

# Specific Chromosomal Aberrations in Mouse Lung Adenocarcinoma Cell Lines Detected by Spectral Karyotyping: A Comparison with Human Lung Adenocarcinoma

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## ABSTRACT

Although adenocarcinoma is rapidly becoming the most common form of lung cancer in the United States, the difficulty in obtaining lung cancer families and representative samples of the various stages of adenocarcinoma progression has led to intense study of mouse models. As a powerful approach to delineating molecular changes, we have analyzed 15 early-passage mouse cell lines by spectral karyotyping. Entire copies of chromosomes 1, 2, 6, 12, 15, and 19 were gained, and entire copies of chromosomes 4, 7, 8, and 14 were lost. Significant gains of portions of chromosome 1 (93% of the tumor cell lines analyzed), chromosome 2 (53%), chromosome 6 (73%), chromosome 7 (80%), chromosome 12 (47%), and chromosome 15 (73%) and partial loss of chromosome 4 (87%), chromosome 7 (80%), chromosome 8 (53%), chromosome 10 (33%), and chromosome 14 (33%) were observed. Recurrent translocations included 10:del(10)(A1::C1), t(4;8)(C4;A1), and der (1;12)(C2;C2). The minimal regions of chromosomal alteration, 1G1, 2F1, 4C4, 6D, 7F1, 8B3, and 12C2, contain putative susceptibility genes for mouse lung adenocarcinoma. Chromosomal regions containing susceptibility genes linked to tumor size were frequently amplified, whereas regions with susceptibility loci linked to tumor multiplicity were deleted. Similar linkage groups are altered in human lung adenocarcinoma, implying that the mouse is a valid genetic model for the study of human lung adenocarcinoma susceptibility.

## INTRODUCTION

Lung cancer, the most common cause of cancer death in the world, is second only to bladder cancer in the number of cases linked to occupational exposure. Non-small cell lung cancer, accounting for ~75% of human lung cancers (1), has a high metastatic potential and is most frequently diagnosed at an advanced stage of disease. Adenocarcinoma is the most common form of non-small cell lung cancer diagnosed in smokers, nonsmoking women, and adults younger than 45 years of age (2). Familial clustering indicates that adenocarcinoma has a strong genetic component (3, 4). The poor prognosis coupled with the difficulty in obtaining useful lung cancer families makes genetic analysis of human adenocarcinoma very difficult. Murine adenocarcinoma has been proposed as a model for human adenocarcinoma (5). Crosses between susceptible and resistant mouse strains and subsequent quantitative trait locus mapping have demonstrated genetic loci that are correlated with tumor multiplicity or tumor progression. Manenti *et al.* (6) have reported the mapping of a lung cancer susceptibility locus, *Pas1*, in the mouse. The homologous human locus was shown to correlate with increased susceptibility for

adenocarcinoma of the lung in population-based studies (7). The high incidence and death rate associated with this cancer indicate the need to identify markers for early detection in individuals at high risk.

Genetic alterations in cancer commonly occur in chromosomal regions that regulate growth and development. Identifying these chromosomal regions is the first step in determining which genes are critical for the initiation and progression of cancer. Human lung adenocarcinoma is characterized by multiple complex chromosomal abnormalities. Lung tumor susceptibility has been studied extensively in the mouse, but karyotypic analysis of mouse lung adenocarcinoma has not been as well characterized. Among inbred strains of mice, the A/J mouse is one of the most susceptible to the development of spontaneous and chemically induced lung cancer (8–10). Quantitative trait locus mapping of mouse hybrids has identified regions of chromosomes that are correlated with susceptibility to lung cancer (11–15). In this study, we demonstrate that mouse lung tumor susceptibility loci are associated with deletions, translocations, and amplifications. Genetic linkage groups that are altered in lung adenocarcinomas from both mice and humans serve to highlight regions of the genome significant in the development of lung cancer and may aid in identifying human susceptibility genes.

