Mouse Models for Xeroderma Pigmentosum Group A and Group C Show Divergent Cancer Phenotypes

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Abstract

The accumulation of DNA damage is a slow but hazardous phenomenon that may lead to cell death, accelerated aging, and cancer. One of the most versatile defense mechanisms against the accumulation of DNA damage is nucleotide excision repair, in which, among others, the Xeroderma pigmentosum group C (XPC) and group A (XPA) proteins are involved. To elucidate differences in the functions of these two proteins, comprehensive survival studies with Xpa−/−, Xpc−/−, and wild-type control female mice in a pure C57BL/6J background were done. The median survival of Xpc−/− mice showed a significant decrease, whereas the median survival of Xpa−/− mice did not. Strikingly, Xpa−/− and Xpc−/− mice also showed a phenotypical difference in terms of tumor spectrum. Xpc−/− mice displayed a significant increase in lung tumors and a trend toward increased liver tumors compared with Xpa-deficient or wild-type mice. Xpa−/− mice showed a significant elevation in liver tumors. Additionally, Xpc-deficient mice exhibited a strong increase in mutant frequency in lung compared with Xpa−/− mice, whereas in both models mutant frequency is increased in liver. Our in vitro data displayed an elevated sensitivity to oxygen in Xpc−/− in mouse embryonic fibroblasts (MEF) when compared with Xpa−/− and wild-type fibroblasts. We believe that XPC plays a role in the removal of oxidative DNA damage and that, therefore, Xpc−/− mice display a significant increase in lung tumors and a significant elevation in mutant frequency in lung, and Xpc-deficient MEFs show greater sensitivity to oxygen when compared with Xpa−/− and wild-type mice. [Cancer Res 2008;68(5):1347–53]

Introduction

Cancer remains one of the main causes of death nowadays in both men and women and is accompanied by a kaleidoscope of unsolved questions about the induction and progress of this disease. An important factor in the development of cancer is the accumulation of somatic DNA damage (1). Normally, several sophisticated defense mechanisms are active to repair the modified DNA to prevent mutations and damage accumulation. Base excision repair (BER), for example, will remove most small base modifications (e.g., oxidative DNA damage). Nucleotide excision repair (NER) has a very broad lesion spectrum and is responsible for the removal of bulky, DNA helix–distorting adducts (2–7).

The autosomal recessive disorder Xeroderma pigmentosum (XP) is an elucidative example of the influence of a DNA repair defect on cancer predisposition. XP patients exhibit extreme UV sensitivity and are predisposed to skin cancer by a 1,000-fold higher risk (8, 9). Until now, seven complementation groups (XPA through XPG) plus a variant form (XPV) were identified. XP disorders arise from a deficiency in one or more of these XP proteins, which belong to the NER pathway, with the notable exception of the XPV protein, which is involved in translesion synthesis of UV-damaged DNA (10).

NER can be subdivided into two subpathways: global genome NER (GG-NER), which covers the complete genome, and transcription-coupled NER (TC-NER), which focuses on repair of the transcribed strand of active genes (11, 12). XPC is associated with the GG-NER whereas XPA plays a role in both GG-NER and TC-NER. The XPC protein, in complex with the HR23B protein, is responsible for DNA damage recognition (6, 13). Following detection of distorted helix structures, the XPC/HR23B complex will initiate the GG-NER process (14). Subsequently, the XPC/HR23B complex will dissociate from the damaged DNA strand when the transcription factor TFIIH in combination with the XPA and RPA protein set the verification of the DNA damage in motion (15). XPC-HR23B is dispensable for TC-NER; the CSA and CSB proteins, together with RNA polymerase II stalled at a lesion, fulfill the role of recognition factor in this pathway. As in GG-NER, DNA damage verification in TC-NER requires the presence of the TFIIH-XPA-RPA complex (16).

When TC-NER components or the more common elements in NER (e.g., XPA) are affected, very complex clinical features are observed (17). Patients with a defect in genes unique to GG-NER (like XPC) exhibit fewer clinical symptoms besides cancer. In general, deficiencies in the TC-NER pathway are related to neurodegenerative disorders, whereas defects in the GG-NER pathway are designated as more cancer prone (18–21).

