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Dual-signal electrochemical sensor for detection of cancer cells by the split primer ligation-triggered catalyzed hairpin assembly

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ABSTRACT

Simultaneous detection of various intracellular biomarkers is promising for early diagnosis and treatment of cancer. Herein, a split primer ligation-triggered catalyzed hairpin assembly-based on dual-signal electrochemical biosensor was constructed for the determination of two pairs of cancer mRNAs: TK1 and c-myc, survivin and GalNAc-T by using ferrocene molecular beacon and hemin molecular beacon as detection signal sources. Each pair of targets exists simultaneously, can release the split primers and ligated as the integral primers, hybridization occurred between the integral primers and part of MBs, causing a double-stranded DNA formed. The probes hybridized with the unfolded MBs and displaced integral primers. Finally, the displaced integral primers again hybridized with the MBs and initiated cycle amplification. Under the optimal conditions, the detection limit of TK1 and c-myc mRNA is as low as 0.022 nM, and that of survivin and GalNAc-T mRNA is 0.029 nM. In addition, two pairs of cancer mRNAs could act as outputs to activate an AND logic gate.

1. Introduction

Cancer is a highly fatal disease that affects the lives of millions of people around the world [1,2]. The survival chance of cancer patients would be greatly improved by early diagnosis and therapy. Early diagnosis largely depends on sensitive detection of some biomarkers, such as mRNA [3,4]. However, diagnosis of tumor or cancer relies on one biomarker would easily causes misreading because of the false negative or positive results. Therefore, the multiple-signal electrochemical sensors, which can simultaneously detect several kinds of mRNAs, are the essential tools for analysis the variation of biomarkers in vivo.

Traditionally, various kinds of methods have been reported, such as real-time polymerase chain reaction (RT-PCR) [5,6], enzyme-linked immunosorbent assay (ELISA) [7,8], fluorescent [9,10] and electrochemical molecular beacons (E-MBs) [11,12]. E-MBs mRNA assays have some advantages of high sensitivity and selectivity, fast and portable, simple instrumentation and low-cost [13,14]. As is well known, such E-MBs are usually designed to be immobilized onto the electrode surface [15,16], which may cause lower available hybridization efficiency and higher background noise, possibly arising from spatial hindrance effects. Hence, it is very important to develop an efficient and convenient E-MBs method for simultaneous detection of multiple mRNAs.

Many signal amplification strategies for sensitive determination of mRNAs have been developed, including strand displacement amplification (SDA) [17,18], hybridization chain reaction (HCR) [19,20] amplification, rolling circle amplification (RCA) [6,21] and catalyzed hairpin assembly (CHA) [22,23] amplification. Among these, CHA amplification shows great potential in signal amplification, because CHA is a kinetics-controlled reaction, without requiring any enzymes, and shows high sensitivity and selectivity toward the detection of the target. Hence, combine with the novel electrochemical active inactive switching molecular beacons (EAIS-MB), such as ferrocene molecular beacon (Fc-MB) [24] and hemin molecular beacon (Hs-MB) [25], which are designed not fixed on the surface of electrode and free in the solution. They can detect the targets directly in homogeneous solution and label-free to provide satisfactory results.

Herein, a split primer ligation-triggered catalyzed hairpin assembly strategy was designed for high specific and ultrasensitive detection of two pairs of cancer mRNAs: TK1 and c-myc, survivin and GalNAc-T which related to breast cancer and liver cancer. Each pair of the released split primers can ligated as the integral primers, hybridized with part of MBs to form a double-stranded DNA. A cascade of hybridization reactions is triggered between MBs and probes to release the integral primers and initiated cycle amplification. Therefore, taking advantage of the dual-signal electrochemical biosensor and the amplification efficiency of the cascade signal amplification strategy, the proposed method provides an ultrasensitive platform for cancer cell detection.

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2. Experimental

2.1. Instrumentation

Electrochemical measurements were obtained on a CHI 660E electrochemistry workstation (CH Instruments, Shanghai, China) with conventional three-electrode electrochemical system which was composed of a platinum wire (ϕ = 0.5 mm) as the counter electrode, an Ag/AgCl (3 mol/L KCl) electrode as the reference electrode and a glassy carbon electrode (GCE) and its modified electrodes were used as the working electrode at ambient temperature (22 ± 2 ˚C). The modified electrode was made according to reference [26], and the concentration of the modified materials were diluted ten times. The electrolyte solution was 0.1 M phosphate buffer solution (pH 7.4) containing 0.1 M NaCl.

