Deletion of XPC leads to lung tumors in mice and is associated with early events in human lung carcinogenesis


*National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; and ‡University of Texas Southwestern Medical Center, Dallas, TX 75390

Edited by Richard B. Setlow, Brookhaven National Laboratory, Upton, NY, and approved August 4, 2005 (received for review April 22, 2005)

Chromosome 3p and 1p deletions are among the most frequent genetic changes in human lung cancer and although candidate tumor suppressor genes have been identified in these regions, no causative correlations have been drawn between deletion or mutation of these and lung carcinogenesis. We identify XPC and Gadd45a as genes within each of these regions involved in lung tumor initiation and progression, respectively. One hundred percent of XPC–/– mice develop multiple spontaneous lung tumors with a minority progressing to non-small cell lung adenocarcinoma, occasionally with metastasis to adjacent lymph nodes. Deletion of Gadd45a alone does not lead to increased lung tumors in mice, but coupled with an XPC deletion, it results in lung tumor progression. Analysis of published data indicated allelic loss of XPC in most human lung tumors and allelic loss of Gadd45a in some human lung and other cancer types. Because DNA repair capacity is compromised in XPC+–/– cells, it is possible that the loss of a single XPC allele in the human lung might confer a mutator phenotype. Coupled with cigarette carcinogens, decreased DNA repair would lead to additional mutations in genes such as p53 that are frequent targets in lung cancer.

Gadd45a | allelic loss | non-small cell lung cancer | metastasis

Lung cancer is the leading cause of cancer deaths for both men and women in the United States with >160,000 estimated U.S. deaths in 2005. The average age at diagnosis is 60, and environmental/lifestyle factors such as cigarette smoke are a major etiological factor. Specific cyogenetic alterations have been associated with lung tumors, whereas more recent investigations have focused on mapping minimal deletions by using genetic markers with known locations. Potential critical target genes in these deleted regions have been identified, but mouse models for these deletions have not shown high incidence of lung cancer thus far.

Several regions of human chromosome 3p exhibit allelic deletion in both small cell (SCLC) and non-small cell (NSCLC) lung cancers (reviewed in ref. 1). Genes on human chromosome 3p that show allelic loss in lung cancer have been identified as potential lung tumor suppressors, and mouse models have been generated for some. For example, fragile histidine triad (FHIT) abrogation occurs in many human tumors through gene deletion or promoter methylation (reviewed in ref. 2), but homozygous and heterozygous deletion of FHIT leads to increased tumorigenesis in a variety of tissues, not including the lung (3). RASSF1A is inactivated by methylation in most lung cancers (4), but homozygous deletion of this gene leads to late onset lung adenomas in only 12% of mice (5). DUTT1/ROBO1 is inactivated by methylation in some tumors (6), and 22% of DUTT1/ROBO1 heterozygous mice developed late onset lymphomas and carcinomas, which were predominantly invasive lung adenocarcinomas (7). Nearly half of Ogg1-deficient mice develop lung tumors (8) but, although allelic loss is associated with human lung cancer (9), few human tumors show mutation of the remaining OGG1 allele (10). It is possible that there are multiple tumor suppressors on chromosome 3p and that combined allelic deletion of multiple genes in the absence of mutation of the remaining allele may act as an initiating event in lung carcinogenesis.

Other mouse models of lung cancer have focused on mapping naturally occurring loci that vary between strains and confer sensitivity or resistance to lung tumors. In the case of the mouse pulmonary adenoma susceptibility (Pas1) locus, K-ras is the most likely candidate, and K-ras mutations are present in a subset of lung tumors from both mice and humans. Mice carrying an activated K-ras gene targeted to the lung develop multiple lung tumors, suggesting that K-ras mutation may be an early event in lung carcinogenesis (11). However, the majority of human lung cancers do not have K-ras mutations, indicating the existence of alternate mechanisms for lung carcinogenesis. Many human lung tumors carry p53 mutations, but mice lacking p53 die early from predominantly sarcoma or lymphoma, although targeted disruption of p53, together with the loss of both Rb1 alleles in the lung, led to SCLC-like tumors in mice (12). In human lung tumors, p53 mutations are more frequent in tumor types associated with smoking, including SCLC, and the mutation spectrum suggests that p53 mutations result from cigarette smoke carcinogens (reviewed in ref. 13).

