Talanta 179 (2018) 501–506

Contents lists available at ScienceDirect

Talanta

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

Ultrasensitive and high specific detection of non-small-cell lung cancer cells in human serum and clinical pleural effusion by aptamer-based fluorescence spectroscopy

Yuhang Wu[a,b], Hong Zhang[a,b,*], Junfeng Xiang[a,b], Zhihua Mao[c], Gang Shen[a,b], Fengmin Yang[a,b], Yan Liu[a], Wei Wang[d], Ning Du[d], Jinghua Zhang[d], Yalin Tang[a,b]

[a] National Laboratory for Molecular Sciences, Center for Molecular Sciences, State Key Laboratory for Structural Chemistry of Unstable and Stable Species, Institute of Chemistry Chinese Academy of Sciences, Beijing 100190, PR China
[b] University of Chinese Academy of Sciences, Beijing 100049, PR China
[c] The 307th Hospital of Chinese People's Liberation Army, Beijing 100071, PR China
[d] Beijing Centre for Physical and Chemical Analysis, Beijing 100089, PR China

ARTICLE INFO

Keywords:
Aptamer
Non-small-cell lung cancer
Human serum
Clinical pleural effusion

ABSTRACT

Lung cancer is the leading cause of cancer-related deaths worldwide, and approximately 85% are diagnosed as non-small-cell lung cancer (NSCLC). However, efficient detection and diagnosis of NSCLC at early stage is still challenging. In this work, we developed a simple, ultrasensitive and high selective strategy for A549 human NSCLC cells detection based on combining the reorganization property of a novel cyanine dye 3,3′-di(3-sulfopropyl)-4,5,4′,5′-dibenzo-9-methyl-thiacarbocyanine triethylammonium salt (**cy-M**) to aptamer **S6** G-quadruplex structure having specific affinity to NSCLC cells, which induced a dramatic fluorescence enhancement (~ 10^4 times). Moreover, this strategy was successfully used for detecting of A549 cancer cells in complex media such as human serum and clinical pleural effusion, which strong indicated that the proposed method could be applied for the early diagnosis of lung cancer.

1. Introduction

Lung cancer is presently the most commonly diagnosed metastatic tumor and a leading cause of cancer-related deaths worldwide [1]. The most aggressive form is non-small-cell lung cancer (NSCLC) which is common type and account for about 85% of all lung cancer cases [2]. Patients with this invasive cancer have a poor prognosis and a five year survival rate less than 20%. Low survival is largely attributed to late-stage diagnoses. If NSCLC is recognized and diagnosed in the early stages, the treatment and the five year survival rate increases to over 60% [3]. Therefore, a highly efficient strategy for early diagnosis of NSCLC is of extreme significance and urgent need in clinical cancer diagnostics and therapy. Up to now, several conventional methods have been explored for the detection of NSCLC and cancer diagnostics [4]. However, most of these methods are not only costly, time-consuming and labor-intensive, but also require sophisticated instruments, hence they may be not suited for the sensitive detection and diagnosis of the NSCLC, especially at the early stage. Thus, the development of highly sensitive, selective, simple, reliable and cost-effective protocols for accurate detection of NSCLC cells is of paramount importance.

Molecular probes with high sensitivity and specificity for tumor cells have emerged as important tools in the field of cell detection. Aptamers are single-stranded DNA or RNA sequences artificially selected through systematic evolution of ligands by exponential enrichment (SELEX), and possess high affinity and binding specificity for their target molecules [5]. In recent years, aptamers have gained increasing popularity in establishing aptasensors for tumor cell detection [6]. Many of these aptasensors have been applied successfully for tumor cells analysis with several sensitive methods, such as colorimetry [7], electrochemistry [8], fluorescence [9], and some signal amplification strategies, such as quantum dots (QDs) [10] and gold nanoparticles (AuNPs) [11] which improved the sensitivity of these aptamer-based approaches in cancer analysis. However, most reported cancer cell detection strategies involved a sophisticated design of aptamer molecules to provide a detectable signal. This process is time-consuming and labor-intensive. What's more, the complicated probes may decrease the performance of the aptamers. Therefore, developing a robust and sensitive cancer cell detection strategy will be more encouraging.