2.2. Materials

All oligonucleotides were synthesized and HPLC-purified by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). The oligonucleotide sequences are listed in Table S1. T4 DNA ligase was purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China). Ferrocenecarboxylic acid, Hemin, 2-(7-aza-1H-benzotriazole-1-yl)-1, 1, 3, 3-tetramethyluronium hexafluorophosphate (HATU), N-hydroxysuccinimide (NHS), N, N-diisopropylethylamine (DIPEA), dimethylsulfoxide (DMSO), N, N-dimethylformamide (DMF), and cucurbit [7] uril were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). MCF-7, MCF-10A, HepG2 and HL-7702 cells were purchased from Shanghai Thermo Fisher Scientific Co., Ltd. (Shanghai, China). Fetal bovine serum, penicillin, and streptomycin were maintained in Dulbecco's modified Eagle's medium (Invitrogen, Paisley, UK). The PAGE Gel Kit was purchased from Beijing Solarbio Science & technology Co., Lid. The ladder and gelred were purchased from Shanghai Thermo Fisher Scientific Co., Ltd. (Shanghai, China). All other chemicals were purchased from Shanghai Chemical Reagents (Shanghai) and could be used without further purification. Milli-Q water (resistance > 18 MΩ cm^{-1}) was used in all experiments.

2.3. Fabrication of Hs-MB and Fc-MB

The hemin active esters and hairpin-shaped oligonucleotides were used to prepare the Hs-MB [25], and the ferrocenecarboxylic acid active esters and hairpin-shaped oligonucleotides were used to prepare the Fc-MB [24].

2.4. Ligation reactions

The 10 × T4 DNA ligase buffer containing 400 mM Tris-HCl, 100 mM $MgCl_2$, 100 mM dithiothreitol and 5 mM ATP (pH = 7.8, at 25 ˚C) was used for both ligation reaction. 1 nM H1/TK1′, 1 nM H2/c-myc', 1 nM H3/survivin' and 1 nM H4/GalNAc-T' were reacted with targets, then 1.5 nM H5, 1.5 nM H6 and 0.1 U/μL T4 DNA ligase were added into the above mixture to produce the H1–H2 and H3–H4 for 30 min at 37 ˚C. Then 2.25 nM H7 and 2.25 nM H8 were added into the resulting mixture to hybridize with H5 and H6 and released the H1–H2 and H3–H4. Finally, produced mixture was heated to 70 ˚C for 5 min and then cooled to room temperature.

2.5. CHA reactions

Each concentration was 10 nM of Fc-MB, Hs-MB, probe1, and probe2 were heated to 95 ˚C for 5 min and then allowed to cool to room temperature before use. Different concentrations of target DNA (H1–H2 and H3–H4 which produced by ligation reactions) were mixed with Fc-MB, Hs-MB, probe1 and probe2 in 10 mM Tris-HCl (pH = 7.5) buffer containing 15 mM KCl and 4 mM $MgCl_2$. Finally the solutions were incubated at 37 ˚C for 40 min.

2.6. Gel electrophoresis

The reliability for the catalyzed hairpin assembly reaction was validated using the PAGE method. After incubation, the mixtures of 8 μL of the enzymatic products and 2 μL of loading buffer were analyzed by 16% nondenaturating polyacrylamide gel electrophoresis (PAGE) in 0.5 × TBE buffer (44.5 mM Tris-boric acid, 1 mM EDTA, pH 8.0) at a 120 V constant voltage at room temperature for 70 min. The gels were stained by gelred.

2.7. Detection of intracellular mRNAs in HepG2, HL-7702, MCF-7 and MCF-10A cells

HepG2, HL-7702, MCF-7 and MCF-10A cells were maintained in accordance with the protocols provided by the American Type Tissue Culture Collection [27]. Firstly, cells were cultured by high glucose Dulbecco's Modified Eagle Medium (DMEM, 4.5 g of glucose/L) containing 10% fetal bovine serum (FBS), $NaHCO_3$ (2 g/L) and 1% antibiotics (penicillin/streptomycin, 100 U/mL). The cultured cells were kept in a humidified incubator at 37 ˚C and 5% CO_2/95% air. All of the total RNA samples extracted from HepG2, HL-7702, MCF-7 and MCF-10A cells were diluted to 20 ng/μL with RNase free TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 7.5), respectively.

Firstly, 1 nM TK1'/H1 duplex, 1 nM c-myc'/H2 duplex, 1 nM survivin'/H3, 1 nM GalNAc-T'/H4 and various concentrations of cell samples were mixed together and incubate for 30 min. Then, 1.5 nM H5, 1.5 nM H6 and 0.1 U/μL T4 DNA ligase were added into the above solution and continuously incubated for 30 min. Next, added 2.25 nM H7 and 2.25 nM H8 into the above solution and continuously incubated for 30 min. The mixture was then heated to 70 ˚C for 5 min and cooled to room temperature. 10 nM of Fc-MB, Hs-MB, probe1, and probe2 were added and incubated for 40 min. Finally, 100 nM CB [7] and phosphate buffer solution (pH 7.4) were performed with volumes of 600 μL for electrochemical measurement. The whole reaction process was at 37 ˚C.