The XPC is the most common type of the XP disease in North America and Europe (22). This form is only defective in the GG-NER pathway. XPA patients are disrupted in both their GG-NER and TC-NER pathways. Mutant frequency analyses at the Hprt locus in mouse models of these two forms of XP previously uncovered striking differences. Hprt mutant frequencies in the spleen of Xpc−/− mice in a mixed genetic background were highly elevated in comparison not only with their wild-type controls but also with Xpa−/−, both in a pure C57BL/6J background (23). This indicates that knockout mouse models of Xpc and Xpa may also exhibit different spontaneous phenotypes. Xpc−/− mice of a mixed background (25% 129, 75% C57BL/6J) were shown to exhibit a
high prevalence of lung tumors (24), but this has not been studied in a pure C57BL/6j background; also, a clean comparison with $Xpa^{-/-}$ mice has not yet been made.

To refine and expand our knowledge on human XPA and XPC, $Xpa^{-/-}$ and $Xpc^{-/-}$ mice were used. To investigate the phenotypic differences between these mice, we carried out a more comprehensive study with both mouse models in a pure C57BL/6j background.

We compared the life span of the two models and determined the pathology with focus on tumor development. In addition, to help explain the differences in tumor outcomes, we carried out mutation analyses in several organs. Our data suggest that Xpc-deficient mice are more sensitive to (oxidative) DNA-damaging agents in the lung compared with the Xpa-deficient mice and wild-type controls. These findings support evidence provided in various studies that XPC, the XPC homologue in mouse lines, is a key factor in the repair of oxidative DNA damage. Therefore, $Xpc^{-/-}$ mice seem to be more sensitive to oxidative stress and lung tumor development than their NER-deficient counterparts, $Xpa^{-/-}$ mice.

**Materials and Methods**

**Mice.** The generation and characterization of $Xpa^{-/-}$ and $Xpc^{-/-}$ mice have been described before (25, 26). To obtain a genetically homogeneous background, $Xpa$- and $Xpc$-deficient mice were back-crossed more than 10 times with C57BL/6j animals (Harlan). To offer the future possibility to monitor genomic instability, the heterozygous mutant mouse strains as well as C57BL/6j controls were crossed with pUR288-lacZ C57BL/6j transgenic mouse line 30, homozygous for lacZ integration on chromosome 11 (27). In the second round of breeding, double heterozygous mice were intercrossed to obtain homozygous mice carrying one locus of the integrated copies of the lacZ marker, used in the third breeding round to generate the experimental animals for the aging and cross-sectional studies as described below. Mice were genotyped by a standard PCR reaction using DNA isolated from tail tips. Primers to amplify the wild-type and targeted alleles, as well as primer sequences for lacZ determination, have previously been described (27, 28). The experimental setup of the studies was examined and agreed on by the institute’s Ethical Committee on Experimental Animals according to national legislation.

**Experimental design.** Female mice were marked and randomized at the day of birth in different groups (i.e., longevity cohorts or cross-sectional cohorts in which the mice were sacrificed at fixed time points). Cross-sectional cohorts of $Xpa^{-/-}$, $Xpc^{-/-}$ mice and their C57BL/6j controls were sacrificed at a fixed age of 13, 52, 78, and 104 weeks. The interim cohorts consisted of at least 15 female mice per time point and genotype. In the longevity cohorts, a total of 45 $Xpa^{-/-}$ and 50 $Xpc^{-/-}$ female mice and 45 or 50 of their wild-type controls (referred to in the text as C57BL/6j 1 and C57BL/6j 2) were monitored during their entire life span. The health state of the mice was checked daily, beginning at the day of weaning. Individual animals were weighed biweekly to determine live weights. During the entire experiment, animals were kept in the same stringently controlled (specific pathogen-free) environment, fed ad libitum, and kept under a normal day/night rhythm. The microbiological status of the cohorts was monitored every 3 months. Animals from the longevity cohort were removed from the study when found dead or moribund. Complete autopsy was done on animals of all cohorts; a total of 45 different tissues were isolated from each animal and stored for further histopathologic analysis (see below). In addition, a selective set of 20 different tissues were snap frozen in liquid $N_2$ for molecular analyses (e.g., lacZ mutant frequency analyses). Total animal weights as well as various organ weights were determined at time of death or when killed.

