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A highly sensitive and facile graphene oxide-based nucleic acid probe: Label-free detection of telomerase activity in cancer patient's urine using AIEgens

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ABSTRACT

Molecular beacon (MB)-based sensing platforms that consist of a fluorogen-quencher pair play an important role in medical and biological researches. However, the synthesis of both fluorogen and quencher in the nucleic acid probes will increase the burden of organic synthesis works and induce the difficulties for precisely controlling the relative distance between fluorogen and quencher, which may lead to false-positive and false-negative results. In this work, initially we report a single labeled MB (FAM-MB, with carboxyfluorescein as fluorogen and without quencher) thus simplifies MBs with the aid of graphene oxide (GO) to detect telomerase activity. To further simplify this structure, namely label-free strategy, we design a facile, sensitive and selective platform using a label-free beacon (AIE-MB, without fluorogen and quencher), based on aggregation-induced emission fluorogen (silole-R). Upon the addition of telomerase, AIE-MB induced comb-like DNA structure leads to high aggregation of silole-R and thus exhibits strong fluorescence emission. By exploitation of this, we can detect telomerase with superior sensitivity and demonstrate their applications in bladder cancer diagnosis. Compared to single-labeled FAM-MB based telomerase activity assay, the label-free AIE-MB induced method could perform the sensitive detection with high signal-to-background ratio.

1. Introduction

Nucleic acid probes, especially those based on DNA, are essential tools for exploring the biological processes of nucleic acid amplification, ligation, duplication and transcription (Wu et al., 2014; Zhao et al., 2008). Many kinds of DNA probes have been developed in recent years through various molecular engineering strategies (Jia et al., 2015; Li et al., 2008). Among them, molecular beacons (MBs), first reported by Tyagi and Kramer in 1996, play an important role in medical and biological researches (Tyagi and Kramer, 1996). It consists of a special short nucleic acid strand with a fluorogen-quencher pair attached to its ends that can report the presence of specific nucleic acids in homogeneous solutions (Zheng et al., 2015). However, the synthesis of both fluorogen and quencher in the nucleic acid probes, not only increase the burden of organic synthesis works, but also induce the difficulties for the designing of the exact positions of fluorogen and quencher in the nucleic acid sequences. In addition, the changes of relative distance between fluorogen and quencher before and after the target/probe bindings needed to be precisely controlled. Thus, there are many factors influence the effect of MBs, which may lead to false-positive and false-negative results.

In the past decade, nanomaterials with unique components, structures and properties are used in constructing high efficient biosensors. As a typical nanomaterials, graphene oxide (GO) is readily available and exhibits exceptional optical, electrical, mechanical and chemical properties (Lin et al., 2014). It has attracted more attention in the study of DNA-based sensors since graphene was discovered in 2004. It's well known that single-stranded DNA (ssDNA) interacts with GO through π-π stacking interaction (Tang et al., 2015). The fluorescence will be quenched after fluorogen labeled ssDNA binds to GO (Lin et al., 2014). Thus, GO can enhance the signal-to-background ratio and improve the detection limit (Cui et al., 2012). Moreover, the GO quenched MBs needs only one fluorogen labeled on the end, which reduces the cost and simplifies the step to synthesis or purification of the beacon comparing with conventional method (Huang and Marti, 2012).

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To further simplify this structure, namely label-free, aggregation-induced emission fluorogen (AIEgen) was favored (Lou et al., 2015). Instead of aggregation-caused quenching, aggregate formation enhances their light emission, turning them from weak fluorogens in solution to strong emitters in the aggregated state (Mei et al., 2014). The discovery of the AIE phenomenon has provided us idea to design label-free biosensors with high emission efficiency in the aggregate state. As a stealthy biomarker, telomerase was chosen to evaluate this design. Telomerase is upregulated in $\geq$85% of cancer cells, providing a telomere maintenance mechanism, but is generally absent in normal somatic cells (Blasco et al., 1997; Cong et al., 2002). Therefore, telomerase could be regarded as a valuable tumor marker, and the evaluation of telomerase activity is of significant importance to cancer diagnosis, therapy, and monitoring (Hoos et al., 1998; Wang et al., 2015).

2. Experimental

2.1. Cell culture

Hela and E-J cells were cultured in 1640 medium with 10% fetal calf serum and 1% penicillin streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2 in a cell culture flask. MCF-7 (HLECs) and HLF cells were cultured similarly in DMEM and MEM medium, respectively.

2.2. Telomerase extension reaction and detection by FAM-MB with GO

Telomerase extracts from a certain number of cells were diluted in 1 × CHAPS lysis buffer and then added into the 50 μL telomerase extension solution containing 1 × NEBuffer 2, 1 mM dNTPs, 5 μM TS primer, 0.5 μM FAM-MB, and 0.4 U/μL RNase inhibitor. The solution was incubated at 37 °C for 60 min, then transferred to 95 °C for 15 min to deactivate the telomerase. Finally, GO was added into this system with a final concentration was 0.05 mg/ml.

