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Enzyme-free and label-free fluorescence sensor for the detection of liver cancer related short gene

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ABSTRACT

Non-invasive early diagnosis of liver cancer is the most effective way to improve the survival rate. In this paper, we developed a label-free and enzyme-free fluorescent biosensor based on target recycling and Thioflavin T (ThT) induced G-quadruplex formation for MXR7 (liver cancer related short gene) detection in human serum. The proposed sensor can detect the target DNA in the concentration range of 0–350 fM with the detection limit as low as 10 fM. Due to the outstanding structural selectivity of ThT for G-quadruplex, this sensor possesses better discrimination ability and higher sensitivity. Furthermore, this enzyme-free and label-free fluorescence sensor has demonstrated to be capable of detecting target DNA in human serum samples because of its high selectivity and sensitivity. The mechanism employed in this study represents a promising path toward directing liver cancer detection in human serum. In addition, this strategy may be extended to detect other cancer related genes by choosing a rational DNA probe according to different sequences of targets.

1. Introduction

Liver cancer, as a lethal disease, is one of the most common cancers worldwide with the rising morbidity year by year (Llovet and Bruix, 2008). Accurate diagnosis of liver cancer is important to determine the extent of disease and to plan appropriate therapies. Especially, the early diagnosis of liver cancer is of great significance for improving the survival rate via early treatment and reducing the suffering and cost for the patients. At present, the conventionally used clinical diagnosis methods for liver cancer are ultrasound elastograph (Kudo, 2014), computed tomography (CT) (Sofocleous et al., 2014), liver puncture needle aspiration cytology (Novak et al., 2014), etc. However, these universal methods have the following drawbacks, damage to patient body, time-consuming, high cost, etc. Tumor markers existing in tumor cells or host body fluids are substances which can be used for diagnosis of tumors based on their biochemical or immune characteristics (Lai et al., 2012). The accurate detection of tumor markers in biosamples has been regarded as an ideal method in early stage due to its higher sensitivity, non-invasiveness, simplicity and low cost. Some tumor markers are always used to evaluate specific cancers, but most are less predictable. For example, an elevation of alpha-fetoprotein (AFP) level in human serum can indicate not only liver cancer but also epithelial ovarian tumors, which bring substantial difficulty for the diagnosis of liver cancer. Hence, it is necessary to explore a more credible and specific diagnostic marker of hepatocellular tumor. Recently, the mitoxantrone resistance7 (MXR7) oncogene has been recognized as an efficient early marker of liver carcinogenesis with great applied prospect (Wang et al., 2006).

The fluorescent biosensor has been proved to be a promising tool for clinical diagnosis owing to its appealing advantages (Chung et al., 2009). However, because of the low concentration of MXR7 and complex background of serum, conventional fluorescent biosensors cannot be available to meet the clinical diagnostic requirement for direct MXR7 detection in human serum. To address this problem, many enzyme-based amplification approaches had been developed (Fu et al., 2011; Weizmann et al., 2006; Wang et al., 2011; Tian et al., 2006; Cheglakov et al., 2007; Cheglakov et al. 2006). However, the applications of these amplified sensors are restricted by the fact that protein enzymes are of high-cost and the enzymatic activity is sensitive to reaction conditions (Zheng et al., 2012). Thus, several enzyme-free sensors have been also proposed based on hybridization and strand displacement (Dirks and Pierce, 2004; Huang et al., 2011; Wang et al., 2011; Zhang et al., 2007; Bi et al., 2011; Yin et al., 2008; Li et al.,

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2011). But these approaches always require specific labels or extra steps to obtain optical or electrochemical signals, which makes them time-consuming or high-cost (Lu et al., 2010). So the development of label-free and enzyme-free methods is a necessity for such amplified sensors to realize their extensive practical application and biomedical utilization in DNA detection.

In this study, an enzyme-free and label-free fluorescence sensor for liver cancer related short gene (MXR7) has been developed. The proposed sensor has been applied to detect the target in the human serum samples with satisfied results. Hence, this study may provide an approach for liver cancer detection, which may also be extended to detect other cancers in the near future.

2. Experimental

2.1. Reagents and apparatus

All the designed DNA sequences in this study were synthesized by Sangon (Shanghai, China), purified by high-performance liquid chromatography (HPLC), and confirmed by mass spectrometry (MS). The concentrations were quantified by OD 260 based on their individual absorption coefficients. Each DNA was heated to 90 °C for 5 min and was slowly cooled down to room temperature before use. The sequences of oligonucleotides used in this study are list as follows:

Hairpin 1 (H1): 5′-**GGG TAG GGC GGG TTG GG** AT *TAG GAA AGG CTG CCA* CATCC CAA CCC ATA-3′
Hairpin 2 (H2): 5′-TATGGG TTGGGATGTGGCAGCCATCCCAAC-3′
Perfectly complementary target (T1, MXR7): 5′-TGGCAGCC TTTCCTA-3′
Single-base-mismatched target (T2): 5′-TGGCAGCGTTTCCTA-3′
Double-base-mismatched target (T3): 5′-TGGCAGGCATTCCTA-3′
Three-base mismatched target (T4): 5′-TGCCAGGCTTTCGTA-3′
Non-complementary target (T5): 5′-ACCATCGGAAAGGAT-3′

The boldfaced letters indicate the sequence that forms a G-quadruplex; the italic letters are the complementary sequence of T1; the underlined letters are the mismatched base sequence.

