Molecular and chemical characterization by Fourier transform infrared spectroscopy of human breast cancer cells with estrogen receptor expressed and not expressed

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Abstract. The present study was designed to identify and compare the infrared absorption spectra of two human breast cancer cell lines: MCF-7 (estrogen receptor expressed, ER+) and SKBr3 (estrogen receptor non-expressed, ER−). Comparison between SKBr3 and MCF-7 cells revealed differences in the following absorption band areas: 1087 cm^{−1} (DNA), 1397 cm^{−1} (CH₃), 1543 cm^{−1} (amide II), 1651 cm^{−1} (amide I), 2924 cm^{−1} (fatty acids). Additionally, peak shifts were observed at 1122 cm^{−1} (RNA), 1397 cm^{−1} (CH₃), 1651 cm^{−1} (amide I), 2851 cm^{−1} (fatty acids) and 2962 cm^{−1} (fatty acids). An analysis of the ratio between band areas was conducted, in order to obtain an index that could effectively distinguish between these two cell lines. The following ratios were found: 1650 cm^{−1}/1540 cm^{−1}, 1650 cm^{−1}/1740 cm^{−1}, 1650 cm^{−1}/1084 cm^{−1} and 1120 cm^{−1}/1084 cm^{−1}. This work demonstrates that it is possible to distinguish between MCF-7 and SKBr3 cells through differences in their FTIR spectra. This work enables distinction between two cell lines from the same breast cancer.

Keywords: FTIR, SKBr3, MCF-7, cell, cancer, vibrational spectroscopy

1. Introduction

Breast cancer is the most frequently diagnosed cancer in women aged between 40 and 55, and it is heterogeneous in its clinical, genetic and biochemical profile. The large majority of the affected women present with a breast mass or mammographic abnormality as the only clinically detectable manifestation of the disease; yet, approximately 30 percent of the women diagnosed with breast cancer go on to develop metastatic disease that is ultimately fatal [1]. Distinct characteristics of the breast carcinoma can be exploited, in order to help determine the lifetime risk of disease development and the overall prognosis after diagnosis of breast carcinoma, as well as the chance of response to a specific therapy. In addition, increased understanding of breast carcinoma pathways may enhance our ability to design

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targeted approaches for prevention or therapy. Thus, the biology of breast carcinoma can contribute vital information regarding many aspects of the disease.

Estrogens induce various physiological effects that allow the normal development and growth of female reproductive tissues, as well as regulation of bone integrity, cardiovascular function and central nervous system [19]. Aberrant expression of estrogen can induce pathophysiological effects that give rise to the growth of tumors, in particular those of the breast. Estrogen receptors are required for estrogen-stimulated growth and proliferation of breast cancer. They are found to some degree in 50–80% of breast tumors [2,6]. Endocrine treatments have been designed, to antagonize the effects of estrogen [1]. Approximately 70–80% of all breast tumors express the ER protein, and therefore they are termed ER positive (ER+). These tumors tend to grow more slowly, are better differentiated, and are associated with a slightly better overall prognosis [9]. Thus, ER expression is one of a few prognostic factors, along with auxiliary lymph node status, tumor size and histological grade [6,7,9]. In this context, the present study involved analysis of the Fourier transform infrared (FTIR) spectra of the breast cancer cell lines MCF-7 (ER+, or estrogen receptor positive) and SKBr3 (ER–, or estrogen receptor negative).

Vibrational spectroscopy has attracted a lot of attention in medicine because it can serve as an auxiliary method in the diagnosis of certain diseases, being a non-invasive technique [5,10,11,24]. This technique has several advantages, such as sensitivity, speed and the reagent-free nature of the measurement. FTIR spectroscopy can detect changes in the chemical composition and morphology of intact cells, including certain differences between different cell populations such as normal and tumor cells [11].

FTIR has several advantages, such as sensitivity, velocity and no requirement for staining or reagents. It is able to detect changes in the chemical composition of intact cells, making it useful for spotting differences in cell populations: for example, to tell the difference between normal and tumoral cells [3,20,23,25]. The absorption spectrum in the infrared spectral range provides information regarding the identification of the covalent bonds of a molecule mainly, but additional information about molecular conformation and intermolecular interactions can be extracted from the spectrum [12,17,27–29].

