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Original Article

Raman spectroscopy of luminal subtype and basal subtype muscle invasive bladder cancer

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Abstract: Bladder cancer is the killer of human health, and its prevalence ranks second among all cancers. In order to improve the survival rate of bladder cancer, in addition to early detection, study on drugs, improvement of surgical protocols, and good prognosis of high-grade bladder cancer are crucial. Surface Enhanced Raman spectroscopy (SERS) is not only a method of diagnosing bladder cancer, but also it can provide mechanisms of cancer and medicine for treatment. This paper explores the Raman spectra of luminal and basal subtype of muscle invasive bladder cancer. A total of 250 SERS spectra from muscle invasive bladder cancer (MIBC) were acquired from 24 luminal subtype subjects and 26 basal subtype subjects who were given a pathological diagnosis. The experimental results demonstrate that two categories of muscle invasive bladder cancer can be distinguished by Raman spectrum. Principal component analysis combined with linear discriminate analysis (PCA-LDA) was used to classify luminal and basal subtype, with an accuracy of 94% and the accuracy of cross validation was 86%. Surface Enhanced Raman spectrum combined with PCA-LDA algorithms is a relatively accurate method to separate different levels of cancer and it can be used to research the mechanism of cancer and medicine for treatment. It lays the foundation for clinical application.

Keywords: Bladder cancer, Raman spectroscopy, PCA-LDA

Introduction

Cancer is the human health killer and more than seven million people dying from cancer each year all over the world [1]. The prevalence rate of bladder cancer is second [2]. It's important for diagnosing and treating bladder cancer in early stage. The first recommended treatment plan for bladder cancer treatment in Europe and America is chemotherapy. Neoadjuvant chemotherapy (NAC) based on platinum, gemcitabine, and paclitaxel can greatly improve the prognosis of muscle invasive bladder cancer (MIBC) patients, reduce tumor recurrence and metastasis, and prolong median survival time [3, 4]. But not all patients benefit from chemotherapy. For those patients who are not sensitive to chemotherapy, this increases the side effects and the cost of medical treatment, and even delays the surgical treatment time [5].

Currently, the main methods for diagnosing bladder cancer include ultrasound imaging [6], magnetic resonance imaging (MRI) [7], computed tomography (CT) [8], endoscopy [9-11], and cytology [12] etc. These technologies are complex and require doctors to have extensive clinical experience and knowledge. The Raman spectrum is a scattering spectrum formed by the interaction of incident light, so the molecular information is concluded in a Raman spectrum as molecular fingerprints with the advantages of no damage, high sensitivity, and efficiency. The technology such as Surface Enhanced Raman spectroscopy (SERS) has been applied to physics, chemistry, material science, biomedicine, etc.

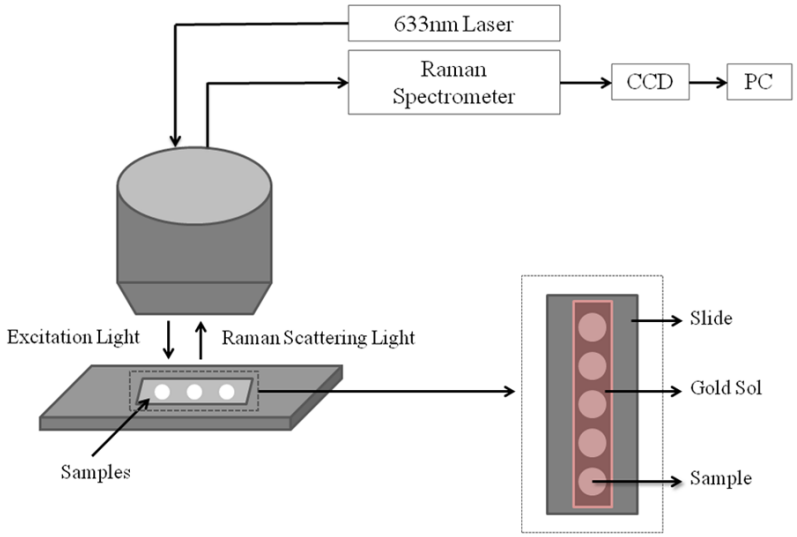

Figure 1. The experimental setup for Raman measurement.

