Raman and Surface Enhanced Raman spectroscopy analysis of breast cancer cell lines with different HER2 expression profiles

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Abstract. Assessing HER2 expression in breast cancer cells holds significant diagnostic and prognostic importance. Traditional methods like immunohistochemistry and in situ hybridization suffer from low sensitivity and misclassification rates. In this frame, techniques such as vibrational microscopies can ensure, together with low costs and analytical speed, both high accuracy and precision. Herein, we propose a combined Raman and SERS approach for characterizing 4 breast cancer cell lines and normal cells with varying HER2 expression levels. We show that Raman spectroscopy offers a promising alternative, providing unique molecular fingerprints for cell types based on their biochemical signatures. Its non-invasive nature and ability to detect subtle changes in cellular metabolism make it ideal for cancer cell analysis. Coupled with machine learning techniques like PCA and LDA, Raman spectroscopy can classify different breast cancer subcategories accurately. Surface Enhanced Raman Scattering (SERS) further enhances sensitivity, allowing the detection of single molecules like HER2 receptors. Overall, our results enable fast screening of cancer subpopulation in terms of HER2 concentration and macromolecule cell content. Integration of Raman spectroscopy with SERS offers precise identification and opens avenues for personalized therapies.

1 Introduction

The accurate detection and identification of breast cancer cells that express epidermal growth factor receptor 2 (HER2) are essential for effective clinical treatment of breast cancer. Cellular HER2 levels are associated with malignancy and aggressiveness of BC cells. Traditional diagnostic tools are based on HER2 recognition by immunohistochemistry (IHC) and hybridization in situ staining from breast biopsies; whose levels of staining are associated with higher level of protein expression and number HER2 gene copies, respectively. These techniques are subjected to high misclassification rate at the lower detection limit, due to low sensitivity and accuracy. Novel techniques for HER2 detection and HER2 positive tumour classification, based on the Raman spectroscopy have been developed during the last decades. By analyzing the Raman scattering of laser light interacting with biological molecules, such as proteins, lipids, nucleic acids, and carbohydrates, Raman spectroscopy can generate unique molecular fingerprints for different cell types. This enables researchers and clinicians to distinguish between normal and tumor cells based on their biochemical signatures. Moreover, Raman spectroscopy can detect subtle changes in cellular metabolism and biomolecular structure associated with tumorigenesis, providing valuable insights into the progression and behavior of cancer cells. Additionally, Raman spectroscopy is non-invasive and label-free, allowing for real-time analysis of live cells without the need for exogenous markers or invasive procedures. Overall, the capability of Raman spectroscopy to probe the molecular composition of cells makes it a promising technique for the identification and classification of tumor cells, with potential applications in cancer diagnosis, prognosis, and personalized therapy. Thus, Raman by itself, coupled with machine learning algorithms, such as principal component analysis (PCA) and linear discriminant analysis (LDA), can extrapolate the biochemical differences in biological samples allowing a precise classification of different BC subcategories [1]. Furthermore, Surface Enhanced Raman Scattering (SERS), which leverages the plasmonic properties of metal nanoparticles, represents a significant enhancement to traditional Raman techniques. By harnessing SERS, researchers can achieve unparalleled sensitivity, capable of detecting even single molecules. This remarkable capability extends to the direct quantification of specific biomarkers, such as the HER2 receptor on cell membranes [2]. Consequently, the integration of Raman spectroscopy with SERS not only enhances the precision and accuracy of cancer cell identification but also opens up new avenues for targeted and personalized therapeutic interventions. Herein, we proposed a combined Raman

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and SERS approach for characterization of BC cell lines with different HER2 expression levels.

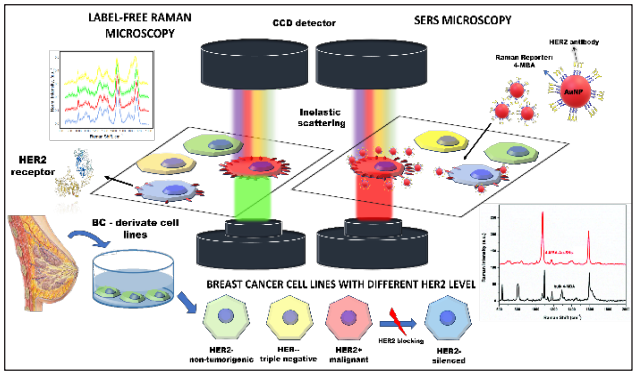

Figure 1. Conceptual scheme of combined SERS-Raman analysis on BC cell lines.

2 Results

Four cell lines have been used in this work:

1. MCF10A normal breast cells
2. MDAMB468 cells with low HER2 levels
3. SKBR3 BC cells with High levels of HER2
4. SKBR3-sh with HER2 silenced expression [3]

Confocal Raman experiments have been performed by using an inverted Raman Microscope Xplora (Horiba Joen Yvonne). Single cell Raman scanning measurements have been performed for each cell line, with an excitation of 532 nm, integration time 0.5 s and the scheme of the Raman experiment is shown in Figure 1. Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) are used for the classification of breast cancer cell spectra. PCA works by transforming high-dimensional data into a lower-dimensional space while preserving the most important variance in the data. It achieves this by identifying orthogonal axes, known as principal components, along which the data varies the most. By projecting the original data onto these principal components, PCA effectively reduces the dimensionality of the data while retaining as much information as possible. In the context of breast cancer cell spectra, PCA can help identify patterns or clusters within the spectral data that correspond to different cell types or states. In our case we show that Raman signal strongly correlates the difference in cell cancer sub-type, in terms of aggressiveness and malignancy, with an increment of lipids and proteins, among the cell lines retrieving the worst prognostic scenarios [4].

On the other hand, LDA is a supervised classification technique that aims to find the linear combinations of features that best separate different classes of data. It does this by maximizing the between-class variance while minimizing the within-class variance. In the case of breast cancer cell spectra, LDA can be used to identify spectral features that are most discriminatory between different types of cancer cells or between cancerous and non-cancerous cells. By finding the optimal linear discriminant functions, LDA can effectively classify new spectra into predefined classes based on their spectral characteristics.

In our case we show that by implementing a machine learning supervisionated approach, even subtle spectral differences are crucial to differentiate with high specificity different breast cancer cell lines, that with an PCA approach are not-easily detectable, allowing even a 100% specificity in BC cell classification.

Ultimately, the Raman measurements were cross-referenced with Surface Enhanced Raman Scattering (SERS) analysis. In the process of preparing the SERS probe, gold nanoparticles of approximately 50 nm in diameter underwent functionalization with two key components: an antibody specific to the HER2 receptor and a Raman reporter molecule, mercaptobenzoic acid (4-MBA). This tailored functionalization enabled the nanoparticles to selectively bind to HER2-positive cells. Moreover, the inclusion of the Raman reporter molecule facilitated the generation of distinct spectral features with heightened sensitivity. This approach not only ensured precise targeting of HER2-positive cells but also provided enhanced detection capabilities, thereby advancing the characterization and identification of these cells with unprecedented accuracy. The scheme of the SERS experiments is shown in **Figure 1**.

3 conclusions

Raman spectroscopy easily demonstrated the strong correlation among differences in BC sub-typologies and their biochemical composition, specifically clearly differentiate among tiple-negative (MDAMB) and HER2 positive cells (SKBR3) tumours. Finally, SERS allows the fast discrimination among different HER2 phenotypes.

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