OPTOPLASMONIC BIOSENSOR FOR ULTRASENSITIVE DETECTION OF INTERLEUKIN 11 (IL-11): TOWARDS SINGLE-CELL ANALYSIS IN LUNG CANCER

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Cancer remains one of the leading causes of premature mortality worldwide [1], with around 20 million new cases every year and over 10 million deaths reported in 2022. Lung cancer is the most frequently diagnosed cancer (12, 4%) followed by female breast cancer (11.6%). Projections indicate an increase of 77% in global cancer incidence by 2050 [2]. Notably, 50% of the cancers are detected at an advanced stage, when the prognosis is poor and treatment options are limited. Therefore, early detection is essential for improving survival rates and outcomes [3].

The development of advanced proteomic technologies for detecting small amounts of proteins at the single-cell level and low abundant proteins circulating in the bloodstream is crucial for deepening our understanding of cellular processes and disease mechanisms [4] and for the early detection of the disease. Traditional methods often lack the sensitivity needed to capture low-abundance proteins or the ability to resolve protein expression heterogeneity within individual cells – factors that are key to studying complex biological systems such as cancer progression [5].

Innovations in single-cell proteomics offer the potential to reveal critical insights into cell-specific protein dynamics, facilitating more accurate diagnostics, targeted therapies, and a greater understanding of molecular biology at an unprecedented resolution. Furthermore, early tumor proteins released into the bloodstream when the tumor is composed of only a few cells are found at very low concentrations (< 1pg/ml), making them undetectable for current proteomic technologies [6, 7] (Figure 1).

IL-11 is a member of the IL-6 cytokine family, which is important in regulating immune responses, inflammation, and hematopoiesis. It exerts its effects by signaling through the Interleukin11 (IL-11) receptor (IL-11Rα) and the gp130 protein, which in turn activates several key downstream pathways, including JAK/STAT, MAPK, and PI3K/AKT. These pathways are often dysregulated in cancer and contribute to tumor growth, metastasis, and resistance to therapies. Notably, IL-11 is overexpressed in bronchoalveolar lavage fluid of patients with lung adenocarcinoma compared to non-cancer controls and other histological subtypes of lung cancer, indicating its potential as a diagnostic biomarker for this disease [8].

In this work, we developed an optoplasmonic sandwich assay for detecting IL-11, using both purified proteins and non-small cell lung carcinoma (NSCLC) cell lines. Specifically, we used the H3122 cell line, which overexpresses IL-11, and the H1975 cell line, which does not express IL-11, serving as the negative control. This selection of cell lines allows for a clear comparison between a positive signal and a baseline or negative signal, helping validate the specificity and sensitivity of the assay in detecting IL-11 in NSCLC contexts.

Immunoreactions were carried out on biofunctionalized silicon wafers coated with capture antibodies, while detection antibodies were conjugated to gold nanoparticles (GNPs), which functioned as plasmonic labels (Figure 2). The optoplasmonic signal is measured using the automated dark field reading system, AVAC, from Mecwins, which allows the automatic measurement of 48 samples in just 40 minutes. For each sample, 119 images are captured to ensure robust statistical analysis of the gold nanoparticle (GNP) distribution on the surface. After the measurement process, the AVAC system automatically analyzes the acquired images. The system optically identifies individual nanoparticles, and their scattering properties are examined to characterize, classify, and count the nanoparticles present on the silicon surface due to IL-11 detection. This method offers high specificity in detecting IL-11, with a precise analysis of nanoparticle scattering contributing to the assay's sensitivity and accuracy.

The optoplasmonic assay was tested using varying concentrations of recombinant IL-11 protein, diluted in fetal bovine serum (FBS), to validate its specific recognition of IL-11. Remarkably, the assay achieved a limit of detection of just 1 x 10^{-18} g/mL (Figure 3).

After successful validation of the sensor, we tested the optoplasmonic biosensor with an IL-11 overexpressing NSCLC cell line (H3122) and an IL-11 knockout NSCLC cell line (H1975). The cells were initially cultured at a density of 10^5 cells/mL and subsequently lysed to prepare serial dilutions ranging from 10^5 cells/mL to 10^{-1} cells/mL. The optoplasmonic assay was then carried out using these dilutions. We successfully differentiated between the two cell lines, even in dilutions corresponding to a single cell or fewer (Figure 4). The observed decrease in the optoplasmonic signal at the highest concentrations is likely due to the high-dose hook effect. This phenomenon occurs when there is an excess of antigen relative to the capture antibodies, leading to falsely lower signal values.