**Histopathology.** Organ samples (45 organs and tissues) of each animal were preserved in a neutral aqueous phosphate-buffered 4% solution of formaldehyde. Tissues required for microscopic examination were processed, embedded in paraffin wax, sectioned at 5 μm, and stained with H&E. Detailed microscopic examination was done on nine major organs of all female mice from the longevity cohort and on all gross lesions suspected of being tumors or representing major pathologic conditions. For each animal, histopathologic abnormalities, tumors as well as nonneoplastic lesions, were recorded using the PATHOS pathology data acquisition software, and if possible, cause of death was established.

**Hprt mutant frequency analyses.** $Xpa^{-/-}$, $Xpc^{-/-}$ mice and their wild-type controls were sacrificed at 13 or 52 weeks and spleens were isolated to determine spontaneous Hprt mutant frequencies. All mice were in pure C57BL/6j background. The number of mice within one genotype and age group varied between 4 and 10.

Priming and cloning of T lymphocytes were done in RPMI 1640 as described by Tates et al. (29) with some minor modifications (30). Mouse T lymphocytes were isolated from the spleen by rubbing the spleen through a sterile 70-μm nylon mesh (Falcon Cell Strainer, 2350). Subsequently, cells were frozen in RPMI 1640 supplemented with 10% DMSO and 40% fetal bovine serum (FBS) by using a Cryomed freezing apparatus (Forma Scientific). When required, frozen cells were thawed and immediately stored on ice. Stimulated T lymphocytes were cultured and selected for Hprt deficiency in the presence of lethal irradiated (30-Gy X-rays) mouse lymphoblastoid Sp2/0 feeder cells (29). Cloning efficiencies and mutant frequencies were calculated as described (29). Further details on the procedure were described by Wijnhoven et al. (30).

**LacZ mutant frequency analyses.** From a selected set of snap frozen tissues, DNA was extracted with a phenol/chloroform/iso-amyl alcohol mixture (25:24:1). Complete protocols for plasmid rescue and mutant frequency determinations with the pUR288 model have been described elsewhere (31). Briefly, between 10 and 20 μg of genomic DNA were digested

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**Figure 1.** Survival curves of female $Xpa^{-/-}$, $Xpa^{+/+}$ and their wild-type control cohorts. C57BL/6j 1 is the control cohort for $Xpa^{-/-}$, C57BL/6j 2 is the control cohort for $Xpc^{-/-}$. Median survival of the cohorts: C57BL/6j 1, 103 wk (light green); C57BL/6j 2, 102.5 wk (dark green); $Xpa^{-/-}$, 94 wk ($P = 0.0023$; red); $Xpc^{-/-}$, 94.5 wk ($P = 0.6023$; blue). For further details, see Materials and Methods.
with HindIII for 1 h in the presence of magnetic beads (Dynal) recoated with lac-lacZ fusion protein. The beads were washed thrice to remove the unbound mouse genomic DNA. Plasmid DNA was subsequently eluted from the beads by isopropyl-β-D-thiogalactopyranoside and circularized with T4 DNA ligase. Next, ethanol-precipitated plasmids were transfected into E. coli C (ΔlacZ, galE−) cells. One thousandth of the transformed cells was plated on the titer plate (with X-gal) and the remainder on the selective plate (with p-gal). The plates were incubated for 15 h at 37°C. Mutant frequencies were determined as the number of colonies on the selective plates versus the number of colonies on the titer plate (times the dilution factor of 1,000). Each mutant frequency is based on at least 300,000 recovered plasmids.

Statistical evaluation. Incidences of tumors were analyzed with the method of Peto (SAS; ref. 32) and with the poly-3 and poly-k method (33). These tests for statistical analysis of tumor incidences take differences in survival of the various groups into account. The poly-3 and poly-k tests were done by Dr. H. Moon (California State University, Long Beach, CA).

