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Comparative study of SPR and ELISA methods based on analysis of CD166/ALCAM levels in cancer and control human sera

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ABSTRACT

Surface plasmon resonance (SPR), as a label free method for analysis of various analytes, has significantly advanced in recent years. However, assessment of the performance of SPR compared to label-based immunoassays such as the commonly used multiplexed enzyme-linked immunosorbent assay (ELISA) is limited, particularly for applications involving complex media. In this work, an optimized SPR assay was implemented and its performance compared with an ELISA assay for CD166/activated cell leukocyte adhesion molecule (ALCAM), as candidate pancreatic cancer marker, based on direct and amplified detection in buffer and in human serum samples from healthy individuals and subjects with cancer. ALCAM antibody was immobilized on the surface of a four-channel SPR sensor via physical adsorption onto charged amine-terminated alkanethiolates to mimic the ELISA plate surface. Excellent correlations between SPR and ELISA results were achieved in buffer and in human serum. SPR detected the target protein with a similar sensitivity to sandwich ELISA, with a detection limit below ng/mL. The detection time, sample consumption, throughput, signal referencing, and surface blocking and washing for detection in human serum were evaluated. It is demonstrated that SPR can distinguish between ALCAM levels in cancer and control sera using direct detection without the need for additional amplification steps.

1. Introduction

The development of simple non-invasive tests for disease detection, based on assays of disease markers in body fluids, is an important goal of biomarker discovery. Furthermore, an outcome may be significantly improved with early detection of diseases such as cancer and thus the development of biomarkers to this effect is of substantial importance (Hanash et al., 2008). Although significant progress has been made in recent years for disease diagnosis utilizing tissue mRNA/DNA assays, this approach is insufficient for early diagnosis at a time when subjects are asymptomatic when disease tissue is not available, hence the importance of developing blood-based assays (Taylor-Papadimitriou and Epenetos, 1994; Sanchez et al., 1997). There is substantial ongoing effort to profile the proteome to discover new protein markers in body fluids and tissues, enabling accurate cancer diagnosis, prognosis, and monitoring of disease progression, regression, or recurrence. A single cancer biomarker does not provide sufficient specificity and sensitivity to identify the disease at an early stage. Therefore, there is an intensive search for a panel of biomarkers specific to a cancer type and at a certain stage (Marrero and Lok, 2004; Quek et al., 2004; Calvo et al., 2005).

Advances in proteomic technologies have helped to overcome challenges associated with the complexity of the proteome as well as with the heterogeneity of disease. Currently used proteomic approaches include mass spectrometry-based methods and affinity-based methods, such as multiplexed enzyme-linked immunosorbent assays (ELISA). Mass spectrometry in connection with liquid chromatography (MS–LC) represents an important tool for biomarker discovery. Surface-enhanced laser desorption/ionisation time of flight mass spectrometry (SELDI-TOF) has been utilized as a biomarker discovery platform due to its simplicity (Hanash et al., 2008). Protein arrays and ELISA are based on immobilization of a specific receptor (either antibody or protein) on a solid surface. Affinity-based methods for biomarker discovery and validation mostly utilize fluorescence- or radioisotope-labeling or enzymatic amplification to visualize target-analyte binding. For label-based immunoassays, where an antibody is immobilized on the surface and the target analyte is bound from solution, a secondary antibody, used as a label transporter, is needed for the visualization of antigen binding. Since the development of high-quality antibodies is laborious and expensive, the need for two specific antibodies with sufficient affinity per one analyte is a key limiting factor in immunoassay development (Phelan and Nock,

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2003; Cretich et al., 2006). Therefore, a robust, highly sensitive, specific and label-free method for the direct detection of target proteins in complex media such as serum or plasma in a high-throughput format will highly benefit the development of biomarkers.

Surface plasmon resonance (SPR) technology represents a promising alternative to current routine immunoassay-based methods. This method has been significantly improved in the last decade in terms of surface chemistry, resolution, microfluidics, sample handling, and data analysis (Homola, 2006). Various platforms of SPR sensors have been developed, including spatial-resolved SPR imaging that enables detection of hundreds of biomolecular interactions in a high-throughput format (Homola, 2006). A large number of studies utilizing SPR in recent years have shown that these sensors enable direct and label-free specific detection of various analytes below the nanomolar level with real-time monitoring of various biomolecular interactions (Keusgen, 2002; Yang et al., 2005; Vaisocherová et al., 2007). However, the sensitivity, reproducibility and robustness of SPR-based protein detection compared to the established and commonly used sandwich-based or other labeling-based methods remains to be critically assessed. Comparative assessment of methodology can be complicated for complex media such as body fluids (Homola, 2008; Kim et al., 2006; Scholler et al., 2006). A comparable sensitivity of ELISA and SPR in the detection of insulin-like growth factor-1 (IGF-1) in buffer and milk was presented by Guidi et al. (2001). A good correlation between ELISA and SPR for the detection of antibody against capsid protein of *Porcine circovirus type 2* in diluted pig sera was described by Cho et al. (2006). A good correlation between SPR imaging and ELISA was also found for the detection of fish iridovirus antibody in diluted bream sera (Cho and Kim, 2007). While diagnostic efficacies were given in these studies, a systematic comparison of these two methods have been lacking in terms of background signal levels, detection limits, reproducibility, and accuracy.