## MATERIALS AND METHODS

**Tumor Cell Lines.** Lung tumors of 0.6 cm or greater in diameter with a pathology of adenocarcinoma were excised from the lungs of 15 A/J mice. Ten of those tumors were spontaneous, and 5 were chemically induced. The tumor tissue was prepared and cultured as described previously (16). When the cells were 70% confluent, 1 mg/ml to 100 mg/ml Colcemid was added to block the cells in metaphase. Cell cultures that were resistant to Colcemid were given the higher dose of Colcemid. After a 10-h incubation, cells were harvested for inverted DAPI<sup>2</sup> chromosome banding, spectral karyotyping, and FISH analysis. Cells were incubated with 0.075 M KCl for 30–90 min and then fixed using 3:1 methanol:acetic acid (v/v) fixative.

**Methods for Spectral Karyotyping.** Spectral karyotyping and inverted DAPI banding were performed by standard methods (17). At least 20 metaphase cells of good banding morphology and spectral hybridization quality were analyzed for each tumor cell line. Chromosome abnormalities were included in the karyotype of a tumor if they occurred in at least 40% of the metaphase spreads. Translocations were confirmed with FISH using direct-labeled chromosome painting probes (Vysis, Inc., Downers Grove, IL).

**Statistics.** The percentages of tumor cell cultures with a specific aberration were determined. SD denotes the variation between tumor cell lines. Chromosome regions with an apparent likelihood of elevated breakage were tested for statistical significance using the  $\chi^2$  test. The *P*s were corrected for multiple comparisons using the Bonferroni correction.

<sup>2</sup> The abbreviations used are: DAPI, 4',6-diamidino-2-phenylindole; FISH, fluorescence *in situ* hybridization; Pas, pulmonary adenoma susceptibility; Sluc, susceptibility to lung cancer.

Received 9/10/01; accepted 12/10/01.

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RESULTS

Molecular cytogenetic analysis of 15 early-passage mouse lung adenocarcinoma cell lines was performed using spectral karyotyping, FISH, and inverted DAPI banding. Chromosomal aberrations in cell lines derived from chemically induced and the spontaneous lung adenocarcinomas were not significantly different, and the two groups were therefore combined for further analysis. Statistically significant gains and losses of chromosomal material are summarized in Table 1. Nonrandom amplifications of chromosomes 1, 2, 6, 12, 15, and 19 were observed (Tables 1 and 2). All or part of chromosome 1 was gained in 93%, 2 in 53%, 6 in 73%, 12 in 47%, 15 in 73%, and 19 in 47% of the tumor cell lines analyzed (Table 1, Fig. 1). Loss of all or part of chromosome 4 was observed in 87% of the tumor cell cultures (Table 1; Fig. 2), with the most frequent breakpoint at the border of bands C4/C5 (Table 1; Fig. 3). An entire copy of chromosome 7 was lost in 80% of the tumor lines (Tables 1 and 2), with the minimal region of deletion at 7F1. Chromosome 8 was deleted in 53% of the tumor cell lines (Table 1), with minimal break of chromosome 8 at 8B3 (Fig. 1). A deletion of chromosome 10 at band B1 was observed in 33% of the cell lines (Table 2). An entire copy of chromosome 14 was lost in 33% of the tumor cell lines (Table 2), with a deletion on 14qE1 as the smallest deletion (Table 1; Fig. 3). There were more amplifications than deletions of chromosomal material, analogous to the greater number of chromosomal amplifications reported in human lung adenocarcinoma by Petersen *et al.* (18).

Ninety-three percent of the cell lines had an amplification of all or part of chromosome 1 (Table 2; Fig. 1), with the most frequent breakpoint and the minimal amplification on chromosome 1 occurring at band G1 (Figs. 1 and 4). All or part of chromosome 2 was gained in 53% of the tumor cell lines (Table 1; Fig. 1). The minimal region of amplification occurred at 2F1. Seventy-three % of the tumor lines had amplifications of chromosome 6, and gain of material from band D1 to the end was the minimal region that was amplified (Table 2; Fig. 1). Seventy-three % of the tumor cell lines had an addition of an entire copy of chromosome 15 (Fig. 1), with the smallest region of alteration occurring at 15D1 (Fig. 1). Thirty-eight % of the tumor cell lines had an extra copy of chromosome 19 (Fig. 1; Tables 1 and 2).

