[CANCER RESEARCH 54, 4007–4010, August 1, 1994]

Advances in Brief

Deletion Mapping of a Putative Tumor Suppressor Gene on Chromosome 4 in Mouse Lung Tumors[1]

Christopher R. Herzog, Roger W. Wiseman, and Ming You[2]

Department of Pathology, Medical College of Ohio, Toledo, Ohio 43699 [C. R. H., M. Y.], and National Institute for Environmental Health Sciences, Research Triangle Park, North Carolina 27709 [R. W. W.]

Abstract

Genetic and molecular studies have implicated the region of the α-interferon gene cluster on mouse chromosome 4 as the location of a putative tumor suppressor gene. A region of homology on human chromosome 9p21–22 that is frequently deleted in multiple human cancers has recently been found to contain a candidate tumor suppressor gene called multiple tumor suppressor-1 (*MTS1*), which was previously shown to encode an inhibitor of cyclin-dependent kinase 4. We performed loss of heterozygosity and deletion analyses to map the most commonly deleted region on chromosome 4 in F_1 hybrid mouse lung tumors. Ten simple sequence length polymorphism markers were analyzed with focus on the α-interferon region. Allelic losses were detected in 29 of 61 (48%) of the lung adenocarcinomas but in only 1 of 38 (3%) of the lung adenomas examined. In most cases, the losses appeared to occur by nondisjunction. However, in three carcinomas, we detected homozygous deletions that overlapped at simple sequence length polymorphism marker *D4MIT77*. These data suggest a critical region of about 2 cM immediately distal to the α-interferon locus as the likely domain of a novel tumor suppressor gene on mouse chromosome 4, the loss of which appears to be involved in the progression of mouse lung tumorigenesis.

Introduction

The frequent deletions of human chromosome 9p21–22 in multiple cancer types, which include melanomas (1), small and non-small cell carcinomas of the lung (2, 3), gliomas (4, 5), acute lymphoblastic leukemias (6), transistional cell carcinomas of the bladder (7), malignant mesotheliomas (8), and head and neck squamous cell carcinomas (9), have implicated this region as the site of a putative tumor suppressor gene. A locus for familial melanoma was also mapped to this region (10). Recently, a candidate tumor suppressor gene termed *MTS1*[3] was identified by virtue of its frequent homozygous deletion in cell lines derived from many different tumor types (11, 12) and its apparent mutational inactivation in melanoma cell lines (11). The *MTS1* gene encodes a previously identified inhibitor (p16) of CDK4 (13).

A region of synteny to human 9p21–22 on mouse chromosome 4 has also been implicated as the site of a putative tumor suppressor gene. Somatic cell hybrid studies first suggested the presence of a tumor suppressor gene at this location, which contains the IFN-α gene cluster (14). More recently, this region was found to display allelic loss in 6 of 8 $B6C3F_1$ mouse lung adenocarcinomas (15) and in 26 of 49 $B6C3F_1$, $C3AF_1$ and $AC3F_1$ lung adenocarcinomas.[4]

In the present study, this region on mouse chromosome 4 was further defined by deletion and LOH analyses using constitutionally heterozygous SSLP markers in F_1 hybrid mouse tumors. Ten dispersed markers were used with emphasis on the region containing the IFN-α gene cluster. By this approach, a distance of ~2 cM was defined as the most frequently deleted region on chromosome 4 in mouse lung adenocarcinomas. Because this critical region is syntenic to where *MTS1* was localized on human chromosome 9p21–22, the involvement of the mouse homologue of *MTS1* in the progression of mouse lung tumorigenesis is implicated by our results.

