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Insights on colorectal cancer relapse by infrared microscopy from anastomosis tissues: Further analysis☆

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ABSTRACT

The relapse rate for colorectal cancer (CRC) is high and has a significant impact on survival and quality of life. CRC relapse can be local, at the anastomosis, or distant, as evidenced by metastasis. Reducing the local recurrence of the disease may improve patient survival and quality of life. This could be achieved by determining safe surgical margins of the removed cancerous lesion during colorectal surgery. The detection of abnormal crypts is essential for diagnosis of the spread of CRC and its effective management.

Our overall goal in this study is to evaluate and suggest which biological molecules in the cell contribute more to the classification procedure. This may provide clues for biologically understanding the principle of CRC recurrence. Longitudinal sections from normal anastomosis tissues obtain from CRC patients during surgery were used. Fourier transform infrared spectroscopy (FTIR) in tandem with multivariate analysis, principal component analysis (PCA), and linear discriminant analysis (LDA) were used to achieve this purpose.

Colonic longitudinal sections are preferable for studying the colonic tissues quantitatively. It was possible to differentiate between the three categories: local recurrence, distant recurrence and control with a 100% success rate.

1. Introduction[1]

CRC is one of the most aggressive cancers in the western world. According to the American Cancer Society's most recent estimates, the number of projected CRC cases in the United States for 2015 are: overall 132,700 new colorectal cancer cases, (93,090 colon and 39,610 rectal), with an estimation of 49,700CRC-related deaths. One of the core cancer treatments is surgery to remove the malignant tissues. However, if the surgeon does not perform a radical resection the cancer can recur from residual malignant cells.

The relapse rate for CRC is high (20–30%), making post-operative care and survival a big challenge for CRC patients [1]. Reducing the number of recurrence cases in CRC patients is vital and in many cases, can save lives. In our previous study [2], we demonstrated that it is possible to classify crypts measured from anastomosis tissues into three distinct groups, (1) distant metastasis without local recurrence, which occurs in approximately 80% of recurrence cases, (2) local (~10%), and (3) local recurrence with distant metastasis (~10%).

If the malignant tissue is completely removed during the surgery, theoretically a complete recovery after five years can be achieved in patients if no evidence of any systemic disease is present at the time of surgery. Colon cancer tumors that have high microvessel density tend to recur or spread compared with those that are less dense [3]. The local recurrence rate also depends on the tumor size and the stage of the disease [4]. Studies have shown that the recurrence rate is ~11% if the tumor is less than 3 cm and ~28% for larger tumors. The recurrence rate is directly proportional to the stage of disease. Five-year recurrence rates for rectal cancer are ~41% for stage 3 and ~10% and ~24% for stages 1 and 2, respectively. The variance in the recurrence rate (up to 55%) within five years from surgery depends on cancer aggressiveness, original tumor size, the stage of the disease when the surgery is performed, and the expertise of the surgical team [4–6].

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[1] Colorectal cancer-CRC, Fourier transform infrared spectroscopy-FTIR, Principal component analysis -PCA, Linear discriminant analysis-LDA, Infrared-IR, FTIR microscopy-FTIR-MSP, Soroka University Medical Center-SUMC, Principal components-PCs, Leave-one-out-LOO, Sensitivity-SE, Specificity-SP, Accuracy-Acc.

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Table 1
Details about the samples included in this study, number of crypts from each category, number of measured crypts from the bottom, middle, and top of each site. The abnormal category is a merger of the local or distant recurrence categories.

	Number of crypts category	Number of bottom measurements	Number of middle measurements	Number of top measurements	Number of all site measurements
Control	21	20	21	19	60
Local recurrence	53	50	48	51	149
Distant recurrence	54	48	53	47	148
Abnormal	107	98	101	98	297

The human intestinal surface is lined with small mucosal crypts with epithelial cells that secrete at the rate of about three liters of mucous fluid per day. The cells in the normal crypt have a large turnover, with a life cycle of approximately 48 h. The epithelial cells in the normal crypt continuously undergo mitosis at the bottom of the crypt, move gradually upward along the basement membrane, and are finally disposed into the intestinal lumen [7–10]. Thus, the maturation and migration of cells in the colonic crypt hold important clues to the origin of the premalignant and malignant stages of cancer [11]. Abnormal cell proliferation and biological changes have been known to be an indicator of the initiation of malignancy [12].

