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Label-free nanoplasmonic sensing of tumor-associate autoantibodies for early diagnosis of colorectal cancer

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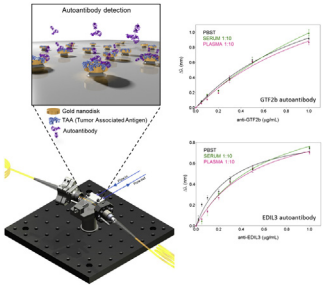
HIGHLIGHTS

- A nanoplasmonic biosensor for colorectal cancer autoantibody detection is proposed.
- Direct, label-free detection of TAA autoantibodies is achieved with LOD around 1 nM.
- The strategy allows the detection in serum and plasma after a 1:10 dilution step.
- Real samples from cancer patients have been analyzed and compared with ELISA.

GRAPHICAL ABSTRACT



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ABSTRACT

Colorectal cancer is treatable and curable when detected at early stages. However there is a lack of less invasive and more specific screening and diagnosis methods which would facilitate its prompt identification. Blood circulating autoantibodies which are immediately produced by the immune system at tumor appearance have become valuable biomarkers for preclinical diagnosis of cancer. In this work, we present the rapid and label-free detection of colorectal cancer autoantibodies directly in blood serum or plasma using a recently developed nanoplasmonic biosensor. Our nanoplasmonic device offers sensitive and real-time quantification of autoantibodies with excellent selectivity and reproducibility, achieving limits of detection around 1 nM (150–160 ng mL^{-1}). A preliminary evaluation of clinical samples of colorectal cancer patients has shown good correlation with ELISA. These results demonstrate the reliability of the nanobiosensor strategy and pave the way towards the achievement of a sensitive diagnostic tool for early detection of colorectal cancer.

1. Introduction

Colorectal cancer (CRC) is a worldwide health problem with an incidence over 1 million annual cases and being a major cause of morbidity and mortality in developed countries [1]. It is the third most common cancer and the fourth most common cause of death

Abbreviations: CRC, colorectal cancer; ELISA, enzyme-linked immunosorbent assay; LSPR, localized surface plasmon resonance; POC, point of care; RI, refractive index; SAM, self-assembled monolayer; TAA, tumor-associate antigen.

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around the world. Despite the exact cause for CRC is not known, several risk factors have been established for the disease, including genetic and epigenetic parameters [2]. Familiar history of colon cancer or inflammatory diseases, age, lifestyle and environmental conditions are strongly associated to CRC development. CRC is easily curable when detected early [2], thereby prevention and regular screening play crucial roles in the fight against this cancer. However, CRC diagnosis is particularly challenging. The most reliable diagnosis technique is *via* sampling of colon biopsies suspected of possible tumor development, which is typically done during colonoscopy or sigmoidoscopy for the distal colon and rectum [3]. These procedures are highly invasive and present important limitations in terms of costs, available resources and low compliance. On the other hand, established non-invasive tests such as the guaiac-based fecal occult blood test (gFOBT) suffer from low specificity leading to inaccurate diagnosis results [4]. There is an evident need for novel screening tools, ideally, analytical techniques based on blood analysis, which permit the early and reliable identification and diagnosis of CRC. Development of blood biomarker assays that could indicate that a cancerous process is triggered would represent a great benefit. However, although a few serum proteins have been described as biomarkers in CRC (carcinoembryonic antigen (CEA), CA19.9 or CA125), none of them are recommended for early clinical diagnosis but for advanced stages and for monitoring recurrence of the disease [5].

