Map kinase activation correlates with K-ras mutation and loss of heterozygosity on chromosome 6 in alveolar bronchiolar carcinomas from B6C3F1 mice exposed to vanadium pentoxide for 2 years

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Previous work showed a correlation between K-ras mutation and loss of heterozygosity (LOH) on chromosome 6 in the region of K-ras in lung carcinomas from B6C3F1 mice. We hypothesized that mitogen-activated protein kinase (MAPK) would be activated only in those lung neoplasms with both K-ras mutation and LOH. As MAPK activity can be correlated directly with signal detection using antibodies to phosphorylated MAPK, we were able to analyze lung carcinomas from B6C3F1 mice for the presence or absence of MAPK activity by western analysis. Vanadium pentoxide-induced mouse lung carcinomas, which had been shown to have a high frequency of K-ras mutations and LOH on chromosome 6 and for which frozen tumor tissue was available, were used for this study. Total MAPK expression levels were similar between normal lung and lung carcinomas. Phospho-MAPK was elevated in five of six lung carcinoma samples examined in which K-ras mutations and chromosome 6 LOH were identified and in four of five carcinomas with K-ras mutations that lacked LOH. Phospho-MAPK was undetectable or weakly expressed in seven carcinomas examined without K-ras mutations and in normal lung. By immunohistochemistry three K-ras positive/LOH negative samples exhibited multifocal areas of nuclear and cytoplasmic staining for phospho-MAPK. Large amounts of non-staining fibroblasts, lymphocytes and macrophages were also observed in these tumors. Two of these lung carcinomas were microdissected and chromosome 6 LOH was detected in regions of phospho-MAPK positive cells. These results suggest that MAPK is activated during vanadium pentoxide-induced B6C3F1 mouse lung tumorigenesis following K-ras mutation and loss of the wild-type K-ras allele.

Introduction

Activation of the Ras/MAPK pathway seems to play an important role in carcinogenesis as indicated by the high frequency of Ras mutations identified in rodent and human cancers, including those of lung (1–3). Mouse lung carcinogenesis has been a standard model for examining the biology of human lung adenocarcinoma, and lung adenocarcinomas from both species often have K-ras mutations (2). In this study we are continuing our investigation into the role of normal and mutant K-ras in mouse lung carcinogenesis.

In the past we assumed that mutation of one allele of a Ras protooncogene family member was sufficient to activate the mitogen-activated protein kinase (MAPK) pathway. However, recent investigations into the mechanisms of Ras action indicate that the different ras isoforms vary in function and role in cell proliferation and differentiation depending on cell and tissue type and species (4). Evidence has been accumulating which points to a tumor suppressive role in mouse lung for normal K-ras p21 protein (5) and mouse lung tumors that exhibited decreased total- and cell membrane-associated K-ras p21 but increased activation of ERK 1 and 2 (6). Additionally, Zhang et al. (7) demonstrated by utilizing heterozygous K-ras knockout mice that wild-type K-ras can be a mouse lung tumor suppressor gene. In lung neoplasms from sensitive mice such as the A/J strain that contain ‘susceptible’ K-ras alleles, the mutant K-ras has a higher expression than the wild-type allele and may contribute substantially to increased MAPK activity and tumor formation (8). However, for the B6C3F1 mouse (C57BL/5×C3H) that contains two ‘resistant’ K-ras alleles, loss of the wild-type allele may be necessary for mutant K-ras to drive MAPK activation and mouse lung carcinogenesis. In this study we investigated whether B6C3F1 mouse lung carcinomas with K-ras mutations and loss of heterozygosity (LOH) in the region of K-ras have higher MAPK activity than those tumors without mutation and LOH.

The detection of LOH at specific chromosomal locations in tumors often indicates the presence of a tumor suppressor gene. While there is little generalized LOH in mouse lung neoplasms, a high frequency of LOH on chromosome 6 in the region of K-ras has been detected in spontaneous and chemically induced lung tumors from B6C3F1 mice (9). In these studies it was almost exclusively the tumors with K-ras mutations that exhibited the LOH, and this finding is supported by similar results with other chemically induced lung neoplasms (7).

