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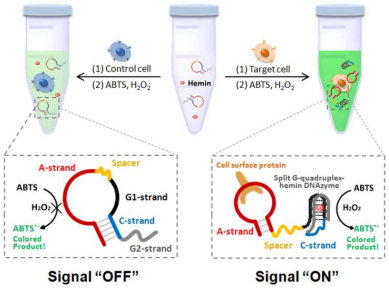

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Label-free activatable aptamer probe for colorimetric detection of cancer cells based on binding-triggered in situ catalysis of split DNAzyme

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A novel label-free tailed hairpin-shaped activitable aptamer probe (THAAP) was developed by rationally integrating aptamer and split G-quadruplex into one sequence. Basing on target recognition-triggered *in situ* catalysis of split DNAzyme, the THAAP strategy achieved a simple, fast, washing-free, specific and quantitative colorimetric assay of human leukemic CCRF-CEM cells.

Development of efficacious molecular probes is of great significance for the progress of cancer diagnostic technologies.[1,2] In recent years, nucleic acid aptamers, which are short single-stranded oligonucleotides with distinct binding properties to diverse targets including cancer markers and cells, have attracted considerable interest in biochemical researches.[3-5] Due to their advantages of high affinity, high specificity, facile synthesis, easy modification and structure-controlled design, aptamers hold a great promise as next-generation molecular probes for cancer detection.[6,7] Using different signal readout techniques, such as fluorescent measurement,[8,9] colorimetric measurement,[10,11] magnetic measurement,[12] electrochemical measurement,[13,14] *etc*., a variety of aptamer-based detection methods have been developed and widely applied in cancer diagnosis and related researches.

Compared with conventional methods that are time-consuming, expensive, and require advanced instrumentation, colorimetric assays exhibit unique properties including simplicity, rapidness and cost-efficiency.[15,16] In particular, no need of any costly or complex apparatus makes colorimetric methods quite favorable for point-of-care cancer diagnostics.[11] For example, Tan group reported a colorimetric detection method for cancer cells using aptamer-conjugated gold nanoparticles (NPs).[17] Specific binding-induced aggregation of gold NPs on target cell surface could cause a distinct colour change, thus indicating the presence of cancer. Then, by further combining a lateral flow device, an aptamer-gold NP strip biosensor for cancer cell detection was developed.[11] Above methods were rapid, specific, sensitive and potential for point-of-care cancer diagnosis, but still had shortcomings. Since the colour change of gold NPs depends on the distance between each other, a relatively low density of target proteins on cancer cell surface might result in invalidation of aptamer-gold NP methods.[18] Moreover, the synthesis and modification of gold NPs inevitably increases the complexity and cost. Hence, it is desirable to design novel label-free aptamer-based strategies for colorimetric assay of cancer cells.

As a kind of new and promising biocatalyst, DNAzymes have been widely used to construct label-free biosensors.[19-21] Particularly, G-quadruplex, which is known as a G-rich sequence and is found to hold horseradish peroxidase (HRP)-mimicking activity after binding with hemin, has been extensively studied.[22-24] Recently, Li group proposed a label-free aptamer strategy to detect human leukemic lymphoblasts (CCRF-CEM cells) by adopting a G-rich sequence as the signal probe.[10] In this strategy, however, the multistep addition of aptamer and G-rich probes, and the necessary centrifugation process to remove cells might make it operation-complicated. Ideal label-free aptamer probes with "signal-on" architectures for cancer cell detection are still in need of exploration, though several excellent studies on such aptamer sensors for biomolecules have been reported.[25-28] Actually, a G-quadruplex can also be folded by two G-rich DNA strands, which is denoted as split G-quadruplex.[29-31] The activity of split G-quadruplex-hemin DNAzymes can be effectively regulated by controlling the accessibility between G-rich fragments.[31] Inspired by the fact, we envisage that the advantage of aptamers in target cell binding-triggered conformational alteration[32] might be smartly utilized to switch the catalysis of split DNAzyme, thus laying a substantial basis for developing a label-free activatable aptamer probe for colorimetric assay of cancer cells.