Materials and Methods

Tumor Induction. CDF_1 (BALB/cJ × DBA/2J F_1) hybrid mouse lung tumors were either induced by IQ or occurred spontaneously as described previously (16). The mice received i.p. injections 1 time per week during the first 4 weeks after birth with a total dose of 16.2 mg/kg body weight. Two IQ-induced adenomas, 11 IQ-induced, and 5 spontaneous adenocarcinomas as well as 48 lesions were harvested when the mice were 22 months of age. Eighteen spontaneous $C3AF_1$ (C3H/HeJ × A/J F_1) hybrid mouse lung adenocarcinomas and three adenomas were collected from untreated 2-year-old mice as described (17). Other $C3AF_1$ lung tumors were either induced by NNK at a dose of 50 mg/kg body weight, 20 or 60 mg/kg VC, or 10 mg/kg dimethylnitrosamine (18). Induction by NNK involved treating 6- to 8-week-old mice by i.p. injection three times per week for 8 weeks, and 13 lung adenocarcinomas and 1 adenoma were used in this study. Induction by VC involved the administration of this compound to 7-week-old mice by a single i.p. injection (17 adenocarcinomas were used). Tumors induced by NNK or VC were harvested when the mice were between 6 and 14 months of age. Induction by dimethylnitrosamine was conducted by a single i.p. injection using 15-day-old mice, and 14 adenomas were harvested after 6 months. Eighteen $AB6F_1$ (A/J × C57BL/6J F_1) hybrid lung adenomas were induced by an i.p. treatment of newborns with 5 mg urethan/kg body weight and were harvested 6 months posttreatment. A portion of lung tumors was fixed in 10% neutral buffered formalin for histopathological examination; 48 of 147 grossly nodular lesions were too small to save sufficient tumor tissues for histopathology, 61 were diagnosed as lung adenocarcinomas, and 38 were lung adenomas.

DNA Isolation. High molecular weight DNA was isolated from normal and tumor tissues by overnight incubation at 37°C with Proteinase K (Sigma Chemical Co., St. Louis, MO) in 10 mM Tris, 400 mM NaCl, 2 mM EDTA (disodium salt), and 10% sodium dodecyl sulfate, followed by salt extraction and ethanol precipitation.

PCR of SSLP Markers. PCR amplification of SSLPs on chromosome 4 of CDF_1, $C3AF_1$, and $AB6F_1$ mice was performed using oligonucleotide primers (*D4MIT15, D4MIT17, D4MIT18, D4MIT27, D4MIT77, D4MIT81, D4MIT45, D4MIT82, D4MIT84,* and *D4MIT31*) purchased from Research Genetics (Huntsville, AL). These primer sequences are reported elsewhere (19–20). The PCR reaction mixture was comprised of 0.5–100 ng genomic DNA, 100 μM of each of the four deoxyribonucleoside triphosphates (dCTP, dATP, dGTP, and dTTP), 40 pmol of each primer, 1.0 unit Taq DNA polymerase (Promega), 50 mM KCl, 10 mM Tris-HCl (pH 9.0), and 0.1% Triton X-100. A reaction volume of 25 μl was overlaid with one drop of sterile mineral oil and subjected to 25–30 cycles of amplification using the DNA Thermal Cycle (Perkins Elmer Cetus). Each cycle consisted of 1 min denaturation (first cycle, 3 min) at 94°C, 1 min reannealing at 55°C, and 2 min of extension at 72°C. Alternatively, prior

Received 5/27/94; accepted 6/16/94.

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

[1] This work was supported by NIH Grant CA58554.

[2] To whom requests for reprints should be addressed, at Department of Pathology, Medical College of Ohio, 3000 Arlington Ave., Toledo, OH 43699.

[3] The abbreviations used are: *MTS1*, multiple tumor suppressor-1; CDK4, cyclin-dependent kinase 4; IFN-α, interferon-α; LOH, loss of heterozygosity; SSLP, simple sequence length polymorphism; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; VC, vinyl carbamate; PCR, polymerase chain reaction.

[4] M. E. Hegi *et al.*, manuscript in preparation.

Table 1 *Pattern of chromosome 4 LOH in F_1 hybrid mouse lung tumors*[a]

