DETECTION OF p53 GENE MUTATION OF BRONCHOSCOPIC SAMPLES IN THE PATIENTS SUSPECTED TO LUNG CANCER*

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ABSTRACT

Objective: To determine the feasibility of detecting p53 gene mutations for early diagnosis of lung cancer using the samples from bronchoscopic examination. Methods: Point mutations of the exon 5 - 8 of p53 gene were detected in 85 bronchoscopic samples of 35 patients suspected to be lung cancer using silver staining PCR-SSCP. Results: p53 gene mutations were founded in 10 of 35 patients(28.6%). The incidence of p53 gene mutations (14.9%) was obviously higher than the cytological positive incidence(2.9%) in samples of sputum, bronchoalveolar lavage and brush, especially for the sputum(27.7%). In the bronchoscopic biopsy specimens, the incidence of p53 gene mutations (12.5%) was lower than that of pathologic positive result (50.0%). However, in view of all the bronchoscopic samples, there was no statistically difference between cytopathologic positive results (11.8%) and the incidence of p53 gene mutations (14.1%). Although the p53 mutations were most common in the samples from the patients bronchoscopically manifested as neoplasm compared with other manifestations, there was no statistical difference. It is valuable to notice that 3 patients with p53 gene mutation merely presented as bronchial inflammation in bronchoscope. Conclusion: Results indicated that the value of detecting p53 gene mutation for the diagnosis of lung cancer using the bronchoscopic samples was more superior to cytological examination and detection of p53 gene mutations in post-bronchoscopic sputum was easy and effective, may be used as a valuable method for early diagnosis of lung cancer.

Key words: Lung cancer, Bronchoscopy, p53 gene, Mutation detection

As other malignant tumors, lung cancer is a disease with alteration of genetic materials. P53 gene mutation is the most common and the early genetic alteration in lung cancer. Previous studies about the p53 gene mutation in lung cancer were mainly focused on the resected tumor tissues, it was not useful to the early diagnosis of lung cancer. In this paper, point mutation of the exon 5 to 8 of p53 gene were detected using PCR-single strand conformation polymorphism(PCR-SSCP) in bronchoscopic samples (biopsy, brushing, bronchial lavage and post-bronchoscopic sputum) in patients suspected to be lung cancer.

MATERIALS AND METHODS

Samples

Thirty-five cases of patients suspected to be lung cancer were involved in this study. 29 cases were male and 6 cases were female. Mean ages was 57.7± 11.1 years old. Clinical symptoms include dry cough, hemoptysis and chest pain. All the patients had one of the following presentations of chest X-ray: (1) Segmental or lobular atelectasis; (2) peripheral nodular shadow; (3) hilar mass; (4) obstructive pneumonitis. Findings in bronchoscopy included: (1)

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Endobronchial neoplasm; (2) Bronchial stenosis and/or thicking of a segmental or subsegmental spur; (3) Mucous inflammation, such as mucosal congestion or edema; (4) no obvious abnormal findings. According to the findings of bronchoscopy, patients were performed with biopsy, brushing, bronchoalveolar lavage (BAL) and collection of post-bronchoscopic sputum. 3 ml peripheral blood was collected from all patients.

DNA Isolation

The samples of sputum (digested with an equal volume of 1 mol/L NaOH) and BAL fluid were centrifugalized at 10000 rpm for 10 min. The precipitates were added with 1 ml digestion buff (10 mmol Tris-Hcl, pH8.2, 400 mM NaCL, 2mmol EDTA, 10%SDS, 2mg/ml protease K). The samples of biopsy, brushing and BAL were directly added with digestion buffer and incubated at 37℃ overnight. Extracted samples twice with an equal volume of phenol/chloroform. Recovered the DNA by centrifugation and rinsed pellets with ethanol. Resuspend DNA in TE buffer. The DNA of peripheral blood was extracted by standard technique.

