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Colorimetric detection of gene transcript by target-induced three-way junction formation

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ABSTRACT

Gene transcript often varies by alternative splicing, which plays different biological role that results in diversity of gene expression. Therefore, a simple and accurate identification of targeted transcript variant is of prime importance to achieve a precise molecular diagnosis. In this work, we presented a three-way junction based system where two split G-quadruplex forming sequences were coupled into two probes. Only upon the introduction of target gene transcript that offering a specific recognizable splicing site did the two probes assembled into three way junction conformation in a devised process, thus providing a functional G-quadruplex conformation that greatly enhanced hemin peroxidation. A notable resolution for gene splicing site detection was achieved. The detection limitation by colorimetric assay was 0.063 μM, and this system has been proved to discriminate even in a single base false level around splicing site (about 3 times of single mismatched analyte to gain an equal signal by perfect analyte). Furthermore, recoveries of 78.1%, 88.1%, 104.6% were obtained with 0.75 μM, 0.25 μM, 0.083 μM of target, respectively, showing a capacity to further exploit a simple equipped device for gene transcript detection.

1. Introduction

Alternative splicing reaction provides a uniquely versatile means of genetic regulation, in which particular exons of a gene may be excluded or included from the produced RNA precursor [1]. This process leads to various transcripts by a single DNA, and gives rise to diversities of gene expression. Moreover, Abnormally spliced mRNAs are also found to relate to numbers of cancers [2,3]. Generally, to detect these gene transcripts, specific recognition for gene splicing site is primarily favored so that the RNA precursor or the gene itself cannot act intrusively. Reverse Transcription Polymerase Chain Reaction (RT-PCR) validates it by designated primers: exon-specific or splice-junction specific primers, and only the extensible polymerized product by these primers is supposed to astride the splicing site [4,5]. It is efficient as well as convenient, yet it requires elaborate design for valid primers and is prone to cross-hybridization that is defined as a false binding between probe and unintended target, avoidance of which is an indispensable requisite [6]. Currently, single mRNA spliced variants splicing site has been identified by robust analytical tools [7,8]. However, discrimination for tiny variations (for instance, single mismatch, which was also verified to have impacts on alternative splicing [9,10]) around splicing site hasn't been attained by these approaches due to their rough site-recognition mode. Besides, requirements for highly specialized and sophisticated equipment also hinder their widespread use. DNA assembly is sought-after in field of nanotechnology. Among which multi-way junction nanostructure has been widely utilized as analytical tool where several nucleic acid fragments would be closed up by another unique continuous sequence [11–16]. Moreover, when coupled with binary probes, the detection system would be better achieved since the detectable signal would be produced only when the both parts of the probe hybridize to the analyte [17]. We thus conceived its capacity in concatenate target discrimination from discontinuous ones such as splicing site detection.

Hemin-DNA aptamer complex was originally investigated by Dipanka Sen and his cooperators in the late 1990s [18], they noticed that this G-quadruplex/hemin complex could enhance hemin peroxidation in significant measure. In later decades, this fascinating structures had been greatly developed in vitro nucleic acid detection [19–24]. Besides, it could lost its peroxidase activity whereby the G-quadruplex sequence was separated, but once in the assistance of designated template, each split fragments could be reconstructed to restore its peroxidation activity [25–29]. Based

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on this controllable peroxidase activity just by G-quadruplex conformation change, we constructed two junction-forming probes flanking each edge of the splicing site that allocated with divided symmetrical G-quadrupex sequence at each of the probes. In the presence of target, an activated G-quadruplex conformation could be generated along with the assembly of the probes. Since the interactions happen between these probes were localized in where the splicing site existed, the peroxidation would be specifically selected.

2. Materials and methods

2.1. Preparation of reagents

The target cDNAs were designed on the basis of the sequence of the prostate cancer antigen 3 (PCA3) gene presented in GenBank (accession number: AF103907) and the structure of the PCA3 gene described before [30,31]. Sequences were synthesized from GenScript Biotech. Co., Ltd. (Nanjing, China). All the other oligonucleotides were synthesized from BGI-Sequencing (Beijing, China) unless otherwise mentioned. Hemin was purchased from GenScript Biotech. Co, Ltd. (Nanjing, China). DEPC-treated water was purchased from Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China). Tris–HCl, KCl, NaCl, $MgCl_2$, Trizol, isopropanol, trichloromethane and ethyl alcohol of analytical grade were obtained from standard commercial sources and used without further purification or treatment. Primescript 1st Strand cDNA Synthesis Kit was obtained from TAKARA Biotech (DALIAN). CO, LTD. Target cDNAs and oligonucleotides were dissolved by DEPC-treated water and diluted to 10 μM, then stored at −20 °C. A hemin stock solution (1 mM) was prepared in dimethylsulphoxide (DMSO) and stored in the dark at room temperature. TMB substrate solution and Stop solution were provided by EUROIMMUN, and kept in the dark at 4 °C. All the sequences used in this work were concluded in Table S1.

