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A simple, fast, label-free colorimetric method for detection of telomerase activity in urine by using hemin-graphene conjugates

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ABSTRACT

Telomerase, a widely accepted cancer biomarker for early cancer diagnostics, is considered as an important therapeutic target. To now, it is still a challenging subject to develop a simple and sensitive strategy for telomerase activity detection. Herein, we reported a simple colorimetric strategy for label-free quantification of human telomerase activity in urine by using hemin-graphene nanomaterial (H-GNs). H-GNs possessed tailored dispersibility in the high salt concentration and highly active biomimetic oxidation catalyst property. In this strategy, H-GNs were adjusted to coagulate to appropriate degree by carefully selecting the contained NaCl amount in the presence of original TS primer. The supernatant of the solution contained few H-GNs and showed light blue color. Under the action of telomerase, TS primer was elongated with repeating sequences of $(TTAGGG)_n$. These negatively charged DNA enhanced individual H-GNs electrostatic repulsion and resisted salt-induced H-GNs coagulation. As a result, the supernate of the corresponding solution containing more dispersed H-GNs and showed dark blue color after chromogenic reaction. Thus, telomerase activity could be quasi-quantified by naked eye and precise quantified by UV spectrometer. The proposed method has the linear range from 100 to 2300 HeLa cells/mL and the detection limit was 60 cells/mL. It has been successfully applied to detect telomerase activity in real urine samples. Obtained results were in good agreement with the clinical diagnosis. Therefore, this colorimetric approach affords simplicity, sensitivity and reliability in telomerase activity detection.

1. Introduction

Human telomerase is a ribonucleoprotein complex that maintains telomere length by adding repetitive nucleotide sequence $(TTAGGG)_n$ onto the end of the human chromosomes using its RNA template, reverse transcriptase and associated protein (Feng et al., 1995; Cohen et al., 2007). A number of studies have indicated that telomerase expression is associated with cell immortalization and tumorigenesis (Rodier and Campisi, 2011). Since over 85% of all known cancer cells telomerase is over-expressed, while there is no detectable telomerase activity in normal cells (Shay and Bacchetti, 1997). Therefore, the simple methods for detection of telomerase activity could be significant for cancer diagnosis, screening of anticancer drugs, and evaluation of cancer therapy (Harley, 2008).

The most widely used method to detect telomerase activity in cell extracts was the polymerase chain reaction (PCR)-based telomeric repeat amplification protocol (TRAP) and its modified assay since the discovery of telomerase in 1985 (Wege et al., 2003; Herbert et al., 2006). However, TRAP assay or other modified TRAP assay are based on polymerase chain reaction(PCR), which suffers from severe limitations such as time-consuming procedures and complicated manipulation (De Cian et al., 2007; Xiao et al., 2010; Hou et al., 2001). To overcome these deficiencies, some PCR-free assays for telomerase activity based on alternatives techniques have been proposed including surface enhanced raman scattering (SERS) (Zong et al., 2014; Xu et al., 2016), chemiluminescence (Li et al., 2010; Zhang et al., 2014a, 2014b; Wang et al., 2013), colorimetry (Wang et al., 2012; Sharon et al., 2014; Freeman et al., 2010; Duan et al., 2014; Zhang et al., 2016), fluorescence (Lou et al., 2015; Gao et al., 2016; Zhuang et al., 2015a, 2016b; Qian et al., 2014; Li et al., 2016; Jia et al., 2016), electrochemistry (Yi et al., 2014; Liu et al., 2015; Wang et al., 2015; Li et al., 2015; Alizadeh-Ghodsi et al., 2016) and so on. Label-free methods (Zhu et al., 2011a, 2011b) and colorimetric methods were most attractive because they usually were simple, and no expensive instruments were needed.

Gold nanoparticles were the mostly used probes to construct colorimetric analytical methods. Xia and his groups developed a

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bidirectional strategy for telomerase activity detection and bladder cancer diagnosis based on four detection-color states of difunctional gold nanoparticle probes (Duan et al., 2014). Telomerase extracted from 293T cancer cells catalyzed the telomerization of its primer, generating the catalytic telomeric hemin/G-quadruplex chains that control the aggregation of AuNPs. The extent of aggregation was dependent on the concentration of telomerase, which could be used to detect telomerase activity with a detection limit of 27 cells/μL (Sharon et al., 2014). Qu reported a colorimetric analysis of telomerase activity by using telomere complementary oligonucleotides modified AuNP probe (TC-AuNPs). The telomerase reaction products, which could hybridize with the TC-AuNPs, were able to protect the AuNPs from the aggregation induced by salt. Telomerase activity in 1000 HeLa cells can be discerned with the naked eye, and down to 100 HeLa cells with the aid of UV–vis spectroscopy (Wang et al., 2014).

The hemin-graphene conjugates (H-GNs) possessed the advantages of both hemin and graphene and exhibited excellent properties (Guo et al., 2011a; Xue et al., 2012). H-GNs showed different dispersibility in the high salt concentration in the presence of single- or double-strand DNA sequences. On the other hand, they possessed the intrinsic peroxidase-like activity that could catalyze the reaction of peroxidase substrate due to the existence of hemin on the graphene surface. Since its highly active biomimetic oxidation catalyst property had been reported by Duan in 2011, several promising colorimetric platforms were constructed. Dong's group constructed a label-free colorimetric method for detection of single nucleotide polymorphism and small molecules (Guo et al., 2011a, 2011b). Then, our group also developed a simple colorimetric method for detection of DNA damage induced by environmental compounds and ultraviolet irradiation by using H-GNs (Wei et al., 2014). Due to the inherent superiorities such as being easily synthesized, stable, reliable and easily to be readout, H-GNs possessed high prospects to be used to construct simple colorimetric analytical methods.

