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New look inside human breast ducts with Raman imaging. Raman candidates as diagnostic markers for breast cancer prognosis: Mammaglobin, palmitic acid and sphingomyelin

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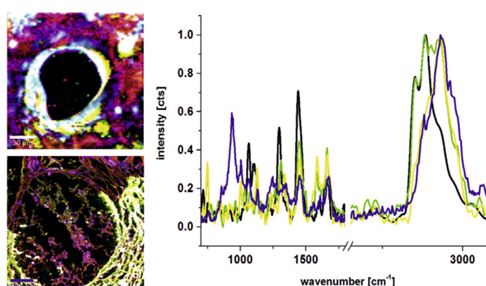
HIGHLIGHTS

- Carotenoids, mammaglobin, palmitic acid, sphingomyelin as key molecular targets in ductal breast cancer have been identified.
- The composition of the epithelial cells surrounding the lumen of the cancerous duct changes in comparison with a normal duct.
- In contrast to the normal duct there is a complete depletion of carotenoids in the cancerous duct.
- The cancerous duct contains smaller amount of monounsaturated fatty acids than the normal duct dominated by oleic acid.
- For the cancerous duct a higher level of saturated lipids and sphingomyelin compared to the normal one is observed.

GRAPHICAL ABSTRACT



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ABSTRACT

Looking inside the human body fascinated mankind for thousands of years. Current diagnostic and therapy methods are often limited by inadequate sensitivity, specificity and spatial resolution. Raman imaging may bring revolution in monitoring of disease and treatment. The main advantage of Raman imaging is that it gives spatial information about various chemical constituents in defined cellular organelles in contrast to conventional methods (liquid chromatography/mass spectrometry, NMR, HPLC) that rely on bulk or fractionated analyses of extracted components. We demonstrated how Raman imaging can drive the progress on breast cancer just unimaginable a few years ago. We looked inside human breast ducts answering fundamental questions about location and distribution of various biochemical components inside the lumen, epithelial cells of the duct and the stroma around the duct during cancer development. We have identified Raman candidates as diagnostic markers for breast cancer prognosis: carotenoids, mammaglobin, palmitic acid and sphingomyelin as key molecular targets in ductal breast cancer in situ, and propose the molecular mechanisms linking oncogenes with lipid programming.

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1. Introduction

The role of cancer treatment is a central target in the medical research. The slowing down in the treatment of cancer observed since the late 1990s and the harmful whole-body secondary effects of chemo/radio therapies emphasize the urgent need to develop different approaches to cancer diagnostics, treatment and monitoring responses to therapy. Current diagnostic and imaging methods are often limited by inadequate sensitivity, specificity and spatial resolution [1]. Cancer diagnosis requires better screening of early stages of pathology and monitoring patient responses to treatment. Better diagnostics requires understanding mechanisms of metabolic alterations for the synthesis of proteins, nucleic acids and lipids related to the development of cancer. Raman imaging may bring revolution in understanding of cancer biology [2–7]. The approach presented in the paper is ideally suited to explore cancer phenotype by monitoring the biochemistry and morphology of cells necessary for survival, proliferation, differentiation, cell death, and expression of many specific functions. There is increasing evidence on important role of altered lipid biosynthesis in cancer metabolism and tumour development [8–16].

This paper focuses on the emerging understanding of the role of lipids and lipogenic pathway regulation in breast cancer. We will provide unique insight into vibrational features of cells/tissues and intracellular processes occurring in cancerous human breast tissue. We will demonstrate that Raman imaging opens a new era as the label-free, minimally-invasive molecular detection tool allowing us to monitor cancer development, because it gives spatial information on molecular compositions of biomolecules in defined cellular compartments of cells without destroying the tissue in contrast to current diagnostic and imaging methods [17].