In order to assess the relationship between K-ras activation and MAPK function in mouse lung carcinogenesis, we examined MAPK protein expression and activity in a set of lung neoplasms from B6C3F1 mice exposed to vanadium pentoxide (V2O5) that had been shown previously to have a high frequency of K-ras mutations and LOH on chromosome 6 (7). Occupational exposure to vanadium compounds occurs in the petrochemical industry, causing asthma and bronchitis (10,11). Previous studies indicate that the Ras compounds may be a mouse lung tumor suppressor gene. In lung neoplasms from sensitive mice such as the A/J strain that contain ‘susceptible’ K-ras alleles, the mutant K-ras has a higher expression than the wild-type allele and may contribute substantially to increased MAPK activity and tumor formation (8). However, for the B6C3F1 mouse (C57BL/5×C3H) that contains two ‘resistant’ K-ras alleles, loss of the wild-type allele may be necessary for mutant K-ras to drive MAPK activation and mouse lung carcinogenesis. In this study we investigated whether B6C3F1 mouse lung carcinomas with K-ras mutations and loss of heterozygosity (LOH) in the region of K-ras have higher MAPK activity than those tumors without mutation and LOH.

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Materials and methods

Lung neoplasms

Male and female B6C3F1 mice were exposed to 0, 1, 2 or 4 mg/m3 V2O5 by inhalation in a two year carcinogenicity study by the National Toxicology

Abstracts: LOH, loss of heterozygosity; MAPK, mitogen-activated protein kinase; SOS, sodium dodecyl sulfate; SSCP, single-strand conformation polymorphism.
Program females (13). The incidence of lung neoplasms increased from 22/50 in unexposed males to 42/50 in exposed males and from 1/50 in control females to 34/50 in exposed. At necropsy, lung neoplasms >5 mm were promptly frozen in liquid nitrogen and representative sections were placed in 10% formalin for histopathological evaluation. Our present study was possible because of the large number of frozen tumors collected, and 18 were evaluated.

We reported previously that 29 of 40 lung adenocarcinomas from B6C3F1 mice exposed to V2O5 had K-ras mutations and 18 of those had LOH in the region of K-ras on chromosome 6 (7). Sixteen of the 18 tumors with LOH also had K-ras mutations, indicating a good concordance between LOH and mutation. In the present study we identified one additional carcinoma from that tumor set with both a K-ras mutation and chromosome 6 LOH (data not shown).

**Western blot analysis of total MAPK protein and phosphorylated MAPK**

To isolate cellular protein, 50–100 mg frozen lung tumor and normal lung tissues were dissolved in radio-immunoprecipitation buffer (50 mM Tris–Cl, 1% Triton X-100 and 150 mM NaCl, 0.25% Na deoxycholate, 1 mM NaF, 1 mM Na3VO4, 4 µg/µl phenylmethylsulfonyl fluoride, 20 µg/µl aprotinin and 20 µg/µl of leupeptin and PIC inhibitors). Fifty micrograms of protein were denatured by boiling in Laemmli sample buffer (50 mM Tris–Cl pH 7.5, 100 mM dithiothreitol, 2% sodium dodecyl sulfate (SDS), 0.1% bromophenol blue and 10% glycerol) then separated by SDS-polyacrylamide gel electrophoresis. Afterwards, the gel was transferred to a nitrocellulose membrane and blocked overnight at 4°C in 5% BSA in Tris-buffered saline-Tween buffer (20 mM Tris, 500 mM NaCl and 0.01% Tween 20). The membrane was incubated for 2 h at room temperature with a 1:5000 dilution of a polyclonal goat anti-rabbit IgG. The immunoblot was visualized by chemiluminescence (Amersham, Arlington Heights, IL). An identical membrane was stored under the same conditions and times using a 1:20 000 dilution of HRP-conjugated anti-goat IgG. The NIH Image program was used to measure densitometry of the phospho-MAPK bands on the western blot.

**Isolation of DNA**

Microdissected phospho-MAPK positive areas from five serial 10 µm sections of two lung carcinomas were dissolved in 50 µl lysis buffer (for 1 ml: 950 µl buffer (0.5% Tween 20, 1 mM EDTA, pH 8.0, 50 mM Tris, pH 8.5) + 50 µl proteinase k (10 mg/ml stock)) at 55°C overnight. The samples were diluted to 100 µl with dh2O, boiled for 10 min, centrifuged for 10 min and stored at −20°C not longer than two days prior to use for PCR.