PCR-SSCP

Primers used for amplifying the exon 5 - 8 of p53 gene were listed as Table 1. PCR reactions were performed in 50 μ l of 10 × buffer: 100 μg of genome DNA, 200 mmol/L dNTP, 1.5 mmol/L MgCL, 0.25 μ mol/L primers and 1 unite of Taq polymerase. The reaction was denatured for 5 min at 95℃ and the DNA was subsequently amplified for 35 cycles at 95 ℃ for 40 sec, 60℃ for 40 sec and 72℃ for 70 sec each step. Final extension was 72℃ for 10 min. 5 μl PCR product was processed by loading buffer and denatured at 95℃ for 5 min and refrigerated on ice immediately, then eletropharesed on 12% polyacrylamide gel for 2–3 hours at 2W followed by silver stained.

Any PCR fragments of bronchoscopic samples with mobility different from normal paired peripheral blood DNA in SSCP were considered to be mutation of the p53 gene.

Table 1. PCR primers

Exon	Primer	Size of fragment
Exon 5	P1 5-TACTCCCCTGCCCTCAACAA-3	
	P2 5-CATCGCTATCTGAGCAGCGC-3	84bp
Exon 6	P1 5-GTCTGGCCCCTCCTCAGCAT-3	
	P2 5-CTCAGGCGGCTCATAGGGCG-3	132bp
Exon 7	P1 5-TCTGACTGTACCACCATCCA-3	
	P2 5-CTGGAGTCTTCCAGTGTGAT-3	110bp
Exon 8	P1 5-TGGTAATCTACTGGGACGGA-3	
	P2 5-CGGAGATTCTCTTTCTTCTGT-3	88bp

RESULTS

The results of the cytology, pathophsiology and the p53 gene mutation examination of 35 patients suspected to be lung cancer were listed on Table 2 and 3. The information of 10 patients with p53 mutation listed on Table 4.

Table 2. The relationship of the cytological/Pathophisiologic results and the p53 mutation in 85 samples from bronchoscopy

Examination	Sputum(n=29)	BAL(n=22)	Brushing(n=18)	Biopsy(n=16)	Total(n=85)
Positive of cytology /Pathophisiology	2(6.9%)	0	0	8(50.0%)	10(11.8%)
Positive of p53 mutation	8(27.6%)	1(4.5%)	1(4.5%)	2(12.5%)	12(14.1%)
P value	<0.05	>0.05	>0.05	<0.05	>0.05

Table 3. The relationship of the bronchoscopy presentations and the results of cytology/pathophisiology as well as p53 mutation

Presentations of bronchoscopy	Number	Positive of cytology/pathophisiology	Positive of p53	P value
Endobronchial neoplasm	11	3 (27.3%)	5 (45.5%)	>0.05
Bronchial stenosis and/or thicking of a segmental or subsegmental spur	12	4 (33.3%)	3 (25.0%)	>0.05
Mucous inflammation	3	3 (37.5%)	2 (25.0%)	>0.05
Abnormal findings	4	0	0	

Table 4. Information of 10 patients with p53 mutation

No	Bronchoscopic presentation	Pathophisiology		Cytology	P53 mutation			
		Biopsy	Tissue		Sputum	Biopsy	BAL fluid	Bushing
1	Neoplasm	PDC	BAC	Epidermoid carcinoma	Exon 7	-	-	-
2	Neoplasm	-	Epidermoid carcinoma	-	Exon 8	Exon 8	Exon 6,8	-
3	Inflammation	Suspected carcinoma	N	Adeno-carcinoma	Exon 6	-	-	-
4	Bronchial stenosis	Epidermis carcinoma	N	-	Exon 6	-	-	-
5	Inflammation	-	BAC	-	Exon 6	-	-	-
6	Neoplasm	Epidermis carcinoma	N	-	Exon 6	-	-	-
7	Neoplasm	-	Epidermis carcinoma	-	Exon 6	-	-	-
8	Bronchial stenosis	-	Adeno-carcinoma	-	-	Exon 5,8	-	-
9	Neoplasm	-	Epidermis carcinoma	-	Exon 5	-	-	-
10	Inflammation	-	Adenocarcinoma	-	-	-	-	Exon 7

BAC means bronchioloalveolar carcinoma. PDC means poorly differentiated carcinoma. N means no information. -means negative result.

