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

Christopher R. Herzog, Roger W. Wiseman, and Ming You

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 (MTSI), 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 F1 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), transitional 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 (11) was identified by virtue of its frequent homozygous deletion in cell lines derived from 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 B6C3F1 mouse lung adenocarcinomas (15) and in 26 of 49 B6C3F1, C3AF1, and AC3F1 lung adenocarcinomas.*

In the present study, this region on mouse chromosome 4 was further defined by deletion and LOH analyses using constitutionally heterozygous SSLP markers in F1 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. CDF1 (BALB/c × DBA/2 F1) 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 C3AF1 (C57BL/6J × A/J F1) hybrid mouse lung adenocarcinomas and three adenomas were collected from untreated 2-year-old mice as described (17). Other C3AF1, 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 AB6F1 (A/J × C57BL/6J F1) hybrid lung adenomas were induced by an i.p. treatment of newborns with 5 mg urethane/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 be 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 CDF1, C3AF1, and AB6F1 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

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

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-amin-3-methylimidazo(4,5-f)quinoline; NNK, 4-(methylamino)-1-(3-pyridyl)-1-butanone; VC, vinyl carbamate; PCR, polymerase chain reaction.

* M. E. Higgi et al., manuscript in preparation.


Table 1  Pattern of chromosome 4 LOH in F1 hybrid mouse lung tumors

<table>
<thead>
<tr>
<th>Tumor</th>
<th>D4MIT77</th>
<th>D4MIT27</th>
<th>D4MIT31</th>
<th>D4MIT15</th>
<th>D4MIT18</th>
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<td>C3A-1 to C3A-9</td>
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<td>C</td>
<td>C</td>
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<td>C3A-10 and C3A-11</td>
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<td>ND</td>
<td>ND</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<td>ND</td>
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<tr>
<td>C3A-14 and C3A-15</td>
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<td></td>
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</table>

a Shown are the retained alleles: A, BALB/c; B, C3H/HeJ; C, DBA/2J.

b Loci are shown in their relative chromosomal order from the centromere at left to the telomere at right.

ND, not done; NI, noninformative; —, homozygous deletion.

Results

A total of 147 hybrid mouse lung lesions were analyzed for LOH on mouse chromosome 4. Upon screening, 23 of 45 C3AF1 and 6 of 16 CDF1 lung adenocarcinomas were detected with allelic losses, as were 12 of 48 small gross CDF1 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, CDF1-18 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). The IFN-α gene cluster is recombinationally inseparable from D4MIT27 and D4MIT45. 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

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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 tumors 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 C3AF1, 18 AB6F1, and 2 CDF1), 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.

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

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Table 2 Frequency of chromosome 4 LOH in F2 hybrid mouse lung tumors

<table>
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<tr>
<th>Hybrid strain</th>
<th>Tumor type</th>
<th>Number with LOH</th>
<th>Number tested</th>
<th>Frequency (%)</th>
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<td>45</td>
<td>51</td>
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<td>18</td>
<td>6</td>
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<td>carcinoma</td>
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<td>38</td>
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<tr>
<td>AB6F1</td>
<td>adenoma</td>
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<td>18</td>
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</tbody>
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LOH OF CHROMOSOME 4 IN MOUSE LUNG TUMORS

References


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