Impact of Global Genome Repair versus Transcription-coupled Repair on Ultraviolet Carcinogenesis in Hairless Mice

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ABSTRACT

The nucleotide excision repair (NER) system is comprised of two subpathways, i.e., transcription-coupled repair (TCR) and global genome repair (GGR). To establish the relative importance of TCR and GGR for UV effects on the skin, we have used hairless knockout mouse strains lacking either TCR (CSB /−/−) or GGR (XPC /−/−). In single exposure experiments, we found GGR to be far more efficient in removing all types of DNA damage produced by UV radiation, with the highest efficiency for UVB-induced CPD2 and 6-4PP. These types of DNA damage are removed from the genome by NER, a versatile repair system responsible for the elimination of a wide variety of DNA lesions, including UVB-induced 6-4PP and cyclobutane pyrimidine dimers. Exposure to 80 J/m2 UV radiation (i.e., suberythemogenic in CSB /−/−) on 10 consecutive days gives rise to epidermal hyperplasia in CSB /−/− and XPC /−/−, whereas repair-proficient controls do not show epidermal hyperplasia from these exposures. In addition, CSB /−/− mice develop marked parakeratosis, whereas XPC /−/− mice and controls do not. Under continued exposure to this daily dose, squamous cell carcinomas appear in CSB /−/−, XPC /−/−, and in the control groups, whereas only in the CSB /−/− animals is a fairly high number of benign papillomas also found. The median latency time of squamous cell carcinomas (diameters ≥1 mm) is 84 days for the XPC /−/− mice, 115 days for the CSB /−/− mice, and 234–238 days for the heterozygous and wild-type control groups. These results indicate that GGR is more important than TCR in protection against UV-induced carcinomas of the skin but not against other UV effects such as sunburn, epithelial thickening, scaling of the stratum corneum, and development of papillomas. These results also indicate that GGR capacity may serve as a better predictor for skin cancer susceptibility than sensitivity to sunburn. The relative cancer susceptibilities of GGR- and TCR-deficient skin could well depend on the balance between an increased mutation rate and the presence (in CSB /−/−) or lack (in XPC /−/−) of a compensatory apoptotic response.

INTRODUCTION

Non-melanoma skin cancer is the most frequently diagnosed type of cancer in the United States. In addition, non-melanoma skin cancer patients have a 20–30% increased mortality from other cancers, suggesting that skin cancer susceptibility relates to a more general type of cancer susceptibility (1). A potential underlying determinant of cancer susceptibility is the efficiency by which genotoxic damage is removed from the genome (1). The dominant types of DNA lesions responsible for induction of skin cancer are UVB (280–315 nm) radiation-induced CPD2 and 6-4PP. These types of DNA damage are removed from the genome by NER, a versatile repair system responsible for the elimination of a wide variety of DNA lesions, including UVB-induced 6-4PP and cyclobutane pyrimidine dimers. Exposure to 80 J/m2 UV radiation (i.e., suberythemogenic in CSB /−/−) on 10 consecutive days gives rise to epidermal hyperplasia in CSB /−/− and XPC /−/−, whereas repair-proficient controls do not show epidermal hyperplasia from these exposures. In addition, CSB /−/− mice develop marked parakeratosis, whereas XPC /−/− mice and controls do not. Under continued exposure to this daily dose, squamous cell carcinomas appear in CSB /−/−, XPC /−/−, and in the control groups, whereas only in the CSB /−/− animals is a fairly high number of benign papillomas also found. The median latency time of squamous cell carcinomas (diameters ≥1 mm) is 84 days for the XPC /−/− mice, 115 days for the CSB /−/− mice, and 234–238 days for the heterozygous and wild-type control groups. These results indicate that GGR is more important than TCR in protection against UV-induced carcinomas of the skin but not against other UV effects such as sunburn, epithelial thickening, scaling of the stratum corneum, and development of papillomas. These results also indicate that GGR capacity may serve as a better predictor for skin cancer susceptibility than sensitivity to sunburn. The relative cancer susceptibilities of GGR- and TCR-deficient skin could well depend on the balance between an increased mutation rate and the presence (in CSB /−/−) or lack (in XPC /−/−) of a compensatory apoptotic response.