DISCUSSION

P53 gene locates on $17P^{13.1}$. Its abnormality of genetic construction and expression have turned out to be the most common molecular alterations yet identified in lung cancer. Many studies has conformed that p53 gene mutation is the early event in the carcinogenesis of lung cancer, although it is not the most original and important event controlling the transformation of normal cells to malignant cells.[1] Now, PCR-SSCP is one of the methods most commonly used to screen the mutation of Oncogene or tumor suppresses gene. Recently, some studies began to focus on the application of p53 mutation detection for non-operative samples in the early diagnosis of lung cancer.[2] Sundarreson[3] studied the loss of heterozygosity at 3p and overexpressions of p53 protein for the preinvasive lesions of the bronchus. However, there were only two samples involved. Hrano and Walker[4-6] studied the p53 mutation in hyperplastic and dysplastic bronchus in patients with lung cancer. However, the method they used was Immunohistochemistry other than the direct detection of genetic alteration. Using PCR-SSCP, Mistudomi[7] found the p53 mutation in bronchial biopsy samples obtained from fluoro-broncoscopy in 4 patients with lung cancer. Mao, et al.[8] found 8 patients had mutations of p53 and ras gene in stored sputum samples collected 13 to 24 months before confirmed diagnosis. However, the complicated techniques of cell culture and cloning used in this

research make it difficult for clinic application.

We found p53 gene mutation in 10 cases of 35 patients suspected to be lung cancer (28.6). Among those, 5 cases brochoscopically showed Endobronchial neoplasm (50.0%); 2 cases showed bronchial stenosis and/or thicking of a segmental or subsegmental spur (20.0%). It was should be noticed that 3 cases merely showed mucous inflammation (30.0%). The frequency of mutation of exon 5~8 were 5.7%, 17.1%, 5.7% and 5.7% respectively. The frequency of exon 6 was highest among them, this was similar with that we found in resected tumor tissues (dates were not be listed). 2 cases had mutations of two exons simultaneously. One patent had p53 gene mutation in both postbronchoscopic sputum and the samples from brushing and BAL.

In our study, we found the positive incidence of p53 gene mutations was obviously higher than the cytological positive incidence in samples of post-bronchoscopic sputum (t=2.0200, $P<0.05$). Although the positive incidence of p53 genemutations was higher than the cytological positive incidence in samples of brushing and BAL. There was no statistic difference (t=0.6108 and 0.6579 respectively, $P>0.05$). In view of the samples of postbronchoscopic sputum, brushing and BAL, the positive incidence of p53 gene mutations was also obviously higher than the cytological positive incidence. In the bronchoscopic biopsy specimens, the incidence of p53 gene mutations (12.5%) was lower than that of pathologic positive result (50.0%). However, in view of all the bronchoscopic samples, there was no statistically difference between cytopathologic positive results and the incidence of p53 gene mutations (t=0.4570, $P>0.05$).

Because the frequency of p53 mutation of post-bronchoscopic sputum was higher than the cytological positive results, especially the frequency of p53 mutation in post-bronchoscopic sputum was higher than that in samples from brushing, biopsy and BAL, and sputum was easy to be collected as well as be handled simply, suggesting it is practicable to merely using sputum for detecting p53 mutation. The frequency of p53 mutation was lower than that of pathologic positive result in the bronchoscopic biopsy specimens might be due to (1) only one tissue was biopsied to detect p53 mutation, and the brochoscopic biopsy place for detecting p53 mutation deviated from the place for pathologic examination. (2) DNA extraction of biopsy tissue may not be good (may not be digested enough).

Although the p53 mutations were most common in the samples from the patients bronchoscopically manifested as neoplasm compared with other manifestations, there was no statistical difference, indicating that the result of p53 mutation was not influenced by the samples from bronchus with different bronchoscopy presentation.

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