Translocations of chromosome 4 were observed in 60% of the cell lines (Fig. 2, Table 2), and translocations of chromosomes 1, 2, and 8 were observed in 33% of the cell lines (Fig. 4, Table 2). An interstitial deletion of chromosome 10:del(10)(A1::C1) was observed in 26% of the cell lines. Two translocations were observed in 20% of the cell lines, t(4;8)(4C4;8A1) (Fig. 2) and der(1;12)(C2;C2) (Fig. 4). The

Table 1 Frequency of chromosomal alterations in mouse lung adenocarcinoma cell lines

This table illustrates common chromosome break sites observed by spectral karyotyping analysis and confirmed by FISH. At least 20 karyotypes were analyzed for each of the 15 early-passage cell lines. Chromosome changes that occurred in at least 40% of the cells from a given cell line were included in the karyotype. The data are expressed as the ratio of the number of cell lines with the specific band changes to the total number of tumor cell lines analyzed. The table is divided into chromosomal deletions and gains.

	% of tumor cell lines with aberration
<b>Chromosome losses</b>	
4	87 ± 5.0
7	80 ± 10.0
8	53 ± 15.0
10	27 ± 20.0
14	33 ± 20.0
<b>Chromosome gains</b>	
1	93 ± 5.0
2	53 ± 13.0
6	73 ± 7.0
12	47 ± 15.0
15	73 ± 7.0
19	47 ± 15.0

Table 2 Modal karyotype of mouse lung cell lines

This table demonstrates the modal karyotype and chromosome number present in the mouse lung adenocarcinoma cell lines. At least 20 metaphase cells of good banding and spectral hybridization quality were analyzed for each tumor cell line. Only aberrations that were observed in at least 40% of the cells were included in the karyotype.

- SP 4<sup>a</sup>**  
del(1)(G1), dup(2)(G1), del(4)(C4), iso(4)(A1), +6, -7, -9, -14, +15, -18, modal number 76.
- SP 6<sup>a</sup>**  
t(1;2)(G1;E3), der(1)t(1;12)(C2;C2), del(1)(1F), 1C5ter-, -4, del(4)(C4/C5), der(4)t(4;8)(C3;A1), del(5)(D5), +6, del(6)(D1), -7, -9, del(9)(E1), del(10)(A1::C1), +15, -16, +19, modal number 71.
- SP 7<sup>a</sup>**  
+1, +2, t(2;4)(A1;A1), +3, +4, der(4)(4;8)(C3;A1), del(4)(C3), +5, +6, +7, -8, del(10)(B5), del(10)(A1::C1), +11, +12, +13, +15, +17, +19, modal number 52.
- Sp 8<sup>a</sup>**  
+1, del(4)(C4/C5), del(7)(E3-F1), del(8)(B3), +12, +12, -13, +15, modal number 82.
- SP 10<sup>a</sup>**  
+1, +2, -4, +6, -7, -14, -16, +19, modal number 57.
- FT 5<sup>a</sup>**  
+3, -4, -14, -16, +19, modal number 59.
- FT 3M<sup>a</sup>**  
+1, t(1;12)(C2;C2), del(1)(E1-G1), Is(1;4)(G1;C4), del(4)(A5), -4, del(7)(E3), del(8)(B3), del(10)(A1::C1), del(11)(B1), +15, modal number 79.
- CL 13<sup>a</sup>**  
+1, del(4)(C6), +6, -7, -8, del(10)(A1::C1), modal number 40.
- CL 20<sup>a</sup>**  
+6, -7, der(9)t(9;8)(E4;C2), -12, modal number 32.
- CI 30<sup>a</sup>**  
iso(1)(A1), +1, +2, +3, del(4)(C4), +6, +12, +15, +19, +19, modal number 48.
- Lm 2<sup>b</sup>**  
dup(2)(E1), -4, iso(6)(A1), +6, del(8)(A3), -16, modal number 78.
- MC 7<sup>b</sup>**  
+1, iso(4)(A1), del(4)(C4), +5, -7, +15, +15, +19, modal number 80.
- MC 14<sup>b</sup>**  
iso(4)(A1), +15, modal number 40.
- NNK 25<sup>b</sup>**  
del(1)(F1), +1, del(4)(C3-C5), iso(4)(A1), del(4)(C4), +15, bimodal 40, 80.
- NNK 30<sup>b</sup>**  
iso(1)(A1), dup(1)(E1), dup(1)(C4), +2, +3, +6, -7, +8, -9, +12, +15, +16, +19, modal number 51.