Mutations in DNA repair genes result in tumor susceptibility in both humans and mice. In addition, specific polymorphisms in genes involved in nucleotide excision repair have recently been suggested to confer risk for specific tumor types (reviewed in ref. 14). In particular, three recent articles describe the association of XPC polymorphisms with increased lung cancer risk (15–17). Although people with germ-line XPC mutations develop primarily sun-induced skin tumors, the presence of other tumor types in this population has not been reported (reviewed in ref. 18).

Both 3p and 1p deletions are common in human lung tumors. Here we identify XPC and Gadd45a as potential critical genes in each of these regions that are involved in lung tumorigenesis and lung tumor progression, respectively, in mice. XPC is localized to human 3p25, and it was found that 100% of mice with deleted XPC develop spontaneous lung tumors, the majority of which are adenomas. Gadd45a is localized to human 1p31.1–31.2, and deletion in mice that also lack XPC leads to progression of lung adenomas to non-small cell carcinomas. Because allelic or total loss of XPC and Gadd45a are associated with lung tumors in humans and mice, respectively, these two genes should be considered critical targets in lung cancer initiation and progression, respectively.
A cohort of 10 male and 11 female +/+ mice were analyzed. All mice were in a strain background of 75% C57BL/6 and 25% 129. At 16–17 mo, 18 male and 11 female XPC−/−, 18 male and 11 female Gadd45a−/−, 18 male and 11 female XPC−/−Gadd45a−/− mice were monitored for up to 24 mo at which time all remaining mice were euthanized. During this period, animals exhibiting obvious tumors or who were moribund, cachectic, or nonresponsive were also euthanized. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

Methods

XPC−/− and Gadd45a−/− mice have been described in refs. 19 and 20 and were maintained in a mixed C57BL/6 (75%) and 129 (25%) background. Mice were housed in Plexiglas cages and given autoclaved National Institutes of Health (NIH) 31 diet and water ad libitum. NIH is an Association for Assessment of Laboratory Animal Care International-accredited animal facility, and all experiments were done under an approved NIH animal study protocol.

A cohort of 10 male and 11 female +/+ mice, 13 male and 14 female Gadd45a−/−, 18 male and 11 female XPC−/−, and 19 male and 14 female XPC−/−Gadd45a−/− mice were monitored for up to 24 mo at which time all remaining mice were euthanized. During this period, animals exhibiting obvious tumors or who were moribund, cachectic, or nonresponsive were also euthanized. Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin.

Scheduled necropsies were also performed at various ages, and the lungs were inflated with 10% neutral buffered formalin. At 16–17 mo, 18 XPC−/− mice, 21 Gadd45a−/− mice, and 21 +/+ mice were analyzed. All mice were in a strain background of 75% C57BL/6J and 25% 129. Gadd45a−/− and +/+ mice were littermates and were unrelated to the XPC−/− mice. For surface lung tumor counting, lungs were inflated and fixed in 10% neutral-buffered formalin. Lung lobes were separated by using a razor blade, and each lobe was examined on all surfaces. Tumor diameter was measured at low power by using a dissecting microscope fitted with a micrometer and calibrated for the same magnification. Most tumors were spherical so tumor volume was determined from the radius. For nonspherical tumors, length and width were measured, and depth was measured from cuts into the tumor with a razor blade. Data were analyzed with PRISM software (GraphPad, San Diego) by using a log-rank test for survival and two-tailed t test for tumor incidence.

DNA for genotyping (for XPC and Gadd45a status) was obtained from 2-mm tail clips that were digested overnight at 45°C with 100 μl of 100 mM Tris, pH 8.0/5 mM EDTA/0.2% SDS/200 mM NaCl/100 μg/ml proteinase K. Samples were then diluted with 300 μl of water and boiled for 5 min before use for PCR. To prepare DNA from paraffin tissue blocks, tumor location was determined from hematoxylin and eosin-stained slides of adjacent tissue. Tumor morphology in paraffin blocks was visually distinct from surrounding normal lung tissue so that tumor tissue could be excised from the block and digested as above. Samples were filtered to remove paraffin after proteinase K digestion. K-ras codon 12 and 61 mutations were determined by sequencing K-ras PCR products. PCR primers for genotyping and marker analysis are listed in Table 1.

