Review Article

DNA repair and cancer: Lessons from mutant mouse models

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DNA damage, if the repair process, especially nucleotide excision repair (NER), is compromised or the lesion is repaired by some other error-prone mechanism, causes mutation and ultimately contributes to neoplastic transformation. Impairment of components of the DNA damage response pathway (e.g., *p53*) is also implicated in carcinogenesis. We currently have considerable knowledge of the role of DNA repair genes as tumor suppressors, both clinically and experimentally. The deleterious clinical consequences of inherited defects in DNA repair system are apparent from several human cancer predisposition syndromes (e.g., NER-compromised xeroderma pigmentosum [XP] and *p53*-deficient Li-Fraumeni syndrome). However, experimental studies to support the clinical evidence are hampered by the lack of powerful animal models. Here, we review *in vivo* experimental data suggesting the protective function of DNA repair machinery in chemical carcinogenesis. We specifically focus on the three DNA repair genes, *O*^{6}-methylguanine-DNA methyltransferase gene (*MGMT*), XP group A gene (*XPA*) and *p53*. First, mice overexpressing *MGMT* display substantial resistance to nitrosamine-induced hepatocarcinogenesis. In addition, a reduction of spontaneous liver tumors and longer survival times were evident. However, there are no known mutations in the human *MGMT* and therefore no associated cancer syndrome. Secondly, *XPA* mutant mice are indeed prone to spontaneous and carcinogen-induced tumorigenesis in internal organs (which are not exposed to sunlight). The concomitant loss of *p53* resulted in accelerated onset of carcinogenesis. Finally, *p53* null mice are predisposed to brain tumors upon transplacental exposure to a carcinogen. Accumulated evidence in these three mutant mouse models firmly supports the notion that the DNA repair system is vital for protection against cancer. (Cancer Sci 2004; 95: 112–117)

The DNA repair system is crucial in genome maintenance. DNA damage arises as a consequence of exposure to environmental mutagens, but can also occur through endogenous mechanisms. To defend organisms against detrimental consequences of DNA damage, a complex network of repair strategies, each focusing on a different class of lesions, is evolutionally conserved from bacteria to man. Alterations in DNA structure, if left unrepaired, cause genome instability that enhances cancer risk. Conversely, maintaining genome integrity has emerged as a major factor in cancer prevention; however, *in vivo* proof in support of this notion has been insufficient until recently, because of the lack of appropriate animal models. Continuous work in our laboratory during the past few years has centered on the possible involvement of DNA repair machinery in chemical carcinogenesis. We used several kinds of mutant mice bearing genetically engineered DNA repair genes. First, a transgenic mouse line overexpressing *Escherichia coli* *O*^{6}-methylguanine-DNA methyltransferase (*MGMT*) gene, *ada* was generated by our group. A study of nitrosamine-induced hepatocarcinogenesis revealed that the tumorigenesis was dramatically suppressed in *ada* mice. We next observed that xeroderma pigmentosum group A gene (*XPA*)-deficient mice with a selective impairment of nucleotide excision repair, an animal model for human XPA, developed skin tumors at high incidence when exposed to 7,12-dimethylbenz[*a*]anthracene, as they did when exposed to ultraviolet (UV)-B irradiation. Furthermore, experimental approaches targeting the liver, lung and tongue of *XPA*-deficient mouse helped to answer the intriguing problem of whether internal organs of XP patients are also prone to cancer. Finally, we found that loss of *p53* might be of direct significance to early events in brain tumorigenesis by transplacental exposure of *p53*-deficient mice to ethylnitrosourea. Collectively, lessons learnt from these three genetic mouse models provide ample evidence that DNA repair genes including *MGMT, XPA* and *p53* actually protect against cancer.

