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Diagnosis of liver cancer based on tissue slice surface enhanced Raman spectroscopy and multivariate analysis

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ABSTRACT

Traditional detection methods can be used to make early diagnosis for the liver cancer patients, but these methods are limited by some factors and cannot to expound the tissue carcinogenesis at the molecular level. With surface-enhanced Raman spectroscopy, the normal (n = 46) and cancerous (n = 56) liver tissue slices from 56 patients were analyzed in the fingerprint region (500 ~ 1800 cm^{-1}). The relative intensities of the characteristic vibration peaks at 838, 1448, and 1585 cm^{-1} are significantly changed in the cancerous tissues. In the preliminary analysis, there are differences in the content of specific biomolecules (such as DNA and glycogen) of cancerous tissues and normal ones. Principal component analysis and linear discriminate analysis were combined to classify cancerous and normal liver tissue slices. The receiver operating characteristic curve can give the sensitivity and specificity of the classification method, and their values were 100% and 100%, respectively. This study demonstrates that the fingerprint SERS of tissue slices have great potential in the clinical detection of liver cancer.

1. Introduction

Cancer statistics reported a 23 percent drop in cancer deaths in 2012 compared to 1992 [1]. Furthermore, the mortality rate of liver cancer is higher than that of other cancers [2]. B-type ultrasonography and tissue biopsy techniques can be used to diagnose liver tumors, but these methods have a lot to do with the operation level and experience of doctors. Magnetic resonance imaging (MRI) and computed tomography (CT) have been implemented in the imaging diagnosis of liver cancer [3,4]. However, they are based on the complex combination of clinical and laboratorial tests, and have a certain degree of destructiveness as well as low sensitivity [3]. Although fluorescence spectroscopy can obtain the structural information of the sample, it is easily interfered by the spectra due to the presence of some universal fluorescence groups, resulting in incomplete structural information [5]. Raman spectroscopy is inelastic scattering based on monochromatic light, and can capture fingerprinting type information about the structure and conformation of biomolecules [6]. Up to now, Raman spectroscopy has brought a revolution in the medical field. Many researchers have made lots of investigation, especially for the applications in the diagnostic of cancers, such as liver [7], colon [8], oral [9], brain [10], and so forth.

However, there are two main drawbacks for conventional Raman signal in actual acquisition process. The first is the faintness of Raman signal. Although choosing a higher power laser and increasing exposure time can compensate for this shortcoming, these methods may do harm to biological samples. The second is the impact of autofluorescence. It is difficult to extract Raman signal in the background of stronger fluorescence when the biological tissue is tested. Fortunately, Fleischman et al. first proposed the surface-enhanced Raman spectroscopy (SERS) in 1974 [11]. The Raman signal increases 10^9-10^{15} orders of magnitude and the fluorescence effect is considerably reduced while detecting molecules on the surfaces of the precious metal [12–18]. In recent years, the application of SERS in clinical medicine has received wide attention, furthermore, metal nanoparticles have been widely used in the detection of tumor [19–24]. Moreover, silver nanoparticles (Ag-NPs) are preferred for many biomedical applications due to favorable physical and chemical properties and biocompatibility [21,25,26]. It is reported that adding Ag-NPs to the top of the tissue section can enhance the spectral signal of tissue slices [26,27].

Tissue slice sample is one of the fundamental materials for liver

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cancer diagnosis, which can be used to obtain accurate information about patients at the time of diagnosis. However, to our knowledge, few work aimed of SERS for liver cancer and normal tissue slices have been reported. Currently, using Raman spectrum on the diagnosis of liver cancer mostly limited to pure liver cell lines [7,28]. However, the composition of the samples used in clinical diagnosis was significantly different from that of single cell lines due to the cell differentiation. Therefore, SERS detection of biomolecules in liver tissue sections is of profound significance in clinical medicine.

In this study, we utilized SERS technique to study tissue sections of normal and cancerous livers, and obtained the information about the changes of biological composition in the liver tissues. Due to the variability from patient to patient, we tested 102 tissue sections, including 56 cancerous slices and 46 normal ones, and established the data set of SERS. The complex spectral data were processed to verify the resolution ability of SERS for cancerous and normal liver tissues with the combining of principal component analysis (PCA) and linear discriminate analysis (LDA). The accuracy of SERS for distinguishing liver tumor tissues from normal ones was examined through a receiver operating characteristic (ROC) curve. The integration area under the ROC curve is closer to 1, which means the higher accuracy of this method. Furthermore, through fingerprint SERS analysis of the tissue slices, the changes of biological components caused by liver cancer can be clearly demonstrated. The insights gained from this study shed new light on the general applicability of fingerprint SERS analysis in clinical trials.