Many studies on bladder cancer have been done by using Raman spectroscopy in various ways. At present, most studies research classification bladder cancer patients and normal people. The Raman spectra of bladder cancer tissue and normal bladder tissue may have more obvious difference in the intensity of characteristic peaks, which means that the specific chemical component changes in the process of cancer [13]. Many types of sample are related to bladder cancer, such as tissue, urine, and serum. Therefore, different methods of Raman spectroscopy are combined with different type of sample. For example, bladder cancer is diagnosed from urine by Raman molecular imaging [14], and bladder cancer cells are detected effectively by Raman spectroscopy with atomic force microscopy [15]. In order to apply Raman spectroscopy to clinic, different levels of tumor can be accurately classified with appropriate algorithms. At the moment, most of studies utilize principal component analysis combined with linear discriminate analysis (PCA-LDA), to support vector machine combined with linear discriminate analysis (SVM-LDA) and genetic algorithms combined with linear discriminate analysis (GAs-LDA). For example, some studies explore that the SERS spectra of serum which acquired from 55 bladder cancer patients and 36 normal people are classified by genetic algorithms (GAs) combined with linear discriminate analysis (LDA), and the sensitivity and specificity are 90.9% and 100% [16].

By the detection and analysis of genomics and transcriptology of tumor tissue, some studies suggest that there are different molecular subtypes in MIBC. At present, the mainstream classification includes basal-like subtype, luminal subtype, and other classification subtypes [17, 18]. The study shows that the luminal subtype is closer to the papillary growth of non-muscle invasive bladder cancer. It is a potential population for bladder preservation. The prognosis of Basal subtype is worse in general and should be operated and supplemented with neoadjuvant chemotherapy actively [19]. For patients with different molecular subtypes, individualized treatment, including surgery, NAC, and adjuvant chemotherapy, or other targeted therapy and immunotherapy, is an important breakthrough in the accurate treatment of bladder cancer. This study used SERS detected muscle invasive bladder cancer tissues and combined with PCA-LDA algorithms to discriminate between luminal subtype and basal-like subtype of MIBC on the resulting Raman spectra. Based on distinguishing luminal subtype from basal-like subtype of muscle invasive bladder cancer, the difference between luminal and basal-like subtype at the molecular level could be researched through SERS. The information of luminal and basal-like subtype in the molecular level could be provided with researching medicine for treatment and improving surgery program and adjuvant therapy.

Material and method

Preparation of the sample

Bladder cancer tissue samples were obtained from Renji Hospital of Shanghai Jiaotong University. The study was approved by the Institution Ethics Committee of Shanghai RenJi Hospital, School of Medicine, Shanghai Jiao Tong University. All of the samples are invasive bladder cancer, and the muscle invasive bladder cancer are divided into two categories:

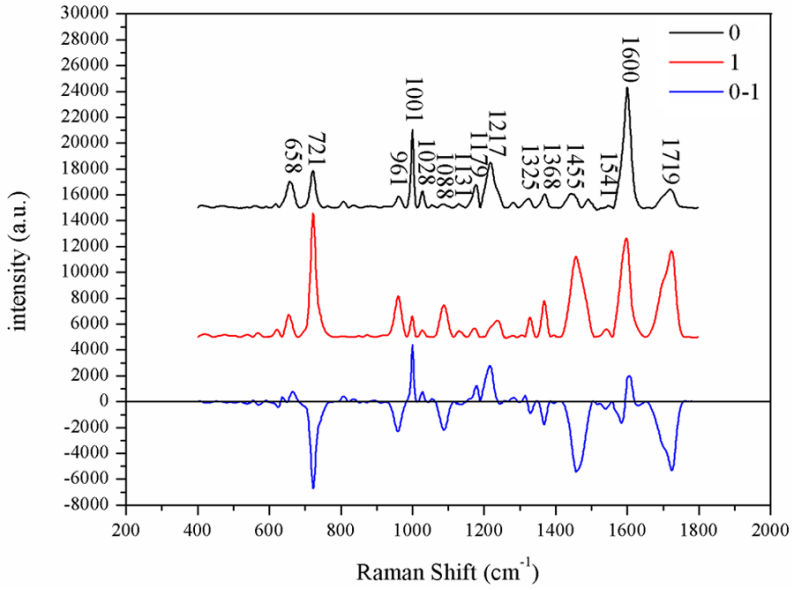

Figure 2. The Raman spectrum which is subtracted of bladder cancer.

