Editorial

Detection of membrane antigens of extracellular vesicles by surface plasmon resonance

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Extracellular vesicles (EVs) are membrane-bound vesicles released from cells to the extracellular milieu, playing an important role in the biological processes, including intercellular communications (1). EVs can be generally divided into exosomes, microvesicles (MVs), and apoptotic bodies largely by their size, despite debate about their definition (2). Recently, the application of EVs as a tool for non-invasive liquid biopsy in tumor specimens has been investigated in prostate cancer (3), glioblastoma (4), and pancreatic cancer (5). Therefore, it is important to develop a better detection technique for the surface antigen of EVs, leading to the discovery of a precise biomarker for the diagnosis and prognosis of tumor. Currently, there are several techniques being used for the detection and characterization of EVs, including nanoparticle tracking analysis, dynamic light scattering analysis, zeta potential analysis, tunable resistive pulse sensing, Raman spectroscopy, electron microscopy, enzyme-linked immunosorbent assay, western blot, and "omics" such as proteomics and RNA-seq (6). In addition, surface plasmon resonance (SPR) sensing has emerged as a widespread biophysical sensing tool for the analysis of membrane molecules, including surface proteins and carbohydrates. Recently, a plethora of research on the detection of EVs by SPR technique has been carried out for various purposes (*Table 1*).

In the paper entitled "*Surface plasmon resonance is an analytically sensitive method for antigen profiling of extracellular vesicles*", Gool EL *et al.* established a sensitive method to detect membrane proteins, such as Her2 and EGFR, in the EVs derived from human breast cancer cell lines, including HS578T, MCF7, and SKBR3. Importantly, the density of surface antigens in these cells and their exosomes was accurately measured via employing surface plasmon resonance imaging (SPRi) and the technique was much sensitive to detect small amount of surface antigens above the limit of detection (LOD) (16). The common Kretschmann configuration setup of the SPR for the detection of EVs has been represented in *Figure 1*.

Gool EL *et al.* established the SPRi device in which the sensor surface was precoated with a gold- and 3D hydrogel-layer, leading to the reduction of nonspecific background. An array of 48 spots functionalized with various antibodies was used to capture EVs, making the conduction of high-throughput experiments much easier. Once the chip is illuminated, light goes through the coupling crystal at a constant incident angle and the alteration of reflected light from the SPR gold surface are transformed into the altered refractive index resulting from the binding of EVs. Furthermore, this whole set of event is recorded by a charge-coupled device (CCD) camera which eventually yields plot as a component of time. The stage is quick and exceptionally subtle and requires a very low volume of samples. For high throughput multiplex estimations, a multichannel fluidic cell framework is necessary, alongside the identification framework incorporated with optics comprising of a laser diode and a CCD camera (16).

In recent studies, various exosomal proteins, including exosomal membrane antigens, have been proposed as diagnostic biomarkers for cancer, such as Glypican-1

Table 1 SPR-based techniques to detect EVs

Technique	Type of EV detected	Peculiarity	Reference
nPLEX (nano-plasmonic exosome) or nanohole-based SPR sensor	Exosomes	Characterization of exosomes	(7)
SPR	EVs derived from inflammation-triggered endothelial cells	Characterization of EV biomarkers in primary cells derived from patients with acute CHD	(8)
LSPR with self-assembly gold nanoislands (SAM-AuNIs)	Exosomes and MVs	Direct detection without functionalization of the LSPR chip	(2)
Dual-Wavelength SPR	Exosomes	Determining the size and concentration of sub-populations of EVs	(9)
SPR	Clinically relevant exosomes	Detection of human epidermal growth factor receptor 2 (Her2)-positive exosomes	(10)
Biacore 3000 instrument (GE Healthcare)	Exosomes	Molecular screening of exosomes derived from cancer	(11)
Aptamer-based SPR	Exosomes	CD63-specific aptamer-based SPR for biosensing exosomes	(12)
SPRi	Exosomes	Quantitative detection of exosomes	(13)
SPR	Exosomes	Determination of concentration of exosome in solution	(14)
SPR	Exosomes	Detection of exosome internalization by prostate cancer cells	(15)

EVs, extracellular vesicles; SPR, surface plasmon resonance; SPRi, surface plasmon resonance imaging; MVs, microvesicles; CHD, coronary heart disease.