This system, the repair machinery is directed preferentially to the template strand of actively transcribed DNA to avoid that unrepaird lesions interfere with transcription by stalling RNA polymerase II (2, 3).

In humans, mutations in NER genes are the cause of the rare genetic diseases XP and CS. Complementation studies have revealed the existence of seven genes involved in XP (XPA through XPG) and two in CS (CSA and CSB; Refs. 2 and 6). All XP complementation groups are defective in both GGR and TCR, with the exception of XPC and XPE, which are defective in GGR only (3, 7, 8). A specific defect in TCR is encountered in CSA and CSB (9–11). Patients within the same complementation group can vary quantitatively in their skin cancer susceptibility, because different mutations in the same gene can lead to different levels of impairment. Because it is very difficult to make well-controlled, qualitative, and quantitative comparisons in cancer susceptibility among the separate XP and CS complementation groups based on the available patient data, mouse models for XP and CS have been developed.

XPA (12, 13) and XPC (14–16) knockout mice show a higher susceptibility to UV-induced skin cancer than their heterozygous and wild-type littermates, and as such resemble human patients with XPA and XPC. In marked contrast to human CS patients, CSB mice are also prone to UV- and chemically induced skin cancer (11), indicating that TCR also protects against carcinogenesis. XPC and CSB mutant mice are very well suited to compare the relative contributions of GGR and TCR to the prevention of skin cancer. However, a direct quantitative comparison of skin cancer susceptibilities between rodent XPC and CSB cannot be made on basis of the published data because: (a) animals of different genetic background were used; (b) experiments were discontinued before the repair-proficient control animals contracted tumors; and (c) different irradiation schedules and different types of lamps were used. To directly compare the effects of defective GGR versus TCR, we have crossed the CSB and the XPC defect into a strain of SKH hairless mice and compared their relative susceptibilities to UV-induced skin cancer under identical experimental conditions. Here we show that GGR deficiency results in a much higher skin cancer susceptibility than TCR deficiency, whereas other UV effects such as sunburn, parakeratosis, and the development of benign papillomas appear to be primarily related to TCR deficiency.

MATERIALS AND METHODS

Generation of Hairless XPC and CSB Mice. The XPC mice (a kind gift of Dr. E. C. Friedberg, Dallas, TX) and CSB mice used in this study have been described in detail previously (11, 14). Shortly, XPC mice were generated by deletion of exon 10 of the XPC gene and in CSB mice a premature stop codon (mimicking a mutation in a CSB patient) was introduced in exon 5 of the CSB gene. The specific GGR and TCR defects in XPC and CSB mice models have been confirmed by analysis of DNA repair characteristics in mouse embryonic fibroblasts (11, 14).

To make a direct comparison of the relative contribution of GGR and TCR to UV carcinogenesis XPC (129/Sv-C57Bl/6 background) and CSB animals (129/Ola-FVB background) were crossed with HRA/SKH albino hairless mice. Hairless animals do not have to be shaved before exposure and, therefore, allow a very accurate UV dosimetry. After one more backcross with HRA/2858

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3 The abbreviations used are: CPD, cyclobutane pyrimidine dimer; 6-4PP, pyrimidine [6-4] pyrimidone photoproducts; NER, nucleotide excision repair; TCR, transcription-coupled repair; GGR, global genome repair; XP, xeroderma pigmentosum; CS, Cockayne syndrome; SCC, squamous cell carcinoma; MEF, minimal erythema/edema dose.

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SKH mice, hairless offspring with albino eyes and heterozygous for XPC and CSB were intercrossed to generate hairless albino XPC and CSB mice. Heterozygous and wild-type littermates were used as controls. Genotyping was performed by PCR analysis of genomic DNA isolated from the tail tips (11, 14). For XPC genotyping, the following three primers were used: (a) XPCex10s 5’-ATTGCCGTCATACCTTCGAC-3’; (b) neo 5’-CGCATCGC-CTTCTATCCGCT-3’; and (c) XPCin10as 5’-TACTTCTTCAAAACCCTGCTC-3’. Primer a + c identifies the wild-type allele as a ±250-bp PCR fragment, and primer b + c identifies the targeted allele as a ±350-bp PCR fragment. For CSB genotyping, the following three primers were used: (a) CSBex5s 5’-GCGTTGATTAAATATCCATCTCC-3’; (b) neo 5’-ATCT- GCGTTGGTGAATCCGCAATG-3’; and (c) CSBin5as 5’-GCTTCTGAT-GAGCTTGAATCTATGAG-3’. Primer a + c identifies the wild-type allele as a ±200-bp PCR fragment, and primer b + c identifies the targeted allele as a ±500-bp PCR fragment. Animal experiments were approved by an ethical committee of Utrecht University, as required by Dutch law.

