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Review

Raman 'optical biopsy' of human breast cancer

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ABSTRACT

Raman imaging (RI) is a novel method of medical diagnostics of human breast cancer and has a potential to become a routine optical biopsy. Up to date the present study is the most statistically reliable Raman analysis based on data of normal, benign, and cancerous breast tissues for 146 patients. This paper present the first Raman 'optical biopsy' images of the normal and cancerous breast tissue of the same patient. The results presented here demonstrate the ability of Raman spectroscopy to accurately characterize cancer tissue and distinguish between normal (noncancerous), and cancerous types. The results provide evidence that carotenoids and lipids composition of cancerous breast tissues differs significantly from that of the surrounding noncancerous breast tissue and may be a key factor responsible for mechanisms of carcinogenesis. We have found that fatty acid composition of the cancerous breast tissue is markedly different from that of the surrounding noncancerous breast tissue. The cancerous breast tissue seems to be dominated by the metabolism products of the arachidonic acid - derived cyclic eicosanoids catalyzed by cyclooxygenase, while the noncancerous breast tissue is dominated by monounsaturated oleic acid and its derivatives.

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1. Introduction

Cancer is a multi-factorial disease and molecular imaging is able to demonstrate various mechanisms and phases of pathogenesis. The combination of information using different modalities, various imaging agents and various biomarkers will improve the sensitivity and specificity of the diagnostic method. As a result there has been an increased use of imaging of biomarkers to monitor metabolism, cell proliferation, cell migration, receptor expression, gene expression, signal transduction, hypoxia, apoptosis, angiogenesis and vascular function (Fass, 2008; Shah et al., 2004). Imaging systems produce images that have differences in contrast. Images of human cells, tissues, organs, body are derived from the interaction of energy from the external field with a human tissue. The energy can be in the form of radiation, magnetic or electric fields, or acoustic energy. Most clinical imaging systems are based on the interaction of electromagnetic radiation with body tissues or fluids.

Abbreviations: RI, Raman imaging; LA, linoleic acid; ALA, alpha-linolenic acid; FA, fatty acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DPA, decosapentaenoic acid; DHA, docosahexaenoic acid; COX, cyclooxygenase; LOX, lipoxygenase; GJC, gap junctional communication.

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Ultrasound is an exception as it is based on the reflection, scattering and frequency shift of acoustic waves.

The differences in contrast can be due to changes in physical properties caused by the endogenous nature of the tissue or by the use of exogenous agents. The later are usually performed with radioactive substances or a fluorescent dyes by contrast agent injection or drinking and waiting until they are distributed in the body in order to show more clearly the boundaries between organs or between organs and tumors. Endogenous methods need no external labeling, and use the properties of the biological tissue as a contrast in producing the image. Raman imaging belongs to this group and the contrast is based on the different cross sections for Raman scattering of various components of the tissue.

Raman spectroscopy is a novel method for research of medical diagnostics of human breast cancer (Abramczyk et al., 2008a,b, 2009; Alfano et al., 1991; Brozek-Pluska et al., 2008; Frank et al., 1994, 1995; Haka et al., 2005; Manoharan et al., 1998; Motz et al., 2005; Redd et al., 1993; Shafer-Peltier et al., 2002a,b) and has a potential as an optical biopsy. As it is well known a biopsy is the next step after the previous investigations (mammography, ultrasound and physical examination) to find abnormal mass tissue. The role of a biopsy is to remove a certain amount of tissue sample for microscopic histopathology investigation. There are several types of biopsies: 1) fine needle aspiration biopsy, 2) core (large needle) biopsy, and 3) surgical biopsy. The decision as to which biopsy type is necessary depends on the characteristics of the suspected cancerous mass (lesion's level of suspicion, the mass/lump size and location, the presence of other symptoms of lesions). The Raman 'optical biopsy' gives a chance to perform the measurement without removing tissue suspected of being cancerous because instead of microscopic investigation, the Raman spectra/imaging investigation via remote fiber coupled catheter to micro-Raman spectrometer can be performed. Histopathology, so far the gold standard of diagnosis, is time consuming and often prone to subjective interpretations, in contrast to Raman investigation that eliminates the human factor because it is based entirely on biochemical analysis of vibrational properties of tissue.

