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Genetics

Three tumor-suppressor regions on chromosome 11p identified by high-resolution deletion mapping in human non-small-cell lung cancer

(chromosome mapping)

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ABSTRACT **Non-small-cell lung cancer is the leading cause of cancer death for men and women in the industrialized nations. Identification of regions for genes involved in its pathogenesis has been difficult. Data presented here show three distinct regions identified on chromosome 11p. Two regions on 11p13 distal to the Wilms tumor gene *WT1* and on 11p15.5 between the markers *HBB* and *D11S860* are described. The third region on the telomere of 11p15.5 has been previously described and is further delineated in this communication. By high-resolution mapping the size of each of these regions was estimated to be 2–3 megabases. The frequency of somatic loss of genetic information in these regions (57%, 71%, and 45%, respectively) was comparable to that seen in heritable tumors such as Wilms tumor (55%) and retinoblastoma (70%) and suggests their involvement in pathogenesis of non-small-cell lung cancer. Gene dosage analyses revealed duplication of the remaining allele in the majority of cases in the 11p13 and the proximal 11p15.5 region but rarely in the distal 11p15.5 region. In tumors with loss of heterozygosity in all three regions any combination of duplication or simple deletion was observed, suggesting that loss of heterozygosity occurs independently and perhaps at different points in time. These results provide a basis for studies directed at cloning potential tumor-suppressor genes in these regions and for assessing their biological and clinical significance in non-small-cell lung cancer.**

Non-small-cell lung cancer (NSCLC) comprises all primary malignant lung tumors except small-cell tumors and accounts for 80% of all lung cancers. Many reasons should place NSCLC in the front line of cancer research. Some of these are as follows: (*i*) the annual incidence of lung cancer is $\approx 3 \times 10^6$ worldwide and continues to rise; (*ii*) the number of young individuals without exposure to tobacco, in particular women, is rising; (*iii*) 87% of patients with NSCLC will die of the disease, and approximately 115,000 people annually succumb to NSCLC in the United States; (*iv*) the 5-year survival rate of 13% has not changed appreciably in the last 20 years; (*v*) screening of high-risk individuals with frequent chest radiographs and sputum cytological examinations was proven unsuccessful; and (*vi*) fresh specimens and well-characterized immortalized cell lines are now available (1). The molecular genetic data presented here will provide a focus for investigations to isolate genetic elements involved in NSCLC development and progression.

The identification of regions for genes involved in the pathogenesis of NSCLC has been difficult because a possible familial risk is obscured by environmental risk factors (2, 3) and because cytogenetic analyses have shown complex aberrations (4, 5). Molecular genetic studies have implicated chromosomes 3 (6, 7), 5 (8), 8 (9), 9 (10, 11), 11 (12, 13), and 17 (12) as possibly carrying disease-related regions. The present study was performed to specifically address whether chromosome 11 harbors NSCLC regions.

Mechanisms by which regions carrying recessive genetic information become unmasked are nondisjunction, mitotic recombination, deletion, and gene conversion (14), which result in homozygosity in tumor cells. The size of such regions may be small and elude cytogenetic detection, as described for the childhood tumor rhabdomyosarcoma (15), or large and visible by cytogenetic studies, as exemplified by small-cell lung cancer (6, 16).

We have used 24 highly polymorphic markers on chromosome 11 with particular focus on bands 11p13 and 11p15.5 and assessed the frequency of loss of heterozygosity (LOH) in 28 pairs of normal and malignant tissue from patients with NSCLC and in 1 pair from a patient with small-cell lung cancer. In this communication, we show that three distinct regions on bands 11p13 and 11p15.5, two of which have not been previously described, are frequently deleted and may therefore identify loci for hitherto unknown tumor-suppressor genes.

MATERIALS AND METHODS

Tissue specimens obtained during surgery were immediately frozen in liquid nitrogen and stored at −135°C or −196°C until use. High molecular weight genomic DNA was extracted by a modified version of the method of Blin and Stafford (17). Powdered tissue was dissolved in 0.05 M Tris/0.05 M EDTA/0.1 M NaCl, pH 8.0. Pronase (0.5 mg/ml) was added, followed 30 min later by SDS (0.5%) and incubation at 42°C for 12 hr with a gentle rocking motion. Phenol extraction was performed with 0.5 volume for 12 hr at room temperature, and DNA was precipitated from the aqueous phase with ethanol. After evaporation of ethanol from the retrieved DNA, the DNA was dissolved in 10 mM Tris/1 mM EDTA, pH 8.0, and incubated at 42°C for 3 hr with RNase A (50 μg/ml). SDS (0.1%) and NaCl (0.1 M) were added, followed by Pronase (0.2 mg/ml). This solution was gently rocked at 42°C for 3 hr before phenol/chloroform extraction. The DNA was then ethanol precipitated and, after evaporation of the ethanol, dissolved in water. The yield was ≈200 μg of DNA per 1 g of tissue or per 5×10^7 cells of lung cancer cell lines.

