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SPECIAL ISSUE ARTICLE

Label-free molecular probe based on G-quadruplex and strand displacement for sensitive and selective detection and naked eye discrimination of exon 2 deletion of AIMP2

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Abstract

Exon 2 deletion of aminoacyl tRNA synthetase complex-interacting multifunctional protein 2 (AIMP2) is a genetic deletion related to various cancers, for instance ovarian and lung cancers. It can be worked as an indicator of cancer for diagnosis of diseases. Here, we developed a label-free method based on the formation of split G-quadruplex in the presence of target DNA combined with strand displacement to detect exon 2 deletion of AIMP2 (DE2) sensitively and selectively. This method is easy-operating and cost-saving. Moreover, it has observed discrimination of gene deletion from wild-types by naked eyes. The results demonstrate that this strategy can be further used for the detection of different gene deletions to achieve early diagnosis of diseases and allow better prognosis.

KEYWORDS

exon 2 deletion of AIMP2, fluorescence biosensor, G-quadruplex, naked eyes, strand displacement

1 | INTRODUCTION

Mutants in human genome may cause disorder of normal function of organisms.[1] Cancer is one severe consequence of mutants in the genetic information. Besides widely researched single nucleotide polymorphism, genetic deletion also associated with several diseases, such as a 3-base pair deletion of CRYAA gene lead to an autosomal dominant congenital perinuclear cataract in large Chinese pedigree.[2] In addition to several bases deletion, exons deletion is also common in genetic mutants. For instance, deletions of chromosome 17p, especially TP53, a widely studied tumour suppressor gene, occur frequently in human cancers.[3] Aminoacyl tRNA synthetase complex-interacting multifunctional protein 2 (AIMP2, also named MSC p38) was discovered that it could be dissociated from the complex and worked as a potent tumour suppressor.[4–6] Through binding to p53, AIMP2 can promote apoptosis under damage. However, exon 2 deletion of AIMP2 was observed to compromise the apoptosis activity of normal AIMP2 via a competitive binding to p53. Because of the increased resistance to apoptosis, organisms show higher opportunity to form tumour under exposed to carcinogen. Exon 2 deletion of AIMP2 was suggested to be related to various cancers, such as ovarian and lung cancers.[7–9] Thus, it can serve as an indicator of cancers for diagnosis. Since the important influence of exon 2 deletion of AIMP2 to cancers,

the sensitive and selective detection of it is essential for early diagnosis and therapy of cancers. Hitherto, most methods reported to detect gene deletion are gene sequences analysis and capillary electrophoresis, which are time-consuming, operation-complicated and expensive.[10–13] Therefore, the development of an easy-operating and sensitive method to detect gene deletion is of great importance.

G-quadruplex, a non-canonical secondary structure of DNA or RNA, composes at least two planar stacks of four guanines linked together by Hoogsteen hydrogen bonds.[14,15] It can be formed by a single strand or several strands containing guanines, and stabilized by cations like potassium ions (K^+) and sodium ion (Na^+).[16,17] Because of its versatility and polymorphism, various methods based on G-quadruplex have been developed to detect DNA,[18] proteins,[19,20] small molecules[21,22] and metal ions, et al.[23,24] Among these methods, label-free fluorescent biosensors received intense attention with the advantages of easy-operating, cost-effective and time-saving [25–28]. For example, *N*-methyl mesoporphyrin IX (NMM), an unsymmetrical anionic porphyrin which could specifically bind to G-quadruplex and generate a dramatic fluorescence increase has been used to construct label-free biosensors for the detection of different targets.[29,30] Furthermore, hemin can bind to G-quadruplex and then constitute a horseradish peroxidase-mimicking DNAzyme, which can catalyse H_2O_2 to oxidize colourless 2,2′-azino-bis (3-eth ylbenzothiazo-line)-6-sulfonate disodium salt ($ABTS^{2-}$) to green $ABTS^{\cdot-}$.[31,32] The G-quadruplex/hemin DNAzyme offers an opportunity to visible detection of targets.

In this paper, we developed a label-free method based on G-quadruplex to detect Exon 2 deletion of AIMP2. As illustrated in Scheme 1, a partly double strand probe with two guanine-rich overhangs which could form a split G-quadrulex was designed as G-Lock. The single strand C was partially complemented with exon 2 deletion of AIMP2 (DE2) and HG. Since there are much more complementary base pairs between DE2 and C than those between HG and C, in the presence of DE2, an effective strand displacement was initiated and HG was released from the double strand, allowing the formation of a split G-quadruplex structure in the presence of K^+ and leading to an obvious fluorescence enhancement. But in the presence of wild type WT1 or WT2, due to thermodynamic stability, C could not be replaced from double strand, following none G-quadruplex formation and weak fluorescence signal of NMM.