2. Material and methods

2.1. Cellular cultures

SKBr3 cells and MCF-7 cells are human breast cancer cell lines (ATCC, Manassas, VA, USA), and thus are presented in Fig. 1. These cells were grown at 37°C in a 5% CO₂ atmosphere, maintained in RPMI 1640 medium without phenol red (RPMI-S) supplemented with 7.5% fetal bovine serum (Gibco-Ivitrogen, BRL). Prior to the experiments, the cells had been washed and centrifuged (1000 rpm, 15 min) in a solution of 0.9% NaCl, to remove all growth medium, according to procedures described by Mostaço-Guidolin et al. [15].

To guarantee that the influence of the number of cell behavior (film/agglomerates) would not affect the spectral difference, a normalization conducted by band area calculation was employed. The band area from one specific compound (in this case, PO₂) was used to normalize all band areas obtained in all spectral data, and the effects related to the quantity of cells or cell agglomeration were minimized. In this work, we have cultured the samples of each cell line independently. This procedure was repeated five times for each cell line, respecting the same conditions of growth, drying procedures and spectral acquisition setups.

Fig. 1. Photos of MCF-7 cells (left) and SKBr3 cells (right) (ATCC, Manassas, VA, USA).

2.2. Spectroscopic measurements

The spectra were acquired on a Nicolet 380 spectrophotometer (Thermo Scientific Nicolet™, Waltham, MA, USA) at a 0.5 cm^{-1} resolution, 32 scans, in the $4000\text{--}900\text{ cm}^{-1}$ spectral range. For the FTIR measurements, cells were deposited on IR transparent CaF_2 windows (approximately $10\text{ }\mu\text{l}$ with $10^7\text{--}10^8$ cells). The samples were dried with N_2 for about 10 min. The fast drying procedure does not remove the entire water content from the sample but, we chose this methodology to preserve the biological characteristics of the cells. Another possibility would have been to lyophilize the entire sample, but this was not applied in this work because we were searching for fast procedures for cell identification and classification. For this reason it is important to remember during the discussion that the water content can introduce some errors to the band area measurement.

2.3. Data analysis

Before spectral analysis, the background signal was removed by using a straight line method from the Origin 8.0 software (OriginLab Corporation, MA, USA). The spectral data sets were analyzed in two ways: first, the spectral shifts in the absorption peaks were evaluated through analysis of the second derivatives of the absorbance spectrum. All the spectra were normalized to the band area positioned at 1240 cm^{-1} (PO_2 asymmetric stretching). Following normalization, the average area of each band was calculated. A method of an algebraic sum of trapezoids was employed for area measurement. The differentiated values were calculated by averaging the slopes of two adjacent points for each data point. Both analyses, namely band area and peak displacement, were conducted by Student *t*-test statistics using $p < 0.05$ as presented in Table 1.

Ratios to discriminate between the two cell lines were selected from the references and from the observations of the present work. A list of ratios that better describe the differences between the two cell lines can be observed in Table 2. The ratio analysis has the objective to achieve biomarkers; i.e., a ratio between two bands that can differentiate between two cell lines. The significance of the ratio represents a relative proportion between a determined compound; e.g., in the first column of Table 2 there is the amide I/amide II ratio, the ratio $1650\text{ cm}^{-1}/1540\text{ cm}^{-1}$ represents the relative composition of amide I to

Table 1

FTIR bands obtained for the SKBr3 and MCF-7 (negative and positive estrogen-receptor breast cancer cells, respectively) cell lines

SKBr3		MCF-7		Chemical assignment
Band area (a.u.)	Peak position (cm ⁻¹)	Band area (a.u.)	Peak position (cm ⁻¹)	
0.35 ± 0.12	1039.80 ± 0.40	0.11 ± 0.01	1042.60 ± 0.10	ν C–O
0.17 ± 0.04	1087.20 ± 0.18	0.29 ± 0.08	1084.80 ± 0.05	ν PO ₂ sym.
0.08 ± 0.02	1122.00 ± 0.40	0.10 ± 0.02	1120.80 ± 0.04	ν C–N and ν C–C
1.00	1232.30 ± 0.14	1.00	1230.20 ± 0.12	ν PO ₂ asym.
0.18 ± 0.01	1309.70 ± 0.12	0.13 ± 0.01	1310.60 ± 0.05	amide III
1.02 ± 0.17	1397.70 ± 0.07	1.36 ± 0.16	1401.40 ± 0.13	$\zeta\delta$ CH ₃ sym.
0.50 ± 0.02	1454.50 ± 0.16	0.30 ± 0.03	1454.80 ± 0.05	$\zeta\delta$ CH ₃ asym.
2.21 ± 0.28	1543.30 ± 0.11	4.15 ± 0.55	1542.60 ± 0.10	ν N–H and ν C–N
10.99 ± 0.86	1641.30 ± 0.18	14.13 ± 0.85	1643.80 ± 0.98	ν C=O
1.22 ± 0.28	1651.80 ± 0.83	3.25 ± 0.55	1650.00 ± 0.63	ν C=O
0.18 ± 0.06	1740.70 ± 0.03	0.14 ± 0.04	1742.20 ± 0.10	ν C=O
0.37 ± 0.05	2851.80 ± 0.09	0.24 ± 0.07	2854.00 ± 0.09	ν CH ₂ sym.
0.59 ± 0.10	2924.30 ± 0.17	0.34 ± 0.02	2923.20 ± 0.04	ν CH ₂ asym.
0.44 ± 0.25	2962.30 ± 0.06	0.14 ± 0.02	2964.60 ± 0.07	ν CH ₃ asym.
0.43 ± 0.14	3072.30 ± 0.40	0.44 ± 0.08	3047.60 ± 0.73	ν CH ₃ sym.

