Distinct Infrared Spectroscopic Patterns of Human Basal Cell Carcinoma of the Skin

Patrick T. T. Wong, Sanford M. Goldstein, Roy C. Grekin, Thomas A. Godwin, Chris Pivik, and Basil Rigas

Steacie Institute for Molecular Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada [P. T. T. W., C. P.]; Department of Dermatology, University of California, San Francisco, California 94143 [S. M. G., R. C. G.]; and Departments of Pathology [T. A. G.] and Medicine [B. R.], Cornell University Medical College, New York, New York 10021

ABSTRACT

Infrared spectroscopy combined with high pressure (pressure-tuning infrared spectroscopy) was applied to the study of paired sections of basal cell carcinomas (BCC) and normal skin from ten patients. Atmospheric pressure IR spectra from BCC were dramatically different from those of their corresponding normal skin. Compared to their normal controls, BCCs displayed increased hydrogen bonding of the phosphodiester group of nucleic acids, decreased hydrogen bonding of the C—OH groups of proteins, increased intensity of the band at 972 cm⁻¹, a decreased intensity ratio between the CH₃ stretching and CH₂ stretching bands, and accumulation of unidentified carbohydrates. Some of these changes are shared by all human epithelial malignancies studied to date, while some others appear as yet unique to basal cell carcinoma. The diagnostic value of infrared spectroscopy in BCC remains to be determined.

INTRODUCTION

IRS, a powerful physical method for the study of not only the structure of chemical compounds but also their relationship to surrounding molecules, has been used recently to study human tissues and exfoliated and cultured cells (1-5). Our work has demonstrated all human epithelial malignancies studied to date, while some others appear as yet unique to basal cell carcinoma. The diagnostic value of infrared spectroscopy in BCC remains to be determined.

MATERIALS AND METHODS

Patients. The 10 patients whose tissue samples were studied underwent excision of BCC by Mohs micrographical surgery at the Department of Dermatology, University of California, San Francisco, CA. Normal skin was obtained from tumor-free margins of the excision defect during repair of tissue protuberances (colloquially referred to as "dog ears"). Tissue samples were frozen in liquid nitrogen and stored at −180°C. Histological evaluation of the tissues studied was performed as described (1, 2).

RESULTS

Fig. 1 depicts typical spectra of a pair of tissue sections from BCC and the corresponding normal tissue obtained from a single patient. There are distinct and significant differences between these two spectra. The band peaking at 970 cm⁻¹ is due to the symmetrical stretching mode of dianionic phosphate monomers corresponding to phospholipidated proteins (7) and cellular nucleic acids (8). Several overlapping bands in the frequency region of 1000 to 1080 cm⁻¹, such as the bands of 1031 cm⁻¹ and 1055 cm⁻¹, are mainly due to the vibrational modes of C—OH groups and the C—O stretching coupled with C—O bend involving the C—OH groups of carbohydrates (9). The band at 1082 cm⁻¹ is due mainly to the symmetrical phosphate (PO₂⁻) stretching mode (8). The band at 1162 cm⁻¹ is due to the C—O stretching mode of the C—OH groups of serine, threonine, and tyrosine residues of cell proteins as well as the C—O groups of carbohydrates (9). The band at 1241 cm⁻¹, due to the asymmetrical phosphate (PO₃⁻) stretching mode, originates mainly in the phosphodiester groups of nucleic acids (8). The bands at 1403 and 1456 cm⁻¹ arise mainly from the symmetrical and asymmetrical CH₂ bending modes, respectively, of the methyl groups of proteins (9).

The tissue sections used in this study varied with respect to their histological composition. The proportions of epidermis and dermis (the fibroelastic connective tissue supporting epidermis) ranged for each between 20 and 80%. Several smaller segments of each sample were examined, and the variation in the spectra was minimal. Several of the regions of the infrared spectrum noted above have already provided significant information about human colon and cervical cancer (1, 2, 4) and were studied in greater detail. These findings are presented below.

