Combined effects of asbestos and cigarette smoke on the development of lung adenocarcinoma: Different carcinogens may cause different genomic changes

KENTARO INAMURA1, HIROHORI NINOMIYA1, KIMIE NOMURA1, EIJU TSUCHIYA1, YUKITOSHI SATOH2, SAKAE OKUMURA3, KEN NAKAGAWA3, AYAKO TAKATA4, NORIHIKO KOHYAMA5 and YUICHI ISHIKAWA1

1Division of Pathology, The Cancer Institute, Japanese Foundation for Cancer Research (JFCR), Koto-ku, Tokyo 135-8550;
2Department of Thoracic Surgery, Kitasato University School of Medicine, Sagamihara, Kanagawa 228-8555;
3Department of Thoracic Surgery, The Cancer Institute Hospital, JFCR, Koto-ku, Tokyo 135-8550;
4Department of Preventive Medicine, St. Marianna University School of Medicine, Miyamae-ku, Kawasaki, Kanagawa 216-8511;
5Faculty of Economics, Toyo University, Bunkyo-ku, Tokyo 12-8608, Japan

Received February 1, 2014; Accepted April 15, 2014

DOI: 10.3892/or.2014.3263

Abstract. The carcinogens in cigarette smoke are distinct from asbestos. However, an understanding of their differential effects on lung adenocarcinoma development remains elusive. We investigated loss of heterozygosity (LOH) and the p53 mutation in 132 lung adenocarcinomas, for which asbestos body burden (AB; in numbers per gram of dry lung) was measured using adjacent normal lung. All cases were classified into 9 groups based on a matrix of cumulative smoking (CS in pack-years; CS=0, 0<CS<25, ≥25 CS) and AB (AB=0, 0<AB<1,000, ≥1,000 AB). AB=0 indicates a lower level than the detection limit of ~100. LOH frequency increased only slightly with the elevation of CS in the AB=0 groups. In the AB>0 groups, LOH frequency increased as AB and/or CS was elevated and was significantly higher in the ≥1,000 AB, ≥25 CS group (p=0.032). p53 mutation frequency was the lowest in the AB=0, CS=0 group, increased as AB and/or CS rose, and was significantly higher in the ≥1,000 AB, ≥25 CS group (p=0.039). p53 mutations characteristic of smoking were frequently observed in the CS>0 groups contrary to non-specific mutations in the CS=0, AB>0 groups. Combined effects of asbestos and smoking were suggested by LOH and p53 analyses. Sole exposure to asbestos did not increase LOH frequency but increased non-specific p53 mutations. These findings indicate that the major carcinogenic mechanism of asbestos may be tumor promotion, acting in an additive or synergistic manner, contributing to the genotoxic effect of smoking. Since this study was based on a general cancer center's experience, the limited sample size did not permit the consideration that the result was conclusive. Further investigation with a large sample size is needed to establish the mechanism of asbestos-induced lung carcinogenesis.

Introduction

Lung cancer is one of the leading causes of cancer-related death in both men and women worldwide, and adenocarcinoma is the most predominant histologic subtype in many parts of the world. Tobacco smoke is clearly the most important factor associated with the development of lung cancer, accounting for 80-90% of all cases. Asbestos is another significant inhaled carcinogen, contributing to the development of ~5-7% of all lung cancers (1). Many studies on asbestos-related lung carcinogenesis have analyzed the genotoxic effects of asbestos; asbestos fibers induce DNA damage, chromosome aberrations, mitotic disturbances and gene mutations (2). In addition, asbestos fibers can stimulate a range of other effects including cell proliferation, chronic inflammation, enhanced gene expression, such as c-fos and c-jun overexpression, and transformation (3,4). Despite these studies, the efficacy of asbestos-exposure as a complete lung carcinogen, independent of tobacco smoke, has not been demonstrated in humans, since lung cancers of asbestos-exposed individuals frequently occur in smokers and ex-smokers. The majority of asbestos-related lung cancers may result from the combined effects of asbestos and carcinogens in tobacco smoke, with the possibility of a synergistic relationship first proposed by Doll (5). Hence, the mechanism of asbestos-induced lung carcinogenesis still remains unclear.