Background

Cancer is caused by multiple irreversible mutations in genes critical for cell growth.[1)] Since the DNA repair network plays a key role in faithful maintenance of the genome, inherited or acquired abrogation of its function is detrimental and potentiates increased genome instability, leading to cancer.[2–8)] To address the importance of DNA repair pathways in carcinogenesis, we have conducted several experiments using mutant mice.[9)] An understanding of the efficacy of DNA repair as a "cancer protector" is very difficult to obtain from humans, but animal models enable us to analyze the stepwise effects of DNA repair genes on multistage carcinogenesis *in vivo*. Owing to space constraints, we will focus on *MGMT*, *XPA* and *p53*. Since a comprehensive overview is beyond the scope of this review, not all significant information on the intricate inter-relationship between DNA repair and cancer can be mentioned. Interested readers may refer to several excellent review articles.[4–8)]

MGMT is a rapid and error-free DNA repair enzyme that eliminate the alkylating lesion of *O*^{6}-methylguanine which is generated both endogenously and by environmental contaminants.[10, 11)] In 1993, transgenic mice overexpressing *E. coli MGMT*, *ada* were established for the first time in our laboratory.[12–14)] *Ada* mice provided new opportunities to examine the relevance of *MGMT* to cancer prevention *in vivo*.[14)]

XP is a rare autosomal recessive hereditary disease arising from deficiency in a DNA repair process, nucleotide excision repair (NER), and is currently divided into seven complemen-

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tary groups (denoted XPA through XPG) and a variant.[15, 16] Of these, XPA exhibits the most severe symptoms, with both skin cancers and neurodevelopmental abnormalities. Following Cleaver's intuitive notion in 1968,[17] XP is considered as the prototype of the NER syndromes, representing the best human example of a direct link between NER deficiency and cancer risk. Twenty-two years later, the NER gene responsible for *XPA* was successfully cloned and *XPA*-deficient mice were subsequently established in 1995.[18] This model mouse for the first time allowed *in vivo* investigation of the role of NER in carcinogenesis. A wealth of information from several *XP*-deficient strains of mice is in line with the epidemiological data that XP results in a marked predisposition to cancer in the sun-exposed skin.[19] However, it was still uncertain whether internal organs are also susceptible to cancer.[7] In order to shed light on this point, we conducted several chemical carcinogenesis studies in internal organs of *XPA*-deficient mice.[9]

p53 acts as a gatekeeper for cellular proofreading in response to many stress signals, including oncogenic DNA damage.[1, 20] Thus, mutation in *p53* is the core event during the multistep process of carcinogenesis. From 1992 to 1994, *p53*-deficient mice were successively developed by several independent groups of scientists.[21] Based on studies with these mutant mice, it has been proposed that loss of *p53* contributes to the later promotion or progression step of carcinogenesis.[21, 22] However, *p53* may have different potential in different tissues.[20] To access the relative contribution of *p53* to brain tumor development, transplacental chemical carcinogenesis in *p53* heterozygous pregnant mice was performed by our group.[23]

In the past several years, considerable progress has been achieved in understanding the implications of DNA repair for cancer control.[4–8] Here, we arrange and present our data gathered from the above three mutant mouse experiments and offer a brief discussion of the importance of the DNA caretaking and gatekeeping systems for cancer prevention. Although these mice are not perfect models for human cancer, the principal findings can be applied to humans.

MGMT as a key enzyme for eliminating *O*^{6}-methylguanine

Alkylating carcinogens produce various kinds of alkylated bases in DNA. Of these, *O*^{6}-methylguanine is the most potent mutagenic lesion and preferentially pairs with thymine, resulting in a G-C to A-T transition mutation.[10, 11] This type of DNA damage can be repaired by a direct reversal process catalyzed by MGMT, which transfers a methyl group from the *O*^{6}-methylguanine moieties of double-stranded DNA to a cysteine residue of the MGMT molecule itself.[4–6, 10, 11] MGMT has been demonstrated to be present in various organisms, including bacteria, yeast, fish, rodents, monkey and humans, at sufficient levels throughout the lifetime, but its activity varies considerably among species. In general, its activity is the highest in liver and is higher in humans than in rodents.[24–26]

