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Activity of Telomerase in Coal Worker's Autopsied Lung

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— Abstract —

탄광부의 폐장내 Telomerase 활성

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Objectives: 석탄분진에 의한 탄광부 진폐증에서 폐의 염증 또는 섬유모세포의 증식에 의한 telomerase의 활성을 알아보고자 하였다.

Methods: 20년 이상 광부 작업력이 있는 탄광부 8명과 폐종양 8명, 대조군으로 교통사고에 의한 사망 6명의 부검을 통한 폐장내 telomerase 활성을 telomeric repeat amplication protocol(TRAP) 분석 방법을 이용하여 측정하였다.

Results: 탄광부 부검폐조직 내의 telomerase 활성도가 대조군에 비하여 유의하게 증가되었으며 이는 폐종양군과 같은 수준이었다.

Conclusions: 탄광부 진폐증에서 폐장내 과도한 세포증식이 telomerase 활성을 증가시키며, 이에 관한 기전에 대해 보다 폭넓은 연구가 필요하다.

Key Words: Coal worker, Telomerase, Lung cancer, TRAP assay

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Introduction

The major effect on respiratory health caused by chronic coal dust exposure is coal worker's pneumoconiosis(CWP), characterized by relatively mild interstitial inflammation and fibrosis of the lungs. Coal dust is composed of carbon and other substances, including quartz and its oxides. Although coal dust is much less fibrogenic than silica, coal dust has been known to play an important role in inducing lung damage through the activation of the fibrotic process.

In Korea, coal seams are mainly composed of anthracite situated between layers of granite and having a high quart contents. Different epidemiological studies reached conflicting conclusions as to whether there was a correlation between coal-dust exposure and the development of lung cancer.

Telomerase is a ribonucleoprotein that synthesizes and directs the telomeric repeats onto the 3' end of existing telomeres using its RNA component as a template(Christopher et al., 1992). Telomerase activity has been shown to be expressed in immortal cells, cancer and germ cells, where it compensates for telomere shortening during DNA replication and thus stabilizes telomere length(Nam et al., 1994).

To evaluate the activity of telomerase using the autopsied lungs of pneumoconiotic patients, we used the TRAP(Telomeric Repeat Amplification Protocol) assay. The purpose of this study was to investigate whether coal dust-induced pulmonary inflammation or fibrosis which could evolve into a tumor and, if so, the possible mechanism by which this might occur.

Materials and Methods

Study Population(Table 1)

Of the 22 autopsied cases in the study group, 8 of those referred for postmortem examination with an occupational history more than 20 years as coal miner were analyzed as Group 1(Table 1). All the cases in Group 1 involved coal workers and were confirmed by the X-rays taken at regular physical exam in St. Mary's Hospital. The pathologic findings of the lung autopsies are summarized in Table 2. An additional 8 subjects were deemed eligible for participation in the study, due to the presence of lung cancer(Group 2). The control group was composed of 6 cases, involving patients who were killed in traffic accidents without any specific respiratory disease(Group 3).

Table 1. Demographic characteristics of the study group

	Group 1 (n=8)	Group 2 (n=8)	Group 3 (n=6)
Age	65.4±8.2	60.2±10.5	48.0±5.4
Sex (M : F)	All male	All male	5:1
Smoking status (pack.yr)	62.1±5.0	53.4±8.4	30.5±3.1
Current : Ex : Non	5:3:0	4:3:1	2:2:2

Group 1: Subjects with coal worker's pneumoconiosis, Group 2: Subjects with lung cancer, Group 3: Subjects who were victims of traffic accidents. Smoking status was categorized as current smoker, ex-smoker or non-smoker.

Table 2. Brief description of the coal worker's autopsied lungs

No.	Name	Occupational History	Pathologic findings
1	한 O O	Coal facer for 20 yrs	Simple pneumoconiosis
2	이 O O	Tunnel driller for 25 yrs	Complicated pneumoconiosis
3	김 O O	Coal facer & Tunnel driller for 20 yrs	〃
4	이 O O	〃 for 23 yrs	〃
5	안 O O	〃 for 22 yrs	Simple pneumoconiosis
6	박 O O	〃 for 20 yrs	Complicated pneumoconiosis
7	김 O O	Tunnel driller for 22 yrs	Complicated pneumoconiosis + bronchopneumonia
8	김 O O	Coal facer & Tunnel driller for 20 yrs	〃

Within 24 hours of death, the focuses of the coal macules were extracted and stored at -70℃(Group 1). Cancerous lesions or peripheral parts of the lung were collected for the 8 patients with lung cancer and the 6 cases of traffic accidents during the autopsy and stored at -70℃ until use.