Both loss of heterozygosity (LOH) and the p53 mutation are genetic alterations. LOH is frequently noted in cancer cells and is thought to occur through genetic instability at the chromosomal level. On the other hand, the p53 mutation is a genetic alteration at the nucleotide level. Mutation in the p53 tumor suppressor gene is the most frequently observed gene...
from patients and their families and presented as cumulative smoking (CS) in pack-years. The study protocol was approved by IRB of CIH and informed consent was obtained from all patients.

Measurement of asbestos-exposure. Asbestos-body burden (AB; in numbers per gram of dry lung tissue) was measured using paraffin blocks of corresponding normal lung tissues by a polarizing microscope (16). The detection limit, which means no AB was found on the measuring filter sample, was ~100 AB/g (dry lung) and expressed as 0 in this study.

A matrix of smoking-exposure and asbestos-exposure. To examine the dose-effect relationship of asbestos-exposure (presented as AB) and smoking-exposure (presented as CS in pack-years) on lung adenocarcinomas, we classified all cases into 9 groups based on a matrix of CS in pack-years: CS=0 (n=54, 41%), 0<CS<25 (n=18, 14%), ≥25 CS (n=60, 45%), and AB: AB=0 (n=64, 48%), 0<AB<1,000 (n=28, 21%), ≥1,000 AB (n=40, 31%). Since the patients were selected consecutively from surgical tumor files in a general cancer center, only 4 cases (3.0%) exceeded 5,000 in AB. To investigate the mechanism of asbestos-induced lung carcinogenesis in

Table I. Clinicopathological data of the patients with lung adenocarcinomas analyzed in this study (n=132).

| Clinicopathological features | No. of patients (%) |
|-----------------------------|---------------------|
| Age (years ± SD)            | 61±11               |
| Gender                      |                     |
| Male                        | 74 (56)             |
| Female                      | 58 (44)             |
| Cumulative smoking          |                     |
| CS=0                        | 54 (41)             |
| 0<CS<25                     | 18 (14)             |
| ≥25 CS                      | 60 (45)             |
| Asbestos burden             |                     |
| AB=0                        | 64 (48)             |
| 0<AB<1,000                  | 28 (21)             |
| ≥1,000 AB <5,000            | 36 (27)             |
| ≥5,000 AB                   | 4 (3)               |
| pStage                      |                     |
| I                           | 63 (48)             |
| II-IV                       | 69 (52)             |
| Differentiation             |                     |
| Well                        | 35 (27)             |
| Moderately                  | 69 (52)             |
| Poorly                      | 28 (21)             |
| Size (mm)                   |                     |
| <30                         | 75 (57)             |
| ≥30                         | 57 (43)             |

CS, cumulative smoking in pack-years; AB, asbestos burden; pStage, pathological stage. Percentages may not total 100, due to rounding.

mutation in cancers. As described below, not only p53 mutations but also LOH spectra differ in different cancer types associated with different etiologies. Previously we compared the frequency of LOH on all autosomal chromosomes among non-small cell lung carcinomas (6,7) as well as p53 mutation patterns with adenocarcinoma cell morphology (8). The frequency of allelic loss on many chromosomal arms was commonly higher in squamous cell carcinomas than in adenocarcinomas. This result suggested that more cumulative genetic changes are associated with tumorigenesis in squamous cell carcinomas than contribute to adenocarcinomas, a pattern which may reflect a difference in the carcinogenic mechanisms responsible for the two histologies. In addition, we observed high frequencies of allelic losses on chromosomes 9p, 9q and 13q in squamous cell carcinomas, the majority of which were from smokers, and higher frequencies of allelic losses on these arms in adenocarcinomas from smokers than those from non-smokers. This loss of specific chromosomes associated with a particular histology is an example of LOH spectra reflecting etiology. The p53 mutational spectra differ among cancers of various organs, and its frequency and mutational spectra can be said to reflect carcinogenic patterns characteristic of exogenous or endogenous factors and thus may be helpful for identification of the responsible agents, including, among others, cigarette smoke, aflatoxin B1 and ultraviolet light. Hence, the analysis of p53 mutation can provide clues to the etiology of diverse tumors and to the function of specific regions of p53 (9,10).