Phosphate Stretching Bands. Fig. 2 compares the spectra of the asymmetric PO₃⁻ stretching band between normal skin and BCC. Fig. 2A shows the original spectra, and Fig. 2B shows the corresponding third power derivative spectra with a break point of 0.4 (10). In the frequency region between 1215 and 1250 cm⁻¹, the spectrum of BCC is resolved into component bands, one peaking at 1220 cm⁻¹ and the other at about 1245 cm⁻¹. In contrast, in the same region the spectrum of the normal tissue has no detectable 1220 cm⁻¹ band, and there is only one band peaking at around 1240 cm⁻¹. The frequency of the
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The asymmetrical PO$_2^-$ stretching band is at about 1220 cm$^{-1}$ when the PO$_2^-$ group is fully hydrogen bonded and at or above 1240 cm$^{-1}$ when it is not hydrogen bonded (11). In BCC, therefore, a significant fraction of the phosphodiester groups are hydrogen bonded, in contrast to those of normal skin.

The pressure dependence of the frequencies of these bands strengthens this conclusion (Fig. 3). The frequency of the low-frequency band decreases with increasing pressure, whereas that of the high-frequency band of cancer and the single PO$_2^-$ band of normal tissue increases with increasing pressure. The response of the low-frequency band to pressure is typical of a hydrogen-bonded group, whereas that of the high-frequency band, common to malignant and normal tissue, is characteristic of a non-hydrogen-bonded functional group (12).

Since the phosphate stretching band originates almost exclusively in the phosphodiester group of nucleic acids (8), these findings indicate that in BCC a significant portion of the phosphodiester groups of nucleic acids are hydrogen bonded, whereas no such hydrogen bonding is detectable in normal tissue. This finding is similar to that observed in tissue sections of all human cancers examined to date by us (to be published elsewhere).

The intensity of the symmetrical PO$_2^-$ stretching band is dramatically increased in BCC as compared to normal skin. This band in normal skin peaks at 1082 cm$^{-1}$ (1082.6 ± 0.28, mean ± SD; range, 1082.2 to 1083.1 cm$^{-1}$), whereas in BCC this peak is shifted to 1086 cm$^{-1}$ (1085.4 ± 0.45, mean ± SD; range, 1085.0 to 1086.4 cm$^{-1}$). Both the increased band intensity and shifted peak position to a higher frequency are common to all human cancers that we have studied, including those of colon, esophagus, stomach, liver, ovary, cervix, vagina, and breast (1, 2, 4, and to be published elsewhere).

The C—O Band. The spectra in the frequency region of 1140 to 1185 cm$^{-1}$ are enlarged and plotted in Fig. 4. Fig. 4A shows the original spectra, and Fig. 4B shows the third power derivative spectra with a break point of 0.4 (10). Even in the original spectrum it is
evident that the C—O band consists of component bands. The derivative spectra confirm this and demonstrate that each consists of two component bands. For normal tissue, these two bands peak at 1163 and 1173 cm$^{-1}$, whereas for BCC they peak at 1152 and 1173 cm$^{-1}$. The band at 1153 cm$^{-1}$ originates from the C—O groups of carbohydrates, and those at 1161 cm$^{-1}$ and 1172 cm$^{-1}$ originate from the C—O groups of cellular proteins (9). These three bands were evaluated further by studying the response of their frequency to high pressure. For demonstrative purposes we present in Fig. 5 such a pressure study from a tissue sample consisting of both normal and malignant elements, where all three bands are present. The frequency of the two lower-frequency bands decreases with increasing pressure, while that of the 1173 cm$^{-1}$ band increases with increasing pressure. Therefore, the bands at 1152 cm$^{-1}$ and 1163 cm$^{-1}$ originate from hydrogen-bonded C—O groups, and that at 1173 cm$^{-1}$ from non-hydrogen-bonded C—O groups (12). These findings indicate that in BCC there is substantial reduction in the amount of hydrogen-bonded C—O groups of amino acid residues of cell proteins, as well as an accumulation of some unidentified carbohydrate(s).

The C—H Stretching Band. As shown in Fig. 6, in BCC the intensity of the CH$_2$ stretching band at 2851 cm$^{-1}$ is increased, whereas that of the CH$_3$ stretching band at 2958 cm$^{-1}$ is decreased.

This indicates that the ratio of the number of methyl groups to that of methylene groups is decreased in malignant tissue, as compared to normal colon tissue. While this ratio is decreased in the malignant tissue of all pairs of normal and malignant tissues that we studied, its absolute value varies from patient to patient. Of note, such a change has also been observed in human colon cancer (1). The 970 cm$^{-1}$ Band. As shown in Fig. 1, in BCC there is a dramatic increase in the intensity of the band at 970 cm$^{-1}$, compared to that of normal skin.

