DETECTION OF TELOMERASE ACTIVITY IN BRONCHOSCOPIC BRUSHING CELLS OF LUNG CANCER PATIENTS

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ABSTRACT

Objective: To investigate the diagnostic significance of the detection of telomerase activity in the brushing cells obtained from fiberobronchoscopy. Methods: The techniques of TRAP-PCR-ELISA and TRAP-silver staining were employed to detect telomerase activity in 42 patients (57 samples) with pulmonary diseases. Results: Telomerase activity in the lesion side of lung cancer patients (N=23) was significantly higher than that in the contralateral side of the same patient ($P<0.05$), and in patients with pneumonia ($P<0.05$). In 23 patients with lung cancer, 21 cases (91.3%) were showed positive in telomerase activity, while only 12 cases (52.3%) were positively diagnosed by cytological smear examination ($P<0.05$). In 6 cases with cytological dysplasia of exfoliated cells, 5 (83.3%) were found to be telomerase activity positive. Conclusion: Detection of telomerase activity in the brushing cells obtained from fiberobronchoscopy may be an effective and sensitive method in the diagnosis of pulmonary malignant diseases.

Key words: Lung cancer, Bronchoscopy, Telomerase activity

Telomeres form the ends of eukaryotic chromosomes, consisting of an array of tandem repeats of the hexanucleotide 5'-TTAGGG-3'.They are believed to protect the ends of chromosomes against exonucleases and ligases, to prevent the activation of DNA-damage checkpoints, and to counter the loss of terminal DNA segments that occurs when linear DNA is replicated. Telomerase, a ribonuleoprotein enzyme and a special reverse transcriptase, utilizes its own RNA as template to add the hexanucleotide to the ends of replicated chromosomes, in order to maintain the length of telomere and to permit the unlimited replication of cells.[1,2] Usually, telomerase activity could be easily detected in the vast majority of human malignant tumors (84%-95%), but only in a few normal somatic cells and benign tumor cells (4%).[3,4] Thus, it is suspected that the activation of telomerase is a key step in the process of carcinogenesis. Here telomerase repeat amplification-polymerase chain reaction-ELISA (TRAP-PCR-ELISA) and TRAP-silver staining are employed to detect the telomerase activity in the brushing cells obtained from fiberobronchoscopy. Its comparison with the cytological smear examination and pathological diagnosis is further discussed.

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MATERIALS AND METHODS

Sample Collection

During May 1996 to October 1997, forty-two in-patients and out-patients of the Second Affiliated Hospital of Zhejiang University were examined by bronchoscopy and involved in this study. The average age for 35 male patients was 59.5 years old, while for 7 female patients how was 39.7 years old. From these 42 patients, a total of fifty-seven samples were collected for detection in this study. Included were 23 lung cancers, 15 from contralateral sides of the lung cancer, 13 cases with pneumonia, and 6 with cytological dysplasia.

Preparation of Cell Extracts

Brushing cells from fiberobronchoscopy were washed in 500 μl sterile saline. Followed the centrifugation at 10,000 r/min for 10 minutes, the

precipitates were resolved in 10 μl to 15 μl lysis buffer and kept at 4°C for 1 or 2 hours. Repeat centrifugation at 12,000 r/min for 15 minutes at 4°C. Supernatants were aliquoted and flash-frozen in -30°C. Protein concentration was determined by the Brtadford assay (Bio-Rad Laboratories, Richmond, CA)

TRAP-PCR-ELISA

TRAP-PCR-ELISA was carried out according to the protocol supplied with the kit (Boehringer Manheim). Twenty-five microliters of TRAP-PCR buffer were added into 1μl to 5μl brushing cell extracts (equal to 50 μg protein). Add DEPC-treated water to a total volume of 50μl. The mixture was mixed gently, incubated for 30 minutes at 25°C, and then warmed to 90°C for 5 minutes. Thirty PCR cycles were carried out, including denaturation for 30 seconds at 94°C, annealing for 30 seconds at 50°C, chain extending for 90 seconds at 72°C, followed by a final ten-minute elongation at 72°C. Five microliters PCR product were mixed with 20μl denaturing buffer, and incubated at room temperature for 10 minutes. Add 225μl hybridization solution to make a total volume of 250 μl. Transfer 100 μl of the mixture per well to the precoated microtiter plate (MTP) modules supplied with the kit and cover the wells with the self-adhesive foil cover to prevent evaporation. Incubate the MTP modules at room temperature for 2 hours. Add peroxidase and TMB substrate for color development for 30 minutes, and finally add stop solution to terminate the reaction. Calculate the OD value at 450 nm using a Microtiter plate reader.

Cell line 293 was used as the positive control (included in the kit) in this study.

SDS-PAGE Electrophoresis

PCR product were loaded on the 12% PAGE, and run at 180 volt for 80 minutes. Sliver staining was employed to show the characteristic ladder-band indicating telomerase activity (Figure 1).

