Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 5099–5103, July 1989
Medical Sciences

Differential DNA sequence deletions from chromosomes 3, 11, 13, and 17 in squamous-cell carcinoma, large-cell carcinoma, and adenocarcinoma of the human lung

(DNA-restriction fragment length polymorphisms/mitotic recombination/tumor suppressor genes)

A. WESTON*, J. C. WILLEY*, R. MODALI*, H. SUGIMURA*, E. M. MCDOWELL†, J. RESAU†, B. LIGHT*, A. HAUGEN‡, D. L. MANN*, B. F. TRUMP†, AND C. C. HARRIS*§

*Laboratory of Human Carcinogenesis, Division of Cancer Etiology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; †Department of Pathology, University of Maryland, 10 South Pine Street, Baltimore, MD 21201; and ‡Institute of Occupational Health, P.O. Box 8149, Dep 0033, Oslo, Norway

Communicated by Theodore T. Puck, February 21, 1989

ABSTRACT **Activation of protooncogenes and inactivation of putative tumor suppressor genes are genetic lesions considered to be important in lung carcinogenesis. Fifty-four cases of non-small-cell lung cancer (23 adenocarcinomas, 23 squamous-cell carcinomas, and 8 large-cell carcinomas) were examined for loss of DNA sequences at 13 polymorphic genetic loci. Loss of heterozygosity was seen more frequently in squamous-cell carcinoma than in adenocarcinoma. The loss of DNA sequences from the short arm of chromosome 17 (D17S1 locus) was detected in 8 of 9 heterozygous cases of squamous-cell carcinoma and in only 2 of 11 heterozygous cases of adenocarcinomas. Furthermore, in 7 of these 8 squamous-cell carcinomas, loss of heterozygosity from chromosome 17 was accompanied by loss of DNA sequences from chromosome 11. The spectrum of allelic sequences lost from chromosome 11 was, however, similar in every type of carcinoma studied, and the data show two regions commonly deleted from chromosome 11 (11pter–p15.5 and 11p13–q13) that may have a role in the pathogenesis of all these types of non-small-cell bronchogenic carcinoma. Loss of DNA sequences from chromosome 3 was seen in 16 of 31 cases where the constitutive DNA was heterozygous—i.e., informative. These data included only 6 of 16 cases where loss of heterozygosity involved a chromosomal locus previously shown to be lost consistently in small-cell lung cancer (DNF15S2). Loss of heterozygosity at the chromosome 13q locus, D13S3, was seen in 9 of 21 informative cases, and in 2 cases, both adenocarcinomas, duplication of the intact DNA sequences suggested the possibility that mitotic recombination had occurred. Frequent DNA sequence deletions, including those from chromosome 17, in squamous-cell carcinomas may reflect the extensive mutagenic and clastogenic effects of tobacco smoke that may lead to inactivation of putative tumor-suppressor genes.**

More than 125,000 deaths each year in the United States can be attributed to carcinomas of the lung (1). The major etiological agent, tobacco smoke, is a complex mixture containing several thousand compounds including chemical and physical carcinogens and clastogenic agents (2). These chemically and functionally diverse agents play an interactive role in the genetic and epigenetic changes occurring during the multistage process of lung carcinogenesis. The genetic changes include the activation of protooncogenes of the *myc*, *ras*, *raf*, and *jun* multigene families (3) as well as the inactivation of putative tumor-suppressor genes (4–9). Activated protooncogenes—i.e., Ha-*ras*, Ki-*ras*, or the combination of c-*myc* and c-*raf-1* have recently been transferred into human bronchial epithelial cells *in vitro*, and neoplastic transformation was subsequently observed by the formation of tumors in athymic nude mice (10–12).

Most reports of DNA sequence deletions in lung cancer are confined to studies of small-cell lung cancer and have described an associated 3p deletion. However, the importance of loss of heterozygosity at the 3p locus (DNF15S2) in non-small-cell lung cancer remains equivocal (5, 6, 13, 14).

This report describes DNA sequence deletion analysis of 13 polymorphic chromosomal loci in squamous-cell carcinomas (23), large-cell carcinomas (8), and adenocarcinomas (23). Clearly, the frequency and spectra of DNA sequence deletions differ between squamous-cell carcinomas and adenocarcinomas of lung cancer, whereas large-cell carcinomas have similar characteristics to adenocarcinomas. Differences were also found between the results reported here in non-small-cell carcinoma and those previously described in small-cell carcinoma of the lung (5–7, 13, 14).