<sup>a</sup> Cell line from spontaneous mouse lung adenocarcinomas.

<sup>b</sup> Cell line derived from chemically induced tumors.

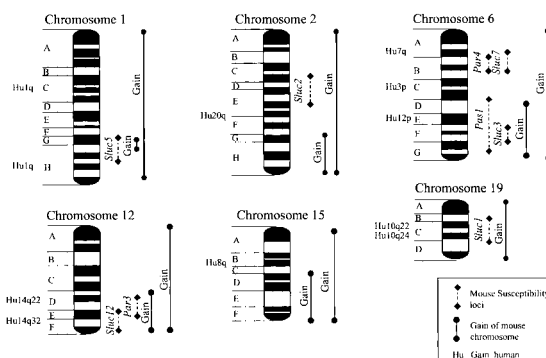


Fig. 1. Ideograms of chromosomes 1, 2, 6, 12, 15, and 19 demonstrate that duplications commonly observed in mouse lung adenocarcinoma cell lines correlate to amplifications observed previously in human lung adenocarcinoma. The mouse chromosome number is indicated at the top of the ideogram, and the homologous human chromosome is indicated to the left of the ideogram as *Hu* with the chromosome number. The common gains observed in the mouse lung cell lines are indicated by a solid bar to the right of the ideogram. The large bar indicates the most common region of alteration, and the small bar indicates the minimal region of alteration. The tumor susceptibility loci, *Sluc 5*, *Sluc 2*, *Pas 1*, *Par 4*, *Sluc 3*, *Sluc 7*, *Sluc 12*, *Par 3*, and *Sluc 1* are indicated to the right of the ideogram by a dashed line.

minimal regions of alteration of chromosomes 1, 2, 4, 6, 7, 8, and 12 contain putative susceptibility genes for mouse lung adenocarcinoma. The syntenic linkage groups are also modified in human adenocarcinoma (Figs. 1 and 3).

DISCUSSION

The chromosomal alterations in mouse chromosomes 1, 2, 4, 6, 7, 8, 12, 14, 15, and 19 are evident in Figs. 1 and 3; similar changes were

