Human Colon Adenocarcinoma Cell Lines Display Infrared Spectroscopic Features of Malignant Colon Tissues

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ABSTRACT

Seven human colon cell lines were studied by infrared spectroscopy including study of several spectral parameters under high pressure (pressure tuning spectroscopy). The results were compared to those obtained from the study of normal and malignant colon tissues (B. Rigas et al., Proc. Natl. Acad. Sci. USA, 87: 8140–8144, 1990; P. T. T. Wong and B. Rigas, Appl. Spectrosc., 44: 1715, 1990). The seven adenocarcinoma cell lines displayed almost all of the important spectroscopic features of colon cancer tissues: (a) increased hydrogen-bonding of the phosphodiester groups of nucleic acids; (b) decreased hydrogen-bonding of the C—OH groups of carbohydrates and proteins; (c) a prominent band at 972 cm⁻¹; and (d) a shift of the band normally appearing at 1082 cm⁻¹ to 1086 cm⁻¹. These cell lines differed spectroscopically from the colon cancer tissues in that: (a) they displayed a band at 991 cm⁻¹, which is weak in colon tissues; and (b) the packing and degree of disorder of membrane lipids were close to those observed in normal colon tissues. These findings (i) establish IR spectroscopy, used in combination with pressure tuning, as a useful method to address problems of tumor biology in cell culture systems, (ii) indicate that these cell lines offer a useful experimental model to explore the origin of the spectroscopic changes that we observed in colon cancer tissues, and (iii) support the idea that the malignant colonocyte is the likely source of all or most spectroscopic abnormalities of human colon cancer.

INTRODUCTION

IR spectroscopy is a powerful method for the study of not only the structure of chemical compounds but also their relationship to surrounding molecules (1). Its application to tissues has been hampered by problems in sample preparation for optimal spectra acquisition and, most importantly, the strong absorption of IR light by water. By resolving technical and methodological problems (2–4) we were able to apply IR spectroscopy to the study of human tissues (4, 5). Thus, we demonstrated that the IR spectra of microtome sections of malignant colon tissues were significantly different from those of corresponding normal tissues. These changes involved the phosphodiester and C—O stretching bands, the CH stretching region, and the pressure dependence of the CH₂ bending and C—O stretching modes.

Our findings indicated that in colon cancer tissues there were (a) changes in the degree of hydrogen bonding of (i) the phosphodiester groups of nucleic acids (increased); (ii) the C—OH groups of serine, tyrosine, and threonine residues (any or all of them) of cell proteins (decreased); and (iii) the C=O groups of the acyl chains of membrane lipids (increased). In addition, they indicated (b) changes in the structure of proteins and membrane lipids, as judged by the changes in their ratio of methyl to methylene groups, and (c) changes in the packing and the conformational structure of the methylene chains of membrane lipids.

Subsequent work showed that some of these spectroscopic changes are not restricted to colon cancer but are manifested by other human cancers (6). These findings generated two important questions. (a) Which one of the cell types comprising the colonic tissue is responsible for these spectroscopic findings? Since our work examined only tissue sections, the possibility exists that cells other than the malignant cell may be responsible for these spectroscopic differences. (b) What is the mechanism whereby these changes develop. To address this question, an easily controlled experimental system such as that provided by cultured colonocytes is needed. We studied, therefore, by IR spectroscopy, combined with high pressure (pressure tuning IR spectroscopy), seven established colon cell lines. This report describes our findings.

MATERIALS AND METHODS

Cell Lines. Seven human colon adenocarcinoma cell lines were studied. Their salient features are summarized in Table 1. All cell lines were cultured following standard methodology and the specific instructions of the American Type Culture Collection (ATCC, Rockville, MD). Cells were harvested from confluent plates by either trypsinization or gentle scraping off of the tissue culture plates. Control experiments revealed that the IR spectra of these cells were not affected by the method of their harvesting. Briefly, cells were washed with PBS, incubated with 3 ml of trypsin/EDTA (Gibco BRL, Gaithersburg, MD) for 1 min at room temperature, and, after trypsin was aspirated, the cells were incubated at 37°C for 6 min. The detached cells were resuspended in 10 ml PBS, washed once with PBS, aliquoted, and pelleted by centrifugation, all at 4°C. The pellet was stored in liquid nitrogen until analyzed by spectroscopy. Scraping of the cells was done in 10 ml of PBS following their washing with PBS, and the same procedure was followed as for the trypsinized cells.

