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In situ labeling and imaging of cellular protein via a bi-functional anticancer aptamer and its fluorescent ligand

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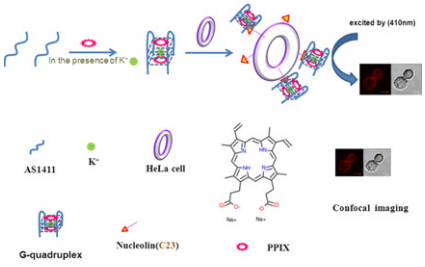
HIGHLIGHTS

- AS1411 bind protoporphyrin IX and enhances the fluorescence signal remarkably.
- According to LSCM experiment, HeLa cells were imagined by AS1411–PPIX.
- Aptamer-based bioimaging plays an important role for need not any covalent modification.

GRAPHICAL ABSTRACT

In this work, we report a novel approach to in situ labeling and imaging of a cellular protein nucleolin utilizing a multifunctional anticancer aptamer combined with its fluorescent ligand.



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ABSTRACT

In this article, we reported a novel approach for in situ labeling and imaging HeLa cancer cells utilizing a bifunctional aptamer (AS1411) and its fluorescent ligand, protoporphyrin IX (PPIX). In the presence of potassium ion, AS1411 folded to G-quadruplex structure, binded fluorescent ligand (PPIX) with fluorescent enhancement, and targeted the nucleolin overexpressed by cancer cells. Consequently, bioimaging of cancer cells specifically were realized by laser scanning confocal microscope. The bioimaging strategy with AS1411–PPIX complex was capable to distinguish HeLa cancer cells from normal cells unambiguously, and fluorescence imaging of cancer cells was also realized in human serum. Moreover, the bioimaging method was very facile, effective and need not any covalent modification. These results illustrated that the useful approach can provide a novel clue for bioimaging based on non-covalent bifunctional aptamer in clinic diagnosis.

1. Introduction

Protein imaging technologies have been attracting increasing attention for their significance in the fields of medicine, cancer therapies and bioanalysis [1–3]. Traditionally, fluorescent organics/nanoparticles and antibodies were widely used as signal indicators and recognition elements, respectively [4]. However, the organic dyes/nanoparticles were usually required to be covalently labeled and may be subject to possible photobleaching over time, which increased the complexity of experimental operation and displayed relatively higher chance to induce false signals. Meanwhile, though antibodies had high affinity to target specific tumor markers, the targeting process had limitations such as possible immune reactions, long detention time in the blood, and difficulties in yield [5]. Therefore, further effort was still required in searching for better alternatives to make the imaging simpler, more efficient, and more accurate.

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Study through the past two decades has proven that an aptamer, a single strand DNA/RNA in vitro selected for a specific target, had special advantages such as immunogenicity-free, smaller size, penetrating tumors better and easy synthesis. These advantages made the aptamer to be one of the promising alternatives to antibody in molecular detection [6] and other biomedical applications [7]. Recently, aptamer-based cancer cell recognition or imaging has also been realized [8]. For example, an aptamer-based Ag nanoparticle probe demonstrated the applications of protein imaging and single nanoparticle spectral analysis in cells [8–11]. Huang et al. synthesized dye-labeled aptamer-modified Au–Ag nanorod for efficient cancer cell targeting [12]. Even though, most of these methods still cannot avoid the process of covalently labeling the fluorescent signal probes onto aptamers.

In this article, in order to solve the above limitations and provide more choices for further cellular recognition and imaging, an aptamer-based method was developed. Human nucleolin, a significant cancer marker abundantly expressed on the surfaces of several kinds of tumor cells [13–16], was employed as a model target to prove the principle. The method was based on the recent exciting finding that the dimeric G-quadruplex anti-nucleolin aptamer (AS1411) had the other tightly binding site for protoporphyrin IX disodium salt (PPIX, $C_{34}H_{34}N_4O_4Na_2$) [17]. And upon binding, the fluorescent emission of PPIX at 634 nm can be significantly enhanced. This phenomenon allowed us to use AS1411 as a bifunctional aptamer serving as both signal probe and recognition elements in bioimaging. Briefly, the AS1411 was firstly incubated with PPIX to form AS1411–PPIX complexes, which could target the nucleolin molecules expressed at the surface of HeLa cells, providing a bright and stable fluorescent image under LEICA TCS SP2 laser scanning confocal microscope (LSCM) and flow cytometer (FCM). Besides, fluorescent binding assay and circular dichroism spectrometer (CD) were used as assistant techniques to investigate some basic principles of AS1411–PPIX binding. This bioimaging method was finally demonstrated to be robust and with high sensitivity, with detection limit of nucleolin expressed on 10,000 HeLa cells. Therefore, it is a promising cancer diagnostic method which motivates further refinement in the future.