2.2. Colorimetric assay

Before use, target cDNA and CpA were incubated at 95 °C for 10 min individually, and then chilled on ice to avoid any intramolecular secondary structure . CpB/inhibitor complex were prepared by mixing them in an equal amount following denaturation for 10 min at 95 °C, whereafter being allowed to cool to room temperature up to 1 h. Standard procedure was as below. Firstly, target cDNA was added into the reaction mix that contained 200 nM CpA, 200 nM CpB/inhibitor complex, 100 mM Tris–HCl, 150 mM NaCl, 12 mM $MgCl_2$ and 12 mM KCl (pH=8.0), which was incubated for 3 h under room temperature. Subsequently, 20 μL TMB and hemin(final concentration of 1.5 μM) were added into 100 μL of above reaction mix for another 20 min photophobic catalyzation. After that, the reaction was terminated by 20 μL Stop solution. The absorbance measurement was finally conducted on a Microplate Reader (BioTek) at 450 nm wavelength. 3 times of replicates were performed in each experiment and showed by error bars in diagrams. All the data were analyzed by SPSS Statistics 19.0.

2.3. Gel electrophoresis

The experimental products were qualitatively analyzed by a 4% agarose gel with adjusted amount of ethidium bromide premixed. Electrophoreses of various reaction samples were carried out at 100 V for 40 min in 1* TAE (pH 8.0) as the running buffer. the gel was then photographed by a Gel Doc XR+ Imaging System (Bio-Rad).

2.4. RNA extraction and reverse transcription

Total RNAs were extracted from cell line and were reverse transcribed to cDNA according to instructions. the cDNAs were then stored in −20 °C for further use.

3. Results and discussion

3.1. Principle of the system

The scheme was shown in Fig. 1, a single strand CpA that binded with exon 1 and a partial double strand CpB/inhibitor binding with exon 3. Each complementary sequences were marked by the same letter with or without asterisk, CpA contained two domains: a and c, while CpB contained domain a*, b* and d, with the inhibitor covered its whole a* and b*. In the absence of the target, the two split G-quadruplex sequence in each probe (CpA and CpB) were kept from interaction by inhibitor and the

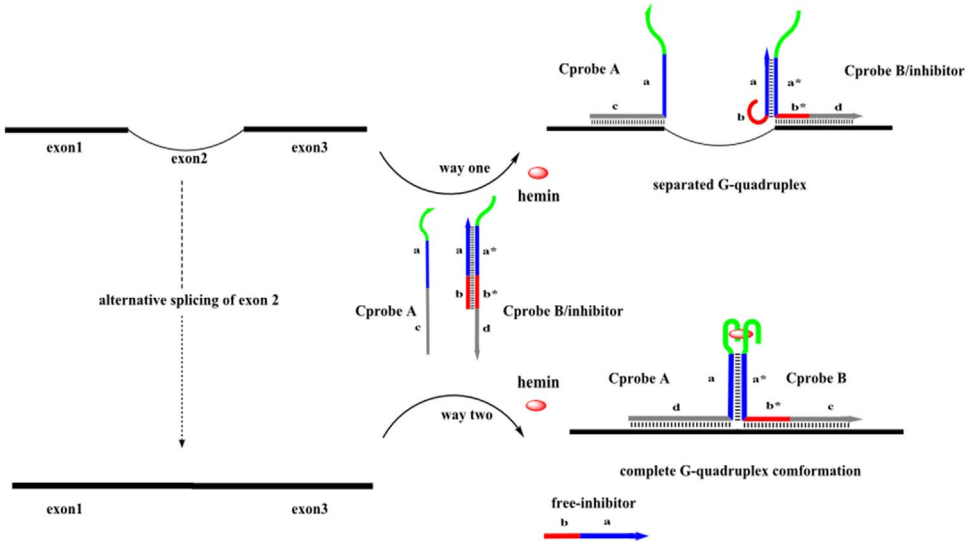

Fig. 1. Scheme of the assembled probes. In the absence of target, the system will go as Way one. While the probes will assemble through Way two in the presence of target to generate an active G-quadruplex conformation. The sequence of the target was designed from one of PCA3 gene transcript variant.