Herein we report such a tailed hairpin-shaped activitable aptamer probe (THAAP) by rationally integrating aptamer and split G-quadruplex into one sequence. As illustrated in **Scheme 1**, the THAAP is a single-stranded oligonucleotide consisting of three parts: (1) a cancer-targeted aptamer sequence (A-strand, red); (2) a split G-quadruplex sequence comprising a G-rich sequence 1 (G1-strand, dark), a G-rich sequence 2 (G2-strand, gray), and a connective DNA sequence (C-strand, blue) complementary to a part of the A-strand; (3) a Spacer18 linker (spacer, yellow) that is composed of PEG and designed to reduce the interference between A-strand and split G-quadruplex. Because of hybridization of the C-strand with its complementary part of the A-strand, the THAAP is tailed-hairpin structured. This conformation keeps G1-strand geographically apart from G2-strand, avoiding the formation of split G-quadruplex-hemin DNAzyme in the absence of a target. However, when the THAAP encounters the target cancer cell, the A-strand is capable of binding with the cell surface protein, causing a spontaneous conformational reorganization of its tailed-hairpin structure and, consequently, freeing the split G-quadruplex part. In the presence of hemin and potassium ion, G1-strand and G2-strand can interact to form a split G-quadruplex-hemin DNAzyme, which can catalyze the oxidation of 2,2'-azino-bis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) into a blue-green radical product ($ABTS^{\bullet+}$) with a maximum absorption at ~418 nm.[33] Finally, a colorimetric signal is activated in response to the successful binding of the THAAP to the target cancer cell. As a label-free and "signal-on" probe, the THAAP is expected to achieve a simple, rapid and direct detection of cancer cells without complicated instruments and laborious wash steps. As proof of concept, an analysis of CCRF-CEM cancer cells has been performed by using the specific aptamer Sgc8c as a demonstration. The Sgc8c was selected by cell-SELEX against CCRF-CEM cells,[34] and identified to interact with the cell membrane protein tyrosine kinase-7 (PTK7),[35] a protein closely associated with a number of cancers.

Before feasibility investigation, the HRP-mimicking activity of split G-quadruplex-hemin DNAzyme was firstly testified. Time-dependent absorbance variation was measured to optimize several relevant parameters, including concentrations of hemin, ABTS and H_2O_2 (**Fig. S1**). Results showed that at the optimum condition (0.5 μM hemin, 6 mM ABTS, and 3 mM H_2O_2), the absorbance gradually increased with the catalysis time extended, and finally reached a plateau. It was clear that the designed split G-quadruplex could effectively bind with hemin and catalyze the oxidation of ABTS, thus serving as a generator of colorimetric signals.

Next, Sgc8c was labelled with fluorescent dye Cy5, and its ability to recognize target CCRF-CEM cells was tested through flow cytometry (**Fig. S2**). It was demonstrated that in HEPES buffer (the buffer used in this work for DNAzyme catalysis), Cy5-Sgc8c could effectively maintain the specific binding to CCRF-CEM cells showing a similar recognition affinity as that in binding buffer (the buffer used in selection process[34]). Thereupon, THAAP was constructed by adopting Sgc8c sequence as the A-strand. Taking into account that hybridization of Sgc8c with the C-strand in split G-quadruplex would strongly affect the hairpin-structure stability of THAAP, three probes-Probe 1, 2 and 3-with different C-strand compositions were designed (**Table S1**). By selecting human Burkitt's lymphoma Ramos cells as the nontarget cell line control, the feasibility of these probes for specific cancer detection was investigated. As displayed in **Fig. 1A**, with the hairpin-structure stability decreased from Probe 1 to Probe 3, the background signal of probe itself was gradually elevated. A similar tendency was observed upon addition of Ramos cells, although the signals were overall raised due to the effect of living cells on the catalytic reaction. In contrast, CCRF-CEM cells exhibited a distinctive response to Probe 2. After subtracting the background signal from probes and cells themselves, the absorbance result revealed that Probe 1 was too stable to change structure to form G-quadruplex, and Probe 3 was unstable so that it was not recognition-switched. In contrast, Probe 2 could effectively interact with CCRF-CEM cells with an activated colorimetric signal, while its response to control Ramos cells was very weak (**Fig. 1B**). It was clearly demonstrated that the novel THAAP strategy based on recognition-switched DNAzyme activity could work well on the living cell surface, thus achieving the specific identification of target cells from nontarget cells.

We next investigated a series of experimental conditions in this strategy, including concentration of Probe 2, time and temperature for incubation of Probe 2 with cells, as well as catalysis time (**Fig. S3**). After optimization, the THAAP strategy was applied to detect CCRF-CEM cells by incubating cells and 100 nM Probe 2 at 25 ℃ for 90 min with a followed 45 min-catalysis. As illustrated in **Fig. 2A**, a quantitative assay of CCRF-CEM cells over a large amount variation from 0 to 1.01×10^5 cells was performed. With the cell number increased, the absorbance was measured to be gradually enhanced. There was a good linear correlation between the colorimetric signal and the cell amount in the range of 3,300~26,900 cells ($R^2 = 0.9965$), with the lowest amount of 3.3×10^3 cells detected. It was shown that the THAAP effectively realized a simple, fast, selective and quantitative detection of target cells without the aid of complicated instruments.