Nitrosamine-induced liver tumors in *ada* mice[14]

We generated transgenic mice on a C3H/HeN background by introducing the *E. coli* MGMT gene, *ada*, attached to the Chinese hamster metallothionein I promoter.[12–14] In the liver of *ada* mice, MGMT activity was 3 times higher than in control littermates, and can be increased up to 8 times by treatment with zinc. We adopted nitrosamine as a hepatocarcinogen because it is a prototype alkylating agent ubiquitously present in our environment. Groups of *ada* and control (non-transgenic) mice received $ZnSO_4$ treatment and then were injected intaperitoneally with various doses of dimethylnitrosamine (DMNA). The most striking differences between *ada* and control mice were observed in females given 5 mg of DMNA and sacrificed at 9 months. With respect to the tumor multiplicity, *ada* mice showed significantly lower values than the control littermates (13% vs. 68%), and no carcinoma was produced. By month 11, a similar result was obtained in males given 1 mg of DMNA, but carcinomas were induced in 4% of *ada* mice. Representative tumor-bearing livers are shown in Fig. 1. In the diethylnitrosamine-treated groups, female *ada* mice given 5 mg had significantly fewer tumors (19% vs. 56%). Soon after, our observations were confirmed by other experiments in which elevated levels of human *MGMT* in thymus efficiently protected mice from lymphomas after treatment with methylnitrosourea (MNU).[27] Further, carcinogenesis experiments involving our laboratory revealed that a large number of tumors developed in *MGMT*-deficient mice exposed to MNU or DMNA, whereas no or few tumors occurred in normal control littermates.[28–31] Thus, the pooled data provide unequivocal evidence that *MGMT* plays a protective role in carcinogenesis by alkylating agents.

XPA as a caretaker for DNA damage: NER and XP

NER is the most flexible of all DNA repair mechanisms and is an absolutely error-free process responsible for the elimination of a broad spectrum of chemically and structurally distinct lesions.[4–8, 19] In general, NER specifically protects against mutations caused by environmental carcinogens, and thus is predominantly responsible for cancer protection.[7, 8] As outlined above, deficiency in a single NER gene has shown to be associated with the human inherited cancer-prone disease XP.[15, 16] Patients with XP are hypersensitive to UV and have a 1000-fold increased risk of skin cancer.[32] The mean age of onset of the first skin cancer is 8 years, which is nearly 50 years earlier than in the general population. The importance of NER in the prevention of UV-induced skin cancer has been well established by several experiments using *XP*-deficient mice.[19] In addition, information gathered from a literature survey suggests that XP patients have a 10- to 20-fold increased risk of cancers in internal organs.[6, 19, 33–37] The relatively lower internal tumor frequency might be due to the fact that XP patients rarely survive beyond the third decade of life, as a consequence of the dramatic development of skin cancer or severe neurological degeneration.[7] Needless to say, an increase in the frequency of internal tumors in XP can not readily be explained in terms of UV-induced DNA damage.

7,12-Dimethylbenz[*a*]anthracene (DMBA)-induced skin tumors[18]

A single UV-B irradiation evoked severe inflammatory edema in *XPA*-deficient mouse skin. In a UV-B-induced skin carcinogenesis study, these mice all animals had squamous cell carcinomas within 34 weeks. Similarly, *XPA*-deficient mice de-