TRAP (Telomeric Repeat Amplification Protocol) Assay

The TRAP assay is the photometric enzyme immunoassay for the detection of telomerase activity, utilizing the Telomeric Repeat Amplification Protocol(TRAP). To prepare the tissue specimens for the measurement of telomerase activity, thin slices of cryostat section are prepared on sterile disposable petridishes that are immediately transferred to homogenization tubes containing 200 ml ice-cold lysis buffer. Each sample was centrifuged at 16,000 g for 20 minutes at 4℃ and the supernatant used for the ELISA kit(Cat. No.1 854666, Boehringer Mannheim, Seoul, Korea) with protein titration. The activity was measured as the absorbance value(A450-690nm) using an ELISA reader(UV MR700, Dynatech, USA). Samples were considered as telomerase-positive if the difference in absorbance was more than twice as high the background activity. For the detection of telomerase-mediated DNA-ladder, Southern-hybridization was performed with the biotin-labeled primer using a Biotin Luminescent Detection Kit(Cat No. 1 811 592, Boehringer Mannheim, Seoul, Korea). Finally, the probe was visualized by virtue of peroxidase metabolizing TMB to form a colored reaction product measured as the absorbance value(A450) reading against a blank(reference wavelength A690) by ELISA.

Statistics

Each experiment was carried out at least in triplicate, and the results of a representative experiment are shown. Data were expressed as mean ± SD. Statistical differences among each group were determined by one-way analysis of variance with Dunnett's test to identify which differences were significant. A p value of less than 0.05 represented a significant difference.

Results

Fig. 1 shows the result of the TRAP assay with the lung samples of the CWP and lung cancer patients. Most of the first 8 lanes(No 1~8) as well as lane 11 were positive, while

the control group was negative. A review of past history was done in order to evaluate the difference in telomerase activity, however no reference information was available except for some general information on job history. We compared the telomerase activity by semiquantitative measurements of telomerase using TRAP PCR ELISA. Fig. 2 represented a significantly increased activity of telomerase in the lung cancer and CWP patients as compared to the control patient. Negative control refers to the internal control that degrades the telomerase-associated RNA by pre-incubating the tissue extract with DNase-free RNase. Positive control was prepared from immortalized human kidney cell(293 cells) line to express telomerase.

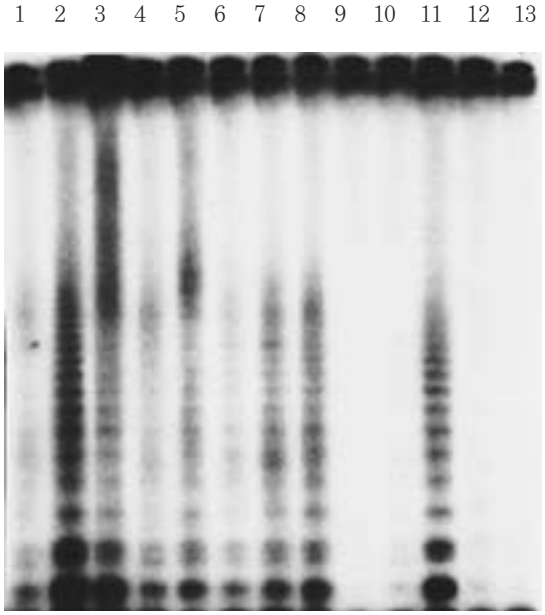

Fig. 1. TRAP assay in lungs of CWP, lung cancer and control patients. lane 1-8: autopsied lung of CWP, lane 9,10: control patients, lane 11: lung cancer lane 12,13: internal (negative) control with RNase treatment.

Discussion

In a nation-wide survey on the incidence of lung cancer in CWP patients, the relative risk of lung cancer was found to be 1.5-3.4 times higher in previous or current coal miners, after taking into consideration the effects of smoking in Korea(Choi et al., 2000). Most cohort studies involving coal miners have some limitations due to the effect of lung carcinogens, such as smoking and radon exposure and to selection bias in the detection of pneumoconiosis cases, all of these factors tending to confound the results. This in turn complicates the epidemiological assessment of silica-containing dust exposure. The balance of evidence indicates that pneumoconiotic patients incur an increased risk of developing lung cancer.

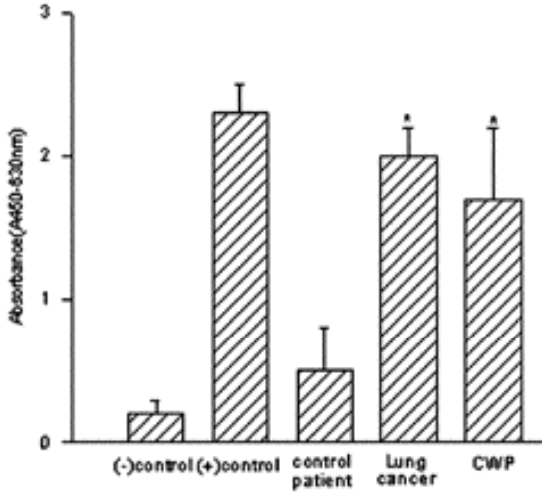

Fig. 2. Specific detection of telomerase activity in CWP. * Significantly different from control patient, $p<0.05$, (-) control: the internal control degrading the telomerase-associated RNA by pre-incubating tissue extract with DNase-free RNase. (+) control: RNA from immortalized human kidney cell (293 cells) line to express telomerase.