**Single Exposure Experiments.** The dossal skin of hairless CSB and hairless XPC mice as well as heterozygous and wild-type littermates was exposed to UVB radiation using an Hanovia Kromayer lamp equipped with a Schott WG305 filter (exposure rate, 135 J/m²/s; 280–400 nm). This hand-held lamp allows short exposures to limited areas of the skin by placing its circular port (~2.5 cm²) in close contact to the skin (17). For all genotypes, two male and two female mice were exposed to 1, 2, 8, 16, and 24 s of UV radiation from the Kromayer lamp. All exposures were given in duplicate on separate mice. The mice were checked independently by two experienced, but noninformed, observers for erythema and/or edema at 8, 24, 36, 48, and 72 h after the exposures.

**Chronic Exposure Experiments.** During the chronic exposures (that lasted ~300 days), the animals were housed separately in macrolon type I cages. Standard mouse chow (Hope Farms RMB-H) and tap water were available ad libitum. The mice were kept at an ambient temperature of 25 ± 1°C. The room was illuminated with yellow fluorescent tubes (Philips TL40W/16) in a 12-h cycle (switched on at 6:00 a.m. and off at 6:00 p.m.). These lamps do not emit any measurable UV radiation. No daylight entered the room. In the irradiation set-up, three fluorescent American Philips F40 sun-lamps were mounted above the animal compartments. A perforated metal sheet was positioned between the lamps and the animals to reduce the exposure rate. The lamps were mounted above the animal compartments. A perforated metal sheet was positioned between the lamps and the animals to reduce the exposure rate at the mouse level. Fine tuning of the exposure was accomplished by electronic dimmer circuits that controlled the radiant output of the lamps. A schematic representation of the exposure set-up and the relative spectral energy output of the F40 lamps have been published earlier (18).

The groups of repair-deficient animals (CSB −/− and XPC −/−) consisted of 14 mice, with each 7 heterozygous (CSB +/− and XPC +/−) and 7 wild-type (CSB +/+ and XPC +/+) littermates as controls. The different genotypes were placed in random order under the lamps, and all animals were exposed daily to 80 J/m² (250–400 nm) UV radiation from the Philips F40 lamps. Exposure time was 6 min/day, between 12:30 and 12:36 p.m. In addition, two CSB −/− mice, two XPC −/− mice, and controls were subjected to this dose regimen for 10 days. Mice were killed by cervical dislocation after ether anesthesia, and skin samples were routinely processed (H&E staining) for histopathological examination.

**Animal Observations, Definitions, and Data Analysis.** Animals were checked weekly, and all deviations from normal skin appearance (redness, scratching, scaling, tumors, and others) were recorded. The tumor locations were mapped and numbered for each animal separately, and lesions were counted only if the observation could be confirmed during the next check-up. During the experiment, we distinguished between morphologically evident papillomas (pedunculated, protruding tumors with a “cauliflower-like” surface) and other tumors. Previous experiments have shown that macroscopic identification of papillomas corresponds very well with the histological findings, and that the “other tumors” almost exclusively consist of SCCs and their precursor lesions, actinic keratoses (18–21). Tumors were also subdivided according to diameter: <1 mm, ≥1 mm, and ≥2 mm. Mice were sacrificed when carrying at least 1 tumor with a diameter ≥4 mm. The day of the first exposure of an experimental group was defined as t = 0 (t0). The median tumor induction time. If a tumor was first seen at t = t0, t = t0 was the previous checkup time, then the induction time was defined as t = t0 + t2/2. Graphical representation of the prevalence versus time is based on an actuarial method described by Kaplan and Meier (22) and adapted to carcinogenesis by Peto et al. (23). This procedure computes the chance of tumor-free survival. The death-corrected tumor prevalence is then given by one minus this chance. Individual latency times of first tumors were fitted by a log-normal distribution using a maximum likelihood method for a concise description of the observations in terms of ln(t50) and the SD in In(time-to-first-tumor/mouse).

