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Short communication

Detection of HLA-B*27 gene using a spectral plasmon resonance imaging system

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ABSTRACT

Detection of HLA-B*27 gene is clinically important due to its strong association with diseases, such as ankylosing spondylitis. Nucleic acid-based biosensors represent a promising clinical tool for gene diagnosis. Surface plasmon resonance (SPR) can be used to monitor DNA–DNA hybridization in real-time and without any prior labeling step. Here, a self-built HLA-B*27 positive PCR products spectral SPR imaging system was used for multichannel direct-detection of a specific sequence of the HLA-B*27 gene. Thiol-labeled single-stranded oligonucleotide probes, which were proved to be superior to amine-labeled probes in immobilization, were immobilized onto the surfaces of the gold spots on the sensor chip to target the specific sequence in the HLA-B*27 gene in blood. SPR measurements were performed with different concentrations of synthetic target DNA sequence. The calibration curve of synthetic target sequence showed a good linear relationship between the concentration of the synthetic target sequence and the shift of the SPR wavelength from 10 nM to 500 nM with a detection limit of 5 nM. The HLA-B*27 gene was isolated from human whole blood and amplified using polymerase chain reaction (PCR). PCR products were measured using the SPR imaging system. HLA-B*27 positive PCR products showed significant SPR wavelength shift, while synthetic non-complementary sequence and negative PCR products showed no significant SPR wavelength shift. The method is high-specific, high-throughput and label-free.

1. Introduction

HLA-B*27 gene is a member of human major histocompatibility complex genes. Their products human leukocyte antigens (HLAs) are important for the immune system to differentiate "self" from "non-self" and are essential for communication between cells of the immune system (Khan et al., 2007). HLA-B*27 is strongly associated with a number of diseases, including ankylosing spondylitis (AS), Reiter's syndrome, acute anterior uveitis, and inflammatory bowel disease (Fan et al., 2011; López-Larrea et al., 1996; Ramos and De Castro, 2002). AS is an inflammatory systemic disease that affects the spine and the sacroiliac and peripheral joints. Previous epidemic-logical studies have indicated that about 90% of AS patients are HLA-B*27-positive (Khan et al., 2007). HLA-B*27-positivity has been commonly used for diagnosis of AS. The earliest method for detection of HLA-B*27 is serologic micro-lymphocyte toxicity test (MLCT), which is gradually replaced by flow cytometry (FC) and enzyme immunoassay (EIA) since it is time-consuming and laborious (Chou et al., 2001). Currently, most HLA-B*27 testing is performed with FC analysis of cell surface antigen. However, antibodies used in cell surface antigen tests may cross-react with surface antigens of other HLA-B family members, especially HLA-B7 and HLA-B40 (Levering et al., 2003). Such cross-reaction can compromise the accuracy of the assay results. Therefore, some molecular methods, which are more accurate and do not require the use of viable cells, have been developed for HLA-B*27 genotyping. Polymerase chain reaction (PCR)-based approaches have been introduced to strengthen the probability of AS diagnosis. These methods include PCR with sequence-specific primers (PCR-SSP), PCR with sequence-specific oligonucleotide hybridization (PCR-SSO), and PCR-sequence-based typing (SBT) (Steffens-Nakken et al., 1995). The real-time PCR method using SYBR Green I dye with melting curve analysis for typing HLA-B*27 is reliable, accurate and time-saving (Kim et al., 2011; Kuzio et al., 2004). However, this method requires a complex fluorescent substance labeling procedure and special molecular biology facilities.

Surface plasmon resonance (SPR) biosensor is a sensitive optical technique for monitoring bimolecular binding events by detecting

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local refractive index changes on a nano-metal (gold) surface of sensor (Homola et al., 1997, 1999). SPR analysis is done in real-time and without fluorophore labeling. It is highly sensitive and time-saving and requires only a small sample volume. Thus, SPR biosensors are increasingly popular in areas such as medical diagnostics, environmental monitoring, drug screening, food safety, and security (Hoa et al., 2007; Homola, 2003). SPR biosensors have also been used to monitor DNA-DNA hybridization for gene diagnosis (Altintas et al., 2012; Binh et al., 2007; Carrascosa et al., 2009; Mariotti et al., 2002; Wang et al., 2004; Zhang et al., 2012). For instance, Bianchi et al. (1997) used a SPR biosensor to determine the PCR-amplified HIV-1 genomic sequence of AIDs patients. However, most experiments using SPR biosensors involve single sample analysis and therefore, are time consuming and not practical for high-throughput applications. Therefore, multichannel SPR sensors are needed for direct, high throughput detection.

SPR imaging (SPRi) technique, also referred to as SPR microscopy, is the most straightforward approach to multichannel SPR sensing (Homola et al., 2005; Piliarik et al., 2005). In SPRi system, the signal at a fixed angle of incidence in a linear part of the SPR curve was simultaneously measured by collecting the reflected light intensity in an image using a Charge-Coupled Device (CCD) camera. The variation in the reflected light intensity is proportional to the change in the refractive index due to the binding of molecules to the gold surface (Boozer et al., 2006; Brockman et al., 2000). SPRi has been applied to hybridization-based sensing, including bacterial genotyping, DNA repair and SNP identification (Gorodkiewicz et al., 2011; Mannelli et al., 2006). However, SPRi provides lower resolution than classical SPR because SPRi measures the intensity variation at a single position in the resonance spectrum rather than throughout the surface plasmon resonance curve (Palumboa et al., 2003). Fang et al. (2011) have presented an array biosensor system based on spectral SPR imaging. It maintained the high resolution of mono-channel SPR sensor by measuring full resonance curve and introduced an array chip to perform multichannel measurement. In the present work, we employed this system for multichannel direct detection of HLA-B*27 gene with an aim to develop a fast screening method for clinical diagnosis of AS.