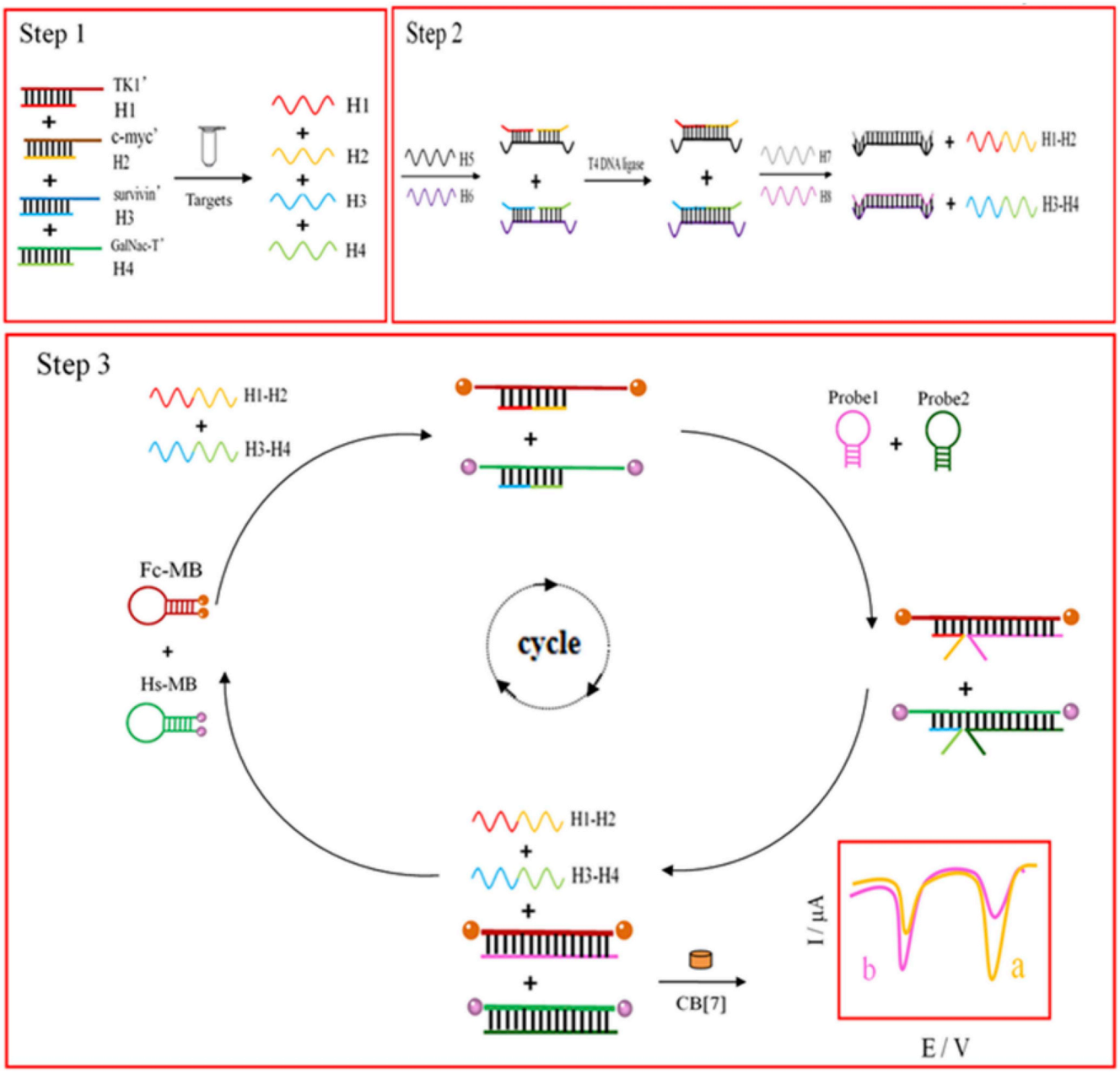
3. Results and discussion

3.1. The schematic illustration of the sensing principle for TK1 and c-myc mRNA detection

Combine with two electrochemical active-inactive switching molecular beacons, ferrocene molecular beacon (Fc-MB) and hemin molecular beacon (Hs-MB), the detection strategy is divided into three steps. First of all, four duplex probes of H1 and TK1' (TK1-binding aptamer sequence), H2 and c-myc' (c-myc-binding aptamer sequence), H3 and survivin' (survivin-binding aptamer sequence), H4 and GalNAc-T' (GalNAc-T-binding aptamer sequence) are designed for capturing four targets and release the split primer H1, H2, H3 and H4. Secondly, each pair of split primer (H1/H2 and H3/H4) hybridizes with their template H5 and H6 to form ligatable nicks. In the presence of T4 DNA ligase, the ligatable nicks are mended to produce integral primers named H1–H2 and H3–H4. With the introduction of H7 and H8, the integral primers could be liberated and go on catalyzed hairpin assembly step.