MATERIALS AND METHODS

Reagents. Chemicals and stock solutions were purchased from either J. T. Baker, Bio-Rad, or Biofluids (Rockville, MD), unless otherwise stated. Restriction endonucleases (*Bam*HI, *Hin*dIII, *Hpa* II, *Msp* I, *Pvu* II, and *Taq* I) and their appropriate digestion buffers were supplied by either Boehringer Mannheim or New England Biolabs. Molecular probes that were used in this study are indicated in Table 1.

Tumor Samples. Portions of tumor tissue and unaffected peripheral lung tissue removed from 8 female and 46 male lung cancer patients at surgical resection were cut into thin slices with a scalpel. The sliced tissue was placed into lysis buffer (0.6% SDS/10 mM EDTA/10 mM Tris·HCl, pH 7.5) containing RNase A (50 μg/ml) and incubated for 1 hr at 37°C. Proteinase K was then added (200 μg/ml), and the mixture was incubated at 37°C for 16–24 hr before extraction with phenol (Bethesda Research Laboratories) and chloroform. DNA was precipitated from the aqueous phase by 2 vol of ethanol and the counter ion sodium acetate (2 M to 10%).

DNA-Restriction Fragment Length Polymorphism (RFLP) Analyses. Tumor and uninvolved lung from each case were coded to disguise their histological identity. Nucleic acid hybridization was performed with appropriate restriction enzymes on 5-μg portions of the DNA samples according to the method of Southern (15). Polymorphic DNA probes were radiolabeled with deoxycytidine-5′-[^{32}P]triphosphate (3000 Ci/mmol; 1 Ci = 37 GBq, Amersham). These probes were

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: RFLP, restriction fragment length polymorphism.

§To whom correspondence should be addressed at: Building 37, Room 2C05, National Cancer Institute, Bethesda, MD 20892.

hybridized to the immobilized DNA samples for 12–16 hr at high stringency (1% SDS/10% wt/vol) dextran sulfate/1 M NaCl at 65°C) with salmon sperm DNA (100–200 μg/ml) (Lofstrand Laboratories, Gaithersburg, MD). After hybridization filters were washed (5 min each) in 300 mM sodium chloride/30 mM sodium citrate, then in 300 mM sodium chloride/30 mM sodium citrate/1% SDS twice for 30 min each at 65°C, and finally in 15 mM sodium chloride/1.5 mM sodium citrate twice for 30 min. X-ray films were exposed to the radiolabeled filters with enhancer screens at −70°C. The resulting autoradiograms were examined to compare differences in signal intensity between constitutive and tumor samples from the same individuals.

Histological Classification of Lung Tumors. Histological classification of the tumors was made independently of RFLP analyses according to the criteria of the World Health Organization (16).

RESULTS

The autoradiograms in Fig. 1 show representative RFLP analyses for lung cancer patients whose constitutive DNA, extracted from unaffected peripheral lung, was found to be heterozygous. In every case DNA restriction fragments of the expected size were seen. The corresponding tumor samples all show significant reduction or loss of autoradiographic signal for one of the DNA restriction fragments (bands), indicating loss of heterozygosity. These data show, therefore, that for each of the 13 polymorphic loci heterozygosity was lost in at least one tumor studied. Loss of heterozygosity was more commonly observed in squamous-cell carcinomas (51%) than adenocarcinomas (32%; $P < 0.02$; Wilcoxon paired-sample testing by ranks).

A summary of the results of 253 RFLP analyses of the constitutive and tumor DNA samples from 23 squamous-cell carcinoma patients at 13 polymorphic loci is given in Table 1. The data show that loss of heterozygosity was seen at the D17S1 locus (17p13) in 8 of 9 informative cases of squamous-cell carcinoma. In addition, loss of heterozygosity from chromosome 17 for 7 of these 8 tumors was accompanied by loss of heterozygosity from chromosome 11. Fourteen of the squamous-cell carcinoma patients were heterozygous for polymorphic loci that map to chromosome 3p and among these 9 tumors showed loss of heterozygosity. Loss of heterozygosity was most frequently observed at the D3S2 locus (6 of 8 informative tests), *RAF1* was lost in half of the tumors (3 of 6), and the DNF15S2 locus, which appears to be consistently lost or deleted in small-cell carcinoma of the lung (5–7), was also lost in half of the informative cases (3 of 6). One case shows loss of heterozygosity at all three of these chromosome 3p loci, and losses of heterozygosity that were detected in six other samples are consistent with terminal deletions of chromosome 3. Heterozygosity at the *RAF1* locus and losses of heterozygosity that were detected in 2 tumors are consistent with interstitial deletions. Because 1 tumor was heterozygous at both the D3S2 and the DNF15S2 loci, and 2 other tumors were heterozygous at the *RAF1* locus, the shortest region of deletion overlap is located between 3p25 and p21.