The mutation pattern in smokers shows an excess of G:C to T:A transversions (34.2%), which are relatively uncommon in non-smokers or passive-smokers (16.6%) (11). These transversions often occur at codons 157, 158, 245, 248 and 273, experimentally identified as sites of adduct formation by benzo(a)pyrene, a single polycyclic aromatic hydrocarbon (PAH)-compound found in cigarette smoke. Other PAH-compounds also have a similar preference for adduct formation in these p53 codons (12,13).

In the present study, to elucidate the combined effects of asbestos-exposure and smoking on development of lung adenocarcinomas, we used 132 lung adenocarcinomas, from which we already obtained all detailed smoking histories, comprehensive LOH data for all autosomal chromosomes (7), and p53 mutation data.

Materials and methods

Patients and sample preparation. A total of 335 cases of lung adenocarcinoma were surgically removed at the Cancer Institute Hospital (CIH), Tokyo, Japan, between September 1989 and August 1996. Among the cases, fresh tumor tissues and corresponding normal lung and detailed smoking histories were successfully collected from 132 patients, which were used as materials in this study. Hence, they were collected semi-randomly without respect to asbestos-exposure status, and therefore provided a representative population for a cancer center in Japan. The clinicopathological data for these samples are summarized in Table I. We used a differentiation grading that was basically according to the former version of the Japanese Lung Cancer Society (14), as previously performed (15). Smoking history was surveyed intensively
a representative population for a cancer center, not a biased population heavily exposed to asbestos, we divided the cases between AB <1,000 and ≥1,000 AB.

**LOH analysis.** For LOH analysis, we performed Southern blotting. Experimental procedures and probes used were essentially the same as previously described (6,7). To facilitate the comparison, we used a fractional allelic loss (FAL) value, defined as: (number of chromosome arms with LOH)/(number of informative arms) for each case. Of 132 patients with adenocarcinomas, LOH data were available for 114 patients.

**p53 mutation analysis.** Analysis of p53 mutation was performed essentially as described elsewhere (8). Genomic DNA from fresh tumor samples was prepared and exons 4-8 and 10 of p53 were analyzed by polymerase chain reaction and DNA sequencing. Of the 132 patients with adenocarcinomas, p53 mutation data were available for 123 patients.

**Statistical analysis.** For statistical analysis, we used the t-test, Fisher's exact test, and Chi-square test, as appropriate. The two-sided significant level was set at p<0.05. Data were analyzed with the statistical software Stata version 11 (StataCorp., College Station, TX, USA).

**Results**

LOH frequency of lung adenocarcinomas classified by CS and AB is shown in Table II and Fig. 1A. LOH frequency increased only slightly correlating with the elevation of CS in the AB=0 groups, whereas, in the AB>0 groups, it increased as AB and/or CS was elevated and was significantly higher in the ≥1,000 AB, ≥25 CS group than in the AB=CS=0 group (p=0.032). Tobacco smoke, one of the most significant exogenous carcinogenic agents has been shown to frequently cause specific p53 mutations, especially G:C to T:A transversion (17) at specific codons described as 'hotspots', such as codon 157, 158, 245, 248 and 273 (13). p53 mutations characteristic of smoking, such as G:C to T:A transversion at the
### Table III. Details of the cases with p53 mutations in lung adenocarcinomas.