* Corresponding author at: National Laboratory for Molecular Sciences, Center for Molecular Sciences, State Key Laboratory for Structural Chemistry of Unstable and Stable Species, Institute of Chemistry Chinese Academy of Sciences, Beijing 100190, PR China.
E-mail addresses: zhanghong@iccas.ac.cn (H. Zhang), tangyl@iccas.ac.cn (Y. Tang).

https://doi.org/10.1016/j.talanta.2017.11.029
Received 5 September 2017; Received in revised form 14 November 2017; Accepted 16 November 2017
Available online 20 November 2017
0039-9140/

Aptamer S6 has been developed for NSCLC by using cell-based SELEX method. S6 is a 45-mer single stranded DNA with the sequence 5′-GTGGCCAGTCACTCAATTGGGTGTAGGGGATTGTGGGTTG-3′ with selective binding to NSCLC cells [12]. Although the NSCLC aptamer have been reported, the detection of NSCLC by using this aptamer (S6) has not been reported yet. In this work, we have developed a novel aptasensors for the sensitive and selective detection of living A549 human adenocarcinoma cells, the typical and most common subtype of NSCLC.

Supramolecular assembly of a cyanine dyes have been used extensively for decades as fluorescent probes for detecting some biomacromolecules. Since the recognition signature comes from the distinct spectral properties of supramolecular arrangements, the spectral changes can be more visible and stronger than those from different states of the same molecule [13]. Herein, a novel, simple, ultrasensitive and high selective strategy for A549 human NSCLC cells detection strategy is proposed based on combining the reorganization property of supramolecule **cy-M** (3,3′-di(3-sulfopropyl)-4,5,4′,5′-dibenzo-9-methylthiacarbocyanine triethylammonium salt) to aptamer **S6** and the specific affinity of **S6** to NSCLC cells. Compared with other publications on sensing of cancer cells (as summaried in Supporting information Table S1), the present work has some unique features: (1) it does not require labeling or modification of oligonucleotides, it uses supermolecule **cy-M** for signal readout. (2) It was highly sensitive for A549 cells with limit of detection down to 8 cells/mL, which is relatively much lower than other fluorescence assays for cancer cells. (3) It was successfully applied for the detection of living A549 cancer cells in complex media such as human serum and clinical pleural effusion, with potential application in clinical analysis.

2. Experimental

2.1. Circular dichroism (CD)spectroscopy

CD spectra were recorded on a JASCO J-815 spectrophotometer equipped with a temperature-controlled circulator with an average of three scans. CD measurements were carried out in the wave length range of 200–400 nm with a response time of 0.5 s, a step size of 1 nm and a 2 nm bandwidth. The scanning speed was set at 1000 nm/min. A 10 mm path length quartz cuvette was used in all experiments. The final concentration of **S6** was 5 μM. The measured sample was prepared by mixing a quantity of **cy-M** solution with **S6** DNA solution, and then being diluted by corresponding buffer solution. The samples were kept in darkness overnight at 4 °C before measurement. CD melting experimental detail was shown in supporting information.

2.2. Absorption spectroscopy

Absorption spectra were acquired with UV-1601PC at room temperature using a quartz cuvette with a path length of 10 mm. Absorption titration experiments were performed by increasing **S6** DNA concentrations from 0 to 35 μM, with the **cy-M** concentration fixed at 5 μM.