Loss of heterozygosity for the chromosome 11 pair was observed in 14 of 22 squamous-cell carcinomas in 25 of 56 informative tests. Loss of heterozygosity was most frequent at the *HBG2*, *INS*, and *HRAS1* gene loci (Table 1). Scrutiny of the data does not define a single chromosomal region that is consistently lost in squamous-cell carcinoma. Five tumors show loss of heterozygosity consistent with loss of the whole p-arm or the whole chromosome. However, two possible regions of interest may be defined in squamous-cell carcinomas when cases are considered in which partial loss of heterozygosity has occurred (Fig. 2). Tumor 759 is heterozygous at the *INT2*, *CAT*, *CALCA*, and *INS* loci but has lost DNA sequences from the *HRAS1* locus, and loss of heterozygosity is consistent with the presence of larger terminal deletions for tumors 553, 683, 727, and 771. Alternatively, tumor 749c is heterozygous at the *HRAS1*, *INS*, and *CAT* loci, having lost DNA sequences from the *INT2* locus. Tumor 965 has also lost an *INT2* locus but is heterozygous at the *INS* and *HRAS1* loci. Tumor 970 is heterozygous for *INT2* and the telomeric locus *INS* but has lost *CAT* and *CAL*, whereas tumor 730 has lost *CAT* and retained *INS*. Taken together, these data suggest that loss of heterozygosity in either of two separate regions of chromosome 11 may be of importance in the pathogenesis of squamous-cell carcinoma of the lung—i.e., 11pter–p15.5 and 11p13–q13. Loss of heterozygosity was also seen for genes located on chromosome 13q, 18, and 20. These chromosomes appear to be affected, at least to the same extent as chromosomes 3 and 11.

The results of 229 RFLP analyses of the constitutive and adenocarcinoma DNA samples from 23 patients at 13 polymorphic loci are summarized in Table 1. These data show that loss of heterozygosity was seen in 18 cases of adenocarcinoma for at least 1 heterozygous locus. For chromosome 17, the region D17S1 was heterozygous in the constitutive DNA of 11 patients; reduction to homozygosity was seen in only 2 tumors. Thirteen of the adenocarcinoma patients were heterozygous for loci on chromosome 3; among these, 7 of the tumors showed loss of heterozygosity. Loss of heterozygosity was most frequent at the *RAF1* locus (3 of 5) as compared with the D3S2 (4 of 9) and the DNF15S2 (3 of 8) loci. Furthermore, 2 tumors that were found to be informative for all studied 3p loci remained heterozygous. Therefore, it is not possible from these data to assign a region of chromosome 3 consistently lost in adenocarcinoma of the lung.

For the chromosome 11 pair, loss of heterozygosity was seen in 10 of 22 tumors in 15 of 55 informative tests. Loss of heterozygosity was most frequent at the *HBG2* (4 of 8), *INS*

