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Simultaneous detection of two hepatocellar carcinoma-related microRNAs using a clever single-labeled fluorescent probe

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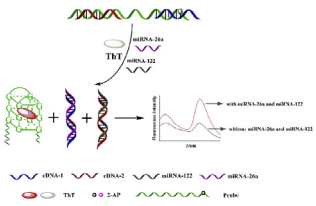
HIGHLIGHTS

- A single-labeled and dual-functional probe was cleverly designed.
- Simple and cost-efficient fluorescence sensor was applied to quantify miRNA.
- Simultaneous detection of miRNA-122 and miRNA-26a was successfully achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

The simultaneous detection of two important microRNAs (miRNAs) can potentially evaluate pathological states. The simultaneous detection of miRNA-26a and miRNA-122 is proposed in this study using a cleverly designed single-labeled, dual-functional fluorescent probe with a 2-aminopurine as a fluorophore, which is a G-quadruplex single-stranded DNA. The probe can partly complement cDNA-1 and cDNA-2. cDNA-1 and cDNA-2 are complementary strands of miRNA-26a and miRNA-122, respectively. When the target miRNAs are added simultaneously, these cDNA (cDNA-1 and cDNA-2) can be competed off from the cDNA\probe duplex to form a cDNA\RNA heteroduplex. The probe is released and the fluorescent signal is increased. The detection limits of miRNA-26a and miRNA-122 are both 2.5 nM, and their wide linear which ranges from 2.5 to 500 nM are achieved using the assay. This method has potential practical applications.

1. Introduction

The aberrant expression of microRNAs (miRNAs) is associated with the progression and development of various human cancers [1–3]. For example, miRNA-26a and miRNA-122 are vital in the diagnosis, prognosis, and treatment of hepatocellar carcinoma (HCC) [4,5]. Thus, the detection of tumor-related miRNAs is crucial to accurately diagnose cancers.

Various methods have been developed to detect single-miRNA, such as electrochemical devices [6,7], surface plasmon resonance [8,9], and fluorescent sensors [10–12]. These methods provide a useful reference for cancer diagnosis. Approaches that can detect several relevant miRNA simultaneously will significantly improve the reliability of a cancer diagnosis [13,14]. These methods have several potential advantages, such as providing simplified assays procedures and reducing costs [15,16]. Studies on the simultaneous detection of miRNAs are still scarce [17–19]. Zhou et al. reported an efficient strategy based on strand-displacement amplification for the detection of miRNA simultaneously [20]. Rhee et al. investigated simultaneous detection of multiple miRNAs in entire exosomes using molecular beacons [21]. The previously mentioned methods are simple and convenient with high specificity. However, these methods still require each terminus of a hairpin to be labeled

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with both a fluorophore and quencher, which renders the labeling and purification expensive and time-consuming [22,23]. In order to reduce labeling, Deiters et al. developed a two input sensor using a fluorophore and quencher [24]. The sensor can simultaneously monitor, image, and respond to cell-specific marker. This strategy is simple, convenient, and ingenious. Based on the above mentioned, quencher-free design for detection has elicited attention. Therefore, a simple, quencher-free, convenient, and cost-efficient fluorescence strategy should be developed for the simultaneous detection of miRNA-26a and miRNA-122 to increase the reliability of evaluating the pathological states in HCC.

MiRNA-26a and miRNA-122 are two tumor suppressors that are reduced in HCC [25,26]. The potential function of miRNA-26a and miRNA-122 in the progression and development of HCC has been investigated recently [4,5,25,26]. Thus, the detection of these tumor suppressors is desired.

