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A portable chemiluminescence imaging immunoassay for simultaneous detection of different isoforms of prostate specific antigen in serum

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ABSTRACT

A multianalyte chemiluminescence (CL) imaging immunoassay strategy for sensitive detection of different isoforms of prostate specific antigen (PSA) was developed. The microtiter plates were fabricated by simultaneously immobilizing of free-PSA (f-PSA) and total-PSA (t-PSA) capture antibody on nitrocellulose (NC) membrane. Each of the array were spotted in replicates of six spots within a spacing of 2 mm. 16 or 48 detection wells were integrated on a single NC membrane and each well could be used as a microreactor and microanalysis chamber. Under a sandwiched immunoassay, the CL signals on each sensing site were collected by a charge-coupled device (CCD), presenting an array-based chemiluminescence imaging. Soybean peroxidase (SBP) was used to label f-PSA or t-PSA monoclonal antibody. With the amplification effects of two enhancers, 3-(10′-phenothiazinyl) propane-1-sulfonate (SPTZ) and 4-morpholinopyridine (MORP), the CL intensity could significantly enhanced, which improved the sensing sensitivity and detection limit. Under the optimal conditions, the linear response to the analyte concentration ranged from 0.01–36.7 ng/mL and 0.02–125 ng/mL for f-PSA and t-PSA, respectively. The results for the detection of forty serum samples from prostate cancer patients and cancer-free patients showed good agreement with the clinical data, suggesting that the proposed assay had acceptable accuracy. The proposed CL imaging immunoassay possess high throughput and acceptable reproducibility, stability and accuracy, which made it great potential to available to distinguish different isoforms of PSA in serum samples.

1. Introduction

Prostate-specific antigen (PSA) that is detectable in serum has proved to be one of the most important markers for diagnosis and monitor the therapeutic efficacy of prostate cancer (Basso et al., 2000; Kavosi et al., 2014; Mikolajczyk et al., 2004). An increased concentration of 10 ng/mL of the total PSA (t-PSA) is often suspected to be associated with prostate cancer (Sarkar et al., 2002). But when the PSA levels fall in the “diagnostic gray zone” of 4–10 ng/mL (Healy et al., 2007), the PSA test is limited by its relative lack of accuracy to distinction between prostate cancer and benign prostatic hyperplasia (Wu et al., 2001). As PSA is not a cancer-specific marker, other prostate diseases can also lead to increased release of PSA into the circulation (Sarkar et al., 2002). The distinction between unbound or free PSA (f-PSA) and complexed PSA (c-PSA) has helped to improve the distinction between prostate cancer and benign prostatic hyperplasia (Basso et al., 2000; Catalona et al., 2000; Healy et al., 2007). Thus, roughly 90–100% of PSA exists as c-PSA in prostate cancer while the complexed-to-total PSA ratio decreases to 75-85% in benign prostatic hyperplasia (Wu et al., 2001). The development of assays that are able to distinguish different isoforms of PSA in serum samples to reduce the false-positive plays a crucial role in clinical diagnosis (Liu et al., 2014). The common method of determination of PSA include enzyme linked immunosorbent assay (ELISA) (Lang et al., 2014), fluorescent immunoassay (Lee et al., 2005), surface plasmon resonance (Uludag and Tothill, 2012; Yu et al., 2004) and electrochemical immunosensor (Kavosi et al., 2014). Nevertheless, these methods can only detect a single marker for one time. In comparison with parallel single-analyte methods, multianalyte immunoassays present excellent advantages, such as shorten analysis time, smaller sample volumes, more accurate diagnosis and lower cost per test (Ouyang et al., 2015; Zhu and Trau, 2015). Sensitive, rapid, high-throughput, cost-effective, point-of-care methods for detecting different isoforms of PSA is still an imperative need for the

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diagnosis of prostate cancer.

As horseradish peroxide (HRP) labeled-antibody to catalyze the luminol-H_2O_2 system with low efficiency, enhancers are added to the solution of luminol-H_2O_2 and the luminescence intensity could be enhanced, which allows chemiluminescence ELISA (CL-ELISA) to have high sensitivity (Ichibangase et al., 2014; Kritikos et al., 2015). To explore new immunoassays with other enzyme-labeled-antibodies, in our previous work (Zhao et al., 2015b), SBP-antibody conjugates were applied in CL-ELISA with addition of co-enhancer, 3-(10′-phenothiazinyl) propane-1-sulfonate (SPTZ) and 4-morpholinopyridine (MORP) to the luminol-H_2O_2 substrate, which enhanced the chemiluminescence intensity nearly 200-fold compared to luminol-H_2O_2 only, suggesting the great potential of SBP-labeled antibodies in CL-ELISA assays. To expand the finding's applications, our another work developed an array-based chemiluminescence imaging assay method for the simultaneous detection of multiple serum biomarkers (Zhao et al., 2015a). We found that HRP catalyzed luminol-H_2O_2 system chemiluminescent reaction is very quickly, the CL intensity reached to the maximum value in the first few minutes and then rapidly decayed over time. However, when using SBP, the recession of luminescence was slower than that using HRP-catalyzed due to the inactivation of the oxidation reaction product, leading to high sensitivity of SBP-based chemiluminescent immunoreaction. Other recent studies also pointed to the same conclusion (Alpeeva and Sakharov, 2005, 2007; Huang et al., 2007; Roda and Guardigli, 2012).