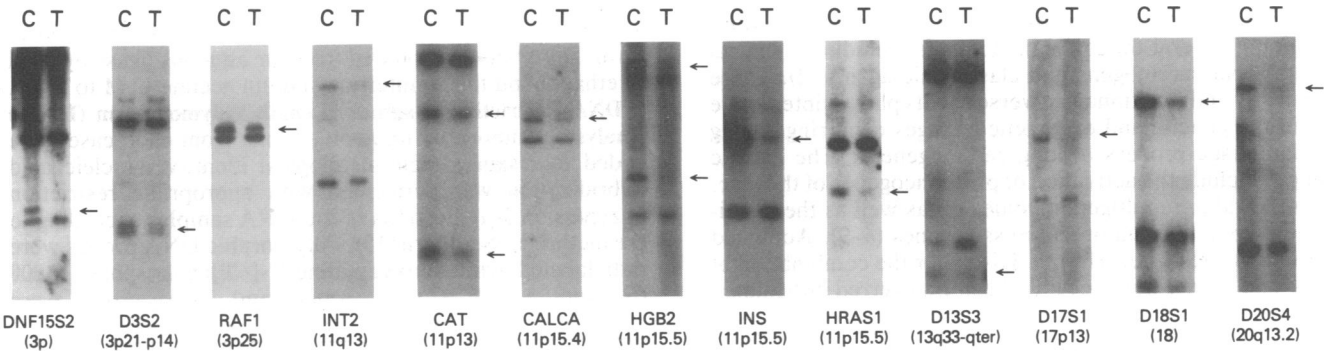

FIG. 1. RLFP analyses of constitutive (C) and tumor (T) samples in non-small-cell lung cancer. Representative Southern hybridization patterns for 13 different polymorphic genetic loci are shown.

Table 1. Analysis of loss of heterozygosity in 54 cases of lung cancer

Histological type	D3S2 (3p21-14)*	DNF15S2 (3p21)	*RAF1* (3p25)	D13S3 (13p33-qter)	D17S1 (17p13)	D18S1 (18)	D20S4 (20q13.2)
Squamous-cell carcinoma (23)†	6/8‡	3/6	3/6	4/9	8/9	2/5	4/10
Adenocarcinoma (23)	4/9	3/8	3/5	4/9	2/11	1/4	2/6
Large-cell carcinoma (8)		0/2	0/2	1/3	1/3	1/1	2/2

	INT2 (11q13)	*CAT* (11p13)	*CALCA* (11p15.4)	*HBG2* (11p15.5)	*INS* (11p15.5)	*HRAS1* (11p15.5)
Squamous-cell carcinoma (23)	3/11	3/8	2/6	4/5	7/14	6/12
Adenocarcinoma (23)	2/11	1/7	0/6	4/8	3/10	5/13
Large-cell carcinoma (8)	1/3	0/2	0/2	0/2	1/5	2/5

*Chromosomal locus in parentheses.
†Number of cases per group.
‡Observed loss of heterozygosity per number of informative cases.

(3 of 10), and *HRAS1* (5 of 13) loci, and loss of heterozygosity was not seen in any of 6 informative cases at the *CALCA* locus. Three tumors that were informative at 3 or more loci on chromosome 11 remained heterozygous. For cases that showed partial loss of heterozygosity the data can be analyzed to establish a region of shortest overlapping loss of heterozygosity in adenocarcinomas of the lung (Fig. 2). The following observations show that the same regions would be defined as those for the squamous cell carcinomas, 11pter–p15.5 and 11p13–q13. One tumor showed loss of all 4 het-