2. Experimental details

2.1. Chemicals and materials

The oligonucleotides, AS1411 (5′-d(GGTGGTGGTGGTTGTGGTGGTGGTGG)-3′), was synthesized and HPLC purified by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Tris (hydroxymethyl) aminomethane (Tris) were obtained from Aladdin Chemistry Co. Ltd (USA). Other reagents and chemicals were at least analytical reagent grade. The ordered oligonucleotide was dissolved with small volume of the Tris–HCl buffer (10 mM Tris–HCl, 0.1 mM EDTA, pH 7.4) as stock solution, and quantified using a UV/Vis/near IR spectrophotometer (Varian, America) by determining the 260 nm UV absorbance and with the following extinction coefficients: A = 15,400, C = 7400, G = 11,500, T = 8700. Before using, the stock solution was diluted with Tris–HCl buffer (20 mM Tris–HCl, 140 mM NaCl, 100 mM KCl, pH = 7.4), heated at 90 °C for 10 min, and gradually cooled to room temperature in water bath. Including oligonucleotide, Tris–HCl buffer, all the reagents and buffers were prepared using distilled water and stored at 4 °C before use. PPIX was purchased from Aldrich (Steinheim, Germany). HeLa cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained in DMEM supplemented with 10% standard FBS (HyClone Laboratories, UT) at 37 °C and in 5% CO_2. Glass chamber slides (14 mm bottom well) were purchased from Hangzhou Sanyou Biotechnology Co. Ltd. (Hangzhou, China).

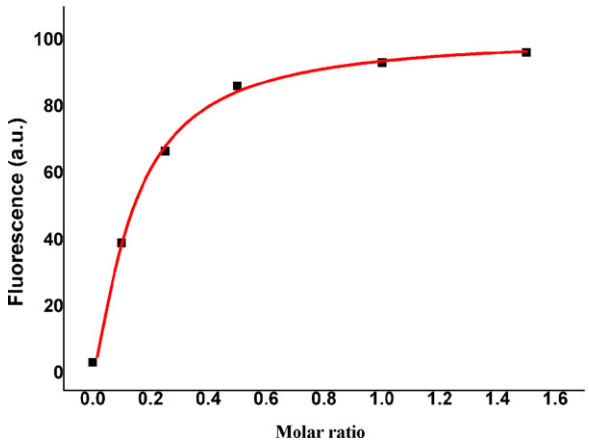

Fig. 1. Stoichiometry (n) of AS1411–PPIX at different molar ratio of AS1411 and PPIX, 1.0 μM of PPIX and AS1411 of increasing concentration of 0, 0.1, 0.25, 0.5, 1.0, 1.5 and 20 μM in 50 mM Tris–HCl buffer (pH = 7.4).

2.2. Instrumentations

UV/Vis absorption spectra were carried out by a CARY 500 UV/Vis/near-IR spectrophotometer (Varian). Fluorescence spectra were recorded at room temperature using a Perkin-Elmer LS 55 luminescence spectrometer (America). CD measurements were performed on a JASCO-820 Circular Dichroism spectrometer (Tokyo, Japan). The sample for cell imaging was prepared by mixing with in 35 mm tissue culture dish (World Precision Instruments) and acquired using LEICA TCS SP2 laser scanning confocal microscope (LSCM) (Germany) with a 100× oil immersion objective. Fluorescence lifetime (FL) delay was gotten from LK1-005 (GL-302 Dye laser, GL-3300 Nitrogen laser, GL-303 Frequency Doubler) (Photon Technology International). Flow cytometry analysis was operated on a FACSAria analyzer (Becton Dickinson Immunochemical Systems, Mountain View, CA). In each analysis, about 10,000 cells were examined.

2.3. Assaying process

2.3.1. Preparation of PPIX–AS1411 complex

As mentioned above, AS1411 oligonucleotides in Tris–HCl buffer were annealed to form stable G-quadruplex structure, mixed with PPIX of different concentration and incubated for 1 h. The fluorescence signal of the prepared AS1411–PPIX solution was measured. A series of CD spectra of AS1411–PPIX complex were gotten from temperature interval measurement, and analyzed by the spectra analysis program. The experimental conditions were as follows: CD measurements from 210 to 350 nm were taken, the data pitch was 0.1 nm, scan speed was 200 nm min^{-1}, response time was 0.5 s, and bandwidth was 1 nm. End temperature was 90 °C and start temperature was 20 °C; temperature interval was 5 °C. The CD spectrum of DNA sample was determined using first derivative analysis by the data-processing software of Origin 8.0.

2.3.2. HeLa Cells incubated with AS1411–PPIX complex

The HeLa cells were plated onto 35 mm glass chamber slides. AS1411–PPIX complex solutions dissolved in Tris–HCl buffer were prepared at concentrations of 5 μM. Diluted solutions in complete growth medium were then freshly prepared and placed over the cells for 1 h. All cells were washed with phosphate buffered saline (abbreviated as PBS) for three times at room temperature. After that, cells imaging was scanned by LSCM.