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Development of double-generation gold nanoparticle chip-based dengue virus detection system combining fluorescence turn-on probes

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ABSTRACT

A sensing platform, combined amino acid labeling kit and a double-generation gold nanoparticle (DG-AuNP) chip, was designed to prove the existence of weak but crucial binding between the DV (dengue virus) and its CLEC5A receptor. At first, we have designed a kit combining the novel fluorescence turn-on sensors for lysine, arginine and cysteine amino acids. Advantages of this kit are that emission on–off switching can increase the signal-to-noise ratio and the virus must be fluorescently labelled with sufficient numbers of fluorophores because the lysine, arginine and cysteine residues of viral proteins are labelled simultaneously. Consequently, this kit can be used to light-on single DV spot both in solution and in cell under fluorescence microscopy. Second, the labeling kit was used to examine the DV binding to the CLEC5A-coated DG-AuNP chip. Based on our study, the double-generation gold nanoparticle construction of chip can support more coating areas for receptor CLEC5A and then, support more binding opportunities for DV. Meanwhile, the grooves between nanohemispheres will provide the extra driving force for DV stacking. We try to give a proof that this sensing platform is very useful for detection of weak binding mechanism.

1. Introduction

Dengue hemorrhagic fever (DHF), is caused by any of four dengue virus serotypes (serotype 1–4); is characterized by hemorrhagic manifestations, thrombocytopenia, and plasma leakage; and has the potential to further develop into dengue shock syndrome (DSS) with a 1–2.5% mortality rate (Back and Lundkvist, 2013; Yacoub et al., 2013). Characterizing the dengue virus (DV) recognition/entry receptors is crucial to illustrate the mechanism of DV pathogenesis to enable the specific treatment of DV infections by developing anti-DV drugs or vaccines. Both the mannose receptor (MR) and Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Nonintegrin (DC-SIGN) receptor have been reported to regulate DV binding and entry (Miller et al., 2008; Navarro-Sanchez et al., 2003; Tassaneetrithep et al., 2003). Furthermore, we showed that CLEC5A (C-type lectin domain family 5, member A) can interact directly with the dengue virion and act as a signaling receptor to stimulate the release of proinflammatory cytokines. That is, although the CLEC5A–DV interaction does not result in viral entry, it can mediate DV-induced proinflammatory cytokine production and pathogenesis (Chen et al., 2008). This major breakthrough in understanding the mechanism of DV pathogenesis may offer a promising strategy to alleviate tissue damage and increase the survival of patients suffering from DHF and dengue shock syndrome.

The interaction between CLEC5A and DV is very weak, although CLEC5A can also be identified by an Enzyme-linked immunosorbent assay (ELISA)-based innate immunity receptor array in the same way as the DV-specific receptors DC-SIGN and DC-SIGNR (Chen et al., 2008; Hsu et al., 2009). Thus, a more sensitive and reliable platform should be developed to overcome the challenge resulting from the weak binding between CLEC5A and DV, and would represent a good contribution to diagnostic developments. In previous work, we combined EIS (electrochemical impedance spectroscopy) and a nanostructured chip to develop an effective method to verify the weak conjunction between the

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glycoprotein at the envelope of DV and CLEC5A (Tung et al., 2014). CLEC5A was immobilized on the gold nanoparticles deposited in a nanohemisphere array on the anodic aluminum oxide (AAO) surface to construct a DV-detecting biosensor chip. The bonding between CLEC5A and DV can be detected by the change in impedance before and after the immobilization of DV on a CLEC5A-coated electrode. In this case, the nanohemisphere array is necessary because no impedance difference was observed when the receptor was coated on a flat gold surface. We proposed that the defect sites (grooves) between nanohemispheres would provide the driving force for ligand binding. The main goal of the current manuscript is to visualize the location of DV on the CLEC5A-coated chip and prove that DV is located in the groove between the nanoparticles.

Fluorescence labeling is a popular and powerful method to visualize viruses, with direct chemical labeling with a small organic fluorescent dye as the most common general strategy. However, because of the small structure of viruses, viruses must be labeled with a sufficient number of fluorophores to enable detection without inhibiting their infectivity (Brandenburg and Zhuang, 2007; Sivaraman et al., 2011; Seisenberger et al., 2001). The DV labeling dyes DiD/DiI and Alexa Fluor 488/594 are known as membrane-fusion lipophilic dyes and amine reactive dyes, respectively (Ayala-Nuñez et al., 2011; Van der Schaar et al., 2007; Zhang et al., 2010). However, these dye molecules either cause a self-quenching effect at high labeling densities or only a limited number of fluorescent chemical labels can be attached to a virus particle (Wojta-Stremayr and Pickl, 2013). Therefore, a fluorescent emission enhancement protocol was designed to label viral proteins. In this study we have designed a kit combining the novel fluorescence turn-on sensors for lysine, arginine and cysteine amino acids. One advantage of this kit is that emission on–off switching can be used to negate the background signal problem and increase the signal-to-noise ratio. Furthermore, the virus must be fluorescently labelled with sufficient numbers of fluorophores because the lysine, arginine and cysteine residues of viral proteins are labelled simultaneously. Thus, this protocol enables the direct visualization of the DV actually binding on the CLEC5A-coated DG-AuNP chip, and we evaluate the diagnostic applications of the biochip. The experimental strategy and process are illustrated in Fig. 1.