The integral primer H1–H2 and H3–H4 will open the hairpin structure of Fc-MB and Hs-MB by toehold binding reaction and release the rest sequences of Fc-MB and Hs-MB which are severally complementary with probe1 and probe2 to become the integral primer-MB-probe intermediates. This intermediate is inherently unstable and the integral primers are released by the probes, and then, the subsequent catalyzed hairpin assembly reaction is initiated. (Scheme 1).

Each pair of targets exist, the split primers will be released and ligated as one integral primer, the opening of the hairpin structure of MB



Scheme 1. The schematic illustration of the sensing principle for two pairs of mRNA targets: TK1 and c-myc, survivin and GalNAc-T detection.

is facilitated. One probe hybridize with one unfolded MB and displace one integral primer. The released integral primer can then interact with another MB, and the cycle starts again. In this way, each pair of targets can generate many MB-probe complexes, which leads to the significant electrochemical signal enhancement.

3.2. The feasibility of the split primer ligation-triggered catalyzed hairpin assembly

First, the series of catalyzed hairpin assembly strategy triggered by the split primer ligation was verified by PAGE experiments. As shown in Fig. 1, the band of lane 1 was MB. With the addition of the split primer H1 (lane 3) or H2 (lane 4), we could see there was no reaction between MB and H1 or H2, respectively. However, when the integral primer H1–H2 being added, a brighter band appeared (lane 5). It demonstrates that only the split primer H1 or H2 cannot change the stem-loop shaped MB into the line structure. Furthermore, without the addition of H1–H2 (lane 2) or added both of the split primer H1 and H2 (lane 6), only a small amount of MB-probe duplex could be found. When integral primer H1–H2 was presented, the bands of amplification product were more obvious (lane 7), indicating the successful catalyzed hairpin assembly reaction. It illustrates that only the split primer H1 and H2 ligated as the integral primer H1–H2, could it be as a template to make the MB unfold and trigger the catalyzed hairpin assembly reaction.

Furthermore, the electrochemical signals of Fc-MB and Hs-MB in the detection process have also changed. As shown in Fig. 2, when the split primer H1, H2, H3 and H4 were added, the reduction peaks of both Fc-MB and Hs-MB (curve b) were almost the same high as that of free Fc-MB and Hs-MB (curve a). When the integral primer H1–H2 and H3–H4 were added, the reduction peak of Fc-MB decreased and that of Hs-MB increased (curve d). It demonstrates that the integral primer can induce MB to extend its hairpin structure. When probe1 and probe 2 were instead of integral primers to add into the detection system, merely a small change on the electrochemical signal of MBs has been observed

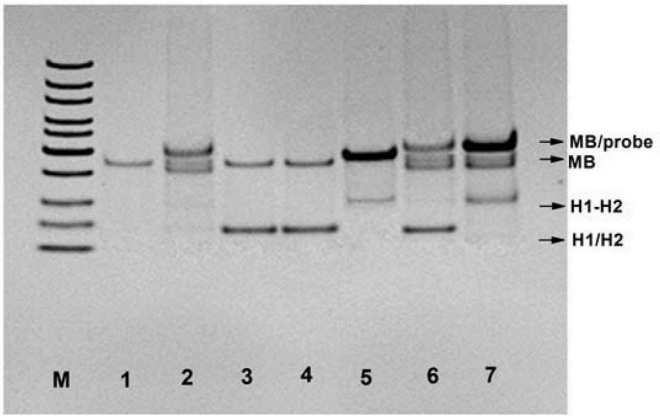


Fig. 1. The enzymatic products were analyzed by 16% nondenaturating polyacrylamide gel electrophoresis (PAGE). Lane 1: MB. Lane 2: MB/probe; Lane 3: MB/H1; Lane 4: MB/H2; Lane 5: MB/H1–H2; Lane 6: MB/H1/H2/probe; Lane 7: MB/H1–H2/probe.

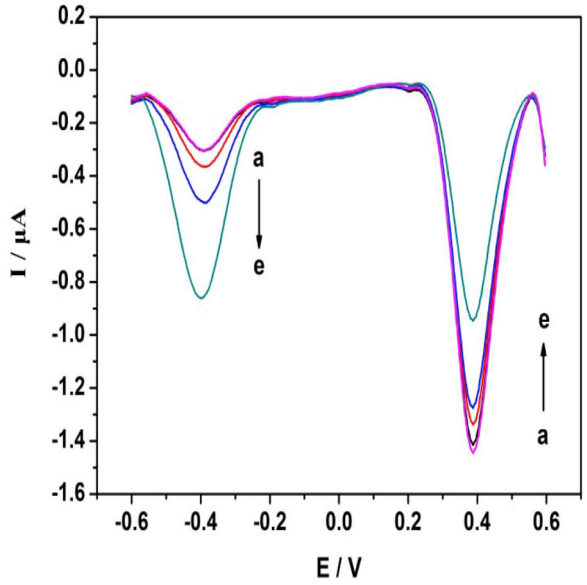


Fig. 2. DPV curves of the hairpin shaped Fc-MB and Hs-MB (a), with split prime H1, H2, H3 and H4 (b), with probe1 and probe2 (c), with the integral prime H1–H2 and H3–H4 (d) and with probe1, probe2, integral prime H1–H2 and H3–H4 (e). Experimental conditions: (A) 10 nM Fc-MB, 10 nM Hs-MB, 10 nM probe1, 10 nM probe2, 100 nM CB [7], 1 nM H1, 1 nM H2, 1 nM H3, 1 nM H4, 1 nM H1–H2, 1 nM H3–H4 in PBS with 0.1 M NaCl at pH 7.4.