**LOH analysis**

The V2O5-induced lung carcinomas contained varying amounts of treatment-associated inflammation and contaminating normal fibroblasts, lymphocytes and macrophages that interfered with LOH analysis. For this reason most of the samples did not exhibit complete LOH, and allelic imbalance was scored by phosphorimage analysis comparing the difference in density of the C57BL/6 allele band to the C3H allele band of the D6MCO12 marker in the tumors to those from normal lung DNA. For the D6MCO12 marker, single-strand conformation polymorphism (SSCP) analysis was utilized that distinguished a single nucleotide polymorphism in the amplified PCR product between...
the C3H and C57BL/6 alleles. This was the only LOH marker that worked well for paraffin embedded tissues, including the microdissected tumors.

A semi-nested PCR technique was used to amplify this marker with outer primers D6MC012-1F and D6MC012-1R [2] for 25 cycles using 2 μl of the DNA prepared from the microdissected samples in a 20 μl reaction, and primers D6MC012-1F and D5MC012-2R (5'-CCGGGTTAGTTCTTCATGATT-3') for an inner reaction of 27 cycles. One microliter of the outer reaction was added to an 11 μl reaction that contained [32P]dATP (Amersham Pharmacia Biotech, Piscataway, NJ). Unlabeled dATP was diluted 1:100 in the inner PCR reaction mix. Upon completion of the amplification, the PCR products were diluted 1:8 in sequencing stop solution, the samples denatured and 5 μl loaded onto an MDE gel. The gel was electrophoresed at 3 W at room temperature for ~18 h to separate the alleles. The gels were dried and exposed to X-ray film overnight.

Statistics

The Cochran Armitage Trend test was used to see if frequency of K-ras mutations and chromosome 6 LOH correlated with the size of the neoplasms.

Results

In order to assess the effect of K-ras mutation and chromosome 6 LOH on MAPK activity in the B6C3F1 mouse lung tumors, we performed western blot analysis using antibodies directed against total MAPK and phospho-MAPK on 17 V2O5-induced alveolar bronchiolar carcinomas and one spontaneous carcinoma that had all been evaluated previously for K-ras mutations and LOH (7). MAPK activity is regulated by phosphorylation of the pathway members, and MAPK activity is directly correlated with the signal detected on the anti-phospho-MAPK immunoblot. Total MAPK expression did not change between normal lung and the lung carcinomas examined (Figure 1). MAPK activity was strongly elevated in five of six tumors evaluated that had both a K-ras mutation and LOH (Table I and Figure 1) and was undetectable or weak in all seven tumors examined in which no K-ras mutation was detected, including the two samples with LOH but no K-ras mutation. In four of five carcinomas with K-ras mutations but no detectable LOH, phospho-MAPK was highly expressed. The average relative density value of phospho-MAPK for the K-ras mutation+/LOH+ (+ = positive, – = negative) tumors was 165.0 + 87.6 and for the K-ras mutation+/LOH– tumors, 130.3 + 68.3 (Figure 1), suggesting that there was little or no difference between these groups. However, staining of the carcinomas with H&E revealed varying presence of infiltrating lymphocytes, macrophages and fibroblasts, all of which do not exhibit the molecular alterations that are characteristic of the tumors, and therefore interfere with the detection of LOH in the tumors (data not shown).

To evaluate the extent and intracellular localization of MAPK activity, a subset of the lung carcinomas was examined by immunohistochemical staining with anti-phospho-MAPK (Table I and Figure 2). Four tumors without detectable K-ras mutation or chromosome 6 LOH showed <5% of the cells staining positive for phospho-MAPK, and the two tumors with LOH but no K-ras mutation also did not stain positive. Non-tumor tissue of all samples was likewise negative. In contrast, five of six carcinomas with both K-ras mutation and LOH exhibited positive staining of phospho-MAPK in nuclei and cytoplasm in multifocal areas of carcinomas cells; ~30–50% of the cells of these tumors stained positive. Similarly, three lung carcinomas with K-ras mutations and no LOH that also demonstrated strongly elevated MAPK activation by western analysis showed multifocal areas of positive staining for phospho-MAPK in both nuclei and cytoplasm. Phospho-MAPK staining in the three K-ras+/LOH– carcinomas, by western analysis, was not significantly different from that in the K-ras+/LOH+ tumors. In general, the immunohistochemical staining of phospho-MAPK reflected similar results with western analysis, although there were multifocal areas that lacked positive staining in carcinomas with K-ras mutations regardless of LOH status (Figure 2). Additionally, the histological pattern of the carcinomas whether papillary, solid or mixed, did not appear to correlate with the phospho-MAPK staining.