IR Spectroscopy. Atmospheric pressure IR spectra were obtained by placing small amounts (about 0.01 mg) of cell samples at room temperature between two BaF₂ windows of non-pressure dependency IR adsorption spectra sample holder (2). High pressure studies were performed by applying small amounts (typically 0.01 mg) of cell samples at room temperature, together with powdered α-quartz, onto a 0.37-mm-diameter hole in a 0.23-mm-thick stainless steel gasket mounted on a diamond anvil cell. Pressures at the sample were determined from the 695 cm⁻¹ IR adsorption band of α-quartz, as described previously (13). Fournier transform-IR spectra at various pressures were measured with a Digilab FTS-60 Fourier transform spectrometer using a liquid nitrogen cooled mercury-cadmium-telluride detector. For each spectrum 512 scans were coadded, at a spectral resolution of 4 cm⁻¹. Data reduction was performed using software developed in our Ottawa laboratory.

RESULTS

Fig. 1 shows superimposed the IR spectra of the HCT15 adenocarcinoma cell line and a section of human colon cancer

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2 Unpublished data.

The abbreviation used is: PBS, phosphate buffered saline.
Table 1  Human colon cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>Morphology</th>
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<tbody>
<tr>
<td>LoVo</td>
<td>Adenocarcinoma</td>
<td>Acinar structures, signet ring cells; EM: desmosomes, terminal bars;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>produces CEA*</td>
</tr>
<tr>
<td>SW1116</td>
<td>Adenocarcinoma</td>
<td>Epithelial-like; EM: colonies show brush borders; produces high levels of</td>
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<tr>
<td></td>
<td></td>
<td>CEA</td>
</tr>
<tr>
<td>HCT-15</td>
<td>Adenocarcinoma</td>
<td>Epithelial-like; EM: prominent</td>
</tr>
<tr>
<td>SW403</td>
<td>Adenocarcinoma</td>
<td>Epithelial-like; EM: prominent</td>
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<tr>
<td>SW480</td>
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<tr>
<td>SKCO1</td>
<td>Adenocarcinoma</td>
<td>Undifferentiated</td>
</tr>
<tr>
<td>HT-29</td>
<td>Adenocarcinoma</td>
<td>Moderately well-differentiated</td>
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* CEA, carcinoembryonic antigen; EM, electron microscopy.

Changes in the C—O Band. The relatively weak band at 1172 cm⁻¹ (Fig. 1) is due to the stretching mode of C—O groups of cell proteins (5, 6). The spectra of the HCT15 cell line and human colon cancer tissue in the frequency region 1145–1185 cm⁻¹ are enlarged and plotted superimposed in Fig. 3. Fig. 3A shows the original spectrum and Fig. 3B shows the third power derivative spectrum with a break point of 0.6 (14). The intensity of the lower frequency component band, observed in normal colon tissue and other tissues, is dramatically decreased compared to that of the higher frequency component band, which peaks at 1174 cm⁻¹. In order to evaluate these bands further, we studied the response of their frequency to high pressure (1). The frequency of the lower frequency band decreases with increasing pressure while that of the 1172 cm⁻¹ band increases.

Phosphate Stretching Bands. Fig. 2 shows superimposed the spectra of the asymmetrical PO₂⁻ stretching band of the HCT15 cell line and human colon cancer tissue. Fig. 2A shows the original spectra and Fig. 2B shows the corresponding third power derivative spectra with a break point of 0.3 (14). The frequency of the asymmetrical PO₂⁻ stretching band is at about 1220 cm⁻¹ when the PO₂ group is fully hydrogen bonded and at or above 1240 cm⁻¹ when it is not hydrogen bonded (15). Both the hydrogen bonded and the non-hydrogen bonded asymmetrical PO₂⁻ bands are observed in the spectra of these cell lines. However, the ratio of the intensity of the hydrogen bonded PO₂⁻ band to that of the non-hydrogen bonded PO₂⁻ band is 1:21, which is similar to that of the colon cancer tissue sections (1:15). The remaining adenocarcinoma cell lines gave results similar to those shown here. Since the phosphate stretching band originates in the phosphodiester group of nucleic acids (16), these findings indicate that in adenocarcinoma cell lines the hydrogen bonding of the phosphodiester groups of nucleic acids is increased. This finding is similar to that observed in tissue sections of colon carcinomas. In normal colon tissue the symmetrical PO₂⁻ stretching band peaks at 1082 cm⁻¹ and in colon cancer it is shifted to 1086 cm⁻¹. In adenocarcinoma cell lines this band peaks at 1086 cm⁻¹.