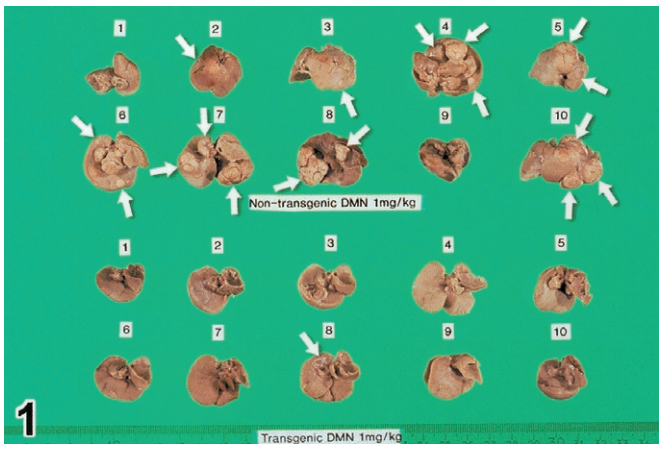

Fig. 1. DMNA-induced liver tumors in male *ada* (transgenic) and control (non-transgenic) mice. Arrows indicate tumors. Tumor-bearing livers are significantly fewer in *ada* mice.

veloped skin ulcers after an application of DMBA. As shown in Fig. 2, repeated painting with 10 μg of DMBA resulted in skin tumor formation in *XPA*-deficient mice. At 18 weeks, 90% of the *XPA*-deficient mice bore tumors. Interestingly, the DMBA-induced skin tumors were papillomas. A high frequency of papillomas was also found at lower daily UV doses.[19]

Benzo[*a*]pyrene (B[*a*]P)-induced lung tumors[38]

B[*a*]P is the most important environmental carcinogen. *XPA*-deficient mice were instilled intratracheally with 0.5 mg of B[*a*]P once a week for 4 weeks. The pulmonary adenoma incidence in *XPA*-deficient mice at month 16 was significantly higher than in control littermates (71% vs. 35%). Similarly, tumor multiplicity was elevated and, in addition, only *XPA*-deficient mice bore carcinomas.

Aflatoxin B_1 (AFB_1)-induced and spontaneous liver tumors[39]

AFB_1 is a potent hepatocarcinogenic mycotoxin which contaminates traditional diets. We recently created a congenic *XPA*-deficient mouse line by repeated back-crosses with the inbred C3H/HeN strain for hepatocarcinogenesis experiments. F10 *XPA*-deficient mice were given an intraperitoneal injection of 0.6 mg of AFB_1 at 7 days of age. By month 11, they had more liver tumors than wild-type mice (83% vs. 55%). Carcinomas were found exclusively in *XPA*-deficient mice. As shown in Fig. 3, the incidence of spontaneous liver tumors at 16 months was also significantly higher in F5 *XPA*-deficient mice than in wild-type littermates (92% vs. 47%). In addition, carcinoma-bearing livers were more frequent in F10 *XPA*-deficient line (51% vs. 12%).

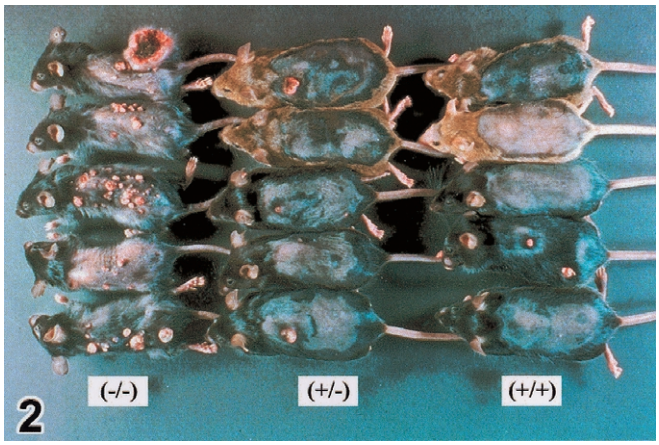

Fig. 2. DMBA-induced skin tumors in *XPA*-deficient (left), heterozygous (middle) and wild-type (right) mice. Tumors are more frequent in *XPA*-deficient mice.

