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FT-IR microimaging spectroscopy: A comparison between healthy and neoplastic human colon tissues

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Abstract

FT-IR microimaging was performed on colon tissues with the aim to characterize spectral 'markers' to distinguish healthy from pathological tissues. Evidences of spectral peculiarities were mainly found in the finger print region even in the presence of a low grade adenocarcinoma. The occurrence of inflammation and necrotic states can also be demonstrated. Through statistical analysis as well as custom map procedures it was possible to reconstruct the topological distribution of different biochemical states and to verify results from the histopathological analysis. Preliminary results from FT-NIR analysis are in substantial agreement with those in the mid infrared region.
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Keywords: FT-IR microspectroscopy; NIR spectroscopy; Colon adenocarcinoma; Imaging

1. Introduction

Colon cancer is one of the main causes of cancer death worldwide and many efforts to improve early diagnostic and therapeutic procedures have been proposed. Radiation therapy, chemotherapy and surgical intervention have improved the life expectancy of cancer patients, but these methods are critically dependent upon the stage of the disease once diagnosed. Imaging techniques, such as radiography and endoscopy are routinely used as diagnostic tools. However, diagnosis is highly subjective and requires the judgment of an expert. Conventional histology and, in particular histopathology, is the current gold standard for diagnosis of cancer but it is a very complex process,

strongly dependent on the opinion of pathologists. Thus, it is interesting to dispose of a technique that allows a simpler, non-subjective and quicker diagnosis of cancer. FT-IR microimaging, a combination between IR spectroscopy and microscopy, is becoming an attractive technique in biomedical spectroscopy, because it provides spatially resolved information on the basis of the biochemistry and the chemical composition of the different structural compartments of cancer, where components like lipids, phospholipids, carbohydrates and phosphates do absorb selectively in the mid-IR, as well as proteins and nucleic acids [1–5]. Compared with other conventional techniques, vibrational spectroscopy offers various advantages: (1) it requires only a small amount of sample that can be analyzed in different forms and physical states [6]; (2) it does not demand the use of dyes or stains and (3) it is a computer-based digital technique and therefore, the method

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of tissue evaluation can be automated. The FT-IR microimaging technique is well suited for characterizing tissue structures and identifying tissue pathology for its capability to highlight biochemical changes in normal and cancer tissues [7–11].

In the present paper, we investigate the application of FT-IR microspectroscopy and multivariate data analysis as screening tools in colon cancer diagnosis. As a first step, healthy and pathological tissues characterized by a low grading were analyzed in order to enhance even subtle differences. In addition, FT-NIR analysis was also attempted to ascertain and identify different spectral patterns.

2. Experimental

2.1. Human colon tissues

Human colon cancer tissues were obtained from the tumour bank of Anticancer Metaxa Hospital, Histopathology Department, Piraeus, Greece. Adjacent thin sections (5 μm) of colon specimens from a number of patients were used for histopathological analysis and for spectroscopic measurements.

2.2. Histological determinations

All the surgical specimens of human colon tumour were placed in 10% neutral buffered-formalin for a maximum of 24 h. The fixation of the specimens was succeeded by placing them in xylene, alcohol and then embedded in paraffin. The whole procedure was automated. The tissues were enclosed with paraffin in a block and two microtome sections of 5 μm thick were cut. The first section was fixed on a glass with hematoxilin–eosin (H&E) for histological examination, to identify the interesting regions, while the second section was deposited on a steel support and used for infrared analysis.

2.3. Spectroscopic measurement

Spectral data were obtained with a Perkin-Elmer Spectrum One FT-IR equipped with a Perkin-Elmer Autoimage microscope. The spectral resolution was 4 cm^{-1}. The spatial resolution was 50×50 μm and reflectance spectra were collected. The average spectra were the co-addition of 128 scans. Background scans were obtained from a region of no sample and rationed against the simple spectrum. Specific areas of interest were identified by means of the microscope television camera. Baseline (polynomial line fit) was performed in all cases while Second Derivative, Fourier Self Deconvolution and Curve Fitting (Gaussian character) procedures were used to determine the absorbance ratio between bands of interest. All spectra were scaled for equal intensity in the Amide I band. For data handling and software packages Spectrum 5.3 (Perkin-Elmer), Grams AI (Galactic Corp.) and Pirouette 4.0 (Infometrix Corp.) for multivariate analysis were used.

Near infrared spectral data were achieved with a PE-Spectrum One NTS FT-NIR spectrometer (spectral resolution 64 cm^{-1}, scans 64, scan rate 1 cm/s).

Assignments of the bands in the middle and near region were carried out according to the literature data [12a,12b].

3. Results and discussion

Fig. 1 reports the photomicrograph of a section which, according to the pathologist, showed normal (N), connective (C) and cancer (K) features, mainly in the highlighted restricted area whose chemical map is also shown.

On a number of 1269 spectra (second derivative) collected on this zone, the HCA and PCA analysis classified four main groups (Fig. 2a and b): N1 and N2 from normal, C from connective and K from neoplastic zones.

Representative spectra of the four subclusters, in absorbance units and in second derivative, are shown in Fig. 3a and b.
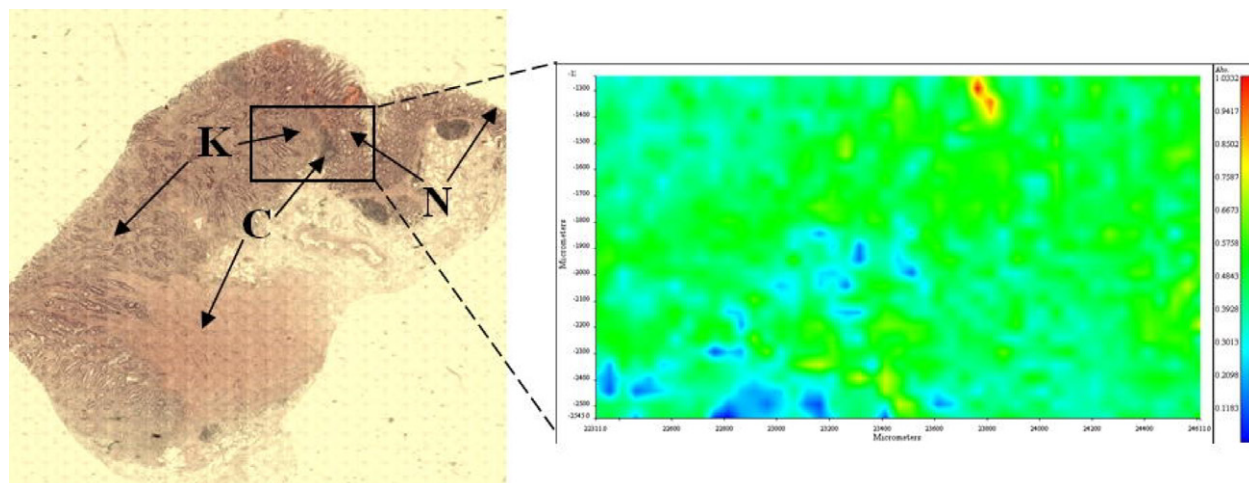

Fig. 1. Photo of an H&H-stained section of a colon adenocarcinoma tissue together with its chemical map.