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A novel electrochemical cytosensor for selective and highly sensitive detection of cancer cells using binding-induced dual catalytic hairpin assembly

Ye Zhang[a,b,1], Shihua Luo[a,b,1], Bo Situ[a,b], Zhixin Chai[a,b], Bo Li[a,b], Jumei Liu[a,b], Lei Zheng[a,b,*]

[a] Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, PR China
[b] Guangdong Engineering and Technology Research Center for Rapid Diagnostic Biosensors, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, PR China

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ABSTRACT

Rare cancer cells in body fluid could be useful biomarkers for noninvasive diagnosis of cancer. However, detection of these rare cells is currently challenging. In this work, a binding-induced dual catalytic hairpin assembly (DCHA) electrochemical cytosensor was developed for highly selective and sensitive detection of cancer cells. The fuel probe, released by hybridization between the capture probe and catalytic hairpin assembly (CHA) products of target cell-responsive reaction, initiated dual CHA recycling, leading to multiple CHA products. Furthermore, the hybridization between fuel probe and capture probe decreased non-specific CHA products, improving the signal-to-noise ratio and detection sensitivity. Under the optimal conditions, the developed cytosensor was able to detect cells down to 30 cells mL^{-1} (S/N = 3) with a linear range from 50 to 100,000 cells mL^{-1} and was capable of distinguishing target cells from normal cells in clinical blood samples.

1. Introduction

Rare cancer cells shed from tumors can travel through the bloodstream to distant sites where they initiate metastatic disease, which is the major cause of death in cancer patients (Fidler, 2003; Pantel and Alix-Panabières, 2010; Hou et al., 2011). Identification and detection of rare cancer cells can provide an easy and effective method for early clinical diagnosis, diseases surveillance, and cancer therapies. Therefore, it is imperative to develop a rapid, simple, sensitive and specific assay for detection of rare cancer cells (Cayrefourcq et al., 2015; Ke et al., 2015; Ge et al., 2017).

The relatively low concentration of rare cancer cells in clinical samples makes their detection quite challenging. Conventional techniques that have been used for their detection include polymerase chain reaction, immunohistochemistry, and flow cytometry. However, the selectivity of these methods is unsatisfactory (Schamhart et al., 2003; Singh et al., 2004; Yoon et al., 2013; Williams, 2013; Plaks et al., 2013). In order to address this problem, aptamers have been used for recognition of cancer cells because of their competitive advantages of high specificity and affinity, high flexibility, ready availability and chemical stability (Zhao et al., 2012; Yin et al., 2013; Chen et al., 2016a, 2016b, 2018a, 2018b; Huang et al., 2016; Lan et al., 2017).

More recently, a variety of amplification techniques have been established to enhance the sensitivity of aptamer recognition-based cytosensors, such as gold nanoparticles (Borghei et al., 2016; Shuai et al., 2017a, 2017b; Chen et al., 2017), hybridization chain reaction (Niu et al., 2016; Lan et al., 2016; Shuai et al., 2017a, 2017b), nanomaterials (Chen et al., 2016a, 2016b; Wang et al., 2017) and so on. Despite their excellent performances, these methods are neither simple nor rapid enough for an easy-to-use detection of cancer cells because of their poor stability, time-consuming operation, and vulnerability to the external environment influence. Catalytic hairpin assembly (CHA) has attracted much attention recently for signal amplification in aptamer systems (Li et al., 2011). This isothermal enzyme-free DNA amplification strategy is highly compatible with aptamer-based cytosensors and does not require enzyme or special laboratory conditions (Yin et al., 2008; Chen et al., 2013; Bhadra and Ellington, 2014; Liao et al., 2014). However, catalytic performance and nonspecific background signals compromise the analytical performances of CHA-based cytosensor.

Aiming to improve the analytical performance of CHA-based biosensing of cancer cells detection, we have established a novel dual catalytic hairpin assembly (DCHA) strategy by introducing an assistant fuel probe that initiates a new CHA cycle and improves signal amplification. The hybridization between fuel probe and capture probe

* Correspondence to: Department of Laboratory Medicine, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong Province, PR China.
E-mail address: nfyyzhenglei@smu.edu.cn (L. Zheng).
[1] These authors contributed equally to this work.