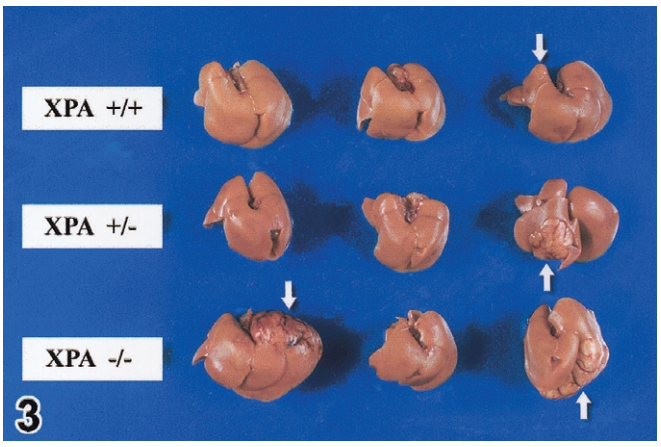

Fig. 3. Spontaneous liver tumors in *XPA*-deficient (lower), heterozygous (middle) and wild-type (upper) mice. Tumors (arrows) are significantly more frequent in *XPA*-deficient mice.

4-Nitroquinoline 1-oxide (4NQO)-induced tongue tumors[40]

XPA-deficient mice were orally given 10 ppm 4NQO in their drinking water. After 50 weeks, tongue squamous cell carcinomas occurred in more than 90% of *XPA*-deficient mice (Fig. 4). *p53* missense mutations were identified in 20% of tongue carcinomas by sequencing analysis. To further address the interplay between NER and *p53*, we then generated an *XPA*-deficient mouse line lacking *p53*. As expected, the *XPA/p53*-deficient tongue showed an aggravated response to 4NQO and interestingly, loss of heterozygosity of the wild-type *p53* was not observed.[41] The importance of NER for preventing cancer in internal organs other than the skin seems clear from our analysis of three independent carcinogenesis experiments.[9]

p53 as a gatekeeper for cancer prevention

p53 is located on chromosome 17 p13 and seems to be a key tumor suppressor gene, because it is mutated in about half of all cancer types arising from a variety of tissues.[1, 20] Furthermore, human Li-Fraumeni patients who carry germline *p53* mutations develop a variety of different tumors at an early age. The most frequent are breast and brain tumors, followed by sarcoma and adrenocortical cancers.[20] Inactivation of *p53* function facilitates genome instability. Consequently, loss of *p53*, through clonal expansion of cells in which unrepaired DNA damage would lead to mutation, creates conditions that drive tumorigenesis.[1] In the previous experiments, astrocytes isolated from *p53*-deficient mouse brain showed an increased growth advantage, resulting in malignant transformation *in vitro*.[23]

Ethylnitrosourea (ENU)-induced brain tumors[23]

We treated groups of pregnant mice heterozygous for *p53* transplacentally with ENU at gestational day 12.5–16.5. A total of 168 offspring were genotyped and monitored for tumor development. In 70.6% of *p53*-deficient offspring, brain tumors were induced (Fig. 5, A and B), whereas only 3.6% of *p53* heterozygous pups bore tumors. There was no evidence of brain tumor in the wild-type mice. Tumors developed in the cerebrum near the hippocampus, just above the lateral ventricles (Fig. 5C) and often showed ventricular and subarachnoidal involvements (Fig. 5D). They were diagnosed histopathologically as glioblastomas because of the intense immunopositivity for glial

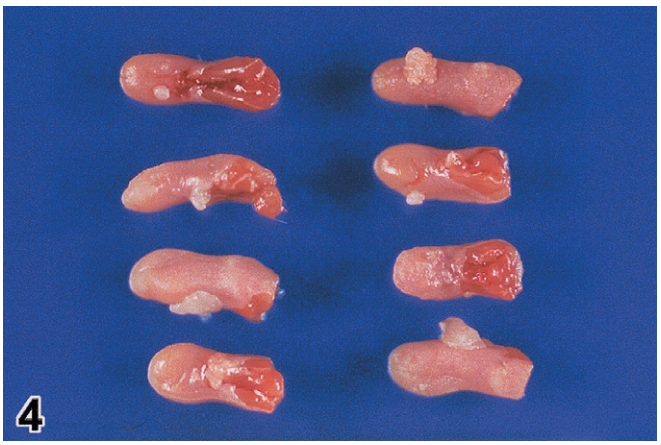

Fig. 4. 4NQO-induced tongue carcinomas in *XPA*-deficient mice.