For analysis of published human lung tumor data, locations of mapped loci, XPC, and Gadd45a were determined by using the MapViewer web page of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/mapview). Allelic loss of XPC or Gadd45a was considered possible if adjacent markers on either or both sides showed allelic loss.

Results

Both XPC and Gadd45a proteins are involved in DNA repair, and Gadd45a−/− mouse embryonic fibroblasts (mef) have a similar global genomic repair defect as XPC−/− mef (21). Mice lacking both Gadd45a and XPC were generated to investigate cooperative effects of these two proteins on DNA repair and spontaneous carcinogenesis. It became apparent that both XPC−/− and XPC−/−Gadd45a−/− mice were developing lung tumors when visible lesions were found on the lungs of all mice that became moribund during the study. One hundred percent of mice from both groups developed lung tumors, including mice with no obvious distress at 24 mo when all surviving mice were necropsied. Spontaneous lung tumorigenesis in older XPC−/− mice was independently found at the University of Texas Southwestern (data not shown) and at the NIH.

XPC−/− mice developed lung tumors histologically classified as adenomas, whereas few had an adenocarcinoma coincident with adenoma(s). In contrast, the majority of XPC−/−Gadd45a−/− mice developed lung adenocarcinoma, also coincident with adenoma(s) (Fig. 1). In addition, 2 of 26 XPC−/−Gadd45a−/− mice had two or more adenocarcinomas each (data not shown). In a serial killing of XPC−/−, Gadd45a−/− and +/+ mice at 16–17 mo of age, 100% of XPC−/− mice had one or more tumors visible on the lung surface, whereas only 1 of 21 +/+ and 2 of 21 Gadd45a−/− died. Because the +/+ cohort was in the same strain background yet unrelated to XPC−/− mice, several markers for lung tumor susceptibility that varied between the two parent strains, C57BL/6J and 129, were investigated. There was no correlation of Pas1, Par1, Par2, Par3, or Par4 alleles with lung tumors in the 16–17 mo cohorts of +/+, Gadd45a−/−, and XPC−/− mice (data not shown and ref. 22).

Table 1. Primers used for genotyping and marker analysis

<table>
<thead>
<tr>
<th>Allele</th>
<th>Size, bp</th>
<th>3′ primer</th>
<th>Sequence</th>
<th>5′ primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>XPC wt</td>
<td>218</td>
<td>CH360</td>
<td>TATCTCTCCCTCCACCCCTCTCC</td>
<td>CH361</td>
<td>ATTCGCCATACCTTTGAC</td>
</tr>
<tr>
<td>XPC del</td>
<td>350</td>
<td>CH218</td>
<td>GACAGCAGATCCCTCTCTCTAT</td>
<td>CH360</td>
<td>TATCTCTCCCTCCACCCCTCTCC</td>
</tr>
<tr>
<td>K-ras2 exon 1</td>
<td>217</td>
<td>CH466</td>
<td>TGATATCATCTACGACATAATCTCTTCT</td>
<td>CH467</td>
<td>GTCCCTTTGACCGCCAGCGCA</td>
</tr>
<tr>
<td>K-ras2 exon 2</td>
<td>217</td>
<td>CH468</td>
<td>GTTCTTCCCTCTCCACAGACT</td>
<td>CH469</td>
<td>ATTCGCCATACCTTTGAC</td>
</tr>
</tbody>
</table>

