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Enhanced spontaneous and aflatoxin-induced liver tumorigenesis in xeroderma pigmentosum group A gene-deficient mice

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Xeroderma pigmentosum (XP) is an autosomal recessive hereditary disease featuring defective nucleotide excision repair (NER). XP patients are highly sensitive to sunlight and develop skin cancer at an early age. While the fact that XP patients have a large increase in mortality from skin cancers has been extensively documented, the relation between XP and internal tumors has received little attention. We therefore analyzed development of spontaneous and aflatoxin B_1 (AFB_1)-induced liver tumors in XPA-deficient congenic mice, originally created by repeated back-crosses with inbred C3H/HeN mice. Spontaneous liver tumors were assessed at the age of 16 months in two separate experiments using F5 and F10 lines. The incidence of and average number of spontaneous tumors per mouse were significantly higher in XPA−/− than in XPA+/+ and +/− mice. Similarly, F10 XPA−/− mice receiving i.p. injection of 0.6 or 1.5 mg/kg b.w. AFB_1 at 7 days of age demonstrated more liver tumors than their heterozygous or homozygous positive counterparts when examined at month 11. These results demonstrate that XPA-deficient mice have increased susceptibility to both spontaneous liver tumor development and AFB_1-induced hepatocarcinogenesis.

Introduction

Cells possess several DNA repair systems to protect themselves from DNA damage (1,2). Nucleotide excision repair (NER) was the first to be discovered (3) and its mechanism has been studied in detail. It entails four steps: (i) recognition of DNA damage; (ii) unwinding of the DNA double strand structure around the damaged site; (iii) an incision in the damaged DNA strand; (iv) excision of the site and *de novo* DNA synthesis. It is known that NER is concerned with the repair of UV (ultraviolet)-induced and adduct-dependent DNA damage.

Several human diseases are known to exist with disordered NER mechanisms (4–6), with xeroderma pigmentosum (XP) as an important representative (4). XP is an autosomal recessive hereditary disease whose bearers are highly sensitive to ultraviolet light and develop skin cancers in early life. The fact that cells of XP patients have abnormality in excision repair was first discovered by Cleaver in 1968 (4). XP is divided into eight types – seven complementary groups (A–G) and a variant. Among them, group A (XPA), the most frequent in Japan, manifests a severe clinical phenotype with neurological complications. XPA protein recognizes various DNA damages by binding to the damaged DNA region through its zinc finger domain (7–9) and functions in the first step of NER.

In 1989, Tanaka *et al.* were successful in cloning the XPA gene for the first time (10) and XPA-deficient mice were created in 1995 using gene targeting techniques (11,12). Skin tumors arise at high incidence when XPA-deficient mice are exposed to UV or 9,10-dimethyl-1,2-benzanthracene (DMBA) and it has been confirmed that they have a similar phenotype to human XP patients (11).

While extensive documentation exists for the link between XP and skin cancers (13), with a 2000 fold increased sensitivity to sunlight, the incidence of non-skin tumors in XP patients is reported to be only 10–20 times higher than in normal people (13). However, there have been limited studies of such malignancies (14–16).

In order to cast light on this point, experiments to examine tumorigenesis of internal organs in XPA-deficient mice are useful. We have focused on liver tumorigenesis in these animals. Since the original genetic background of our XPA-deficient mice was hybrid in nature, we repeatedly back-crossed XPA-deficient mice with inbred C3H /HeN mice to establish a congenic line for studies of both spontaneous liver tumors development and aflatoxin B_1 (AFB_1)-induction of hepatocellular lesions. In the present paper, we document that XPA deficient mice have a higher susceptibility than wild type mice and heterozygotes in both models.

Abbreviations: AFB_1, aflatoxin B_1; BrdU, 5-bromo-2′-deoxyuridine; DMBA, 9,10-dimethyl-1,2-benzanthracene; DMSO, dimethyl sulfoxide; NER, nucleotide excision repair; 8-oxo-dGTP, 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-triphosphate; PCR, polymerase chain reaction; UV, ultraviolet; XP, xeroderma pigmentosum; XPA, xeroderma pigmentosum group A.

