FTIR spectroscopic comparison of serum from lung cancer patients and healthy persons

Xin Wang\textsuperscript{a}, Xiang Shen\textsuperscript{b}, Daping Sheng\textsuperscript{b,⇑}, Xianliang Chen\textsuperscript{a}, Xingcun Liu\textsuperscript{b,⇑}

\textsuperscript{a}School of Basic Medical Sciences, Anhui Medical University, Hefei, Anhui 230032, China
\textsuperscript{b}The First Affiliated Hospital, Anhui Medical University, Hefei, Anhui 230032, China

HIGHLIGHTS

• IR spectra of lung cancer patients' and healthy persons' serum were investigated.
• IR spectra of malignant serum and healthy serum were similar.
• A1080/A1170 might be potentially useful for identifying malignant and healthy serum.
• The protein secondary structure was different between malignant and healthy serum.
• FTIR spectroscopy can indentify serum from lung cancer patients and healthy persons.

GRAPHICAL ABSTRACT

IR spectra of lung cancer patients' serum (A) and healthy persons' serum (B) were compared.

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ABSTRACT

The incidence and mortality of lung cancer remains so high that it is very urgent to develop an effective method for early detection of lung cancer. Serum can reflect physiological and pathological changes of human body, so FTIR spectroscopy was used to compare lung cancer patients' and healthy persons' serum in this study. The A1080/A1170 ratio might be potentially useful for distinguishing lung cancer patients' serum from healthy persons' serum. Moreover, the result of curve fitting indicated that the ratios of \( \alpha \)-helix/antiparallel \( \beta \)-sheet were lower for lung cancer patients' serum than those for healthy persons' serum. These results indicated that IR spectra of serum might be potentially useful for detecting lung cancer.

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Introduction

Lung cancer is one of the most prevalent malignant tumors worldwide. Due to the increase of smokers and deterioration of environment, the incidence and mortality of lung cancer have increased gradually in recent years [1–3]. Now the 5-year survival rate of lung cancer patients is less than 15% [4,5], indicating that most of lung cancer patients were diagnosed at the late stage [6]. If lung cancer can be detected at early stage and then treated properly, its survival rate will improve largely [7,8]. At present three methods such as chest X-ray, CT and bronchoscope are widely used for clinical diagnosis of lung cancer [8,9]. Although these methods can improve the ability to diagnose lung cancer, they are still less
effective for detecting lung cancer at early stage [6,9]. So it is very urgent to develop an effective method for early detection of lung cancer.

All disease processes are accompanied with biochemical changes in infected cells, tissues and organs, and these changes usually occur before the changes of cell morphology and the appearance of body symptoms. Since FTIR spectroscopy can reflect these changes at molecular level, it can be used as a tool for early detection of diseases [10]. From the late 1980s till now, FTIR spectroscopy has been used to explore a number of diseases, including colon cancer [11], basal cell carcinoma [12], preclinical scrapie [13], β-thalassemia [14], laryngeal cancer [15], gastric cancer [16] and so on [17]. FTIR spectroscopy was also applicable for lung cancer research [2,6,8–23]. For example, Lewis et al. studied IR and so on [17]. FTIR spectroscopy was also applicable for lung cancer patients' serum samples for 6 different types of cancer); 2. The strong absorption bands of water at 3300 cm⁻¹ will influence the whole spectrum [26]. In this study, the sample size increased largely compared with Yang's study, moreover, the influence of water was removed by drying the serum on BaF₂ window under vacuum. The goal of our study is to explore the possibility of discriminating lung cancer patients from healthy persons using IR spectra of their serum.

Materials and methods

Sample preparation

Twenty-four lung cancer patients' (before surgery and definite diagnosis) and twenty-two healthy persons' serum samples (Table 1) were obtained from The First Affiliated Hospital of Anhui Medical University. Additionally, another ten serum samples (containing six lung cancer patients' and four healthy persons' serum) were used for a blind test. The samples for FTIR spectroscopic study were prepared in accordance with Ref. [25].

FTIR spectroscopic measurements and data procession

All serum samples were measured by an IRAffinity-1 FTIR spectrometer (SHIMADZU Corporation) in the 4000–1000 cm⁻¹ region. The parameters were set as 64 accumulations and an 8 cm⁻¹ spectral resolution. The spectral data were collected using IRsolution software and then transformed into JCAMP format. OPUS5.5 software was used to deal with the spectral data subsequently. After cut between the 3700 and 1000 cm⁻¹ range, all IR spectra were baseline corrected (rubberband correction with 64 baseline points) and then min–max normalized to the amide I band. For statistics, the data were analyzed by origin6.0 software and expressed as mean ± SD. Independent t-test was applied to compare the differences between control group and cancerous group, and the accepted level of significant difference was \( P < 0.05 \). The statistic data were exhibited at Table 2.

