Human breast carcinomal tissues display distinctive FTIR spectra: implication for the histological characterization of carcinomas

Tiyu Gao, Jun Feng and Yunxiang Ci *

Department of Chemistry, Peking University, Beijing 100871, China

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Fourier transform infrared spectroscopic study of human breast normal and carcinomal tissues has been carried out. Some distinctive spectral differences which are mainly due to nucleic acids and proteins are observed between normal and carcinomal tissues. This method of analysis results in nearly 100% diagnostic accuracy of carcinomal tissues from normal tissues. The spectral patterns of well-differentiated carcinomal tissues exhibit marked heterogeneity, however that of poorly differentiated carcinomas demonstrate significant similarity. Apocrine, tubular, intraductal and mucinous carcinomas and invasive infiltrating ductal carcinomal tissues can be discriminated based on their characteristic spectra. The spectral differences confirm the possibility of using FTIR as an accurate and rapid technique to distinguish between normal and malignant breast tissues and classify breast carcinomas in different subtypes.

Keywords: Infrared spectroscopic features, breast carcinomal tissues, clinical classification

1. Introduction

Breast cancer is a world-wide and frequent disease among women. Histopathological examination, mammographic or ultrasonic imaging, computerized tomography, magnetic resonance imaging [1], flow cytometry [2], and biological markers [3] have been used to detect the malignancy of breast tissues. However, these methods are either subjective, imprecise, labour intensive or costly. Alternative detecting methods which are accurate, inexpensive and rapid are required. And in fact, other techniques have been under investigation for reducing the current reliance on the traditional diagnostic methods. Fourier transform infrared (FTIR) spectroscopic analysis represents a powerful tool since it can furnish important indications of the biological and physicological alterations of tissues or cells which are related to the changes of components, molecular structures and intra-/intermolecular interactions. This physical method has been applied to examine normal and neoplastic cells or tissues: leukemic lymphocytes and lymphoblasts [4–7], human cervical cells during carcinogenesis [8–13], human colon adenocarcinoma cell lines [14,15], lung tumour cells [16,17], skin tumour [18] and human breast tumours [19,20]. Among them, the spectral differences between normal and malignant cells or tissues were intensively studied. Breast cancer presents varied histological types, but the spectral differences among the histological types of breast carcinomal tissues are rarely reported. The purpose of this study is to assess the possibility of using FTIR spectroscopy to distinguish between normal and malignant breast tissues and to classify the carcinomal tissues in different subtypes.

2. Experimental

2.1. Materials and procedures

Forty two surgical specimens of human breast tissues were obtained from breast resection (lumpectomy and mastectomy) in the First People's Hospital, Beijing Medical University. Each sample was cut into two pieces, the first was sent to the pathologists for evaluation by standard pathological methods; the second was washed with 0.9% NaCl solution and frozen

*Corresponding author: Department of Chemistry, Peking University, Beijing, China, 100871. Tel.: 0086 10 62751499; Fax: 0086 10 62751708; E-mail: ciyx@chemms.chem.pku.edu.cn.

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in liquid nitrogen immediately after collection until used for IR spectroscopic studies. Small amounts of the frozen tissue sample was minced, then dispersed, and finally centrifuged [16]. The cell suspension was evenly spread over the surface of a specially designed infrared window of BaF_2, then the sample was placed in a vacuum desiccator and allowed to dry to form a even film. Among them, 11 samples were diagnosed cytologically as invasive infiltrating ductal carcinoma (IIDC) tissues; 8 samples were diagnosed as IIDC with a predominant intraductal component; 3, 4, 4, 5, 3 samples were diagnosed as aprocine, tubular, mucious, simplex and medullary carcinomal tissues, respectively. Normal breast tissues were from 6 noncancerous patients.

2.2. Apparatus and data analysis

Infrared spectra in the 400–4000 cm^{-1} region were recorded on a Bruker Vector 22 Fourier transform spectrometer equipped with an air-cooled DTGS detector. Infrared window of BaF_2 without cell suspension was scanned as the background for each spectrum; 512 scans were co-added at a spectral resolution of 2 cm^{-1}. Residual water vapor was interactively subtracted. To minimize problems from avoidable baseline shifts, the spectra were baseline-corrected.