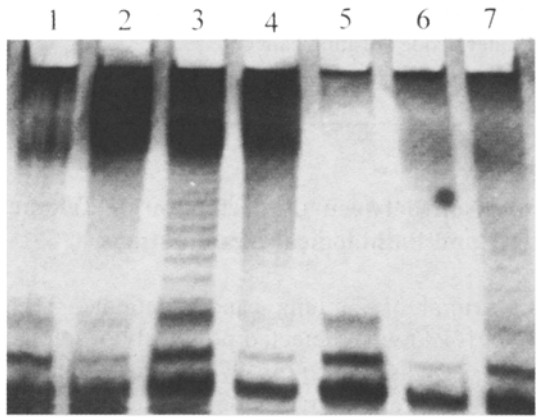

Fig. 1. Telomerase activity in brushing cells obtained from fiberobronchoscopy.
1: Cancer cell line;
2,4,6: Contralateral sides of lung cancer;
3,5,7: Lesion sides of lung cancer.

RESUTLS

Qualitative Analysis of Telomerase Activity

Telomerase activity was detected positively in 91.3% (21 out of 23) of the lesion side of lung cancer patients, while only in 20.0% (3 out of 15) of the contralateral side of the same patients. An obvious difference was observed ($P<0.05$). In the patients with pneumonia, the positive rate with telomerase activity was 15% (2 out of 13), much lower than that in the patients with lung cancer ($P<0.05$). Based on the pathological examination, 83.3% (5 out of 6) of the cases with obvious cytological dysplasia showed positive telomerase activity. The positive rate was similar to that of patients with lung cancer ($P>0.05$ Table 1).

Table 1. Qualitative analysis of telomerase activity in brushing cells obtained from fiberobronchoscopy

	No. of cases	Positive cases	Positive rate(%)
Lesion sides of lung cancer	23	21	91.3
Contralateral sides of lung cancer	15	3	20.0
Cytological dysplasia	6	5	83.3
Pneumonia	13	2	15.0

Quantitative Analysis of Telomerase Activity

The average OD_{450nm} value in the patients with lung cancer is 0.106, while in the conralateral side of the same patients and in the patients with pneumonia is 0.038 and 0.018, respectively ($P<0.05$). As compared with patients with cytological dysplasia, no obviously statistic difference could be observed (0.016 v.s. 0.135, $P<0.05$). (Table 2)

Table 2. Quantitative analysis of telomerase activity in brushing cells obtained from fiberobronchoscopy

	No. of cases	$OD_{450\ nm}$ value	Average $OD_{450\ nm}$
Lesion sides of lung cancer	23	0.027~ 0.452	0.106
Contralateral sides of lung cancer	15	0.012~ 0.110	0.038
Cytological dysplasia	6	0.058~ 0.312	0.135
Pneumonia	13	0.001~ 0.052	0.018

Comparison between the Detection of Telomerase Activity and Pathological Examinations

In a total of 23 lung cancer patients, 21 cases (21/23, 91.3%) were detected positively of telomerase activity, 22 cases (22/23, 97.9%) were positively diagnosed as cancer by tissue pathologic diagnosis (P>0.05). Twelve cases (12/23, 52.2%) were diagnosed as lung cancer by cytological smear examination. An obvious statistic difference could be available as compared to the above two(P<0.01).

DISCUSSION

In this study, we detected telomerase activity in 57 samples from 42 patients suffering from pulmonary diseases. In 23 patients with lung cancer, the majority of the cases (21 out of 23) were positive with telomerase activity in the brushing cells obtained from fiberobronchoscopy, supporting the view that the activation of telomerase is closely related with cancer development.[5] Compared to the tissue biopsy, a similar positive rate is available by detecting telomerase activity. Meanwhile, the finding of a much lower positive rate of cytological smear examination (52.2%) in the same group of patients indicated that the detection of telomerase activity might be a more sensitive technique than cytological smear examination in the clinical diagnosis of cancer.

In 1996, Califano, et al. reported that telomerase was activated in the early stage of the malignant transformation.[6] In this study, we found the positive rate and OD_{450nm} value of telomerase activity in patients with cytological dysplasia is similar to those in patients with lung cancer, implying that the telomerase activity is increased in the rather early stage of carcinogenesis of lung cancer.

Usually, telomerase activity is absent or low in benign tumor cells.[7,8] Here, two out of thirteen cases with pneumonia showed a low-level telomerase activity, which might be due to the vast infiltration of inflammatory cells. Similar results reported by Counter, et al. showed the inflammatory cells have a certain proliferative ability, displaying a low expression of telomerase.[9] For the two samples with telomerase activity in 15 contralateral side of lung cancers, the possible explanation might be the existence of potential micrometastases or multiple primary lesions.

Above all, based on this study, we suggest that detection of telomerase activity in the brushing cells obtained from fiberobronchoscopy might serve as a new, effective and sensitive method in the diagnosis of pulmonary malignant diseases.

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