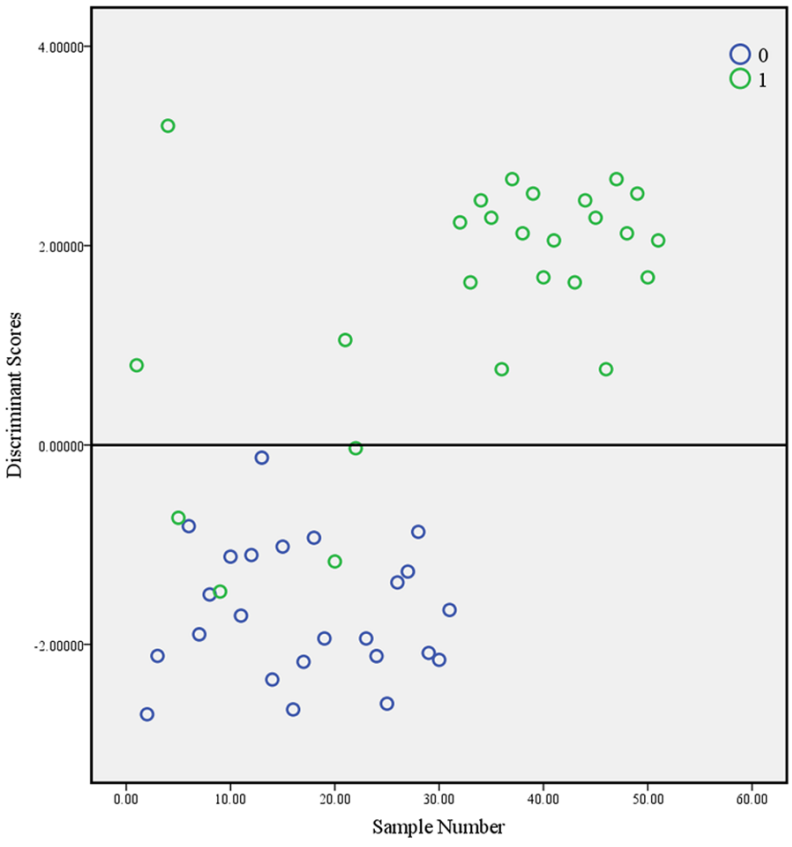

Figure 3. The classification result between luminal and basal-like subtype.

luminal subtype (0), basal-like subtype (1). A total of 50 muscle invasive bladder cancer tissue samples were used in this experiment. There were 24 groups with luminal subtype and 26 groups with basal-like subtype. The bladder cancer tissue samples were cut into slices for 20 μm by freezing microtome and the slices were soaked in gold sol.

SERS measurement

A Raman microscope with a 50×objective and 633-nm excitation was utilized to obtain the spectrum with a 10 second integration time over the spectral range of 400-1800 cm^{-1}. Each sample was measured at five times to reduce noise and the side effect due to any instability in the spectrometer. The experimental setup is shown in **Figure 1**.

Results and discussion

The Raman spectra were obtained from 24 patients of luminal subtype (0) and 26 patients of basal-like subtype (1). Two sets of spectra are removed the fluorescence background and averaged respectively and the results are shown in **Figure 2**. The black and red curves represent the Raman spectrum of luminal subtype and basal-like subtype respectively, and the blue curve represents the difference between black and red curves.

To distinguish luminal subtype from basal-like subtype further, the two sets of data were classified by PCA-LDA algorithm and the accuracy was 94% (**Figure 3**). As displayed in **Table 2**, the accuracy of cross validation was 86%. It can be seen that

Table 1. The Raman characteristic peak of invasive bladder cancer

Raman shift (cm^{-1})	Peak position	Reference number
658	C-S stretching mode of cystine (collagen type I)	[20, 21]
721	DNA	[22]
961	Calcium-phosphate stretch band (high quantities of cholesterol)	[23, 24]
1001	Symmetric ring breathing mode of phenylalanine	[20, 25]
1028	C-H in-plane bending mode of phenylalanine	[26]
1088	C-C stretch, O-P-O^- stretch	[21]
1131	(C-N) proteins (protein assignment), C-O stretching (carbohydrates)	[26, 27]
1179	Cytosine, guanine, adenine	[25]
1217	Stretching of C-N	[28]
1325	CH_3CH_2 wagging mode in purine bases of nucleic acids	[29]
1368	Guanine, TRP (protein), porphyrins, lipids	[25]
1455	Structural protein modes of tumors	[30]
1541	Tryptophan	[31]
1600	C=C in-plane bending mode of phenylalanine and tyrosine	[31]
1719	C=O	[32]

Table 2. Classification between luminal and basal subtype

Classification		Expected Classification		Total
		Luminal Subtype	Basal-like Subtype	
Original label	Luminal Subtype	24	0	24
	Basal-like Subtype	3	23	26
Cross validation	Luminal Subtype	21	3	24
	Basal-like Subtype	4	22	26