2. Materials and methods

2.1. Reagents

Tis(2-carboxyethyl)-Phosphine Hydrochloride (TCEP), 6-mercapto-1-hexanol (MCH), 11-mercaptoundecanoicacid (MUA), *N*-hydroxysuccinimide (NHS), and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimidehydrochloride (EDC) were purchased from Sigma Company (China). QIAmp DNA Blood Mini kit was from QIAGEN (Beijing, China). The primers used to amplify the HLA-B*27 gene and the DNA probe complimentary to the target sequence of the HLA-B*27 gene were designed using the BLAST search engine. All oligonucleotides (Table 1 in Supplementary Information) were synthesized by Sangon Biotech (Shanghai, China). DNA immobilization buffer (10.0 mM Tris–HCl and 0.1 mol/l NaCl, pH 7.4) was used to immobilize the probe on the gold surface of the biosensor chip, hybridization buffer (NaCl 150 mM, Na_2 HPO_4 20 mM, EDTA 0.1 mM, pH 7.4) was used for the hybridization of the probe DNA and the target DNA, and PBS (0.01 M, pH 7.4) was used to dilute the reagents when necessary Table 1.

2.2. PCR amplification

DNA samples were extracted from human peripheral blood using a DNA extract kit and dissolved in TE buffer. HLA-B*27 positive and negative blood samples were provided by the First Affiliated Hospital of Medical College of Xi'an Jiaotong University, China. The concentration of the extracted DNA was determined by measuring the optical density (OD) at 260 nm using NanoDrop (Thermo, USA). A 150 bp fragment of HLA-B*27 was amplified by using the sense primer (5′-GTGGGCTACGTGGACGACACGCT-3′) and the anti-sense primer (5′-GTCAGTCTGTGCCTTGGCCTTGC-3′). The PCR details are described in Supplementary Information

2.3. SPRi system and measurements

The SPR spectral of 12 samples were measured in parallel using the self-built spectral SPRi instrument (Fang et al., 2011), and the resonance wavelength shift was used to characterize the DNA hybridization on the sensor surface. The analytical signal was expressed as the shift of the resonance wavelength ($\delta\lambda$) (Fig. S1 in Supplementary Information). $\delta\lambda$ was related only to the amount of the HLA-B*27 gene which interacted with the probe DNA on the gold spot surface of the sensor chip.

2.4. Preparation of sensor chip

BK7 Glass slides were cleaned by sequentially using deionized water, acetone, and ethanol in an ultrasonic bath for 5 min, respectively. Then, the slides were sufficiently rinsed with deionized water and dried with a stream of nitrogen. After the washing procedure, a 2 nm-thick chromium film was coated on the slide surface to form an adhesion layer. Then the sensor chip with a 3×4 gold spot array (diameter of the spot: 1.0 mm) was fabricated by depositing a 50 nm-thick Au film on the masked slide using a vacuum sputtering apparatus. Finally, the sensor chip containing 12 gold spots was masked by a 12-cell polydimethylsiloxane (PDMS) plate to confine target solutions on the respective gold spots. The SPR image of the chip array obtained by the CCD camera is shown in Fig. 1.

2.5. Immobilization of DNA probes on gold spot surfaces of sensor chip

Two different types of DNA probes were examined. An equal amount of thiol-labeled or amine-labeled probe was pipetted onto the surfaces of the gold spots A1–A4, B1–B4, and C2–C4 on the array chip, respectively (Fig. 1). The bare spot C1 was used as a reference for calculating the SPR resonance wavelength shift ($\delta\lambda$) of other spots. The immobilization details for each probe are described in Supplementary Information. Since the amount of the thiol-labeled probe immobilized on the sensor chip was larger than the amount of amine-labeled probe (see Supplementary Information), the thiol-labeled probe was immobilized on the gold surface of sensor chip in the following study. All the spots (A1–A4, B1–B4, and C1–C4) were modified with same concentration (2.0 μM) of thiol-modified HLA-B*27 probe DNA and then blocked with MCH (1 mM, pH8.0) solution.

2.6. Hybridization reactions on the sensor array

Ten μl synthetic target oligonucleotide solutions with different concentrations (0 nM to 2000 nM) were pipetted onto the gold spots (A1–A4, B1–B4, and C2–C4), respectively, and incubated for 30 min at 37 °C. The specificity of the interaction was tested by pipetting 500 nM of non-complementary sequence on a spot. Unbound DNA materials were removed by washing with hybridization buffer.

10 μl of denatured PCR products of HLA-B*27 (see Supplementary Information) were pipetted onto the gold spot surfaces and the hybridization reaction was allowed to proceed for 30 min at 37 °C.