**RESULTS**

**Sunburn Is Associated with TCR Deficiency.** Exposure of the mammalian skin to UV light results in a vascular response consisting of erythema and edema (generally known as sunburn). In humans, the just perceptible response at low UV doses peaks between 8 and 24 h after exposure and is dominated by erythema (24, 25). Probably because of differences in the cutaneous vascular density, the peak in the murine skin response occurs between 24 and 48 h after exposure, and this threshold response is dominated by edema (26, 27). In the present study, using the Kromayer lamp as UVB source, all CSB −/− mice showed erythema and edema at 48 and 72 h after exposures of 2 s and higher, whereas 1-s exposures did not result in a visible effect on the skin at any of the time points. The CSB +/+ and +/+ mice showed erythema and edema at 48 and 72 h after 16- and 24-s exposures only. Hence, the MEF for the CSB −/− mice is between 1 and 2 s (corresponding to 135–270 J/m²), whereas for wild-type and heterozygous mice, the MEF is between 8 and 16 s (corresponding to 1080–2160 J/m²). In agreement with our previous data for another XPC mouse model (27), we did not observe a difference in response between the XPC −/−, +/+ and +/+ animals. All three genotypes had an MEF between 8 and 16 s (corresponding to 1080–2160 J/m²).

We have established earlier the MEF for the Kromayer lamp for hairless XPA knockout mice, which are defective in both GGR and TCR. These results parallel our present findings for the CSB mice, i.e., an MEF between 1 and 2 s for XPA −/− and between 8 and 16 s for XPA +/+ and +/+ (18). Apparently, erythema and edema are triggered by persistent photoproducts in the template strand of transcriptionally active DNA, rather than by lesions in nontranscribed DNA.

**Epidermal Hyperplasia Is Associated with TCR and GGR Deficiency.** Epidermal hyperplasia can be considered as a protective reaction against carcinogenesis, because the increase in the number of epidermal cells dilutes unrepaired DNA damage and the thickening of the stratum corneum and stratum spinosum reduces the penetration of UV radiation to the basal cell layer. On the other hand, it can also be argued that the epidermal cell proliferation leading to hyperplasia enhances the mutation rate and thus stimulates carcinogenesis (28). To establish the importance of TCR and GGR for development of epidermal hyperplasia, we sacrificed two XPC −/− mice, two CSB −/− mice, and control animals after 10 days of exposure to 80 J/m²/day (Philips F40 lamps; the daily dose used in the chronic exposure experiment). This dose corresponds to about 70% of the MED for CSB −/− mice but is <10% of the MED for XPC −/− mice and repair-proficient control animals. Fig. 1 shows representative samples of the epidermis of XPC −/−, CSB −/−, and control mice after 10 days of exposure. It is evident that both TCR-deficient animals and GGR-deficient animals are prone to epidermal hyperplasia at an exposure level that does not induce a hyperplastic reaction in repair-proficient control animals. Hence, unlike erythema and edema, epidermal hyperplasia appears to be triggered by persistent photoproducts in both transcriptionally active and inactive DNA.

**Parakeratosis and Papillomatosis Are Associated with TCR Deficiency.** Hairless CSB −/− mice show parakeratosis after 10 days of daily UV exposure (Fig. 1B), which is in agreement with earlier findings in shaved CSB −/− mice (11). Under the same experimental conditions XPC −/− mice do not show parakeratosis (Fig. 1A). The parakeratosis is macroscopically visible as scaling of the stratum
of 34 papillomas were found. Evidently, the induction of papillomas is related to a TCR deficiency, rather than a GGR deficiency.