Tumor	*D4MIT* SSLP loci[b]									
	18	17	84	82	81	45	27	77	15	31
C3A-1 to C3A-9	C	NI[c]	C	NI	C	ND	C	C	NI	C
C3A-10 and C3A-11	ND	NI	C	NI	C	ND	C	C	NI	C
C3A-12	C	NI	ND	NI	C	ND	C	C	NI	C
C3A-13	ND	NI	ND	NI	C	ND	C	C	NI	C
C3A-14 and C3A-15	C	NI	C	NI	ND	ND	C	C	NI	C
C3A-16	A, C	NI	A, C	NI	C	ND	C	C	NI	C
C3A-17	A, C	NI	A	NI	A	ND	A	A	NI	A
C3A-18	A, C	NI	A, C	NI	ND	ND	A	A	NI	A
C3A-19	A, C	NI	A, C	NI	A, C	A, C	A, C	C	NI	C
C3A-20	A	NI	A	NI	A	A	−	−	NI	A
C3A-21	A	NI	A	NI	A	A	A	−	NI	A
C3A-22	C	NI	C	NI	C	C	A, C	C	NI	A, C
C3A-23	A, C	NI	A	NI	A	ND	A	A	NI	A, C
C3A-24	A, C	NI	A	NI	A	A	−	−	NI	A
CDF1-1 to CDF1-11	B	B	ND	B	NI	ND	B	B	B	B
CDF1-12 to CDF1-14	B	B	ND	ND	NI	ND	B	B	B	B
CDF1-15	ND	B	ND	B	NI	ND	B	B	B	B
CDF1-16	ND	B	ND	ND	NI	ND	B	B	B	B
CDF1-17	ND	ND	ND	B	NI	ND	B	B	B	B
CDF1-18	B, D	B, D	ND	B, D	NI	B, D	B, D	B	B, D	B, D

[a] Shown are the retained alleles: A, A/J; B, BALB/cJ; C, C3H/HeJ; D, DBA/2J.
[b] Loci are shown in their relative chromosomal order from the centromere at left to the telomere at right.
[c] ND, not done; NI, noninformative; −, homozygous deletion.

to PCR, 1 primer of a pair was end-labeled with [γ^{32}P]ATP (ICN) using T4 DNA kinase (United States Biochemical).

Multiplex PCR Analysis. A control primer pair (*D10MIT3*) from outside the critical homozygously deleted region was used as an internal quantitative standard in comparative multiplex PCR. PCR was carried out as described above. Primer pairs from the control locus and the locus suspected to be homozygously deleted were included in each reaction at equivalent concentrations (40 pmol).

Gel Electrophoresis and DNA Polymorphism Analysis. Amplified target DNAs were either resolved in MetaPhor agarose gels (FMC BioProducts) and stained with ethidium bromide or in 8% denaturing polyacrylamide gels followed by autoradiography. High speed Polaroid film was used to photograph the stained agarose gels. Allelic losses were scored visually. A reduction of at least 50% in the tumor *versus* normal tissue allele ratio was considered to represent loss of heterozygosity. The mapping of LOH was done relative to linkage maps provided by the MIT database (20).[5]

Results

A total of 147 hybrid mouse lung lesions were analyzed for LOH on mouse chromosome 4. Upon screening, 23 of 45 $C3AF_1$ and 6 of 16 CDF_1 lung adenocarcinomas were detected with allelic losses, as were 12 of 48 small gross CDF_1 lung lesions. Eighty-one % (34 of 42) of the tumors with LOH displayed losses at all of the markers analyzed, including the centromeric *D4MIT18*, suggesting a high incidence of nondisjunction. Of eight with more local patterns of LOH, all were detected with losses of marker *D4MIT77*. Of these, CDF_1-18 lost only this marker while retaining heterozygosity at the nearest informative markers, *D4MIT27* and *D4MIT15*. Tumor C3A-19 also displayed LOH at marker *D4MIT77* with retention of heterozygosity at the nearest flanking informative markers, *D4MIT31* and *D4MIT27* (Table 1; Fig. 1). Thus, the LOH data localized a critical region to SSLP marker *D4MIT77*. Linkage analysis has placed this marker at a position that is ~1.0 cM distal to marker *D4MIT27* and ~1.1 cM proximal to *D4MIT15* (19, 20).[5, 6] The IFN-α gene cluster is recombinationally inseparable from *D4MIT27* and *D4MIT45*.[6] Our results excluded these nearby markers from the minimum region of deletion and have therefore defined a candidate region of ~2.0 cM.