Notes: The assignment was based on different works in the literature [13,15,16]. The symbols concerning the chemical assignment are related to the stretching vibrational mode (ν), scissoring (δ), bending (ζ), and symmetrical (sym.) and asymmetrical (asym.) modes. Band area and peak positions in bold are the values which presented significant differences, considering $p < 0.05$.

amide II in the sample. To differentiate between the two cell lines it is not necessary to have the means of the final ratio, because this is a mathematical index that will help tumor diagnosis.

3. Results

Figure 2 provides a complete view of the spectral region between 3800 and 1100 cm⁻¹. Figures 3 and 4 represent a detailed view of the spectra and the second derivative from both cell lines studied in this work.

Figure 3 represents a detailed view of the spectra and the second derivative from both cell lines studied in this work, and it is related to the region between 1800 and 1100 cm⁻¹. According to Fig. 3 the spectra of the MCF-7 and SKBr3 cell lines are dominated by two bands, assigned to the absorption modes of proteins. The most intense is the amide I band, centered near 1640 cm⁻¹, which corresponds to the C–O stretching coupled with the N–H bending and the C–N stretching modes of peptide linkages. The second band, centered at 1540 cm⁻¹, corresponds to amide II – predominantly a C–N stretching mode [13,15,16].

In Fig. 4 it is possible to observe in detail the C–H absorption bands, presenting the region located between 3200 and 2700 cm⁻¹; the position and area values for both cell lines can be visualized in Table 1.

The absorption spectra of both cell lines evaluated here contain absorption bands similar to those of other cell lines previously studied by Mostaço-Guidolin et al. [15]. Table 1 presents the absorption bands identified for each cell line; the band areas and peak positions which presented significant statistical differences ($p < 0.05$) are in bold. In the region of 1000–1300 cm⁻¹, the main spectral features are the

Fig. 2. Absorption infrared spectra of the SKBr3 cell (non-estrogen receptors expressed) and the MCF-7 cell (estrogen receptor expressed) in the entire spectral range 4000–1000 cm^{-1} . The spectra is composed of two intense absorbance regions which will better exposed in the following figures.

Fig. 3. (A) Infrared absorption spectra of the SKBr3 cell and the MCF-7 cell in the 1800–1100 cm^{-1} spectral range, and (B) the second derivative for the same cell lines and spectral range.

vibrations of the nucleic acid PO_2 stretching mode and the C–O stretching vibrations from the glycogen carbohydrate residues in the glycogen. Other bands related to nucleic acids, originated by C–O polysaccharides stretching (1039 cm^{-1}), C–OH stretching (1087 cm^{-1}) from DNA sugar and other compounds, and C–N and C–C stretching (1122 cm^{-1}) were also detected [13,16].

The band located at 1239 cm^{-1} is assigned to the asymmetric PO_2 stretching from phosphodiester bonds [13,16]. This band was used as the basis for spectral normalization, compensating for any effects from the difference in the number of cells in the films deposited on the CaF_2 windows [15]. This band was chosen because the phosphate moiety is found only within phosphodiester connections and phospholipids (in the membrane), and its intensity is generally unaffected by changes in the environment (washing, dry film creation, etc.). The amide I band is often used for normalization, but this absorption

Fig. 4. (A) Infrared absorption spectra of the SKBr3 cell and the MCF-7 cell in the 3200–2700 cm^{-1} spectral range. (B) The second derivative spectra for the same cell lines and spectral region.

