Journal of Pharmaceutical and Biomedical Analysis 152 (2018) 81–88

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

A novel BRCA1 gene deletion detection in human breast carcinoma MCF-7 cells through FRET between quantum dots and silver nanoclusters

Yasaman-Sadat Borghei[a], Morteza Hosseini[a,b,*], Mohammad Reza Ganjali[c,d], Saman Hosseinkhani[e]

[a] Department of Life Science Engineering, Faculty of New Sciences & Technologies, University of Tehran, Tehran, Iran
[b] Department of Pharmaceutical Biomaterials and Medical Biomaterials Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[c] Center of Excellence in Electrochemistry, Faculty of Chemistry, University of Tehran, Tehran, Iran
[d] Biosensor Research Center, Endocrinology & Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
[e] Department of Biochemistry, Tarbiat Modares University, Tehran, Iran

ARTICLE INFO

Article history:
Received 28 November 2017
Received in revised form
31 December 2017
Accepted 8 January 2018
Available online 9 January 2018

Keywords:
Deletion
Fluorescence
Nanobiosensor
Nanoclusters
Quantum dots
Mutations

ABSTRACT

BRCA1 (breast cancer 1) genomic deletions are the most important founder mutations in breast cancer patients and can be passed to you from your mother or father. Herein, we report a silver nanoclusters-based (AgNCs-based) fluorescence resonance energy transfer (FRET) method for detection of *BRCA1* gene deletion. The method relies on the specific hybridization of DNA-AgNCs fluorescent probe to deleted genes and interaction between double stranded DNA-AgNCs and QD, and the signal amplification through energy transfer from fluorescent AgNCs to QDs during FRET. Such fabricated QDs/DNA-AgNCs interaction might be beneficial for the nanomaterials based biosensing methods Under best possible conditions a linear correlation was established between the fluorescence intensity and the concentration of deletion sequence in the range of 5.0×10^{-13}–1.0×10^{-9} M with a detection limit of 1.2×10^{-13} M. Using this method, we could effectively determine gene deletions by using the nonamplified genomic DNAs that were extracted from the MCF-7 as a breast cancer cell line.

1. Introduction

Women with a first-degree female relative who've been an abnormal *BRCA1* or *BRCA2* gene with breast cancer have a higher risk of developing breast cancer [1]. The function of the *BRCA* genes is to produce Tumor Suppressor Gene (TSG) proteins and keep breast, ovarian, and other cells growing normally. But when *BRCA1* or *BRCA2* genes contain mutations, they don't function wild normally and the risk of different cancers increases. These abnormal two types of gene may account for up to 10% of all breast cancers and can be passed on to the next generation [2,3]. Families with a strong history of breast cancer can be occurred mostly by different large genomic mutations such as point mutations, both duplications and deletions of one or more exons and large genomic rearrangements in *BRCA1* [4]. Deleterious mutations in either the *BRCA1* or *BRCA2* genes such as the deletion of exons 21–22 removes at least 43 amino acids of the wild type *BRCA1* protein at codon 1770 or deletion of the whole exon 5 removes 26 amino acids from the regular protein [4].

As a result, methods for detecting the genetic mutations for common diseases, especially breast cancer that is inherited genetically is important. Traditional methods for detecting of gene deletion include sequencing [5], Southern blot analysis [6], capillary electrophoresis [7], polymerase chain reaction [8] and rolling circle amplification [9]. But, these methods require sample preparation protocols were often clumsy, time-consuming and required the use of expensive instruments and reagents.

Förster resonance energy transfer (FRET) is one of the most reliable techniques which is widely used in bimolecular detection to improve its efficiency and performance [10]. The application of colloidal semiconductor quantum dots (QDs) in FRET-based biosensing possess ideal properties for diagnostic, for example the fluorescence of QDs is highly sensitive to the states of their sur-

* Corresponding author at: Medical Biomaterials Research Center, Tehran University of Medical Sciences, Tehran, Iran.
E-mail address: smhosseini@khayam.ut.ac.ir (M. Hosseini).

https://doi.org/10.1016/j.jpba.2018.01.014
0731-7085/

face and physico-chemical interactions between surface QDs and analytes [11]. These QDs – analyte interactions show a vital role in the photoluminescence changes. Direct interaction between the QDs surfaces and analytes can be used to cause those changes, such as luminescence activation or quenching, which makes it possible to have a selective detection in complex mixtures [12]. In the previous studies a strong interaction between CdTe QDs and DNA has been reported. The chalcogenide QDs represented electrostatic interaction between metal centers Zn or Cd and the N or O site of single stranded DNA bases (with cytosine showing the strongest binding). On the other hand, the positively-charged sites (the metal ions) of chalcogenide QDs interact with accessible complimentary electronegative sites of double stranded DNA [13]. Noble metal nanoclusters (NCs) because of their unique optical properties and potential applications in biosensing, as a fluorescent nanomaterials (with ultra-small size (<2 nm)), have been reported as useful reporters [14]. Silver nanoclusters are bright and have easy synthesis procedures by using several "encapsulation agents" or "templates", such as DNA oligomers, amino acids, polymer and etc. [15–18].

