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High-throughput identification of telomere-binding ligands based on the fluorescence regulation of DNA-copper nanoparticles

Luzhu Yang, Yanjun Wang, Baoxin Li, Yan Jin*

Key Laboratory of Applied Surface and Colloid Chemistry, Ministry of Education, Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an 710062, China

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ABSTRACT

Formation of the G-quadruplex in the human telomeric DNA is an effective way to inhibit telomerase activity. Therefore, screening ligands of G-quadruplex has potential applications in the treatment of cancer by inhibit telomerase activity. Although several techniques have been explored for screening of telomeric G-quadruplexes ligands, high-throughput screening method for fast screening telomere-binding ligands from the large compound library is still urgently needed. Herein, a label-free fluorescence strategy has been proposed for high-throughput screening telomere-binding ligands by using DNA-copper nanoparticles (DNA-CuNPs) as a signal probe. In the absence of ligands, human telomeric DNA (GDNA) hybridized with its complementary DNA (cDNA) to form double stranded DNA (dsDNA) which can act as an efficient template for the formation of DNA-CuNPs, leading to the high fluorescence of DNA-CuNPs. In the presence of ligands, GDNA folded into G-quadruplex. Single-strdanded cDNA does not support the formation of DNA-CuNP, resulting in low fluorescence of DNA-CuNPs. Therefore, telomere-binding ligands can be high-throughput screened by monitoring the change in the fluorescence of DNA-CuNPs. Thirteen traditional chinese medicines were screened. Circular dichroism (CD) measurements demonstrated that the selected ligands could induce single-stranded telomeric DNA to form G-quadruplex. The telomere repeat amplification protocol (TRAP) assay demonstrated that the selected ligands can effectively inhibit telomerase activity. Therefore, it offers a cost-effective, label-free and reliable high-throughput way to identify G-quadruplex ligands, which holds great potential in discovering telomerase-targeted anticancer drugs.

1. Introduction

Human telomerase is a ribonucleoprotein reverse transcriptase that uses its integral RNA as a template to synthesize telomeric repeats sequence TTAGGG onto chromosome ends (Xiaojun et al., 2006). It is ensuring the maintenance of telomere length in tumor cells commensurate with successive rounds of cell division. Researches have shown that approximately 85–90% of all human cancers are positive for telomerase, whereas most normal cells appear to lack detectable levels of telomerase (Kim et al., 1994; Gerald et al., 2003; Holt et al., 1996). What's more, folding of telomeric DNA into G-quadruplex is one of the effective ways for inhibiting telomerase activity by G-quadruplex formation at the 3′ end of telomere DNA (Shay and Bacchetti, 1997; Shay and Wright, 2005; Pedroso et al., 2007a, 2007b; Stephen and Thurston, 2005; Zahler et al., 1991; Sun et al., 1997; Ken-Ichi et al., 2010; Fletcher et al., 1998). Therefore, screening ligands of G-quadruplex is important on account of quadruplex-binding ligands have potential applications in the treatment of cancer such as malignant gliomas (Pennarun et al., 2005) by inhibit telomerase activity.

Several methods have been established to study and screen ligands of human telomeric G-quadruplexes, including fluorescence resonance energy transfer (FRET)-based melting (Simonsson and SjÖBack, 1999; Koeppel et al., 2001), G4-fluorescent intercalator displacement (G4-FID) (Monchaud and Teulade-Fichou, 2010; Tran et al., 2011), nuclear magnetic resonance (NMR) spectroscopy (Zhou et al., 2009), isothermal titration calorimetry (ITC) (Trotta et al., 2011), electrospray ionization mass spectrometry (ESI-MS) assays (Pagano et al., 2012), surface plasmon resonance (SPR) (Pillet et al., 2011), affinity chromatography-based assay (Domenica et al., 2014), etc. Mergny's group (Mergny et al., 2001) used a fluorescence resonance energy transfer (FRET) between a donor (fluorescein) and an acceptor (tetramethylrhodamine) to judge the G-quadruplex-binding ligands. It uses double-labeled DNA probe, which is costly. To solve this problem, nanomaterials were employed as signal transfer element, such as gold nanoparticle (Yan et al., 2009), gold nanorod (Jin et al., 2011), graphene oxide (Haibo et al., 2012), etc. However, DNA probe was single-labeled with

* Corresponding author.
E-mail address: jinyan@snnu.edu.cn (Y. Jin).

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fluorescent dyes. Moreover, those methods did not perform high-throughput screening. Virtual screening methods have been employed. Castillo-González D et al. applied a virtual screening strategy based on the application of QSAR to screen telomeric G-quadruplex stabilizing ligands (Castillogonzález et al., 2015), but without a corresponding high-through experimental method to validate the predictions generated. So, a real sense of high-throughput method for screening telomeric G-quadruplex stabilizing ligands is still very rare. Shi et al. presented a fast method to realize high-throughput screening of G-quadruplex-binding ligands using carboxyfluorescein-labeled hairpin DNA as a molecular recognition probe (Shi et al., 2016). To ensure high specificity, hairpin DNA probe needs to be ingeniously designed. Therefore, it is significant to establish label-free and more universal strategy for high-throughput screening G-quadruplex-binding ligands from large chemical libraries, which can fast screen the potential ligands applied to the next step research.