Inspired by previous working that the CL signal in SBP-labeled immunoassay can efficiently increase with the addition of co-enhancers (SPTZ and MORP), a portable and high-throughput multiplex chemiluminescence imaging immunoassay for simultaneous detection of different isoforms of PSA was developed. The preparation of anti-t-PSA-SBP and anti-f-PSA-SBP bioconjugates was introduced. The sensitivity, specificity and high-throughput capacity of this method were discussed by using a homemade microarray platform. As shown in Fig. 1, two kinds of capture antibody was simultaneously immobilized on disposable NC membrane, consecutive addition of target antigens and corresponding anti-PSA-SBP bioconjugates triggered the formation of sandwich immunocomplex. The capture antibody and detection antibody of f-PSA or t-PSA could specifically recognize target antigens f-PSA or t-PSA respectively. Thus, different isoforms of PSA in the samples can be distinguished. After adding chemiluminescent substrate solution, the CL reaction was triggered, presenting an array-based chemiluminescence imaging assay method for simultaneous detection of two kinds of target antigens.

2. Experimental section

2.1. Materials and reagents

Soybean peroxidase (SBP, EC 1.11.1.7, RZ ≤ 3.29, 2229 IU per mg dry weight) was purchased from Bio-Research Products, Inc. (USA). Horseradish peroxide (HRP, EC 1.11.1.7, RZ ≤ 3.4, 300 IU per mg dry weight) was obtained from BBI Life Science Corporation (Shanghai, China). Carbohydrate antigen 19-9 (CA 19-9), neuron specific enolase (NSE), carcinoembryonic antigen (CEA), cancer antigen 242 (CA242), β-human chorionic gonadotropin (β-HCG), ferritin, alpha-fetoprotein (AFP), cancer antigen 125 (CA125), cancer antigen 15-3 (CA15-3), human chorionic gonadotropin (HCG), total prostate-specific antigen (t-PSA), free prostate-specific antigen (f-PSA), and their primary antibodies (mouse monoclonal antibody, 1 mg/mL): anti-f-PSA antibody and anti-t-PSA antibody, were purchased from Fitzgerald Industries Int. (USA); secondary antibodies (goat polyclonal antibody, 1 mg/mL): anti-f-PSA antibody and anti-t-PSA antibody, were purchased from Fujirebio Diagnostics Inc. (USA). Sodium periodate, sodium borohydride, saturated ammonium sulfate, ethylene glycol, glacial acetic acid, tris (hydroxymethyl) amino-methane (Tris) and hydrogen peroxide were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). 3-(10′-phenothiazinyl) propane-1-sulfonate (SPTZ), 4-morpholinopyridine (MORP) and luminol were received from Sigma-Aladdin (Shanghai, China). Nitrocellulose membrane (Aperture 0.45 μm, Whatman, England), bovine serum albumin (BSA, ≥96%) and Tween-20 were obtained from Sunshine Biotechnology Co. Ltd. (Nanjing, China). Double distilled water was

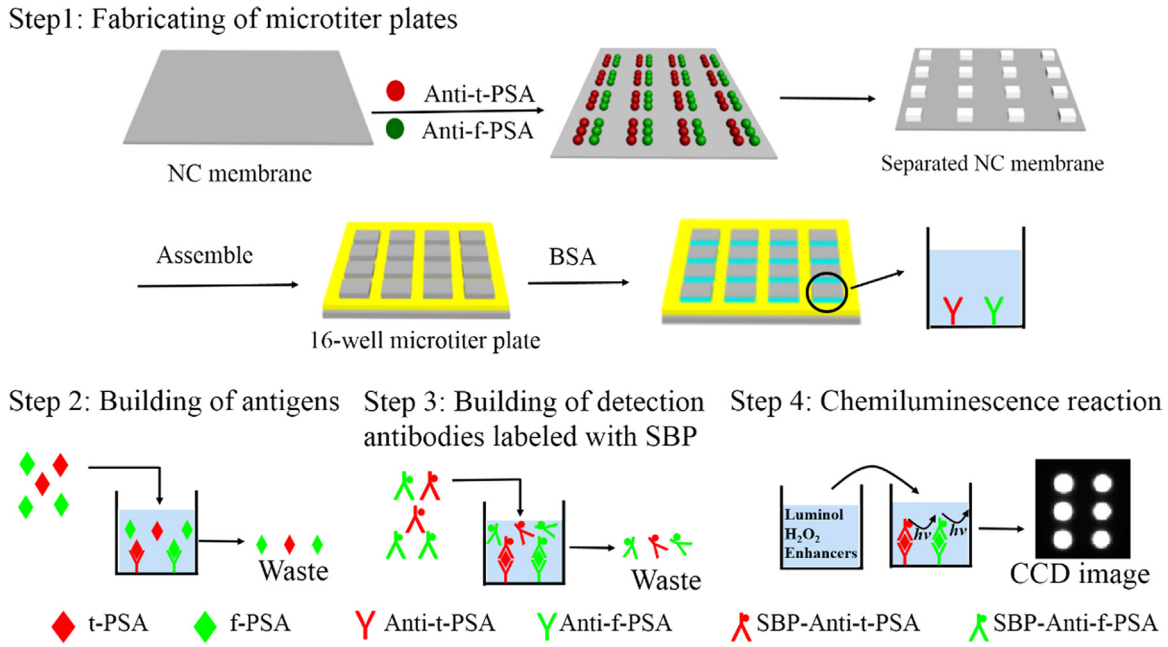

Fig. 1. Schematic illustration of the microarray manufacture and CL imaging immunoassay procedure. Step 1 shows the fabrication process of microtiter plates, the capture antibody: Anti-f-PSA and Anti-t-PSA were spotted on surface of the NC membrane to form the microarray spots. Then, the NC membrane was well separated from each other and coupled to a 16-well bottomless microtiter plate. After incubation with the sample and then the tag (steps 2-3), the CL substrates were dropped in the wells to collect the CL image by CCD (step 4).