The Combined Effects of Xeroderma Pigmentosum C Deficiency and Mutagens on Mutation Rates in the Mouse Germ Line

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Abstract

Spontaneous and induced mutation rates at two expanded simple tandem repeat (ESTR) loci were studied in the germ line of xeroderma pigmentosum group C (Xpc) knockout mice defective in global genome nucleotide excision repair. Spontaneous and radiation-induced mutation rates in homozygous Xpc−/− males were significantly higher than those in isogenic wild-type (Xpc+/+) and heterozygous (Xpc+/−) mice. In contrast, exposure to the monofunctional alkylating agent ethylnitrosourea resulted in similar increases in ESTR mutation rates across all genotypes. ESTR mutation spectra in the germ line of Xpc−/−, Xpc+/− and Xpc+/+ did not differ. Considering these data and the results of other publications, we propose that the Xpc-deficient mice possess a mutator phenotype in their germ line and somatic tissues that may significantly enhance carcinogenesis across multiple tissues. [Cancer Res 2007;67(10):4695–9]

Introduction

Nucleotide excision repair (NER) is a highly efficient repair system capable of removing a wide range of DNA lesions disrupting base pairing (1, 2). There are two subpathways of NER: transcription-coupled repair and global genome repair (GGR), acting on the transcribed genes and the entire genome, respectively (2). Given that NER is involved in the removal of UV-induced DNA damage, mutations inactivating this pathway result in variety of photosensitive disorders in humans, such as xeroderma pigmentosum (XP) and Cockayne syndrome (3). To further elucidate the role of genes involved in NER, a number of mouse knockout mice deficient in NER have been generated (4). In line with our previous studies (5–8, 11, 15, 16), all cases of germline mutational mosaicism with denovo mutations resulting from cryptic germ cells were scored as germline.

Materials and Methods

Mice. The Xpc knockout mice on (129/Sv × C57BL/6) mixed background, generated by Cheo et al. (9), were used in this study. Isogenic wild-type (Xpc+/+), heterozygous (Xpc+/−), and homozygous (Xpc−/−) males were generated by mating the Xpc−/− knockout parents. To obtain control offspring, non-exposed Xpc+/+, Xpc+/−, and Xpc−/− males were crossed to untreated CBA/J females (Elevage Janvier).

Male mice were given whole-body acute irradiation of 1 Gy of γ-rays delivered at 1.97 Gy min−1 (Cs-137 source, IBL 637 CisBio International). Male mice were given a single i.p. dose of 50 mg/kg ethylnitrosourea (ENU; CAS No. 759-73-9, Sigma), dissolved in 0.9% NaCl. All exposed males were mated to untreated CBA/J females 10 weeks after irradiation, ensuring that the litters generated were conceived with sperm derived from irradiated A5 spermatogonia (10). The animal procedures were carried out under the guidance issued by the French government ("Décret 87-848 du 19 octobre 1987 modifié") and under the supervision of an authorized investigator (No. 92-163 09/09/2002 to L.M.).

DNA isolation and ESTR typing. Genomic DNA was extracted from tails using a standard phenol-chloroform technique and digested to completion with AluI. All parents and offspring were profiled using two mouse-specific hypervariable single-locus ESTR probes Ms6-hm and Hm-2, as described previously (11). Following Southern blot hybridization, autoradiographs were scored by two independent observers. DNA fragment sizes were estimated by the method of Southern (12), using a 1-kb DNA ladder (Invitrogen) included on all gels.

The maternal CBA/J inbred strain was selected because of the non-overlapping size range of alleles for two known ESTR loci in the wild-type and Xpc knockout male mice. The mean progenitor allele sizes in Xpc-deficient strain were ~3 and 4.5 kb for Ms6-hm and Hm-2, respectively, whereas in the CBA/J strain, they were ~2 and 4 kb. This substantially facilitated the scoring of mutations and allowed unambiguous establishment of the parental origin of mutant bands identified by gel electrophoresis. ESTR mutants were identified as novel DNA fragments present in offspring, which cannot be ascribed to either parent. Only bands showing a shift of at least 1 mm relative to the progenitor allele were scored as mutants. Somatic mosaics with a third non-parental allele (13, 14) have not been included in the analysis. As in our previous studies (5–8, 11, 15, 16), all cases of germline mosaicism with de novo mutations shared by more than one offspring in the litter were recorded and treated separately (Table 1).