(curve c), which reflected that effective extension of MB only occurs in the case of the integral primer existed. A significant electrochemical signals change of MBs (curve e) obtained in the existence of both integral primers and probes. The integral primers induce hairpin structure MBs elongation to hybridize with probes, meanwhile, releases themselves based on the mechanism of strand displacement. The released integral primers could induce other hairpin shaped MBs extension to initiate the cycles of strand displacement resulting in amplification of electrochemical signal. Based on the cyclic strand displacement amplification strategy, the electrochemical signal change of curve e is nearly 2.5 times as much as curve d at the same concentration of the integral primers. The PAGE and DPV experimental results demonstrate that the split primer ligation-triggered catalyzed hairpin assembly method is reasonable and feasible.

In order to obtain the optimum sensing performance, the reaction time of ligation and catalyzed hairpin assembly, as well as the amount of T4 DNA ligase and probe were then further optimized (Fig. S1 A–D). Thus, the proposed amplified method was carried out using a 0.1 U/μL T4 DNA ligase, 10 nM probe, the reaction time of ligation was 30 min and the time of catalyzed hairpin assembly was 40 min, respectively. The whole reaction process was at 37 °C.

3.3. Selectivity and quantitation detection of two pairs of cancer mRNA targets

The feasibility of simultaneous detection was also investigated by the dual-signal electrochemical sensor. As shown in Fig. 3A, the peak current of Hs-MB gradually enhanced with increasing concentration of survivin and GalNAc-T mRNAs, whereas the peak current of Fc-MB was suppressed by degrees with the concentrations of TK1 and c-myc mRNAs increased. As expected, the variation of Fc-MB electrochemical responses ($\Delta I = I_{target} - I_{no\ target}$) was linear with the increasing TK1 and c-myc mRNAs concentration in the range of 0.1 nM–10 nM (shown in Fig. 3B). The calibration curve is ΔI_{Fc} (μA) = $-0.070 - 0.295\ C_{TK1/c\text{-}myc}$ (nM), $R^2 = 0.982$ and the detection limit is 0.022 nM (based on 3δ/slopes) with 2.2% RSD for 10 independent tests. Similarly, the variation of Hs-MB electrochemical responses ($\Delta I = I_{target} - I_{no\ target}$) was linear with the increasing survivin and GalNAc-T mRNAs concentration in the range of 0.1 nM–10 nM (shown in Fig. 3C). The calibration curve is ΔI_{Hs} (μA) = $0.075 + 0.400\ C_{survivin/GalNAc\text{-}T}$ (nM), $R^2 = 0.969$ and the detection limit is 0.029 nM (based on 3δ/slopes) with 3.9% RSD for 10 independent tests. These results indicated that sensitive analysis of two pairs of cancer mRNA targets: TK1 and c-myc, survivin and GalNAc-T were achieved via the dual-signal electrochemical biosensor based on the split primer ligation-triggered catalyzed hairpin assembly.

The specificity of the two pairs of cancer mRNA targets: TK1 and c-myc, survivin and GalNAc-T simultaneous sensing strategy was basically determined by the aptameric recognition function. The selectivity of the method was tested. It is no significant influence on the detection of two pairs of cancer mRNA targets when 10,000 times of some single-base mismatched targets, such as s-TK1, s-*c*-myc, s-survivin and s-GalNAc-T existed (in Fig. 4). When only TK1 and c-myc existed simultaneously, ΔI of Fc-MB increased, whereas when only survivin and GalNAc-T existed simultaneously, ΔI of Hs-MB increased. The results indicated that the dual-signal electrochemical biosensor can sensitive and selective detect the biomarkers, which might support its potential in clinical applications.

3.4. Real sample analysis

The method was further applied to simultaneous detection mRNAs in cancer cells: MCF-7 and HepG2 cells and distinguish between themselves and their normal cells: MCF-10A and HL-7702. The diluted samples (total mRNA 20 ng/μL) were diluted again into different concentrations (0.3, 0.5, 0.7 ng/μL), respectively. Then, the electrochemical detection was performed under the same conditions as described in 2.7 section. The results were shown in Fig. 5. In MCF-7 and HepG2 cells, the variation of both Fc-MB and Hs-MB electrochemical response (ΔI) were increased, demonstrating that two pairs of mRNA were expressed in cancer cells. However, in HL-7702 cell, there were just the pair of survivin and GalNAc-T expressed, so that only did the ΔI of Hs-MB increased. In MCF-10A cell, two pairs of mRNA were no expression, therefore, there were no changes exhibit in the responses of Fc-MB and Hs-MB. It demonstrated that the proposed method could be effectively and successfully applied to distinguish and detect the cancer cells such as MCF-7 and HepG2 and their normal cells, MCF-10A and HL-7702.

