FT-IR SPECTROSCOPY FOR HUMAN COLON TISSUE DIAGNOSTIC

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Abstract. The human colon carcinoma and normal tissues were investigated using FT-IR spectroscopy. The preliminary studies demonstrate that it is possible to probe individual chemical species and molecular signaling within the normal and colon carcinoma tissue, drawing first step toward cancer molecular profiling. The molecular structural changes induced by the disease in the studied samples were identified based on the relative intensity of the bands assigned to the lipid/protein content. The results obtained in this study are in good agreement with the recent reports.

Key words: FT-IR, colon tissue, adenocarcinoma.

INTRODUCTION

Adenocarcinoma is a malignant epithelial tumor, originating from the glandular epithelium of the colorectal mucosa. Colon cancer is currently the third leading cause of cancer related deaths in the Western world with 655.000 deceased worldwide per year. It is believed that most colorectal cancers arise from adenomatous polyps in the colon, mushroom-like growths which are usually benign and develop into cancer over time.

Another study of colon cancer shows specific changes in nucleic acid, protein lipid and carbohydrate quantities and/or conformation, characterizing the neoplastic cells [9].

Current cancer diagnosis is based on various expensive and time-consuming medical imaging techniques such as magnetic resonance imaging, computerized tomography, ultrasonography, which are followed by histopathological

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examination of a biopsy specimen taken from the patient. More accurate analysis and elucidation of cancer mechanism could be achieved by using spectroscopic techniques which probe the molecular content of the investigated samples.

Several studies on normal, premalignant (polyp) and malignant human colonic tissues from patients with different stages of malignancy, were reported [2]. These studies used a method which is based on microscopic infrared study (FT-IR-microscopy) of thin tissue specimens and a direct comparison with traditional histopathological analysis, which serves as a "gold" reference [7].

In this study we employ FT-IR spectroscopy for examining cancerous and normal colon tissues in order to characterize the spectral changes that could differentiate between them.

FT-IR spectroscopy employs a unique approach to optical diagnosis of tissue pathology based on its characteristic vibrational spectrum. The biomolecular changes in the cellular and sub-cellular levels developing in abnormal tissue, including a majority of cancer forms, manifest themselves in different optical signatures, which can be detected in infrared microspectroscopy [8].

FT-IR spectroscopy has proven to be a potent analytical tool for studying complex biological materials such as tissues, body fluid and cell cultures [3, 4, 5, 6, 10, 11]. IR spectroscopy is based on the absorption of radiation in the 400 – 4000 cm^{-1} range which excites molecular vibrations. Biological macromolecules such as proteins, nucleic acids, and lipids have specific, fingerprint-like IR spectra in the wavenumber range 950–1800 cm^{-1}, directly determined by molecular structures and cellular chemistry. FT-IR spectroscopy has been shown to have promising potential to detect abnormal changes in cells and tissues. Therefore, FT-IR spectroscopy has been recognized as an analytical tool in medical diagnostics.

MATERIALS AND METHODS

SAMPLE PREPARATION

Tissue samples were isolated from human ascending colon specimen obtained within 2 h after resection from a patient (only one patient) that underwent surgery. Ethical approval has been obtained in order to study human sample tissues. Standard histopathological examination confirmed adenocarcinoma. Freshly collected carcinoma tissues were preserved in phosphate-buffered formalin solution (10%) about 2 h, up to the spectroscopic measurements. Samples were fixed in formalin and it was shown that this procedure did not alter the observed Raman peaks of the tissue, and was therefore a suitable method of fixation. Thin cross-sectioned pieces from normal and adenocarcinoma colon tissue were selected

for spectral data acquisition. The samples were transversely sectioned to the colon wall, and revealed three distinct zones with brown, yellow, and white aspect, respectively, corresponding to the outer to inner colon wall, where the epithelial layer distortion due to carcinogenesis was evident.

INSTRUMENTATION

The FT-IR spectra were obtained by using an Equinox 55 Bruker spectrometer with an integrated Raman module (Bruker, FRA106), fiber optic coupled to a Ramanscope II module. The spectral data were analyzed using the OPUS 2.0.5 and Origin 6.0 software.

RESULTS

The samples transversally sectioned (Fig. 1.a, b, c) of the colon wall revealed three distinct regions with "brown", "yellow" and "white" aspect. These regions are assigned to the corresponding muscularis, submucosa and mucosa layer of the human colon tissue.

(a) **(b)** **(c)**

Fig. 1. Digital images of the tissues: a) normal colon cross section with the main anatomical layers under study; b) adenocarcinoma colon cross section where the carcinoma tissue is prominent; c) back cross section from b), where the adipose tissue agglomeration is abundant.