Seemingly inconsistent with our LOH data, four adenocarcinomas with large regions of allelic loss were detected to retain heterozygosity at either *D4MIT77* (C3A-21), *D4MIT27* (C3A-22), or at both of these markers (C3A-20 and C3A-24). This suggested the possibility of homozygous deletion in these tumors. As our LOH analysis was PCR based, these apparent retentions of heterozygosity would have been attributable to target amplification of DNA from normal cells that contaminate these tumors. To address this issue, multiplex PCR was used to comparatively amplify target loci and a marker locus outside the region of interest. This marker, *D10MIT3*, was found to PCR-amplify to an equivalent extent in both tumor and normal tissues and was used in these experiments as an internal control. The coamplification of the control and the loci of interest in tumor and normal tissues provided a means to control for PCR amplification and enabled the relative level of the target sequences to be quantified. When the

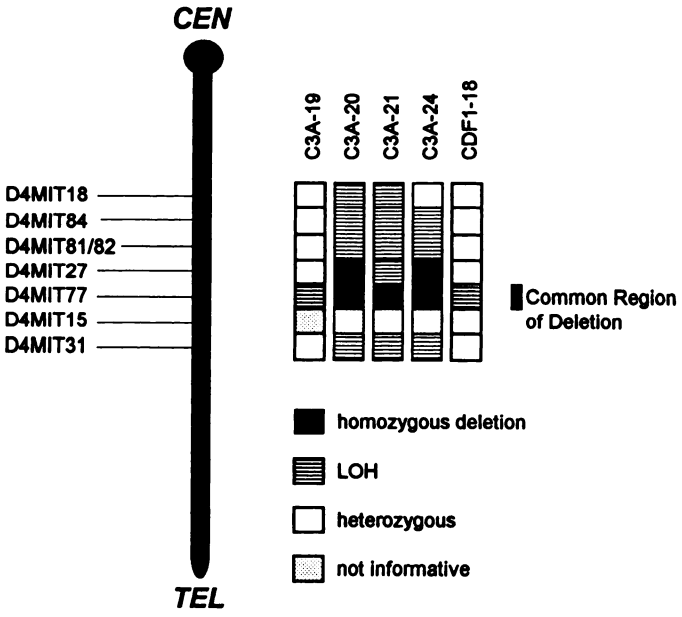

Fig. 1. Deletion map of chromosome 4. The relative distances between SSLP markers are as follows: *D4MIT18* —— ~25.2 cM —— *D4MIT84* —— ~1.1 cM —— *D4MIT81/D4MIT82* —— ~3.0 cM —— *D4MIT27* —— ~1.0 cM —— *D4MIT77* —— ~1.1 cM —— *D4MIT15* —— ~11.4 cM —— *D4MIT31*.

[5] Supplemented by additional markers in the Whitehead Institute/MIT Center for Genome Research, Genetic Maps of the Mouse, Database release 6, April 5, 1994.
[6] W. Dietrich, personal communication.

autoradiographic signal of the *D10MIT3* alleles from normal and tumor DNA were of equal intensity, the *D4MIT77* alleles of tumors C3A-20, C3A-21, and C3A-24 were observed to be significantly diminished (Fig. 2).

To quantify the extent of diminution, a dilution assay was performed in which normal DNA was diluted up to 100-fold prior to PCR of either *D4MIT77* (Fig. 2) or *D4MIT27* (data not shown). A dilution factor of >10 was necessary to attain an autoradiographic signal equivalent to that of tumors C3A-20, C3A-21, or C3A-24 (Fig. 2). Tumors C3A-20 and C3A-24 displayed equal diminution of both *D4MIT77* and *D4MIT27*, whereas tumor C3A-24 did not show diminution at marker *D4MIT27*. These results suggest that homozygous deletions had occurred and spanned marker *D4MIT77* in tumor C3A-21 and both *D4MIT77* and *D4MIT27* in tumors C3A-20 and C3A-24. Tumor C3A-22 appeared not to undergo homozygous deletion but evidently retained heterozygosity at marker *D4MIT27* while displaying LOH at *D4MIT77*. These data implicate the *D4MIT77* marker region extending between, but non-inclusive of, *D4MIT27* and *D4MIT15* as the location of a putative tumor suppressor gene.