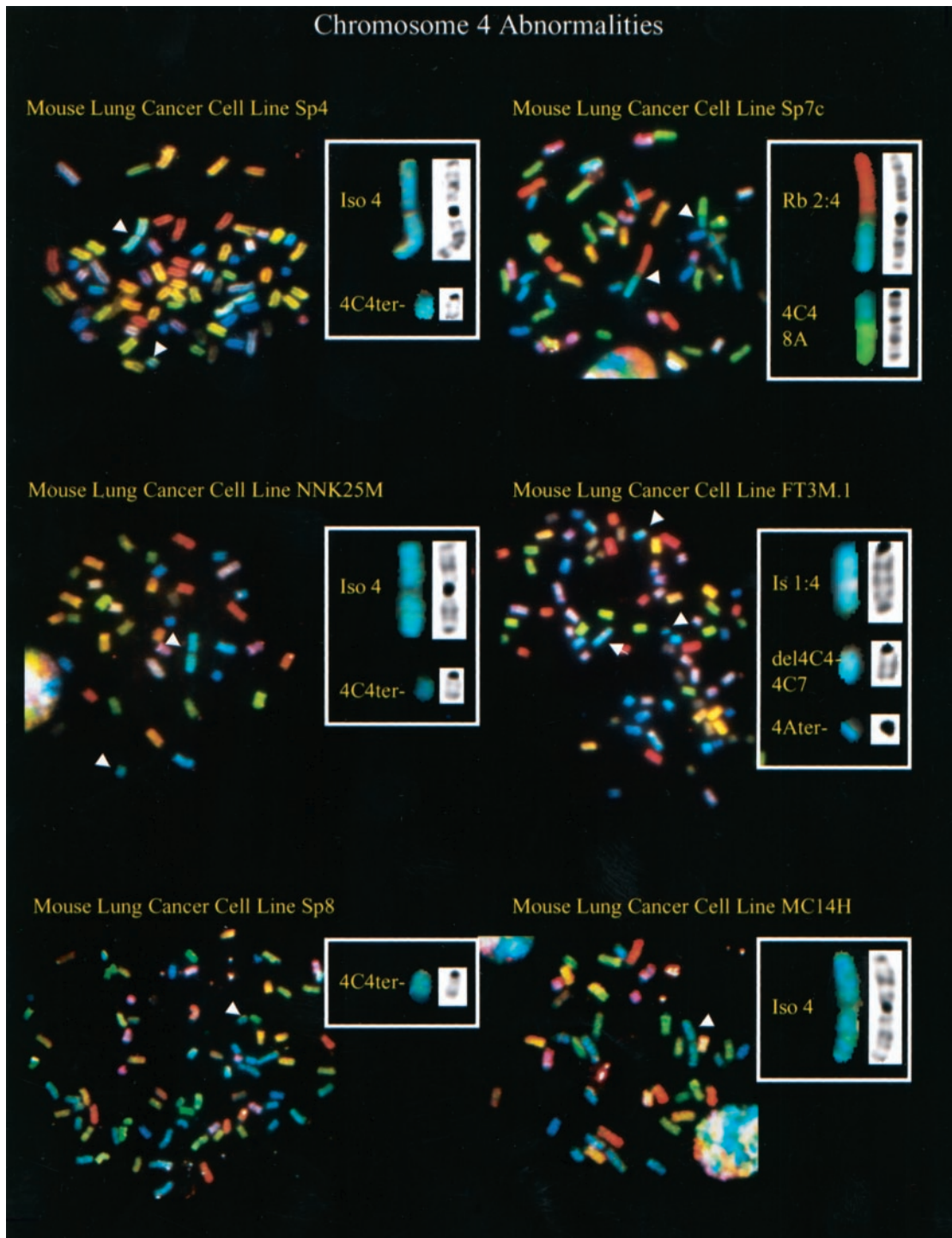


Fig. 2. This is a composite of the spectral images from cell lines with deletions of chromosome 4. The banded image is in the *white box* to the *right* of the spectral image. The breakpoint at 4C4 was the minimal region of deletion. Insertion of chromosome 1 into 4:ls(1;4)(G1;C4) was observed in FT3M.1.

observed in the homologous human linkage groups in human lung adenocarcinoma. The correlation between the chromosome alterations observed in mouse lung adenocarcinoma cell lines and where on these chromosomes that susceptibility loci for adenocarcinoma development map supports the importance of susceptibility loci in the development of lung cancer. Susceptibility loci associated with increased tumor size were amplified, whereas loci affecting tumor number were deleted in these mouse lung cell lines. This suggests selection for tumors with a rapid growth rate. The region of chromosome 1 that was frequently amplified in the cell lines contains a locus that confers lung

tumor susceptibility (12). Distal chromosome 1 also has genetic linkage groups that confer susceptibility to liver and intestinal tumors (19, 20). The tyrosine kinase *Abl 1* (21), *RXR* $\gamma$  (22), a kinetochore protein (23), and a microtubule-associated protein (24) also map to this locus. *Scfr1* is an important predictor of the size of the hematopoietic cell stem cell population and is located in the fragile region of mouse chromosome 1 (25). Amplification of the corresponding region of the human chromosome, 1q32–41, is a frequent change in human lung adenocarcinoma (18, 26). The minimal region of mouse chromosome 2 that is amplified is 2F1. The corresponding region of

