Objective: To analyze the characteristics and difference of the laser-induced autofluorescence spectra of normal bladder tissue and bladder carcinoma tissue and find a method for discrimination among normal bladder tissue and bladder carcinoma tissue. Methods: Laser-induced autofluorescence detection analytical system was adopted to collect 47 autofluorescence spectra of cutting normal bladder tissue and bladder carcinoma tissue in surgery in 300-800 nm. A total of 25 bladder specimens of rats were involved to detect collagen IV and cathepsin B expression in each group: normal bladder tissue and bladder carcinoma tissue from the rat colorectal carcinoma model. The collagen IV and cathepsin B was detected with immunohistochemical staining. The correlation of the results was studied. Cathepsin B RNA expression was detected with reverse transcription-polymerase chain reaction (R-TPCR). Results: Of 94 specimens detected by laser-induced autofluorescence, 47 were normal and 47 carcinoma tissues. All normal tissue fluorescence intensity was high than carcinoma tissue on the same wave length. There was a char wave crest at 452 nm respectively. Normal tissue of peak intensity was obviously high than carcinoma tissue ($P < 0.05$). Collagen IV expression existed in all specimens in different degrees, it was decreased carcinoma group, and there was significant difference compared with the normal group and hyperplasia group. The expression of cathepsin B in carcinoma group was increased compared with normal group ($P < 0.05$). Conclusions: Normal tissue and carcinoma tissue of autofluorescence intensity are different, laser-induced bladder tissue autofluorescence spectra may be used in distinguishing Normal tissue and carcinoma tissue. The expression of collagen IV is decreased in bladder carcinoma compared with the normal bladder tissue. The expression of cathepsin B is increased in bladder carcinoma compared with the normal bladder tissue, and possibly induces difference of one of reason for autofluorescence in normal bladder tissue and bladder carcinoma tissue.

1. Introduction

Bladder cancer is the most common malignancy in urology. The incidence of males is much higher than that of women, which is 3-4 times that of women[1]. Laser-induced tissue autofluorescence spectroscopy has provided new insights for the diagnosis of bladder cancer[2-4]. We studied the autofluorescence spectrum characteristics of normal and cancerous bladder tissues. Its mechanism provides an experimental basis for early detection of bladder tumors by fluorescence cystoscopy.

2. Materials and methods

2.1. Experimental specimens

BALB/c nude mice (SPF grade), 3 to 9 weeks old, weighing 18 to
22 g, a total of 50 were selected. A certain concentration of bladder cancer single cell suspension was inoculated into the right axillary fossa of BALB/c nude mice, and the tumor tissue and normal bladder tissue were taken on the 7th day.

2.2. Laser induced autofluorescence detection system

Excited neodymium-doped yttrium aluminum garnet lasers with a final excitation wavelength of 355 nm, a pulse width of 6 ns, a single-pulse probe output energy of 1.5 mL and a frequency of 5 Hz. Autofluorescence was generated (Figure 1) and autofluorescence spectra and analysis of 47 specimens of bladder cancer and normal tissue were collected. Some tissue was placed in liquid nitrogen and the remaining tissue was examined by pathological examination.

2.3. Specimen origin and immunohistochemical staining detection of expression of collagen IV and cathepsin B

Specimens Source Paraffin blocks and liquid nitrogen-preserving tissues derived from the bladder tissue of rat bladder cancer animal models. Immunohistochemical staining was used to detect the expression of collagen IV and cathepsin B in 25 specimens of the normal group and the bladder cancer group. The differences between the groups were compared and the correlation analysis was performed. Using β-actin gene as a reference, semi-quantitative RT-PCR method was used to detect the expression of cathepsin B mRNA in each group, and the differences in expression between the groups were compared.

Collagen IV antibody positive semi-quantitative grading: Absence (-); 25% of basement membrane positive fragments were weakly positive (+); 25% to 50% Discontinuous positive fragments were positive (++); basement membranes were more than 50% positive, and continuous lines were strongly positive (+++).

Determination criteria of Cathepsin B according to the Shimizu method, the number of positive cells: cell staining=0, <1/3 cell staining=1, multifocal (<2/3 cell) stainings=2; diffuse (>2/3) = 3; staining positive degree: no coloring 0, strong staining = 2, moderate staining (between O-2) = 1 . Adding the scores from the previous two is the final result, where 0 is (-), 2 is (+), 3 is (++), and 4 and 5 are (+++). > (+) means positive.

2.4. Statistical processing

The data of this group were statistically analyzed using SPSS 16.0 statistical software. All data were expressed as mean± standard deviation. The paired sample t-test was used to compare the wavelengths of body tissue and ex vivo tissue: between the groups collagen IV, Cathepsin for the comparison of B expression differences, non-parametric tests (Kruskal- Wallis H test) were used overall. The expression level of Cathepsin B among groups was compared using the group design t-test, and the correlation analysis was based on bivariate Spearmma rank correlation analysis. P<0.05 is considered as statistically significant.

