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A sensitive assay of telomerase activity based on the controllable aggregation of quantum dots

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ABSTRACT

In order to overcome the shortcomings of traditional methods for telomerase activity detection of time-consuming and susceptibility to interferences in cell extracts, here, we develop a semiconductor quantum dots-streptavidin conjugates (QDs-SA)-based biosensor for sensitive detection of telomerase activity. In the absence of telomerase, the aggregation and thus the fluorescence quenching of QDs-SA can be observed in buffer solution due to charge shielding effect. In the presence of telomerase and biotin-dATP, QDs-SA will bind to telomerase elongation products due to the high affinity between the biotin and streptavidin, protecting the QDs-SA from aggregation and retaining their strong fluorescence. This sensor exhibited good fluorescence responses toward telomerase activity from HeLa cells in the range of 40–1000 cells with a detection limit of 13 cells or A549 cells within the linear range of 20–500 cells with a detection limit of 9 cells. Furthermore, the sensor can be used to efficiently monitor the evolution of telomerase activity in living cells, demonstrating great potential in tumor diagnosis and inhibitor drug screening.

1. Introduction

Telomere is a small fraction of DNA-protein compound located at the end of the linear chromosome of eukaryotic cells, which maintains chromosome integrity and controls the cell division cycle [1]. During cell proliferation, the length of telomere will be gradually shortened on account of chromosome replication, leading to cell ageing and apoptosis [2]. However, this shortened telomere can be supplemented by telomerase [3]. Telomerase as a complex of RNA and protein, is mainly composed of two parts: telomerase catalytic subunit (human telomerase reverse transciptase, hTERT) and telomere RNA (human telomerase RNA, hTR) [4–6], which can catalyze and supplement telomeric repeats TTAGGG onto the 3′ end of telomeres, preventing the shortening of the natural telomere and leading to immortalization of cells [7]. Currently, studies have shown that telomerase is highly expressed in over 85% cancer cells, but with low expression in normal somatic cells [8,9]. The close association between telomerase and human malignancies makes it one of the most common tumor molecular markers, and thus it is of great significance to measure telomerase activity in tumor cells [10].

Since telomerase was discovered in 1985 [11], a great deal of assays have been presented for telomerase activity determination. Among them, the most widespread method is the telomere repeat amplification protocol (TRAP) [12]. This method depends on polymerase chain reaction (PCR) process and hence it is susceptible to inhibition by defined interferences present in cell extracts, leading to false negative or positive results [13]. To overcome the above shortcomings, alternative nanoparticle-based approaches have been proposed, including fluorescence methods [14–17], electrochemical methods [18–20], colorimetric methods [21,22], chemiluminescence methods [23,24], surface enhanced Raman scattering (SERS) assays [25], and so on. However, most of the nanosensors suffer from difficult nanoprobe synthesis, complicated pretreating procedures and tedious probe immobilization. For instance, Jin's group [26] exploited a fluorescence method for measurement of telomerase activity based on FRET between FAM-labelled DNA and gold nanorods (GNRs), which could detect telomerase activity down to 1 HeLa cell. But the time-consuming synthesis of GNRs might hinder their practical applications. Zhang's group [14] developed a semiconductor quantum dots-based FRET nanosensor for telomerase activity detection with poor sensitivity without amplification, and the detection limit was high to 185 cells. Thus there is urgent need to establish a simple and sensitive fluorescent method for the telomerase determination.

Herein we construct a semiconductor quantum dots streptavidin conjugates (QDs-SA)-based biosensor for telomerase determination

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with high sensitivity and good selectivity. The detection mechanism is based on the biotin-labeled extended product of TS primer triggered by telomerase that can prevent the aggregation of QDs-SA *via* the special affinity between biotin and streptavidin. By employing the controllable aggregation of QDs-SA as well as their fluorescence evolution, simple and reliable quantification of telomerase activity can be realized. Meanwhile, it can be applied to fluorescence imaging of telomerase activity in living cells and screening anticancer drugs.

2. Experimental section

2.1. Materials and reagents

Quantum dots streptavidin conjugates (QDs-SA, 605 nm) were commercially obtained from Wuhan Jiayuan Quantum Dots Co. Ltd. (Wuhan, China). The telomerase primer DNA (5′-AATCCGTCGAGCAG AGTT-3′) and ACX primer (5′-GCGCGGCTTACCCTTACCCTTACCCTA ACC-3′) was purchased from Sangon Biological Engineering Technology Co. Ltd. (Shanghai, China). NEBuffer4 (50 mM KAc, 20 mM tris-HAc, 10 mM MgAc, 1 mM DTT, pH 7.9) was obtained from New England Biolabs (NEB). dGTP and dTTP were purchased from Invitrogen Corporation (California, U.S.A.). N6-(6-Amino) hexyl-2′deoxyadenosine-5′triphosphate-Biotin (biotin-7-dATP) was purchased from Jena Bioscience (Germany). The telomerase ELISA kit was obtained from Jiangsu Meimian industrial Co. Ltd. Epigallocatechin gallate (EGCG) and N,N1,3-phenylenebis-[2,3-dihydroxy-benzamide] (MST) were procured from Sigma-Aldrich (Shanghai, China). Bull serum albumin (BSA) was bought from BoAo. Co. Ltd. (Shanghai, China). All other chemicals were of analytical reagent grade. Ultrapure water ($\geq$18 MΩ.cm, Milli-Q, Millipore) was used to prepare to all aqueous solutions.