For Southern blotting (18), 5 μg from each sample was digested with 50 units of restriction endonucleases as specified by the manufacturer (New England Biolabs), electrophoresed in 0.9% agarose in 40 mM Tris acetate/1 mM EDTA, pH 7.2, at room temperature, and transferred to nylon membranes (Nytran, Schleicher & Schuell) by capillary transfer in 0.8 M Tris base/3 M NaCl. Membranes were

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Abbreviations: LOH, loss of heterozygosity; NSCLC, non-small-cell lung cancer.

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hybridized at 65°C for 12 hr in 3× standard saline citrate (SSC)/1 mM EDTA/0.1% SDS/10× Denhardt's solution/7% dextran sulfate containing salmon testis DNA (0.1 mg/ml) and random-primer-labeled (19) probes (1–5 × 10^6 cpm/ml), washed several times at variable stringencies to obtain the best results (0.05–0.5 × SSC/0.1% SDS/1 mM EDTA, 58–72°C), and exposed to x-ray film with an intensifier screen at −80°C for 24–96 hr.

Probes used (20) were pbc-N1 [American Type Culture Collection (ATCC)], which detects an *Msp* I polymorphism at the *HRAS* locus; phins311 (ATCC), which detects a *Bam*HI polymorphism at the *IGF2* locus; phins310 (ATCC), which detects a *Pst* I polymorphism at the *INS* locus; pADJ762 (ATCC), which detects an *Msp* I polymorphism at the *D11S12* locus; p1596 (provided by Jack Lichy, National Cancer Institute, Bethesda, MD) (21), which detects an *Eco*RI polymorphism at the *ST5* locus; p20.36 (ATCC), which detects a *Taq* I polymorphism at the *PTH* locus; pTT42 (provided by Berry Nelkin, Johns Hopkins Oncology Center, Baltimore) which detects a *Taq* I polymorphism at the *CALCA* locus; p56H2.4 (provided by Vicki Huff, M. D. Anderson Cancer Center, University of Texas, Houston), which detects a *Pst* I polymorphism at the *D11S151* locus; p32-1 (ATCC), which detects an *Msp* I polymorphism at the *D11S16* locus; p5S1.6 (Vicki Huff), which detects a *Pvu* II polymorphism at the *D11S323* locus; p8B1.25 (Vicki Huff), which detects a *Bgl* II polymorphism at the *D11S325* locus; pINT-800 (ATCC), which detects a *Taq* I polymorphism at the *CAT* locus; pTHH26 (ATCC), which detects a *Pvu* II polymorphism at the *D11S149* locus; pMEL34 (ATCC), which detects a *Bgl* II polymorphism at the *TYR* locus; and pHE5.4 (ATCC), which detects a *Sac* I polymorphism at the *ETS1* locus.

Polymerase chain reaction (PCR) was performed in 25–100 μl with 25–100 ng of template DNA. Primers synthesized with a model 392 DNA/RNA synthesizer (Applied Biosystems) were D4-3 and D4-42, which detect a minisatellite at the *DRD4* locus (22); 1020 and 1019, which detect a microsatellite at the *D11S988* locus (Genome Database identification no., G00-195-012); BS48R and BS48L, which detect a microsatellite at the *D11S860* locus (23); KZ53 and SP11, which detect a *Hin*fI polymorphism at the *HBB* locus (24); AFM240vh2a and AFM240vh2m, which detect a microsatellite at the *D11S932* locus (25); MIT-A136-1 and MIT-A136-2, which detect a microsatellite at the *D11S861* locus (26); and WT1.PCR1.1 and WT1.PCR1.2, which detect a *Hin*fI polymorphism at the *WT1* locus (20). Primers obtained from ATCC included HUMTH01[AATG].1 and .2, which detect a microsatellite at the *TH* locus (27); and Mfd69L and -R, which detect a microsatellite at the *CD3D* locus (28). PCR products were separated in 2–4% Metaphor agarose (FMC) in 90 mM Tris borate/2 mM EDTA at room temperature and visualized by ethidium bromide under UV light.