3. Results

Fluorescence intensity of normal tissues at the same wavelength was higher than that of cancer tissues, and the difference was statistically significant (P<0.05) (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>452 nm maximum peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer tissue</td>
<td>47</td>
<td>275.5±32.2</td>
</tr>
<tr>
<td>Normal tissue</td>
<td>47</td>
<td>316.6±34.1</td>
</tr>
<tr>
<td>t</td>
<td>14.082</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

The expression of collagen IV was decreased in the cancer group and the normal group, with significant differences (Table 2).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Collagen IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Normal group</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Cancer group</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>

Comparison of two groups χ²=19.353, P<0.05.

The expression of tissue-specific expression of cathepsin B was increased in the cancer group compared with the normal group. The difference was significant (Table 3).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Cathepsin B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Normal Group</td>
<td>25</td>
<td>21</td>
</tr>
<tr>
<td>Cancer Group</td>
<td>25</td>
<td>4</td>
</tr>
</tbody>
</table>

Comparison of two groups χ²=26.125, P<0.05.
There was a negative correlation between the expression of collagen IV and the expression of woven cathepsin B in 25 cases of bladder cancer (correlation coefficient = -0.721, \( P<0.05 \)). The expression of collagen IV enhanced by woven expression of cathepsin B was weakened (Figure 2). Tissue cathepsin B mRNA expression levels are higher than normal tissue (Table 4).

![Figure 2. Electrophoresis](image)

Lanes 01, 02, 03, 04, 05, and 06 were normal controls, and lanes 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 were tumors.

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Tissue</td>
<td>0.2239±0.0121</td>
</tr>
<tr>
<td>Cancer Tissue</td>
<td>0.4201±0.0264</td>
</tr>
</tbody>
</table>

Comparison of two groups \( t=15.746, \ P<0.05 \).

### 4. Discussion

Since the beginning of the 1920s, experts from various countries have put forward many methods and methods for detecting diseased tissue. It has been found that the use of autofluorescence to identify cancer is one of the most promising and most effective means [5]. Laser-induced tissue autofluorescence spectroscopy, through computer-wide control and real-time analysis, can detect early small and flat lesions that cannot be detected under conventional cystoscopy, which will help improve the early diagnosis of bladder cancer (small) and determine the boundaries of the lesions. The accuracy of cystoscopy improves the early diagnosis of bladder cancer. However, due to the current detection methods are difficult to find early bladder cancer lesions, so the clinical significance of autofluorescence diagnosis of early bladder cancer in the real sense is minimal, mainly in vitro bladder cancer tissue and a small amount of sample in vivo bladder tissue autofluorescence spectroscopy Differences from normal organizations. Some studies have shown that compared with normal mucosa, the papilloma tissue has a wavelength in the range of 309 nm to 450 nm with a low signal. In this study, the fluorescence intensity of normal bladder tissue in the study group after treatment was significantly higher than that in the cancer tissue (\( P<0.05 \)). Collagen IV is the main component of the basement membrane and extracellular matrix. In recent years, many studies have found that the invasion and metastasis of malignant tumor cells are related to collagen IV. The basement membrane composed of collagen IV constitutes the invasion and metastasis of malignant tumors. An important barrier, the study found that in the process of invasion of malignant tumors, collagen IV have different degrees of degradation[6]. This study found that the expression of collagen IV in bladder cancer tissues was significantly lower than that in normal tissues. It indicates that the collagen of bladder is gradually degraded during the development of bladder cancer, and the invasion process of bladder cancer is closely related to the degradation of collagen IV.

Cathepsin B is a lysosome cysteine protein hydrolase in vivo that degrades cell substrates such as collagen IV and participates in tumor metastasis, indicating that BC is overexpressed and has increased activity in tumor cells. Invasion and metastasis are related [7]. Studies have shown that the autofluorescence spectrum of cancerous tissue and normal tissue is similar in shape, but the intensity is significantly different[8-11]. At the same time, the mechanism of autofluorescence difference between bladder cancer tissues and normal tissues remains unclear. It was found that cathepsin B can degrade a variety of extracellular matrix components (such as fibronectin, type IV collagen, etc.) [12-14], and then undermine a series of organizational barriers involved in tumor invasion and metastasis.

In this experiment, the expression of cathepsin B in the bladder cancer group was greater than that in the normal tissues by the detection of normal bladder tissues and cancer specimens by molecular biology methods. The difference was significant. At the same time, it was found that cathepsin B diffused in the bladder wall, and cancer cells were strongly positively expressed. Podgorski [15] thought that the cathepsin B mRNA protein and activity levels increased in tumors; Khan et al [16] found that cathepsin B expression and activity in cancer cells increased. Helps the degradation of the basement membrane. This experiment indicates that the expression of collagen IV in bladder cancer tissue is negatively correlated with the expression of cathepsin B, indicating that cathepsin B may be an important proteolytic enzyme leading to the degradation of collagen IV, which may lead to the difference of autofluorescence between normal bladder tissue and cancer tissue. Through this experimental study, it was found that the autofluorescence characteristics of bladder normal tissues and cancer cystitis tissues are different and there are significant differences. Applying this technique to fluorescence cystoscopy will provide early evidence of cancer tissue and provide a theoretical basis.

### References


[2] Aantor AF, Hartge P, Hoover RN. Epidemiological characteristics of