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* CEA, carcinoembryonic antigen; EM, electron microscopy.
Pressure Dependence of the CH$_2$ Bending Modes. The pressure dependence of the frequency of the CH$_2$ bending mode has been used to study interchain packing and order/disorder properties of lipid bilayers (1, 17—19). Fig. 4 displays the pressure dependence of the frequency of the CH$_2$ bending mode of the HCT15 adenocarcinoma cell line. The pressure dependence of the CH$_2$ bending mode is very close to that displayed by normal colon tissues (5). For instance, in the adenocarcinoma cell line discussed here, the atmospheric pressure frequency of the CH$_2$ bending mode increases about 9 cm$^{-1}$ in response to 20 kbar pressure. In normal colon tissue this increase to the same pressure is also about 11 cm$^{-1}$ but in colon cancer tissue it is only 4 cm$^{-1}$.

Changes in the Region 950—1010 cm$^{-1}$. As shown in Fig. 1, there are two bands in this region of the IR spectra of adenocarcinoma colon cell lines, one at 972 cm$^{-1}$ and the other at 991 cm$^{-1}$. Fig. 5A shows enlarged the original spectra of the HCT15 adenocarcinoma cell line. Fig. 5B shows the corresponding third power derivative spectrum with a break point of 0.3 (14). In the spectra of colon tissue the 991 cm$^{-1}$ band is very weak. The band at 972 cm$^{-1}$ is due to nucleic acids (16) and the symmetrical stretching mode of dianionic phosphate with increasing pressure. The response of the 1164 cm$^{-1}$ band to pressure is typical of a hydrogen bonded group: pressure enhances the strength of hydrogen bonds and thereby decreases the frequency of the C—O stretching vibration. On the other hand, the response of the 1172 cm$^{-1}$ band indicates that this band originates from non-hydrogen bonded C—O groups.

Fig. 4. Pressure dependence of the frequency of the CH$_2$ bending mode of membrane lipids of the HCT15 adenocarcinoma cell line and human colon cancer tissue.

Fig. 5. IR spectra of the HCT15 adenocarcinoma cell line and human colon cancer tissue in the region 950—1010 cm$^{-1}$. A, original spectra; B, the corresponding third power derivative spectra.

Fig. 6. IR spectra of four adenocarcinoma colon cell lines in the regions 1145—1185 cm$^{-1}$ (A) and 950—1050 cm$^{-1}$ (B).
monoesters of phosphorylated proteins (20), whereas the band at 991 cm\(^{-1}\) has not been assigned yet.

**IR Spectra in Regions 1145-1185 cm\(^{-1}\) and 950-1050 cm\(^{-1}\).**

The spectra of four cell lines are shown in these two regions of the IR spectrum (Fig. 6). The band near 1152 cm\(^{-1}\), due to carbohydrates (6, 21, 22), varies in intensity among these four cell lines, indicating that different amounts of carbohydrates are present in each one of them. Similarly, the band at 991 cm\(^{-1}\) varies among these cell lines. Since this band is not yet assigned with certainty, the significance of this limited variation is unknown.

The bands at 1172 cm\(^{-1}\) and 972 cm\(^{-1}\) (HCT15 and SKCO1) are shifted to lower frequencies in the HT29 and SW1116 cell lines. The spectra of the cell lines LoVo, SW403, and SW480 are similar to those of the HCT15 and SKCO1 cell lines.