RESULTS

Region Identification. Pairs of specimens from 28 previously untreated patients with histologically confirmed NSCLC and from one patient with small-cell lung cancer, each consisting of one sample from normal lung tissue and one sample from tumor tissue, were obtained immediately after surgical resection. The histological NSCLC subtypes included 15 epidermoid (squamous-cell) carcinomas, 10 adenocarcinomas, 2 large-cell carcinomas, and one carcinoid tumor. High molecular weight DNA was extracted and analyzed for restriction fragment length polymorphisms, minisatellites, and microsatellites by Southern blotting and PCR techniques for 24 polymorphic markers on chromosome 11 (Fig. 1).

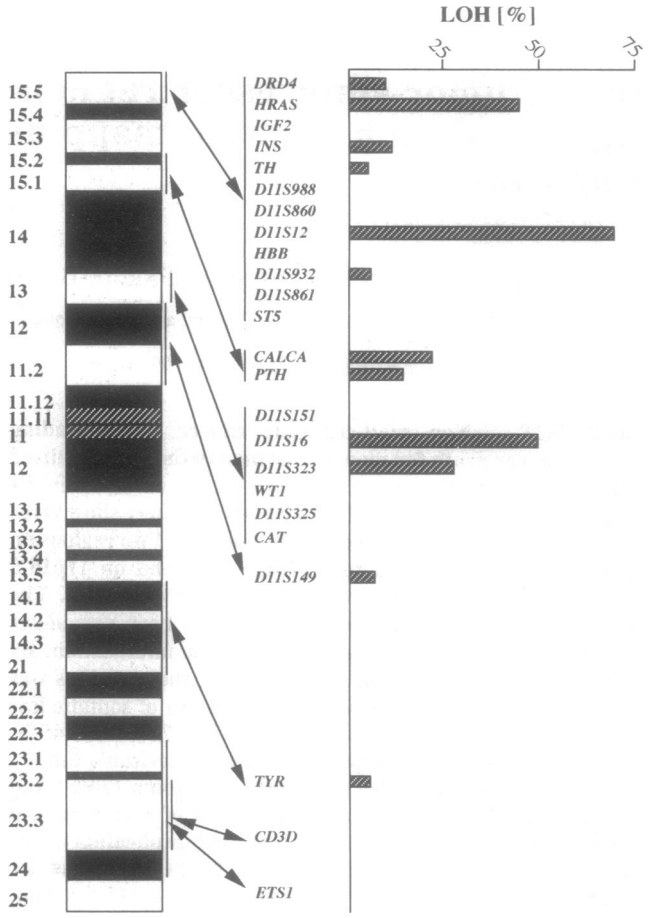

FIG. 1. Diagram of human chromosome 11 showing a G-banding pattern representative for a karyotype at a 550-band resolution and the frequency of LOH for each marker in percent. The location of markers used is given in genetic order or, where known, in physical order. The marker *ST5* is located proximal to *HBB* on band 15.5. Its location relative to *D11S861* and *D11S932*, however, is unknown.

Three distinct regions showed frequent LOH, one on band 11p13 and two on band 11p15.5 (Figs. 1–3). The marker *D11S16* on band 11p13 had LOH in 53% (10/19) of cases with heterozygosity in the normal lung specimen (Figs. 2 and 3). *D11S323*, located centromeric to *D11S16*, showed LOH in 2 of 7 cases, which were both homozygous for *D11S16* and thus uninformative. This suggests that loss of somatic genetic information at this locus occurs in at least 57% of NSCLC. *WT1*, located between the markers *D11S325* and *D11S323* (29), had no LOH, which suggests that another tumor-suppressor gene is located on band 13 between *D11S323* and *D11S151*, an area of ≈2.5 megabases (Mb) (29).

D11S12 on band 15.5 had LOH in 71% (12/17) of cases. *HBB* and *D11S860*, the closest markers tested, were not deleted and therefore define the borders of this second NSCLC region (Figs. 1–3). The exact size of this area is unknown but is estimated to be ≈2 Mb (30). Restriction fragment length polymorphism analyses on 18 NSCLC cell lines revealed homozygosity in this region more frequently than statistically expected (83% in cell lines for *D11S12*, compared with 33% in the general population), which extends the described finding to currently available *in vitro* models. The tumor-suppressor gene locus *ST5* (21), located proximal to *HBB*, had no LOH, suggesting that it is not involved in NSCLC pathogenesis.