Cell culture. Primary mouse embryonic fibroblasts (MEF) were isolated from E13.5 day embryos, all in C57BL/6J background, and genotyped as previously described (27, 28). MEFs were cultured as described before (34) in DMEM (Life Technologies, Inc.) supplemented with 10% FBS (fetal calf serum, Biocell), 1% nonessential amino acids (Life Technologies), penicillin (0.6 μg/mL), and streptomycin (1 μg/mL) at 37°C, 5% CO2. MEFS were cultured 3 days per passage at 3% or 20% O2. Cell survival was determined by blue/white screening using trypan blue stain 0.4% (Life Technologies; 1:1), counting a minimum of 200 cells per sample. A minimum of three different embryos were used per genotype, plus a technical replica of all samples was used.

Results

To determine the average life span of Xpa−/− and Xpc−/− mice, 45 to 50 mutant females and corresponding C57BL/6J control mice were followed during aging. The survival curves for the longevity cohorts of female Xpa−/−, Xpc−/− and both matching wild-type control groups are depicted in Fig. 1. The median survival of female Xpc−/− mice (94 weeks) was significantly reduced (P = 0.0023, Kaplan-Meier with log-rank test) compared with the median survival of their female wild-type C57BL/6J controls (C57BL/6J 1, 103 weeks). This difference in significance is mainly caused by the fact that a certain fraction (±10%) of the Xpa−/− animals survive extremely long (see Fig. 1). Survival curves of both wild-type control studies show a very similar median survival and shape of the curve.

Of all groups ~30 or more animals were histopathologically examined. Neoplasms and inflammation (mainly ulcerative dermatitis) are the most common cause of demise in all groups, ranging from 72% in wild-type controls to 87% in Xpc−/− mice (data not shown). Occasionally more than one pathologic condition might have contributed to death, in which case the most pronounced condition was taken as cause of demise. Incidences of major tumor types at the time of death are listed in Table 1. Xpa−/− and Xpc−/− mice lived shorter than their respective wild-type controls. Accordingly, lower tumor incidences were expected in the mutant animals because they had less time to develop tumors. Statistical methods that take differences in survival into account were therefore used in this case to evaluate the significance of differences in tumor incidences. Nevertheless, some tumor types were increased in either Xpa−/− or Xpc−/− mice. Xpa−/− mice show a significant increase (P = 0.02) in bronchioloalveolar lung tumors and near significant elevation (P = 0.054) in hepatocellular tumors. Xpa−/− mice solely show a significant increase (P < 0.01) in hepatocellular tumors when compared with their matching wild-type control group. Furthermore, female Xpc−/− mice also show a significant increase in acidophilic macrophage pneumonia (P = 0.032, data not shown), which is not apparent in Xpa−/− mice.

The percentage of tumor-bearing NER-deficient animals (all types together) is surprisingly lower than the corresponding NER-proficient control groups. In addition to the shorter life span, the strong and significant decrease in benign pituitary adenomas in both Xpa−/− and Xpc−/− mice is mostly responsible for this lower percentage in tumor-bearing animals. The increase in number of tumor-bearing animals (excluding the pituitary adenomas and taking into account the survival distribution) reaches significance for Xpc−/− mice (P < 0.01) and approaches significance for Xpa−/− animals (P = 0.05) using the Peto test.

Although a variety of nonneoplastic changes were observed in the organs of mutant as well as wild-type mice, there were no

Table 1. Tumor incidences and P values for difference between mutant and its wild-type group