Optical methods, including the methods of laser-induced fluorescence, Raman spectroscopy, and mid-IR spectroscopy, offer several significant advantages over the above mentioned conventional imaging methods, such as: a) high spatial resolution, b) **non-invasiveness** through the use of safe, non-ionizing radiation without the need to remove the biological material, c) display of contrast between soft tissues based on optical properties, d) a facility for continuous bedside monitoring. Raman spectroscopy and Raman imaging has now reached a level of sophistication that makes it competitive with more classical methods of confocal fluorescence microscopy. In contrast to the fluorescence microscopy in which there is a limited number of tissue components such as flavins, phorphyrins, and structural proteins (collagen and elastin), the wealth of information provided in the Raman spectra with respect to biochemical composition and structure can be used in medical diagnostics. Raman spectra of most important constituents of the biological tissue such as proteins, nucleic acids, lipids represent the specific pattern of vibrational behavior and distinctive molecular and structural features, and may overcome some of the limitations of fluorescence spectroscopy. Several groups have indicated the potential of vibrational spectroscopy for cancer diagnosis in different organs (Baker et al., 2008; Gazi et al., 2007; Kendall et al., 2009; Mahadevan-Jansen and Richards., 1997). Breast cancer research is one of the most widespread application of Raman spectroscopy (Abramczyk et al., 2008a,b, 2009; Alfano et al., 1991; Brozek-Pluska et al., 2008; Frank et al., 1994, 1995; Haka et al., 2005, 2006; Kumar et al., 2008; Manoharan et al., 1998; Motz et al., 2005; Redd et al., 1993; Shafer-Peltier et al., 2002a,b).

The aim of the present study is to demonstrate that the label-free Raman imaging has ability to accurately characterize breast cancer tissue and distinguish between normal (noncancerous), and cancerous types. We will demonstrate that RI has a potential to replace the conventional biopsy and to give a new insight into mechanisms that drive transformation of a normal breast tissue into a cancerous one.

2. Experimental

2.1. Materials: patients and samples

All procedures were conducted under a protocol approved by the Bioethical Committee at the Medical University of Lodz (RNN/30/11/KE/15/02/2011 and RNN/29/11/KE/15/02/2011). We used Raman spectroscopy and Raman imaging to analyze human breast specimens taken during a surgical operation. The use of the samples for research did not affect the course of the operation or treatment of the patients. We have studied ductal and lobular carcinoma (in situ and infiltrating) as well as various benign changes including benign dysplasia and ductal-lobular hyperplasia. Total number of patients was 146.

The samples were processed as fast as possible with a well-established protocol. The fresh tissue sample from the tumor mass and the tissue from the safety margins outside of the tumor mass were cryosectioned with a microtome into 2 μm-thick sections for histopathological and Raman analysis. The thin sections were put on microscopic glasses and stained with hematoxylin and eosin to provide histopathological diagnosis of suspected areas. The adjacent sections of 2 μm layers without staining and paraffin embedding have been examined by Raman spectroscopy and Raman imaging. After Raman measurements the sections were stained and histologically examined to check if the results are identical to those obtained for the adjacent sections. Histological analysis was performed by pathologists from the Medical University of Lodz, Department of Pathology, Chair of Oncology. We have also examined the bulk tissues taken from the safety margins outside of the tumor mass and directly from the tumor mass without any fixation in formalin. The paraffin-embedding procedure has been avoided in the Raman measurements because it affects important indicators of cancer pathology: 1) vax has its own vibrational spectra of lipids that overlap those from the breast tissue, 2) the standard procedures of deparafinization with various agents (xylene) and rehydration with aqueous solutions of ethanol lead to washing out fatty acids and artificial hydration of the tissue.

2.2. Instrumentation

All Raman images and spectra reported here were acquired using a Raman spectrometer Ramanor U1000 (Jobin Yvon, JY), CCD, ion Ar laser (488, 514 nm) and alpha500 RA (WITec, Ulm, Germany), SHG (NdYAG 532 nm) equipped with an UHTS spectrometer and a Newton-CCD camera (operated in standard mode 1027 × 127 pixels at temperature −64 °C, read out: full vertical binning). Raman acquisition modes used for the samples were taken with a SHG frequency of the NdYAG laser (532 nm), and a Olympus microscope. The spot is focused with a 50× objective with a numerical aperture NA of 0.50 to the spot of 200 nm. The average laser excitation power was 10 mW. Before recording a spectrum, the fluorescence in the sample was quenched during 500 ms at each point. Variation in fluorescence intensity has been observed over the sample area.