Table 2
Absorption band ratios selected for differentiation between the SKBr3 and MCF-7 cells line

	Biochemical assignment ratio							
	Amide I Amide II	RNA DNA	Amide I DNA	Amide I Lipids	Protein RNA	Protein Lipids	Protein Lipids	Protein RNA
Wavenumber (cm^{-1})	1650 1540	1120 1085	1650 1085	1650 1740	1651 1120	1651 2851	1651 2962	2851 1120
								2962 1120

Notes: These ratios were selected according to the literature and evidences from this work. The first and second lines correspond to the biochemical ratio, and the two following lines correspond to the wavenumber used for ratio calculation.

profile is very sensitive to conformation and hydrogen-bonding. Since we observed significant shifts in the amide I absorption maximum, as well as additional changes that indicate modifications in the proportion of secondary structures, we believe that the amide I band is not the best possible reference for normalizations.

Through the second derivative spectra of the SKBr3 and MCF-7 cells, it was possible to verify the presence of peak shifts, related to the bands located at 1122 cm^{-1} (RNA), 1397 cm^{-1} (CH_3 from proteins and lipids), 1651 cm^{-1} (amide I- β sheet), 2851 cm^{-1} (fatty acids) and 2962 cm^{-1} (stretching of fatty acids). There were significant differences in the absorption band areas of the bands located in the regions of 1087 cm^{-1} (C–OH stretching), 1397 cm^{-1} (CH_3 from proteins and lipids), 1543 cm^{-1} (amide II stretching), 1651 cm^{-1} (amide I- β sheet) and 2924 cm^{-1} (stretching of fatty acids), as shown in Table 2.

In this study two human breast cancer cell lines were analyzed, namely MCF-7 (estrogen receptor expressed, ER+) and SKBr3 (estrogen receptor non-expressed, ER–). Estrogen receptor (ER) is a member of the nuclear hormone family of intracellular receptors that is activated by the hormone 17 β -estradiol (estrogen). It binds directly to estrogen response elements (EREs), to regulate gene transcription, according to Peng et al. [18]. However, the estrogen receptor also has additional functions independent of DNA binding.

Pollock et al. [19] suggest that two mechanisms contribute to explaining the causes of tumorigenesis. Firstly, the binding of estrogen to the ER stimulates proliferation of mammary cells, thereby increasing

cell division and DNA replication. Secondly, cells that present expressed estrogen are able to synthesize certain biomolecules, causing a rise in the number of genes and proteins. In our study, different band areas were found in the region related to proteins and DNA, specifically at 1085 cm^{-1} (DNA), 1542 cm^{-1} (amide II) and 1650 cm^{-1} (amide I), where the MCF-7 cell line (ER+) displays a larger absorbance intensity compared with the SKBr3 cell line (ER-). Specifically, the protein and DNA band areas in the spectrum of the MCF-7 cell line (ER+) have a value almost twice the value obtained for the SKBr3 cell line (ER-).

A closer look at the lipids region between 2800 and 3000 cm^{-1} , as shown in Fig. 4, revealed differences with relation to band area (2924 and 2962 cm^{-1}) and peak displacement (2962 cm^{-1}). Phosphatidylcholines are a class of phospholipids which incorporate choline as a head group. They are a major component of biological membranes and are called “essential phospholipids” [14]. Rohlf et al. [22] and Rillema et al. [21] suggest the choline is supplied by the diet and considered an essential nutrient in mammals, and mammary epithelial cells accumulate choline from the plasma during lactation and secrete high levels of choline metabolites, namely phosphocholine (PCho) and glycerophosphocholine (GPCho). Our results demonstrate that the SKBr3 cell line (ER-) has a larger band area compared with the MCF-7 cell line (ER+) in all the lipids region. Although we do not have more evidences to correlate C-H content and Pcho content, our hypothesis is that our results (higher C-H content) can be explained by the fact that SKBr3 cells have increased ChoK expression and, consequently, greater generation of PCho [8].

Additionally, we looked for biomarker ratios that would enable us to discriminate between lesions and normal cells. We selected absorption bands that represent each macromolecule, as shown in Table 2. With respect to DNA, the absorption bands related to the PO_2 symmetric stretching vibrational mode (1084 cm^{-1}) were considered, because this bond is abundant in this macromolecule in the same way that C-N and C-C stretchings (1120 cm^{-1}) are abundant in the RNA molecule. The bands which represent the protein region (1650 and 1540 cm^{-1}) are well applied in ratio analysis [13,15,16]. The lipids were represented by the sebum absorption band (1740 cm^{-1}), because according to Movasaghi et al. [16], and Mantsch and Chapman [13], this band represents a lipid macromolecule well, without influence of the protein vibrational modes. Having defined the absorption bands, we calculated the ratio between their areas and the obtained indices that are shown in Figs 5 and 6.