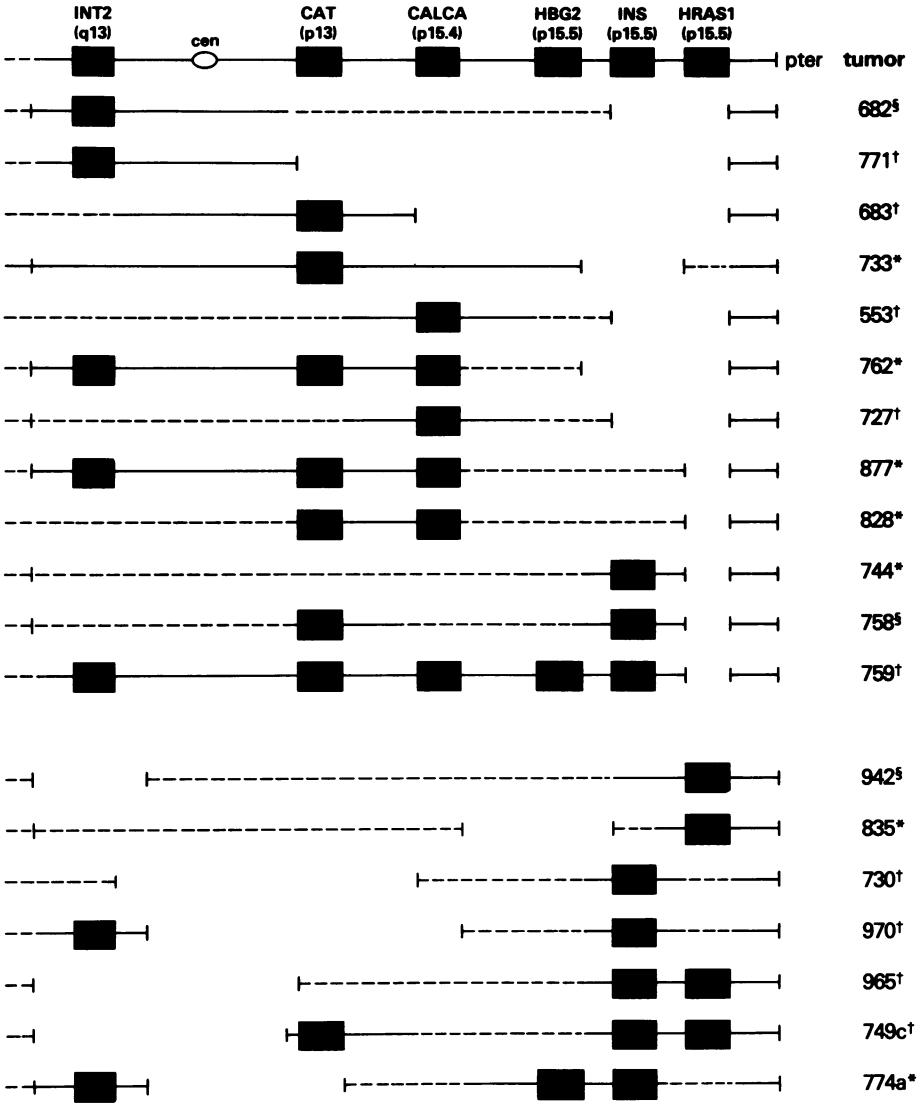

FIG. 2. Idiographic representation of shortest region of overlapping DNA sequence deletion analysis for nine squamous-cell carcinomas (†), seven adenocarcinomas (*), and three large-cell carcinomas (§) of the lung. Solid lines show intact genetic loci, dashed lines show regions for which no information is available, and gaps show regions of gene deletion. Tumors that either lost all heterozygous markers or showed no loss of heterozygosity are not included.

erozygous loci including *INT2* and *HRAS1*, suggesting complete chromosomal loss, 2 tumors (762 and 877) show loss of only telomeric regions, and tumor 774a shows maintenance of heterozygosity at *INS* and *HBG2* but loss of heterozygosity at *CAT*. Tumor 835 shows loss of *HBG2*, whereas *HRAS1* remains intact; this could indicate the presence of a locus of importance at 11p15.5. However, since the *CAT, CAL*, and *INT2* loci were homozygous, these data could also result from a large interstitial deletion. Loss of heterozygosity was also seen for 4 of 9 cases of adenocarcinoma at the 13q locus (D13S3). In 2 of these cases the remaining restriction fragment was determined to be relatively more intense than either of those in the constitutional DNA by a factor of approximately 2 (e.g., Fig. 1, D13S3). This suggests duplication of the preserved DNA sequence by either mitotic recombination or a replicative duplication of the sequences. This phenomenon was also seen for 1 tumor at the *RAF1* locus and another at the *INT2* locus. A similar observation was made for tumor 877 at the *INT2* locus; however, the smaller *INT2* restriction fragment was over-represented by >10-fold, suggesting amplification—i.e., the presence of a homogeneously staining region. Relatively modest loss of heterozygosity in adenocarcinomas was seen for chromosomes 18 and 20.

The results of 71 RFLP analyses of the constitutive and tumor DNA samples from eight large-cell carcinoma patients at 13 polymorphic loci are summarized in Table 1. These data show that loss of heterozygosity was seen in 5 cases of large-cell carcinoma for at least 1 heterozygous locus. For chromosome 17, the D17S1 region was lost in 1 of 3 informative tests; 3 of 7 informative large-cell carcinomas showed loss of heterozygosity from regions of chromosome 11. Among these 3 tumors, determination of shortest region of overlapping deletion (Fig. 2) showed the same pattern as described above for both squamous-cell carcinomas and adenocarcinomas—i.e., examples of either telomeric or pericentric deletions. Loss of heterozygosity in 3 informative cases of large-cell carcinoma at 4 polymorphic loci on chromosome 3, including DNF15S2, was not seen.