<table>
<thead>
<tr>
<th>Organ</th>
<th>C57BL/6J 1</th>
<th>Xpa−/−</th>
<th>P</th>
<th>C57BL/6J 2</th>
<th>Xpc−/−</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All organs</td>
<td>40</td>
<td>31</td>
<td></td>
<td>29</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Tumor-bearing animals</td>
<td>30 (75)</td>
<td>21 (68)</td>
<td></td>
<td>24 (83)</td>
<td>23 (61)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0 (0)</td>
<td>3 (10)</td>
<td>0.0002</td>
<td>2 (7)</td>
<td>5 (13)</td>
<td></td>
</tr>
<tr>
<td>Hepatocellular tumor</td>
<td>0 (0)</td>
<td>2 (6)</td>
<td></td>
<td>1 (3)</td>
<td>6 (16)</td>
<td>0.02</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchioloalveolar</td>
<td>20 (50)</td>
<td>8 (26)</td>
<td>*</td>
<td>20 (70)</td>
<td>9 (24)</td>
<td>0.01</td>
</tr>
<tr>
<td>Pituitary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pars distalis adenoma</td>
<td>18 (45)</td>
<td>19 (61)</td>
<td>0.001</td>
<td>21 (72)</td>
<td>23 (61)</td>
<td></td>
</tr>
<tr>
<td>Pituitary adenomas excluded</td>
<td></td>
<td></td>
<td></td>
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NOTE: Data are absolute values with percentages in brackets. Statistics: poly-k test (poly-3 test and Peto test generally gave similar significances, although exact P values may differ).

* Approaches significance with poly-3 test only (P = 0.08 for Xpc−/− liver and P = 0.054 for Xpa−/− pituitary).
† Not reaching significance with poly-k test, but P = 0.05 (positive trend) by the Peto test in this case.
obvious genotype-specific pathologies typical for Xpc<sup>-/-</sup> or Xpa<sup>-/-</sup> mice. The observed changes belonged to the normal background pathology of C57BL/6J mice and generally occurred to about the same degree in all groups. Because mutant animals died slightly earlier than wild-type mice, it may well be that these spontaneous aging lesions occurred slightly earlier in Xpc<sup>-/-</sup> and Xpa<sup>-/-</sup> mice than in wild-type animals. However, this aspect was not explicitly investigated in this study.

**Mutant frequency analyses.** A comprehensive analysis of spontaneous Hprt mutant frequency was done with Xpc<sup>-/-</sup>, Xpc<sup>-/-</sup> mice and their wild-type controls in a pure C57BL/6J background (Fig. 2). At the age of 52 weeks, Hprt mutant frequencies (0.9 × 10<sup>-5</sup>) in wild-type mice were in the same range as previously reported (23). At this age, mutant frequencies in Xpc<sup>-/-</sup> and Xpc<sup>-/-</sup> mice were elevated 15- and 3-fold, respectively, when compared with their age-matched wild-type controls. In addition, 13-week-old Xpc<sup>-/-</sup> mice already exhibited a 5-fold increase in mutant frequency compared with the 52-week-old wild-type control animals.

In addition, we determined mutant frequencies in several tissues of C57BL/6J, Xpc<sup>-/-</sup>, and Xpa<sup>-/-</sup> mice using the lacZ recovery system. Samples were taken from spleen, liver, and lung of 13-, 52-, and 78-week-old animals. Results are shown in Fig. 3. Table 2 depicts the P values of the compared genotypes in all three tissues at different ages. Mutant frequencies increased over time in spleen, liver, and lung of Xpc<sup>-/-</sup> mice. In Xpa<sup>-/-</sup> and C57BL/6J wild-type mice, an increase over time was visible in liver and lung, but not in spleen. Mutant frequencies in spleen showed a significant increase in mutants in 52- and 78-week-old Xpc<sup>-/-</sup> mice compared with their age-matched Xpa<sup>-/-</sup> and wild-type samples (Fig. 3A). Samples of Xpa<sup>-/-</sup> exhibited a similar lacZ mutant frequency pattern in the spleen as the wild-type samples over the entire life span. The liver of Xpc<sup>-/-</sup> and Xpa<sup>-/-</sup> mice showed a strong increase in mutant frequency compared with that of wild-type at 52 and 78 weeks of age (Fig. 3B). Xpc<sup>-/-</sup> liver samples exhibited slightly higher values on average than Xpa<sup>-/-</sup> samples at those time points.

The mutant frequency in Xpc<sup>-/-</sup> lung samples was significantly elevated in comparison with wild-type controls at all time points. Xpa<sup>-/-</sup> mice only exhibited this increase at 13 and 52 weeks of age.

**Figure 2.** Hprt mutant frequencies including SDs of Xpc<sup>-/-</sup>, Xpc<sup>-/-</sup> and their wild-type controls at ages 13 and 52 wk in spleen. The numbers of biological replicas are between 4 and 10. For further details, see Materials and Methods.

