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Screening and staging for non-small cell lung cancer by serum laser Raman spectroscopy

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ABSTRACT

Objective: Lung cancer is the leading cause of cancer-related death worldwide. Current clinical screening methods to detect lung cancer are expensive and associated with many complications. Raman spectroscopy is a spectroscopic technique that offers a convenient method to gain molecular information about biological samples. In this study, we measured the serum Raman spectral intensity of healthy volunteers and patients with different stages of non-small cell lung cancer. The purpose of this study was to evaluate the application of serum laser Raman spectroscopy as a low cost alternative method in the screening and staging of non-small cell lung cancer (NSCLC).

Methods: The Raman spectra of the sera of peripheral venous blood were measured with a LabRAM HR 800 confocal Micro Raman spectrometer for individuals from five groups including 14 healthy volunteers (control group), 23 patients with stage I NSCLC (stage I group), 24 patients with stage II NSCLC (stage II group), 19 patients with stage III NSCLC (stage III group), 11 patients with stage IV NSCLC (stage IV group). Each serum sample was measured 3 times at different spots and the average spectra represented the signal of Raman spectra in each case. The Raman spectrum signal data of the five groups were statistically analyzed by analysis of variance (ANOVA), principal component analysis (PCA), linear discriminant analysis (LDA), and cross-validation.

Results: Raman spectral intensity was sequentially reduced in serum samples from control group, stage I group, stage II group and stage III/IV group. The strongest peak intensity was observed in the control group, and the weakest one was found in the stage III/IV group at bands of 848 cm^{-1}, 999 cm^{-1}, 1152 cm^{-1}, 1446 cm^{-1} and 1658 cm^{-1} ($P < 0.05$). Linear discriminant analysis showed that the sensitivity to identify healthy people, stage I, stage II, and stage III/IV NSCLC was 86%, 65%, 75%, and 87%, respectively; the specificity was 95%, 94%, 88%, and 93%, respectively; and the overall accuracy rate was 92% (71/77).

Conclusion: The laser Raman spectroscopy can effectively identify patients with stage I, stage II or stage III/IV Non-Small Cell Lung cancer using patient serum samples.

1. Introduction

Lung cancer is the leading cause of cancer-related death worldwide; Non-small cell lung cancer (NSCLC) is the major types of lung cancer and accounts for 85% of lung cancer cases [1]. The TNM (tumor, node and metastasis) system is used to subdivide NSCLC into Stage I, II, III and IV [2,3]. Stage I and stage II NSCLC are considered as early stages of cancer. They can exist and progress without causing any significant physical symptom. Therefore, most of the patients diagnosed of NSCLC were unfortunately often at stage III or IV. The delayed diagnosis of NSCLC adversely limits treatment options for patients and is frequently associated with poor prognosis. The early diagnosis of lung cancer is of great significance for better treatment and prognosis of patients. Currently there are several screening methods for lung cancer including sputum cytology, chest X-ray and CT scan [4]. However, those screening methods have not been applied prevalently due to controversy over their effectiveness and high cost as screening methods [5–8]. Accurate staging is important because treatment options and prognosis differ significantly by stages. Current clinical staging of NSCLC is majorly done through the chest radiograph, X-ray computed tomography (CT), emission computed tomography (ECT), positron emission computed tomography (PET-CT), magnetic resonance imaging (MRI) and invasive fiberoptic Bronchoscopy [9–11]. These tests are expensive and associated with complications. Therefore, there is a great need for developing methods that are simple and reliable to help identify lung cancer and facilitate staging.

Raman scattering is the inelastic scattering of photons which can reflect the vibrational or rotational modes of the interacting molecules

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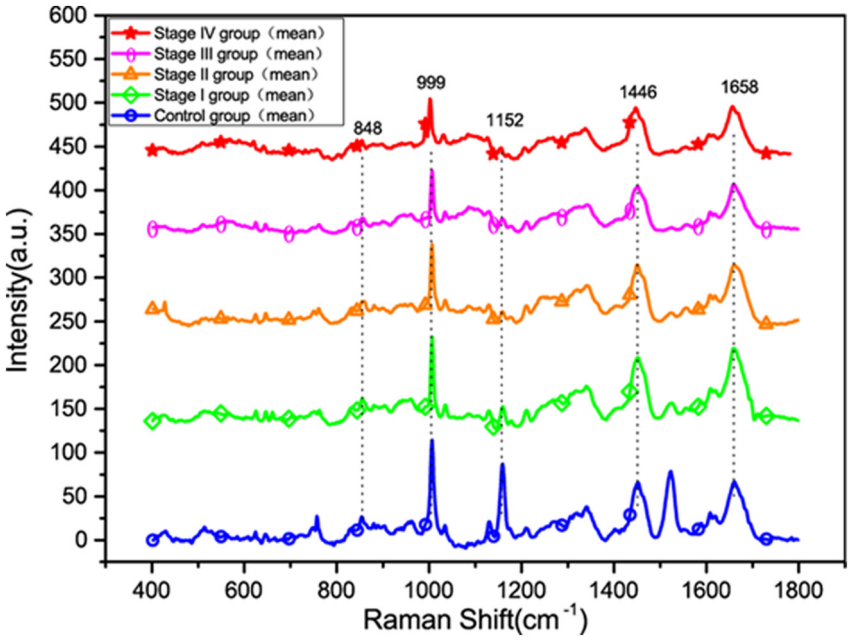

Fig. 1. Rama spectra of serum from normal people (control group), NSCLC stage I, stage II, stage III, and stage IV groups. Data were presented as mean ± SD.