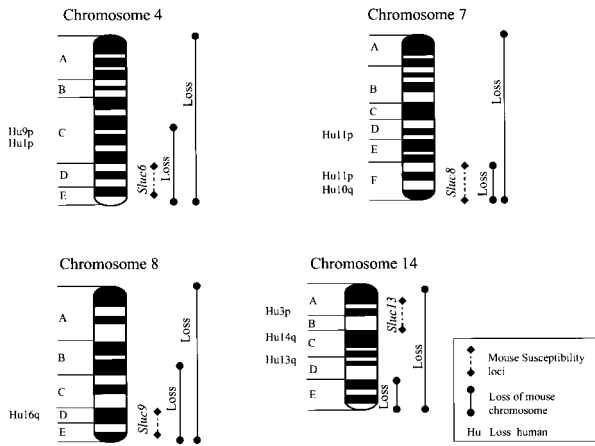


Fig. 3. Ideograms of chromosomes 4, 7, 8, and 14 demonstrate that the consistent losses observed in these mouse lung adenocarcinoma cell lines correlate with losses demonstrated previously in human lung adenocarcinoma. The mouse chromosome number is at the top of the ideogram. The homologous human chromosome is indicated to the left of the ideogram such as Hu with the chromosome number. The losses that were observed in the mouse lung cell lines are indicated by a solid bar to the right of the ideogram. The large bar indicates the most common region of deletion, and the small bar indicates the minimal region of deletion. Tumor susceptibility loci, *Sluc 6*, *Sluc 8*, *Sluc 9*, and *Sluc 13*, identified in mouse lung tumor models are indicated by a dashed line to the right.

human chromosome 20 is amplified in human lung adenocarcinoma (26, 27). This region of mouse chromosome 2 is not only increased in copy number but is also translocated to other chromosomes. The cell cycle regulatory gene, *E2f1*, is located within this amplified region. *E2f1* plays a critical role in the G<sub>1</sub> to S transition of the cell cycle and is therefore a reasonable candidate for amplification in rapidly dividing tumor cells. *E2f1* is amplified in human leukemia cells, which also demonstrate both amplification and translocation of this region (28). Amplification of *E2f1* cooperates with an activated *ras* oncogene that induces formation of morphologically transformed foci in primary rat embryo fibroblast cultures (29) and dermal epithelial cells (30). Many mouse and human lung adenocarcinomas have been observed to have mutations of the *K-ras* gene located on mouse chromosome 6. All of the cell lines that had a duplication of chromosome 2 also had a duplication of chromosome 6. We are currently examining the association between increased copy number of chromosome 6, mutation of *K-ras*, and the alteration of *E2f1*.

The duplicated region of chromosome 6 contains the two tumor susceptibility loci, *pulmonary adenoma susceptibility loci (Pas 1)*, and *susceptibility to lung cancer loci 3 (Sluc 3; Refs. 6, 13)*. The mutant form *K-ras* oncogene has been proposed as a candidate gene for *Pas 1* (31). The oncogenic action of mutant *K-ras* can result from a loss