2 | METHODS AND MATERIALS

2.1 | Materials

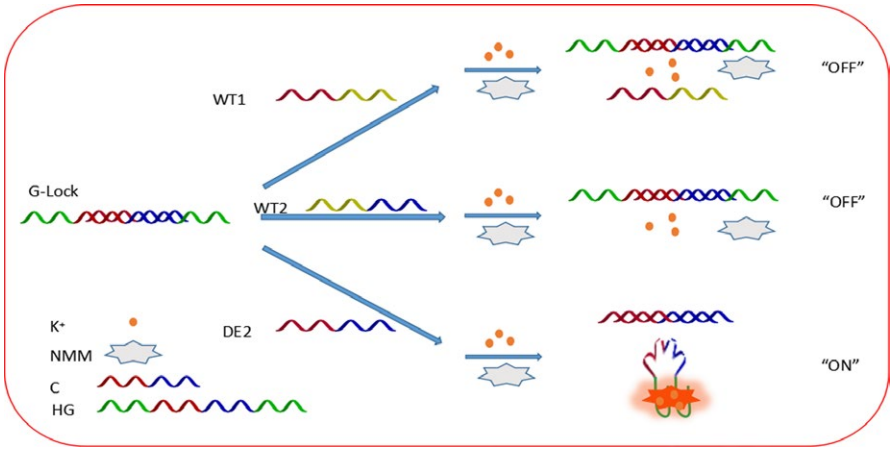
Oligonucleotides were synthesized and purified by Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. (Shanghai, China) and sequences were listed in Supporting Information Table S1. All oligonucleotides (100 μM) were dissolved in Tris-HCl Buffer (10 mM Tris, pH 7.4) and annealed by heating at 95°C for 5 min, then cooled to room temperature and incubated at 4°C for overnight.

N-methyl mesoporphyrin IX, 2,2-Azinobis (3-ehtylbenzo thiazolin-6-sulfnicacid) diammonium salt (ABTS), Tris (hydroxymethyl) aminomethane (Tris), potassium chloride (KCl) and hydrochloric acid (HCl) were purchased from Sinopharm chemical reagent Co., Ltd. 30% H_2O_2, triton X-100, DMSO and hemin were obtained from Aladdin (USA). A549 cell lysate was obtained from Shanghai Sangon Biological Engineering Technology & Service Co., Ltd. (Shanghai, China).

The stock solution of NMM (5 mM) and hemin (5 mM) were prepared in DMSO, respectively, and stored in the dark at −20°C. The stock solution of ABTS (100 mM) was prepared in 0.1 mM NaOH and stored in the dark. Other solutions were prepared with ultrapure water.

2.2 | Design of the molecular probe

A label-free molecular probe, G-Lock, was designed for the detection of exon 2 deletion of AIMP2. G-Lock was



SCHEME 1 Schematic illustration of the strategy of detection of exon 2 deletion of AIMP2 based on split G-quadruplex

designed as a duplex composed of HG and C and described in Supplementary Information.

2.3 | Fluorescence assay for exon 2 deletion of AIMP2

G-Lock probe (10 μl, a mixture containing HG (100 μM) and C (100 μM)) was incubated with DE2 (10 μl) of indicated concentrations at 37°C for 2 hr. After the addition of 960 μl Tris-KCl buffer (10 mM Tris, 50 mM KCl, pH 7.4) and standing for 3 hr, NMM (20 μl, 100 μM) was added. Emission spectra were recorded 40 min later. Fluorescence spectra were measured on a Hitachi F-7000 fluorescence spectrophotometer at room temperature. The excitation wavelength was set at 399 nm and the emission spectrum was collected from 550 to 750 nm. The excitation and emission slits were both set at 10 nm.

In order to detect DE2 in cell lyate, G-Lock probe (10 μl, a mixture containing HG (100 μM) and C (100 μM)) was incubated with DE2 (10 μl, 100 μM) at 37°C. After 2 hr, 10 μl A549 cell lysate (1% v/v[33]) and 950 μl Tris-KCl buffer (10 mM Tris, 50 mM KCl, pH 7.4) were added into the mixture, and the following procedure was the same as that mentioned above.