3.5. Establishment of two pairs of cancer mRNAs triggered and logic gate

On the basis of the above experimental results, we designed an AND logic gate by using the electrochemical signals of Fc-MB and Hs-MB as inputs and two pairs of cancer mRNAs (H1–H2 and H3–H4) as outputs.

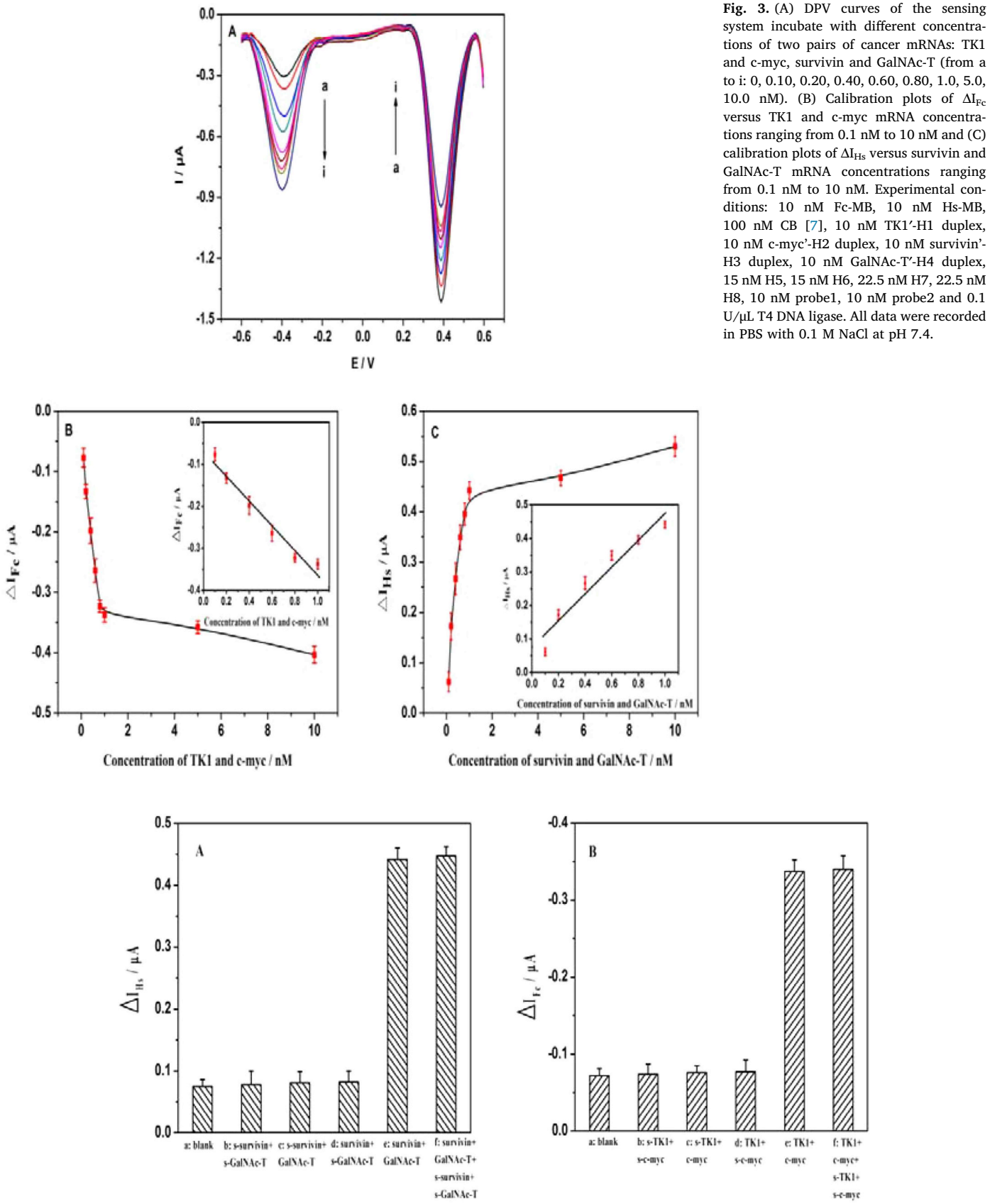


Fig. 3. (A) DPV curves of the sensing system incubate with different concentrations of two pairs of cancer mRNAs: TK1 and c-myc, survivin and GalNAc-T (from a to i: 0, 0.10, 0.20, 0.40, 0.60, 0.80, 1.0, 5.0, 10.0 nM). (B) Calibration plots of ΔI_{Fc} versus TK1 and c-myc mRNA concentrations ranging from 0.1 nM to 10 nM and (C) calibration plots of ΔI_{Hs} versus survivin and GalNAc-T mRNA concentrations ranging from 0.1 nM to 10 nM. Experimental conditions: 10 nM Fc-MB, 10 nM Hs-MB, 100 nM CB [7], 10 nM TK1′-H1 duplex, 10 nM c-myc'-H2 duplex, 10 nM survivin'-H3 duplex, 10 nM GalNAc-T′-H4 duplex, 15 nM H5, 15 nM H6, 22.5 nM H7, 22.5 nM H8, 10 nM probe1, 10 nM probe2 and 0.1 U/μL T4 DNA ligase. All data were recorded in PBS with 0.1 M NaCl at pH 7.4.