Fig. 1. XPC−/− mice develop lung tumors and additional deletion of Gadd45a results in increased malignancy of lung tumors. (A) 26 XPC−/− and 26 XPC−/−Gadd45a−/− mice were allowed to live out their normal lifespan for up to 24 mo. Lungs sections were analyzed for tumor diagnosis. XPC−/− vs. XPC−/−Gadd45a−/−, P < 0.0001. (B) 18 XPC−/−, 21 Gadd45a−/−, and 21 +/+ mice were necropsied at 16–17 mo. Lung surfaces were examined for tumors. No histological analysis of tumors was done for these mice.
Lung tumors in XPC−/− and XPC−/−Gadd45a−/− mice were sometimes quite large and led to a moribund state characterized by labored breathing. A full range of lesions was observed in lungs from both genotypes ranging from mild atypia and hyperplasia to malignant adenocarcinoma with metastasis to adjacent lymph nodes (Fig. 2). Most lung tumors present in XPC−/− mice were classified as adenomas with a smaller percentage of adenocarcinomas, all were NSCLCs, whereas none of the mouse tumors had SCLC-like histology. Characteristically, all adenocarcinomas revealed heterogeneous growth patterns, but only one of them was poorly differentiated. In addition, many mice also exhibited areas of atypical adenomatous hyperplasia in the lung (Fig. 2C), which is a putative precursor lesion for lung adenocarcinoma in humans (23), and XPC−/−Gadd45a−/− mice demonstrated enhanced airway cell atypia (Fig. 2B). Notably, most of the XPC−/−Gadd45a−/− mice also demonstrated increased cellularity and moderate atypia throughout the airway epithelium.

Lung tumors did not lead to a decreased lifespan in either XPC−/− or XPC−/−Gadd45a−/− compared with +/+ mice (Fig. 3). In fact, XPC−/−Gadd45a−/− mice lived longer than Gadd45a−/− mice. This result is due to a significant (P = 0.0074) increase in lifespan of XPC−/−Gadd45a−/− females compared with Gadd45a−/− females, whereas lifespan for males did not differ between these groups (P = 0.9814). However, at 24 mo, 43% of +/+ mice were alive, whereas only 35% of XPC−/− and 28% of XPC−/−Gadd45a−/− mice were. In longer-term studies, it is possible that deletion of XPC might lead to a decrease in the maximum lifespan of mice.

In a separate cohort, lung surfaces from XPC−/− mice were examined at various ages to allow a more quantitative analysis of lung tumorigenesis. Lung tumors were observed in mice as young as 6 mo. At 16–17 mo, 18 of 18 XPC−/− mice had lung tumors, averaging nearly six tumors per mouse. This finding is compared with 1 of 21 +/+ and 2 of 21 Gadd45a−/− mice at the same age with lung tumors (one tumor each) (Fig. 4). Although tumors from this group of XPC−/− mice were not histologically typed, the appearance of tumors on the surface of the lung made them easily distinguishable from adjacent normal lung tissue. After fixation, normal lung tissue appeared pink and smooth, whereas tumors were observed as raised, white nodules.

In a serial killing of XPC−/− mice at various ages, 26% (5 of 19) mice had tumors visible on the surface of the lungs at 6 mo (Fig. 4A). At 12 mo, eight of eight mice had lung tumors. The number of visible lung tumors per mouse increased significantly between 6 and 12 mo (P = 0.0005) from an average of 0.4 to 3.8 per mouse (Fig. 4B). The increase in tumors per mouse from 12 to 16–17 mo was not significant (P = 0.10), perhaps due to the small sample size (n = 8) at 12 mo. Although there appeared to be a progressive increase in tumor diameter with age, these differences were not significant (P > 0.2 for all pairs) (Fig. 4C). The total surface lung tumor load for XPC−/− mice increased with age and averaged 4.8 mm3 for 16- to 17-mo-old mice compared with 1.6 mm3 for 12-mo-old mice (P = 0.048) (data not shown). This significant increase in tumor load likely results from increases in both number and size of lung tumors during this period.

K-ras mutations are common in both human and mouse lung tumors but are absent in normal lung tissue (24). Deletion of DNA repair genes Msh and Ogg1 in mice leads to a 75% mutation rate of K-ras in lung tumors (25). Because XPC is involved in DNA repair, it was thought that increased K-ras mutation might provide a mechanism for lung tumorigenesis in XPC−/− mice, whereas XPC−/−Gadd45a−/− mice were analyzed for the common K-ras codon 12 and 61 mutations. Only three of eight of these lung tumors had codon 12 mutations, whereas none had codon 61 mutations (data not shown). Therefore, lung tumorigenesis in XPC−/− mice does not result from decreased DNA repair leading to increased K-ras mutation.