2.4 | Visual distinguish for exon 2 deletion of AIMP

G-Lock probe (10 μl, a mixture containing HG (100 μM) and C (100 μM)) was incubated with WT1 (10 μl, 100 μM), WT2 (10 μl, 100 μM) and DE2 (10 μl, 100 μM) at 37°C for 2 hr, respectively. (a) After the addition of 960 μl Tris-KCl buffer and standing for 3 hr, NMM (20 μl, 100 μM) was added. Forty minutes later, the luminescence intensity difference can be observed under the UV-transillumination in 254 nm. (b) After the addition of 920 μl Tris-KCl buffer and standing for 3 hr, hemin (10 μl, 100 μM) was added into the mixture and incubated for 1 hr to form the G-quadruplex/Hemin DNAzyme. ABTS (30 μl, 100 mM) and H_2O_2 (20 μl, 100 mM) were added in turn, the color of the mixtures turned to green in varying degrees rapidly, and visualization realized.

2.5 | Cytotoxicity assays

Human breast cancer A549 cells were seeded in 96-well plates and incubated overnight. G-Lock probe were added to cells at final concentrations ranging from 20 to 1,000 nM. 50 μl of 1× MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-dipheny ltetrazolium bromide) reagent was added to each well after 24 or 48 hr later. After incubating 4 hr in dark, cytotoxicity was assessed by SpectraMax M5 microplate reader at a wavelength of 570 nm.

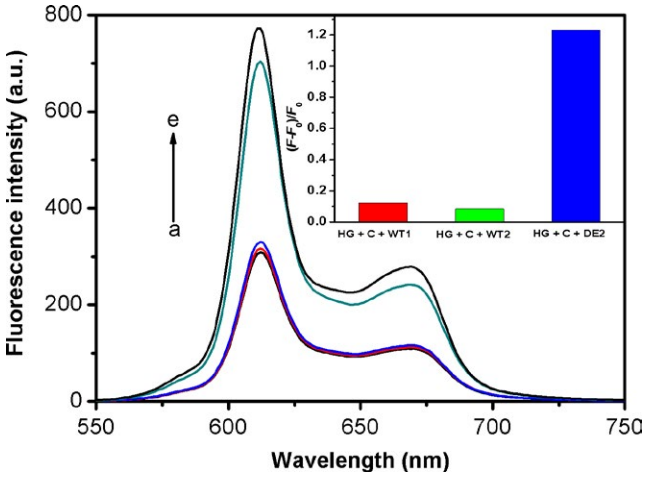


FIGURE 1 Fluorescence spectra of NMM in the presence of (a) HG and C; (b) HG, C and WT1; (c) HG, C and WT2; (d) HG, C and DE2, and (e) single HG respectively (each 1 μM)

3 | RESULTS

3.1 | Design of the molecular probe

For purpose of proving the feasibility of this method, effect of the related strands on the fluorescence signal is tested. As shown in Figure 1, HG, containing guanine-rich sequences, formed a split G-quadruplex in the presence of potassium and then generated a prominent rise of the fluorescence intensity of NMM. When the complementary strand C was added, the fluorescence of NMM decreased obviously. The tension of double strand lengthened the spatial separation of two guanine-rich sequences, which consequently prevented the formation of split G-quadruplex and generated slightly rise of the fluorescence of NMM. Upon the addition of target strand DE2, C was displaced from the double strand, generating obviously rise of the fluorescence of NMM. Meanwhile the addition of WT1 or WT2 made almost no difference on the fluorescence of NMM. The background is moderate, this method can evidently distinguish DE2 from WT1 and WT2. So it is completely and potentially capable of being further developed to detect exon 2 deletion of AIMP2.

In order to obtain a better performance of target detection, several parameters of this system were investigated and optimized. According to the scheme of this method, the background is mainly caused by the spatial distance of guanine-rich overhangs in G-Lock, and the number of complementary bases between HG and C is a dominant element. Meanwhile the amount of C displaced from the double strand is also associated with the length and sequence of C. So the optimization of C is crucial. As shown in Supporting Information Figure S1, several sequences were selected, and fluorescence intensities incubated with WT1, WT2 and DE2 were detected. All these sequences were partially complementary with WT1,

WT2 and HG, while completely complementary with DE2. As the number of bases in C which is complemented with WT2 increased, the fluorescence differential related to G-Lock was also enhanced, but with no obviously distinguish of DE2 and WT2. When there were nine bases complemented with WT2 in C, it obtained an outstanding distinction between DE2 and wild types. Similarly, with the increase of the number of bases in C that complemented with WT1, the fluorescence differential of DE2 was enhanced, and the fluorescence differential of WT1 was improved at the same time. In order to clearly discriminate and sensitive detect DE2, we choose C2 as C for further detection of target, which contains nine bases complemented with WT2 and six bases complemented with WT1.