**Amide I Band.** Fig. 7 shows the deconvoluted amide I band spectra of the HCT15 adenocarcinoma cell line and human normal colon and colon cancer tissue. The amide I band in the frequency region 1600–1700 cm\(^{-1}\) is due to the in-plane C=O stretching vibration weakly coupled with C—N stretching and in-plane N—H bending of the amide groups in proteins (21, 23). The peak maximum of the amide I band is sensitive to the secondary structure of proteins (21, 23). The \(\alpha\)-helical structure has its peak maximum near 1650 cm\(^{-1}\), the parallel \(\beta\)-sheet near 1635 cm\(^{-1}\), and the antiparallel \(\beta\)-sheet near 1683 cm\(^{-1}\). The amide I band of the unordered random coil is at around 1645 cm\(^{-1}\) and that of turns at around 1665 cm\(^{-1}\). Since each molecule of a globular protein contains segments with different substructures, the amide I band appears as a broad band with several maxima. The changes in the relative intensities of these maxima are used to monitor changes in the secondary structure of globular proteins.

The spectra in Fig. 7 represent the amide I bands of the entire population of cellular proteins. They show that the cellular proteins are to a large extent \(\alpha\)-helices with considerable segments of \(\beta\)-sheet. The relative amount of \(\beta\)-sheet segment with respect to that of the \(\alpha\)-helical segment is larger in both adenocarcinoma cells and malignant tissue than in normal tissue.

**DISCUSSION**

The understanding of cancer has been greatly facilitated by the study of cultured cells, since their environment can be both defined and manipulated. Our work demonstrates that high quality IR spectra can be obtained reproducibly from human colon cell lines cultured as monolayers and, by extension, from other cells in culture.

Four of the important spectroscopic features observed in colon cancer are also shared by the colon cancer cell lines that we studied: (a) the presence of extensive hydrogen bonding of the phosphodiester groups of nucleic acids (all adenocarcinoma cell lines displayed this phenomenon to essentially the same extent). (b) the hydrogen bonding of C—OH groups of carbohydrates and proteins is reduced in the adenocarcinoma cell lines to roughly the same extent as in colon cancer tissues; (c) the band normally appearing at 1082 cm\(^{-1}\) is shifted to 1086 cm\(^{-1}\); and (d) a prominent band is present at 972 cm\(^{-1}\) in both adenocarcinoma cell lines and colon cancer tissue. It is of note that the changes in the degree of hydrogen bonding of both the phosphodiester and C—OH groups are common to all human malignancies examined to date; they may reflect either a common epiphenomenon or a shared common pathway associated with cancer.

The finding that the degree of disorder of methylene chains of lipids is (a) the same between all of the colon adenocarcinoma cell lines and (b) close to that exhibited by the normal colon tissue indicates one of two possibilities: either this property is not critical to malignant transformation; or the cultured colonocytes that we studied have dispensed with such a requirement.

In this study we explored regions of the IR spectrum not evaluated in our original study of colon cancer, such as the peaks at 972 cm\(^{-1}\) and 991 cm\(^{-1}\) and the amide I band. These findings prompted us to reexamine the spectra of human tissues. Our data show that the 972 cm\(^{-1}\) band, present in normal and malignant tissues, is probably not important to the process of malignant transformation in the colon [although the situation may be different in cervical cancer (6)]. The significance of the findings from the study of the amide I band, which reflect changes in protein structure, which are not apparent at this time, is being studied. Nevertheless, these results suggest that study of the amide I band in cancer tissues may yield useful information.

The precise cell type among those comprising the colon cancer tissue that gives rise to the spectral abnormalities that we have described remains unknown. However, the present data demonstrate that an extensive set of spectroscopic changes are common between the malignant tissue and colon adenocarcinoma cell lines. This observation, therefore, strengthens the argument that the malignant colonocyte is responsible, if not for all, at least for most of the spectroscopic findings associated with colon cancer.

In summary, the colon cancer cell lines studied here demonstrate several of the spectroscopic features displayed by colon cancer tissues. Given the advantages of working with cultured cells, these cell lines may be an appropriate experimental system not only to study the origin of the spectroscopic changes observed hitherto but also to apply the IR technology to other aspects of cancer biology.
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REFERENCES

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