A third NSCLC region is defined by *HRAS*, which had LOH in 45% (10/22) of cases (Figs. 1–3). The regions identified by *D11S12* and *HRAS* are not contiguous, since

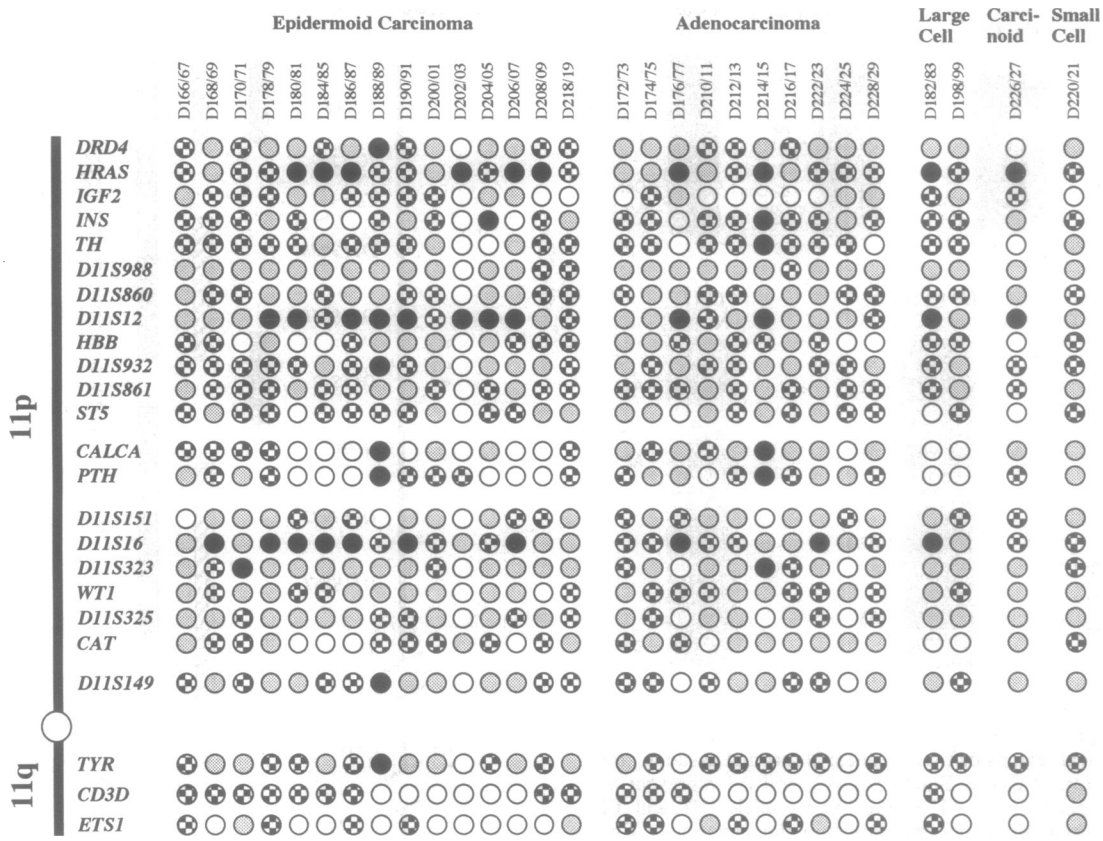

FIG. 2. Detailed allelotyping for 24 polymorphic markers in tissue pairs from 29 patients with lung cancer. DNA from tissue pairs is labeled with the prefix D followed by a pair of laboratory numbers given to tissue samples in consecutive order of receipt in the laboratory. ⊗, Heterozygosity in normal and tumor tissue; ●, LOH in the tumor tissue; ◎, homozygosity in normal and tumor tissue; ○, no available data.

five markers, *D11S860, D11S988, TH, INS*, and *IGF2*, located between these loci had LOH in only one tumor (D214/15).

The tumor/normal tissue pairs tested included only one case of small-cell lung cancer (D220/21), which had no LOH in any of the tested markers on chromosome 11. The total number of NSCLC cases examined in this study is too small to allow meaningful conclusions on the frequency and distribution of the described NSCLC regions among the various pathological subtypes and clinical-outcome groups.

Gene Dosage Analysis. To assess whether LOH at the individual loci resulted in hemizygosity or homozygosity, densitometric scanning of autoradiographic hybridization signals for corresponding alleles from *D11S16, D11S12*, and *HRAS* was performed. An approximate doubling of the signal intensity for the remaining allele was observed in four of seven interpretable cases for *D11S16*, six of nine for *D11S12*, and one of five for *HRAS*, which is consistent with genetic duplication. In all other cases LOH appeared to be the result of simple genetic deletion.