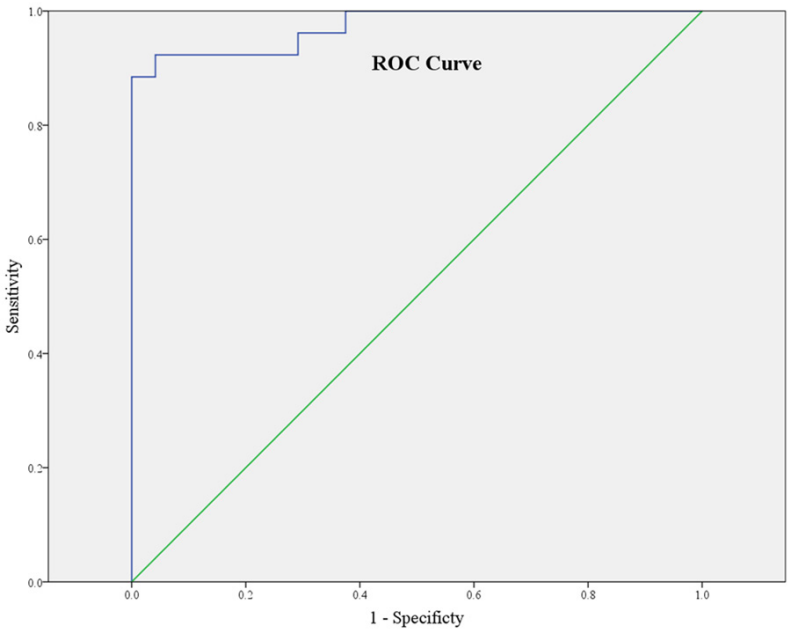

Figure 4. ROC curve of the result of distinguishing luminal subtype from basal-like subtype by PCA-LDA.

the luminal subtype can be differentiated from basal subtype by Raman spectrum preliminarily.

According to the model of PCA-LDA, the ROC curve was drawn to evaluate the ability of classifying two categories of samples by PCA-LDA (**Figure 4**). The integral of the area under the curve was 0.973. Raman spectra of invasive bladder cancer tissue can be distributed by PCA-LDA well.

As shown in **Figure 2**, the distribution of Raman characteristic peaks of the two sets of spectra is very similar. The characteristic peaks on the Raman spectra of the invasive bladder cancer were located at 658, 721, 961, 1001, 1028, 1088, 1131, 1179, 1217, 1325, 1368, 1455, 1541, 1600 and 1719 cm^{-1}, as detailed in **Table 1**. The Raman peaks belong to biological molecules, such as proteins, lipid, DNA.

Although the peaks of the Raman spectrum were similar between luminal and basal subtype, the relative intensities of the peaks and peaks change. According to spectral range of 650-750 cm^{-1}, 950-1100 cm^{-1} and 1400-1750 cm^{-1}, it can separate two sets of data. 658/721 cm^{-1} (the ratio of 658 to 721 cm^{-1}), 961/1001 cm^{-1}, 1088/1217 cm^{-1}, 1368/1455 cm^{-1} and 1600/1719 cm^{-1} all change. Subtle changes occurred in some biological molecules. In the luminal subtype group, the intensity of 658, 1001, 1028, 1179, 1217 and 1600 cm^{-1} was higher than the other group. In the basal subtype group, the intensity of 721, 961, 1088, 1325, 1368, 1455 and 1719 cm^{-1} was higher than the group of basal subtype. These differences can divide samples into two types potentially. The characteristic peak in 1455 cm^{-1} represents the structural protein modes of tumors, which indicates the concentration of the protein participating in reproduction, division of cancer cell in the group of basal subtype is higher than the other group. The peaks in 721, 1088, 1325 and 1368 cm^{-1} represent DNA, cytomembrane, which means that the rate of the reproduction, division of cancer cell in basal subtype is faster than luminal subtype.

Conclusion

In this study, Raman spectroscopy-molecular typing-chemosensitivity predictive system was established by using Surface-Enhanced Raman spectroscopy to explore the difference of different molecular typing and chemosensitivity of bladder tumor. The results show that higher level of tumor, luminal subtype, and basal subtype of muscle invasive bladder cancer can be distinguished by Raman spectrum preliminarily. The prediction system is an important reference indicator if muscle invasive bladder cancer need for neoadjuvant chemotherapy preoperative. It provides important evidence to the individual patient's precise treatment. Moreover, by using PCA-LDA algorithm to classify, the accuracy was 94% and the accuracy of cross validation was 86%. It indicates benefit to study the mechanism of cancers and drugs screening for treatment of cancers at present. In addition, it will be used in clinic basing on more precise algorithms and plenty of databases.

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