Over the past decade, cancer research has made major advances in understanding the causes of developing CRC as well as the molecular mechanisms involved in the disease [6]. For instance many solid tumors such as breast, lung or colon cancer have revealed to be immunogenic. These tumors express aberrant levels of mutated or modified proteins known as tumor-associated antigens (TAA), which are related to the malignant growth. Such proteins can stimulate cellular and humoral immune response, triggering specific autoantibody production [7,8]. The role of autoantibodies in cancer is still unclear. It is not well-known whether they play a cancer-promoting role, an anti-tumor effect or if they are an epiphenomenon associated to inflammation and tumor progression [9]. Nevertheless, autoantibody responses to TAAs hold promising characteristics to consider them as blood biomarkers for cancer detection and they are currently being investigated as potential diagnostic tools in multiple cancer types. Some reports have described the use of autoantibodies for early and preclinical detection of cancer, such as lung [10,11] or breast cancer [12]. The analysis of autoantibodies offers significant benefits when compared to direct determination of protein antigens associated to the tumor. Whereas detection of directly tumor-shed proteins in serum may be challenging due to their low abundance or to the difficulty of identifying simple mutations or structural modifications, serum autoantibodies are highly stable biomolecules and are produced in large quantities even after stimulation by a minimal amount of tumor antigen [12,13]. As a result, TAA-specific serum autoantibodies can constitute excellent circulating reporters for early and preclinical cancer diagnosis [14,15]. In the particular case of colorectal cancer, over 100 individual TAAs have been identified as target for autoantibody production, including full-length proteins, peptides, phage-peptides or glyco-peptides [14,16–18]. Current efforts in CRC research are directed not only to define specific TAA panels but also to develop efficient and highly sensitive analytical methods capable of detecting TAA autoantibodies in serum with optimum accuracy and reliability [19,20]. Most commonly employed methodologies are based on ELISA or protein microarrays [21] which are usually aimed at finding relative cut-off values, so far providing qualitative or semi-quantitative results. Optical biosensors can offer a valuable alternative in terms of time and sample consumption and can provide accurate quantification, which may result useful from a diagnosis point of view. Photonic and plasmonic biosensors in particular have shown great promise for the development of high-throughput and miniaturized platforms capable of carrying out label-free and highly sensitive biochemical analysis [22,23].

In this paper, we show the design and optimization of a nanoplasmonic-based biosensor for the direct detection and quantification of specific CRC-related TAA autoantibodies. We employ a refractometric nanoplasmonic biosensor whose configuration is based on the Localized Surface Plasmon Resonance (LSPR) of gold nanodisks [24]. The gold nanodisks are fabricated by hole-mask colloidal lithography [25] which is an easy, fast, low-cost and well-established methodology which leads to reproducible results, with controlled density of disks on the surface. This device is highly sensitive to local refractive index (RI) changes occurring in close proximity to the surface of the transducer (in this case the gold nanodisks), such as the ones originated from biomolecular interactions. These RI changes can be detected as variations of the LSPR, which permits the real-time monitoring of the biorecognition events under label-free conditions. This nanoplasmonic biosensor has demonstrated excellent capabilities in terms of RI sensitivity improvement and signal-to-noise ratio enhancement, compared for instance with conventional Surface Plasmon Resonance [26] and more specifically its performance has also been validated for accurate detection of clinical biomarkers and antibodies in blood serum in few minutes [26,27]. The proposed biosensor strategy would allow rapid and simple analysis of TAA autoantibodies, providing a unique and innovative tool for CRC diagnosis.

2. Experimental

2.1. Materials

Main chemical reagents and salts for buffer preparation and biofunctionalization procedure were acquired from Sigma–Aldrich (Germany): alkanethiols for self-assembled monolayer (SAM) formation (16-mercaptohexadecanoic acid (MHDA) and 11-mercaptoundecanol (MUOH)), reagents for carboxylate group activation (1-ethyl-4 (3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and N-hydroxysulfosuccinimide (s-NHS)), ethanolamine and Tween 20. Poly-L-Lysine-graft-PEG MW~70000 g mol^{-1} was purchased to SuSoS (Switzerland). Commercial serum was obtained from Sigma–Aldrich (Germany) and commercial plasma was purchased to Innovative Research (USA). cDNA encoding for full-length human genes EDIL3 and GTF2B in pDONR221 were obtained from the PlasmId repository (Harvard Institute of Proteomics) and, then, subcloned into pET28a (Novagen) for protein expression. TAAs were expressed in bacteria and purified according to previous studies [16,28]. The Institutional Ethical Review Boards of the Centro de Investigaciones Biológicas (CIB), the Spanish National Research Council (CSIC) and Hospital de Cabueñes (Gijón) approved this study on biomarker discovery in colorectal cancer. Serum samples were obtained from the Hospital of Cabueñes previous informed consent of the patients. Antibodies anti-GTF2b and anti-EDIL3 were purchased to Santa Cruz Biotechnology (USA) and Abcam (UK), respectively.

2.2. Description of the nanoplasmonic biosensor

The nanoplasmonic device is based on a recently implemented LSPR sensing scheme based on a waveguided electromagnetic mode that arises in thin monolayers of sparse and randomly distributed plasmonic nanoparticles. Nanoplasmonic chips consist of short-range ordered arrays of gold nanodisks (diameter D = 100 nm, height H = 20 nm (Ti/Au 1/19 nm), surface density