase 7(PTK7), which is over-expressed on the membrane of approximately 70% of T cell acute lymphocytic leukemia (T-ALL). Our approach relies on significant signal enhancement by the target self-assembly of DNA oligonucleotides and formation of DNA nanostructures on cell surfaces. As shown in Fig. 1, a DNA nanostructure was formed on the cell surface, inspired by sticky-ended self-assembled three-way junction [42,43] and DNA concatemers [9]. The three-way junction DNA nanostructures carried G-rich sequences at 3′ terminus that could form peroxidase-active G-quadruplex with the hemin molecules. This enzyme- and label-free method can dramatically increase the simplicity and cost-efficiency of cancer cell detection and provides a highly promising tool for point-of-care diagnostics. It also allows for the large-scale screening of particular diseases prior to the use of invasive procedures.

2. Materials and methods

2.1. Reagents

Hemin, 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), bovine serum albumin (BSA), 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES), and yeast tRNA were purchased from Sigma-Aldrich (USA). Dimethyl sulfoxide (DMSO) was achieved from Merck Co. (Germany). Fetal bovine serum (FBS), RPMI 1640 medium, penicillin–streptomycin, glutamine, trypsin, and Dulbecco's minimal essential medium (DMEM) were purchased from Biosera (France). The DNA oligonucleotides (purified by PAGE) used in this study were synthesized by Generay Biotech Co. (China). HEPES buffer was prepared by mixing 25 mM HEPES, 20 mM KCl, 200 mM NaCl and DMSO (1%) (pH 6.8). Binding buffer was prepared by adding 1 mg mL^{-1} bovine serum albumin (BSA) and 0.1 mg mL^{-1} yeast tRNA into washing buffer (Dulbecco's PBS containing 4.5 g L^{-1} glucose and 5 mM $MgCl_2$). Fifty micromolar hemin stock solution was prepared in DMSO (1%) and stored in a dark glass bottle at −20 °C. All solutions were prepared with deionized water (resistivity $\geq$ 18 MΩcm).

2.2. Oligonucleotides design

In this work, six DNA oligonucleotides, including m, n, p, q, r, and a chimeric aptamer were designed. The m and n strands could hybridize to each other and form a DNA concatemer. Three DNA oligonucleotides p, q, and r were designed in order to assemble into a stable DNA three-way junction. These two DNA nanostructures could hybridize to each other through s* domain and form nanoassemblies. Chimeric aptamer consists of two domains, including the sgc8 aptamer that specifically binds to CCRF-CEM and an initiator domain which can guide these nanoassemblies to the cell surface. Each three-way junction bears two guanine-rich domains (domain g) that can interact with hemin molecules to form active peroxidase-mimicking DNAzymes. In order to avoid the non-related self-assembly, the oligonucleotide sequences were designed using NUPACK web server (nupack.org). Domains and