We then microdissected two of three K-ras mutation+/LOH– carcinomas, which exhibited strong map kinase activation by western analysis (Figure 1, lanes 7, 9 and 10) and immunohistochemistry, for evaluation of LOH in regions of high MAPK activity. The third sample showed areas of fibrosis throughout the tumor and was not analyzed. Microdissection approaches

### Table I. Correlation of MAPK activity (phospho-MAPK expression) to K-ras mutation and chromosome 6 LOH status in lung carcinomas from B6C3F1 mice exposed to vanadium pentoxide

<table>
<thead>
<tr>
<th>Sample</th>
<th>K-ras/LOH status</th>
<th>Immunohistochemistry for phospho-MAPK</th>
<th>Lane-Fig. 1</th>
<th>Western</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No K-ras/no LOH</td>
<td>Negative</td>
<td>17</td>
<td>+/-</td>
</tr>
<tr>
<td>2</td>
<td>No K-ras/no LOH</td>
<td>&lt;5% cells weak positive</td>
<td>19</td>
<td>+/-</td>
</tr>
<tr>
<td>3</td>
<td>No K-ras/no LOH</td>
<td>Negative</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>No K-ras/no LOH</td>
<td>Negative</td>
<td>-</td>
<td>Not done</td>
</tr>
<tr>
<td>5</td>
<td>No K-ras/no LOH</td>
<td>Not done</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>6b</td>
<td>No K-ras/no LOH</td>
<td>Not done</td>
<td>14</td>
<td>+/-</td>
</tr>
<tr>
<td>7</td>
<td>No K-ras+/LOH</td>
<td>Negative</td>
<td>5</td>
<td>+/-</td>
</tr>
<tr>
<td>8</td>
<td>No K-ras+/LOH</td>
<td>Negative—small areas at edge of tumor positive (&lt;1%)</td>
<td>8</td>
<td>+/-</td>
</tr>
<tr>
<td>9</td>
<td>K-ras/no LOH</td>
<td>30% scattered cells and multifocal areas strong positive</td>
<td>9</td>
<td>++++</td>
</tr>
<tr>
<td>10</td>
<td>K-ras/no LOH</td>
<td>50% strong positive in multifocal areas</td>
<td>10</td>
<td>++++</td>
</tr>
<tr>
<td>11</td>
<td>K-ras/no LOH</td>
<td>50% strong positive, some weak positive areas</td>
<td>7</td>
<td>++++</td>
</tr>
<tr>
<td>12</td>
<td>K-ras/no LOH</td>
<td>Small area with a few positive cells (&lt;5% positive)</td>
<td>2</td>
<td>+/-</td>
</tr>
<tr>
<td>13</td>
<td>K-ras/no LOH</td>
<td>Not done</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>K-ras+/LOH</td>
<td>50% positive cells</td>
<td>-</td>
<td>Not done</td>
</tr>
<tr>
<td>15</td>
<td>K-ras+/LOH</td>
<td>30–50% multifocal areas strong</td>
<td>18</td>
<td>++++</td>
</tr>
<tr>
<td>16</td>
<td>K-ras+/LOH</td>
<td>50% positive—many multifocal areas in middle of tumor</td>
<td>15</td>
<td>++++</td>
</tr>
<tr>
<td>17</td>
<td>K-ras+/LOH</td>
<td>30–50% scattered weak to moderate staining</td>
<td>16</td>
<td>++++</td>
</tr>
<tr>
<td>18</td>
<td>K-ras+/LOH</td>
<td>30–50% of cells with wk-mod staining</td>
<td>13</td>
<td>+/-</td>
</tr>
<tr>
<td>19</td>
<td>K-ras+/LOH</td>
<td>10% positive-wk-mod staining only around edge big tumor</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>20</td>
<td>K-ras+/LOH</td>
<td>Not done</td>
<td>3</td>
<td>++++</td>
</tr>
</tbody>
</table>

*Phospho-MAPK staining was localized to the nucleus and cytoplasm of carcinoma cells (see Fig. 2).

*Sample 6 was a spontaneous B6C3F1 lung carcinoma from this study.