In human lung tumors, the loss of portions of chromosomes 3p and 1p are among the most frequent cytogenetic alterations. XPC is located at human 3p25 and, therefore, possibly exists within chromosomal regions frequently deleted in lung cancer. Two studies of human lung tumors used genetic markers on

---

Fig. 2. Lungs from XPC−/− mice show a range of lesions covering the range from early atypia and hyperplasia to metastatic lung adenocarcinoma. (A) Normal lung. (B) Atypia. (C) Atypical adenomatous hyperplasia. (D) Adenoma. (E) Carcinoma in situ. (F and G) Adenocarcinoma. (H) Metastases to adjacent lymph nodes.

Fig. 3. Lung tumors in XPC−/− and XPC−/−Gadd45a−/− do not lead to decreased lifespan compared with +/+ mice.
chromosome 3p, which coincidently flank XPC, to investigate allelic loss at this locus. Allelic loss for individual markers in relation to XPC and Gadd45a are highlighted in Table 2. In one study, 75% of primary NSCLC showed potential XPC loss due to allelic loss at one or two of the closely flanking loci (D3S1263 and D3S2338) (26). The other study found allelic loss at one or two flanking loci (D3S1263 and D3S2338) in 100% of SCLC and 73% of NSCLC cell lines. This same study found deletion of 1p31.1 in the vicinity of Gadd45a in 59% of NSCLC but not in SCLC (between D1S230 and D1S2841) (27). Gadd45a is also <100 kbp from the D1S2829 marker deleted in most ovarian tumors (28) and some stomach, colorectal, endometrial, testis, renal, thyroid, sarcoma, and breast tumors as well (29). Therefore, although XPC allelic loss may occur in the overwhelming majority of human lung cancer, Gadd45a allelic loss is associated with a greater variety of cancer types.

Discussion
Both XPC and Gadd45a gene products are involved in nucleotide excision repair, which repairs bulky DNA lesions. XPC in particular is important for repair of UV radiation-induced DNA damage, and xeroderma pigmentosum patients with inactivating XPC mutations have a high incidence of sun-induced skin cancers that often result in early mortality (reviewed in ref. 30). Mice lacking XPC are also prone to UV radiation-induced skin tumors (31). Although spontaneous tumors have not been reported in any tissue, drug treatment can lead to lung and liver tumors in XPC−/− mice (32). Increased incidence of tumors, other than skin, has not been reported in the limited number of human patients with XPC mutations. However, the late onset of adverse health effects due to lung tumors in XPC−/− mice suggests that lung tumors would be apparent only in older XPC patients.

Lung tumors were observed in XPC−/− mice as early as 6 mo (Fig. 4). However, in a cohort of 29 XPC−/− mice, there were no deaths before 14 mo (Fig. 3), and a cohort of XPC−/− mice serially killed at 6 and 12 mo had no apparent adverse health effects due to lung tumors. Deletion of XPC does not shorten lifespan relative to +/+ mice, perhaps due to late onset, slow growth, and/or slow progression of tumors. In support of this interpretation, most tumors in XPC−/− mice were benign adenomas with few progressing to malignant carcinomas (Fig. 1). In addition, there was only a small, non-statistically significant increase in tumor size with age (Fig. 4C), suggesting that additional events are required for tumor progression. Strain A mice, which have been extensively used as a model for pulmonary adenoma susceptibility, showed an intermediate lifespan when compared with other inbred strains of mice (33).

Table 2. Allelic loss of XPC occurs in the majority of human SCLC and NSCLC tumors, whereas allelic loss of Gadd45a occurs in various tumor types