3. Results

3.1. The spectra and the assignment of the absorption bands of carcinomal tissues

At the beginning of this investigation, many tests were carried out to obtain samples giving reliable and reproducible spectra. Two or three spectroscopic samples were prepared and determined for each tissue. As shown in Fig. 1 (A and B), the infrared spectral patterns of the human breast normal and IIDC tissues exhibit an appreciable degree of reproducibility in our experiments. The representative superimposed spectra of normal and IIDC are shown in Fig. 2. The representative infrared spectra in the frequency region 950 to 1500 cm^{-1} of the well-differentiated and poorly differentiated carcinomal tissues are shown in Fig. 3 and Fig. 4, respectively. The typical infrared spectra in the frequency region 2800 to 3050 cm^{-1} of carcinomal tissues are shown in Fig. 5. Based on the previous IR studies on cellular organelles and macromolecules [4,14–16,20,21], the assignment of infrared bands of breast tissues can be established. As shown in Figs 1–4, the weak and broad peak near 970 cm^{-1} is generally assigned to the symmetric stretching mode of dianionic phosphate monoester of cellular nucleic acids. The bands near 1032 cm^{-1} and 1052 cm^{-1} are mainly from the vibrational modes of $–CH_2OH$ groups and the C–O stretching coupled with C–O bending of the C–OH groups of carbohydrates and carbohydrate residues. The peaks near 1083 cm^{-1} and 1238 cm^{-1} are from both collagen and nucleic acid. The bands at 1083 cm^{-1} is due mainly to the symmetric phosphate (PO_2^-) stretching modes (ν s PO_2^-) of nucleic acids and the vibrational modes of collagen carbohydrates residues, and 1238 cm^{-1} is due to the asymmetric phosphate (PO_2^-) stretching modes (ν as PO_2^-) of nucleic acids and the amide III/CH_2 wagging vibrations of collagen. As for the absorption band at 1095 cm^{-1} in the spectrum of the tubular carcinomal tissue, it can be assigned to the degenerate stretching of dianionic phosphate monoester [21]. The peaks near 1205, 1281 and 1338 cm^{-1} arise from the amide III/CH_2 wagging vibrations of collagen [20]. The band near 1164 cm^{-1} can be assigned to the C–O stretching modes of the C–OH groups of cell proteins and the C–O groups of carbohydrates. The weak peaks near 1400 cm^{-1} and 1456 cm^{-1} mainly arise from the symmetric and asymmetric bending vibrational modes of methyl groups of proteins (δ s CH_3 and δ as CH_3), respectively. Peaks near 2853 $cm^{-1}$and 2925 cm^{-1} originate from ν s CH_2 and ν as CH_2, 2958 cm^{-1} is from ν as CH_3 of proteins, lipids and DNA.

3.2. The spectroscopic differences between normal and IIDC tissues

As shown in Fig. 2, some remarkable spectral differences can be observed between the normal and IIDC tissue samples. The predominant differences include the following findings. Firstly, the peak at 970 cm^{-1} is sharper and stronger, the intensity of the peak near 1032 cm^{-1} is decreased sharply and the peak almost disappears; the serious bands at 1205, 1281 and 1338 cm^{-1} are weaker and broader for carcinomal tissue whereas the band near 970 cm^{-1} is weaker and almost disappears. The serious peaks of collagen are sharper and stronger for normal tissues. These results imply the increase of nucleic acids and the decrease of the relative content of collagen in carcinomal tissues. Secondly, the band near 1164 cm^{-1} band in normal tissues shifts to 1169 cm^{-1} in carcinomal tissue. The shift of about 5 cm^{-1} of the frequency in the IIDC

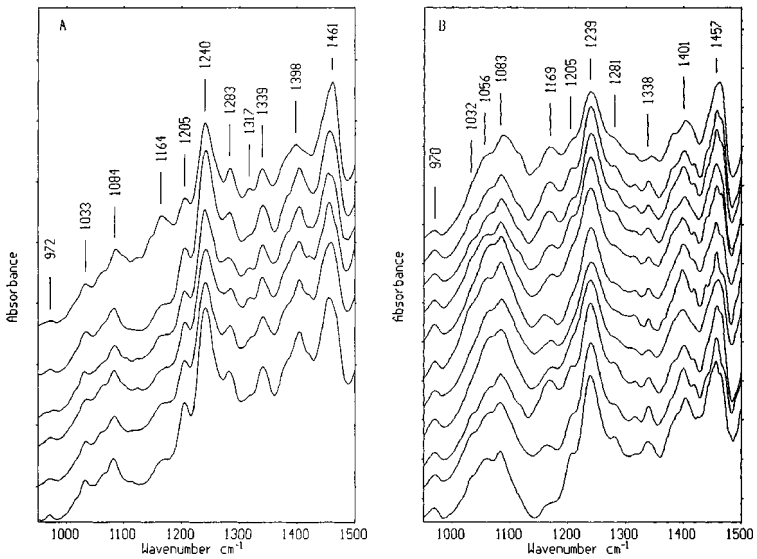

Fig. 1. This figure shows the appreciable reproducibility of the FTIR spectra in the frequency region 950 to 1500 cm^{-1} of human breast normal tissues (A) and invasive infiltrating ductal carcinomal tissues (B).