Materials and methods

XPA-deficient congenic mice with C3H/HeN genetic background

The generation of our XPA-deficient mice by gene targeting in embryonic stem cells was described previously (11). Their genetic background is a hybrid of CBA, C57BL/6 and CD-1. Inbred C3H/HeN mice used for mating were purchased from Japan SLC Laboratory (Hamamatsu, Japan). The original XPA-deficient mice were mated with inbred C3H/HeN mice from generation to generation to establish inbred XPA-deficient mice with a C3H/HeN genetic background.

The mice were genotyped by polymerase chain reaction (PCR) analysis of tail DNA using four primers: W1 (5′-GTG GGT GCT GGG CTG TCT AA-3′), W2 (5′-ATG GCG TGG GTT CTT CTT CA-3′), M1 (5′-ATG GCC GCT TTT CTG GAT TC-3′), M2 (5′-ATG GCG TGG GTT CTT CTT CA-3′). The wild type allele was identified as a 205 bp PCR fragment created by W1, which is situated in intron 3, and W2, which is situated in intron 4 (11). The targeted allele was identified as a 279-bp PCR fragment created by M1, which is situated in intron 3, and M2, which is situated in intron 4 (11). A gel showing the genotype analysis is shown in Figure 1.

Spontaneous liver tumor development was assessed using F5 and F10 lines (F5 and F10 refer to the number of backcrosses), and the AFB_1-induced liver tumor induction was performed with the F10 line. All animals were housed in a controlled environment at 23°C and fed on NMF diet (Oriental Yeast, Tokyo, Japan) and tap water ad libitum.

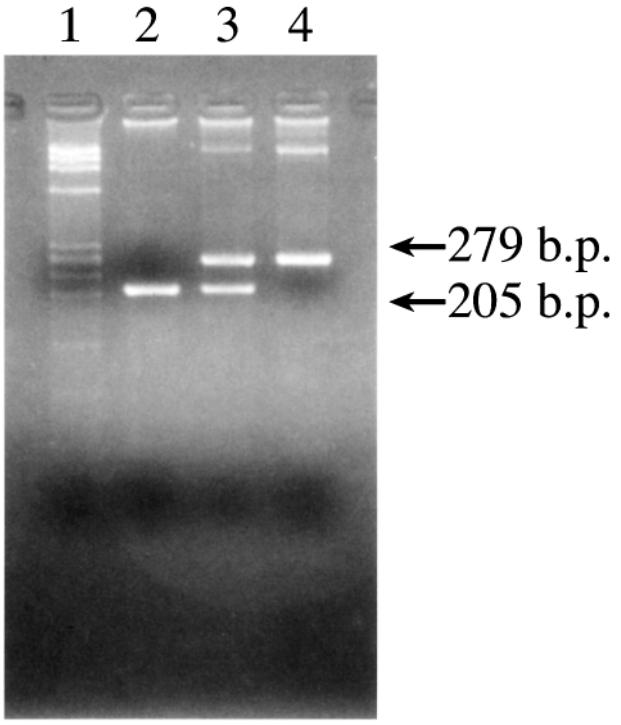

Fig. 1. A gel showing the genotype analysis. The wild type allele is identified as a 205 bp PCR fragment and the targeted allele is identified as a 279 bp PCR fragment. The first lane: DNA molecular size marker (d X174RF DNA/HaeIII fragments), the second lane: XPA+/+, the third lane: XPA+/−, the fourth lane: XPA−/−.