Herein, a simple label-free colorimetric sensor for telomerase activity based on the advantages of H-GNs was developed. In the presence of TS primer, H-GNs were adjusted to coagulate to appropriate degree by carefully selecting the presented NaCl concentration. Then, the supernate of the corresponding solution contained little H-GNs and showed light blue color after chromogenic reaction. Then, repeating sequences of $(TTAGGG)_n$ were extended on TS primer by telomerase. These negatively charged DNA enhanced individual H-GNs electrostatic repulsion and resisted salt-induced H-GNs coagulation. As a result, the supernate of the corresponding solution containing more dispersed H-GNs and showed dark blue color after chromogenic reaction. The proposed method has the linear range from 100 to 2300 HeLa cells/mL and the detection limit was 60 cells/mL. The method had good selectivity, accuracy and applicability. It has been successfully applied to detect telomerase activity in real urine samples and the obtained results were in good agreement with the clinical diagnosis. Therefore, this colorimetric approach affords simplicity, sensitivity and reliability in telomerase activity detection.

2. Experiment section

2.1. Chemicals and materials

The oligonucleotides used in this paper were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (Shanghai, China). The sequences were listed as follow: 5′-AATCCGTCGAGCAGAGTT-3′. Hemin and 3,3′,5,5′-tetramethylbenzidine (TMB) were purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). 2-[(E)-3-naphthalen-2-ylbut-2-enoylamino]benzoic acid (BIBR 1532) was received from Selleck Chemicals (Houston, USA). Curcumin was obtained from J & K Chemical Ltd. (Shanghai, China). Hydrazine hydrate (≥ 85.0%) was obtained from Shanghai Linfeng Chemical Reagent Co. Ltd. (Shanghai, China). All other chemicals were of analytical reagent grade and used without any purification. Ultrapure water (18.2 MΩ cm at 25 °C, Barnstead, Thermo Scientific, USA) was used in all experiments.

In this study, the buffer solutions were employed as follows: telomerase extension reaction buffer (20 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 1 mM EGTA, 63 mM KCl, 0.005% (v/v) Tween 20); 1 × PBS (pH 7.2–7.4, 136.89 mM NaCl, 2.67 mM KCl, 8.24 mM Na_2HPO_4, 1.76 mM NaH_2PO_4); Lysis buffer (0.5% (w/v) CHAPS, 10 mM Tris-HCl, pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 10% (v/v) glycerol, 0.1 mM PMSF).

2.2. Apparatus

The UV–vis absorption spectra was recorded on a Cary 100 UV–vis spectrophotometer (Agilent, Singapore). Raman spectra analysis was performed on a Labram HR800 Laser Raman spectrophotometer (JobinYvon, France). Dynamic light scattering (DLS) was measured by Zetasizer Nano S/ZS (Malvern, UK).

2.3. Synthesis of H-GNs

Graphene oxide (GO) was synthesized from natural graphite by Hummers' method with minor modification (Zhang et al., 2012). H-GNs were prepared as follows: Briefly, the 20 mL as-prepared graphene oxide dispersion was mixed with 20 mL of 0.5 mg/mL hemin solution followed by vigorously shaking for several minutes. 200 μL ammonia solution and 30 μL hydrazine in sequence were added to the solution. After being stirred for 1 h, the mixture was heated to 60 °C for 24 h. Then, the dispersion rinsed with ultrapure water was centrifuged at 13000 rpm for 30 mins for several times to obtain H-GNs. The obtained H-GNs could be easily redispersed in water by ultrasonication.

2.4. Telomerase extraction

Briefly, HeLa cervical cancer cells, A549 lung cancer cells, MCF-7 breast cancer cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% fetal bovine serum (FBS), penicillin (100 μg/mL), and streptomycin (100 μg/mL) in 5% CO_2, 37 °C incubator. All kinds of cells were collected during the exponential phase of growth, and 1×10^6 cells were dispersed in a 1.5 mL EP tube, washed twice with ice-cold 1 × PBS by centrifugation at 1800 rpm for 5 mins. After discarding the supernatant carefully, the cells were resuspended in ice-cold lysis buffer. The cells were incubated for 30 mins on ice and then centrifuged for 20 min at 12000 rpm, 4 °C. Without disturbing the pellet, the cleared lysate was carefully collected and transferred to a fresh RNase-free tube, flash frozen, and stored at −80 °C before assay.

For extraction of telomerase from urine samples, 200 mL fresh urine were collected and centrifuged for 10 min (1 000 rpm 4 °C) and washed once using 1 × PBS. The above samples were centrifuged at 1800 rpm for 5 mins at 4 °C. The precipitate was resuspended in 2 mL of ice-cold lysis buffer and then incubated on ice for 30 mins. The mixture was centrifuged at 12000 rpm for 20 min at 4 °C. The supernatant was transferred, aliquoted, and stored at −80 °C before analysis.

2.5. Telomerization reaction

1 μL of the HeLa cell extracts were added to 12 μL telomerase extension reaction buffer containing 1 mM dNTPs, 1 μM telomerase substrate (TS) primer. The telomerization reaction was