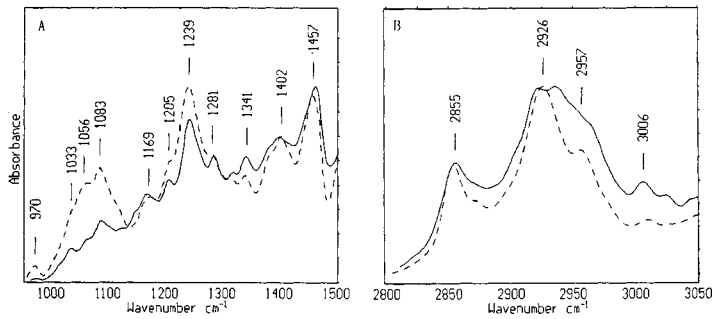

Fig. 2. This figure shows the remarkable spectral differences between human breast normal tissues (—) and invasive infiltrating ductal carcinomal tissues (- - -) in the frequency region 950 to 1500 cm^{-1} (A) and 2800 to 3050 cm^{-1} (B): the peak at 970 cm^{-1} is sharper and stronger, the serious bands at 1205, 1281 and 1338 cm^{-1} are weaker and broader for carcinomal tissue than that for normal tissues; the band near 1164 cm^{-1} band in normal tissues shifts to 1169 cm^{-1} in carcinomal tissue; the absorbance ratio A_{2958}/A_{2853} in carcinomal tissue is smaller than that of normal tissue. These results imply the increase of nucleic acids and the decrease of the relative content of collagen in carcinomal tissues.

samples was observed, and the shift indicates the increase of malignant cells in carcinomal tissue, because the band near 1169 cm^{-1} band is mainly attributed to the C–O stretching mode of phosphorylated proteins in parenchymal cells [14] or is mainly from malignant epithelial cells [15]. Thirdly, infrared spectra in the frequency region 2800 to 3050 cm^{-1} of normal and carcinomal tissues are shown in Fig. 2B. The band at 2853 cm^{-1} is mainly due to the ν as CH_2 mode, the 2958 cm^{-1} band is due to the ν as CH_3 mode. The absorbance ratio between the methyl stretching band and methylene band A_{2958}/A_{2853} in carcinomal tissue is smaller than that of normal tissue. This trend indicates a decrease in number of methyl groups compared to methylene groups in IIDC tissue. The slight decrease of A_{2958}/A_{2853} ratio from normal epithelium to malignant epithelium or to malignant tissues was observed [10,15] and explained as the results of hypomethylation [22]. However, compared to normal and malignant epithelial tissues, the A_{2958}/A_{2853} ratio increases dramatically in connective tissue and this result was explained as the main contribution of the large number of methyl groups in protein fibers [15]. Therefore, the decrease of A_{2958}/A_{2853} ratios in carcinomal tissues may be contributed from three factors: hypomethylation of DNA [22], the increase of malignant epithelial components and the decrease of protein fibers in carcinomas, but the latter two are the main factors.

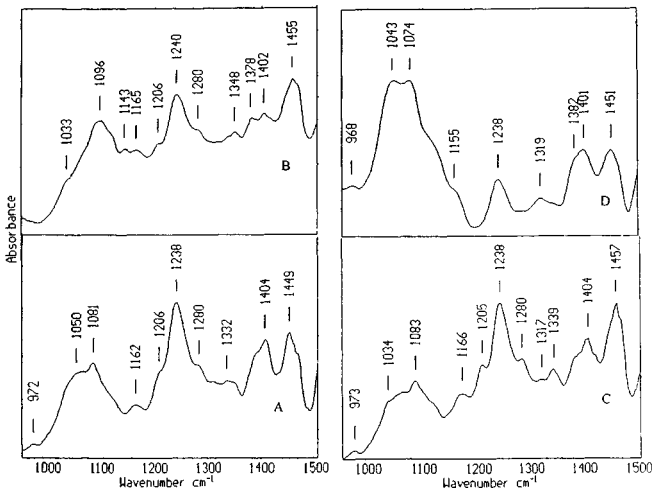

Fig. 3. This figure shows the significant differences among the representative FTIR spectra in the frequency region 950 to 1500 cm^{-1} of human breast (A) apocrine, (B) tubular, (C) intraductal, (D) mucinous carcinoma: the spectral patterns of apocrine and mucinous carcinomas are completely different from others; $A_{1449}/A_{1404} \approx 1.0$ in apocrine carcinoma whereas $A_{1457}/A_{1404} \gg 1.0$ in intraductal carcinoma.