At 78 weeks, the mutant frequency of Xpa<sup>-/-</sup> lungs was comparable to that of wild-type controls. Strikingly, Xpc<sup>-/-</sup> lungs showed a significant elevation in mutant frequency at ages of 52 and 78 weeks as compared with their NER-deficient counterpart Xpa<sup>-/-</sup>. A 2-fold increase was visible in Xpc<sup>-/-</sup> lung samples at 78 weeks in relation to both wild-type controls and Xpa-deficient samples (see Fig. 3C).

**Cell survival under oxygen exposure.** In view of the increased tumor incidence and elevated spontaneous mutant frequency in lung, we suspected that the Xpc<sup>-/-</sup> animals were more susceptible to oxidative stress. To directly test this hypothesis, we investigated the effects of exposure to different levels of oxygen, 3% and 20%, on Xpa<sup>-/-</sup>, Xpc<sup>-/-</sup> and wild-type MEFs. Results are shown in

**Figure 3.** LacZ mutant frequencies including SDs of Xpc<sup>-/-</sup>, Xpa<sup>-/-</sup> and their wild-type controls at ages 13, 52, and 78 wk in spleen (A), liver (B), and lung (C). The numbers of biological replicas are between 5 and 6. For further details, see Materials and Methods.
Fig. 4. Xpc<sup>−/−</sup> fibroblasts subjected to 3% oxygen level seemed to be slightly more sensitive than Xpa<sup>−/−</sup> and wild-type MEFs in the first passage. Survival of Xpc<sup>−/−</sup> fibroblasts was 68% after 3 days of culturing (first passage in Fig. 4), whereas Xpa<sup>−/−</sup> and wild-type fibroblasts exhibited 80% and 82% survival, respectively. After two more passages, at 3% oxygen pressure, 62% survival was visible in fibroblasts. The survival of Xpa<sup>−/−</sup> and wild-type MEFs, at this point and under 3% oxygen pressure, was 79%. When, after the initial passage of 3% oxygen, the fibroblasts were cultured for two more passages at 20% oxygen pressure, a severe decrease in survival was exhibited in Xpc<sup>−/−</sup> fibroblasts. The percentage of survival dropped to a mere 39%. This dramatic decrease was not observed for Xpa<sup>−/−</sup> and wild-type (67% and 74% survival, respectively).

Discussion

Previous results obtained in separate studies in which patterns of survival, tumor formation, and mutation accumulation were determined in Xpc- and Xpa-deficient mice were difficult to interpret because of variation in genetic background and the use of different mutational reporter genes. Here we carried out a side-by-side comprehensive study of these end points in the same genetic background using both the Hprt and lacZ mutational target genes. NEC-deficient mouse models lacking a functional XPA or XPC protein show phenotypic differences when compared with their wild-type controls. A significant reduction in spontaneous survival was observed in our comprehensive longevity studies for female Xpc-deficient mice in a pure C57BL/6J background. Xpa-deficient female mice also showed a decrease in survival when compared with their wild-type control, although not statistically significant. Previously reported survival studies of Xpc<sup>−/−</sup> mice in a mixed background did not exhibit a decrease in survival (24). Female Xpa<sup>−/−</sup> and Xpc<sup>−/−</sup> mice in our studies reached an average median age of 94.5 and 94 weeks, respectively, whereas their matching wild-type control mice attained a median survival of 103 and 102.5 weeks. Both the wild-type control cohorts show a similar survival pattern and a comparable median age of 50% survival. As in humans, a deficiency in one of these NEC proteins leads to a reduced life span, and therefore XPA and XPC prove to be part of delicate and important processes that have a substantial effect on survival.