The first calculated ratio corresponds to the protein region: amide I (1650 cm^{-1})/amide II (1540 cm^{-1}). The index was 1.88 ± 0.17 for the SKBr3 cell line and 0.53 ± 0.09 for MCF-7 cells. This index allows for evaluation of the protein degradation level [26], since the amide absorptions are sensitive to protein conformation. In other words, an increase or decrease in this ratio could be attributed to changes in the composition of the whole protein pattern.

The ratio between proteins (in this case, the amide I absorption band area) and DNA can be related to malignant transformations; that is, this ratio increases as the tumor malignancy level rises [4]. The MCF-7 cell line had an index of around 0.35 ± 0.03 , whereas SKBr3 had a higher value, 0.55 ± 0.04 , which could indicate that SKBr3 cell line is derived from a more aggressive tumor compared with MCF-7. The ratio value considering the DNA and RNA absorption band are close (2.26 ± 0.12 for SKBr3 cell line and 2.83 ± 0.16 for the MCF-7 cell line), which is not useful to distinguish between these cell lines. Finally, the index between amide I and lipids was 22.78 ± 0.72 for the SKBr3 cell line and 16.00 ± 0.31 for MCF-7 cells.

The bands presenting peak displacement were also considered here. The absorption bands with some significant peak displacement were 1120 cm^{-1} (related to RNA), 1651 cm^{-1} (amide I α helix structure), 2851 cm^{-1} (related to lipids – CH_2 stretching) and 2962 cm^{-1} (related to lipids – CH_3 stretching). The

Fig. 5. Average ratio value of SKBr3 and MCF-7 cell lines that show statistical differences ($p < 0.05$), considering different absorption band areas: DNA (1084 cm^{-1}), RNA (1120 cm^{-1}), proteins (1650 cm^{-1} and 1540 cm^{-1} , amide I and amide II bands, respectively) and lipids (1740 cm^{-1}).

Fig. 6. Average ratio value of SKBr3 (breast cancer with negative estrogen receptor) and MCF-7 (breast cancer with positive estrogen receptor) cell lines considering other absorption band areas: 1120 cm^{-1} (RNA), 1651 cm^{-1} (protein), 2851 cm^{-1} (lipids) and 2962 cm^{-1} (lipids).

peak displacement can be assigned to changes in the environment in which the chemical bonds are immersed, causing changes in the vibrational frequencies. These bands could be employed to differentiate cell groups. Looking for the bands that show peak displacement, we selected more ratios and observed different ratio values for the two cell lines (see Fig. 6).

The first calculated ratio, referent to proteins and RNA, had an index value around 15.25 ± 1.09 for the SKBr3 cell line and 32.50 ± 3.66 for MCF-7 cells. The first ratio between proteins and lipids had

an index around 32.97 ± 2.29 (SKBr3) and 13.54 ± 1.09 (MCF-7) considering the absorption band located at 2851 cm^{-1} ; the second ratio between proteins and lipids (considering the absorption band area located at 2962 cm^{-1}) led to values around 2.77 ± 0.30 (SKBr3) and 23.21 ± 1.87 (MCF-7). Two more ratios relating both absorption band areas from lipids (2851 and 2962 cm^{-1}) and RNA (1120 cm^{-1}) were calculated: the first ratio was 4.62 ± 0.51 (SKBr3) and 2.40 ± 1.63 (MCF-7), considering the lipids absorption band located at 2851 cm^{-1} , and the second was 5.50 ± 0.48 (SKBr3) and 1.40 ± 0.09 (MCF-7), located at 2962 cm^{-1} .

4. Conclusions

This work demonstrates that it is possible to distinguish between MCF-7 and SKBr3 cells through differences in their FTIR spectra. Although both cell lines are derived from the same type of breast cancer (both are adenocarcinomas from a mammary gland, derived from metastatic pleural effusion), this methodology enables distinction between two cell lines, where the main difference between them, is the expression or not of estrogen receptors. It was possible to observe statistical differences ($p < 0.05$) by analyzing the absorption band areas and peak shifts. The molecular mechanisms and changes therein, which lead to the development and progression of breast carcinoma, are extremely complex, so the significant differences observed between the two cells lines can lead to future work on the identification of different types of breast cancer.

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