DISCUSSION

The analyses described provide evidence for the existence of three independent regions on chromosome 11p with frequent LOH in NSCLC and implicate gene conversion or double mitotic recombination and simple deletion as the mechanisms leading to genetic loss.

Two previous studies have suggested the existence of NSCLC regions on chromosome 11. Weston *et al.* (12) studied 54 NSCLC pairs with six markers located on chromosome 11 (*CAT, CALCA, HBB, INS*, and *HRAS* on 11p and *INT2* on 11q). Results suggested frequent LOH at the *HRAS* locus (13 of 30 cases, 43%) which could stretch from *INS* to the telomere on chromosome band 15.5. A second area of LOH was bordered by *INT2* on 11q13 and *CAT* on 11p13, and the frequency of LOH in this area appeared to be in the 25% range. Skinner *et al.* (13) studied 45 NSCLC pairs for LOH on 11p15.5 by using the markers *HBB, INS*, and *HRAS*. Nine of 40 cancers had LOH at *HRAS* (23%), 7 of these 9 also had LOH at the *INS* locus, and 8 of these were heterozygous for *HBB*. Both groups of authors concluded that LOH for *HRAS* is a common finding in primary NSCLC and that the spectrum of LOH is similar in the different NSCLC subtypes. These reports favor a telomeric (*INS*–pter) location of a NSCLC tumor-suppressor gene, with LOH being observed in 23–43% of cases, and a possible second location for a tumor-suppressor gene between 11q13 and 11p13, albeit with low-frequency LOH (≈25%). We studied three markers within or adjacent to the latter region (*CAT, D11S149*, and *TYR*) and found LOH in only one tumor (D188/89; see Fig. 2), which accounts for 5–10% of informative specimens. This low number suggests that this region is not of general importance for NSCLC pathogenesis. The differences in LOH frequencies among these studies are most likely caused by contamination of tumor specimens with normal diploid cells, resulting in a falsely positive heterozygosity of the tumor. Our data concur with the authors of refs. 12 and 13 in the location of a tumor-suppressor gene in the vicinity of the *HRAS* marker (LOH was found in 45% of tumors). However the extent of this region (Fig. 4, *HRAS* locus) was unclear, and the current work shows it not to extend beyond *IGF2* and *DRD4*. Although *DRD4* has been mapped telomeric to *HRAS* by genetic linkage analysis (39), its exact physical location is unknown and may perhaps be centromeric to *HRAS*. Thus it remains unclear whether this LOH region includes the entire telomere. The same region has also been implicated in the pathogenesis of breast cancer (31), rhabdomyosarcoma (15),

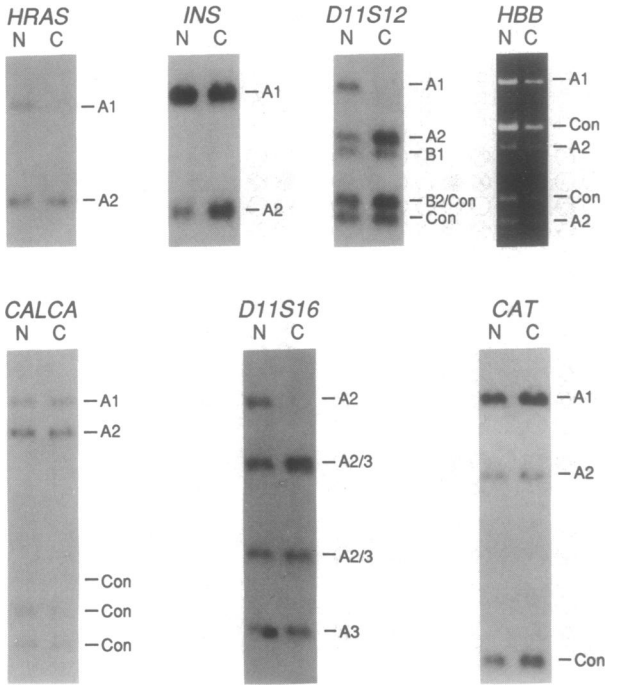

FIG. 3. Representative Southern blots or electropherograms of digested PCR products of DNA from tissue pairs in Fig. 2, highlighting the critical regions of LOH. Lanes: N, normal tissue; C, the corresponding tumor tissue. Bands A1, A2, and A3 represent an allelic polymorphism with A1 as the largest allele. B1 and B2 represent a different allelic polymorphism for the same locus. Con represents a constant band. For *HRAS*, LOH for allele A1 in the tumor tissue of pair D226/27 is shown; for *INS*, heterozygosity in the tumor and normal tissue of pair D228/29 is shown; for *D11S12*, LOH for allele A1 in the tumor tissue of pair D182/83 is shown; for *HBB* and *CALCA*, heterozygosity in the tumor and normal tissue of pair D218/19 is shown; for *D11S16*, LOH for allele A2 in the tumor tissue of pair D184/85 is shown; and for *CAT*, heterozygosity in the tumor and normal tissue of pair D220/21 is shown.