2. Experimental methods

All procedures were conducted under a protocol approved by the institutional Bioethical Committee at the Medical University of Lodz, Poland (RNN/45/14/KE/11/03/2014). We have studied ductal in situ carcinoma (G1, G2) and normal human breast tissue. All tissue samples were snap frozen and stored at −80 °C. One part of each type was cryosectioned with a microtome (Microm HM 550, Sermed) into 6 μm-thick sections for Raman analysis. The thin cryosectioned tissue samples (without staining and paraffin embedding) have been examined by Raman imaging. After spectroscopic analysis these sections were stained and histologically examined. The adjacent part of the tissue was paraffin embedded and also cut into 6 μm-thick sections for conventional histological analysis. Raman spectra and images were obtained with an alpha 300 RA (WITec, Ulm, Germany) model equipped with an Olympus microscope coupled via the fibre of a 50 μm core diameter with an UHTS (Ultra High Throughput Spectrometer) spectrometer and a CCD Camera Andor Newton DU970N-UVB-353 operating in standard mode with 1600 × 200 pixels at −60 °C with full vertical binning. The incident laser beam (SHG of the Nd:YAG laser (532 nm)) of alpha 300 RA was focused on the sample through a 40× dry objective (Nikon, objective type CFI Plan Fluor C ELWD DIC-M, numerical aperture (NA) of 0.60, and a 3.6–2.8 mm working distance) to the spot of 1 μm. The average laser excitation power was 40 mW, with an integration time of 0.2 s. Rayleigh scattered light was removed using an edge filter. The samples were irradiated by a laser at 532 nm. Spectra were collected at one acquisition per pixel and a 600 lines/mm diffraction grating. Prior to the basis analysis, each spectrum was processed to remove cosmic rays, increase the signal-to-noise ratio via spectral smoothing (Savitzky–Golay method [18]), and correct for biological autofluorescence. The large number of spectra collected in this study required the use of automated removal method for all of the spectra, which is critical to remove sources of variability arising from autofluorescence and substrate contamination. After baseline removal, the dominant remaining source of distinction between spectra is the intensity of the Raman features, arising from the variable amount of biological material within the sample. Data acquisition and processing was performed using WITec Project 2.10. The 2D array images of tens of thousands of individual Raman spectra were evaluated by the basis analysis method. In this method, each measured spectrum of the 2D spectral array is compared to basis spectra using a least squares fit. Such basis spectra are created as the average spectra from different areas in the sample. The weight factor at each point is represented as a 2D image of the corresponding colour and mixed colouring component. The colour code of Raman maps were based on the integrated Raman intensities in specific regions (sum option in the filter manager in the Witec project Plus 2.10). Using a lookup table, bright colours indicate the highest intensities, whereas dark colours indicate the lowest intensities of the chosen region [19].

3. Results and discussion

Raman images allow to look inside the biochemical composition of cancerous cells in the lumen, the duct and the extracellular matrix surrounding the duct. To understand information that is provided from Raman vibrational spectra of the normal and cancerous breast tissues, we need to associate these features with the breast morphology. Briefly, the normal organization of ducts in the human breast demonstrates lumen surrounded by epithelial cells aligned in a polar manner so their apical side faces the lumen. These cells are surrounded by the basement membrane. Fibroblasts align the basement membrane and this entire structure is surrounded by the stroma, which is predominantly, but not exclusively, composed of connective tissue and adipose tissue. Schematic basic structure of epithelial tissue, stromal and adipocyte cells around the normal breast duct is presented in Fig 1 and compared with a microscopy image of the normal human duct that will be analysed in the paper.

Most cancers, including breast cancer, begin in the epithelium cells. During cancer development in the duct (ductal carcinoma in situ (DCIS)), the normal polar organization of the luminal epithelial cells is lost, as these cells proliferate. The epithelial cells completely fill the lumen. In invasive, or infiltrating, cancer, the epithelial cells migrate and invade through the basement membrane and into the surrounding stroma. We will analyse the normal and cancerous ducts (DCIS) by Raman imaging to learn if this method is capable of identifying morphological and biochemical alteration in the human breast duct in cancer development.

Fig. 2 shows the Raman image of the normal breast duct (P123) compared with the H&E-stained histological image, and microscopy image, as well as the characteristic vibrational Raman spectra for different areas of the breast tissue.

Fig. 3 shows the Raman image of the cancerous breast duct (DCIS, G1 and G2, P115) compared with the H&E-stained histological image, microscopy image and the characteristic vibrational spectra for different areas of the breast tissue. According to the histopathological assessment, the pathology in Fig. 3 represents the early stage of cancer development in situ ductal carcinoma, where, in contrast to infiltrating carcinoma, the epithelial cells do not migrate yet through the basement membrane into the surrounding stroma.

One can see that in both cases there is an almost perfect match between the morphological features obtained from histological images, microscopy images and Raman images.

The Raman image presented in Fig. 2A reveals all morphological features of the normal duct. One can see (white-blue line around

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