DISCUSSION

Carcinomas of the lung appear to be the result of the gross DNA and chromosomal damage that follows exposure to clastogenic agents found primarily in cigarette smoke and the urban environment (17). Karyotyping data for adenocarcinomas, large-cell carcinomas, and squamous-cell carcinomas of the lung are scarce. The available data show a considerable degree of complexity with regard to the presence of deletions, translocations, marker chromosomes, and aneuploidy (18). These changes are consistent with the extensive molecular karyotypic changes seen in the experiments reported here for six chromosomes, and particularly with respect to molecular changes seen in >50% of informative tests for squamous-cell carcinomas. Moreover, for each chromosome studied, with the exception of 13q, loss of heterozygosity was more extensive for squamous-cell carcinomas than for adenocarcinomas. Interestingly, the risk of squamous-cell carcinoma is greater in heavy smokers (> one pack per day) than is the risk of adenocarcinoma (19, 20). Additional studies are required to determine the relationship between the clastogenic effects of tobacco smoke and the widespread genetic deletions found in non-small-cell carcinomas and the time of the occurrence of the deletions during the multistage process of carcinogenesis.

Previous investigations (5, 6, 13, 14, 21) of lung cancer have focused on small-cell carcinoma and only a limited number of non-small-cell carcinomas have been studied by DNA sequence deletion analysis. An extensive analysis of a sufficient number of non-small-cell carcinomas so that comparisons of DNA sequence deletions in different histological types are feasible is reported here. For example, the data that are presented here consistently show loss of heterozygosity at 17p13 in squamous-cell carcinomas but not in adenocarcinomas of the lung. Frequent loss of heterozygosity of this region of chromosome 17 has also been seen in colorectal cancer (22) and small-cell lung cancer (14).

Detection of loss of heterozygosity for markers on chromosome 3 (3p25-p21) agrees with other reports that use DNA RFLPs to examine genetic loci on chromosome 3 in non-small-cell lung cancer (5, 6, 14). However, loss of heterozygosity was found to be substantially <100%, which does not agree with one previous report (13) and may be explained by differences in methods.

In small-cell lung cancer, loss of DNA sequences from the retinoblastoma locus has also been suggested as a critical factor in the development of this disease (23). Previously, loss of sequences from chromosome 13q has been implicated in both sporadic and familial retinoblastoma and osteosarcoma (24–26). However, loss of genes from the so-called retinoblastoma locus might be a more general component of the carcinogenesis process involved in cancers at a variety of body sites including, for example, the lung. The expected distribution of restriction fragments among the human population for the polymorphic probes used were found to be similar among lung cancer populations studied. Therefore, these studies are unable to address hereditary predisposition. Furthermore, studies of a lung cancer population as small as that reported here cannot be expected to do so. A more direct approach to this question would be to examine segregation of polymorphic DNA sequences in families.

Among the three different histological types that were studied, two commonly deleted regions for chromosome 11 were demonstrated—11pter–p15.5 and 11p13–q13. These findings are consistent with observations in pediatric tumors that describe two separate gene regions on chromosome 11 that may harbor distinctly different putative tumor-suppressor genes—11p13 in Wilms tumor (27, 28) and 11pter–15.5 in rhabdomyosarcoma (29). The data of Shiraishi *et al.* (21) that examined loss-of-heterozygosity at six polymorphic loci for chromosome 11 (*CAT, PTH, HBBC*, D11S12, *IGF2*, and *HRAS1*) failed to circumscribe a specific region of this chromosome that might be involved in tumor suppression due to insufficient numbers of tumors and informative tests.

Other studies have defined discrete genomic regions where loss of heterozygosity consistently associates with a specific disease (inferring loss of a specific gene or genes that may be required to suppress the tumor phenotype). These situations have been described for some adult tumors—renal cell carcinoma (30, 31), bladder cancer (32), small-cell lung cancer (4–7), osteosarcoma (26), and adenopolyposis coli (22, 33, 34), as well as a wide range of pediatric tumors (9, 27–29, 35–40). The results presented here for non-small-cell lung cancer are complex and suggest that some differences may occur in the spectrum of genetic deletions occurring in the various histological types of lung cancer. These genetic changes might also be involved in the pathogenesis of lung cancer within a "multi-hit" framework similar to the specific changes that have been described in other cancers (41, 42), which may include loss of genes, loss of elements that affect gene expression, or loss of DNA sequences that affect chromatin structure. Of the 54 tumors studied here, only 20 did not show any loss of heterozygosity for chromosome 11, and, of those, 10 showed deletions at other chromosomal loci. In addition, for squamous-cell carcinomas, loss of DNA sequences from chromosome 17 was associated with loss of DNA sequences from chromosome 11 in seven of eight cases informative for both chromosomes. Similarly, loss of DNA sequences on chromosome 17 was associated with loss of DNA sequences on chromosome 3 in five of seven cases. Therefore, consistent loss of specific genes (putative tumor-

suppressor genes) that have been recognized in other diseases may have a role independently or in combination in non-small-cell lung cancer.