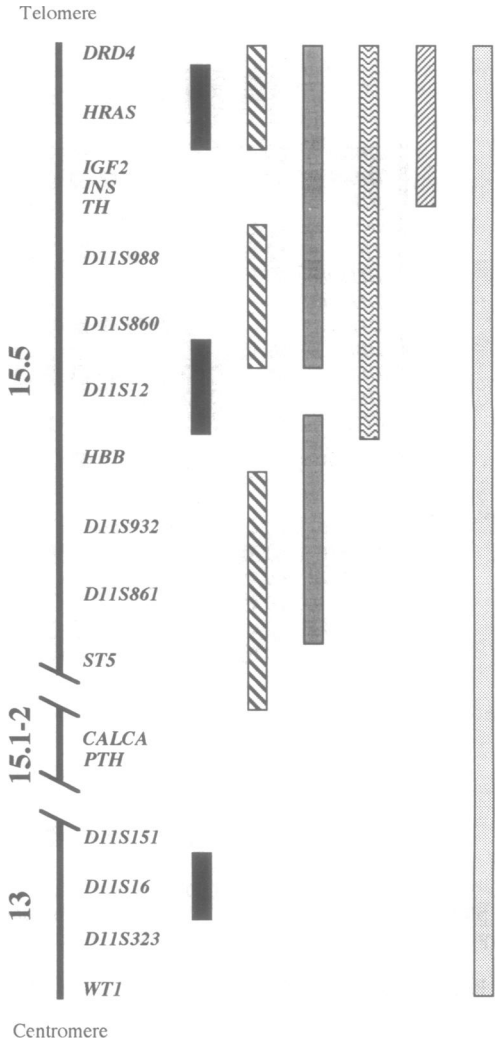

FIG. 4. Schematic representation of regions on chromosome 11p13–15.5 harboring potential tumor-suppressor genes. ■, NSCLC regions described in this communication; ▨, breast cancer regions (31–34); ■, rhabdomyosarcoma regions (15, 30); ▨, Wilms tumor 2 regions (35, 36); ▨, bladder cancer region (37); ▨, testicular cancer region (38).

Wilms tumor (35, 36), bladder cancer (37), and testicular cancer (38). These data are schematically summarized in Fig. 4.

We have found two NSCLC regions, one identified by *D11S16* on band 11p13 (Fig. 4, *D11S16*) and the other identified by *D11S12* on band 11p15.5 (Fig. 4, *D11S12*). The first region, on band 11p13, has not been previously described and is clearly distal to the *WT1* gene (29, 40) and outside of the region described by Weston *et al.* (12). This and the frequency of LOH (57%), which is comparable to the one in Wilms tumor on band 11p13 (41), strongly suggest the presence of a tumor-suppressor gene within this region. Radice *et al.* (38) have described LOH in 4 of 12 testicular cancers (Fig. 4). Because many of the markers used by those authors were uninformative, the minimal region of LOH could not be determined but extended from the telomere (*HRAS* locus) to perhaps as far as band 11q13 (*INT2* locus).

The second region, identified by the *D11S12* locus, is of particular interest because of its high frequency (71%) of LOH, which is comparable to that found in retinoblastoma (14). All polymorphic markers evaluated adjacent to this locus did not reveal LOH, which suggests that the area of interest is ≈2 Mb in size. Interestingly, data by Reeve *et al.* (35) and Wadey *et al.* (36) suggest the presence of a second Wilms tumor locus (*WT2*) in this region (Fig. 4). The recent description of loss of genetic imprinting for *IGF2* in Wilms tumor (42, 43), however, suggests that loss of genetic control elements adjacent to the *IGF2/H19* genes could be the important event within this Wilms tumor LOH domain. There also appears to be some overlap of the second NSCLC region with known areas of frequent LOH in breast cancer (32–34) and rhabdomyosarcoma (15) (Fig. 4). In addition, the centromeric part of this region overlaps with a chromosomal fragment which was recently shown to encode a tumor-suppressor activity when transferred to a rhabdomyosarcoma cell line (30) (Fig. 4).