**Development of SCC Is Predominantly Associated with GGR Deficiency.** In a previous study, we have established the susceptibilities of hairless, totally NER-deficient XPA knockout mice and their heterozygous and wild-type littermates to UV-induced carcinogenesis (18). In the present study, we have measured the relative skin cancer susceptibility of hairless mice with a specific defect in TCR (CSB knockouts) or GGR (XPC knockouts). Histological analysis of an aselect sample of tumors (from XPC knockouts, CSB knockouts, and controls) that were macroscopically classified as “nonpapillomas” confirmed that these tumors were SCCs and their precursors, actinic keratoses (data not shown). If the prevalence of SCC (excluding benign papillomas from further quantitative analysis) is plotted on a probability scale versus time on a logarithmic scale, the points fall along a straight line, a cumulative log-normal distribution. Fig. 3 shows these plots and the log-normal distributions for XPC −/−, CSB −/−, and the control animals for both strains, fitted according to a maximum likelihood method. The optimum values for the median tumor induction time \( t_{50} \), \( \mu = \ln(t_{50}) \), and the SD (\( \sigma \)) of the fitted log-normal distributions are shown in Table 1. The prevalence curves for XPC −/−, CSB −/−, and for the repair-proficient controls run roughly parallel (i.e., \( \sigma \) values do not differ significantly). Most susceptible to UV carcinogenesis is the XPC knockout strain, which has a median tumor induction time \( t_{50} \) of 1-mm tumors of 84 days (cumulative UV dose of 6.7 kJ/m²), whereas the CSB knockout strain has a \( t_{50} \) of 115 days (cumulative UV dose of 9.2 kJ/m²). The appearance of skin carcinomas in the XPC controls (both 1/2 and 1/1 mice; \( t_{50} \) of 238 days; cumulative UV dose of 19.0 kJ/m²) does...
not differ significantly from that in the CSB controls (both +/– and +/+ mice; $t_{50}$ of 234 days; cumulative UV dose of 18.7 kJ/m²), as tested by the nonparametric trend analysis as described by Peto et al. (23). A separate data analysis of $XPC$ +/– mice versus $XPC$ +/+ mice and of CSB +/– mice versus CSB +/+ mice does not show a statistically significant difference between heterozygotes and wild-type animals (data not shown). This is in line with our data published previously showing that XPA +/– mice do not have a higher susceptibility to UV carcinogenesis than XPA +/+ animals (18). In conclusion, our data demonstrate that defective GGR contributes more prominently to SCC development than defective TCR.

**DISCUSSION**

In a previous study, we have established the UV-induced skin cancer susceptibility of hairless XPA mice, completely deficient in NER. We have found that mice lacking functional XPA genes develop skin cancer with a latency time that is reduced by a factor $4.2 \pm 0.2$ compared with heterozygote and wild-type littersmates (18). Here, using the same irradiation set-up and exposure times, we have compared UV carcinogenesis in XPC (GGR-deficient) and CSB (TCR-deficient) mice to assess the contributions of GGR and TCR to prevention of skin cancer. Inactivation of XPC and CSB genes appears to speed up UV-induced skin carcinogenesis by a factor of $2.8 \pm 0.2$ (238 days/84 days) and $2.0 \pm 0.2$ (234 days/115 days), respectively.

In the earlier experiment, 1-mm SCCs in the XPA –/– mice appeared with a $t_{50}$ of 78 days ($\mu = 4.36 \pm 0.04$), which is not significantly different from the tumors appearance in XPC –/– mice in the present experiment ($t_{50} = 84$ days; $\mu = 4.43 \pm 0.03$). However, a direct comparison of $t_{50}$s is difficult to achieve because in the XPA study, the tumor appearance in heterozygote and wild-type controls was much later ($t_{50}$ between 313 and 331 days) than in the present study ($t_{50}$ between 234 and 238 days). Because the experimental conditions (set-up and irradiation protocol) were completely identical, this difference in response of control animals is most likely attributable to a difference in genetic background (i.e., the contribution of residual 129/Sv, 129/Ola, C57Bl/6, and FVB DNA in the genome of the F₂ hairless mice). This finding underlines the importance of littersmates as appropriate controls whereas various mutant lines are not strictly congenic. A comparison between the study with XPA mice and the present data can still be made by determining the factor by which skin carcinoma development is accelerated in the respective NER mutants. Thus, a complete NER deficiency leads to a larger increase in susceptibility to UV-induced carcinogenesis (4.2 times faster than controls) than an isolated deficiency in GGR (2.8 times faster) or TCR (2.0 times faster).