The samples were investigated on the three different areas, the FT-IR spectroscopic study allowing at the same time the differentiation between normal and cancerous tissues of the same area.

In Fig. 2 are presented the FT-IR spectra acquired from the yellow areas of both normal and cancerous samples, this representing the inner part of the human colon.

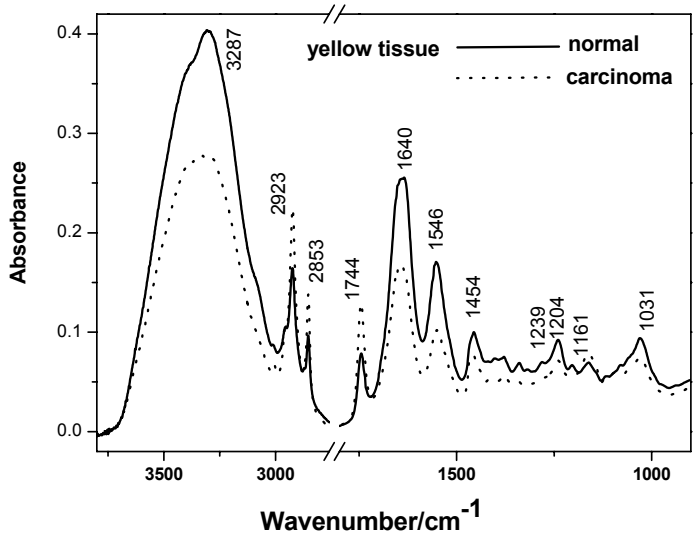

Fig. 2. FTIR spectra of the normal (upper) and carcinoma colon section (lower) acquired from yellow tissues.

In the big wavenumber spectral range, slight differences in the amide A band assigned to N–H stretching mode of proteins can be seen at 3287 cm^{-1} in the normal tissue. In the small wavenumber range the amide I and II bands appear in the normal spectrum and carcinoma spectrum at 1640 cm^{-1}. The bands are more intense in the spectrum of normal samples in comparison with the carcinoma spectrum. Also, the contributions from lipids at 1454 cm^{-1} and from nucleic acids at 1239 cm^{-1} are more prominent in the spectrum characteristic to the cancerous samples.

In Fig. 3, the FT-IR spectra of the white part characteristic to the mucosa layer from normal and cancerous samples are presented. In the big wavenumber range (3600–2800 cm^{-1}) differences in the relative intensity of the present bands in the two spectra are observed. The amide A band present at 3287 cm^{-1} is more intense in the spectrum of normal tissues than the one corresponding to cancerous samples. The CH_2 antisymmetric stretching modes of the main lipids is seen at 2926 cm^{-1} in the cancerous tissue while in the normal mucosa this mode appears shifted at 2923 cm^{-1}. This mode and the CH_2 symmetric stretching mode of lipids which remains constant in both samples at 2853 cm^{-1} are less intense in the normal tissue compared to the cancerous one.

In the fingerprint region, 1800–650 cm^{-1}, the differences in the two spectra lie in the relative intensity of the present bands, as well as of new weak bands which appear in the spectrum of cancerous samples. The C=O stretching mode of lipids at 1744 cm^{-1} in the spectrum of normal tissue is less intense and slightly shifted towards bigger wavenumbers, when compared to the one in the spectrum of

carcinoma samples. The bands at 1640 cm^{-1} and 1546 cm^{-1} corresponding to amide I C=O stretching mode and amide II N–H bending and C–N stretching in proteins [1] have a higher intensity in the spectrum of normal tissues than in the one of cancerous ones.

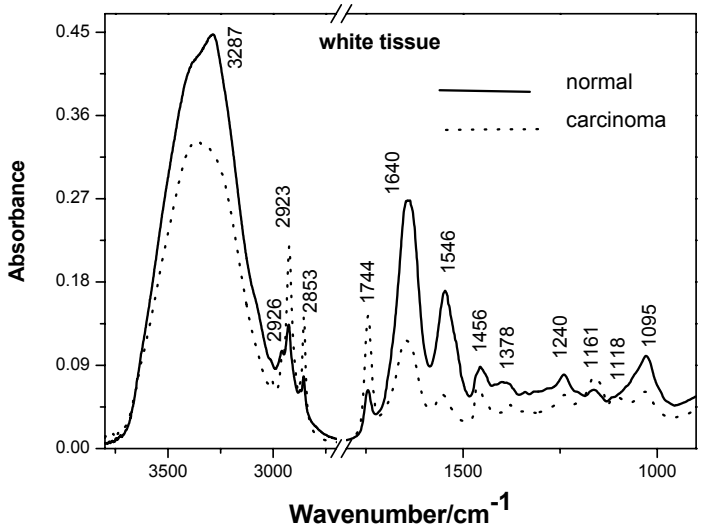

Fig. 3. FTIR spectra of normal (upper) and carcinoma (lower) white tissues from human colon.