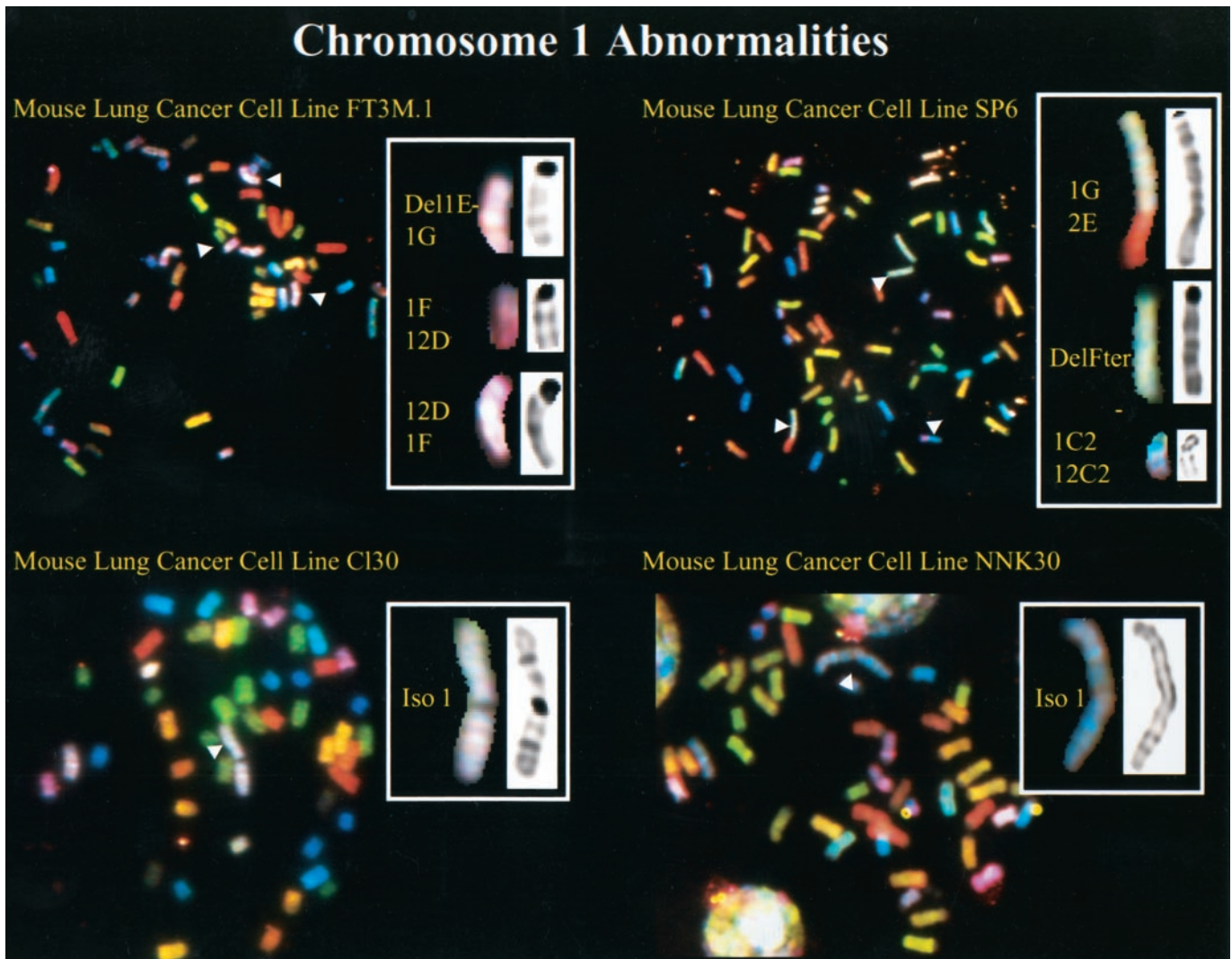


Fig. 4. A composite of the spectral images from cell lines that had duplications of chromosome 1. The banded image is in the white box to the right of the spectral image. The translocation between chromosomes 1 and 2 in cell line SP6: t(1;2)(G1;E3) is also involved 1G1. A duplication of chromosome 1 iso1(A1) was observed in cell lines CL30 and NNK30. Two translocations of 12 are in this figure. FT3M.1 had a balanced translocation, t(1;12)(C2;C2), and SP6 had an unbalanced translocation, der(1;12)(C2;C2).

of the wild-type allele or overexpression of the mutant allele (31). Polymorphisms of *K-ras 2* and *Pthl* loci were associated with an increased risk of human adenocarcinoma (7). The data demonstrating a putative lung cancer susceptibility gene in humans were the first demonstration of a potential link between the genetics of mouse lung tumor susceptibility and that of the human. In human lung adenocarcinoma, the homologous region of human chromosome 12p12 is amplified (18, 32).