Spontaneous liver tumors

The first experiment was carried out with the F5 line (31/32 with a C3H/HeN genetic background). Male and female XPA+/− mice were mated and resulting male offspring (15 XPA+/+, 40 XPA+/− and 12 XPA−/− mice) were reared, and subjected to complete autopsies at month 16. Similarly, F10 (1023/1024 of the genetic background is C3H/HeN) male and female XPA+/− mice were mated and male offspring (17 XPA+/+, 18 XPA+/− and 14 XPA−/− mice) were killed at month 16. Only male mice were used because it is generally known that C3H strain male mice are more susceptible than female mice with respect to spontaneous and chemically induced carcinogenesis (17–19). Male mice develop spontaneous liver tumors nearly 100% in 1.5 to 2 years, but the incidence in female is far less. This is considered due to sex hormonal (androgen) influence in mice (20). Inbred male C3H mice develop not only hepatocellular adenomas but also hepatocellular carcinomas (21–23). In our previous study using transgenic mice, O^6-methylguanine-DNA-methyltransferase was shown to protect C3H mice from dimethylnitrosamine- and diethylnitrosamine-induced liver tumors (24).

At autopsy, livers were grossly examined and nodules larger than 1 mm in diameter were counted as tumors. Diameters were also measured with calipers. After fixation in 10% formaldehyde solution, each liver lobe was completely cut into 2.0 mm thick slices and routinely processed for light microscopy. Diagnosis of hepatocellular adenomas and carcinomas was made microscopically based on established diagnostic criteria (25). Hepatocellular adenomas are at most 5–10 mm in diameter and clearly demarcated. Relatively small and monotonous tumor cells proliferate, forming a thin trabecular pattern. Hepatocellular carcinomas show a thick trabecular growth pattern with dilated sinusoids. Hemorrhage and necrosis are often observed and cellular atypia is marked.

Group differences were assessed for statistical significance using the χ^2 test for incidences of benign and malignant lesions and the *t*-test for numbers of tumors per mouse and diameters.

AFB_1-induced liver tumors

F10 male and female XPA+/− mice, as well as F10 male and female XPA−/− mice were mated and male offspring received an i.p. injection of 0.6 mg/kg b.w. (11 XPA +/+, 30 XPA +/− and 12 XPA−/− mice) or 1.5 mg/kg b.w. (16 XPA +/+, 31 XPA +/− and 16 XPA−/− mice) AFB_1 (Makor Chemicals, Israel) dissolved in DMSO (dimethyl sulfoxide, Kanto Chemical, Tokyo, Japan) at 7 days of age. All survivors were killed and subjected to a complete autopsy at the end of month 11. As a control group, 17 XPA +/+, 20 XPA +/− and 7 XPA−/− mice received i.p. injection of DMSO (0.05 ml per mouse) at 7 days of age and were similarly killed. The autopsy and material processing was the same as described for the spontaneous liver tumors.

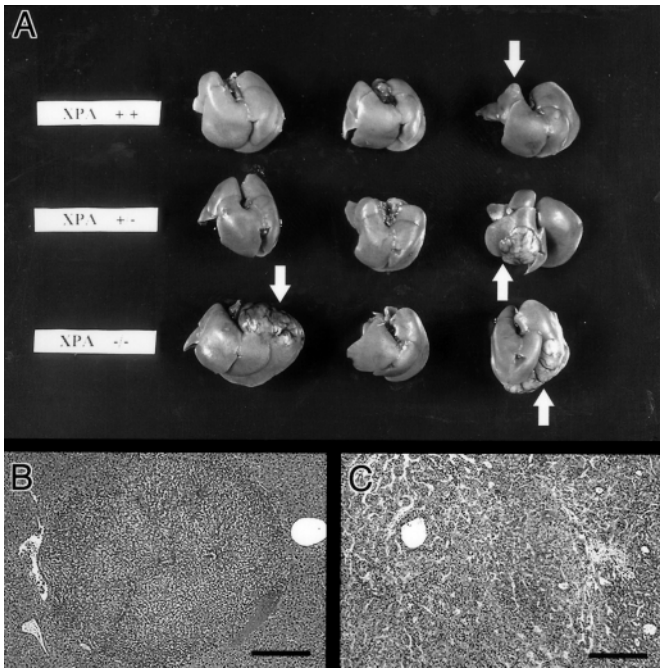

Fig. 2. (**A**) Macroscopic view of the livers of representative XPA+/+, +/− and −/− mice with spontaneous development of liver tumors. Tumors are shown by arrows. (**B–C**) Representative histological appearance of an aflatoxin-induced hepatocellular adenoma (B) and hepatocellular carcinoma (C) (×20). The length of scale bars is 0.25 mm.