Besides the length and sequence of C, the conditions of the total reaction also influence the performance of the detection. So the incubation time and temperature of strand displacement and G-quadruplex formation were optimized, too. As shown in Supporting Information Figure S2, when temperature ranged from 25 to 55°C, the fluorescence differential showed a rise first, followed by a decline, and achieved best performance at 37°C. Then G-Lock was incubated with DE2 and wild types for 10–180 min to displace C from it, and later incubated for 20–240 min to form G-quadruplex. From the result (Supporting Information Figures S3 and S4), 120 and 180 min were chosen as sufficient incubate-time for displacement and G-quadruplex formation. So in the following displacement experiment, G-Lock was cultured with DE2 for 2 hr. And then K^+ was introduced into the system and cultured for 3 hr. After all, NMM was mixed with the mixture and the fluorescence was detected. All these operations were carried out at 37°C.

3.2 | Sensitive and selective detection of exon 2 deletion of AIMP2

Based on above optimizations, the sensitive detection of DE2 was carried out. DNA mixture solution (mixed previously) contained HG and C was incubated with different concentrations of DE2. Encouragingly, as shown in Figure 2, we obtained that the fluorescence intensity of NMM in the system was enhanced as the concentration of DE2 increased, further indicating that DE2 can exchange C from G-Lock and release HG to form G-quadruplex structure. The luminescence response of this system to DE2 showed a good linear correction ($R^2 = 0.999$) with the concentration of DE2 in a wide range, from 20 to 400 nM. And a detection limit of 3.53 nM was achieved on the principle of S/N > 3. In addition to sensitive detection of target, it found that this strategy can selectively detect DE2 as well. As shown in Figure 3, we chose several single strands as interferential targets, and the result indicated that this method can differentiate DE2 from wild types and other interferential DNAs obviously.

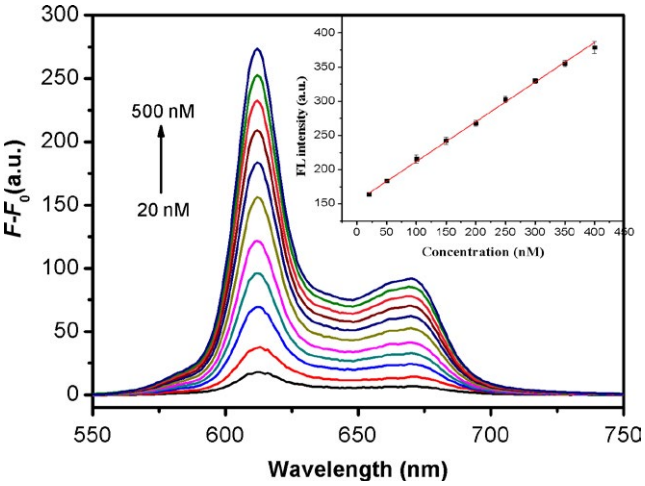


FIGURE 2 Fluorescence spectrum of NMM with HG and C (both 500 nM) in the presence of different concentrations of DE2 (0, 20, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500 nM); and there is a significant linear relationship between fluorescence intensity at λ = 612 nm and DE2 concentrations

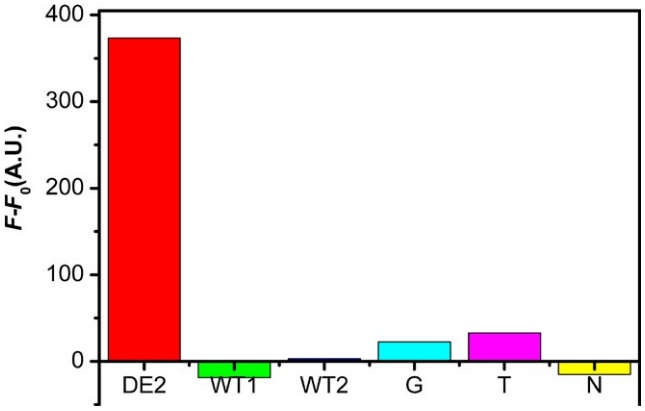


FIGURE 3 Selectivity of this strategy for exon 2 deletion from several single strands (1 μM for all), with HG (1 μM), C (1 μM) and NMM (2 μM) in the reaction system