Note Added in Proof. Genetic changes in colorectal tumors were recently shown to include loss of heterozygosity for the short arm of chromosome 17 that included the p53 gene in addition to mutation of the remaining copy of p53 Baker *et al.* (43). Genetic lesion in p53 may also be involved in human lung carcinogenesis.

We are indebted to Drs. R. White, W. K. Cavenee, H. H. Kazazian, W. F. Benedict, B. Carritt, G. Bell, and B. D. Nelkin for providing molecular probes. We also thank Mr. Robert Julia for his skilled editorial assistance. In addition, we wish to acknowledge the support of the pathology department at the University of Maryland, Baltimore Veterans' Administration Medical Center, and Union Memorial Hospital for providing lung tumor specimens. Finally, we acknowledge Dr. N. Caporaso for helpful discussions and statistical analyses. H.S. is a postdoctoral fellow supported by the Uehara Memorial Foundation, Tokyo, Japan.

1. Koop, C. E. (1985) *The Health Consequences of Smoking: Cancer and Chronic Lung Disease in the Workplace* (GPO, Washington, DC).
2. Grafstrom, R. C., Willey, J. C., Sundqvist, K. & Harris, C. C. (1986) in *Banbury Report 23: Mechanisms of Tobacco Carcinogenesis*, eds. Hoffman, D. & Harris, C. C. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 273–285.
3. Minna, J. D. (1988) *Lung Cancer* **4**, Suppl. P6-P10.
4. Dobrovic, A., Houle, B., Belouchi, A. & Bradley, W. E. (1988) *Cancer Res.* **48**, 682–685.
5. Brauch, H., Johnson, B., Hovis, J., Yano, T., Gazdar, A., Pettengill, O. S., Graziano, S., Sorenson, G. D., Poiesz, B. J., Minna, J., Linehan, M. & Zbar, B. (1987) *N. Engl. J. Med.* **317**, 1109–1113.
6. Naylor, S. L., Johnson, B. E., Minna, J. D. & Sakaguchi, A. Y. (1987) *Nature (London)* **329**, 451–454.
7. Johnson, B. E., Sakaguchi, A. Y., Gazdar, A. F., Minna, J. D., Burch, D., Marshall, A. & Naylor, S. L. (1988) *J. Clin. Invest.* **82**, 502–507.
8. Knudson, A. G. (1986) *Annu. Rev. Genet.* **20**, 231–251.
9. Dumanski, J. P., Carlbom, E., Collins, V. P. & Nordenskjold, M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 9275–9279.
10. Amstad, P., Reddel, R. R., Pfeifer, A., Malan-Shibley, L., Mark, G. E. & Harris, C. C. (1988) *Mol. Carcinogen.* **1**, 151–160.
11. Reddel, R. R., Ke, Y., Kaighn, M. E., Malan-Shibley, L., Lechner, J. F., Rhim, J. S. & Harris, C. C. (1988) *Oncogene Res.* **3**, 401–408.
12. Pfeifer, A., Mark, G. E., Reddel, R. R., Mann, D. L., Amstad, P., Malan-Shibley, L. & Harris, C. C. (1988) *Proc. Am. Assoc. Cancer Res.* **29**, A676 (abstr.).
13. Kok, K., Osinga, J., Carritt, B., Davis, M. B., van der Hout, A. H., van der Veen, A. Y., Landsvater, R. M., de Leij, L. F., Berendsen, H. H., Postmus, P. E., Poppema, S. & Buys, C. H. (1987) *Nature (London)* **330**, 578–581.
14. Yokota, J., Wada, M., Shimosato, Y., Terada, M. & Sugimura, T. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 9252–9256.
15. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
16. World Health Organization (1981) *Histological Typing of Lung Tumours* (WHO, Geneva).
17. Doll, R. (1985) in *Biochemical and Molecular Epidemiology of Cancer*, ed. Harris, C. C. (Liss, New York), pp. 111–125.
18. Zech, L., Bergh, J. & Nilsson, K. (1985) *Cancer Genet. Cytogenet.* **15**, 335–347.