Fig. 4. The selectivity of the two pairs of cancer mRNA targets-sensing method. Experimental conditions: 1 nM TK1, 1 nM c-myc, 1 nM survivin, 1 nM GalNAc-T, 10 μM s-TK1 (TK1 single-base mismatched target), 10 μM s-c-myc (c-myc single-base mismatched target), 10 μM s-survivin (survivin single-base mismatched target), 10 μM s-GalNAc-T (GalNAc-T single-base mismatched target), 10 nM Fc-MB, 10 nM Hs-MB, 100 nM CB [7], 1 nM TK1′-H1 duplex, 1 nM c-myc'-H2 duplex, 1 nM survivin'-H3 duplex, 1 nM GalNAc-T′-H4 duplex, 1.5 nM H5, 1.5 nM H6, 2.25 nM H7, 2.25 nM H8, 10 nM probe1, 10 nM probe2 and 0.1 U/μL T4 DNA ligase. All data were recorded in PBS with 0.1 M NaCl at pH 7.4.

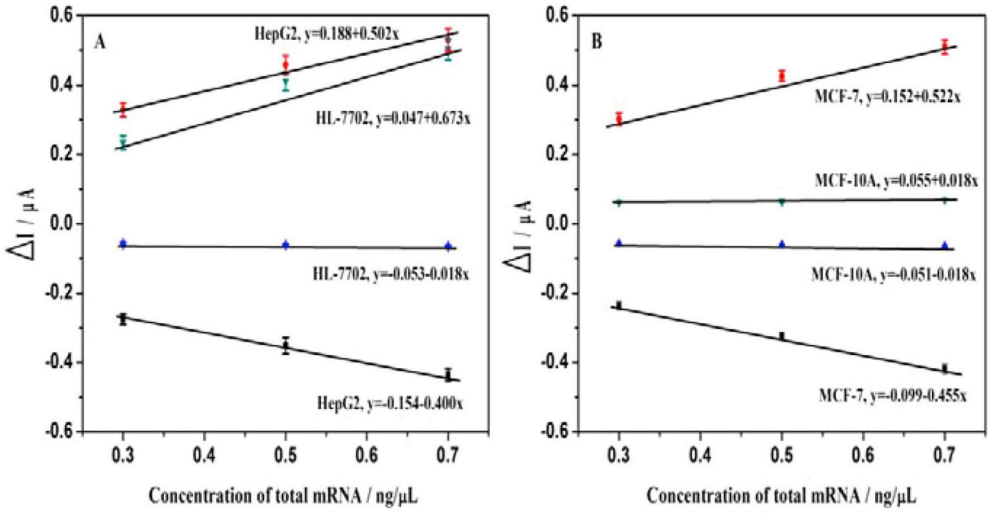
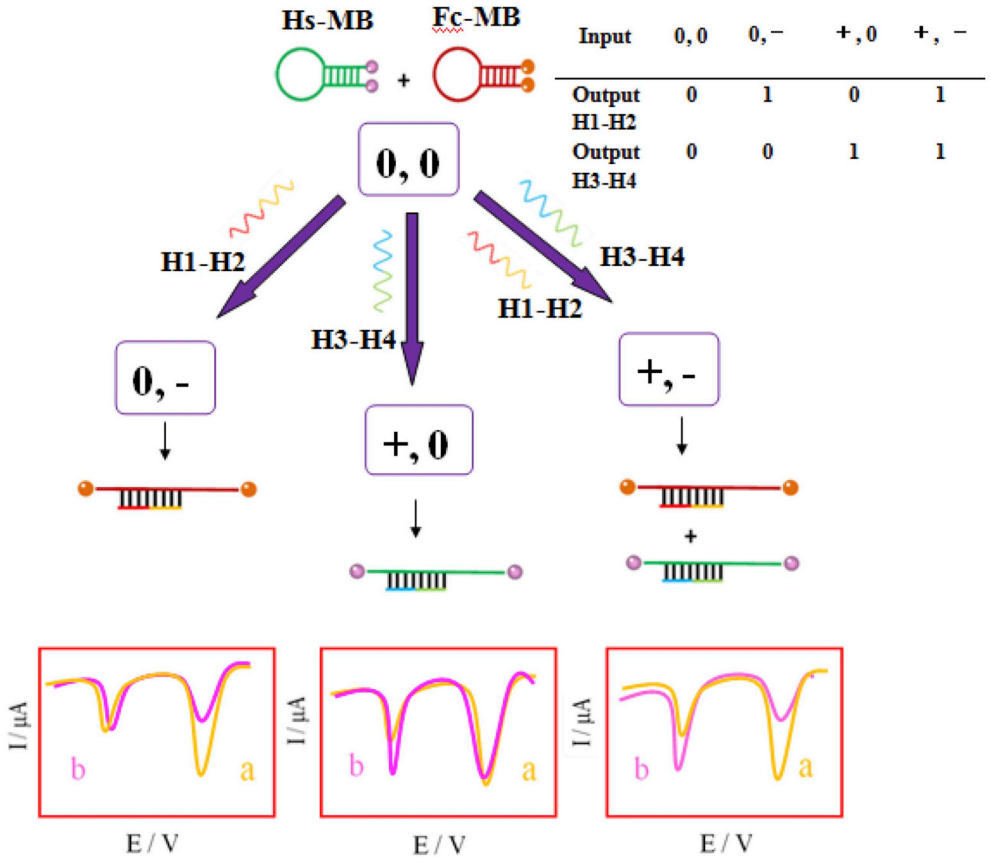