<table>
<thead>
<tr>
<th>Marker/gene</th>
<th>Location/distance</th>
<th>Allelic loss</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3S1263</td>
<td>3/11,492</td>
<td>100% in 14 SCLC, 41% in 22 NSCLC</td>
<td>16</td>
</tr>
<tr>
<td>D3S1263</td>
<td>3/11,492</td>
<td>54% of 79 NSCLC</td>
<td>15</td>
</tr>
<tr>
<td>XPC</td>
<td>3/14,178</td>
<td>89% in 14 SCLC, 58% in 22 NSCLC</td>
<td>16</td>
</tr>
<tr>
<td>D3S2338</td>
<td>3/16,824</td>
<td>53% in 79 NSCLC</td>
<td>15</td>
</tr>
<tr>
<td>D3S2338</td>
<td>3/16,824</td>
<td>100% in 14 SCLC, 73% in 22 NSCLC</td>
<td>16</td>
</tr>
<tr>
<td>D3S1263 or D3S2338*</td>
<td>3/16,824</td>
<td>89% in 14 SCLC, 41% in 22 NSCLC</td>
<td>16</td>
</tr>
<tr>
<td>D3S1263 and D3S2338*</td>
<td>3/16,824</td>
<td>75% of 79 NSCLC</td>
<td>15</td>
</tr>
<tr>
<td>D3S1263 or D3S2338*</td>
<td>3/16,824</td>
<td>35% of 79 NSCLC</td>
<td>15</td>
</tr>
<tr>
<td>D1S209</td>
<td>1/61,342</td>
<td>16% of 471 tumors (various tissues)</td>
<td>18</td>
</tr>
<tr>
<td>D1S230</td>
<td>1/61,972</td>
<td>0% in 14 SCLC, 59% in 22 NSCLC</td>
<td>16</td>
</tr>
<tr>
<td>Gadd45a</td>
<td>1/67,522</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1S2829</td>
<td>1/67,622</td>
<td>42% in 38 ovarian and breast tumors</td>
<td>17</td>
</tr>
<tr>
<td>D1S216</td>
<td>1/77,078</td>
<td>17% in 342 tumors (various tissues)</td>
<td>18</td>
</tr>
<tr>
<td>D1S2841</td>
<td>1/78,910</td>
<td>0% in 14 SCLC, 43% in 22 NSCLC</td>
<td>16</td>
</tr>
</tbody>
</table>

Published data were extended to include XPC and Gadd45a genes. Location is designated as chromosome no./distance from telomere (in kbp). Distance from telomere was obtained from www.ncbi.nlm.nih.gov/mapview or http://genome.ucsc.edu.

*Potential XPC allelic loss in two independent studies. Allelic loss of either flanking marker indicating potential XPC loss, or of both flanking makers indicating near certain XPC loss.
Although mouse lung tumors often do not fit the classifications for human lung tumors (34), mouse models have been used extensively as models for lung carcinogenesis (35, 36). Most spontaneously and chemically induced lung tumors in mice are adenomas, rarely adenocarcinomas, and they originate mainly from alveolar type II cells (37), whereas most human lung cancers are derived from airway epithelium and can be divided into several histological categories (38). Proof that deletion of XPC and Gadd45a genes leads to increased lung tumorigenesis in mice comes from multiple observations: (i) 100% of mice have lung tumors, which is highly unusual even in older mice in a relatively resistant background, (ii) multiplicity of tumors increases over time, and (iii) microscopic analysis revealed a field effect with possible precursor lesions and histologic progression from adenomas to adenocarcinomas (Fig. 2). In this respect, XPC−/− and XPC−/−Gadd45a−/− mice may be an attractive model for human NSCLC because of a common metastatic phenotype.

One caveat to lung tumorigenesis in XPC−/− mice is the role of known pulmonary adenoma and resistance alleles in lung tumor susceptibility, which varies widely between different inbred strains of mice. However, several known lung tumor susceptibility alleles from each of the strains contributing to the mixed genetic background of the mice in this study did not correlate with lung tumors in +/+, Gadd45a−/−, or XPC−/−/− mice (data not shown). A previous study reported that 24% of B6;129 mice develop lung tumors by 24 mo with 3% developing lung carcinomas (39). This result is far less than observed for XPC−/−/− mice in the same strain background and is not inconsistent with our observation of 5% lung tumors in +/+ mice at 16–17 mo. In addition, the widely used strain A mice, which are highly sensitive to spontaneous lung tumors, were reported to develop ~6-fold fewer spontaneous lung tumors than XPC−/−/− mice at 16 mo (40). This finding highlights that XPC is a previously uncharacterized lung tumor suppressor gene in mice.

Chromosome 3p deletions are frequent in human lung cancer (reviewed in ref. 1). Numerous studies have delineated specific regions deleted by using markers whose localization can be pinpointed now that the human genome sequence is available. We have extended published analyses to show that XPC is a target for allelic loss in the majority of human lung cancers, both NSCLC and SCLC (Table 2). Losses of 3p are seen in early events in lung tumorigenesis such as lung hyperplasia, dysplasia, and noninvasive tumors (41), suggesting that XPC allelic loss might also be an early event.