2. Materials and methods

2.1. Preparation of tissue slices

In this study, two groups of tissue slices with thickness of 5 μm for normal (n = 46) and cancerous (n = 56) liver tissues from 56 patients were analyzed. The age range of patients was 30–65 years. All samples were diagnosed by two professional pathologists. All of the tissue sections were approved by the medical ethics committee of Qilu Hospital of Shandong University, and had got the permission from patients and doctors. Table 1 provides more detailed information about the clinical and histopathological diagnoses of patients with cancer.

2.2. Preparation of silver nanoparticles

Ag-NPs were synthesized by means of the reduction of silver nitrate, according to the method described by Zhang et al. [29]. Firstly, 0.05 g silver nitrate, 0.25 g polyvinylpyrrolidone (PVP) and 20 mL glycol were added to the volumetric flask to be stirred and heated for 1 h in oil bath, and the heating temperature is 135 ℃. Then, the mixture was cooled and added acetone. Finally, the mixture was centrifuged six times in a high-speed centrifuge to obtain uniform Ag-NPs. The speed of the centrifugation was set to 12,000 rpm and the centrifuging time was 5 min in each process. Fig. 1a is the scanning electron microscope (SEM, ZEISS Gemini Sigma 500) photograph of a tissue slice covered with Ag-NPs. The histograms exhibited (see Fig. 1b) diameter distributions of the Ag-NPs is complied with a typical Gaussian curve. The size of Ag-NPs is relatively uniform and the diameter is about 70 nm. The absorption spectrum of Ag-NPs was characterized by UV–vis absorption spectrometer, and the absorption maximum peak was at 425 nm, as shown in Fig. 1c. Fig. 1d shows an energy dispersive spectrum (EDS) image of the prepared tissue samples based on Ag-NPs. It can be seen that Ag-NPs have better covering effect on tissue sections. In order to reduce the impact of different batches of silver colloid on the experimental results, we used the same batch of silver colloid to complete the work.

2.3. SERS measurements

In the experiment, SERS spectra in the range of 500–1800 cm^{-1} (fingerprint region) were acquired by using a confocal microscopic Raman spectrometer (Horiba HR evolution, France) with an excitation wavelength of 532 nm [8,30]. Schematic configuration of the real-time Raman system for diagnosis of liver cancer was shown in Fig. 2. Laser beam is focused on the surface of the sample by a 50 × objective (N.A. = 0.50). The Raman scattering light was collected with the same objective and directed through the fiber bundle to a spectrometer equipped by using a back-illuminated charge coupled device (CCD) camera (1024 × 256 pixels). The instrument works in a standard working environment (-60 ℃). Spectral acquisition of each sample (80 μm × 80 μm, 8 × 8 points) was performed with an integration time of 4 s. The laser spot on the sample surface is 1 μm, the diffraction grating is 600gr/mm, and the laser power is 0.5 mW. All data of SERS spectra were acquired under the same experimental conditions.

Table 1
Clinical diagnosis of patients with liver cancer.

Patient no.	Age	Gender	Clinical staging
1	59	Female	III
2	41	Female	III
3	65	Male	IV
4	60	Male	III
5	57	Male	IV
6	69	Male	IV
7	65	Male	IV
8	53	Male	III
9	61	Male	III
10	79	Male	III
11	40	Male	IV
12	44	Female	IV
13	60	Female	IV
14	39	Female	III
15	50	Female	III
16	55	Male	III
17	62	Male	IV
18	56	Male	III
19	61	Male	IV
20	48	Male	IV
21	61	Male	IV
22	62	Male	IV
23	59	Male	III
24	30	Female	IV
25	53	Male	III
26	54	Male	IV
27	57	Female	IV
28	43	Male	IV
29	58	Male	IV
30	58	Male	III
31	50	Female	IV
32	62	Male	IV
33	52	Male	III
34	48	Male	III
35	60	Female	III
36	44	Male	IV
37	65	Female	III
38	64	Female	III
39	57	Female	III
40	63	Male	IV
41	43	Female	III
42	59	Male	IV
43	74	Female	III
44	77	Female	IV
45	53	Male	IV
46	54	Female	IV
47	52	Female	III
48	57	Male	IV
49	51	Female	IV
50	48	Female	III
51	37	Male	III
52	62	Female	IV
53	48	Female	III
54	57	Male	III
55	58	Female	IV
56	63	Female	IV