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Short communication

Protein microarrays and quantum dot probes for early cancer detection

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Abstract

We describe here a novel approach for detection of cancer markers using quantum dot protein microarrays. Both relatively new technologies; quantum dots and protein microarrays, offer very unique features that together allow detection of cancer markers in biological specimens (serum, plasma, body fluids) at pg/ml concentration. Quantum dots offer remarkable photostability and brightness. They do not exhibit photobleaching common to organic fluorophores. Moreover, the high emission amplitude for QDs results in a marked improvement in the signal to noise ratio of the final image. Protein microarrays allow highly parallel quantitation of specific proteins in a rapid, low-cost and low sample volume format. Furthermore the multiplexed assay enables detection of many proteins at once in one sample, making it a powerful tool for biomarker analysis and early cancer diagnostics.

In a series of multiplexing experiments we investigated ability of the platform to detect six different cytokines in protein solution. We were able to detect TNF-α, IL-8, IL-6, MIP-1β, IL-13 and IL-1β down to picomolar concentration, demonstrating high sensitivity of the investigated detection system.

We have also constructed and investigated two different models of quantum dot probes. One by conjugation of nanocrystals to antibody specific to the selected marker—IL-10, and the second by use of streptavidin coated quantum dots and biotinylated detector antibody. Comparison of those two models showed better performance of streptavidin QD–biotinylated detector antibody model. Data quantitated using custom designed computer program (CDAS) show that proposed methodology allows monitoring of changes in biomarker concentration in physiological range.

Keywords: Quantum dots; Protein microarrays; Biomarkers; Cytokines; Cancer detection

1. Introduction

According to American Cancer Society, lung cancer continues to be the most lethal malignancy, accounting for an estimated 160,000 deaths per year in the United States [1]. In 2007 lung cancer will account for 30% of all cancer death in the U.S. Although there is an ongoing research in many medical fields to improve patients' outcome, early detection seems to be the key for cancer survival.

It is believed that availability of multiple biomarkers is extremely important in the diagnosis of complex diseases like cancer [2,3], and tests for single markers like for example CA125 for ovarian cancer are not adequate. Unfortunately, only 80% of patients have elevated levels of CA125 [4]. The situation is even worse for patients with early stage disease, where less than 50% of cases have elevated levels [5]. Patterns of multiple cancer markers might provide sufficient information of disease diagnosis in its early stages [3]. However, ability to use this approach in disease diagnosis is dependent on the technology that will allow healthcare professionals for multiplexed and fast detection of many biomarkers simultaneously with high sensitivity and specificity. We believe that proposed platform of quantum dot protein microarrays offers unique features making it possible to fulfill the task.

1.1. Quantum dots

Quantum dots are semiconductor nanocrystals structures widely used in bioimaging applications. They can be made from a variety of inorganic compounds dependent upon their mode of

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application. QDs usually consist of <10-nm CdSe semiconductor core surrounded by an inorganic shell composed of ZnS. The core-shell complex is coated with a polymer to make the particle water soluble, followed by functionalization with, for example, streptavidin to prepare QDs for use in immunochemistry. The final size of immunochemically functional QDs is ca. 10–15 nm, which is in size range of macromolecules [6,7].

QDs are characterized by broad absorption band and narrow symmetric emission band. Importantly for use as biological probes, QDs absorb and emit light through wide spectrum of wavelengths, from visible to NIR [8], and can be excited with any wavelength from UV to red [9,10]. In comparison to organic fluorophores, QDs absorb excitation photons in wider spectral range and emit photons more efficiently due to their higher quantum yield which is an extremely important feature for sensitive fluorescence imaging [8,10–12]. Quantum dots are resistant to photobleaching and approximately thousand times more photostable than organic dyes. They also exhibit much longer life time [10,13–15] than organic dyes. High signal to noise ratio results in good contrast images, allowing easy separation of QDs from background fluorescence.

Quantum dots have large Stokes shift values (∼300–400 nm, depending on the excitation wavelength), which leads to improved sensitivity of detection [10,16].