19. World Health Organization (1986) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Tobacco Smoking* (IARC, Lyon, France).
20. Fraumeni, J. F. & Blot, W. J. (1982) in *Cancer Epidemiology and Prevention*, eds. Schottenfeld, D. & Fraumeni, J. F. (Saunders, Philadelphia), pp. 564–582.
21. Shiraishi, M., Morinaga, S., Noguchi, M., Shimosato, Y. & Sekiya, T. (1987) *Jpn. J. Cancer Res.* **78**, 1302–1308.
22. Fearon, E. R., Hamilton, S. R. & Vogelstein, B. (1987) *Science* **238**, 193–197.
23. Harbour, J. W., Lai, S. L., Whang-Peng, J., Gazdar, A. F., Minna, J. D. & Kaye, F. J. (1988) *Science* **241**, 353–357.
24. Friend, S. H., Bernards, R., Rogelj, S., Weinberg, R. A., Rapaport, J. M., Albert, D. M. & Dryja, T. P. (1986) *Nature (London)* **323**, 643–646.
25. Lee, W. H., Bookstein, R., Hong, F., Young, L. J., Shew, J. Y. & Lee, E. Y. (1987) *Science* **235**, 1394–1399.
26. Hansen, M. F., Koufos, A., Gallie, B. L., Phillips, R. A., Fodstad, O., Brogger, A., Gedde-Dahl, T. & Cavenee, W. K. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6216–6220.
27. Turleau, C., de Grouchy, J., Dufier, J. L., Phuc, L. H., Schmelck, P. H., Rappaport, R., Nihoul-Fekete, C. & Diebold, N. (1981) *Hum. Genet.* **57**, 300–306.
28. van Heyningen, V., Boyd, P. A., Seawright, A., Fletcher, J. M., Fantes, J. A., Buckton, K. E., Spowart, G., Porteous, D. J., Hill, R. E., Newton, M. S. & Hastie, N. D. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8592–8596.
29. Scrable, H. J., Witte, D. P., Lampkin, B. C. & Cavenee, W. K. (1987) *Nature (London)* **329**, 645–647.
30. Kovacs, G., Erlandsson, R., Boldog, F., Ingvarsson, S., Müller-Brechlin, R., Klein, G. & Sumegi, J. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1571–1575.
31. Zbar, B., Brauch, H., Talmadge, C. & Linehan, M. (1987) *Nature (London)* **327**, 721–724.
32. Fearon, E. R., Feinberg, A. P., Hamilton, S. H. & Vogelstein, B. (1985) *Nature (London)* **318**, 377–380.
33. Solomon, E., Voss, R., Hall, V., Bodmer, W. F., Jass, J. R., Jeffreys, A. J., Lucibello, F. C., Patel, I. & Rider, S. H. (1987) *Nature (London)* **328**, 616–619.
34. Okamoto, M., Sasaki, M., Sugio, K., Sato, C., Iwama, T., Ikeuchi, T., Tonomura, A., Sasazuki, T. & Miyaki, M. (1988) *Nature (London)* **331**, 273–277.
35. de Martinville, B. & Francke, U. (1983) *Nature (London)* **305**, 641–643.
36. Koufos, A., Hansen, M. F., Copeland, N. G., Jenkins, N. A., Lampkin, B. C. & Cavenee, W. K. (1985) *Nature (London)* **316**, 330–334.
37. Larsson, C., Skogseid, B., Oberg, K., Nakamura, Y. & Nordenskjold, M. (1988) *Nature (London)* **332**, 85–87.
38. Schroeder, W. T., Chao, L. Y., Dao, D. D., Strong, L. C., Pathak, S., Riccardi, V., Lewis, W. H. & Saunders, G. F. (1987) *Am. J. Hum. Genet.* **40**, 413–420.
39. Seizinger, B. R., Martuza, R. L. & Gusella, J. F. (1986) *Nature (London)* **322**, 644–647.
40. Seizinger, B. R., de la Monte, S., Atkins, L., Gusella, J. F. & Martuza, R. L. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5419–5423.
41. Gusella, J. F. (1986) *Annu. Rev. Biochem.* **55**, 831–854.
42. Hansen, M. F. & Cavenee, W. K. (1987) *Cancer Res.* **47**, 5518–5527.
43. Baker, S. J., Fearon, E. R., Nigro, J. M., Hamilton, S. R., Preisinger, A. C., Jessup, J. M., vanTuinen, P., Ledbetter, D. H., Barker, D. F., Nakamura, Y., White, R. & Vogelstein, B. (1989) *Science* **244**, 217–221.