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Research Paper

Quantum dots-Ru complex assembling dyads for cancer cell detection and cellular imaging based on hybridization chain reaction

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ABSTRACT

An easy and fluorescent strategy is developed for the detection of cancer cells and cellular imaging. This assay relies on the quenching of Ru complex toward quantum dots (QDs) and the amplification of hybridization chain reaction (HCR). In the presence of target cancer cells, folate-modified single-stranded DNA (F-DNA) links on the cell membranes through high affinity of folate and receptors. Hybridization reaction between pre-prepared HCR products and F-DNA immobilizes HCR products on the surfaces of target cells, resulting in large amount of Ru complex into HCR products and strong red fluorescence on the surfaces of target cells. Meanwhile, QDs-Ru complex assembling dyads in supernate are reduced, leading to the fluorescence restoration of QDs. Taking human cells lines HeLa as a model analyst, the linear response for HeLa cells in a concentration range from 100 to 10^5 cells mL^{-1} is obtained with a detection limit of 100 cells mL^{-1}. The specificity of this assay for the detection of cancer cells is demonstrated against several cell competitors. Additionally, this assay is successfully applied to inhibitor screening for cancer cells in combination with cellular imaging, demonstrating the university of this design.

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1. Introduction

Strategies that enable sensitive and accurate detection of cancer cell are highly desired as the importance in effective diagnosis and therapy [1–3]. In this regard, a variety of detection methods have been developed, such as electrochemiluminescence [4,5], colorimetric assay [6,7], fluorescence [8,9], mass spectrum [10,11] and microcantilever biosensor [12]. Wherein, fluorescence has attracted great attention because of the desirable simplicity, sensitivity and detectability caused by simple preparation and high signal-to-noise ratio [13,14]. However, conventional fluorescent materials usually have a low quantum yield and weak resistance to photobleaching, restricting their further application. Hence, it is in ever increasing demand to develop a desired fluorescence method which provides high sensitivity and accuracy yet requires simple process.

Quantum dots (QDs) as a promising fluorescence materials, have been widely used in various fields with high sensitivity due to the superior properties compared with conventional organic fluorescent dyes, such as high fluorescence quantum yields, tunable emission spectra, and excellent photostability [15–19]. In recent years, Ru complex ($Ru(bpy)_2(dppx)^{2+}$, bpy = 2,2′-bipyridine; dppx = 7,8-dimethyl-dipyrido[3,2-a:2′ 3′-c] phenazine) has been found that could effectively quench the fluorescence of QDs by electrostatic adsorption to form QDs-Ru complex assembling dyads. Moreover, Ru complex is typical DNA molecular "light switch", which can intercalate into double-stranded DNA (dsDNA) with high affinity, exhibiting strong and stable red luminescence, but almost nonemissive in dsDNA-free aqueous solution. Based on these unique properties, Zhao et al. first used QDs and Ru complex (QDs-Ru complex) as assembling dyads for dsDNA detection [20]. Zhang et al. further selected red fluorescence QDs to form QDs-Ru system for highly sensitive dsDNA detection based on single-color fluorescence switch [21]. Although QDs and Ru complex are label-free, low-cost and facile to obtain, the application of QDs-Ru complex assembling dyads is still restricted owing to the non-selection of Ru complex toward DNA and the difficulty in labeling between biomolecules and Ru complex. With the aid of magnetic separation, nuclease and other techniques, the application of QDs-Ru complex assembling dyads in the field of biosensor has been expanded [22,23]. However, the further exploration of QDs-Ru complex assembling dyads for cancer cell detection, especially with synchronous cellular imaging, has not been reported yet.

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To improve the sensitivity, many amplified methods have been introduced into fluorescence detection, including Exo III-assisted signal amplification [24,25], strand displacement amplification [26,27], rolling circle amplification [28,29], etc. But the involvement of enzyme greatly increases the complexity. Hybridization chain reaction (HCR) is proposed as a new amplification method and draws wide attention because of its enzyme-free, operation at mild conditions and DNA self-assembly to induce signal amplification [30–32]. In HCR, it products a comparable reduced pseudo-positive result, which is favorable to obtain low background. Moreover, each copy of the initiators can induce a HCR event, resulting in the linkage of many oligonucleotides, demonstrating great potential in signal amplification for target detection. These specific advantages make HCR that can be widely used in biomolecule sensing applications. Therefore, it is possible that the detection sensitivity of cancer cells could also be enhanced by combining HCR amplification with QDs-Ru complex assembling dyads.

Because folate receptors (FRs) are a kind of membrane protein and overexpress in many cancer cells including ovarian cancer, endometrial cancer and cervical cancer, etc, but they are rare or almost no found in normal cells [33]. Herein, a folate-linked single-stranded DNA (F-DNA), bound to the receptors (FRs) on the cancer cell membranes, is first designed and synthesized. Two single-stranded DNA (H1 and H2) are premixed to yield long dsDNA with numerous repeats as HCR products. Having high affinity to FRs, F-DNA is used as the effective bridge to quantitatively connected FRs-rich cancer cells, thus contributing to a novel and versatile fluorescent analysis platform. The introduction of cancer cells would trigger the mobilization of HCR products and capture more Ru complex to the surfaces of cells through the hybridization of F-DNA and HCR products. As a result, the formed QDs-Ru complex assembling dyads in supernate decrease that one biological binding event convert into the fluorescence quenching assay of Ru complex toward QDs. Combining with HCR, a novel, simple, highly sensitive fluorescent strategy for cancer cells detection and cellular imaging could be developed through QDs-Ru complex assembling dyads due to the intercalation and high quenching capability of Ru complex toward DNA and QDs, respectively.