An interesting question is whether the skin cancer susceptibilities of XPC mice (GGR-deficient) and CSB mice (TCR-deficient) “add up” to the susceptibility of XPA mice (GGR + TCR-deficient). This might be an oversimplification because the fact that both XPC/CSB double knockout mice and XPA/CSB double knockout mice die before weaning (29) suggests that there is another function for the CSB protein, besides its role in NER. To answer this question properly, we are currently determining the dose-response relationships for UV carcinogenesis in the hairless XPA, XPC, and CSB mouse strains.

Although important in protection against skin cancer, GGR appears not to play a role in the development of UV-induced erythema/edema, parakeratosis, and papillomatosis. We have shown that these phenomena relate exclusively to the capacity to perform TCR. Erythema and edema occur within 48 h after a single exposure and are therefore not expected to originate from mutations in active genes. A plausible explanation, put forward by Ljungman and Zhang (30), is that persisting photolesions in the template strand of active genes block the vital process of transcription by RNA polymerase II, which triggers apoptosis. They have demonstrated that skin fibroblasts from XPA and CSB patients are highly prone to UV-induced apoptosis, whereas cells from XPC patients are not. Recently, these findings have been confirmed for the XPA, XPC, and CSB hairless mouse models. The increased sensitivity to UV-induced apoptosis in XPA and CSB, but not in XPC, correlates well with the sensitivities to sunburn found in the present experiment, suggesting that blockage of transcription is also a key event in the development of sunburn. The same holds for the parakeratosis observed in CSB –/– mice (Figs. 1 and 2). In TCR-deficient animals, parakeratosis can already be found after 10 days of exposure (Fig. 1B), whereas GGR-deficient animals do not show parakeratosis, not even at the tumor-bearing stage (Fig. 2A). Disturbed epidermal differentiation in TCR-deficient animals might well be triggered by blockage of RNA polymerase II by persisting photolesions in transcriptionally active DNA.

We have shown that under chronic UVB exposure, CSB –/– mice get, in addition to SCCs, a fair amount of benign papillomas, the first of which appear after about 9 weeks of exposure. This enhanced formation of papillomas is related to the TCR defect, because XPC

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Table 1 The log-normal parameters, $\mu$ [i.e., $ln(t_{50})$ in days] and $\sigma$, derived from the prevalence for different SCC diameters

<table>
<thead>
<tr>
<th>Group</th>
<th>$\leq 1$ mm</th>
<th>$\geq 1$ mm</th>
<th>$\geq 2$ mm</th>
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<tbody>
<tr>
<td>$XPC$ –/–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{50}$</td>
<td>59</td>
<td>84</td>
<td>111</td>
</tr>
<tr>
<td>$\mu$</td>
<td>4.07 ± 0.03</td>
<td>4.43 ± 0.03</td>
<td>4.71 ± 0.03</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>0.12 ± 0.02</td>
<td>0.10 ± 0.02</td>
<td>0.10 ± 0.02</td>
</tr>
<tr>
<td>$CSB$ –/–</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{50}$</td>
<td>98</td>
<td>115</td>
<td>133</td>
</tr>
<tr>
<td>$\mu$</td>
<td>4.59 ± 0.05</td>
<td>4.75 ± 0.05</td>
<td>4.89 ± 0.05</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>0.19 ± 0.04</td>
<td>0.18 ± 0.04</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>$XPC$ +/– and +/+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$t_{50}$</td>
<td>217</td>
<td>238</td>
<td>271</td>
</tr>
<tr>
<td>$\mu$</td>
<td>5.38 ± 0.05</td>
<td>5.47 ± 0.04</td>
<td>5.60 ± 0.04</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>0.18 ± 0.03</td>
<td>0.14 ± 0.03</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>$CSB$ +/– and +/+</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>$t_{50}$</td>
<td>210</td>
<td>234</td>
<td>261</td>
</tr>
<tr>
<td>$\mu$</td>
<td>5.35 ± 0.03</td>
<td>5.45 ± 0.02</td>
<td>5.56 ± 0.03</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>0.11 ± 0.02</td>
<td>0.08 ± 0.02</td>
<td>0.12 ± 0.02</td>
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mice develop these benign tumors at wild-type levels. We have reported recently that hairless XPA mice also develop papillomas under chronic UVB exposure, and that these tumors carry a G → A transition at codon 12 of the H-ras gene (31). We hypothesized that these mutations are caused by a UVB-induced C → T transition at this dipyrimidine site in the transcribed strand as a result of the TCR defect rather than the GGR defect in XPA mice (31). The association of papillomatosis with a TCR deficiency, as found in the present study, strongly supports this inference.