A loss of chromosome 4 was detected in 87% of the tumor cell lines. The minimal region of deletion was band C4/C5 to the end of the chromosome. *Sluc 6* and the *pulmonary adenoma progression gene 1* (*pap1*) are located within this minimal region of deletion (Refs. 6, 13; Fig. 3). Wu *et al.* (33) reported a loss of mouse chromosome 4 in 9 of 10 lung tumor cell lines prepared from *p53* transgenic mice. The homologous region of rat chromosome 5 is frequently deleted in rat lung adenocarcinoma (34). Many investigators have shown that the *Cdkn2a* and *Cdkn2b* genes are deleted in mouse and human lung tumors (35–37). *Cdkn2a* and *Cdkn2b* down-regulate cell growth and are located within the chromosome 4 deleted region. Increased deletion of the maternal chromosome in both mouse and human lung tumors would indicate a loss of imprinted genes not yet identified (38). Homologous regions of human 9p21 and 1p36 are deleted in lung adenocarcinoma as indicated in Fig. 3 (18, 26).

The region of mouse chromosome 7 deleted in the mouse lung tumor cell lines corresponds to human 11p15.5 and 11p13, and rearrangements have been reported in human lung adenocarcinoma (39, 40). Fijneman *et al.* (13) identified a tumor susceptibility locus on mouse chromosome 7 near the imprinted locus of insulin-like growth factor II, *H19*, and *p57*, as well the *H-ras* gene. The maternal copy of *H19* on human chromosome 11 is lost in lung adenocarcinoma (39). Microcell fusion with human chromosome 11 inhibits the growth of human lung adenocarcinoma cells (41).

The amplified site on mouse chromosome 12 contains *Sluc 12* and *Par 3* (13). The orthologous region on human chromosome 14q32 is frequently altered in human lung adenocarcinoma (18).

Deletion of chromosome 14 was common in the tumor cell lines. Chromosomal rearrangements of mouse chromosome 14 have been associated with the later stages of mouse neoplasia (42). The homologous chromosome is deleted in rat lung adenocarcinomas (34). The retinoblastoma gene, urokinase plasminogen activator (43–45), *Tg737* tumor suppressor gene (46), and retinoic acid receptor-related gene (22, 47) are candidates located in this deleted region. The corresponding region on human 13q is often deleted without mutation of the retinoblastoma gene in human lung neoplasia, chronic lymphocytic leukemia, mammary cancer, and liver cancer (48–50). Expression of other tumor suppressor genes is decreased in mouse and human lung tumors, including *APC*, *MCC*, and *p53* (51). Although there are many reports of mutations of *p53* in human lung adenocarcinoma (52) and few reports of similar mutations in mouse adenocarcinoma (53), *p53* expression is reduced in both mouse and human lung adenocarcinoma. Alterations of chromosomes 1, 4, 5, 6, 7, 8, 12, and 14 occurred at high frequency in mouse lung adenocarcinoma cell lines. The minimal regions of alteration of these chromosomes contain tumor susceptibility loci. The breakpoints on these chromosomes correspond to human 1q, 1p and 9p, 12p, 11p15.5 and 11p12–13, 8q, 14q32, and 13q, respectively, shown previously to be altered in human lung adenocarcinoma. Because the breakpoints on chromosomes 1, 2, 4, 5, 6, 7, 8, 12, and 14 correspond to lung tumor susceptibility genes in mouse and exhibit rearrangement and aneuploidy in mouse and human lung adenocarcinoma, these results indicate the importance of identifying tumor susceptibility genes in mice and then analyzing corresponding loci in humans.

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