ThT (3,6-dimethyl-2-(4-dimethylaminophenyl)benzo-thiazoliumcation), obtained from Sigma-Aldrich, was purified by column chromatography using a silica gel column and mildly acidic methanol as the eluent. All other chemicals were purchased from Sigma-Aldrich and used without further purification. The water used was obtained by purification of distilled water with a Millipore Milli-Q system. All measurements were performed in reaction buffer (50 mM Tris–HCl, 50 mM KCl, 10 mM $MgCl_2$, pH 7.5) unless otherwise stated.

The human serum samples of liver cancer patients (confirmed by pathological examinations) were obtained from the First Affiliated Hospital of Fujian Medical University (Fujian, China).

Fluorescence spectra (Varian Cary Eclipse Fluorimeter, Varian, Inc., Agilent Technologies) were measured with a Peltier block, using quartz fluorescence cuvettes (4 mm × 10 mm, Sub-micro, 50 μL), and with the following settings: λ_{ex}=441 nm, λ_{em} =485 nm, 5 nm slit, PMT detector voltage=650 V. UV–vis absorbance spectra were measured with a UV-2501 PC spectrophotometer (SHIMADZU, Japan) using a quartz cell with 1.0 cm optical pathway. The circular dichroism (CD) spectra were studied based on a JascoJ-810 CD spectropolarimeter (Tokyo, Japan). The optical chamber (1 cm path length, 500 μL volume) was deoxygenated with dry purified nitrogen (99.99%) before use and kept in the nitrogen atmosphere during experiments. Three scans (100 nm min^{-1}) from 220 nm to 330 nm at 0.1 nm intervals were accumulated and averaged. The background of the buffer solution was subtracted from the CD data.

2.2. Preparation of the fluorescent biosensor for T1 detection

Different concentrations of T1 were mixed and reacted with 50 μL of reaction buffer containing 200 nM H1, 300 nM H2, and 3 μM ThT at 38 °C for 2 h, respectively. The fluorescence spectra of the above resulted solutions were recorded in the wavelength range from 445 nm to 580 nm with the excitation of 441 nm at room temperature.

2.3. Preparation of human serum sample

The human serum samples were centrifuged at 603*g* for 10 min at 4 °C. Then, the 1 mL of supernatant liquid was taken and diluted to 100 mL with buffer solution. The different concentrations of T1 were then added into the diluted serum to prepare artificial serum samples and the concentrations of T1 in the samples were determined with our biosensor.

3. Results and discussion

3.1. Principle of the biosensor

It has been reported that the G-rich nucleic acid sequences under certain conditions can form G-quadruplexes, which are unique higher-order structures of stacked arrays of G-quartets connected by Hoogsteen-type base pairing (Phan et al., 2006; Amrane et al., 2009; Kong et al., 2009). Water soluble ThT is weakly fluorescent by itself, but can exhibit greatly enhanced fluorescence upon inducing DNA folding into G-quadruplex under physiological salt conditions, which owns pronounced structural selectivity for G-quadruplexes but not for single, double or triplexes stranded DNA. (Mohanty et al., 2013) This character is applied to design the sensitive fluorescence sensor for MXR7 in this study. As shown in Fig. 1, this DNA detection system contains ThT and two hairpin DNAs (hairpins) termed as H1 and H2. H1 contains three domains termed as 1, 2, and 3, while H2 contains two domains termed as regions 4 and 5. The region 3 of the partially oligonucleotides of H1 is guanine-rich sequence (G-rich sequence). In addition, the region 2 is complementary to the target MXR7 (T1), and the region 4 is complementary to region 1 and 2. Thus, in the presence of target MXR7, the region 2 of H1 will hybridize with the target, leading to the release of regions 1 and 3. When both H1 and H2 exist at the same time, the target is replaced with the sequence 4 of H2 because the region 4 can form more stable DNA structure with the regions 1 and 2. Subsequently, the released target further hybridizes with another H1, which induces the cycling of G-rich sequence folding into quadruplex. After numerous cycles, all regions 3 of the H1 hairpins are released, and then fold into quadruplex and bind with ThT, resulting in obvious enhancement of the fluorescent signal. Herein, an enzyme-free and label-free fluorescence sensor for DNA detection is developed.

3.2. Optimization of experimental conditions

To achieve the best sensing performance of the fluorescence sensor, the main experimental variables, such as hybridization temperature, concentration of ThT, incubation time and pH, were optimized (as shown is Fig. 2). The temperature of the detection system is a key factor which directly affects the hybridization efficiency of H1/H2/T1. Hence, the temperature was firstly investigated. As shown in Fig. 2A, with the rise of the hybridization temperature, the fluorescence intensity increases firstly and then