Additional pathologic analyses of female Xpa<sup>−/−</sup> and Xpc<sup>−/−</sup> mice showed that the cause of death in many of these animals was not accountable to the presence of neoplasms. Another main cause of death of the NEC-deficient mice was in fact the occurrence of inflammation, mainly ulcerative dermatitis. For the C57BL/6J 1 group (which was simultaneously executed with the Xpa<sup>−/−</sup> study) and Xpa<sup>−/−</sup>, high incidences of inflammation were apparent (50% and 61%, respectively). The C57BL/6J 2 and the Xpc<sup>−/−</sup> studies showed a somewhat lower incidence of inflammation (24% and 55%, respectively). High incidences of ulcerative dermatitis in control C57BL/6J mice are considered an inevitable byproduct of handling the animals during their entire life span and are a result of Staphylococcus aureus infection. In addition, the type of diet seems to play a role in the severity of this skin phenotype. These findings are, however, clearly in line with our earlier findings (35) and those of others (36, 37) working particularly with female C57BL/6J mice.

To our surprise, the percentage of tumor-bearing animals was higher in NEC-deficient mice than in their NEC-deficient counterparts. This observation is, next to the longer life span observed in wild-type mice, mostly attributable to a dramatic decrease in pituitary tumor development in Xpc<sup>−/−</sup> mice (from 70% to 24% occurrence) and in Xpa<sup>−/−</sup> mice (from 51% to 26% occurrence). A similar strong reduction in pituitary tumor development was observed in another NEC-deficient mouse model, Xpa<sup>TTD</sup> (35). Here a significant decrease from 50% to 9% occurred. Interestingly, no such a decrease in pituitary tumor development was found in the TC-NEC–deficient Csb mouse model (data not shown). Apparently, a defect in GG-NEC is accompanied by suppression of a specific set of tumor types. Further studies are needed to substantiate this hypothesis.

A distinct difference between the two NEC-deficient mouse models is the observed tumor spectrum. Female Xpa<sup>−/−</sup> mice exhibit a significant increase in hepatocellular adenomas compared with wild-types. Female Xpc<sup>−/−</sup> mice also show an elevation in the number of hepatocellular neoplasms, which approaches significance. Additionally, female Xpc<sup>−/−</sup> mice do show a significant increase in bronchioalveolar neoplasms compared with their proficient controls. Such an increase is absent in female Xpa<sup>−/−</sup> mice.

Our results showed that female Xpc<sup>−/−</sup> mice in a pure C57BL/6J background are susceptible to lung cancer and support the previous findings of the lung cancer susceptibility in Xpc<sup>−/−</sup> mice in the more sensitive mixed background (24). However, in this previous study, the lung tumors observed and diagnosed as adenomas and adenocarcinomas seemed to be more malignant and were accompanied by a higher incidence of atypical hyperplasia. Possibly, depending on the genetic background and spontaneous (oxidative) DNA damage levels, tumors will progress earlier to a more malignant state. Next, several studies provide information that Xpc polymorphisms in humans may also contribute to genetic susceptibility for lung cancer (38–40). Exposure of Xpc-deficient mice (in a mixed genetic background) to the harmful genotoxic carcinogen 2-acetylaminofluorene (2-AAF) resulted in a significant elevation in the number of liver and lung tumors compared with wild-type animals (18, 41). In contrast, Xpa<sup>−/−</sup> mice in a pure C57BL/6J background only show an elevation in liver tumors after exposure to 2-AAF, and no increase in lung tumors was apparent in that study (18). The occurrence of lung tumors in Xpc<sup>−/−</sup> mice in

<table>
<thead>
<tr>
<th>Table 2. P values of mutant frequency comparisons between genotypes, depicted per age per tissue</th>
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<tr>
<td></td>
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<tr>
<td>Xpc vs WT</td>
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<tr>
<td>Xpc vs Xpa</td>
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<td>Xpa vs WT</td>
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Note: Open cells represent nonsignificant (P > 0.05) differences.
pure C57BL/6J background after exposure to 2-AAF has not yet been assessed. The difference in tumor spectrum between Xpc−/− mice and Xpa−/− mice in our study indicates that the XPC protein is, besides active in NER, possibly also involved other repair systems, most likely including BER. This idea was recently also put forward by others (42). The outcome of an increase in lung tumors in Xpc−/− mice could, therefore, point to an involvement of the XPC protein in the repair of oxidative DNA damage.