S phase hepatocytes

In order to determine levels of hepatocyte division, BrdU (5-bromo-2′-deoxyuridine) immunohistochemical staining was performed. Seven-day-old and 60-day-old XPA+/+ and XPA−/− F10 male mice (four mice in each group) received an i.p. injection of 100 mg/kg b.w. BrdU (SIGMA, USA) dissolved in saline. One hour later, they were killed and their livers were fixed in 10% neutral buffered formaldehyde and embedded in paraffin blocks. Immunohistochemical staining was performed by means of the streptoavidin–biotin–peroxidase complex method using monoclonal antibody against BrdU (Becton Dickinson Immunocytometry Systems, USA). Total numbers of liver cells (>500) and BrdU-positive liver cells were counted in randomly selected areas of liver sections.

S phase hepatocytes and apoptotic hepatocytes in the early stage after AFB_1 administration

In order to ascertain whether there might be variation in cell division and apoptosis induction, 7-day-old XPA+/+ and XPA−/− F10 male mice received an i.p. injection of 1.5 mg/kg b.w. AFB_1 and 2, 4, 8, 16, 24, 48 and 72 h later, four mice of each genotype were killed and subjected to autopsies. One hundred mg/kg b.w. BrdU was injected intraperitoneally 1 h before sacrifice and immunohistochemical staining was performed and BrdU-positive hepatocytes were counted as described above. TUNEL staining was performed using 10% neutral buffered formaldehyde-fixed, paraffin-embedded sections with an Apop Taq Plus Peroxidase *in situ* Apoptosis Detection Kit (Oncor, USA).

Results

Spontaneous liver tumors

At autopsy, the only neoplasms observed were liver tumors. No metastasis was found. Only a few mice died during the experiments and liver tumors were not the cause of death in most of cases. Macroscopic views of representative livers of the three genotypes at autopsy are shown in Figure 2(A). Histologically, hepatocellular adenomas and carcinomas were diagnosed (Figure 2B–C).

Tables I and II summarize the tumorigenesis results of the

Table I. Spontaneous liver tumors in F5 line (C3H/HeN congenic line established by repeated backcrosses for five generations). The experiment was terminated at the age of 16 months

	Genotype		
	+/+	+/−	−/−
No. of mice in experiment	15	40	12
No. of tumor-bearing mice (%)	7 (47%)	21 (53%)	11 (92%)[a,b]
No. of benign tumor-bearing mice (%)	6 (40%)	20 (50%)	9 (75%)
No. of carcinoma-bearing mice (%)	3 (20%)	7 (18%)	7 (58%)[b]
No. of tumors per mouse (average ± SD)	0.9 ± 1.1	0.9 ± 1.1	2.2 ± 1.7[a,b]
No. of benign tumors per mouse (average ± SD	0.6 ± 0.8	0.7 ± 0.9	1.2 ± 0.9
No. of carcinomas per mouse (average ± SD)	0.3 ± 0.6	0.2 ± 0.5	1.0 ± 1.1[a,b]
Diameter of tumors (mm, average ± SD)	6.4 ± 7.1	5.8 ± 5.3	10.2 ± 7.1[b]
Diameter of benign tumors (mm, average ± SD)	2.6 ± 1.7	3.6 ± 2.4	5.2 ± 2.4[a,b]
Diameter of carcinomas (mm, average ± SD)	15.0 ± 7.0	13.8 ± 4.9	16.0 ± 6.2

[a]Significantly different from the +/+ case ($P < 0.05$).
[b]Significantly different from the +/− case ($P < 0.05$).