Also analyzed were 38 mouse lung adenomas (18 $C3AF_1$, 18 $AB6F_1$, and 2 CDF_1), of which 1 (C3A-14) was detected with LOH on chromosome 4. In this case, losses were revealed at all five markers tested (Table 1). As shown in Table 2, 29 of 61 (48%) adenocarcinomas and 1 of 38 (3%) adenomas had LOH on chromosome 4.

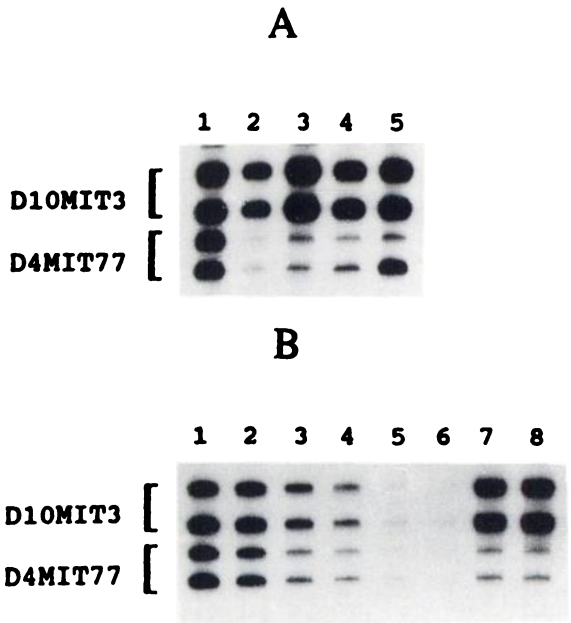

Fig. 2. *A*, comparative multiplex PCR analysis of mouse lung tumor DNA. *Lane 1*, normal DNA; *Lane 2*, tumor C3A-20 DNA; *Lane 3*, tumor C3A-21 DNA; *Lane 4*, tumor C3A-24 DNA; *Lane 5*, tumor C3A-19. Diminution of both *D4MIT77* alleles is evident for tumors C3A-20, C3A-21, and C3A-24. Loss of the upper *D4MIT77* allele is seen for tumor C3A-19. *B*, normal template DNA dilution followed by multiplex PCR for the relative quantification of *D4MIT77* diminution in tumor samples with evident homozygous deletions. *Lane 1*, 50 ng normal DNA; *Lane 2*, 25 ng normal DNA; *Lane 3*, 10 ng normal DNA; *Lane 4*, 5 ng normal DNA; *Lane 5*, 1 ng normal DNA; *Lane 6*, 0.5 ng normal DNA; *Lane 7*, 50 ng tumor C3A-20 DNA; *Lane 8*, 50 ng tumor C3A-21 DNA.

Table 2 *Frequency of chromosome 4 LOH in F_1 hybrid mouse lung tumors*

Hybrid strain	Tumor type	Number with LOH	Number tested	Frequency (%)
$C3AF_1$	carcinoma	23	45	51
$C3AF_1$	adenoma	1	18	6
CDF_1	carcinoma	6	16	38
CDF_1	adenoma	0	2	0
CDF_1	undiagnosed	12	48	25
$AB6F_1$	adenoma	0	18	0

Discussion

The presence of tumor suppressor gene on human chromosome 9p21–22 has been implicated by its frequent deletion in several tumor types, including lung carcinomas, leukemias, gliomas, bladder carcinomas, melanomas, mesotheliomas, and head and neck squamous cell carcinomas (1–10). The culmination of detailed deletion mapping and positional cloning efforts have uncovered the *MTS1*, a potential tumor suppressor gene in this region that was shown to undergo frequent homozygous deletions in cell lines derived from multiple cancer types and in some primary tumors (11, 12). We used a PCR-based SSLP analysis to screen mouse lung tumors for LOH on chromosome 4 with attention paid to the region encompassing the INF-α region which is syntenic to human 9p21–22. In all, 42 lesions were detected with allelic losses, 81% of which displayed LOH of all markers tested, suggesting nondisjunction in these cases. All allelic losses were observed to involve marker *D4MIT77* (Table 1; Fig. 1). Moreover, two cases exhibited LOH exclusive to this marker. Three additional tumors which initially appeared to retain heterozygosity at *D4MIT77* were subsequently revealed to possess homozygous deletions affecting this marker (Fig. 1). The critical region likely to contain a novel tumor suppressor gene was therefore localized to marker *D4MIT77* and the flanking ~2 cM extending from the nearest informative markers.