To analyze the mutation spectrum in repair-deficient mice in the different organs, we conducted additional mutant frequency analyses on several tissues of wild-type, Xpa−/−, and Xpc−/− female mice. Initial spontaneous Hprt mutant analyses in spleen showed a strong increase of mutant frequency in T lymphocytes of 52-week-old Xpc−/− mice compared with their age-matched Xpa−/− and C57BL/6J controls. However, in an earlier study, Xpc−/− mice with a mixed background were used (23). More comprehensive Hprt analyses using Xpc-deficient mice in a pure C57BL/6J background were done here. The strong increase in mutant frequency compared with the wild-type control was reproducible in Xpc−/− in a pure C57BL/6J background, albeit lower than in a mixed background. The results obtained with lacZ mutant analyses show a more moderate response in spleen when Xpc−/− is compared with wild-type. This can be explained by the fact that the background level of the lacZ mutant analyses is higher than that of Hprt. Although Hprt analysis is a more sensitive method than lacZ analyses, its drawback is that it is only applicable to the spleen. Other previous studies conducted here showed an elevation of mutant frequencies in Xpa−/− in liver and kidney using lacZ analyses (43, 44).

Our results of the lacZ mutant analyses show a striking increase of mutant frequency in lung tissue of Xpc−/− mice when compared with wild-type and Xpa−/− mice, especially at the age of 78 weeks. The fact that no severe increase in lung is observed in Xpa−/− mice but is distinctly present in Xpc−/− mice supports the hypothesis that the XPC protein might be involved in the removal of oxidative DNA damage, because the level of oxidative stress is higher in lungs compared with other tissues due to the constant exposure of this tissue to oxygen (45). In the spleen, Xpc−/− mice also show a significant increase in mutant frequency compared with wild-type and Xpa−/− at 52 and 78 weeks. In liver, on the other hand, the mutant frequencies of Xpa−/− and Xpc−/− liver are virtually equal over all time points. DNA damage in the liver most likely arises as a result of genotoxic bulky byproducts of metabolism. Compared with wild-type, 78-week-old NER-deficient mouse livers do show a significant elevation of mutant frequency. This illustrates the sensitivity of the NER-deficient mouse strains to DNA damage. Over time and in all investigated tissues, Xpc−/− mice show the highest mutant frequency, indicating an even higher sensitivity to DNA damage than Xpa−/− animals.

The putative role in the removal of oxidative damage is supported by our in vitro data, in which Xpc−/− MEFs exhibit a severe decrease in survival when cultured at 20% oxygen compared with 3% oxygen pressure. We believe that the excess of oxidative damage is most likely the cause of death because oxygen pressure is the only variable that was changed in culturing. Xpc-deficient fibroblasts seem to be impaired in the removal of this damage. Even at a low oxygen level of 3%, growth of Xpc−/− cells in oxygen-rich surroundings and the severe decrease in cell survival under 20% oxygen pressure are concurrent with the present understandings and recent discoveries about the XPC protein. Several recent studies provide information about the involvement of XPC in BER and the putative role in the repair of oxidative DNA damage. BER is mainly responsible for mending the DNA damage caused by oxidative stress (46). XPC can physically and functionally interact with thymine DNA glycosylase, which plays a role in BER (47). XPC-HR23B also has been assigned as a cofactor for BER of 8-hydroxyguanine by stimulating the activity of its specific DNA glycosylase OGG1 (48). In addition, a recent study has shown a
deficient BER in XPC fibroblasts after oxidative DNA damage induced by methylene blue plus visible light (49). Methylene blue and visible light produce oxidative DNA damage, among others 8-hydroxyguanine.

Our results indicate the importance of the XPC protein in vivo, where the absence of the protein is responsible for susceptibility to lung tumors in mice compared with wild-type and their NER-deficient counterpart Xpa−/−. Mutant frequency analyses show an additional sensitivity in spleen compared with Xpa-deficient animals. In accordance with Xpa deficiency, Xpc−/− mice also show a high mutant frequency in liver. Therefore, results, together with the accumulating evidence provided by others, support the theory of XPC involvement in BER or additional pathways and the removal of oxidative DNA damage. A subsequent consequence of this engagement could explain the difference in tumor spectrum between Xpc−/− and Xpc+/− mice.

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References


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