Given the prevalence of lung tumors in XPC−/−/− mice, it is possible that a single copy of XPC would not be sufficient to prevent lung tumorigenesis either. In support of this concept, XPC+/− mice are more susceptible to UV-induced skin tumors but with a longer latency than in XPC−/−/− mice, and XPC+/−/− cells have increased mutation frequency (42, 43). If XPC+/−/− mice develop spontaneous lung tumors, this result would support XPC haploinsufficiency or XPC allelic loss in mouse and human lung carcinogenesis, respectively.

Gadd45a−/−/− mice do not develop spontaneous tumors (19), and there are no known human conditions, as for XPC, with germ-line mutations in Gadd45a. However, increased lung tumor malignancy in mice lacking both XPC and Gadd45a supports a role for Gadd45a deletion in tumor progression. This finding is consistent with previous studies wherein dimethylbenzanthracene-induced internal tumors and UV-induced skin tumors in Gadd45a−/−/− mice were more likely to be higher grade, malignant and/or metastatic than those in +/+ mice (44, 45). Allelic loss of Gadd45a (on chromosome 1p) is less prevalent in human lung tumors than XPC allelic loss but may exist in a broader range of tumor types (Table 2). Allelic loss at this locus in breast and colorectal tumors is associated with poor prognosis for survival (29), which is supportive of a role for Gadd45a loss in tumor progression.

The XPC protein is a critical component of a multiprotein complex required for DNA damage recognition in global genomic repair, the subtype of nucleotide excision repair required for repair of nontranscribed DNA strands. XPC is critical for repair of UV-induced lesions because humans and mice lacking XPC develop sunlight/UV-induced skin tumors (18, 46). However, lymphocytes from 12-mo-old XPC−/−/− mice accumulated 30-fold more spontaneous Hprt lesions than +/+ mice (43). Coupled with the high frequency of spontaneous lung tumors in XPC−/−/−, this increased mutation rate implicates XPC in the repair of endogenous oxidative lesions in the lung as well. The most frequent mutation in XPC−/−/− lymphocytes was a G to T transversion, which can result from 8-oxodeoxyguanine (8-oxodG) produced during endogenous oxidative processes (43). Whereas XPC is not known to specifically bind 8-oxodG, XPC-containing nucleotide excision repair complexes excised 8-oxoG lesions from DNA in vitro (47), further suggesting that XPC complexes can repair oxidative lesions that may be particularly important in lung carcinogenesis.

There are several known polymorphisms of the human XPC gene. A polymorphism in the splice acceptor site within intron 11 leads to increased skipping of exon 12, a message whose product has both reduced DNA repair capacity and can elicit a dominant negative effect on DNA repair capacity (48). An exon 9 poly AT polymorphism is associated with increased risk of lung cancer in a Spanish population (15) as well as squamous cell carcinoma of the head and neck (49). Two recent articles describe association of XPC polymorphisms with increased lung cancer risk (16, 17). The majority of human lung cancer may be attributable to smoking, likely resulting, at least in part, from tobacco carcinogen-induced DNA damage in the lung. These studies therefore suggest that alterations in XPC function may result in decreased repair of tobacco-induced DNA lesions that lead to cancer in the lung.

Allelic loss of XPC in human lung tumors, coupled with lung tumors in 100% of XPC−/−/− mice, suggests that XPC allelic loss may be an etiologic factor in lung tumorigenesis in humans. Because XPC−/−/− mice develop multiple benign lung tumors that progress infrequently to malignancy, deletion of XPC may be involved in the initiation of lung tumors with additional events required for progression. Based on mouse studies, loss of a single XPC allele is likely to result in decreased DNA repair capacity in humans as well, perhaps conferring a mutator phenotype. Coupled with carcinogen-exposure in particular, this mutator phenotype would allow further mutations required for carcinogenic progression. In this respect, XPC−/−/− mice are an attractive model for carcinogen-induced lung carcinogenesis. The late onset of tumors, low frequency of malignancy, and occasional metastasis leaves a margin to determine the influence of carcinogens on these parameters.

This research was supported by the Intramural Research Program of the NIH, National Cancer Institute.