Ability of being excited by single source makes QDs really unique label for multiplexed experiment, where different species of QDs can simultaneously track various biomarkers and unlike organic dyes, be excited with the same source [17]. Bioconjugated quantum dot probes have demonstrated their potential to be useful for cancer diagnosis through diverse approaches, like, e.g., in vitro diagnostic assays (protein biomarker detection, nucleic acid biomarker detection, high-throughput multiplexing); cellular labeling (fixed cells and tissues, live cell imaging) and in vivo imaging (vascular imaging, lymph node tracking, tumor targeting and imaging) [8].

1.2. Protein microarrays

Mark Schena—author of the first paper demonstrating usefulness of microarrays [18], describes them as analytical devices that possess four distinct characteristics: (a) microscopic target elements or spots, (b) planar substrates, (c) rows and columns of elements, and (d) specific binding between microarray target elements on the substrate and probe molecules in the solution [19]. It is a miniaturized assay where each spot contains "bait" molecules (antibody in protein/antibody microarrays), which are probed with unknown biologic sample containing analytes of interest [20]. By processing the microarrays with detector antibody tagged with fluorescent label, each spot produces fluorescent signal proportional to the analyte of interest present in the solution and captured/bound to the "bait" molecule [21].

Applications of functional protein microarrays include: expression profiling for identification and quantitation of proteins present in the solution; protein-protein interactions for examination of binding activity and binding partners of proteins across entire proteome; drugs for identification of drugs activity, targets, cross-reactivity, and diagnostics to measure proteins expressed in serum samples [19].

Production of microarrays consists of the following steps: printing and immobilization of capture antibodies on a functionalized surface (usually glass slide covered with poly-L-lysine, aldehyde, epoxy or nitrocellulose) [21]; incubation with the sample, detection with fluorescent probe, image capture and analysis. The most sensitive method for protein microarrays processing is the "sandwich assay" based on Elisa technique. It utilizes two antibodies that simultaneously bind to the same antigen: "capture" immobilized onto the surface and "detector" fluorescently labeled, producing a fluorescent signal.

Intensity of the fluorescent signal is the representation of biomarker concentration in the solution.

Bridging together unique features of quantum dots and protein microarrays can lead to design of very sensitive, robust and feasible assay for early detection of cancer. Massive multiplexing capabilities of quantum dots for detection of many cancer biomarkers simultaneously, their exceptional brightness and stability together with biochip approach of protein microarrays, hold tremendous promise for unraveling complex gene expression profiles of cancers and for accurate early clinical diagnosis [8].

2. Materials and methods

2.1. Probe evaluation

To choose the best QD probe, we constructed and evaluated two models. One by conjugation of nanocrystals to antibody specific to the selected marker—IL-10, and the second by use of streptavidin coated quantum dots and biotinylated detector antibody. In our evaluation experiments we chose only one QD size (655); however, for multiplexing purposes it is possible to use also other sizes. Monoclonal capture (clone JES3-9D7) rat anti-human interleukin-10 antibody, detector (clone JES3-12G8) rat anti-human interleukin-10 antibody, and recombinant human interleukin-10 were purchased form Serotec (Raleigh, NC). Microarray nitrocellulose coated glass slides were purchased from Whatman (Sanford, ME) and Quantum Dot 655 antibody conjugation kit was purchased from Invitrogen (Carlsbad, California). The conjugation of detector rat anti-IL-10 antibody to QD655 was performed according to the protocol. "Probe evaluation" array was spotted with BioRobotics MicroGrid microarrayer from Genomic Solutions (Ann Arbor, MI) using Stealth printing pins (TeleChem International, Sunnyvale, CA). Six arrays of capture rat anti-IL-10 antibody at 0.5 mg/ml (1:2 dilution in printing buffer (TeleChem International, Sunnyvale, CA)) were printed on nitrocellulose coated glass slides. After spotting the slides were placed in a box at 4 °C overnight. The next morning slides were rinsed with PBS (Sigma–Aldrich, Germany) and blocked (Whatman; Sanford, ME) for 1 h. After rinsing again with PBS slides were incubated for 2 h with human IL-10 solutions in PBS at 500 ng/ml (two arrays), 1000 pg/ml (two arrays) and 100 pg/ml (two arrays) and then rinsed again with PBS. Half of the arrays was incubated with detector rat anti-IL-10 antibody conjugated to QD655 (20 nM) and the remaining