Results and discussion

Analysis of spectra

The average IR spectra of lung cancer patients' and healthy persons' serum were displayed in Fig. 1. The assignments of the main peaks were given in Table 3 [25–30].

From Table 3, it could be observed that the spectral difference between lung cancer patients' and healthy persons' serum was not obvious.

Analysis of peak area ratios

To distinguish lung cancer patients' serum from healthy persons' serum, five peak areas were measured (Fig. 2 and Table 4).

Table 1

<table>
<thead>
<tr>
<th>Ratios</th>
<th>Lung cancer patient</th>
<th>Healthy person</th>
<th>Independent t-test (( P ) value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2959/A1545</td>
<td>0.2737 ± 0.0461</td>
<td>0.2451 ± 0.0224</td>
<td>1.146 × 10⁻²</td>
</tr>
<tr>
<td>A1650/A1545</td>
<td>1.9486 ± 0.0681</td>
<td>2.0115 ± 0.0733</td>
<td>4.25 × 10⁻³</td>
</tr>
<tr>
<td>A1080/A1545</td>
<td>0.1875 ± 0.0295</td>
<td>0.1540 ± 0.0103</td>
<td>8.0947 × 10⁻⁶</td>
</tr>
<tr>
<td>A1080/A1243</td>
<td>4.7264 ± 0.5652</td>
<td>4.5832 ± 0.6464</td>
<td>4.2723 × 10⁻¹</td>
</tr>
<tr>
<td>A1080/A1170</td>
<td>11.5921 ± 1.3950</td>
<td>9.9561 ± 0.6091</td>
<td>7.5992 × 10⁻⁶</td>
</tr>
<tr>
<td>α-Helix/β-sheet</td>
<td>19.7710 ± 15.8333</td>
<td>43.9050 ± 17.8767</td>
<td>2.9763 × 10⁻³</td>
</tr>
</tbody>
</table>

Fig. 1. Average IR spectra of lung cancer patients' serum (A) and healthy persons' serum (B).
Then the ratios of A2959/A1545, A1650/A1545, A1080/A1545, A1080/A1243 and A1080/A1170 were calculated. Since the 2997–2887 cm⁻¹ region is the asymmetric C–H stretching region (lipsids) and the 1593–1480 cm⁻¹ region is the amide II (proteins) [13], A2959/A1545 can indicate lipids/proteins. In this work, the average of A2959/A1545 was 0.2737 for lung cancer patients while 0.2451 for healthy persons, suggesting the content of lipids increased relative to the content of proteins in lung cancer patients’ serum. The P value of A2959/A1545 was 0.01146 (P < 0.05), making it clear the A2959/A1545 ratio was significantly higher for lung cancer patients’ serum than this for healthy persons’ serum. Using 0.257 as a criterion, 15 lung cancer patients’ ratios were lower than 0.1595 (percentage 81.8%) while 18 healthy persons’ ratios were higher than 0.1595 (percentage 94.7%).

The band around 1080 cm⁻¹ is PO₂ symmetric stretching of nucleic acids, and A1080/A1545 can evaluate the DNA content [31]. In this work, the average of A1080/A1545 was 0.8175 for lung cancer patients while 0.548 for healthy persons, suggesting the DNA content increased in lung cancer patients’ serum. The increase of DNA content may be related to necrosis and apoptosis of lung cancer cells and/or DNA released by lung cancer cells [32], which was accordance with the result of Wang’s study [34]. The P value of A1080/A1545 was 8.0947 × 10⁻⁶ (P < 0.05), making it clear the A1080/A1545 ratio was significantly higher for lung cancer patients’ serum than this for healthy persons’ serum. Using 0.1595 as a criterion, 19 lung cancer patients’ ratios were higher than 0.1595 (percentage 79.2%) while 18 healthy persons’ ratios were lower than 0.1595 (percentage 81.8%).

The band around 1243 cm⁻¹ is PO₂ asymmetric stretching of nucleic acids, and A1080/A1243 can indicate structural changes of nucleic acids [26]. In this work, the average of A1080/A1243 was 4.7264 for lung cancer patients while 4.5832 for healthy persons. The P value of A1080/A1243 was 0.4272 (P > 0.05), making it clear the A1080/A1243 ratio was not significantly different between lung cancer patients’ serum and healthy persons’ serum. Furthermore, the lung cancer patients’ and healthy persons’ ratios were all in the range of 3.4–5.6, so A1080/A1243 could not identify lung cancer patients’ serum and healthy persons’ serum.

The band around 1170 cm⁻¹ receives major contributions from C–O (H) groups of threonine, tyrosine and serine residues in proteins [27], and A1080/A1170 can describe the relative content of nucleic acids [26]. In this work, the average of A1080/A1170 was 11.5921 for lung cancer patients while 9.5961 for healthy persons, suggesting the content of nucleic acids increased in lung cancer patients’ serum. The P value of A1080/A1170 was 7.5992 × 10⁻⁶ (P < 0.05), making it clear the A1080/A1170 ratio was significantly higher for lung cancer patients’ serum than this for healthy persons’ serum. Using 10.4 as a criterion, 19 lung cancer patients’ ratios were higher than 10.4 (percentage 79.2%) while 18 healthy persons’ ratios were lower than 10.4 (percentage 81.8%).