| Classified by | Case no. | Gender | Age (years) | Diff. | Size (mm) | pStage | AB | CS | FAL type | Codon | Base change | Amino acid |
|---------------|---------|--------|-------------|-------|-----------|--------|----|----|----------|--------|-------------|------------|
| CS=0          | 33      | F      | 49          | Mod   | 27        | IIIB   | 0  | 0  | 0.13     | ts     | CGT to CAT  | Asp→His    |
| AB=0          | 70      | F      | 26          | W     | 43        | IIIB   | 0  | 0  | 0.53     | tv     | TGC to TTC  | Cys→Phe    |
| 73            | M       | 44     | P            | 35    | IIIA      | 0      | 0  | 0  | 0.35     | ts     | AAG to AGG  | Lys→Arg    |
| 74            | F       | 70     | W            | 20    | IA        | 0      | 0  | 0  | 0.06     | ts     | CGG to CAG  | Arg→Glu    |
| CS=0          | 27      | F      | 77          | Mod   | 38        | IIIB   | 187| 0  | 0.18     | tv     | ATG to ATT  | Met→Ile    |
| 0<AB<1,000    | 39      | F      | 51          | Mod   | 24        | IIIB   | 214| 0  | 0.05     | ts     | GCC to AGC  | Gly→Ser    |
| 47            | F       | 65     | W            | 24    | IA        | 333   | 0  | 0  | 0.06     | ts     | CGT to CAT  | Arg→His    |
| 81            | F       | 68     | Mod          | 21    | IA        | 671   | 0  | 0  | 0.05     | tv     | CGT to CTG  | Asp→Leu    |
| CS=0          | 3       | F      | 51          | Mod   | 33        | IIIA   | 1,715| 0 | 0.14     | del    | TTC to T_C  | Frameshift |
| ≥1,000 AB     | 7       | F      | 72          | Mod   | 60        | IV     | 3,939| 0 | 0.58     | ts     | GCC to GTC  | Ala→Val    |
| 14            | F       | 57     | Mod          | 40    | IIIA      | 2,305 | 0  | 0  | 0.29     | tv     | GCC to CCC  | Ala→Pro    |
| 84            | F       | 63     | Mod          | 25    | IIIB      | 1,000 | 0  | 0  | 0.21     | ts     | CGG to TGG  | Arg→Trp    |
| 113           | F       | 67     | Mod          | 32    | IIIB      | 1,949 | 0  | 0  | 0.13     | ts     | AAG to AGG  | Lys→Arg    |
| 114           | F       | 49     | Mod          | 33    | IB        | 6,998 | 0  | 0  | 0.1     | ts     | CGA to TGA  | Arg→Stop   |
| 0<CS<25       | 55      | F      | 68          | Mod   | 42        | IIIB   | 0  | 3.8 | 0.17     | ts     | TGC to TAC  | Cys→Tyr    |
| AB=0          | 126     | M      | 66          | Mod   | 30        | IA     | 0  | 8  | NA       | ts     | ATG to ATA  | Met→Ile    |
| ≥25 CS        | 2       | M      | 73          | P     | 53        | IIIB   | 0  | 39.4| 0.25     | ts     | GAC to ΔAC  | Asp→Ile    |
| AB=0          | 12      | M      | 69          | Mod   | 28        | IA     | 0  | 42.3| 0.04     | del    | Del of 19 bp Frameshift |
| 21            | M       | 47     | P            | 39    | IIIA      | 0      | 32.5| 0  | tv       | 245    | GCC to TGC  | Gly→Cys    |
| 42            | M       | 58     | P            | 24    | IIIA      | 0      | 80  | 0.36 | ts       | 223    | CGT to TGT  | Asp→Cys    |
| 46            | M       | 56     | Mod          | 20    | IIIB      | 0      | 31  | 0.1  | del      | 159    | GCC to _C   | Frameshift |
| 54            | M       | 54     | Mod          | 25    | IIIA      | 0      | 48  | 0.38 | tv       | 198    | GAA to TAA  | Glu→Stop   |
| 56            | M       | 74     | W            | 17    | IA        | 0      | 42  | 0.24 | ts       | 175    | CGC to CAC  | Arg→His    |
| 58            | M       | 61     | P            | 23    | IV        | 0      | 80  | 0.33 | tv       | 135    | TGC to TTC  | Cys→Phe    |
| 83            | M       | 72     | Mod          | 75    | IV        | 0      | 126 | 0.44 | del      | 274    | GTT to _T   | Frameshift |
| 88            | M       | 50     | Mod          | 48    | IV        | 0      | 115.5| 0.2 | del      | 189    | GCC to G_C  | Frameshift |
| 96            | M       | 54     | Mod          | 27    | IA        | 0      | 34  | 0.25 | tv       | 158    | CGC to CT_C | Arg→Leu    |
| 102           | M       | 56     | W            | 16    | IA        | 0      | 37.5| 0.06 | ts       | 273    | CGT to TGT  | Asp→Cys    |
| 116           | M       | 50     | P            | 60    | IIIA      | 0      | 32  | NA  | tv       | 245    | GCC to TGC  | Gly→Cys    |
| 0<CS<25       | 17      | M      | 58          | W     | 27        | IA     | 560 | 1.3 | 0.13     | ts     | TAC to TGC  | Tyr→Cys    |
| 0<AB<1,000    | 128     | F      | 69          | P     | 60        | IB     | 980 | 20  | NA       | ts     | GCC to GAC  | Gly→Asp    |
Table III. Continued.