Table II. Spontaneous liver tumors in F10 line (C3H/HeN congenic line established by repeated backcrosses for 10 generations). The experiment was terminated at the age of 16 months

	Genotype		
	+/+	+/−	−/−
No. of mice in experiment	17	18	14
No. of tumor-bearing mice (%)	8 (47%)	6 (33%)	11 (79%)[b]
No. of benign tumor-bearing mice (%)	7 (41%)	3 (17%)	6 (43%)
No. of carcinoma-bearing mice (%)	2 (12%)	3 (17%)	8 (57%)[a,b]
No. of tumors per mouse (average ± SD)	0.6 ± 0.8	0.3 ± 0.5	1.3 ± 0.9[a,b]
No. of benign tumors per mouse (average ± SD	0.5 ± 0.6	0.2 ± 0.4	0.6 ± 0.9[b]
No. of carcinomas per mouse (average ± SD)	0.1 ± 0.3	0.2 ± 0.4	0.6 ± 0.6[a,b]
Diameter of tumors (mm, average ± SD)	6.8 ± 5.2	9.3 ± 9.0	9.9 ± 8.3
Diameter of benign tumors (mm, average ± SD)	4.9 ± 1.0	1.7 ± 0.6	3.2 ± 1.9
Diameter of carcinomas (mm, average ± SD)	14.5 ± 9.2	17.0 ± 5.3	16.7 ± 6.4

[a]Significantly different from the +/+ case ($P < 0.05$).
[b]Significantly different from the +/− case ($P < 0.05$).

two separate experiments using F5 and F10 lines. The incidences of liver tumors in XPA−/− mice were clearly higher than in their XPA+/+ or XPA+/− counterparts. Similarly, the average number of tumors per mouse was significantly greater (*t*-test, $P < 0.05$). Tumor size also had a tendency to be larger in the XPA−/− mice than in XPA+/+ and +/− mice. When benign and malignant tumors were analyzed separately, the difference in the incidence and multiplicity of benign liver nodules was slight in the different genotypes. But the incidence and multiplicity of malignant liver tumors in XPA−/− mice were significantly higher than in XPA+/+ and XPA+/− mice ($P < 0.05$).

AFB_1-induced liver tumors

Tables III–V summarize the data for liver tumorigenesis. With respect to benign liver tumors, the difference of incidence between XPA−/− mice and other genotypes was slight when 1.5 mg/kg AFB_1 was administered, but the incidence was higher in XPA−/− mice than in XPA+/+ or +/− mice when 0.6 mg/kg AFB_1 was administered. The average number of hepatocellular adenomas per mouse was 2- to 6-fold higher in XPA−/− mice than in XPA+/+ or +/− mice. With respect to malignant liver tumors, the incidence and average number of tumors per mouse were clearly higher in XPA−/− mice than in XPA+/+ or +/− mice. Only a small number of liver tumors occurred in the mice that were administered DMSO only. The DMSO treated control mice at 11 months did not show an effect of XPA−/−, but aflatoxin treatment did increase the frequency of tumors at 11 months in all genotypes.

S phase hepatocytes

Percentages of BrdU positive hepatocytes in 7-day-old XPA+/+ (7.0%) and XPA−/− (6.5%) mice were almost the same. No BrdU positive hepatocytes were observed in 60-day-old XPA+/+ or XPA−/− mice.

S phase hepatocytes and apoptosis of hepatocytes after AFB_1 administration

Percentages of BrdU-positive hepatocyte decreased after AFB_1 administration, showing minimum values at 16 and 8 h after AFB_1 administration in 7-day-old XPA+/+ and −/− mice respectively, thereafter increasing. No apparent differences in the pattern of S phase hepatocytes were observed between XPA+/+ and XPA−/− mice (Figure 3).