A single-labeled, dual-functional fluorescent probe was designed for the construction of miRNA sensor (Scheme 1). The probe has a 2-aminopurine (2-AP) as a fluorophore, which is G-quadruplex single-stranded DNA. The nucleotide of 2-AP is an analogue of adenine, which exhibits good fluorescence properties [27,28]. The base-stacking interaction makes its fluorescence in single-stranded DNA higher than that in double-stranded DNA [11,29]. G-quadruplex DNA, which is a G-rich nucleic acid sequence, can significantly improve the fluorescence signals of small molecules [30,31]. The structural selectivity of small molecule such as Thioflavin T (ThT) for G-quadruplex other than duplexes, triplexes, or single-stranded forms may enhance the specificity of sensing [32,33]. First, the probe partly complements cDNA-1 and cDNA-2. The cDNA-1 and cDNA-2 are completely complementary strands of miRNA-26a and miRNA-122, respectively. When the target miRNA-26a was added, the cDNA-1 can be competed off from the cDNA-1\probe duplex to form a cDNA-1\RNA heteroduplex, thereby significantly enhancing the fluorescence emission signal of 2-AP. With the target miRNA-122, the cDNA-2 can be competed off from the cDNA-2\probe duplex to form a cDNA-2\RNA heteroduplex, which enhances the signal because of the accumulation of released G-quadruplexs. When two miRNAs are added simultaneously, the fluorescence intensity is increased at two different fluorescence emission peaks. The proposed sensing protocol is simple, easily labeled, and does not require any additional quenchers. The smart design of the single-labeled, double-functional probe reduces detection costs. Therefore, this detection platform offers significant potential for further applications in analysis.

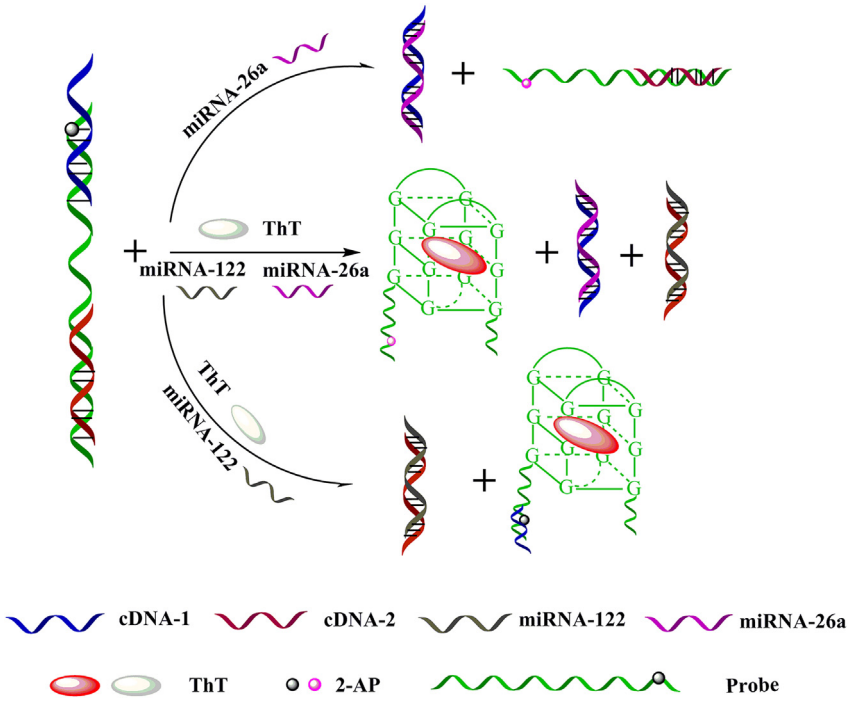
2. Materials and methods

2.1. Materials

cDNA, probe, miRNAs, 1 × TE (10 mM Tris and 1 mM EDTA) buffer, diethylpyrocarbonate (DEPC) treated water, and 10 × TM (500 mM Tris and 80 mM $MgSO_4$) buffer were obtained from Shanghai Sangon Co. Ltd. (Shanghai, China). The sequences of the oligonucleotiedes and miRNAs are listed here (Table 1).

2.2. Fluorescent miRNAs assay

cDNA and the probe were dissolved in 1 × TE buffer. MiRNAs were dissolved in DEPC-treated water. The 500 nM probe was incubated with 500 nM cDNA-1 and cDNA-2 in 10 × TM buffer solution (50 mM Tris, 8 mM $MgSO_4$, and pH 7.5) at 45 °C for 60 min. Up to 10 μL of different concentrations of target miRNAs were added, and the solution was incubated at 45 °C for 40 min. Fluorescence spectra were measured on a spectrofluorophotometer (RF-5301pc, SHIMAPZU, Japan) at room temperature. An excitation wavelength of 300 nm was used for the fluorescence measurement. The fluorescence signal of 2-AP was observed at 367 nm, whereas the fluorescence of ThT was measured with an emission of 482 nm. The excitation and emission slits were set to 5.0 and 10.0 nm, respectively.



Scheme 1. Schematic of Detection Mechanism.