FT-IR spectroscopic analysis of normal and cancerous tissues of esophagus

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AIM: To investigate the special Fourier transform infrared spectroscopy (FT-IR) spectra in normal and cancerous tissues of esophagus.

METHODS: Twenty-seven pairs of normal and cancerous tissues of esophagus were studied by using FT-IR and the special spectra characteristics were analyzed in different tissues.

RESULTS: Different spectra were found in normal and cancerous tissues. The peak at 1550/cm was weak and wide in cancerous tissues but strong and high in normal tissues. The ratio of 1453/cm was 2.0 in normal tissues and 2.36 in cancerous tissues. The peak at 1453/cm was higher than at 1550/cm in cancerous tissues.

CONCLUSION: The results indicate that FTIR may be used in clinical diagnosis.

Spectral measurements

The spectra were recorded on a Nicolet Magna 750 FT-IR spectrometer with a mercury cadmium telluride (MCT) detector. No special sample preparation was needed in the experiment. The sample was defrosted at room temperature, and the measurements were carried out by putting the mucosal surface and cancer side (the section side of cancer tissue) of tissue on the attenuated total reflectance (ATR) probe. A total of 64 scans were recorded at a resolution of 4/cm at the region of 900 to 4000/cm. The samples were frozen again for reexamination after FT-IR scanning.

RESULTS

Significant differences were found between normal and malignant esophageal tissues in the FT-IR spectra except one sample. At the range of 1800-1500/cm, the amide band II at about 1550/cm was weak and broad in malignant tissues, and was diagnosed by this technology because it is limited by the experience of the operator and affected by the atypical clinical manifestations of the disease. Thus, it is the very goal to develop an accurate, quick, convenient, and inexpensive method for detecting early cancer of esophagus at molecular level. With the development of vibrational spectroscopic technologies, their application in cancer research has been reported. Among these technologies, Fourier transform infrared spectroscopy (FT-IR) has been extensively employed in the fields of biology and chemical engineering to get information about the structural and chemical/physical properties at molecular level. Recently, some researches about the application of FT-IR in cancer have been reported. In fact, the results of our previous research have already shown its advantages in clinical and scientific applications. But the study on the spectra of esophageal tissues is still preliminary. In the present work, we examined the cancerous and normal tissues of esophagus with FT-IR in order to put some light on the combination of FT-IR and endoscopy.

MATERIALS AND METHODS

Tissue preparation

Twenty-seven fresh tissues of esophageal cancer were obtained from the First Hospital of Xi’an Jiaotong University. The tissues were washed with normal saline solution and sampled immediately after removed during the operation. Two pieces of the tissues about 1 cm in diameter were taken, one was cut off from the center of the lesion and the other was from the distant edge of the removed tissues. Each tissue was divided into two equal parts, one was frozen with liquid nitrogen and the other was fixed with 10 % formalin, embedded in paraffin and stained with hematoxylin and eosin for pathological examination. All the 27 patients were diagnosed before operation as squamous cell carcinoma with endoscopic biopsy. There were 15 males and 12 females, aged 41 to 71 years old, and averaged 58 years.

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The relative intensity of I 1 647/cm-1 550 of malignant tissues was higher than that of normal tissues (Figure 2). The ratio of I 1 647/cm-1 550 was 2.0 in malignant tissues and 2.36 in normal tissues, with a statistically significant difference (P<0.05). The wave number of amide band I in normal tissues was about 1-6/cm higher than that in malignant tissues, without statistically significant difference (P>0.05).

Figures 1, 2, 3, and 4 show the spectra of the normal and cancer tissues at 1 800/cm-1 500/cm area.

Significant differences between normal and malignant esophageal tissues were seen in FT-IR spectra in this study. They might be caused by the changes of content and space array of protein, nuclear acid, sugar and fat in cells. For the bands of protein, amide bands I and II, the amide band II at about 1 550/cm was weak and broad in malignant tissues and normal tissues. At the range of 1 500-1 400/cm, the relative intensity of I 1 540/cm of malignant tissues was higher than that of normal tissues. At the range of 1 500-1 400/cm, the relative intensity of I 1 454/cm of malignant tissues was higher than that of normal tissues. For these 5 cases, the ratio of I 1 226/I 3 380 was higher in normal tissues. For a pair of samples, in which the same spectra were found by FT-IR, the regular pathological examination showed that the two samples were normal tissues of esophagus.

**DISCUSSION**

The pathogenesis and development of esophageal carcinoma are a multi-step process that is controlled by many genes and affected by many factors[15-24]. The cooperation of oncogene’s activation and anti-oncogene’s inactivation causes the relative changes of contents and structures of protein, nuclear acid, sugar and fat in cells[25-31]. FT-IR could provide information about molecular structure, which makes it possible to reflect the changes of protein, nuclear acid, sugar and fat in cells and the structure changes of space array of the molecule, thus the diagnosis of cancer cells could be made at the molecular level[32-34].

Significant differences between normal and malignant esophageal tissues were seen in FT-IR spectra in this study. They might be caused by the changes of content and space array of protein, nuclear acid, sugar and fat in cells. For the bands of protein, amide bands I and II, the amide band II at about 1 550/cm was weak and broad in malignant tissues and was strong and sharp in normal tissues. The relative intensity of I 1 647/cm-1 550 of malignant tissues was higher than that of normal tissues. At the range of 1 500-1 400/cm, the relative intensity of I 1 454/cm was higher in normal tissues than that in malignant tissues.

At the peak at 1 745/cm was caused by the stretching vibration of carbonyl, the peaks at 2 852/cm and 2 930/cm were absorption spectra of the symmetry and asymmetry stretching vibrations of methylene, the peaks at 2 873/cm and 2 958/cm were absorption spectra of the symmetry and asymmetry stretching vibrations of methyl. Because triglyceride contains lots of methyl, methylene and carbonyl, so the differences of FT-IR spectra in normal and malignant tissues at those area were caused by the different contents of lipid substance in the tissues. The decreased lipid substance in malignant tissues might be related to the following factors. Firstly, the fast growth of cancer needs more nutrition and energy, therefore it might utilize lipid to supply nutrition and energy, which cause the lipid substance not to be accumulated in cancer cells and tissues. The second reason is that normal cell and tissues, including fat cells, are excluded mechanically by proliferating malignant tissues of the tumor.

One of the most important characteristics of cancer cells is the increased DNA content in cells caused by the endless replication of DNA, which is presented with enlarged and dark stained nuclei under light microscopy. The peak at 1 080/cm was caused by symmetry stretching vibrations of phospholipids. In this study, we found the peak at 1 080/cm was stronger and higher in malignant tissues than that in normal tissues and the ratio of I 1 080/I 1 540 was higher in malignant tissues, indicating that DNA content in malignant tissues is significantly higher than that in normal tissues.
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