Results

Experimental design. Here, we analyzed the effects of NER deficiency on spontaneous and induced mutation in the germ line of Xpc knockout mice. Given that the NER pathway is involved in the removal a wide range of endogenous DNA lesions (1, 2), their compromised repair may affect spontaneous mutation rate in the germ line of Xpc-deficient mice. To verify that the effects of XPC deficiency on mutation rate germ line were attributed to the compromised GGR, ESTR mutation rates were also analyzed in mice exposed to two well-recognized mutagens: ionizing radiation (IR) and ENU. Exposure to IR results in the accumulation of base damage.
damage, single- and double-strand DNA breaks (SSB and DSB), DNA-protein links, and bulky adducts (17). As GRR removes a wide range of bulky DNA lesions similar to those induced by UV radiation (1, 2), some of IR-induced bulky adducts with disrupted base pairing may be substrate for this DNA-repair pathway. Besides, the results of two recent studies show that the XPC protein is also involved in the removal of oxidative DNA damage (18), the amount of which is substantially increased after irradiation, and that long-term Xpc silencing substantially reduces the efficiency of DSB repair (19). These data may provide a plausible explanation for the clinical and cellular radiosensitivity of some XPC patients (20, 21). We therefore reasoned that Xpc knockout mice may be hypersensitive to IR. On the other hand, exposure to ENU mainly results in alkylation of DNA bases at the O6-position (22), which cannot be removed by GRR and are almost exclusively repaired by O6-alkyl-guanine-DNA-alkyltransferase (1). If the effects of XPC deficiency in the germ line of irradiated mice were attributed to the compromised GRR and cannot be related to other functions of this protein, then this deficiency should not affect the pattern of mutation induction in the ENU-exposed males.

Table 1. Summary of mutation data

<table>
<thead>
<tr>
<th>Genotype, treatment</th>
<th>No. males</th>
<th>No. litters</th>
<th>No. offspring</th>
<th>Ms6-hm</th>
<th>Hm-2</th>
<th>Total</th>
<th>Rate</th>
<th>Ratio</th>
<th>P</th>
<th>Type of mutants</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Rate b</td>
<td></td>
<td></td>
<td></td>
<td>Gainsc</td>
<td>Losses</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xpc+/+</td>
<td></td>
<td></td>
<td></td>
<td>Rate b</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>27</td>
<td>137</td>
<td>9 (9)</td>
<td>3 (3)</td>
<td>12 (12)</td>
<td>0.0438</td>
<td>—</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>1 Gy</td>
<td>5</td>
<td>19</td>
<td>125</td>
<td>15 (15)</td>
<td>9 (5)</td>
<td>24 (20)</td>
<td>0.0960</td>
<td>2.19</td>
<td>0.0282</td>
<td>9</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>4</td>
<td>19</td>
<td>129</td>
<td>26 (18)</td>
<td>15 (13)</td>
<td>41 (31)</td>
<td>0.1589</td>
<td>3.63</td>
<td>1.24 × 10⁻⁵</td>
<td>21</td>
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<tr>
<td></td>
<td>χ², df: 2</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
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<td>Rate b</td>
<td></td>
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<tr>
<td>Control</td>
<td>10</td>
<td>25</td>
<td>127</td>
<td>8 (8)</td>
<td>2 (2)</td>
<td>10 (10)</td>
<td>0.0394</td>
<td>0.90</td>
<td>0.9737</td>
<td>5</td>
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<tr>
<td>1 Gy</td>
<td>5</td>
<td>28</td>
<td>124</td>
<td>15 (15)</td>
<td>9 (7)</td>
<td>24 (22)</td>
<td>0.0968</td>
<td>2.21</td>
<td>0.0264</td>
<td>12</td>
</tr>
<tr>
<td>50 mg/kg</td>
<td>4</td>
<td>20</td>
<td>93</td>
<td>18 (16)</td>
<td>9 (7)</td>
<td>27 (23)</td>
<td>0.1436</td>
<td>3.31</td>
<td>0.0003</td>
<td>12</td>
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<tr>
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<td>0.18</td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>9</td>
<td>22</td>
<td>132</td>
<td>14 (10)</td>
<td>9 (7)</td>
<td>23 (17)</td>
<td>0.0871</td>
<td>1.99</td>
<td>0.0616</td>
<td>11</td>
</tr>
<tr>
<td>1 Gy</td>
<td>5</td>
<td>17</td>
<td>113</td>
<td>19 (13)</td>
<td>16 (11)</td>
<td>35 (24)</td>
<td>0.1549</td>
<td>3.54</td>
<td>3.86 × 10⁻⁵</td>
<td>21</td>
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<tr>
<td>50 mg/kg</td>
<td>4</td>
<td>20</td>
<td>140</td>
<td>34 (30)</td>
<td>13 (13)</td>
<td>47 (43)</td>
<td>0.1679</td>
<td>3.83</td>
<td>2.32 × 10⁻⁶</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>χ², df: 2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.99</td>
</tr>
</tbody>
</table>