Western analysis scoring taken from Fig. 1 band density image analysis (+/- = 0–50, + = 51–100, ++ = 101–150, +++ = 151+).
Fig. 2. Immunohistochemical staining for map kinase activity with anti-phospho-MAPK antibody. (A) Staining of K-ras mutation+/LOH+ V2O5-induced lung carcinoma with anti-phospho-MAPK (200×). Note very strong brown staining of phospho-MAPK in most nuclei. (B) Serial section of same carcinoma as in (A) with non-immune serum instead of primary antibody (200×). Note some background staining in luminal areas but not in carcinoma cells. (C) Area of K-ras+/LOH– lung carcinoma with strong staining for map kinase activity (200×) in nuclei and cytoplasm. This sample is represented in lane 10 in the western blot in Fig. 1. (D) Negative staining in another area of same lung carcinoma as (C) (200×). (E) Another lung carcinoma (K-ras+/LOH–) showing an area with strong nuclear staining but little cytoplasmic staining of phospho-MAPK (200×). Also note the areas of fibroblasts and focus of inflammatory cells that are negative for map kinase activity (arrows). (F) Lung carcinoma (K-ras+/LOH–) with no positive staining with anti-phospho-MAPK (200×). This sample is represented in lane 8 in Fig. 1.
Discussion

We have demonstrated for the first time the presence of high levels of MAPK activity in B6C3F1 mouse lung carcinomas, and this activity is associated with K-ras mutation and LOH in the K-ras region on distal chromosome 6. During lung carcinogenesis in the B6C3F1 mouse strain that contains two ‘resistant’ K-ras alleles, loss of wild-type K-ras is probably important for the activation of MAPK. Early studies implicated mutant Ras action as being dominant oncogene activation of the MAPK pathway. However, several recent studies point to a possible major role for loss of normal K-ras p21 protein in mouse lung tumorigenesis. Ramakrishna et al. (5) found a correlation between increased expression and activity of K-ras p21 and growth arrest in mouse pulmonary alveolar type II cells, the cell type most associated with adenocarcinoma formation (14). Other results from that lab demonstrated lower total and membrane-associated K-ras p21 protein but higher MAPK activation in chemically induced mouse lung tumors than in normal lung (6).

Those studies are consistent with data showing that wild-type K-ras can be a mouse lung tumor suppressor gene and wild-type K-ras inhibits MAPK activity (7). It appears that activation of MAPK plays a critical role in the development of some mouse lung carcinomas and that loss of normal K-ras may be as important or more important than K-ras mutation in driving this pathway.

While there is growing evidence that normal K-ras p21 may be growth inhibitory in mouse pulmonary type II cells (5), it is possible that loss of wild-type K-ras p21 function and/or expression occurs by different means in different cell types, tissues, strains or species. For lung carcinogenesis in the B6C3F1 mouse, this change in K-ras activity appears to be due to mutation and LOH. However, in almost all lung carcinomas examined with K-ras mutations from hybrid mice that contain one susceptible K-ras allele, including AB6F1 (A/J×C57BL/6), AC3F1 (A/J×C3H) or B6CF1 (C57BL/6×BALB/c) strains, the mutation was almost always identified in the susceptible allele (15,16). It was also observed in one study that the mutant A/J K-ras allele had higher expression than the wild-type normal C3H allele in lung carcinomas from the AC3F1 mice (8). For those F1 mice it is possible that the map kinase pathway can be activated sufficiently by mutant K-ras without loss of the wild-type allele. Indeed, we have not detected chromosome 6 LOH in lung tumors from any of those hybrid mice (T.R.Devreux, unpublished data). However, a contradictory report by Jones-Bolin et al. (17) did not find consistent differences in K-ras expression between normal cells or tumors from A/J compared with C3H mice. The complete understanding of the function and relative expression of wild-type and mutant K-ras in carcinogenesis are yet to be discerned. Expression differences between the wild-type and mutant K-ras genes and proteins may not directly relate to their roles in carcinogenesis. Shields et al. (4) in their review on understanding Ras function discussed the emerging evidence that the different Ras isoforms probably do not have identical functions as first assumed. Most earlier studies analyzed H-ras in functional studies as a paradigm for Ras function, but recent investigations are finding functional differences in Ras signaling by the different isoforms (18). Now that we know that wild-type and mutant K-ras p21 function differently, at least in mouse lung, we should direct our efforts to investigating the different isoforms and normal and mutant Ras p21 as distinct proteins.

While the mechanism by which wild-type K-ras inhibits mouse lung carcinogenesis is not yet clear, Zhang et al. (7) demonstrated that wild-type K-ras inhibited MAPK activation in mouse lung carcinoma cells. In addition, their in vitro experiments showed that transfer of a wild-type K-ras into cell lines carrying a mutant K-ras inhibited tumor cell growth and focus formation. For lung carcinogenesis in the B6C3F1 mouse, the wild-type K-ras suppressor gene appears to be dominant over the oncogenic mutant K-ras.