The incidence of TUNEL-positive hepatocytes was consistently <0.1% before and after AFB_1 administration in both 7-day-old XPA+/+ and XPA−/− mice. Thus the AFB_1 dose used in this carcinogenesis experiment did not appear to exert a major influence in either the cell cycle or apoptosis.

Table III. Liver tumors induced by 0.6 mg/kg b.w. AFB_1 in F10 line (C3H/HeN congenic line established by repeated backcrosses for 10 generations). The experiment was terminated at the age of 11 months

	Genotype		
	+/+	+/−	−/−
No. of mice in experiment	11	30	12
No. of tumor-bearing mice (%)	6 (55%)	15(50%)	10 (83%)[b]
No. of benign tumor-bearing mice (%)	6 (55%)	15 (50%)	10 (83%)[b]
No. of carcinoma-bearing mice (%)	0 (0%)	4 (13%)	6 (50%)[a,b]
No. of tumors per mouse (average ± SD)	0.7 ± 0.8	1.2 ± 1.7	4.5 ± 3.2[a,b]
No. of benign tumors per mouse (average ± SD	0.7 ± 0.8	0.9 ± 1.3	3.9 ± 2.9[a,b]
No.of carcinomas per mouse (average ± SD)	0	0.3 ± 0.8	0.6 ± 0.7[a]
Diameter of tumors (mm, average ± SD)	3.0 ± 1.7	4.6 ± 4.3	3.9 ± 2.8
Diameter of benign tumors (mm, average ± SD)	3.0 ± 1.7	2.7 ± 2.2	3.1 ± 1.9
Diameter of carcinomas (mm, average ± SD)	–	11.1 ± 3.1	9.0 ± 2.3

[a]Significantly different from the +/+ case ($P < 0.05$).
[b]Significantly different from the +/− case ($P < 0.05$).

Table IV. Liver tumors induced by 1.5 mg/kg b.w. AFB_1 in F10 line (C3H/HeN congenic line established by repeated backcrosses for 10 generations). The experiment was terminated at the age of 11 months

	Genotype		
	+/+	+/−	−/−
No. of mice in experiment	16	31	16
No. of tumor-bearing mice (%)	12 (75%)	21 (68%)	13 (81%)
No. of benign tumor-bearing mice (%)	12 (75%)	21 (68%)	13 (81%)
No. of carcinoma-bearing mice (%)	1 (6%)	2 (6%)	6 (38%)[b]
No. of tumors per mouse (average ± SD)	1.5 ± 1.2	1.8 ± 2.0	4.3 ± 3.3[a,b]
No. of benign tumors per mouse (average ± SD	1.4 ± 1.2	1.7 ± 1.9	3.7 ± 2.7[a,b]
No. of carcinomas per mouse (average ± SD)	0.1 ± 0.3	0.1 ± 0.4	0.6 ± 1.0[a,b]
Diameter of tumors (mm, average ± SD)	1.9 ± 1.5	2.8 ± 2.1	4.5 ± 4.3[a,b]
Diameter of benign tumors (mm, average ± SD)	1.7 ± 0.7	2.5 ± 1.7	3.1 ± 1.8[a,b]
Diameter of carcinomas (mm, average ± SD)	8.0 ± 0.0	8.0 ± 1.7	13.1 ± 5.9

[a]Significantly different from the +/+ case ($P < 0.05$).
[b]Significantly different from the +/− case ($P < 0.05$).