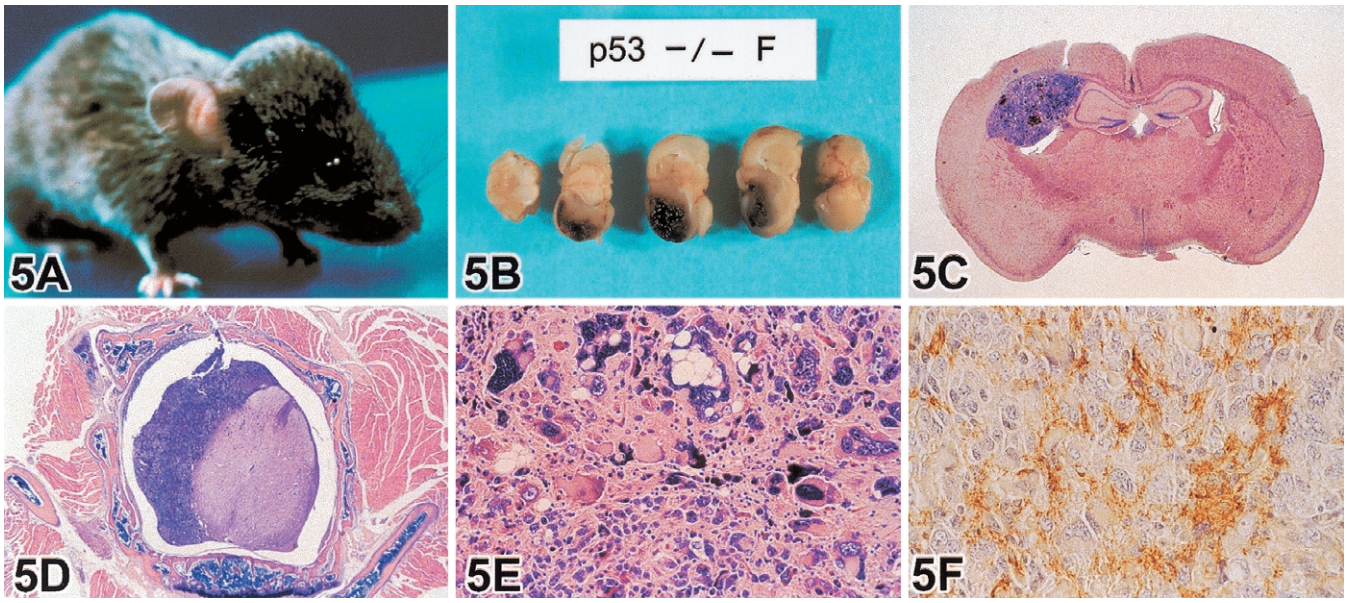

Fig. 5. ENU-induced brain tumors in *p53*-deficient mice. (A) Gross appearance of a tumor-bearing mouse. (B) Brain tumors showing hemorrhage and necrosis. (C) Tumor developed in the cerebrum near hippocampus just above the ventricle (H&E). (D) Tumor invading the subarachnoidal space of the spinal cord (H&E). (E) Tumor showing typical features of glioblastoma (H&E). (F) Tumor cells are immunopositive for glial fibrillary acidic protein (immunohistochemistry).

fibrillary acidic protein and S-100 protein (Fig. 5, E and F). All brain tumors developed in heterozygous mice had already lost the wild-type allele of *p53* by PCR analysis. These results indicate that loss of *p53* may be a prerequisite for brain tumor development.