[12]. Raman spectroscopy has many unique features compared to other spectroscopic techniques. For example, the frequency shifts of Raman scattering can provide "fingerprint" information of analyte's chemical structure. Besides, Raman peaks are very narrow, which facilitate multi-component analysis and quantification [13]. Raman spectroscopy can distinguish the composition and spatial structure of biochemical molecules, and provide chemical fingerprints of biomaterials [14]. It is a spectroscopic technique used to detect vibrational, rotational, and other states in a molecular system, capable of probing the chemical composition of tumor and diagnosis at molecular level. This non-invasive technique can reduce the suffering of patients, and provide a rapid diagnosis method, thus it has a broad scope in future clinical application [15].

It has been shown that Raman technique can distinguish between normal tissue, atypical hyperplasia tissue (precancerous), and cancer tissue of skin cancer [16], lung cancer [17], oral cancer [18], gastric cancer [19], esophagus cancer [20], liver cancer [21], colon cancer [22], prostatic cancer [23], renal cancer [24], cervical cancer [25], and breast cancer [26], by identifying the characteristic structural and component changes of lipid, nucleic acid, and protein. There is a study using the Raman spectroscopy of saliva to distinguish people with lung cancer from normal people [27]; there are also studies using Raman spectroscopy to stage chronic inflammatory diseases such as asthma [28]. However, these studies mainly use surface-enhanced Raman spectroscopy (SERS) that requires surface modification of the samples [29]. As a sub discipline of Raman spectroscopy, SERS offers a resolution to reduce the effects of inherent fluorescence while increasing the intensity of the Raman response of the sample. But, in comparison to normal Raman spectroscopy, SERS additionally requires the presence of metal nanostructures (metal colloids) as an integral component. It needs to consider not only the interaction between light and molecules, but also that between light and metal nanostructures. Therefore, in complicated matrix the "fingerprint" recognition capability of SERS may fail due to the multiple interferences. Bioanalytical applications of SERS set even more stringent constraints on metal colloids with respect to stability under physiologically relevant conditions, bioconjugation to a diverse set of ligands, and biocompatibility. While analytical chemists often prefer to work under well-defined conditions with purified systems, biologists and medical doctors usually do not consider this to be biologically or clinically relevant [10,11].

Serum is the liquid portion of the blood after it has been allowed to clot. It is free of clotting proteins but contains the clotting metabolites that result from the clotting process. It is a cleaner sample typically free of cells and platelets because they are trapped in the fibrin meshwork of the clot. It includes all proteins not used in blood clotting (coagulation) and all the electrolytes, carbohydrates, nucleic acids, lipids, antibodies, antigens, hormones, and any exogenous substances (e.g., drugs and microorganisms). In this study we used serum samples that are easy to obtain and require no additional modification to test the serum of NSCLC patients, and explored the role of Raman spectroscopy of serum in NSCLC screening and staging.

2. Materials and Methods

2.1. Research Objects

The recruitment of patients for this study was approved by the ethical committee of The First Affiliated Hospital of Guangdong

Table 1
Comparison of peak intensities of Raman spectra of serum.

Group	n	848 cm^{-1}	999 cm^{-1}	1152 cm^{-1}	1446 cm^{-1}	1658 cm^{-1}
Control group	14	1521.87 ± 231.29	7622.99 ± 1394.90	4911.49 ± 2272.07	4572.21 ± 1253.99	4617.46 ± 910.73
Stage I group	23	1174.80 ± 178.25	5832.27 ± 804.96	2141.93 ± 713.83	3842.62 ± 824.20	3901.66 ± 536.12
Stage II group	24	835.05 ± 239.45	4439.07 ± 623.64	955.07 ± 869.08	3242.77 ± 434.46	3389.31 ± 435.84
Stage III group	19	659.56 ± 159.86	3602.65 ± 599.66	487.67 ± 323.44	2539.01 ± 733.04	2580.97 ± 700.57
Stage IV group	11	608.91 ± 330.40	3297.83 ± 788.19	531.32 ± 511.66	2668.49 ± 363.48	2534.32 ± 412.75
F		37.884	66.307	20.097	13.925	35.189
P		3.027×10^{-18}	1.769×10^{-25}	1.031×10^{-11}	8.464×10^{-9}	2.171×10^{-17}