In conclusion, we have successfully developed an optoplasmonic immunoassay for the ultrasensitive detection of IL-11. The assay achieved an

impressive detection limit of 1 attogram/mL using recombinant IL-11 diluted in fetal bovine serum (FBS). Additionally, the optoplasmonic immunoassay was successfully applied to cell lines with and without IL-11 expression, demonstrating its potential for detecting IL-11 with single-cell level sensitivity.

References

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Figures

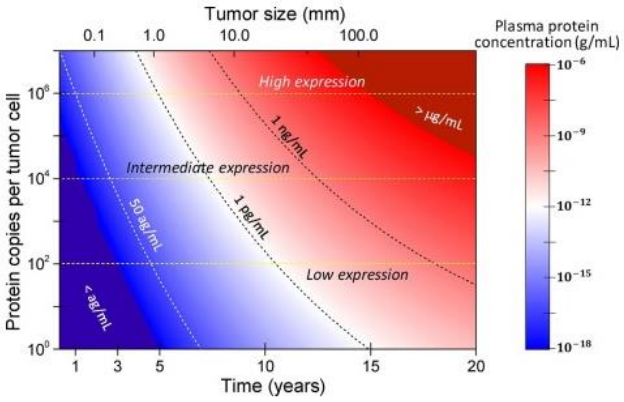

Fig 1. Mathematical prediction of the plasma concentration of proteins shed by the tumor as a function of the time and protein abundance in the tumor cells [7].

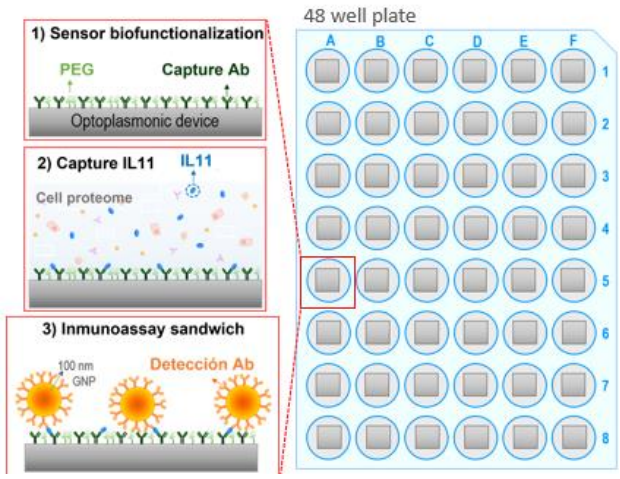

Fig 2. Scheme of optoplasmonic immunoassay procedure.

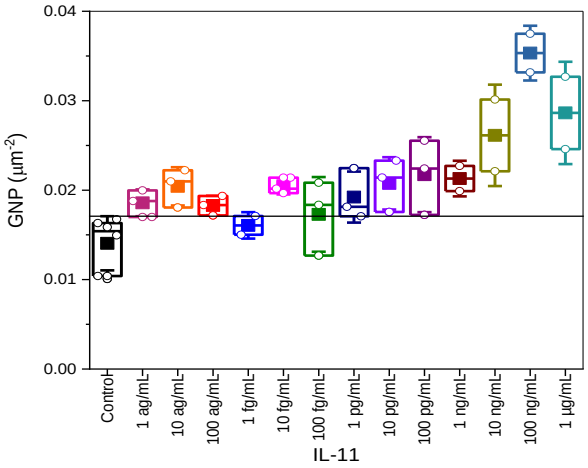

Fig 3. Successful detection of recombinant IL-11, with a limit of detection of 1 attogram/ml.

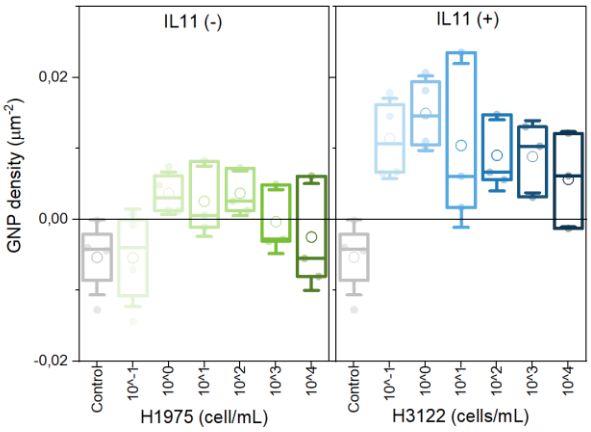

Fig 4. GNP density is significantly higher in cell line H3122 that overexpresses IL-11.