| Classified by CS and AB | Case no. | Gender | Age (years) | Diff. | Size (mm) | pStage | AB | CS | FAL | Mut type | Codon | Base change | Amino acid |
|-------------------------|---------|--------|-------------|-------|-----------|--------|----|----|-----|----------|--------|-------------|-----------|
| ≥25 CS                  | 11      | M      | 64          | Mod   | 20        | IA     | 333| 33 | 0.17| tv       | Donor  | AGgt to AGtt | Splicing  |
| 0<AB<1,000              | 49      | M      | 41          | P     | 105       | IB     | 446| 37.5| 0.22| tv       | Acceptor| agG to aG | Splicing  |
| 97                      | M       | 72     | Mod         | 16    | IIIB      | 929    | 25.5| 0.26| 273 | Cyt to CAT | Asp→His|
| 131                     | M       | 51     | P           | 28    | IIIA      | 339    | 31 | NA | tv   | 244      | GGC to TGC| Gly→Cys    |
| 0<CS<25                 | 23      | M      | 59          | Mod   | 24        | IA     | 1,538| 24 | 0.12| tv       | 274    | GTT to TTT | Val→Phe   |
| ≥1,000 AB               | 64      | F      | 74          | W     | 37        | IIIB   | 1,477| 12 | 0.11| tv       | 209    | ΔGA to TGA | Arg→Stop  |
| 86                      | M       | 49     | Mod         | 23    | IIIA      | 2,039  | 1   | 0.45| tv   | 238      | TGT to AAT | Cys→Ser   |
| ≥25 CS                  | 16      | M      | 72          | P     | 35        | IIIA   | 2,490| 40 | 0.31| ts      | 158    | CGC to CAC | Arg→His   |
| ≥1,000 AB               | 53      | M      | 60          | P     | 28        | IIIA   | 1,750| 40 | 0.64| ts      | 158    | CGC to CAC | Arg→His   |
| 85                      | M       | 65     | Mod         | 28    | IA        | 2,337  | 45  | 0.55| ts   | 275      | TGT to TAT | Cys→Tyr   |
| 103                     | M       | 67     | Mod         | 28    | IIIB      | 1,293  | 30.6| 0.2 | ins  | 305-306 | Ins of 23 bp| Frameshift|
| 104                     | M       | 74     | Mod         | 32    | IB        | 2,378  | 53  | 0.05| ts   | Donor   | A Ggt to AG | Splicing  |
| 105                     | M       | 50     | Mod         | 24    | IA        | 2,212  | 58  | 0.29| del  | 179-185 | Del of 18 bp| Frameshift|
| 109                     | M       | 47     | P           | 64    | IIIA      | 3,207  | 81  | 0.64| tv   | 158      | CGC to CCC | Arg→Pro   |
| 110                     | M       | 55     | Mod         | 15    | IA        | 3,881  | 35  | 0   | ins  | 46       | GTC to TTC | Val→Phe   |
| 115                     | M       | 71     | Mod         | 20    | IIIA      | 5,308  | 48  | 0.46| tv   | 157      | GTC to TTC | Val→Phe   |

