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Raman imaging of human tumor tissue and tumor and normal cell cultures

Abstract

The doctoral dissertation is devoted to identification of Raman biomarkers differentiating between tissue from safety margin and that from tumor mass and monitoring proteomic, glycomic, lipidomic, metabolic and epigenetic changes occurring during cancer development in biological systems.

The experiments were carried out using Raman spectroscopy, Raman imaging, AFM (atomic force microscopy) and SNOM (scanning near field optical microscopy) techniques. Presented research gave the opportunity to determine biomarkers differentiating between tissue from safety margin and that from tumor mass. The conventional techniques such as: mammography and ultrasonography used for cancer detection have a lack of adequate spatial resolution, sensitivity, specificity and don't provide biochemical information. The Raman spectroscopy methods presented in the dissertation enable fast and precise analysis that is crucial in case of the measurements of living cells as such systems exhibit dynamical changes and variability. Spectroscopic methods also provide information on the spatial localization of biochemical components based on the analysis of vibrational spectra. Moreover, the analysis can be carried out under ambient conditions without time-consuming sample preparation. The analysis of polarized Raman images and Raman spectra provided additional information on the symmetry of molecular vibrations and allow to obtain higher sensitivity and specificity.

The studied samples included sections of human breast tissue, sections of human salivary gland and human cell lines MCF10A, MCF7 and MDA-MB-231. The research was carried out thanks to the cooperation with Medical University in Lodz, Nicolaus Copernicus

Hospital in Lodz and Institute of Applied Radiation Chemistry Technical University of Lodz. All tissue 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 and RNN/323/17/KE/17/10/2017).

In the first part of the dissertation human breast sections were analyzed. Samples from 62 patients were tested. Particular attention was paid to the following issues: angiogenesis process in cancerous tissue, glycosylation metabolism, acetylation and distribution in the tissue of photosensitizer used in photodynamic therapy. The presented results demonstrate that the main biomarkers which differentiate between tissue from safety margin and that tumor mass are carotenoids (1158 cm^{-1}, 1520 cm^{-1}), lipids (1444 cm^{-1}, 1656 cm^{-1}, 2854 cm^{-1}), fatty acids (1248 cm^{-1}), carbohydrates (582 cm^{-1}, 1484 cm^{-1}) and proteins (1670 cm^{-1}, 2917 cm^{-1}). The application of Raman spectroscopy and Raman imaging allowed to tracking spatial distribution of the photosensitizer for example hematoporphyrin. The presented results show that hematoporphyrin concentration in the cancerous breast tissue is higher in comparison to that of the noncancerous breast tissue. Another issue concentrated on using polarized Raman spectroscopy and polarized Raman imaging which allowed to obtain higher sensitivity and specificity and additional information about the symmetry of vibrations.

Next chapter of the thesis presents the analysis of metabolic changes in sections of the human salivary gland. The results indicate that proteome profile plays a very important role in the differentiation between healthy tissue and tumor mass. It has been observed that tissue from the safety margin is dominated by α-helix structure while cancerous tissue is dominated by the presence of a β-sheet.

The last part of the dissertation presents the results obtained for cell lines with varying degree of aggressiveness. It has been shown that the number of lipid droplets in individual cell lines depends on the cell line type. The highest number of lipid droplets are identified in the highly aggressive MDA-MB-231 cell line, while the lowest in the normal cells MCF10A, thus showing a correlation between the number of lipid droplets in cells and the aggressiveness of those cell lines. It has been shown that lipid droplets differ from adipocytes not only in size but also in biochemical composition. The Raman spectra of arachidonic acid and adipocytes are in high spectral correspondence, while the Raman spectrum of adipocytes is in high spectral correspondence with that of oleic acid. The

epigenetic changes were also investigated in cell lines. The stretching vibrations of the methyl group are markedly blue shifted in the function of the aggressiveness of cell line.

The results presented in doctoral dissertation unequivocally confirm that tumor changes in sections of human breast, human salivary gland and human cell lines such as MCF10A, MCF7, MDA-MB-231 can be identified by means of spectroscopic methods. Raman spectroscopy creates a new perspective in the field of cancer diagnostics, enabling the identification of pathological changes with a spatial resolution of a micrometer.

The AFM and SNOM techniques were used to high-resolution visualization of topographies of tissues and cells in a nanometric scale.

The presented results confirm the potential applications of vibrational spectroscopy methods in oncological diagnostics.

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