(GPC1), Apbblip, Daf2, Foxp1, Incenp, BCO31781, Aspn, and Gng2 in pancreatic cancer, CD34 in acute myeloid leukemia (AML), phosphatase and tensin homolog (PTEN) in prostate cancer, and EDIL-3/Del1 in bladder cancer (6). However, the membrane of EVs generally contains small level of antigens associated with tumors, resulting in the challenge of the phenotyping of EVs by various biosensor tools, including flow cytometry (FCM). Gool EL *et al.* in their paper demonstrated that SPRi had high sensitivity to detect 31 out of 33 antibody-EV combinations above the LOD by the reduction of the background signals via using hydrogel on the sensor as well as the increase of the "back-and-forth" flow. However, only 5 among 33 antibody-EV combinations exceeded the LOD by FCM, suggesting its low sensitivity for phenotyping of EVs. Particularly, in comparison with FCM, SPRi can be efficiently used as a high-throughput screening platform for the discovery of novel exosomal biomarkers in various diseases, including tumors. However, FCM can only analyze a number of biomarkers ranging from 6 to 11 at a time on a routine basis, nonetheless various types of liquid biopsies still can be conducted by FCM via using the samples from plasma, cell culture supernatant, urine, and cerebrospinal fluid (18).

Importantly, Gool EL *et al.* demonstrated that the SPRi response was proportional to the antigen (Her2) density of SKBR3 cells and their EVs, which was correlated with the specific fluorescence intensity (SFI) of SKBR3 cells by FCM, confirming the precise detection of exosomal Her2 by SPRi. In addition, the sensitivity of the detection by the SPRi also was determined; for example, 2×10^5 cells/mL were necessary on Her2 spots to surpass the LOD within 10 min of binding, whereas 2×10^8 SKBR3 EVs/mL were necessary to surpass the LOD.

Concluding remarks

Gool EL *et al.* (16) clearly demonstrated the strength of SPRi in the accurate detection of exosomal biomarkers, such as Her2, through the experimental tests of the specificity and sensitivity for various exosomal proteins. Qualitative determination of exosomal antigen could be achieved by the SPRi, however, quantitative phenotyping of

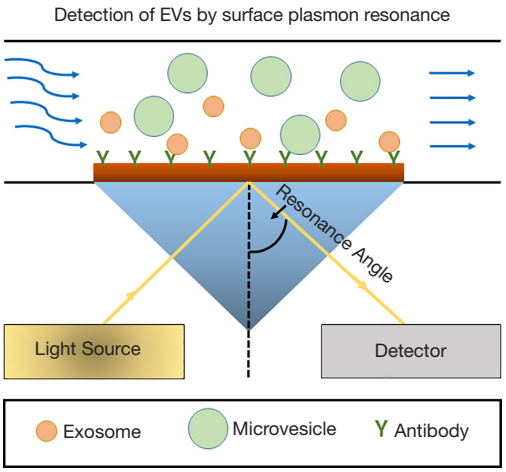

Figure 1 A representative schematic diagram of a setup for the detection of EVs by SPR. The surface of the SPR chip is functionalized by an antibody to capture the antigen present in the surface of EVs. When p-polarized light illuminates an electrically conducting surface, the excitation of surface plasmon occurs. At a specific angle of illumination, excitation of maximum plasmon and minimal internal reflection take place, which is referred as the resonance angle [Adapted from Sabban S *et al.*, 2011 (17) with author's permission]. EVs, extracellular vesicles; SPR, surface plasmon resonance.

EVs was not clearly demonstrated because the contribution of the concentrations and diameters of the EV particles on the SPRi response could not be determined. Finding both optimal detection methods and targets of membrane antigens of EVs might be critical for conducting better quantitative analysis of EVs by SPRi. Nonetheless, in future, quantitative analysis of exosomal biomarkers in disease progression, including tumors, can be a promising biosensing method as a liquid biopsy in the laboratories as well as the clinics.

One of the major concerns in the research of EVs is the dearth of appropriate terminology and techniques for the description of experimental results. Although the authors have referred EVs to all the vesicles in the aforementioned article, accumulated evidence has demonstrated a clear distinction between three major types of EVs, exosomes, MVs, and apoptotic bodies. Each type of EVs are synthesized and released by different biological pathways. Importantly, different type of EVs has different membrane property (2) and it contains different components, prompting us to investigate their specific role in physiological- and pathological- conditions. Therefore, the detection of specific type of EVs by SPRi might be a good diagnostic method and it also can provide a great insight into the underlying mechanism of each type of EVs in the progression of disease, such as tumor metastasis.

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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