**Curve fitting**

The amide I band receives major contribution from carbonyl stretching of proteins, and it is often used for analysis of protein secondary structure [35]. The secondary derivative IR spectra were calculated to gain the curves for curve fitting in the region of 1725–1593 cm⁻¹ (Fig. 3), and two peaks could be observed at 1686 cm⁻¹ (antiparallel β-sheet) [36] and 1655 cm⁻¹ (α-helix) [27]. Then curve fitting was carried out by using Gaussian formula (Fig. 3). The A1655/A1686 ratios were calculated on the basis of curve fitting. In this paper, the average of A1655/A1686 was 19.771 for lung cancer patients while 43.905 for healthy persons, suggesting protein secondary structure was different between lung cancer patients’ serum and healthy persons’ serum, moreover, the relative content of α-helix decreased in lung cancer patients’ serum. The P value of A1655/A1686 was 2.9763 × 10⁻⁵ (P < 0.05), which indicated the A1655/A1686 ratio was significantly lower for lung cancer patients’ serum than this for healthy persons’ serum. Using 32

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### Table 3

Preliminary assignments of serum’s average IR spectra.

<table>
<thead>
<tr>
<th>Frequency (cm⁻¹)</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>3293</td>
<td>The amide A band</td>
</tr>
<tr>
<td>2958 and 2872</td>
<td>CH₂ asymmetric and symmetric</td>
</tr>
<tr>
<td>2931</td>
<td>CH₂ asymmetric stretching</td>
</tr>
<tr>
<td>1743</td>
<td>C=O stretching</td>
</tr>
<tr>
<td>1650 and 1546</td>
<td>The amide I and amide II bands</td>
</tr>
<tr>
<td>1451</td>
<td>CH₂ deformation</td>
</tr>
<tr>
<td>1399</td>
<td>CH₂ bending</td>
</tr>
<tr>
<td>1313</td>
<td>The amide III band</td>
</tr>
<tr>
<td>1243 and 1079</td>
<td>PO₂ asymmetric and symmetric</td>
</tr>
<tr>
<td>1169</td>
<td>C–O (H) stretching in proteins</td>
</tr>
</tbody>
</table>

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### Table 4

The baselines of selected areas.

<table>
<thead>
<tr>
<th>Selected areas</th>
<th>Baselines</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2959</td>
<td>2997–2887 cm⁻¹</td>
</tr>
<tr>
<td>A1650</td>
<td>1725–1593 cm⁻¹</td>
</tr>
<tr>
<td>A1545</td>
<td>1593–1480 cm⁻¹</td>
</tr>
<tr>
<td>A1243</td>
<td>1258–1203 cm⁻¹</td>
</tr>
<tr>
<td>A1170</td>
<td>1184–1140 cm⁻¹</td>
</tr>
<tr>
<td>A1080</td>
<td>1140–1000 cm⁻¹</td>
</tr>
</tbody>
</table>
as a criterion, 18 lung cancer patients' ratios were lower than 32 (percentage 75%) while 19 healthy persons' ratios were higher than 10.4 (percentage 86.4%).

Analysis of blind study cases

Totally, six area ratios such as A2959/A1545, A1650/A1545, A1080/A1545, A1080/A1243, A1080/A1170 and A1655/A1686 were calculated to compare lung cancer patients' serum with healthy persons' serum. The $P$ value of A1080/A1170 was the lowest among these ratios, suggesting that the ratio of A1080/A1170 was the most significantly different [20]. So the A1080/A1170 ratio might distinguish lung cancer patients' serum from healthy persons' serum efficiently.

A single blind test was carried out with 10 serum samples to prove if the A1080/A1170 ratio could discriminate lung cancer patients' and healthy persons' serum. A criterion was set at A1080/A1170 = 10.4. Five lung cancer patients' and four healthy persons' serum samples were identified correctly (sensitivity 83.3% and specificity 100%). The result showed that the A1080/A1170 ratio might be a useful factor for distinguishing lung cancer serum from healthy serum.

Conclusion

Lung cancer patients' and healthy persons' serum samples were compared by FTIR spectroscopy. IR spectra of serum above were similar and consisted of the protein, lipid and nucleic acid absorption bands. The A1080/A1170 ratio might be potentially useful for identifying lung cancer patients' and healthy persons' serum. What is more, the protein secondary structures were different between malignant serum and healthy serum, and the relative content of $\alpha$-helix might decrease when lung cancer occurred. The above results indicate IR spectra of serum may be potentially useful for diagnosing lung cancer.

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