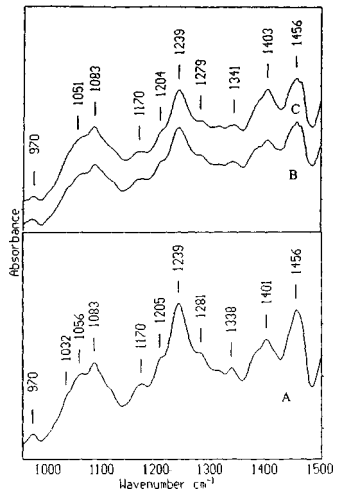

Fig. 4. This figure shows the differences among the representative FTIR spectra in the frequency region 950 to 1500 cm^{-1} of human breast (A) invasive infiltrating ductal carcinoma (IIDC), (B) simplex, (C) medullary carcinomas: this band near 1170 cm^{-1} in IIDC sample is sharper and stronger than that in simplex and medullary carcinomas; the ratio of A_{1238}/A_{1403} in the spectrum of simplex carcinomal tissue is lower than that of medullary carcinoma.

3.3. The spectroscopic comparison of carcinomal tissues

Figures 3 and 4 represent the spectra of the well and poorly differentiated carcinomas, respectively. These spectra exhibit their characteristic differences and the important spectroscopic parameters are listed in Table 1. In the spectrum of apocrine carcinomal tissue, the intensity of band at 1404 cm^{-1} is almost similar to that of at 1449 cm^{-1}, but they are lower than that at 1238 cm^{-1}. The peak at 970 cm^{-1} disappears, while a strong absorption band at 1096 cm^{-1} from the degenerate stretching of dianionic phosphate monoester emerges and overlaps the band of ν s PO_2^- at 1083 cm^{-1} in the spectrum of tubular carcinomal tissues. In the intraductal carcinomal tissue, the relative intensities of bands at 1205 and 1280 cm^{-1}, which are from the collagen, are the strongest in all the carcinoma samples, the intensity of the peak at 1034 cm^{-1} is almost similar to that at 1050 cm^{-1}. As for the mucinous carcinomal tissue, the main peaks between 1025–1180 cm^{-1} appearing at 1043, 1076 and 1155 cm^{-1}, are completely different from the bands appearing at 1032, 1055, 1083 and 1166 cm^{-1} in other carcinoma samples. The collagen absorption bands at 1204 and 1280 cm^{-1} disappear in mucinous carcinomal tissue. This result indicates the relative content of collagen is very low in mucinous carcinomal tissue. In poorly differentiated carcinomas, the most remarkable feature is the appearance of the band at 1170 cm^{-1}, which positively shifts 4–10 cm^{-1} compared to that of other carcinoma samples, but this band in IIDC sample is sharper and stronger than that in simplex and medullary carcinomas. The spectroscopic profile of simplex carcinomal tissue is similar to that of medullary carcinomal tissue, but the relative intensity ratios of main bands are considerably different. The ratio of A_{1238}/A_{1403} in the spectrum of simplex carcinomal tissue is much higher than that in medullary carcinoma. As shown in Fig. 5, among the intensity ratios of A_{2958}/A_{2853} of carcino-

Table 1
The FTIR spectroscopic parameters of breast carcinomal tissues

Tissue/Parameter	1025∼1100			1160	A_{1453}/A_{1240}	A_{1453}/A_{1403}
Aprocrine	1031	1050	1081	1162	<1.0	≈ 1.0
Tubular	1033		1096	1165	>1.0	$\gg 1.0$
Intraductal	1034	1055	1083	1166	≈ 1.0	$\gg 1.0$
Mucinous	1043	1076		1155	>1.0	≈ 1.0
IIDC	1032	1056	1083	1170	<1.0	>1.0
Simplex	1033	1051	1083	1170	>1.0	$\gg 1.0$
Medullary	1033	1051	1083	1170	>1.0	>1.0