IR spectroscopy has been widely used in medicine. It is a noninvasive, nondestructive technique that can detect changes in cells and tissues that are caused by different disorders, including cancer [7,13,14]. FTIR-MSP has been shown to provide important clues to the changes in the biochemical composition of cells and tissues, especially during carcinogenesis [15–18]. The beginnings of colorectal malignancy can be detected by FTIR due to the subtle biological changes resulting from abnormal cell proliferation, even though tissues exhibit normal morphology [11,19]. Our previous studies have shown that IR spectroscopy can detect early development of diseases or cell transformation at a stage when the standard microscopic evaluation is still normal [20,21].

Multivariate analyses, PCA, and LDA were used for the classification and differentiation among the three categories using different ranges of the mid-IR vibrational spectra of the measured tissues.

2. Material and methods

Biopsies in this study were obtained from anastomoses areas of colorectal sections of eight patients from SUMC. One hundred twenty eight crypts were measured from these patients. One patient had no recurrence and was used as the control. Four patients had local recurrences, and three patients had distant recurrences (Table 1).

2.1. Preparation of samples

All the samples were supplied from the histopathology files of SUMC in Beer-Sheva, Israel. The colorectal tissues were obtained from CRC patients who have undergone surgery since 2007. The records of these patients were retrieved with their consent, after the SUMC Institutional Review Helsinki Board approval. The samples were prepared from formalin-fixed paraffin embedded human colonic tissues. The section used for FTIR measurements was deparaffinized using xylol and alcohol before measurements as previously reported and examined for any contaminants by evaluating the spectra in the higher wavenumber region [22].

Two consecutive longitudinal tissues of 10 microns thick were cut from each biopsy. The first was placed on ZnSe crystal, which is transparent to IR radiation that is used for IR measurements.

For the preparation of the samples for the data acquisition, we used the method of Argov et al [16]. The second tissue section was placed on a glass slide and stained with hematoxylin and eosin, and was used by an expert pathologist for histology review. From each crypt three measurements were acquired from the three different sites that are presented in Fig. 1. The first measurement was taken from the crypt's bottom, while the second and third measurements were taken from its middle and top sites, respectively (Table 1). Average normal crypt diameter in colonic mucosa is about 0.1 mm. Crypts that were 100 microns wide were selected [23] to avoid any variation due to extraneous contribution or any other effect like Mie scattering and maintain uniformity across samples.

2.2. FTIR measurements and spectral manipulation

We used Equinox 55 infrared spectrometer (BRUKER Germany) which is attached to an infrared/visible microscope. The microscope is coupled to liquid nitrogen cooled MCT detector. Using the Equinox 55 infrared spectrometer, it is possible to select different circular apertures. We look at the sample in the visible mode of the microscope in order to carefully choose the measured spot. We ensured that the selected spots were not in the edge sites. Then we switched the microscope to IR mode and performed the measurement. The measurements were performed in the transmission mode at 4 cm^{-1} spectral resolution from 4000 to 600 cm^{-1}. Each measurement takes about one minute to acquire 128 co-added scans to increase the signal to noise ratio. The measured spot has a circular shape with a diameter of 100 μm.

All the spectra measured were manipulated using OPUS7 software (BRUKER Germany). All the spectra were bisected into two regions; in the 3010–2800 cm^{-1} and 1762–877 cm^{-1}. The two regions underwent baseline correction, were normalized using vector normalization, and then underwent offset correction. We used "concave rubberband" method with 64 sections and 5 iterations to correct the baseline. In this method the infrared spectrum is divided to 64 ranges that have the same size. A polynomial fit was generated based on the minima of y-values in

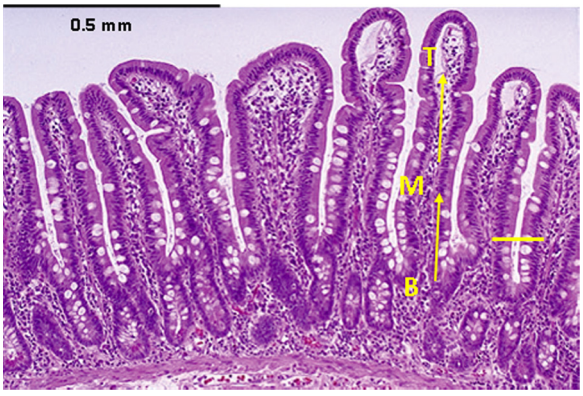

Fig. 1. Longitudinal cross section of human colonic tissue obtained from a normal colon. Three measurements were performed from the bottom (labeled B), middle (labeled M), and top (labeled T) along each crypt. The horizontal bar indicates a width of 100 μm.