Aryl hydrocarbon receptor (*AhR*) acts as a modulator in carcinogenesis

Other interesting issues includes the proposed involvement of *AhR* in the process of carcinogenesis. Although *AhR* itself is strictly not a DNA repair gene, a direct or indirect contribution of *AhR* to carcinogenesis, especially to polycyclic aromatic hydrocarbon (PAH)-induced carcinogenesis has been repeatedly proposed.[42] Therefore, information obtained in a related model will be discussed here. Briefly, skin carcinogenicity of B[*a*]P was completely lost in *AhR*-deficient mice and there was no transcriptional expression of CYP1A1 in the B[*a*]P-treated *AhR*-deficient mouse skin.[43] Thus, B[*a*]P carcinogenicity may be determined primarily by CYP1A1 through the *AhR* pathway. Our ongoing work highlights that skin carcinogenicity of airborne particle extracts is mediated by *AhR*, but its contribution to PAH carcinogenesis may vary greatly depending on the PAH tested, including DMBA and dibenzo[*a,l*]pyrene (unpublished results).

Conclusions

MGMT and cancer

It is generally recognized that the tumor incidence increases gradually with advancing age.[44] In explanation, possible age-related differences in DNA repair capability have been suggested; however, most studies have failed to detect age-related changes in DNA repair, especially NER activity.[45] Our measurement of MGMT in both human lung[26] and mouse liver[25] at various ages also gave similar results. To examine the effect of lifetime elevation of MGMT activity on spontaneous liver tumors, we compared *ada* mice and control littermates along with H-*ras* mutants.[46] There was no significant difference in the tumor frequency or mutation spectrum, but the incidence of carcinomas was far less in *ada* mice. This interesting observation suggests an additional role of *MGMT* in protecting malignant conversion of liver tumors. Similar results were obtained in *MGMT* transgenic mouse skin carcinogenesis by MNU.[47]

Unfortunately, no human analogue of *MGMT*-deficient mice is known at present.[48] If a population with *MGMT* deficiency or decreased MGMT activity does exist, affected individuals would be expected to suffer from cancer at higher frequency. Thus, we searched for germ line polymorphism of *MGMT* in young patients with cancer of adult type.[49] A nucleotide difference at codon 160 in exon 5 was detected in 25% of patients, and also in 10% of the control group, suggesting that this is a normal polymorphism among Japanese. This is in keeping with the finding that *MGMT*-deficient mice do not show any cancer-prone phenotype under natural conditions.[28–31]

XPA and internal cancer

Study of the importance of NER for internal tumor development in XP has been hampered by limited epidemiological data.[7, 19, 33–37] Mouse models for XP can serve to solve this long-standing problem; however, no firm conclusion has so far been drawn.[19] There have been a few reports on chemical carcinogenesis in internal organs of *XP*-deficient mice.[7, 19, 38–40] Briefly, *XPA*-deficient mice are predisposed to B[*a*]P induction of lymphomas, and 2-acetylaminofluorene induced liver and bladder tumors at high incidence.[19] Adenomas of the small intestine were induced in these mice by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), although at low frequency. Another experimental study showed that *XPC*-deficient mice were highly sensitive to liver and lung carcinogenesis after treatment with 2-acetylaminofluorene.[19] In our study, B[*a*]P- or AFB_1-treated *XPA*-deficient mice had a statistically significant increase of lung or liver tumors.[38, 39] It is of interest that human XP cells were hypersensitive to killing and mutation by B[*a*]P and AFB_1.[4] We also found that 4NQO exposure led to tongue carcinomas in *XPA*-deficient mice.[40, 41] Epidemiologically, the estimated incidence of squamous cell carcinoma of the tongue is elevated more than 100,000-fold in XP.[7] Our finding correlates well with the *in vitro* observations that 4NQO is a UV-mimetic agent and UV-sensitive cells from XP could not remove 4NQO-DNA adducts. Accumulating evidence suggests that a