Abbreviation: df, degree of freedom.
*Total number of paternal mutations per group (number of singleton mutations is given in parentheses).
†Mutation rate for total number of paternal mutations.
‡Ratio to mutation rate in the non-exposed XPC+/+ males and probability of difference from the Xpc+/- control males (Fisher’s exact test, two tailed).
§χ² test for homogeneity of the type of mutants between control and exposed males.

ESTR mutation rates. Table 1 presents a summary of ESTR mutation data. ESTR mutation rates per locus in the germ line of males were estimated by dividing the total number of mutations scored in the offspring by the total number of offspring and the total number of loci. The spontaneous ESTR mutation rate in wild-type Xpc+/- and heterozygous Xpc+/- males were similar, with the mean mutation rate for these two genotypes of 0.0417 per locus. In contrast, ESTR mutation rate in the germ line of non-exposed Xpc-/- males was 2-fold higher than in Xpc+/- and Xpc+/- animals (P = 0.0170, Fisher’s exact test).

A comparison of ESTR mutation rates in the germ line of irradiated males (Table 1; Fig. 1) revealed that exposure to IR resulted in a similar 2.2-fold increase in mutation rates in the germ line of wild-type and heterozygous males (mean mutation rate = 0.0964). However, in the irradiated Xpc-/- males, ESTR mutation rate significantly exceeded that in the irradiated Xpc+/- and Xpc+/- animals (P = 0.0355). The results of ANOVA analysis for the combined effects of both factors confirmed that mutation rates differed significantly between non-irradiated and irradiated males as well as between males with different Xpc genotypes (Table 2).

Considering these data, we therefore conclude that the loss of XPC resulted in a similar 2.2-fold increase in mutation rates in the germ line of wild-type and heterozygous males (mean mutation rate = 0.0964). However, in the irradiated Xpc-/- males, ESTR mutation rate significantly exceeded that in the irradiated Xpc+/- and Xpc+/- animals (P = 0.0355). The results of ANOVA analysis for the combined effects of both factors confirmed that mutation rates differed significantly between non-irradiated and irradiated males as well as between males with different Xpc genotypes (Table 2).

Figure 1. ESTR mutation rates in the germ line of male mice with different Xpc genotypes. The 95% confidence intervals (95% CI) for mutation rate estimated from the Poisson distribution. * significant difference in mutation rate between Xpc-/- and (Xpc+/- + Xpc+/-) males within each group.
function affects spontaneous and radiation-induced mutation rates in the mouse germ line.