Other contributions seen in the two spectra collected from the white zones of the samples are nucleic acids (1161 cm^{-1}), phospholipids (1240 cm^{-1}). Also, in the cancerous spectrum a new weak band appears at 1118 cm^{-1}. On the other hand, the vibrational modes 1378 and 1095 cm^{-1}, respectively, appear more intense in the normal tissue [11].

The spectroscopic investigations of the two samples characteristic to the mucosa layer revealed a higher lipid content in the cancerous tissue and a higher protein content in the normal tissue.

The FT-IR spectra collected from the brown areas corresponding to the muscularis layer of the normal and cancerous samples are presented in Fig.4. These areas are consistent with the walls of the colon section and they provided similar spectral response from both normal and cancerous samples. In the big wavenumber region, the amide A band seen at 3305 cm^{-1} is more intense in the spectrum of the normal samples. The asymmetric and symmetric contributions from lipids present at 2923 and 2854 cm^{-1}, respectively, have smaller intensity in the normal than in the cancerous samples. In the fingerprint region the C=O stretching mode of lipids seen at 1744 cm^{-1} is less intense in the normal spectrum, while the contributions from amide I and II observed at 1640 and 1551 cm^{-1} are more intense when compared to the spectrum of the carcinoma samples, reminding of the same situation seen in the spectra collected from the white areas.

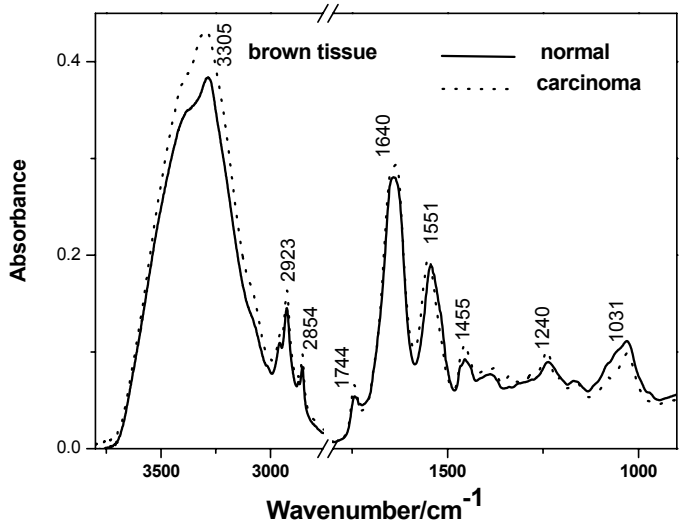

Fig. 4. FTIR spectra of normal (lower) and cancerous (upper) tissues of human colon collected from the brown areas.

The spectra collected from yellow and white tissue revealed large differences on passing from normal to the carcinoma sample, whereas those corresponding to the outer colon wall, represented by the brown areas, were barely distinct. This could be an indicator of the fact that the cancer did not spread in the entire colon, being more intense in the inner part of it.

The characteristic bands of amide A, I and II of proteins were found to be of higher intensity in the spectra collected from the normal tissues, while the bands assigned to lipid contributions were more intense in the spectra acquired from cancerous areas.

CONCLUSIONS

This vibrational spectroscopic analysis identified the principal molecular compounds in the studied samples as well as molecular changes, allowing thus for differentiation between normal and cancerous human colon tissues.

The FT-IR results indicated quantitative differences of lipids and proteins content between normal and cancerous sample. These content differences were best seen in the case of protein bands like the amide A, amide I and II. Differences in the lipid content were also seen, especially in the C=O stretching mode at 1744 cm^{-1}. Considering these observations, the normal tissues were characterized as having higher protein content, while the cancerous tissues were characterized as having lower protein content and higher lipid concentration.

The samples were analysed based on the area of provenience, dividing them into muscularis, submucosa and mucosa layers. Following the spectroscopic investigation it was proved that the samples collected from the inner walls of the colon tissues presented distinct spectral response when passing from the normal to the carcinoma tissue, allowing thus for differentiation, while the ones collected from the outer part (brown areas) provided similar vibrational spectra either of normal or carcinoma tissue.

In principle, basing our assumptions on the given data, it is possible to conclude that FT-IR spectroscopy shows great promise in cancer diagnostic methods and could be used as an automated tissue grading system.

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