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## DETECTION OF GENE MUTATION IN SPUTUM OF LUNG CANCER PATIENT

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Lung cancer is a common malignant tumor, which has a high incidence and mortality rate. Therefore, it is necessary to seek a new method for the diagnosis, especially the early diagnosis of lung cancer. The development of molecular biology makes the gene diagnosis of lung cancer possible. PCR-SSCP was applied to detect p53 gene mutation of lung cancer patients' sputum cells and we have achieved good results.

### MATERIALS AND METHODS

Third-six lung cancer inpatients in the Second Hospital Affiliated to the Fourth Military Medical University were chosen, among whom were 24 males and 12 females with a average age of 58±8.2 (41~72). According to TNM differential criterion, there were 14 patients with stage II lung cancer and 22 with stage III lung cancer. And there were 18 patients with squamous cell cancer, 12 with adenocarcinoma, 4 with small cell lung cancer and 2 with large cell lung cancer. Samples were taken from the patients' resected fresh lung cancer tissues. Sputa were collected before operation (1~2 mouthful/day, 3 days altogether). And there were 20 controls groups, whose samples were the normal lung tissues resected with lung cancer mass as well as sputa taken from pneumonia and bronchitis patients. DNA were extracted with saturated phenol-chloroform and ethanol precipitated. PCR-SSCP was conducted (p53 primers were aimed at exon 5~8 of p53 and were composed by Shanghai Biochemistry Institute of Chinese Academy of Sciences). Sputa were washed and centrifugalized with normal saline for three times. After the sediment was smeared and dyed, common sputum cytology detection was conducted under light micro-cope.

### RESULTS

Of the 36 lung cancer patients, 19 were detected to have p53 mutation in severed lung cancer tissues (stage II 6, stage III 13), with a positive rate of 52.78%, and 15 were

detected to have p53 mutation in sputa with a 41.67% positive rate and all the 15 patients were also among the former 19 patients. To those patients who had p53 mutation in tissues, the positive rate of sputum cell gene mutation detection with PCR-SSCP was 78.95%, and 55.56% (20/36) for common sputum cytology detection. No p53 gene mutation was detected in tissues or sputa of control samples.

### DISCUSSION

The genesis of lung cancer is related to many kinds of oncogenes and suppressor genes. The diagnosis of lung cancer can be conducted by detecting these genes. About 60% of lung cancer patients were found to have p53 gene mutation. This kind of mutation is mainly point mutation, whose site is mainly in exon 5~8. PCR-SSCP is commonly used to detect gene mutation at present. It is sensitive, comparatively simple and able to detect many samples simultaneously. The detection of the resected lung cancer tissues showed that over 50% of the lung cancer patients had p53 gene mutation, which suggested that the p53 mutation is common in lung cancer. However, the detection of the gene mutation in resected tissues of lung cancer is not applicable to the clinical diagnosis before operation and non-operative patients, and has little significance in practical clinical diagnosis. Sputum cytology detection is commonly used in the diagnosis of lung cancer, which is easy to operate and collect samples but not so sensitive. PCR could be used to detect the very few cancer cells in the sputa of lung cancer patients, thus improving the sensitivity of sputum cytology detection. In this study, the p53 gene mutation of sputum cells was detected with PCR-SSCP and the total positive rate was 41.67%, lower than that of common sputum cytology detection. But of those 19 patients who had p53 mutations in lung cancer tissues, the positive rate of sputum detection with PCR-SSCP was 78.95%, obviously higher than that of common sputum cytology detection ( $P<0.01$ ). The positive rate of PCR-SSCP was low mainly because the p53 gene mutation rate in lung cancer is not high enough as well as the defect of the method itself.