deficiency in NER predisposes mice to chemical carcinogenesis in internal organs.[19, 37] In addition to experimentally induced internal tumors, *XPA*-deficient mice have a high frequency of developing spontaneous liver tumors, indicative of high levels of NER lesions accumulated in the metabolically active liver.[19, 39] However, spontaneously occurring extrahepatic tumors were not noticed in these mice. Several possible explanations can be put forward. Most importantly, in the mouse, there is simply not enough time for internal cancers to develop in the short lifespan, since time is a critical factor for onset. Although internal tumors in XP may be closely associated with exogenous or endogenous NER-linked DNA damaging agents,[6–8, 19, 37] it should be kept in mind that DNA damage capable of initiating internal tumors may be preferentially eliminated by repair processes other than NER.[4]

p53 and brain tumors

Since its discovery more than 20 years ago, *p53* has been assigned a central status in the molecular mechanisms of cancer.[1, 20] The most striking finding in our study of *p53*-deficient mouse carcinogenesis is the induction of glioblastomas.[23] As described above, brain tumors are very frequently present in the Li-Fraumeni syndrome, a human cancer syndrome *p53* deficiency.[20] It is well-known that transplacental exposure to ENU caused several types of brain tumors in rats. However, there have been few reports on the experimental induction of malignant brain tumors in mice.[21] Our results also provide a new insight into *p53* functions in carcinogenesis. As already mentioned, previous experiments using *p53*-deficient mice revealed that *p53* alterations are late events in the carcinogenic process, generally occurring along with tumor progression.[21, 22] To the contrary, brain tumors developed exclusively in a *p53*-null environment, thus suggesting that loss of *p53* is an early event.[22] This finding is seemingly in accordance with human clinical data. Comparable with previous findings on transplacental teratogenesis using B[*a*]P, *p53* may protect embryos from DNA damage in the brain induced by ENU.[23] As in tongue carcinogenesis studies in *XPA/p53*-deficient mice,[41] it is likely that in the absence of NER, haploinsufficient status of *p53* may be a predisposing factor for brain tumor development. This will be a focus of future studies.

Concluding remarks and future directions

An ever-growing number of investigations of mutant mouse models has culminated in a breakthrough in our insight into the role of DNA damage and repair in the multistep process of carcinogenesis *in vivo*.[7, 9, 19, 44, 48] When translating the observations from mice to humans, one should take into account that mouse-human differences exist in certain aspects of DNA repair characteristics, metabolic rate and life span. As a whole, human cells are more proficient in the repair of DNA damage.[19] Moreover, the high carcinogen exposure used in animal experiments is not realistic in the human situation. Yet, the main conclusions about the importance of DNA repair in maintaining the fidelity of genome information and in controlling the induction of cancer gathered from mouse models seem valid for humans too. Human cancer is a complicated genetic disease caused by complex exposure to very low daily doses of endogenous and exogenous DNA damaging agents over a long period.[48] Considering the plethora of types of lesions, no single DNA repair process can cope with all kinds of DNA damage. To date, four main, partly overlapping systems have been found in mammals; direct reversal, excision repair (NER, base excision repair and mismatch repair), homologous recombination and end-joining, and replication bypass.[4–6] Unfortunately, it remains a matter of debate whether the different DNA repair processes actually co-operate, and backup pathways do exist for preventing cancer *in vivo*. Thus, further studies are required to identify whether mutant mice defective in two or more of the DNA repair genes are more susceptible to cancer.[19, 21, 50] Such groundwork may help to decipher which pathway is indeed critical for suppressing cancer in mice and, by extrapolation, in humans. These powerful mouse models will also help to uncover the complexity of the interactions between the above genome maintenance systems in the near future.

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