2.2. Apparatus

The fluorescence analysis was conducted on a Hitachi F-7000 spectrometer (Tokyo, Japan) furnished with a Xenon lamp. The UV–vis absorption spectrum was obtained by an UV-2450 spectrophotometer (Shimadzu, Japan). Gel electrophoresis images were obtained by using a Tanon-3500 digital gel imaging system (Tanon Science & Technology, China). Atomic force microscopy (AFM) images were mapped with the ScanAsyst mode of Bruker MultiMode-8 (Bruker, USA). The fluorescence images were obtained on a Zesis LSM 710 (Germany).

2.3. Cell culture and extraction of telomerase

HeLa and A549 cancer cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum, 100 U/mL penicillin and streptomycin. All the cells were maintained at 37 °C in humidified atmosphere (95% air and 5% CO_2), and telomerase was extracted according to the literature [27]. Firstly, HeLa cells and A549 cells were digested with trypsin for 5 min to remove the cells from the substrate and then transferred to a 1.5 mL centrifuge tube, washed twice with PBS (10 mM PB, 0.3 mM NaCl, pH 7.4), centrifuged at 2000 rpm for 2 min at 4 °C. Then the supernatant was discarded carefully, and the cells were suspended in 200 μL of ice-cold lysis buffer. After incubation for 30 min on ice, the mixture was then centrifuged at 12,000 rpm for 20 min at 4 °C, and finally the supernatant was transferred to a microcentrifuge tube and stored at −80 °C before analysis.

2.4. Telomerase extension reaction and detection by QDs-SA

1 μL of HeLa cell extracts (1000 cells.μL^{-1}) were mixed with 9 μL of telomerase elongation reaction solution containing 1 μL 2 μM TS primer, 1 μL NEBuffer4, 0.5 μL 1 mM biotin-7-dATP, 0.5 μL 1 mM dGTP, 0.5 μL 1 mM dTTP, and 5.5 μL ultrapure water. The above solution was then incubated at 37 °C for 1.5 h. For telomerase inhibition, different concentrations of MST or EGCG were added to immobilized concentration of 1000 HeLa cells at 37 °C for 90 min. For negative controls, cell extracts were heated at 95 °C for 10 min.

After the telomerase extension reaction, the reaction products were added to 60 μL H_2O, 30 μL 0.02 μM QDs-SA solution and incubated at 37 °C for 30 min. The fluorescence measurements were carried out with an excitation wavelength at 365 nm by scanning the spectra between 550 and 690 nm. The bandwidth for excitation and emission spectra was 5 nm.

2.5. Fluorescence imaging of telomerase activity in living cells and inhibitor-treated cells

A volume of 100 μL of HeLa cells were seeded in 900 μL culture medium in a confocal dish for 24 h, then washed twice with PBS, followed by supplementation of 921 μL fresh DMEM and 79 μL of the prepared QDs-SA probe which consists of 10 μL 2 μM TS, 4 μL NEBuffer4, 5 μL 1 mM biotin-dATP, 5 μL 1 mM dGTP, 5 μL 1 mM dTTP, and 50 μL 0.02 μM QDs-SA. After incubation at 37 °C for 2 h, the cells were imaged by confocal laser scanning microscopy (CLSM). In control groups, HeLa cells were incubated without TS primer or with common dNTPs instead of biotin-dATP. In order to monitor telomerase activity in inhibitor-treated living HeLa cells, the cells were pretreated with EGCG in confocal dishes for 24 h and then imaged by the QDs-SA probe.

3. Results and discussion

3.1. Assay principle

The principle of fluorescence detection of telomerase activity based on QDs-SA biosensor was schematically illustrated in Scheme 1. When QDs-SA was incubated with inactive telomerase, biotin-dATP, dGTP, and dTTP in salt buffer, the abundant cations led to charge shielding effect of negatively charged groups on QDs-SA surfaces [28], and subsequently the repulsion between QDs-SA was weakened accompanied by the formation of large aggregates as well as the fluorescence quenching of QDs-SA. With the addition of active telomerase, the repetitive sequences of TTAGGG will be added onto the 3′ end of the primer and then a long negative charged biotin-labeled single-stranded DNA (ssDNA) can be generated. The long ssDNA can be attached onto the surfaces of QDs-SA through the biotin-streptavidin interaction, increasing the negative charge of QDs-SA, enhancing the electrostatic repulsion between QDs-SA, improving the QDs-SA stability, and thus protecting the QDs-SA from aggregation.

First, the optical properties of QDs-SA were characterized by UV–vis absorption and fluorescence excitation and emission spectra. As shown in Fig. S1, the commercial QDs-SA show a strong fluorescence emission at 605 nm under the excitation of 365 nm and an obvious absorption peak at about 590 nm. Next, to demonstrate the feasibility of QDs-SA-based biosensor for the telomerase activity detection, we measured the fluorescence responses in the presence of cancer cell extracts and heat-inactivated extracts, respectively. As shown in Fig. 1, when telomerase extension reaction occurred, the QDs-SA maintained dispersing state with enhanced fluorescence signal (curve b). However, remarkable fluorescence quenching of QDs-SA can be observed in the absence of telomerase (curve c) or in the presence of inactive telomerase (curve d) due to the aggregation of QDs-SA in the buffer solution. The atomic force microscope (AFM) images confirmed the controllable aggregation of QDs-SA. As it described in Fig. 2, upon addition of telomerase, the QDs-SA were well dispersed in the salt buffer solution with a typical height of 3–4 nm (Fig. 2B) that was comparable to that of initial QDs-SA (Fig. 2A). Upon incubation with inactive telomerase, the aggregation of QDs-SA can be observed with an increased topographic height of 10 nm (Fig. 2C). These observations supported the proposal that the fluorescence quenching/recovery can be attributed to the controllable aggregation of QDs-SA.