2.3. Fluorescence spectroscopy

Fluorescence spectra were acquired with a Hitachi F-4500 spectrophotometer equipped with a temperature-controlled circulator. A 10 mm path length quartz cuvette was used in all experiments. The excitation wavelength was 570 nm. For fluorescence measurements, both excitation and emission slits were 5 nm, and the scan speed was set at 1200 nm/min. **cy-M** was titrated with **S6** DNA for measurement of the binding constant, with the fluorescence intensity at 605 nm plotted as a function of the DNA concentration. The titration experiment was performed by increasing **S6** DNA concentration from 0 to 35 μM, with the **cy-M** concentration fixed at 5 μM.

2.4. Cells incubation and imaging

Seven established cell lines were used in this experiment: A549, NCI-H446, Hela, MCF-7, SMMC-7721, MRC5, HUVEC. All of above cell lines were obtained from Cancer Research Institute of Beijing, China. They were cultured in Dulbecco's Modified Eagle's Medium that contains 5% fetal bovine serum, streptomycin (0.1 mg/mL) and penicillin (100 U/mL), under 5% carbon dioxide at 37 °C. The cells were washed thrice with sterile buffer solution and observed under a confocal laser scanning microscope (CLSM) (OLYMPUS FV1000-IX81) equipped with an oil immersion 100X objective lens. CLSM images of **cy-M** were collected under excitation of 559 nm. To validate the location of the aptasensor in the cells, additional experiments were carried out making A549 cells incubated with **S6** modified with Cy3 (2 μM) for 0.5 h.

2.5. Flow cytometry assays

FACScan flow cytometry (FACScalibur, BD Bioscience) was used to demonstrate the targeting capabilities of our strategy toward cells and quantitatively detect target cells. About 10^6 of pure cells or mixed cells were obtained from the culture medium and incubated with the aptasensor (35 μM **S6**, 5 μM **cy-M)** for 40 min at 37 °C then for another 10 min at 4 °C in darkness. After incubation, the cells were centrifuged at 1000 rpm for 5 min to separate from the binding buffer then washed once with sterile buffer solution. The cell sediment was re-suspended in the buffer solution. The final volume of the mixture was 500 μL and placed in individual test tubes. The mean fluorescence was determined by counting 10,000 events. The signal was monitored in channel 3.

2.6. Human serum and clinical pleural effusion cell preparation

Samples with A549 cells ranging from 0 to 3×10^5 were used for the quantitative assay in the 5-fold-diluted healthy human serum. The A549 cells in human serum were derived from the standard curve and the regression equation. The test was performed by using the standard addition method. Fresh pleural effusion of patients with diagnosed NSCLC, other cancer and no cancer (including breast cancer, pneumonia and etc.) collected from 307th Hospital of PLA were centrifuged to remove impurity and resuspended in buffer solution for analyzing. All experimental protocols were approved by the ethics committee of 307th Hospital of PLA. All informed consents of the samples were signed by the patients or their family.

2.7. Statistical analysis

The SPSS software for Windows Release 17.0 was used. All values in the text and figures are presented as mean ± SD. Values of $p < 0.05$ were taken to show a significant difference between means.

3. Results and discussion

Fig. 1A shows the mechanism of the aptamer strategy for sensing cancer cells. Initially, for the first time, we found that the aptamer **S6** folds into a stable parallel-stranded G-quadruplex structure under physiological conditions with 140 mM K^+, 10 mM Na^+ and 2 mM Mg^{2+}. As shown in Fig. 1B, the CD spectrum of **S6** exhibits a characteristic positive peak at 263 nm and a negative peak at 240 nm, corresponding to special feature of a parallel G-quadruplex structure. To further investigate the stability of **S6** G-quadruplex structure, the T_m value was determined to 75 °C using CD melting curves at 263 nm (Fig. S1) at 5 μM G-quadruplex concentration.

Moreover, in order to demonstrate the feasibility of using **cy-M** as a novel probe for NSCLC detection, we firstly evaluated the interaction between **S6** G-quadruplex and **cy-M**. Our previous work revealed that the cyanine dyes supermolecules selectively recognized special G-quadruplex structure and showed drastic changes in spectroscopy [14].