To examine the robustness of the system, the stability, cytotoxicity and the performance of the nanoplatform on the detection of DE2 in cell lysate were also investigated. In the presence and absence of the targets after cultured for 3, 24, 48, 72 and 120 hr, neglectable fluorescent changes (Supporting Information Figure S5) indicated that the probe was stable for at least 120 hr, which was sufficient enough for the detection of DE2. The result of cytotoxicity (Supporting Information Figure S6) indicated that the G-Lock probe was non-toxic under experimental conditions, which showed the potential for the detection of exon 2 deletion of AIMP2 in cell lysate. Whereas in cell lysate, F/F_0

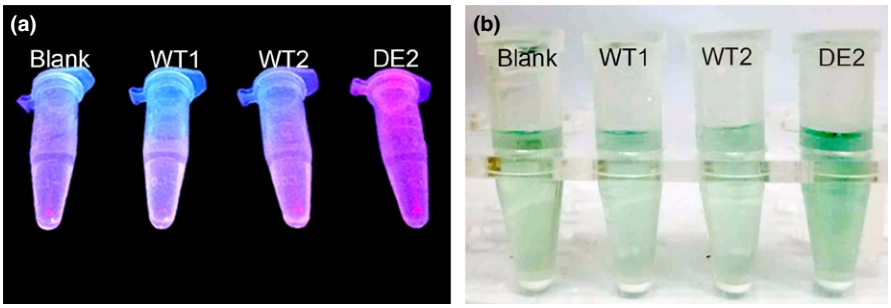


FIGURE 4 (a) UV-transillumination of NMM and (b) colour of $ABTS^{2-}$ catalyzed by mimicking enzymes in the presence of HG and C; HG, C and WT1; HG, C and WT2; HG, C and DE2, respectively (each 1 μM)

of the detection of DE2 in diluted cell lysate was 1.58 as shown in Supporting Information Figure S7, indicating that the method reported in this paper could distinguish DE2 in cell lysate.

3.3 | Visual distinguish for exon 2 deletion of AIMP

Besides fluorescence detection, this strategy can detect target gene visually. As shown in Figure 4a, under 254 nm UV-transillumination, the fluorescence differential of NMM cultured with wild types and mutant gene could be distinguished easily by naked eyes. Instead of NMM, hemin can associate with G-quadruplex to form a mimicking peroxidase enzyme, which can catalyse the oxidation of the colourless $ABTS^{2-}$ to green $ABTS^{\cdot-}$ by H_2O_2. In the result (Figure 4b), there was an obvious colour difference among DE2, wild types and blank, making it is possible to naked discrimination with strand displacement. Applying label-free molecular probe, this method is easy-operating and cost-saving. Moreover, it has observed discrimination of gene deletion from wild-types by naked eyes. These promising results demonstrate that this strategy can be further used for detection of gene deletion difference to achieve early disease detection and allow better prognosis.

4 | DISCUSSION

As a kind of mutants, gene deletions were relatively less studied, not to mention the sensitive and selective detection. Researches have reported that the exon 2 deletion of AIMP2 is related to many types of cancers and can be worked as a biomarker for cancers, especially lung and ovarian cancers.[34] As a deadly cancer, lung cancer is the most common cancer with highest mortality rate, meanwhile biomarkers of lung cancer are few. At this point, a simple and easy-operation method for the sensitive and selective detection of exon 2 deletion of AIMP2 as a biomarker is very imperative and vital for the diagnose and therapy for lung cancer. In this paper, a label-free strategy based on DNA comformational transition and G-quadruplex was developed. A detection limit of 3.53 nm was obtained, which is much lower than other method related to gene deletion (as shown in Supporting Information Table S2). And it realized the obvious distinguish of the deletion gene with wild type genes both under fluorescence detection and naked-eye visualization.

5 | CONCLUSIONS

In summary, a label-free strategy was developed for selective and sensitive detection of gene deletion. This method is based on split G-quadruplex formation in the presence of deleted DNA combined with strand displacement. Applying label-free molecular probe, this method is easy-operating and cost-saving. Moreover, it has observed discrimination of gene deletion from wild types by naked eyes. These promising results demonstrate that this strategy can be further used for detection of gene deletion difference to achieve early disease detection and allow better prognosis.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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