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A biolayer interferometry-based enzyme-linked aptamer sorbent assay for real-time and highly sensitive detection of PDGF-BB

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ABSTRACT

Accurate, fast and sensitive detection of disease-specific protein biomarkers, especially in blood, urine, or other bodily fluids, is an important approach to achieve early disease diagnosis. Platelet-derived growth factor-BB (PDGF-BB), a widely used biomarker, is involved in a substantial number of serious diseases, such as hepatic fibrosis, atherosclerosis, age-related macular degeneration and diabetic eye disease and is often over-expressed in human malignant tumors. Therefore, the development of sensitive and specific detection methods for PDGF-BB is of great importance for the early diagnosis of disease and assessments of patient recovery. In the current study, a biolayer interferometry-based enzyme-linked aptamer sorbent assay (BLI-ELASA) was successfully established for rapid (20–25 min), high-throughput (8 or 16 samples) and real-time monitoring of PDGF-BB in clinical samples. The method exhibited a broad detection range from 0.5 to 1000 ng/mL of PDGF-BB (good linear range from 0.5 to 10 ng/mL), with a low detection limit of 0.08 ng/mL. Moreover, BLI-ELASA was applied to the detection of PDGF-BB in spiked serum and urine samples and showed a high degree of selectivity for PDGF-BB, good reproducibility, and stability. We believe that the methodology in this work can be easily adapted to detect other biomolecules in clinical samples, including viruses, pathogens and toxins, in a rapid, sensitive, high-throughput and real-time manner.

1. Introduction

Accurate, fast and sensitive detection of disease-specific protein biomarkers, especially in blood, urine, or other bodily fluids, is an important approach to achieve early disease diagnosis (Nimse et al., 2016). Platelet-derived growth factor-BB (PDGF-BB) is an important cytokine in serum which regulates cell growth and division (Goldring and Goldring, 1991). As a widely used biomarker, it is often over-expressed in human malignant tumors, is implicated in pathogenic angiogenesis, and influences the formation and metastasis of various cancers (Cimpean et al., 2016; Hellberg et al., 2010). Furthermore, PDGF-BB is also involved in other serious diseases, such as hepatic fibrosis, atherosclerosis, age-related macular degeneration and diabetic eye disease (Dong et al., 2014; Fiset, 2013; Ishii et al., 2017; Kameda et al., 2007). Therefore, the development of sensitive and specific detection methods for PDGF-BB is of great importance for early disease diagnosis and the assessment of patient recovery. To date, various methods, including chemiluminescence immunoassays, radioreceptor immunoassays and immunosensors, have been developed for the detection of PDGF-BB (Bowen-Pope et al., 1984; Leitzel et al., 1991; Qu et al., 2011). Specifically, enzyme-linked immunosorbent assay (ELISA) is the most commonly used technique to detect PDGF-BB, where two antibodies simultaneously bind with the PDGF-BB to form a sandwich structure, and a detectable signal is generated (Leitzel et al., 1991). Though these traditional immunological methods give invaluable insight into PDGF-BB detection, they have some limitations including tedious sample preparation, long detection times, and the need for special storage and handling conditions. While antibodies have long been considered to be the standard in molecular recognition and the use of antibodies as recognition probes for the development of detection methods as early as the 1950s, the high cost of the antibodies, their limited stability, complicated in vivo production and typically undefined cross-reactivity are challenges still facing immunological technologies (Jayasena, 1999).

Aptamers are functional single-stranded deoxyribonucleic acid or ribonucleic acid molecules, which are obtained through an evolution-

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like process known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX)(Ellington and Szostak, 1990; Tuerk and Gold, 1990). They can bind a variety of targets such as proteins (Bock et al., 1992; Green et al., 1995, 1996), small molecules (Gao et al., 2016; Huizenga and Szostak, 1995; Zheng et al., 2015), tissues (Li et al., 2009) and even living cells (Catuogno et al., 2016; Shangguan et al., 2006) with high affinity and specificity. Compared with antibodies, aptamers have some advantages such as ready chemical synthesis with very low cost, high stability and high reproducibility in target recognition, and ease of labeling and chemical modification. Antibodies are generated via in vivo immunoreaction in animals which raises ethical concerns, while aptamers are obtained via SELEX, an affinity enrichment based in vitro screening technique. Therefore, aptamers, as available biorecognition molecules, can be used as the development and supplement in the field of antibody applications. In recent years, the use of aptasensors has allowed several high sensitivity and selectivity detection methods for PDGF-BB, as promising alternatives to traditional immunological techniques (Hong et al., 2017; Zhang et al., 2017b). Significant efforts have been devoted to the development of aptasensors for PDGF-BB using different transducers such as biolayer interferometry (BLI), surface-enhanced Raman scattering, surface plasma resonance, and electrochemical detection (Gao et al., 2017b; Soontornworajit et al., 2014; Wang et al., 2013b; Ye et al., 2016). Among these, BLI-based biosensors are favorable because they can automatically monitor the binding of target molecules to biorecognition molecule directly in real time utilizing inexpensive disposable fiber tips that analyze the samples from an open shaking microplate without any microfluidics (Auer et al., 2015; Gao et al., 2017a; Mechaly et al., 2016; Zhang et al., 2017a, 2013).