Table V. Liver tumors of F10 line (C3H/HeN congenic line established by repeated backcrosses for 10 generations) administered only by vehicle, DMSO. The experiment was terminated at the age of 11 months

	Genotype		
	+/+	+/−	−/−
No. of mice in experiment	17	20	7
No. of tumor-bearing mice (%)	2 (12%)	4 (20%)	2 (29%)
No. of benign tumor-bearing mice (%)	2 (12%)	3 (15%)	2 (29%)
No. of carcinoma-bearing mice (%)	0 (0%)	1 (5%)	1 (14%)
No. of tumors per mouse (average ± SD)	0.1 ± 0.3	0.2 ± 0.4	0.6 ± 1.1
No. of benign tumors per mouse (average ± SD)	0.1 ± 0.3	0.2 ± 0.4	0.4 ± 0.8
No. of carcinomas per mouse (average ± SD)	0.0 ± 0.0	0.1 ± 0.2	0.1 ± 0.4
Diameter of tumors (mm, average ± SD)	4.0 ± 2.8	3.5 ± 4.4	5.0 ± 3.6
Diameter of benign tumors (mm, average ± SD)	4.0 ± 2.8	1.3 ± 0.6	3.3 ± 1.5
Diameter of carcinomas (mm, average ± SD)	–	10.0 ± 0.0	10.0 ± 0.0

Discussion

The present study showed that XPA-deficient mice have a higher susceptibility to both spontaneous and AFB_1-induced liver tumor development than wild type mice or heterozygote littermates. The results are in line with the report by de Vries *et al.* in 1997 that XPA+/+ and XPA+/− mice (genetic background: Ola 129 50%, C57BL/6 50%) had no spontaneous liver tumors, while four out of 24 XPA−/− mice developed spontaneous hepatocellular adenomas between 15 and 20 months of age (26). In our present experiments, the increased susceptibility of XPA mutant mice to spontaneous liver tumors was reproducible, being observed in two separate experiments with F5 and F10 lines.

Several candidate genes that might determine liver tumor susceptibility in C3H/HeN mice have already been mapped on chromosomes 2, 5, 7, 8, 12, 19 by linkage analysis (27,28). The functions of those genes are not clear yet, but there is the possibility that they regulate the growth of preneoplastic lesions

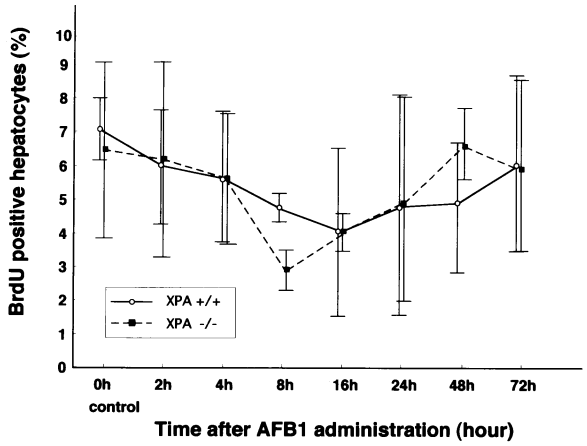

Fig. 3. Change in percentage of BrdU positive hepatocytes with time after AFB_1 administration. Seven-day-old XPA+/+ and XPA−/− mice received an i.p. injection of 1.5 mg/kg b.w. AFB_1 and four mice of each genotype were killed at various time points. BrdU was administered 1 h before killing and immunohistochemical staining was performed. Bars mean SD.

(29). The present results suggest that the XPA gene protects mice against spontaneous liver tumorigenesis through repair processes of DNA damage. Various carcinogens present in the environment may be the cause of DNA damage. Furthermore, reactive oxygen species are generated during metabolic processes in living organisms and it is known that NER is operative in the repair of associated DNA damage (30,31). However, there is no direct evidence that oxidative stress is actually involved in liver tumorigenesis in XPA-deficient mice. The difference in the incidence and multiplicity in different genotypes was more remarkable in malignant liver tumors than in benign tumors. This fact implies that XPA gene has some relation to promotion or progression step of liver carcinogenesis. XPA deficiency might cause mutations in the genes that regulate the promotion or progression of liver carcinogenesis. Several oncogenes and suppressor oncogenes, such as H-*ras*, K-*ras*, N-*ras*, c-*myc*, c-*raf*, c-*fos*, *p53* are thought to be candidate genes for mouse hepatocarcinogenesis (32,33). The lack of any appreciable difference between XPA-deficient and wild type mice in terms of BrdU incorporation at 7 and 60 days of age means that an influence of the cell cycle is unlikely.