Fig. 5. Detect the two pairs of mRNAs in (A) HepG2 and HL-7702 cells, (B) MCF-7 and MCF-10A cells. Experimental conditions: 10 nM Fc-MB, 10 nM Hs-MB, 100 nM CB [7], 10 nM TK1′-H1 duplex, 10 nM c-myc'-H2 duplex, 10 nM survivin'-H3 duplex, 10 nM GalNAc-T'-H4 duplex, 15 nM H5, 15 nM H6, 22.5 nM H7, 22.5 nM H8, 10 nM probe1, 10 nM probe2 and 0.1 U/μL T4 DNA ligase. All data were recorded in PBS with 0.1 M NaCl at pH 7.4.



Scheme 2. Schematic description of the dual-signal electrochemical biosensor AND logic gate for determination of two pairs of cancer mRNA targets: TK1 and c-myc, survivin and GalNAc-T.

This logic gate was dependent on the structural conversion of the Fc-MB and Hs-MB, which were triggered by H1–H2 and H3–H4. We defined the individual peak current enhancement of Hs-MB as "+" input and the suppression of Fc-MB as "-" input (Scheme 2). From the inset table, two "1" outputs of H1–H2 and H3–H4 were achieved only when both inputs were "+" and "-", inferring they were cancer cells: MCF-7 and HepG2. When there were no inputs "0, 0", two "0" outputs of H1–H2 and H3–H4 were achieved, inferring it was normal cell: MCF-10A. When there was only one inputs "+", a "1" outputs of H3–H4 was achieved, inferring it was normal cell: HL-7702 cell. Thus, the dual-signal electrochemical biosensor could also serve as an "AND" gate. It demonstrates that it could be used to detect the mRNAs in cancer cells: MCF-7 and HepG2 cells and distinguish between themselves and their normal cells: MCF-10A and HL-7702.

4. Conclusion

In this work, we have developed a dual-signal electrochemical biosensor for multiplexed detection of intracellular mRNAs by the split primer ligation-triggered catalyzed hairpin assembly. Compared with the conventional detection system, the application of split primer ligation ensures that only a pair of targets existed, could the

electrochemical signal change with the advantage of high sensitivity and stability. The Fc-MB and Hs-MB possess high electrochemical activity, well-biocompatibility and good thermal stability. They exhibit a higher reproducibility and lower background noise by non-fixation on the electrode surface to avoid spatial hindrance effects. The use of the amplification strategy such as catalyzed hairpin assembly could immensely amplify the variation of electrochemical signals and greatly improve the detection sensitivity. Under the optimal conditions, the detection limit of TK1 and c-myc mRNA is as low as 0.022 nM, and that of survivin and GalNAc-T mRNA is 0.029 nM. Furthermore, the real sample experiments show the method has successfully achieved the detection of two pairs of mRNAs in total mRNA samples extracted from MCF-7 and HepG2 cells and perfect distinguish MCF-7 and HepG2 from their normal cells, MCF-10A and HL-7702. It demonstrates that our proposed method has a latent capacity to monitor the biomarkers variation in vivo. In addition, two pairs of cancer mRNAs could act as inputs to activate an AND logic gate.

CRediT authorship contribution statement

Shiyan Dai: Writing - original draft, Conceptualization, Methodology, Data curation. **Yuting Zhou:** Writing - original draft, Conceptualization, Methodology, Data curation. **Guifang Cheng:** Writing - original draft, Conceptualization, Methodology, Data curation. **Pingang He:** Writing - original draft, Conceptualization, Methodology, Data curation. **Yuzhi Fang:** Writing - original draft, Conceptualization, Methodology, Data curation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2020.121079.

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