We report a comparative study of detection of a candidate cancer protein marker in human serum samples by optimized SPR methodology and by conventional ELISA. Activated cell leukocyte adhesion molecule (ALCAM, CD166) is a promising candidate biomarker for pancreatic cancer (Nummer et al., 2007; Faca et al., 2008) which we have chosen as a model protein analyte. Real-time monitoring of each individual step in the sandwich ELISA detection of ALCAM, including binding of capture monoclonal antibody, ALCAM in serum, biotin-tagged detection polyclonal antibody, and streptavidin labeled with horseradish peroxidase, has been performed. The sensitivity, reproducibility, and specificity of ALCAM direct and amplified detection in buffer and in diluted serum by SPR were determined and compared with those for ELISA. Detection time, sample consumption and throughput were also evaluated.

2. Materials and methods

2.1. Reagents

Human ALCAM monoclonal antibody (clone 105901) (capture antibody in ELISA kit), human recombinant ALCAM/Fc chimera (standards in ELISA kit), human ALCAM affinity purified biotinylated polyclonal antibody (detection antibody in ELISA kit), and streptavidin labeled with horseradish peroxidase (Streptavidin/HRP) were purchased from R&D Systems. ELISA Substrate Solution, i.e., 1:1 mixture of Color Reagent A (H_2O_2) and Color Reagent B (tetramethylbenzidine), and the ELISA Stop Solution (2 N H_2SO_4) were also purchased by R&D Systems. Rabbit polyclonal antibody to Salmonella species (reference antibody) was purchased from Meridian Life Science. Bovine serum albumin (BSA) was purchased from Sigma–Aldrich. Amine-terminated alkanethiol was purchased from Dojindo Molecular Technologies. Pooled, male only, human serum was purchased from Biochemed. Caseine, fish skin gelatin, 10 mM phosphate buffer, pH 7.0 (PB) and phosphate buffered saline (PBS), pH 7.4 compounds were purchased from Sigma–Aldrich. Tween-20 was purchased from Sigma–Aldrich. Absolute ethanol was purchased from Aaper Alcohol and Chemical Co. Triethylamine and ammonium hydroxide were purchased from J.T. Baker.

Eight serum samples were provided by Dr. Samir Hanash from the Fred Hutchinson Cancer Research Center, Seattle. Serum samples from four pancreatic cancer patients were obtained at the time of diagnosis following the informed consent using IRB-approved guidelines. Four normal healthy controls were collected under the same protocol.

2.2. SPR sensor

A four-channel SPR sensor developed at the Institute of Photonics and Electronics of the Academy of Sciences of the Czech Republic was used. This sensor is based on the spectroscopy of surface plasmons (Homola et al., 2001). The shift in resonant wavelength is proportional to the refractive index change at the sensor surface which can be caused by the binding of an analyte to a receptor immobilized on the surface. Broadband light from a halogen lamp was collimated, polarized, and then introduced into the SPR sensing element interfaced with a glass chip coated with an adhesion-promoting titanium film (thickness, 2 nm) and a gold film (thickness, 48 nm). Upon the incidence of the thin gold film, each light beam excited a surface plasmon at a certain wavelength (~750 nm). The reflected light was collected into four optical fibers and coupled to a four-channel spectrograph. Acquired spectra were analyzed in real time by a laboratory-made software package that allowed determination of the resonant wavelength for each sensing channel (Nenninger et al., 2002). The RI resolution of the SPR sensor was 3×10^{-7} RIU. This SPR sensor has the sensitivity of 6500 nm/RIU and wavelength resolution of 0.001 nm. A flow-cell with four separate flow chambers, one facing each of the four sensing spots, was interfaced with the chip to confine the different samples during the experiment. The flow chambers were precisely aligned with the zones where the surface plasmons were excited in each sensing channel. The depth of each flow cell was about 50 μm and the volume of each flow-cell chamber was 1 μL. A peristaltic pump was used to flow liquid samples over the sensing spots. The microfluidic configuration enabled circulation of the sample over the sensing areas with a minimal sample volume of 300 μL. This sensor was equipped with a temperature controller which enabled measurements of the studied interactions with high accuracy in the range of temperatures of 5–40 °C.

2.3. Functionalization of the SPR sensor with capture antibody

The gold-coated sensor chip was cleaned in a UV ozone cleaner for 20 min. The chip was then rinsed with Millipore (Q) water and absolute ethanol, and then dried with filtered air. The chip was immersed in an amine-terminated alkanethiol solution with a concentration of 200 μM, to which triethylamine (2%) was added in ethanol. After incubation for 1 day at room temperature, the chip was washed with an ethanolic solution of acetic acid (10%, v/v) and with absolute ethanol, dried with filtered air and immediately mounted into the SPR sensor. Immobilization of antibodies was performed via physical adsorption to the positively charged SAM surface. To control the amount of immobilized antibody molecules, the immobilization of antibody was carried out in the sensor and monitored in real time. The antibody against ALCAM was immobilized in duplicate onto two spots of the SPR chip (measuring channels) and the antibody against Salmonella was immobilized in duplicate onto the other two spots (reference channels). Fig. S-1