2. Experiment

2.1. Materials and apparatus

The general chemicals employed in this study were of the best grade available and were obtained from Acros Organic Co., Merck Ltd., or Aldrich Chemical Co. and used without further purification. All solvents were of spectrometric grade. The sensing probes (CLEC5A, DC-SIGN) were constructed by Dr. Hsieh's lab, which contained a human IgG1 Fc region and lectin ligands as its Fab region (Chen et al., 2008). The FreeStyle 293 Expression System (Invitrogen) were used in overexpressed both sensing probes followed by purifying with protein A beads (GE Healthcare). Dengue virus (DV2/PL046) was kindly provided by Dr. Lin's lab according to Shih's method (Shih et al., 2004). The dengue virus was propagated in C6/36 cells. The viral titers were measured by plaque-forming-assays with BHK-21 cells.

Absorption spectra were generated using a Thermo^{TM} Genesys^{TM}-6 UV–visible spectrophotometer, and fluorescence spectra were recorded using a HORIBA JOBIN-YVON Fluoromas-4 spectrofluorometer with a 1-nm band-pass in a 1-cm cell length at room temperature. The fluorescence images were taken under Leica AF6000 confocal fluorescence microscopy with DFC310 FX Digital color camera. Atomic force microscopy (AFM) measurements were performed under a liquid environment (with a liquid

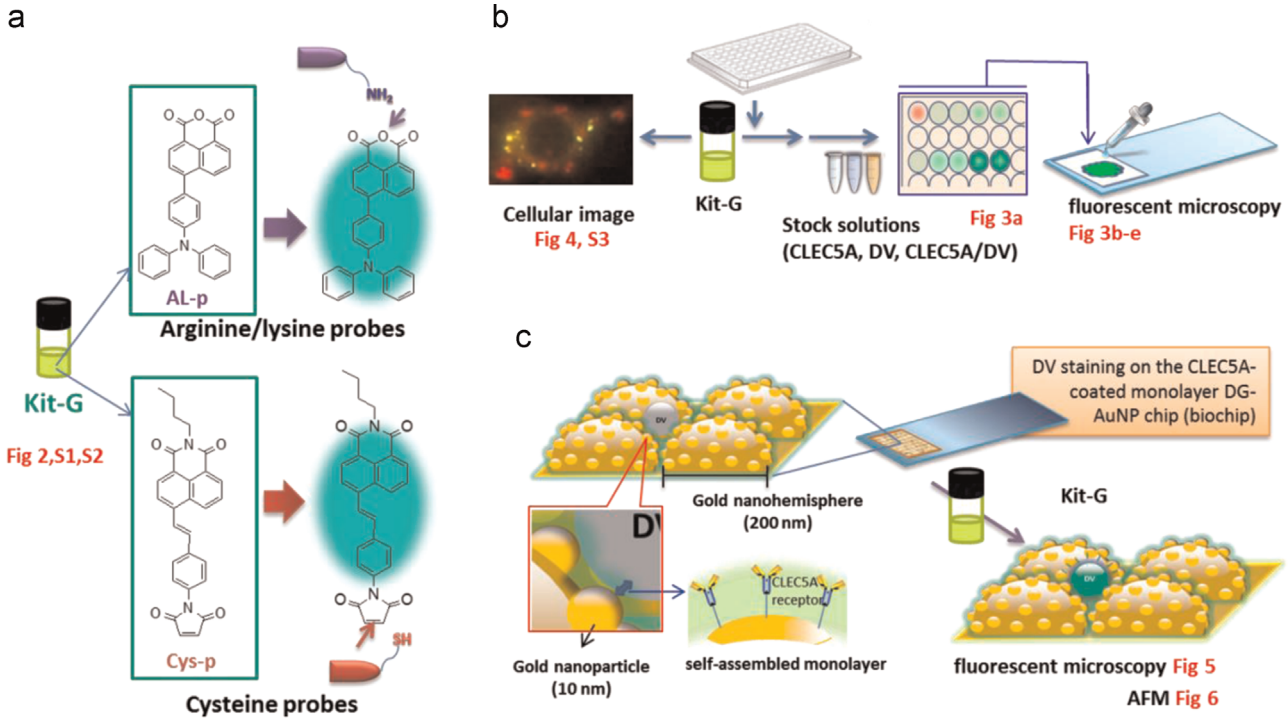

Fig. 1. (a) The proposed fluorescence turn-on mechanisms of the designed arginine/lysine probe (up) and cysteine-probe (down) and the components of kit-G (emission turn-onto a bright green color). (b, c) Illustration of the molecular imaging strategy. (b) Fluorescent labeling of the receptor and virus using kit. (c) The construction of a double-generation gold nanoparticle (DG-AuNP) chip and the fluorescent labeling of this chip using kit. In this case, the virus is bound to the receptor-coated Au surface. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)