Cell imaging assay demonstrates that the assembling dyads could also target cancer cells. Very high fluorescence from HCR-induced Ru complex emission is observed in FRs-positive cancer cells, while low fluorescence are found in FRs-negative HepG2 cells and normal cells (Hacat and Ges cells), which are used as the control. Furthermore, the inhibitor screening for cancer cells could be also realized by cellular imaging. Ru complex possesses good biocompatibility, as demonstrated by cell viability assay. QDs-Ru complex assembling dyads-based fluorescence method provides a new approach for its application in biological fields, such as cellular imaging and targeting of cancer cells. To our best knowledge, this proposed method is the first example that combined the use of QDs-Ru complex assembling dyads with HCR amplification to fabricate a sensitive fluorescent cancer cell sensor, which allows us to detect the target cells simply and rapidly, exhibiting a significant specificity for cancer cells.

2. Experimental section

2.1. Apparatus and materials

Fluorescence emission spectroscopy was performed on F-7000 fluorescence spectrophotometer (Hitachi, Japan). The luminescence intensity was monitored by exciting the sample at 390 nm and measuring the emission at 534 nm. The slits for excitation and emission were set at 10 and 10 nm, respectively. UV–vis absorption spectra data were performed on a DU800 Spectrophotometer (Beckman, Germany). Cell imaging was recorded on Olympus IX 71 inverted fluorescence microscope.

$Ru(bpy)_2(dppx)^{2+}$ complex was obtained according to previous reports [20]. The stock solution of Ru complex (10^{-4} M) was prepared in ultrapure water. Dulbecco's modified eagle media (DMEM), minimum essential medium (MEM), fetal bovine serum (FBS) and penicillin-streptomycin were purchased from Gibco (USA). 3-(4,5-dimethylthiahizol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and trypsin were obtained from Yeasen (Shanghai, China). HPLC-purified oligonucleotides with specific sequences were obtained from Sangon Biotechnology Co. Ltd. (Shanghai, China) and the sequences were as follows:

H1. 5′-TACTCCCCCAGGTGCCCCTCAGACCCTTTTAGT-3′

H2. 5′-GCACCTGGGGGAGTAACTAAAAGGGTCTGAGGG-3′

F-DNA:5′-ACTAAAAGGGTCTGAGGG-Folate-3′.

The buffer solution for HCR products formation contained 10 mM Tris and 500 mM NaCl with a pH of 7.4 regulated by HCl. All chemicals were of analytical grade or better and were used without further purification.

2.2. Preparation of CdTe:Zn^{2+} QDs

CdTe:Zn^{2+} QDs were prepared as follows according to reported method [34]: Firstly, 25 mg tellurium powder was reacted with 20 mg sodium borohydride in 1 mL ultrapure water to produce sodium hydrogen tellurium (NaHTe). By dissolving $CdCl_2 \cdot 2.5H_2O$, $ZnCl_2$ and NAC in deionized water, the precursor solution of Cd^{2+}-NAC and Zn^{2+}-NAC were obtained. The pH of solution was adjusted to 9.0 by dropwise addition of 1 M NaOH. Then the fresh NaHTe solution (400 μL) was injected into the above precursor solution with vigorous stirring under the protection of N_2. The molar ratio of Cd, Zn, Te, and NAC introduced was 1:2:0.2:3.6 in a total volume of 40 mL with 6.25 mM of Cd^{2+} concentration. Finally, the mixture was moved into a Teflon-lined stainless steel autoclave and the reaction was carried out at 200 °C for 40 min. The solution was cooled to the room temperature. The final product was obtained by ultrafiltration using an Amicon Ultra-4 centrifugal filter device with a MW 30 kDa (Millipore Corp) and kept at 4 °C for further experiments.

2.3. Preparation of HCR products

H1 (10 μL, 5 μM) and H2 (10 μL, 5 μM) were mixed with 80 μL Tris-HCl buffer at 90 °C for 10 min and then incubated for 2 h at room temperature. The HCR products were easily formed for further experiments.

2.4. Gel electrophoresis

H1 and H2 were heated to 95 °C for 15 min and cooled to room temperature for 1 h. Then the solution was mixed with fluorescent dyes (SYBR Green I) for incubation 10 min. The 3% agarose gel was prepared and run at 100 V for 30 min. The results were imaged by Gel Documentation Systems.

2.5. Cell culture

Four kinds of cancer cells (HeLa, HepG2, Ges-1, Hacat cells) were cultured in a humidified atmosphere (37 °C, 95% air and 5% CO_2). HeLa, Ges-1 and Hacat cells were cultured in DMEM supplemented with 10% FBS and 100 IU/mL penicillin-streptomycin. HepG2 cells were cultured in MEM supplemented with 10% FBS and 100 IU/mL penicillin-streptomycin. Cell density was determined using Bio-Rad automated cell counter prior to each experiment.

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