2.3. Telomerase detection by AIE-MB with GO

In the same way as detection by FAM-MB, an appropriate telomerase extracts was added into the 50 μL telomerase extension solution containing 1 × NEBuffer 2, 1 mM dNTPs, 5 μM TS primer, 0.5 μM lable-free beacon, 20 μM silole-R and 0.4 U/μL RNase inhibitor. The solution was incubated at 37 °C for 60 min, and then transferred to 95 °C for 15 min to deactivate the telomerase. Finally, GO was added into this system with a final concentration was 0.05 mg/ml.

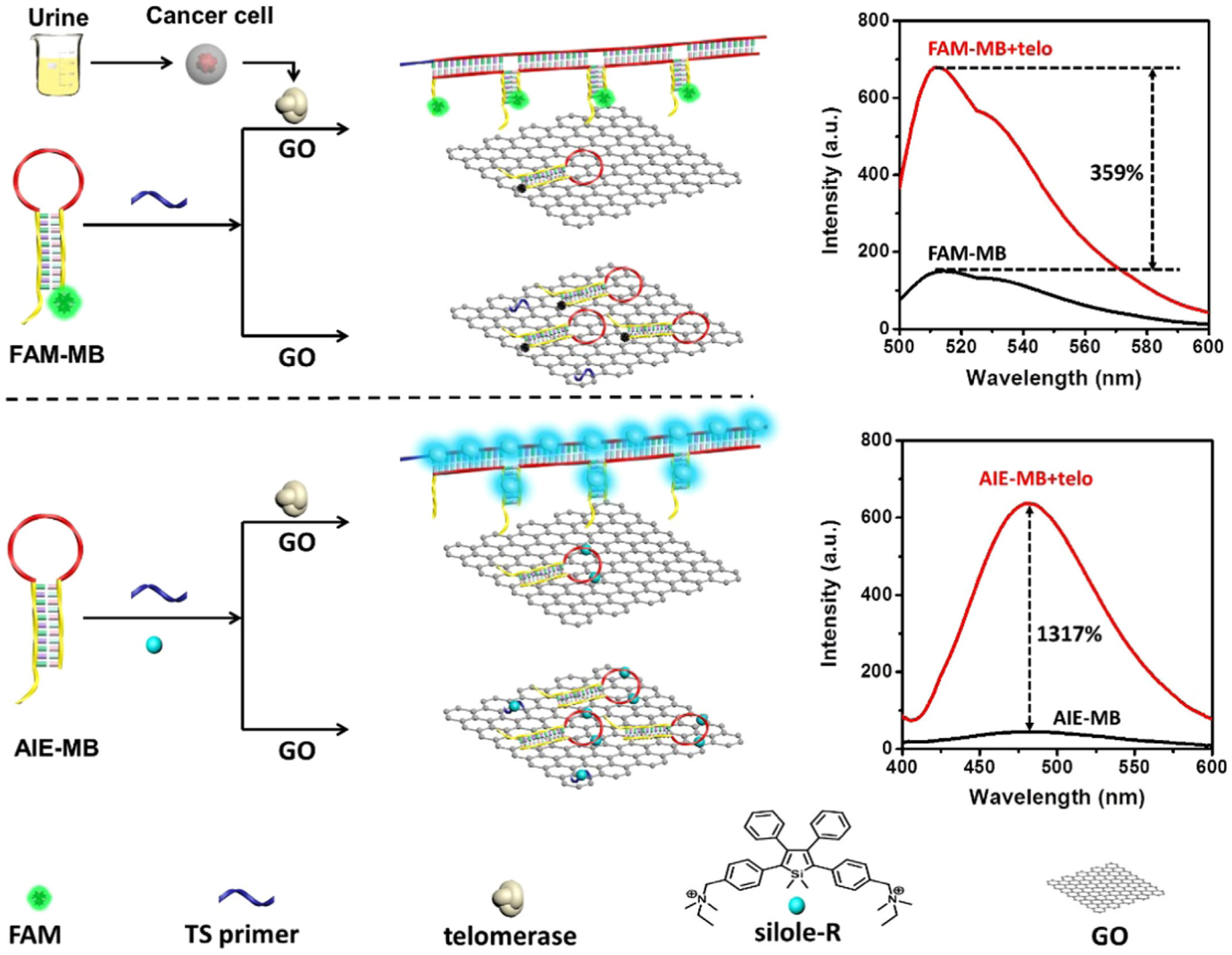

Fig. 1. Schematic illustration of GO-based platform for telomerase activity detection using single-labeled beacon (FAM-MB) and label-free beacon (AIE-MB). The fluorescence emission spectra of FAM (right upper) or silole-R (right bottom) in the presence (red line) or absence (black line) of telomerase extracts from 10,000 Hela cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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