CS, cumulative smoking in pack-years; AB, asbestos burden; Diff., differentiation; pStage, pathological stage; FAL, fractional allelic loss; Mut, mutation; F, female; M, Male; Mod, moderately; W, well; P, poorly; ts, transition; tv, transversion; del, deletion; ins, insertion; NA, not analyzed. Specific codons in p53 mutations characteristic of smoking are underlined.
tobacco-specific codons were frequently observed in the CS>0 groups, whereas non-specific mutations were often detected in the CS=0, AB>0 groups (Tables III and IV). In the ≥1,000 AB, CS=0 group, there was only one transversion and no tobacco-specific codons for the six p53 mutations. In contrast, in the AB=0, ≥25 CS group, there were five G:C to T:A transversions and five tobacco-specific codons among 13 p53 mutations. Fig. 2 shows p53 mutation spectra in lung adenocarcinomas, classified as smokers (A, n=33) or non-smokers (B, n=14) and asbestos-exposed (C, n=28) or not (D, n=19). Although p53 mutation spectra varied depending on the status of smoking history, they showed little difference between asbestos-exposed or non-exposed. Whereas smokers had frequent G:C to T:A transversions, which are smoking-associated p53 mutations, non-smokers had frequent G:C to A:T transitions at CpG sites associated with spontaneous mutations, consistent with previous reports (9,17).

With respect to tumor differentiation grade, a heavier smoking habit was associated with less-differentiated adenocarcinomas (Fig. 3A, p=0.0010, Chi-square test), in line with a previous study (18). On the other hand, there was no correlation between asbestos deposition and the differentiation grade (Fig. 3B, p=0.75).

Discussion

Both tobacco smoke and asbestos fibers are significant inhaled carcinogens which contribute significantly to lung adenocarcinoma development. We previously revealed that chromosome instability and LOH, rather than minisatellite and microsatellite instability, play major roles in the development of lung adenocarcinomas (19). The LOH and p53 spectra provide clues concerning the etiology and nature of carcinogenesis. To elucidate the carcinogenic mechanisms of two different inhaled carcinogens, asbestos and cigarette smoke, we investigated LOH on all autosomal chromosomes and measured asbestos burden (AB; asbestos body per gram of dry lung tissue) using corresponding normal lung tissue and investigated p53 mutation employing fresh tumor samples.

The p53 mutational spectra may be helpful for identification of the origins of the mutations that give rise to human
Asbestos may work in a promoter-like manner. Production of reactive oxygen species and/or induction of tissue regeneration may be relevant.

- **Asbestos-Exposure**
  - **Tandem Mutations**: CC to TT double mutation, most characteristic.
  - **Specific Mutations**: G:C to T:A transversions, characteristic of cancers.

- **Smoking-Exposure**
  - **LOH Frequency**: Increased as smoking-exposure was elevated.
  - **Carcinoma Types**:
    - Squamous Cell Carcinomas (SCC)
    - Adenocarcinomas
      - **Differentiation Grade**: Poorly differentiated (PD) have higher LOH frequency than differentiated adenocarcinomas.
      - **Smoking Association**: PD adenocarcinomas have a relatively weaker association with smoking.