Our results also show that the inactivation of the putative tumor suppressor gene, localized herein to the region around *D4MIT77*, occurs almost exclusively in lung adenocarcinomas. This suggests that these occurrences are contributory to the progression and not the initial formation of these mouse lung tumors. Consistent with these findings have been the observed recurrence of human 9p21 deletions in late grade glioblastomas (5, 6), and in the progression of head and neck squamous cell carcinomas (10).

The *MTS1* is a potentially important tumor suppressor gene based on: (*a*) frequent homozygous deletions of this locus have recurred in cell lines from multiple cancer types such as those of bone, brain, breast, kidney, lung, ovary, bladder, skin, and lymphocytes (11, 12); and (*b*) the *MTS1* gene encodes an inhibitor of CDK4, one of several cyclin-dependent kinases that potentiate cell division by regulating the phosphorylation state of key substrates that control the transition of cells through defined checkpoints in the cell cycle (13). CDK4 is functional, in this regard, when associated with cyclin D1, which has been shown to act as an oncogene (21). The loss of inhibitory control of CDK4 would appear, therefore, to permit the constitutive activation of CDK4 by cyclin D1 and the manifestation of growth changes seen in cancer. In the present study, we define a ~2 cM critical region of frequent deletion in mouse lung adenocarcinomas on chromosome 4 within a region syntenic to where *MTS1* resides on human chromosome 9p21–22. This suggests the location of a putative tumor suppressor gene that is important in the progression of mouse lung adenomas to adenocarcinomas and which may represent the mouse homologue of *MTS1*. Efforts are currently being made to clone this mouse gene and to further define its tumor suppressor function.

Acknowledgments

We are grateful to Dr. Herman Schut for providing CDF_1 mouse lung tumors, to Drs. Marshall Anderson and Robert Maronpot for the $C3AF_1$ mouse lung tumors, and to Dr. Alvin Malkinson for the $AB6F_1$ mouse lung tumors. We thank Drs. John Rapp, Robert Blumenthal, and Robert Sills for their critical reading of the manuscript. We are indebted to Dr. Bill Dietrich for reviewing the placement of *D4MIT77* and to Kathy Deanda for her help in the preparation of the manuscript.