In contrast to the effects of IR, pre-meiotic exposure to ENU caused similar increases in ESTR mutation rates across all three genotypes (Table 1; Fig. 1). This result was further verified by the ANOVA analysis (Table 2).

**ESTR mutation spectrum.** The germline length change was defined for 243 de novo paternal mutations found in the offspring of all control and exposed animals. Within each genotype, the incidence of mutations involving gain or loss of repeat units was essentially the same in control and exposed groups (Table 1). For each genotype, the data for control and exposed males were therefore combined for further analyses. The frequency of gains and losses did not significantly differ between the three genotypes (Xpc+/− males: 35 gains versus 42 losses; Xpc+/−/− males: 29 gains versus 32 losses; Xpc−/− males: 56 gains versus 49 losses; χ² = 1.20; degree of freedom, 2; P = 0.5477).

We next determined the spectra of ESTR mutations. Again, within each genotype, the mutation spectra for the exposed and non-irradiated males did not significantly differ (Kruskal-Wallis test, P > 0.15; data not shown). The combined distributions of length changes were indistinguishable among the three genotypes (Fig. 2). We therefore conclude that neither the loss of XPC function nor exposure to IR or ENU affects the spectrum of ESTR mutations.

**Discussion**

NER in mammalian cells involves recognition of DNA lesion, incision of the damaged strand, DNA re-synthesis, and, finally, ligation to replace an excised sequence (1, 2). Among the many components of NER, the XPC protein is unique, as it is involved in the recognition of DNA damage that can only be repaired by GGR (23). The damage-recognition complex XPC-hHR23B specifically binds to certain DNA lesions, thus acting as the initiator of GGR (24–26). The assembly of XPC-hHR23B complex at the sites of DNA damage occurs within ~15 min and remains elevated until 1 h after exposure (23). In mammals, the Xpc gene is fully expressed, and the XPC protein is functional, during spermatogenesis (27). The results of a recent study show that in mice, all spermatogenic cell types display good repair of (6–4)pyrimidine photoproducts, which are substrates for GGR by the XPC-hHR23B complex (28).

NER is essential for the control of genome stability, and NER deficiencies are associated with a variety of human heritable disorders (1–3). The mouse knockout mutants affecting this pathway recapitulate many characteristics of the human disorders (4). As far as the Xpc knockout mice are concerned, the results of some publications provide strong evidence for elevated cancer incidence (29–31) and increased somatic mutation rate (32, 33) among these mice. Our data showing the 2-fold increase in ESTR mutation rates in the germ line of non-exposed Xpc−/− mice are therefore in line with the results of these studies. It should be noted that elevated spontaneous mutation rate detected in Xpc−/− mice may not be entirely attributed to the compromised GRR. As already mentioned, the XPC protein is partially involved in the removal of oxidative DNA damage (18) and DSB repair (19). Besides, it has been suggested that the XPC-hHR23B complex may also participate in base excision repair (34).

In this study, ESTR mutation rates were analyzed in the germ line of Xpc-deficient mice exposed either to IR or ENU, two mutants with different DNA reactivity. Thus, exposure to IR results in the accumulation of base damage, SSB and DSB, DNA-protein links, and bulky adducts (17). Some bulky adducts with disrupted base pairing, together with oxidatively damaged nucleotides (18), may be removed by GGR. In contrast to IR, ENU reacts with a variety of nucleophilic sites in DNA and proteins, which mostly leads to alkylation of DNA at the N- and O-positions (22). The XPC-hHR23B complex does not bind to these DNA lesions (26), and that is why exposure to ENU resulted in the similar increases in ESTR mutation rates across all Xpc genotypes. A comparison of mutation induction in the two groups of exposed males, therefore, indicates that the compromised repair of bulky adducts and some other types of DNA damage may alone explain the elevated spontaneous and radiation-induced mutation rates in Xpc−/− homozygotes.