In previous studies we have attempted without success to detect mutations in another Ras pathway gene, Raf, in mouse lung neoplasms that lacked K-ras mutations. K-ras mutations were not detected in 25% of the V\textsubscript{O\textsubscript{2}}-induced neoplasms in this study or in 70–80% of spontaneous or methylene chloride-induced lung neoplasms, including high numbers of carcinomas, from B6C3F1 mice (9,19). These new results showing low levels of phospho-MAPK in the lung carcinomas without K-ras mutations provides evidence that activation of the Ras/MAPK pathway does not play a significant role in lung tumor progression in B6C3F1 mouse when K-ras is not mutated. Our
data suggest that B6C3F1 mouse lung tumor formation without K-ras mutation involves other mechanisms or pathways, and we are now engaged in studies to identify these pathways.

Previously, we demonstrated LOH near K-ras on chromosome 6 in lung tumors from the B6C3F1 mouse (7, 9). In our early study the incidence of LOH near K-ras was highly correlated with the presence of K-ras mutations, although the proportion of spontaneous or methylene chloride-induced lung tumors with K-ras mutations was low (9). More recently, we examined lung tumors from B6C3F1 mice exposed to chloroprene and V_2O_5, and these tumors had high frequencies of both K-ras mutations and LOH on chromosome 6 (7). In addition, it was clear in the sequencing of K-ras in DNA from several chloroprene lung tumors that one allele was mutated and the other allele was lost (20). This was not observed for the neoplasms from mice exposed to V_2O_5 presumably because of the presence of non-mutated infiltrating lymphocytes, macrophages and fibroblasts associated with the treatment. It now seems reasonable to believe that loss of heterozygosity on chromosome 6 involves loss of wild-type K-ras, and that this contributes to lung carcinogenesis. Also, it is probable that a higher than previously detected proportion of the V_2O_5-induced lung carcinomas with K-ras mutations also have chromosome 6 LOH as suggested by the microdissection experiment. The high degree of correlation between K-ras mutation and chromosome 6 LOH in the chloroprene lung neoplasms that have very little non-tumor cell contamination supports this conclusion (7, 20).

Another question that remains to be answered is what drives the specific LOH of chromosome 6 in the lung carcinomas. Because alterations in p53 can lead to genomic instability, we examined V_2O_5-induced lung carcinomas for changes in p53 expression as an indicator of mutation; however, we found no evidence of altered expression in 10 of the tumors (13). The chromosome 6 LOH associated with K-ras mutation does not appear to be due to generalized chromosomal instability as evidenced by only a low level of random LOH on other chromosomes detected in methylene chloride-induced lung neoplasms from B6C3F1 mice (9). Moreover, in that study only two of 10 of the methylene chloride-induced carcinomas with K-ras mutations were associated with LOH on chromosomes other than 4 and 6. Additionally, of the 19 V_2O_5-induced lung carcinomas that exhibited LOH on chromosome 6, 14 lost the paternal C3H allele, suggesting parental bias in allele loss. This was unexpected as both C57BL/6 and C3H strains that were bred to create the B6C3F1 hybrid contain identical ‘resistant’ K-ras alleles. While bias in allele loss of different chromosomal regions has been observed before in lung carcinomas (9), at present we do not have an explanation for the bias in allele loss or for the high incidence of chromosome 6 LOH in some lung carcinomas from B6C3F1 mice.

We were surprised initially to find high levels of MAPK activity in the lung carcinomas, thinking that it would be an early event following K-ras mutation (21, 22) and possibly not sustained during tumor progression. However, a recent report by Fisher et al. (23) indicates that continued production of mutant K-ras is necessary to maintain a malignant lung adenocarcinoma phenotype. This suggests that the Ras/MAPK pathway would be up-regulated throughout malignancy following K-ras activation.

K-ras mutations have been identified in 30–50% of human lung adenocarcinomas (24), and activation of the Ras/MAPK pathway is thought to play an important role in the development of these tumors. LOH on chromosome 12p, the region containing human K-ras, has also been observed in a similar fraction of lung adenocarcinomas (25), although the connection between LOH and loss of the wild-type K-ras has not been reported. The findings in this study and our recent report indicating that wild-type K-ras is a mouse lung tumor suppressor gene (7) suggest that this merits more thorough exploration in human lung carcinogenesis.

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