AFB_1, a mycotoxin produced by *Aspergillus flavus*, is considered to be an important factor for liver cancers in Africa and Southeast Asia. It is activated into AFB_1 exo-8,9-epoxide by cytochrome p450s (p450 3A4, p450 1A2), prostaglandin synthase and lipoxygenases (34–36) and reacts with DNA to form guanyl N7 adducts (37,38). Cell culture studies have confirmed that such aflatoxin-DNA adducts are rapidly removed by NER in normal cells, while persisting in cells of XP patients (39). Hepatocarcinogenicity of aflatoxin has been clearly demonstrated in rats (40), ducks (41) and rainbow trout (42–44) even when administered to adult animals. On the other hand, in the case of mice, adult animals are extremely resistant to its carcinogenicity (45). In 1972, Vesselinovitch *et al.* discovered that AFB_1 causes liver tumors in mice when administered to newborn animals (46), and this model is now well established (47,48). In the present experiments, when a single 0.6 mg/kg b.w. or 1.5 mg/kg b.w. dose of AFB_1 was administered to 7-day-old animals, XPA−/− mice had a higher multiplicity of liver tumors (both benign and malignant) than XPA+/+ and +/− mice at month 11. When 1.5 mg/kg AFB_1 was administered, the incidence of liver tumors in XPA−/− mice was not remarkably higher than in XPA+/+ or +/− mice. However, when 0.6 mg/kg AFB_1 was administered, the incidence of liver tumors in XPA−/− mice was higher than in XPA+/+ or +/− mice and the average number of liver tumors per mouse was significantly higher in XPA−/− mice than in XPA+/+ or +/− mice in both AFB_1 dosage. The aflatoxin dose used appeared to be too small to elicit necrosis or apoptosis.

The majority of malignant tumors that arise in XP patients are skin cancers, and the incidence of skin cancer of XP patients is estimated to be >2000 times that of normal people (49,50). While, internal tumors are much less common (13). However, as shown here, NER is also involved in the repair of DNA damage caused by factors other than UV.

Earlier, no difference in lesion incidence was found between XPA +/− and +/+ mice in terms of skin tumor induction by UV and DMBA (11), lymphoma induction by benzopyrene (26) and spontaneous tumors (26). Our experiments also suggest no apparent difference between XPA+/+ and +/− mice regarding either spontaneous or AFB_1-induced liver tumors. Thus gene dosage effects were not manifested.

There have been several indications of carcinogenesis in internal organ using XPA-deficient mice. De Vries *et al.* (26) demonstrated that XPA−/− mice are predisposed to induction of lymphomas by exposure to benzo[*a*]pyrene. The same group briefly demonstrated that XPA−/− mice treated with 2-acetylaminofluorene developed tumors in 100% of female livers and in 90% of male bladders (51). They also observed 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine (PhIP)-induced adenomas of the small intestine in XPA−/− mice, although at low frequency. Another experimental study showed that XPC-deficient mice, which are also defective in NER, are highly sensitive with regard to liver and lung carcinogenesis on treatment with 2-acetylaminofluorene (52). In our own study, XPA−/− mice treated with an intratracheal instillation of benzo[*a*]pyrene had a statistically significant increase of lung tumors (53). We also found that chronic 4-nitroquinoline 1-oxide exposure leads to tongue carcinomas, with high frequency, only in XPA−/− mice (54).

In summary, XPA-deficient congenic mice lines constructed with a C3H/HeN genetic background by back-crossing hybrid XPA-deficient mice with inbred C3H/HeN mice, are more susceptible to development of both spontaneous and carcinogen-induced liver tumors than wild type mice and heterozygotes.

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