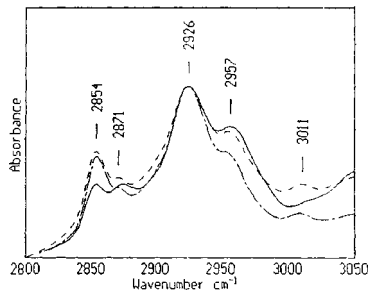

Fig. 5. This figure shows the differences of A_{2958}/A_{2853}: the ratio is the highest in (a) tubular carcinoma (—), almost similar in (b) other carcinomal tissues such as invasive infiltrating ductal medullary carcinomal tissues (- - -), the smallest in (c) mucinous carcinoma (— - — -).

mal tissues, the ratio in tubular carcinomal tissue is the highest, that in mucinous carcinoma is the smallest, but the ratios in other carcinomas are almost similar. The results are consistent with the changes of the relative content of collagen histologically observed in breast carcinomal tissues [23]. Thus, the differences of A_{2953}/A_{2853} ratio in tubular, mucinous and IIDC carcinomal tissues are mainly from the differences of the relative content of collagen in these tissues. Summarily, the spectra of carcinomal tissues show some distinct differences in spectroscopic parameters such as the profile, the band frequencies, the relative intensity ratio, and these spectroscopic differences can be used to classify the carcinomal tissues.

Furthermore, the spectra of well-differentiated and poorly differentiated carcinomas also demonstrate some different spectral features. The absorption bands between 1032–1180 cm^{-1} in well-differentiated carcinomal tissues exhibit marked heterogeneity, whereas the spectral profiles in poorly differentiated carcinomas are almost similar. Figure 6 shows the absorption band in the frequency region 1140 to 1180 cm^{-1}. For well differentiated carcinomas, the band emerges peaking below 1167 cm^{-1}; for poorly differentiated carcinoma, the band is broader, and is composed two bands

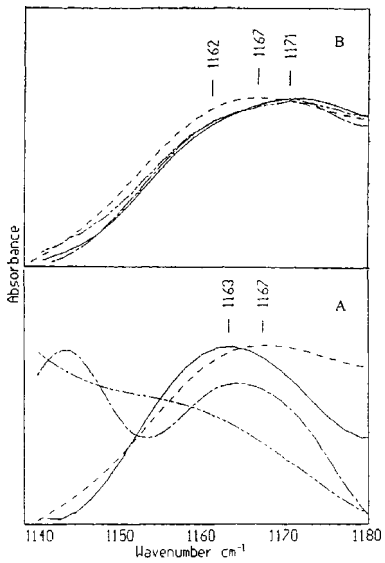

Fig. 6. This figure shows the spectral differences in the region of 1140–1180 cm^{-1} between (A) well differentiated carcinomas: (a) apocrine (—), (b) tubular (— - — -), (c) intraductal (- - -), (d) mucinous (— - - — - -) and (B) poorly differentiated carcinomas: (a) invasive infiltrating ductal (—), (b) simplex (— - — -), (c) medullary carcinoma (— - - — - -), (d) intraductal (- - -): the band of well differentiated carcinoma emerges peaking below 1167 cm^{-1}, the band of poorly differentiated carcinoma is broader, and is composed two bands peaking at 1162 cm^{-1} and 1170 cm^{-1}.

peaking at 1162 and 1170 cm^{-1}. These results may reflect the heterogeneity in well-differentiated carcinomas and the higher relative content malignant epithelial cells in poorly differentiated carcinomas.

4. Discussion

Breast carcinoma is a very heterogeneous disease clinically and pathologically. The differences of the character or composition of carcinoma result in variation of the chemical compositions of the carcinomas. Some distinctive spectral differences which are

mainly due to nucleic acids and proteins are observed between normal and malignant breast tissues. This method of analysis results in nearly 100% diagnostic accuracy of carcinomal tissues from normal tissues. The spectral patterns of well-differentiated carcinomal tissues exhibit marked heterogeneity, but the profiles of poorly differentiated carcinomas demonstrate significant similarity. Apocrine, tubular, intraductal, mucinous, IIDC carcinomal tissues can be discriminated from each other based on their characteristic spectra. However, more cases and effort are needed to distinguish between simplex and medullary carcinomal tissues. Nonetheless, FTIR spectroscopic analysis at the molecular level demonstrate a significance in aiding histological characterization of breast carcinomal tissues. This method is inexpensive, possible to automate, and requires minimal amounts of samples and can be of great assistance to the practicing clinician. The establishment of an analytical system of breast carcinomal tissues is in progress in our laboratory.

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