References

1. Fountain, J. W., Karayiorgou, M., Ernstoff, M. S., Kirkwood, J. M., Vlock, D. R., Titus-Ernstoff, L., Bouchard, B., Vijayasaradhi, S., Houghton, A. N., Lahti, J., Kidd, V. J., Housman, D. E., and Dracopoli, N. D. Homozygous deletions within human chromosome band 9p21 in melanoma. Proc. Natl. Acad. Sci. USA, *89:* 10557–10561, 1992.
2. Olopade, O. I., Bachhagen, D. L., Malik, K., Sherman, J., Nobori, T., Bader, S., Nau, M. M., Gazdar, A. F., Minna, J. D., and Diaz, M. O. Homozygous loss of the interferon genes defines the critical region on 9p that is deleted in lung cancers. Cancer Res., *53:* 2410–2415, 1993.
3. Marlo, A., Gabrielson, E., Askin, F., and Sidransky, D. Frequent loss of chromosome 9 in human primary non-small cell lung cancer. Cancer Res., *54:* 640–642, 1994.
4. James, D. C., He, J., Carlborm, E., Nordenskjold, M., Cavenee, W. K., and Collins, P. V. Chromosome 9 deletion mapping reveals interferon α and interferon β-1 gene deletions in human glial tumors. Cancer Res., *51:* 1684–1688, 1991.
5. Olopade, O. I. Jenkins, R. B., Ransom, D. T., Malik, K., Panykala, H., Nobori, T., Cowan, J. M., Rowkey, J. D., and Diaz, M. O. Molecular analysis of deletions of the short arm of chromosome 9 in human gliomas. Cancer Res., *52:* 2523–2529, 1992.
6. Diaz, M. O., Ziemin, S., LeBeau, M. M., Pitha, P., Smith, S. D., Chilcote, R. R., and Rowley, J. D. Homozygous deletion of the α and β-1 interferon genes in human leukemia and derived cell lines. Proc. Natl. Acad. Sci. USA, *85:* 5259–5263, 1988.
7. Cairns, P., Tofino, K., Eby, Y., and Sidransky, D. Homozygous deletions of 9p21 in primary human bladder tumors detected by comparative multiplex polymerase chain reaction. Cancer Res., *54:* 1422–1424, 1994.
8. Cheng, J. Q., Jhanwar, S. D., Lu, Y. Y., and Testa, J. R. Homozygous deletions within 9p21–22 identify a small critical region of chromosomal loss in human malignant mesotheliomas. Cancer Res., *53:* 4761–4763, 1993.
9. van der Riet, P., Nawroz, H., Hruban, R. N., Corio, R., Toking, K., Koch, W., and Sidransky, D. Frequent loss of chromosome 9p21–22 early in head and neck cancer progression. Cancer Res., *54:* 1156–1158, 1994.
10. Cannon-Albright, L. A., Goldgar, D. E., Heyer, L. J., *et al.* Assignment of a locus for familial melanoma, MLM, to chromosome 9p13-p22. Science (Washington DC), *258:* 1148–1152, 1992.
11. Kamb, A., Gruis, N. A., Weaver-Feldhaus, J., Liu, Q., Harshman, K., Tavtigian, S. V., Stockert, E., Day, R. S., III, Johnson, B. E., and Skolnick, M. A cell cycle regulator potentially involved in genesis of many tumor types. Science (Washington DC), *264:* 436–440, 1994.
12. Nobori, T., Miura, K., Wu, D. J., Lois, A., Takabayashi, K., and Carson, D. A. Deletions of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers. Nature (Lond.), *368:* 753–756, 1994.
13. Serrano, M., Hannon, G. J., and Beach, D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature (Lond.), *36:* 704–707, 1993.
14. Jonasson, J., Poveym S., and Harris, H. The analysis of malignancy by cell fusion. VII. Cytogenetic analysis of hybrids between malignant and diploid cells and of tumors derived from them. J. Cell Sci., *24:* 217–254, 1977.
15. Wiseman, R. W., Cochran, C., Dietrich, W., Lander, E. S., and Soderkvist, P. Allelotyping of butadiene-induced lung and mammary adenocarcinomas of B6C3F_1 mice: frequent losses of heterozygosity in regions homologous to human tumor-suppressor genes. Proc. Natl. Acad. Sci. USA, *91:* 3759–3763, 1994.
16. Herzog, C. R., Schut, H. A. J., Maronpot, R. R., and You, M. Ras mutations in 2-amino-3-methylimidazo-[4,5-*f*]quinoline-induced tumors in the CDF$_1$ mouse. Mol. Carcinog., *8:* 202–207, 1993.
17. You, M. Wang, Y., Stoner, G., You, L., Maronpot, R., Reynolds, S. H., and Anderson, M. Parental bias of Ki-*ras* oncogenes detected in lung tumors from mouse hybrids. Proc. Natl. Acad. Sci. USA, *89:* 5804–5808, 1992.
18. Chen, B., You, L., Wang, Y., Stoner, G. D., and You, M. Allele-specific activation and expression of the K-*ras* gene in hybrid mouse lung tumors induced by chemical carcinogens. Carcinogenesis (Lond.), in press, 1994.
19. Copeland, N. G., Jenkins, N. A., Gilbert, D. J., *et al.* A genetic linkage map of the mouse: current applications and future prospects. Science (Washington DC), *262:* 57–66, 1993.
20. Dietrich, W. F., Miller, J. C., Steen, R. G., *et al.* A genetic map of the mouse with 4,006 simple sequence length polymorphisms. Nature Genetics (Lond.), *7:* 220–245, 1994.
21. Motokura, T., Bloom, T., Kim, H. G., Jüppner, H., Ruderman, J. V., Kronenbert, H. M., and Arnold, A. A novel cyclin encoded by a *bcl1*-linked candidate oncogene. Nature (Lond.), *350:* 512–515, 1991.