BLI is a label-free and real-time optical analytical technology for measuring biomolecular interactions (Concepcion et al., 2009). It analyzes the interference pattern of white light reflected from two surfaces: a layer of immobilized molecular recognition elements on the sensor tip, and an internal reference layer. Initially, white light is launched into the optical fiber sensor top, and some of the light is reflected into the fiber. Other light continues through the fiber and is reflected when it encounters molecules that form a monomolecular layer immobilized on the top of the sensor. Secondly, both beams of reflected light can interfere with each other, which results in a wavelength shift, which is a direct measure of the change in thickness of the biological layer. Therefore, any change in the number of molecules bound to the sensor surface causes a shift in the interference pattern that can be measured in real-time. In this system (ForteBio, OctetRED96), PDGF-BB that interacts with aptamers immobilized on a streptavidin-coated (SA) biosensor surface causes a proportional shift in the interference spectrum of the reflected light, which directly measures the change in the optical thickness of the biosensor as PDGF-BB binds to it. While BLI is a label-free measurement platform, the detection signal can be significantly enhanced by introducing a signal amplification step that induces precipitation after oxidation by horseradish peroxidase directly on the biosensor surface (Auer et al., 2015; Gao et al., 2017a).

In the present study, we harness an enzyme-linked aptamer sorbent assay (ELASA) incorporated onto BLI biosensors to perform real-time, rapid, and cost-effective detection of PDGF-BB. Biotin-labeled aptamers (Biotin-aptamer) immobilized on the biosensor surface were used as capture probes to bind with the free PDGF-BB in the sample. The biotin-aptamer: PDGF-BB complex on the biosensor surface was then incubated with horseradish peroxidase-labeled aptamers (HRP-aptamer) which were used as detection probes to form sandwich complexes. Finally, the biotin-aptamer: PDGF-BB: HRP-aptamer complex was submerged in a diaminobenzidine (DAB) solution to create precipitation on the biosensor surface, thereby inducing a significant wavelength interference shift. Indeed, the coupling of BLI with ELASA (BLI-ELASA) can enable highly sensitive (limit of detection 80 pg/mL), rapid (23 min) and specific detection of PDGF-BB in serum and urine samples.

2. Materials and methods

2.1. Materials and reagents

All the aptamers used in this study were synthesized and purified through HPLC either by Sangon Biotech Co., Ltd. (Shanghai, China) or by Takara (Dalian, China). The 5′ terminal of the aptamer sequences were respectively labeled using biotin or HRP: 5′-CACAGGCTACGGCACGTAGAGCATCACCATGATCCTGTGT-3′ (Green et al., 1996). Recombinant human platelet-derived growth factor BB (PDGF-BB) and recombinant human serum albumin (HSA) were purchased from Novoprotein (Shanghai, China). Bovine serum albumin (BSA), thrombin, adenosine 5′-triphosphate (ATP) and biocytin were purchased from Sigma-Aldrich Co. LLC (USA). An EZ-Link™ Sulfo-NHS-Biotin kit was obtained from Invitrogen, USA. An ELISA kit for PDGF-BB was purchased from Wuhan USCN Business Co., Ltd. (Wuhan, China). A diaminobenzidine kit (20×, 10 μg/mL DAB solution) was obtained from Beijing Solarbio Science and Technology Co., Ltd. (Beijing, China). SA-coated sensors were obtained from ForteBio (Menlo Park, CA). Binding buffer (137.93 mM NaCl, 8.06 mM Na_2HPO_4, 1.47 mM KH_2PO_4, 2.67 mM KCl, pH 7.4) was purchased from Tiandz (Beijing, China). All solutions were prepared using Milli-Q ultrapure water (Millipore, Billerica, MA).

2.2. Biolayer interferometry assay

BLI-based analytical system (OctetRED 96 or 384) is ideally suited for characterization of protein-protein, protein-nucleic acid and protein-small molecule binding kinetics, and for the determination of protein concentrations and titer with high level of throughput, speed, and flexibility. Accurate kinetic analyses of large protein molecules (e.g. PDGF-BB) to small molecule fragments such as oligonucleotides combined with 8-well or 16-well simultaneous read-out gives the results within minutes in a microplate through a simple, semi-automated dip and read assay. The high detection sensitivity and impressive throughput of Octet system has made it a favorite for developing accurate, fast and real-time detection methods of disease-specific protein biomarkers.

The kinetic performance of the PDGF-BB aptamers was determined through BLI using an OctetRED 96 system. The assay process includes five steps: (1) baseline (1 min); (2) loading (3 min); (3) baseline (1 min); (4) association (3 min) and (5) dissociation (3 min). All steps were performed at 25 °C with shaking at 1000 rpm in a 96-well plate containing 200 μl of binding buffer in each well. The response data obtained from the reaction surface were normalized by subtracting the signal simultaneously acquired from the reference surface to eliminate nonspecific binding and buffer-induced interferometry spectrum shift using the Octet Data Analysis Software CFR Part 11 Version 6.x; the affinity parameter K_D was then obtained. A 1:1 binding mode with mass transfer fitting was used to obtain the kinetic data.

2.3. Preparation and detection of PDGF-BB samples

The collected clinical serum and urine samples were diluted 10 times using binding buffer. Free PDGF-BB was spiked in clinical samples at three levels with the concentrations of 5, 50, and 500 ng/mL. Before the determination, 200 μl binding buffer which was used to activate the SA biosensor for 10 min was added to the wells of a 96-well, black microtiter plate. Clinical samples and the related reagents were added into the corresponding wells in another 96-well microtiter plate, respectively. The procedure includes 8 steps: (1) baseline (1 min, 200 μl binding buffer); (2) loading (5 min, 200 μl of 1 μM biotin-labeled aptamers in the binding buffer); (3) blocking (1 min, 200 μl of 5 μM biocytin in the binding buffer); (4) association (5 min, 200 μl of serum and urine samples); (5) washing (0.5 min, 200 μl binding buffer); (6) association (5 min, 200 μl of 50 nM HRP-labeled aptamers in the