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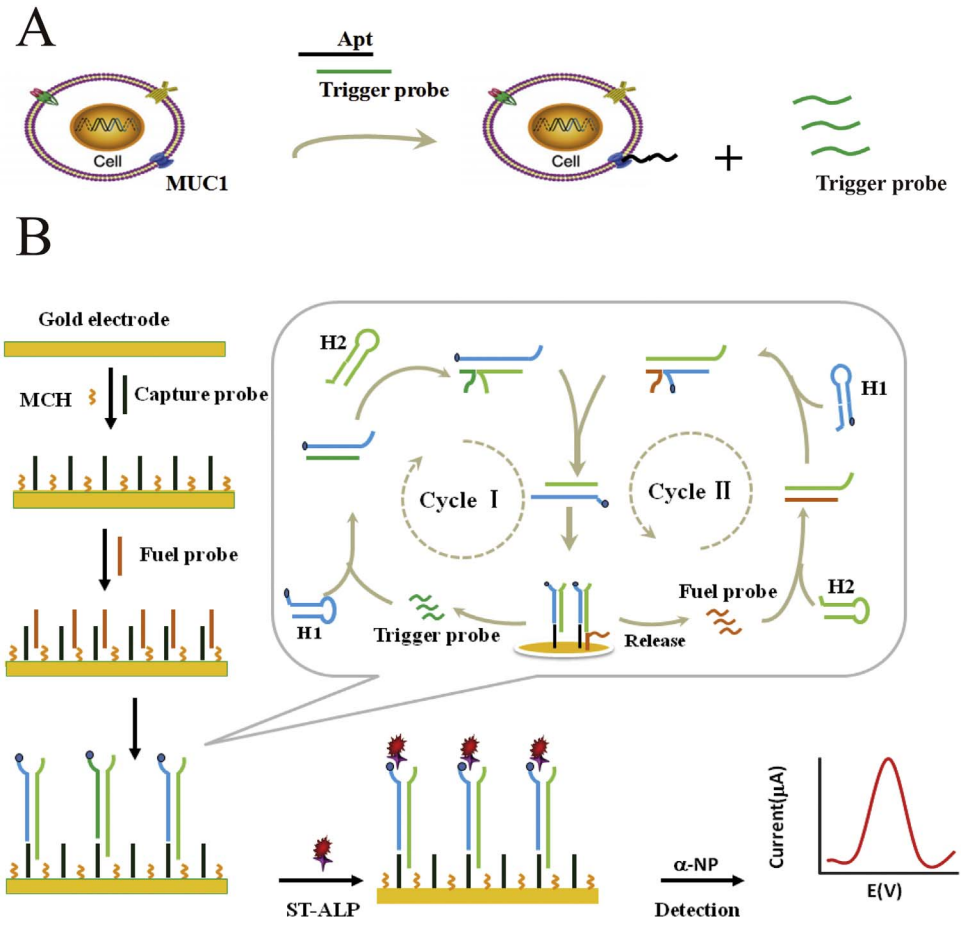

Scheme 1. Schematic representation of the cancer cell cytosensor based on DCHA.

decreased the background signal caused by breathing sites. Thus, a simple, selective and highly sensitive electrochemical cytosensor for cancer cells detection was built by combining the specificity of an aptamer with the signal amplification of DCHA for the first time. As a proof-of-concept, the human non-small cell lung cancer (NSCLC) cell line A549, was used as a model. One of the glycoproteins expressed on the apical surface of normal epithelial cells, MUC1, is often associated with NSCLC, so the MUC1 aptamer was adopted as the recognition elements. Finally, using CHA products as signal probes, an electrochemical method was employed (Scheme 1). The proposed cytosensor appeared significant analytical performance toward cancer cells detection, which provides a powerful and convenient platform for bioanalysis and early clinical diagnosis.

2. Experiment section

2.1. Reagents

DNA oligonucleotides were synthesized and purified by Sangon Inc. The base sequences are listed in Table 1. α-Naphthyl phosphate (α-NP), 6-mer-capto-1-hexanol (MCH), streptavidin-alkaline phosphatase (ST-AP), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). DL500DNA Marker and agarose were from TakaRa Biotech (Dalian, China). All other reagents were of analytical reagent grade. All aqueous solutions used in the experiments were prepared with Milli-Q water (≥ 18 MΩ, Milli-Q,Millipore), Diethanolamine (DEA) buffer (pH 9.6) contained 0.1 M DEA, 1 M $MgCl_2$ and 0.1 M KCl. Tris-HCl buffer used as washing buffer (pH 7.40) contained 20 mM Tris, 0.1 M NaCl, 5.0 mM $MgCl_2$ and 0.05% Tween-20. Reaction buffer (pH 7.4) contained 10 mM Tris, 0.1 mM NaCl, 5.0 mM $MgCl_2$.

2.2. Apparatus

Electrochemical measurements including electrochemical impedance spectroscopy (EIS), square wave voltammetry (SWV) and differential pulse voltammetry (DPV) measurements were carried out on a CHI660D electrochemical workstation (Shanghai Chenhua Instruments Co. Ltd., China). The gel electrophoresis was conducted on a Bio-Rad electrophoresis analyzer (Bio-Rad, USA) and imaged on a Bio-Rad ChemDoc XRS (Bio-Rad, USA).

2.3. Preparation of probes

Hairpin probe 1 (H1) and hairpin probe 2 (H2) were designed based on the principle of an enzyme-free strand-displacement nucleic acid circuits system. All DNA oligonucleotides were denatured at 95 °C for 5 min and then cooled down 5 °C per minute to the room temperature. The probes were stored at 4 °C until used.

2.4. Cell culture and preparation

Human non-small cell lung cancer A549 cells were used as target cells. The human bronchial epithelial 16 HBE and human cervical carcinoma (HeLa) cell lines were chosen as control cells. The cells were obtained from the committee on Type Culture Collection of the Chinese Academy of Sciences. Cells were cultured in RPMI 1640 medium containing 10% fetal bovine serum, penicillin and streptomycin at 37 °C in a humidified atmosphere of 5% CO_2. Cells in logarithmic growth phase were separated from the medium by centrifugation at 1000 rpm for 3 min, and then incubated in sterile phosphate-buffered saline (PBS) to obtain a homogeneous cell suspension. The number of the cells was determined using a Neubauer hemocytometer. Cells were kept in an ice