Pressure Dependence of the CH$_2$ Bending Mode. 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 (12, 13). Fig. 7 displays the pressure dependence of the frequency of the CH$_2$ bending mode of a BCC and its corresponding normal skin tissue. A discontinuous increase in the frequency of the CH$_2$ bending mode is observed at about 6 kbar for cancer tissue and at about 11 kbar for normal tissue. This increase in frequency with pressure results from the conformational and orientational ordering of the methylene chains of lipids (13). These pressure behaviors of the CH$_2$ bending mode are quite different from those observed in the normal and cancerous colon tissues and cervical cells (1, 4). However, similar pressure dependencies of the CH$_2$ bending mode in the skin tissues have been observed in the control liver tissue and liver tumor tissue of mice (14). It was known that large amounts of triacyl glycerol were accumulated in these liver tissues. The behavior of the CH$_2$
bending frequency under pressure and the pattern of the pressure-induced frequency shift observed in these liver tissues resemble those of unsaturated lipids (13). Therefore, the properties of the lipid CH$_2$ chains observed in these liver tissues were largely originated from the accumulated triacyl glycerol. Large amounts of triacyl glycerol are also accumulated in the skin samples for the present work. These accumulated lipids can be easily seen under the microscope as fine oil drops. Consequently, the observed pressure behaviors of the CH$_2$ bending mode of the cellular lipids are undoubtedly contributed mainly from the accumulated triacyl glycerol. Fig. 8 demonstrates that the discontinuities at about 6 kbar for the cancer tissue and at about 11 kbar for the normal tissue in the pressure-induced frequency shifts are also observed for the 1454 cm$^{-1}$ band, which is due to the asymmetrical bending mode of methyl groups (9). These methyl groups could be either the end methyl groups and the branched methyl groups of the accumulated lipids or the methyl side groups of proteins. They are most likely the end methyl groups and the branched methyl groups of the lipids, since the pressure dependencies of the methyl bending mode are similar to those of the CH$_2$ bending mode of the accumulated triacyl glycerol.

**DISCUSSION**

Our data demonstrate that the IR spectra of BCCs of the skin differ from those of normal skin. These spectroscopic differences suggest that there are significant structural changes associated with BCC, which encompass several components of the cell, in agreement with the expected complexity of the malignant phenotype. BCCs displayed invariably increased hydrogen bonding of the phosphodiester groups of nucleic acids and decreased hydrogen bonding of the C—OH groups of proteins. BCCs also showed a significant shift of the 1082 cm$^{-1}$ peak. These three changes are common to all human cancers studied to date. The decreased intensity ratio between the CH$_3$ stretching and CH$_2$ stretching bands is also present in colon cancer. In contrast, the increase in the intensity of the 970 cm$^{-1}$ band, present in cervical cancer and some adenocarcinoma cell lines, is not observed in colon cancer tissue. The accumulation of carbohydrates and the response of its membrane lipids to high pressures appear limited to BCC.

It is not apparent how the changes associated with BCC are caused within the cell. We have already speculated that the C—O changes may arise from phosphorylation of cellular proteins by oncogenes (1). Whatever their mechanism, our findings indicate that there are a number of chemical changes, reflected in the IR spectra, which are common to several human cancers. Whether these changes represent a common epiphenomenon or part of a shared pathway in carcinogenesis remains to be evaluated.

IRS, as these findings suggest, can provide a useful way of looking at important aspects of the malignant cell. Not only can it provide structural information on intact tissues and individual cells, but it can also evaluate physical-chemical parameters of potentially critical molecules in their native state. For example, the changes in carbohydrates, lipids, and nucleic acids that we have observed could lead to several testable hypotheses that may be relevant to cancer biology.

From the point of view of its application to tissues, IRS has three distinct advantages: (a) it requires samples of very small size (e.g., 1 × 1 × 1 mm) to obtain spectra with a favorable signal-to-noise ratio and a high information content; (b) no preparation of the tissue or the cells by using fixatives or other means is necessary; and (c) evaluation of tissue or cells by IRS can be extremely fast (in general, it takes less than 10 min to obtain an IR spectrum from the time the sample is acquired).

The spectroscopic changes, displayed by all samples tested, are of sufficient magnitude to distinguish clearly the malignant from the benign tissues. These findings raise the possibility that one or more IR spectroscopic parameters may become useful in the diagnostic evaluation of skin tissue sections. However, such a possibility requires further exploration, especially in view of the relatively small number of patients studied and the need to also evaluate other skin diseases.

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