Conclusions

Herein, for the first time, a label-free THAAP strategy was proposed based on target binding-switched activity of split G-quadruplex-hemin DNAzyme on living cell surface. In this design, the THAAP not only acted as a molecular recognition probe but also served as a transducer in generating an activated colorimetric signal. The perfect integration of target specificity of aptamer with signal generation of split G-quadruplex endowed the THAAP a great promise for *in vitro* cancer cell assays. As proof of concept, a convenient, rapid, quantitative and direct detection of CCRF-CEM cancer cells was realized with satisfying selectivity. The colour change susceptive by naked eyes might further make this strategy simple and operation-convenient. Moreover, in view of the expanding aptamer discovery for varying cancer targets,[4] the THAAP design might hold great potential as a versatile strategy for colorimetric assay of cancer cells.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Table S1 and Figures S1-3]. See DOI: 10.1039/c000000x/

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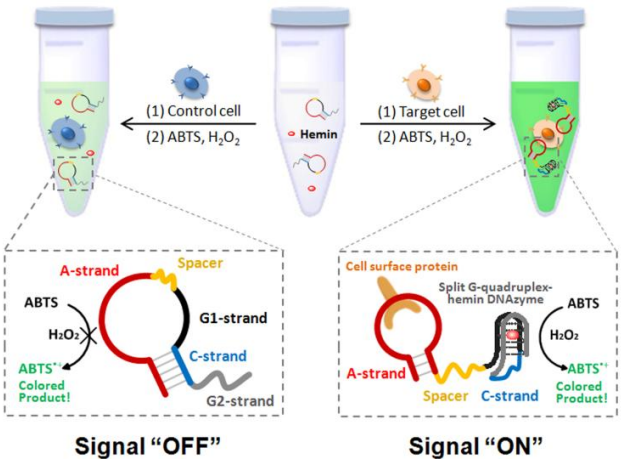

Scheme 1. Schematic representation of the novel strategy for cancer cell detection using a tailed hairpin-shaped activitable aptamer probe (THAAP) based on binding-triggered *in situ* catalysis of split G-quadruplex-hemin DNAzyme.

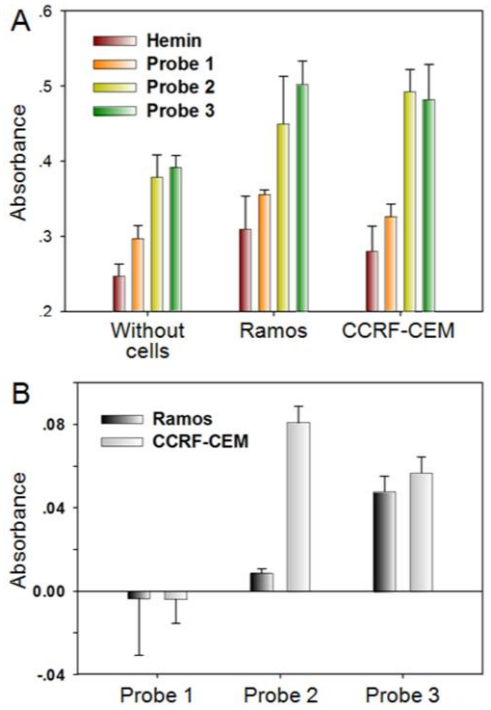

Fig. 1. Feasibility investigation of the THAAP strategy for selective detection of CCRF-CEM cells. (A) Absorbance histogram of the $ABTS^{\bullet+}$ product catalyzed by different probes after incubation with different cells for 60 min. (Probe concentration was 125 nM; Cell number was 2×10^5; Catalysis time was 60 min.) (B) Histogram for analyzing the feasibility of selective cancer cell detection using different probes. (The absorbance in Y-axis was calculated by subtracting the background signal from probes and cells themselves.)

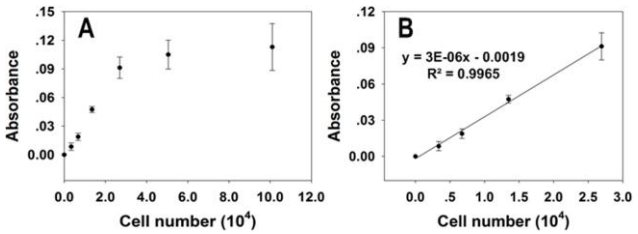

Fig. 2. Quantitative detection of CCRF-CEM cells with the THAAP strategy. (A) The corresponding calibration curve, plotted with the absorbance enhancement versus the cell number increase. (B) The linear response at low concentrations of cells. (Error bars represent standard deviations from three repeated experiments.)