The data presented here, together with the results of our previous publications (5–8), provide further insights into mechanisms underlying the effects of DNA repair deficiencies on the

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**Table 2. ANOVA analysis for effects of the XPC deficiency and mutagens on ESTR mutation rate**

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>F statistics</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiation Genotype</td>
<td>2, 133</td>
<td>3.72</td>
<td>0.0268</td>
</tr>
<tr>
<td>Dose</td>
<td>1, 133</td>
<td>18.88</td>
<td>2.70 × 10⁻⁵</td>
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<tr>
<td>Interaction</td>
<td>2, 133</td>
<td>0.27</td>
<td>0.7621</td>
</tr>
<tr>
<td>ENU Genotype</td>
<td>2, 126</td>
<td>1.77</td>
<td>0.1749</td>
</tr>
<tr>
<td>Dose</td>
<td>1, 126</td>
<td>47.22</td>
<td>&lt;10⁻⁶</td>
</tr>
<tr>
<td>Interaction</td>
<td>2, 126</td>
<td>1.16</td>
<td>0.3179</td>
</tr>
</tbody>
</table>

NOTE: ESTR paternal mutation frequencies (p) were estimated for all litters conceived by exposed and control males; arcsine transformed values (θ = arcsin √p) were used in all estimates.
show any detectable increases in their mutation rate. Meanwhile, the loss of p53 function does not affect mutation rate in the mouse germ line. We have previously suggested that the lack of mutation induction in the irradiated scid, PARP-1−/−, and Msh2−/− mice can be explained by the high killing effects of radiation on the germ line of these mice (5, 8). Given that un repaired DSBs, SSBs, and mismatched DNA pairs, the recognition of which in scid, PARP-1−/−, and Msh2−/− is substantially compromised, are highly deleterious as they are not compatible with DNA replication, a substantial proportion of cells in the germ line of deficient males, containing radiation-induced DNA damage may therefore be eliminated by apoptosis. In contrast, among the variety of radiation-induced lesions, only bulky adducts are preferentially targeted by NER. It should be noted that in Xpc−/− knockout mice, the removal of cyclobutane pyrimidine dimers, the main target for NER, is not completely compromised (9). It would therefore seem that XPC deficiency may only partially affect the repair of radiation-induced damage and thus the survival of irradiated germ cells. The lack of measurable differences in ESTR mutation rates in the germ line of irradiated Xpc−/− heterozygotes and wild-type homozygotes supports this suggestion. Thus, in contrast to our data, the results of previous studies show a considerably elevated predisposition to UV-induced skin cancers among Xpc−/− heterozygotes (29, 30). Given that the photoproducts are almost exclusively repaired by NER, the recessive phenotype of XPC deficiency in irradiated mice implies that the majority of radiation-induced DNA damage in knockout animals is fully repaired by other pathways.

Finally, our data and the results other studies (32, 33) showing elevated mutation rates in the germ line and somatic tissues of Xpc−/− homozygotes can provide a plausible explanation for the increased incidence of cancer among these animals. Given that the development of cancer is a multistep process in which cells acquire mutations in a specific clonal lineage (40), it would therefore seem that the manifestation of instability attributed to the loss of XPC function may significantly enhance carcinogenesis across multiple tissues. Indeed, the results of a recent study showing that 100% of Xpc−/− mice develop spontaneous lung tumors (31) clearly indicate that XPC deficiency can confer cancer risk for a variety of tissues. Moreover, given that the XPC protein plays a unique role in the recognition of DNA lesions with disrupted pairing, as well as in the repair of other types of DNA damage (oxidative damage, double-strand DNA breaks, etc.), it seems that exposure not only to UV, but also to some mutagens, including IR, may further predispose the carriers of mutations affecting the function of this protein to cancer. The data presented here, showing elevated radiosensitivity of Xpc−/− homozygotes, indicate that this deficiency could enhance radiation-induced carcinogenesis.

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References


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