Similar to human XPC (7, 32), XPC mice are proficient in the removal of 6-4PP and CPDs from the transcribed strand of active genes by TCR (14, 27). Also in agreement with human XPC (32), the XPC mouse model is deficient in the removal of 6-4PP from inactive DNA and from the nontranscribed strand of active genes (14, 27). However, in contrast to the situation in humans, rodents are very inefficient in removing CPDs from nontranscribed DNA (33, 34). This is probably because of the lack of expression of the p48 gene, which is required for GGR of CPDs in human cells (8). As a consequence, the effect of the lack of XPC genes is negligible in removal of CPDs in rodent cells, whereas in human XPC removal of CPDs is strongly affected. The high susceptibility of XPC mice to UV carcinogenesis must, therefore, be attributable to a GGR deficiency of DNA lesions other than CPDs, such as 6-4PP. In human XPC, in addition to nonrepaired 6-4PP, nonrepaired CPDs are likely to contribute to the enhanced carcinogenesis. This is in line with the observation that 85% of skin tumors from XPC patients (35) and only 2% of UV tumors from XPC mice (36) contain CPD-derived CC → TT tandem transitions in the nontranscribed strand of the p53 tumor-suppressor gene. Thus, it is to be expected that the observed increased in UV-induced skin cancer proneness in XPC mice represents an overestimation of the susceptibility of human XPC.

The CSB mouse model is deficient in TCR of 6-4PP and CPD. Because mice have efficient GGR of 6-4PP but not of CPD, 6-4PP are removed from the transcribed strand of active genes by GGR. In human CSB, however, both 6-4PP and CPDs are removed from the transcribed strand of active genes by GGR. Hence, we expect that the skin cancer proneness in CSB mice represents an overestimation of the susceptibility of human CSB. This may explain the absence of skin tumors in CSB patients, whereas CSB mice are clearly skin cancer prone (11).

An explanation for the higher carcinogenic effect of a GGR deficiency compared with that of a TCR deficiency could lie in differential mutational activation of transcriptionally inactive cancer genes. It is conceivable that GGR removes DNA photoproducts from transcriptionally inactive proto-oncogenes, thereby preventing mutations by replication of damage-containing sequences. Thus, enhanced mutagenesis attributable to GGR deficiency can lead to increased conversion of proto-oncogenes into oncogenes, which subsequently can stimulate uncontrolled cell divisions. In addition, the importance of GGR relative to TCR can be explained by the finding that TCR deficiency leads to increased apoptosis, whereas GGR deficiency does not. In summary, stimulation or inhibition of carcinogenesis depends on the balance between mutational activation of proto-oncogenes/mutational inactivation of tumor suppressor genes on the one hand and removal of precarcinogenic cells by apoptosis on the other hand. A TCR deficiency can lead to mutational inactivation of actively transcribed tumor suppressor genes but also to increased apoptosis, the net effect of which appears to be mildly procarcinogenic, at least in rodents. A GGR deficiency can also lead to mutational inactivation of actively transcribed tumor suppressor genes (via the nontranscribed strand) as well as to mutational activation of nontranscribed proto-oncogenes, but it does not influence apoptosis. Apparently, the net effect of GGR deficiency is more severely procarcinogenic.

Sensitivity to sunburn is generally considered to be associated with susceptibility to non-melanoma skin cancer (37). The present study shows that sensitivity to sunburn is related to the capacity to perform TCR, whereas susceptibility to SCC is predominantly associated with the capacity to perform GGR. This implies that the GGR capacity may serve as a better predictor for skin cancer susceptibility than sensitivity to sunburn. Determination of MEDs and GGR capacities in non-melanoma skin cancer patients versus healthy controls will show whether this prediction, based on a study with genetically modified mice, holds true for humans.

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