- **Mutational Spectra**
  - **p53 Mutations**: One of the most intriguing recent discoveries.
    - **Characteristics**:
      - Aflatoxin B1-associated hepatocellular carcinomas frequently have specific p53 mutations: G:C to T:A transversions at the 3rd base of codon 249, AGG to AGT (Arg to Ser) (20).
      - **Other Mutations**:
        - CC to TT double mutation
        - G:C to T:A transversions

- **Other Genetic Alterations**
  - **Chromosomal Regions**: 19p13 (28), 9q33.1 (29), 2p16 (30)
  - **Candidate Mutations**:
    - Driver mutations in lung adenocarcinomas
    - EGFR mutations
    - ALK fusion

- **Molecular Targets**
  - **Drug Sensitivity**:
    - Patients harboring such simple oncogenic mutations represent good candidates for molecular-targeted drugs.
  - **Exposure Effects**:
    - In adenocarcinomas with asbestos-exposure, smoking-exposure increases LOH frequency.
    - In adenocarcinomas without asbestos-exposure, smoking-exposure elevates LOH frequency.

- **Histological Grade**
  - **Analysis**:
    - Significant relationship between CS and differentiation grade.
    - No correlation between AB and differentiation grade.

- **Exposure-Related Mutations**
  - **p53 Mutation Rate**
    - Lowest in non-exposed cases.
    - Increased as asbestos-exposure and/or smoking-exposure was elevated.
  - **Driver Mutations**:
    - EGFR, KRAS, MET, ALK, and HER2

- **Molecular Classification**
  - **Adenocarcinomas**:
    - Non-smokers are considered to be less genetically complex.
    - In adenocarcinomas, the relationship with asbestos-exposure remains unclear.

**Figure 3**
- Cumulative smoking (CS) and asbestos burden (AB) with reference to the histological differentiation grade.
- Although there was a significant relationship between CS and the differentiation grade (p=0.0010, Chi-square test), there was no correlation between AB and the differentiation grade (p=0.75).
- Well-diff., well-differentiated; mod-diff., moderately differentiated; poorly-diff., poorly differentiated.
ation that the result is conclusive. Further investigation with a large sample size is required to establish the mechanism of asbestos-induced lung carcinogenesis.

Acknowledgements

The authors thank Ms. Miyuki Kogure, Mr. Motoyoshi Iwakoshi, Ms. Tomoyo Kakita, and Ms. Shizue Kurimori for their technical assistance, and Ms. Yuki Takano and Ms. Yumiko Toriyama for secretarial assistance. Parts of this study were supported financially by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, from the Japan Society for the Promotion of Science including Grant-in-Aid for Young Scientists (B), and by grants from the Ministry of Health, Labour and Welfare, the Smoking Research Foundation, and the Vehicle Racing Commemorative Foundation.

References

1. LaDou J: The asbestos cancer epidemic. Environ Health Perspect 112: 285-290, 2004.
2. Jaurand MC: Mechanisms of fiber-induced genotoxicity. Environ Health Perspect 105 (Suppl 5): 1073-1084, 1997.
3. Heintz NH, Janssen YM and Mossman BT: Persistent induction of c-fos and c-jun expression by asbestos. Proc Natl Acad Sci USA 90: 3299-3303, 1993.
4. Timblin CR, Janssen YW and Mossman BT: Transcriptional activation of the proto-oncogene c-jun by asbestos and H2O2 is directly related to increased proliferation and transformation of tracheal epithelial cells. Cancer Res 55: 2723-2726, 1995.
5. Doll R: Mortality from lung cancer in asbestos workers. Br J Ind Med 12: 81-86, 1955.
6. Tsuji E, Nakamura Y, Weng SY, et al: Allelotype of non-small cell lung carcinoma - comparison between loss of heterozygosity in squamous cell carcinoma and adenocarcinoma. Cancer Res 52: 2478-2481, 1992.
7. Sato S, Nakamura Y and Tsuji E: Difference of allelotype between squamous cell carcinoma and adenocarcinoma of the lung. Cancer Res 54: 5652-5655, 1994.
8. Hashimoto T, Tokuchi Y, Hayashi M, et al: Different subtypes of human lung adenocarcinoma caused by different etiological factors. Evidence from p53 mutational spectra. Am J Pathol 157: 2133-2141, 2000.
9. Hollstein M, Sidransky D, Vogelstein B and Harris CC: p53 in human lung cancer: correlation with clinical response to gefitinib therapy. J Natl Cancer Inst 92: 803-811, 2000.