Several mechanisms leading to loss of genetic information in two or more noncontiguous regions in NSCLC are possible. Our gene dosage studies make it unlikely that the LOH events would have occurred concurrently through a complex rearrangement of a single chromosome, since LOH at different loci in the same tumor resulted from any combination of deletion and duplication, suggesting that LOH at various loci occurs independently and perhaps at different points in time. This can be addressed by studies assessing the pattern of LOH in premalignant bronchoepithelial tissue compared with carcinoma *in situ* and invasive cancer. Whether LOH at different loci within one tumor occurs on the same chromosome 11p cannot be determined from our studies. Studies addressing the parental origin of the remaining alleles could help answer this question. Previous studies have demonstrated a paternal origin of the remaining alleles in rhabdomyosarcoma (44) and Wilms tumor (45), which suggests that LOH occurs on the same chromosome 11p.

The data presented provide a focus for investigations to identify and characterize tumor-suppressor genes on chromosome 11p involved in NSCLC development and progression.

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1. Boring, C. C., Squires, T. S. & Tong, T. (1993) *Calif. Cancer J. Clin.* **43**, 7–26.
2. Ooi, W. L., Elston, R. C., Chen, V. W., Bailey-Wilson, J. E. & Rothschild, H. (1986) *J. Natl. Cancer Inst.* **76**, 217–222.
3. Sellers, T. A., Bailey-Wilson, J. E., Elston, R. C., Wilson, A. F., Elston, G. Z., Ooi, W. L. & Rothschild, H. (1990) *J. Natl. Cancer Inst.* **82**, 1272–1279.
4. Lukeis, R., Irving, L., Garson, M. & Hasthorpe, S. (1990) *Genes Chromosomes Cancer* **2**, 116–124.
5. Whang-Peng, J., Knutsen, T., Gazdar, A., Steinberg, S. M., Oie, H., Linnoila, I., Mulshine, J., Nau, M. & Minna, J. D. (1991) *Genes Chromosomes Cancer* **3**, 168–188.
6. 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.
7. Yokoyama, S., Yamakawa, K., Tsuchiya, E., Murata, M., Sakiyama, S. & Nakamura, Y. (1992) *Cancer Res.* **52**, 873–877.
8. D'Amico, D., Carbone, D. P., Johnson, B. E., Meltzer, S. J. & Minna, J. D. (1992) *Cancer Res.* **52**, 1996–1999.
9. Emi, M., Fujiwara, Y., Nakajima, T., Tsuchiya, E., Tsuda, H., Hirohashi, S., Maeda, Y., Tsuruta, K., Miyaki, M. & Nakamura, Y. (1992) *Cancer Res.* **52**, 5368–5372.
10. Center, R., Lukeis, R., Dietzsch, E., Gillespie, M. & Garson, O. M. (1993) *Genes Chromosomes Cancer* **7**, 47–53.
11. Olopade, O. I., Buchhagen, D. L., Malik, K., Sherman, J., Nobori, T., Bader, S., Nau, M. M., Gazdar, A. F., Minna, J. D. & Diaz, O. (1993) *Cancer Res.* **53**, 2410–2415.
12. Weston, A., Willey, J. C., Modali, R., Sugimura, H., McDowell, E. M., Resau, J., Light, B., Haugen, A., Mann, D. L., Trump, B. F. & Harris, C. C. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 5099–5103.
13. Skinner, M. A., Vollmer, R., Huper, G., Abbott, P. & Iglehart, J. D. (1990) *Cancer Res.* **50**, 2303–2306.
14. Cavenee, W. K., Dryja, T. P., Phillips, R. A., Benedict, W. F., Godbout, R., Gallie, B. L., Murphree, A. L., Strong, L. C. & White, R. L. (1983) *Nature (London)* **305**, 779–784.
15. Scrable, H. J., Witte, D. P., Lampkin, B. C. & Cavenee, W. K. (1987) *Nature (London)* **329**, 645–647.
16. Naylor, S. L., Johnson, B. E., Minna, J. D. & Sakaguchi, A. Y. (1987) *Nature (London)* **329**, 451–454.
17. Blin, N. & Stafford, D. W. (1976) *Nucleic Acids Res.* **3**, 2303–2308.
18. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
19. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **137**, 266–267.
20. Williamson, R., Bowcock, A., Kidd, K., Pearson, P., Schmidtke, J., Ceverha, P., Chipperfield, M., Cooper, D. N., Coutelle, C., Hewitt, J., Klinger, K., Langley, K., Beckmann, J., Tolley, M. & Maidak, B. (1991) *Cytogenet. Cell Genet.* **58**, 1190–1832.
21. Lichy, J. H., Modi, W. S., Seuanez, H. N. & Howley, P. M. (1992) *Cell Growth Differ.* **3**, 541–548.
22. Lichter, J. B., Barr, C. L., Kennedy, J. L., Van Tol, H. H. M., Kidd, K. K. & Livak, K. J. (1993) *Hum. Mol. Genet.* **2**, 767–773.
23. McNoe, L. A., Eccles, M. R. & Reeve, A. E. (1992) *Nucleic Acids Res.* **20**, 1161.
24. Semenza, G. L., Dowling, C. E. & Kazazian, H. H. (1989) *Nucleic Acids Res.* **17**, 2376.
25. Weissenbach, J., Gyapay, G., Dib, C., Vignal, A., Morissette, J., Millasseau, P., Vaysseix, G. & Lathrop, M. (1992) *Nature (London)* **359**, 794–801.
26. Hudson, T. J., Engelstein, M., Lee, M. K., Ho, E. C., Rubenfield, M. J., Adams, C. P., Housman, D. E. & Dracopoli, N. C. (1992) *Genomics* **13**, 622–629.
27. Edwards, A., Civitello, A., Hammond, H. A. & Caskey, C. T. (1991) *Am. J. Hum. Genet.* **49**, 746–756.
28. Weber, J. L., Kwitek, A. E. & May, P. E. (1990) *Nucleic Acids Res.* **18**, 4036.
29. Huff, V., Compton, D. A., Strong, L. C. & Saunders, G. F. (1990) *Hum. Genet.* **84**, 253–257.
30. Koi, M., Johnson, L. A., Kalikin, L. M., Little, P. F. R., Nakamura, Y. & Feinberg, A. P. (1993) *Science* **260**, 361–364.
31. Theillet, C., Lidereau, R., Escot, C., Hutzell, P., Brunet, M., Gest, J., Schlom, J. & Callahan, R. (1986) *Cancer Res.* **46**, 4776–4781.
32. Ali, I. U., Lidereau, R., Theillet, C. & Callahan, R. (1987) *Science* **238**, 185–188.
33. Miyagi, M., Inazawa, J., Takita, K.-I. & Nakamura, Y. (1992) *Hum. Mol. Genet.* **9**, 705–708.
34. Winqvist, R., Mannermaa, A., Alavaikko, M., Blanco, G., Taskinen, P. J., Kiviniemi, H., Newsham, I. & Cavenee, W. (1993) *Cancer Res.* **53**, 4486–4488.
35. Reeve, A. E., Sih, S. A., Raizis, A. M. & Feinberg, A. P. (1989) *Mol. Cell. Biol.* **9**, 1799–1803.
36. Wadey, R. B., Pal, N., Buckle, B., Yeomans, E., Pritchard, J. & Cowell, J. K. (1990) *Oncogene* **5**, 901–907.
37. Fearon, E. R., Feinberg, A. P., Hamilton, S. H. & Vogelstein, B. (1985) *Nature (London)* **318**, 377–380.
38. Radice, P., Pierotti, M. A., Lacerenza, S., Mondini, P., Radice, M. T., Pilotti, S. & Della Porta, G. (1989) *Cytogenet. Cell Genet.* **52**, 72–76.
39. Junien, C. & van Heyningen, V. (1991) *Cytogenet. Cell Genet.* **58**, 459–554.
40. Call, K. M., Glaser, T., Ito, C. Y., Buckler, A. J., Pelletier, J., Haber, D. A., Rose, E. A., Krai, A., Yeger, H., Lewis, W. H., Jones, C. & Housman, D. E. (1990) *Cell* **60**, 509–520.
41. Fearon, E. R., Vogelstein, B. & Feinberg, A. P. (1984) *Nature (London)* **309**, 176–178.
42. Rainier, S., Johnson, L. A., Dobry, C. J., Ping, A. J., Grundy, P. E. & Feinberg, A. P. (1993) *Nature (London)* **362**, 747–749.
43. Ogawa, O., Eccles, M. R., Szeto, J., McNoe, L. A., Yun, K., Maw, M. A., Smith, P. J. & Reeve, A. E. (1993) *Nature (London)* **362**, 749–751.
44. Scrable, H., Cavenee, W., Ghavimi, F., Lovell, M., Morgan, K. & Sapienza, C. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7480–7484.
45. Williams, J. C., Brown, K. W., Mott, M. G. & Maitland, W. J. (1989) *Lancet* **i**, 283–284.