In mammals, the telomere is composed of a simple repetitive DNA sequence $(TTAGGG)_n$ and its length is maintained by an enzyme, telomerase, which has been linked to cellular immortality and tumor progression. After a finite number of cell divisions, mammalian cells ultimately enter a state of non-division, which results in telomere shortening(Bodnar et al., 1998).

In a previous study, we reported that the proliferation of fibroblasts was one of the typical characteristics of pneumoconiosis and that this accelerated proliferation might trigger the activation of telomerase in the experimental silicosis model of the rat(Kim et al., 2000).

The maintenance of represented a significant difference represented a significant difference the epithelial barrier is central to normal lung function, and the loss of epithelial integrity could be an important factor in precipitating the early events that lead to pneumoconiosis. Also, many toxicologists emphasized the association between inflammatory and neoplastic processes after exposure to particulates and the type II epithelial cells are regarded as a target for the mutation subsequent to the recruitment of neutrophiles in the pulmonary system. In this study, there were many limitations involved in the collection of information about the autopsied cases. For example, certain confounding factors, such as the previous smoking habits and minor job history of the patients, could not comprehensively evaluated.

Fig. 1 demonstrates that most samples obtained from pneumoconiotic nodules show strong telomerase activity, similar to that of the samples taken from the lung cancer patients, while no such activity was found in the patients without any respiratory diseases. Even represented a significant difference after taking into consideration the confounding factors, such as age and smoking history, the difference of telomerase activity in the dust-exposure group was quite striking, although a lack of telomerase activity is not enough to confirm the length of the telomere. According to numerous publications, the presence of telomerase activity is highly suggestive of the presence of tumorigenesis. Semiquantitative measurement of telomerase also indicated telomerase activity in the CWP group, as well as in the lung cancer group. Whereas normal cells with relative long telomeres and a senescent phenotype contain little or no telomerase activity, tumor cells with short telomeres usually have significant telomerase activity(Calvin et al., 1990).

The presence of coal macules is characteristic of coal worker's pneumoconiosis pathologically and consists of fibroblasts, plasma cells and lymphocytes with many inflammatory cells. There is no evidence yet as to what kind of cells are central to the activity of telomerase and/or tumorigenesis caused by coal dust. Recently, Nozaki et al.(2000) published a study, which confirms the presence of telomerase activity in fibroblasts from bleomycin-injured lungs. But the measurement of the length of the telomere and the characterization of the cells involved may help to explain the possible mechanism more concretely in the future.

Based on the results of this study, we suggest that repetitive inflammation, with subsequent fibrotic change, might have an effect on telomerase activity, leading to the occurrence of immortalization and tumorigenesis in the case of coal workers pneumoconiosis.

Summary

Objective: We measured the activity of telomerase in coal workers lung tissue and found a significant increase in telomerase activity compared to the control group. Pneumoconiosis has the characteristics of fibroblast proliferation and the accumulation of collagen, thus finally causing the pathologic changes, which lead to the irreversible and progressive fibrosis of the lungs. We hypothesized that this cellular proliferation causing irreversible fibrosis may induce some elongation of the life cycle in the chromosomes and lead to further cellular immortalization.

Method: 8 postmortem(within 24 hours) pneumoconiotic cases were examined and their telomerase activity was compared with that of the autopsied lungs of lung cancer patients and of accident victims without any respiratory diseases. Using the extracted ribo-nucleoprotein from pneumoconiotic nodules, telomeric repeat amplification assay(TRAP) was done.

Result: The pneumoconiotic lungs showed strong telomerase activity, similar to that of the lung cancer patients, while the control group showed no such activity.

Conclusion: Based on the results of this study, we found that coal dust-induced cellular proliferation affects telomerase-activity clinically.

참고문헌

Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP et al. Extension of life-span by introduction telomerase into normal human cells. Science 1998;279(5349):349-352.

Calvin BH, Bruce AF, Carol WG. Telomere shorten during aging of human fibroblasts. Nature 1990;345:458-460.

Choi BS, Choi JK, Bae GH, Kim SJ, Kim JH et al, Lung cancer incidence in pneumoconiosis. Chest 2000;118(4):252s.

Christopher MC, Ariel AA, Catherine EL, Nancy GS, Carol WG et al. Telomere shortening associated with chromosome instability is arrested in immortal cells, which express telomerase activity. The EMBO Journal 1992;11(5):1921-1929.

Kim JK, Lim Y, Kim KA, Seo MS, Kim JD et al. Activation of Telomerase by Silica in Rat Lung, Toxicol Lett 2000;111(3):263-270.

Nam WK, Mieczslaw AP, Karen RP, Calvin BH, Michael DW et al. Specific association of human telomerase activity with immortal cells and cancer. Science 1994;266:2011-2014.

Nozaki Y, Liu T, Hatano K, Kermani MG, Phan SH. Induction of telomerase activity in fibroblasts from bleomycin-injured lungs. Am J Respir Cell Mol Biol 2000;23:460-465.