Herein, a fluorescence regulation strategy was proposed for label-free and high-throughput screening G-quadruplex binding ligands by utilizing DNA-copper nanoparticles (DNA-CuNPs) as a signal probe. DNA-CuNPs based on double-stranded DNA (dsDNA) template exhibits facile synthesis and good repeatability (Zhengui et al., 2015), and has been applied in bioimaging (Das et al., 2015), biosensing (Zhihe et al., 2013; Junhua et al., 2012; Yan et al., 2006), and chemical sensing (Zhang et al., 2015) because of safer than other heavy-metal nanoparticles and quantum dots. The G-rich telomeric DNA (GDNA) can be induced to fold into G-quadruplex by ligands or hybridized with its complementary DNA (c-DNA) to form dsDNA. In the absence of ligands, GDNA hybridized with c-DNA to form dsDNA, leading to high fluorescence of DNA-CuNPs since most dsDNA can act as an efficient template for synthesis of DNA-CuNPs. In the presence of ligands, GDNA folded into G-quadruplex, resulting in low fluorescence of DNA-CuNPs because the c-DNA does not support the formation of DNA-CuNPs. By monitoring fluorescence change of DNA-CuNPs, telomere-binding ligands can be easily high-throughput screened. Moreover, the synthesis conditions of DNA-CuNPs are mild in room temperature, without vigorous agitation and dark treatment, resulting in good repeatability. To the best of our knowledge, our report represents the first example of using DNA-CuNPs as fluorescent probe to high-throughput screen human G-quadruplex binding ligands.

2. Experimental section

2.1. Chemicals

The DNA sequences (listed in Table S1) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Ascorbic acid (98%), Tris-(hydroxymethyl) aminomethane (Tris, 99.9%), fetal bovine serum and dimethyl sulphoxide (DMSO) were from Sigma-Aldrich Inc. Copper sulfate (98%) was obtained from J & K® (Beijing, China). Traditional chinese medicine monomer, such as matrine, colchicine, emodin, luteolin, apigenin, chrysin, chrysophanic acid, rhein, kaempferol, quercetin, aloe-emodin, were purchased from Nanjing TCM Institute of Chinese Material Medical (Nanjing, China). Genistein was synthesized by the group of professor Zhang (Pan et al., 2004). Aloe-emodin derivative 3 (AED3) was synthesized by own group (Zhao et al., 2013) and chemical structural formulas of all drugs are listed in Fig. S1. Human cervical carcinoma (HeLa) was purchased from KeyGen Biotech. Co. Ltd. (Nanjing, China). Dulbecco's modified Eagle's medium (DMEM), and phosphate buffer saline (PBS, pH 7.4) were purchased from HyClone Thermofisher (Beijing, China). Drugs and other reagents were of analytical reagent grade and used without further purification. The oligonucleotide stock solutions were prepared with 10 mM Tris-hydrochloric acid (Tris-HCl) buffer (pH 7.5). All aqueous solutions were freshly prepared in ultrapure water (=18.2 MΩ, Milli-Q, Millipore).

2.2. Instruments

All fluorescence measurements were performed on Hitachi F-7000 fluorescence spectrophotometer (Kyoto, Japan). Circular Dichroism spectras were measured on a Chirascan Circular Dichroism Spectrometer (Applied Photophysics Ltd, England, UK). Transmission electron microscopy (TEM) images were collected on a Field Transmittance Electron microscope (FEI, Hillsboro, America). The vertical electrophoresis system was purchased from Bio-Rad Laboratories, Inc. Cell number was determined using a Petroff-Hausser cell counter.

2.3. Preparation of DNA-copper nanoparticles

Briefly, the GDNA (3 μM) was first hybridized with 3 μM complementary DNA (c-DNA) in 10 mM Tris-HCl buffer (containing 1 mM $MgCl_2$, pH=7.5) for 10 min to form double strand DNA (dsDNA). Ascorbate acid (2 mM) was added, the solution was incubated for 10 min. $CuSO_4$ (300 μM) was introduced into the above solution to synthesize DNA-CuNPs. The fluorescence intensities of DNA-CuNPs were detected 20 min after $CuSO_4$ was added into the above solution. The all prepared samples of DNA-CuNPs were recorded at 340 nm/565 nm of the excitation/emission wavelengths. All process was operated at room temperature. CuNPs synthesized with other DNA sequences listed in Table S1 were synthesized by similar procedure. The morphology of DNA-CuNPs was characterized by transmission electron microscope.

2.4. Circular dichroism spectroscopy

Firstly, the GDNA was premixed with c-DNA6 for 10 min. Then, different concentrations of genistein were added for 15 min. The total volume of reaction is 200 μL. The sample concentrations of GDNA and c-DNA6 are 3 μM, respectively. The CD spectra were performed using an optical chamber (1 mm optical path length) in a response time of 2 s, an instrument scanning speed of 100 nm/min and were accumulated by taking the average of three scans made from 200 to 320 nm at room temperature.

2.5. High-throughput screening G-quadruplex-binding ligands

Firstly, the GDNA (3 μM) was premixed with 20 μM different drugs for 15 min. Then, 3 μM c-DNA was added for 10 min. Ascorbate acid (2 mM) and $CuSO_4$ (300 μM) were introduced similar to that mentioned above. The fluorescence was recorded in a 96-well microplate, which can undertake a variety of drugs at the same time.

2.6. Evaluation and validation of high throughput screening assay

The procedures were similar to that high throughput screening ligands of G-quadruplex. Briefly, after GDNA (3 μM) mixed with 20 μM genistein for 15 min, 3 μM c-DNA was added for 10 min. Ascorbate acid (2 mM) and $CuSO_4$ (300 μM) were introduced to the system. The fluorescence was recorded in a 96-well microplate.

2.7. Conventional telomere repeat amplification protocol assays

Hela cell culture, preparation of telomerase extracts were performed according to our previous work (Zhang et al., 2016). Firstly, a series of the selected ligands solutions were first added to the TRAP buffer. Telomerase extracts accounting to 500 Hela cells were used to accomplish telomerase extension reaction. Then, all telomerase extension reaction was performed just like the procedures described in previous work (Zhang et al., 2016). The PCR process and the gel electrophoresis were performed according to previous report (Zhang et al., 2016).