In this paper, a novel FRET biosensor based on interaction between DNA and CdTe QDs were developed for gene deletion detection. Scheme 1 depicts the proposed sensing principle of this assay with two target oligonucleotides: wild-type and mutant. Upon the addition of wild-type target, the two oligonucleotide overhangs are separated and hybridization between ssDNA-AgNCs probe and wild type target doesn't occur, Hence fluorescent single strand DNA-AgNCs probe are adsorbed to the surfaces of QDs because of electrostatic interaction, so nanoclusters and QDs emissions quenched via silver atom (Ag°). On the other hand, upon addition of deletion target, the two oligonucleotide overhangs are closed and hybridization between ssDNA-AgNCs probe and deletion type target occur. Then incorporation of CdTe QDs to double stranded DNA causes the DNA-AgNCs and CdTe QDs to be in close proximity in the presence of deleted target and thus the CdTe QDs display a strong emission.

2. Experimental

2.1. Apparatus

All fluorescence evaluations were conducted using a Perkin Elmer LS-55 fluorescence spectrometer with a xenon lamp as excitation source with the spectral band widths of 10 nm for monochromators for both excitation and emission. (Buckinghamshire, UK). The size and morphology of nanoparticles were analyzed by transmission electron microscopy (TEM) (Zeiss, EM10C, 80 KV, Germany). UV–vis spectroscopy measurements were carried out by a Specord 250 spectrophotometer (Analytik Jena, Germany).

2.2. Materials and reagents

Fetal bovine serum (FBS), Dulbecco's modified eagle medium (DMEM), and penicillin/streptomycin were purchased from Gibco (USA). Silver nitrate ($AgNO_3$), $Cd(NO_3)_2$, tellurium powder, thioglycolic acid (TGA) and sodium borohydride (NaBH4) were bought from Merck. Cell Culture Lysis Reagent (CCLR), 5X was obtained from Promega and all other commercially available substances were bought from Aldrich, Merck and Across and used with no further purification steps.

Synthesis and purification of oligonucleotides were carried out by Pishgam Biotech Co (Tehran, Iran), and their sequences are listed in Table 1. All DNA samples were prepared in TE buffer (1 M Tris-HCl, 0.5 M EDTA). All other reagents were of analytical reagent grade and ultrapure water (Milli-Q plus, Millipore Inc., Bedford, MA) was used throughout the reactions.

Cells and cell culture human cell lines used in this study were MCF-7 cells (human breast cancer cell line) and HEK 293 cells (from normal human embryonic kidney cell line).

2.3. Cell culture

MCF-7 cells (cell line of human breast cancer) and HEK 293 cells (from normal human embryonic kidney) were cultured in 25 cm^2 tissue culture flasks (SPL, Korea) with 5 ml Dulbecco's modified Eagle's medium (Sigma, UK) containing 10% heat inactivated fetal bovine serum (Gibco), 100 U/ml penicillin (Sigma, UK). Cell lines were allowed to grow at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air for 5 days until the cell monolayer became confluent. Replacement of growth medium with fresh media was carried out every 2 days or as required, indicated by color change arising from production of lactic acid and CO_2, which leads to reduced pH. Cell washing with phosphate buffered saline (PBS) was conducted upon reaching at least 80% confluence, followed bytrypsinization for 10 min at 37 °C with 0.05% trypsin.

2.4. Total RNA& DNA extraction

Cell samples were disrupted and total RNA and genome DNA was extracted from MCF-7 cell line and HEK 293 cell line using Cell Culture Lysis Reagent (CCLR) (Scheme 2A). Approximately 2.0×10^6 cells collected by centrifugation at 3000 rpm for 5 min. Culture medium was carefully removed and the pellet was washed twice using PBS. Carefully removed the PBS and add 600 μl CCLR buffer. Then resuspended cells in CCLR buffer with a vortex and incubate for 20 min. Subsequently, 0.2 ml chloroform was added and vortex mixture 20 s violently. Next, the mixture was incubated on ice for 20 min and then centrifuged for 20 min at 13,000 rpm at 4 °C by refrigerated centrifugation. Following centrifugation, you should have three layers: top: aqueous phase: DNAs & RNAs, middle: debris and proteins, bottom: chloroform. And then transfer the upper aqueous phase (600 μl) to new microtube.

2.5. Probe design

In order to carry out the hybridization process, a probe with target DNA recognition sequence was designed to hybridize with a specific region (7 nucleotide upstream (+7) and 8 nucleotide downstream (−8) of the deletion area). Therefore, in the presence of deletion type, the two oligonucleotide overhangs are closed and hybridization between ssDNA-AgNCs probe and deletion type target occurs, leading to the formation of double stranded DNA. We have demonstrated a structural analysis of the DNA-AgNCs/DNA hybridization with *UNAfold* software (Scheme 2B). The *UNAFold* software predicts structure of nucleic acid folding using energy-based methods ($\Delta G = \Delta H - T\Delta S$) and dynamic programming which is a general computational technique [19].

2.6. DNA/AgNCs fluorescent probe preparation

Synthesis of ssDNA-AgNCs was carried out based on a previous study with a few minor modifications [16]. $AgNO_3$ solution (1 mM, 6 μl) was added to DNA solution (100 μM, 10 μl) prepared in phosphate buffer (20 mM, 100 μl, pH 7.0) to obtain an Ag^+-to-DNA molar ratio of 6:1. The mixture was incubated for 30 min in an ice bath and then reduced by introducing freshly prepared $NaBH_4$ (1 mM, 6 μl) under vigorous shaking. After that, the mixture was reacted for 1 h in an ice bath. The molar ratio of Ag-DNA-$NaBH_4$ in the solution was 6: 1: 6. The reaction mixture was kept at room temperature in the dark for 12 h before use. When the AgNCs were excited with a