Synergistic interactions between *XPC* and *p53* mutations in double-mutant mice: neural tube abnormalities and accelerated UV radiation-induced skin cancer

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The significance of DNA repair to human health has been well documented by studies on xeroderma pigmentosum (XP) patients, who suffer a dramatically increased risk of cancer in sun-exposed areas of their skin [1,2]. This autosomal recessive disorder has been directly associated with a defect in nucleotide excision–repair (NER) [1,2]. Like human XP individuals, mice carrying homozygous mutations in XP genes manifest a predisposition to skin carcinogenesis following exposure to ultraviolet (UV) radiation [3–5]. Recent studies have suggested that, in addition to roles in apoptosis [6] and cell-cycle checkpoint control [7] in response to DNA damage, p53 protein may modulate NER [8]. Mutations in the *p53* gene have been observed in 50 % of all human tumors [9] and have been implicated in both the early [10] and late [11] stages of skin cancer. To examine the consequences of a combined deficiency of the XPC and the p53 proteins in mice, we generated double-mutant animals. We document a spectrum of neural tube defects in *XPC p53* mutant embryos. Additionally, we show that, following exposure to UV-B radiation, *XPC p53* mutant mice have more severe solar keratosis and suffer accelerated skin cancer compared with *XPC* mutant mice that are wild-type with respect to *p53*.

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Received: 6 September 1996
Revised: 28 October 1996
Accepted: 28 October 1996

Current Biology 1996, Vol 6 No 12:1691–1694

Results and discussion

The pathogenesis of many human cancers is believed to involve a series of sequential events, in which multiple genes are implicated. In order to examine the consequences of defective NER in the context of a mammalian organism, as well as possible interactions between defects in NER and in other processes that normally safeguard the integrity of the genome, we have generated mice that are homozygously defective in the *XPC* gene. A targeting vector was constructed to replace a fragment of the mouse genomic *XPC* gene containing exon 10 and a portion of each of the flanking introns with a neomycin phosphotransferase expression cassette. Details of the generation of *XPC* mutant mice and their phenotypic characterization with respect to defects in both global and strand-specific NER will be reported elsewhere.

Recent studies have suggested that p53 protein may directly or indirectly modulate the activity of cellular DNA-repair pathways [8]. In order to examine the consequences of combined defects in the *p53* gene and in NER, we crossed our *XPC* mutant mice with *p53* mutant animals. Crosses between mice heterozygous for both the *XPC* and *p53* genes generated progeny with all nine possible combined genotypes. Subsequent crosses of *p53* heterozygous mice were established to compare the segregation of *p53* alleles between *XPC* wild-type (+/+), heterozygous (+/–) and homozygous (–/–) mutant progeny (Table 1). Consistent with previous studies, inheritance of the *p53* alleles deviated from normal Mendelian segregation [12,13]. However, we detected no significant differences in the inheritance of *p53* alleles between the three different *XPC* genotypic groups. Among a total of 132 $XPC^{+/+}$ mice identified by genotyping, 36 (27 %) were also $p53^{+/+}$, 74 (56 %)

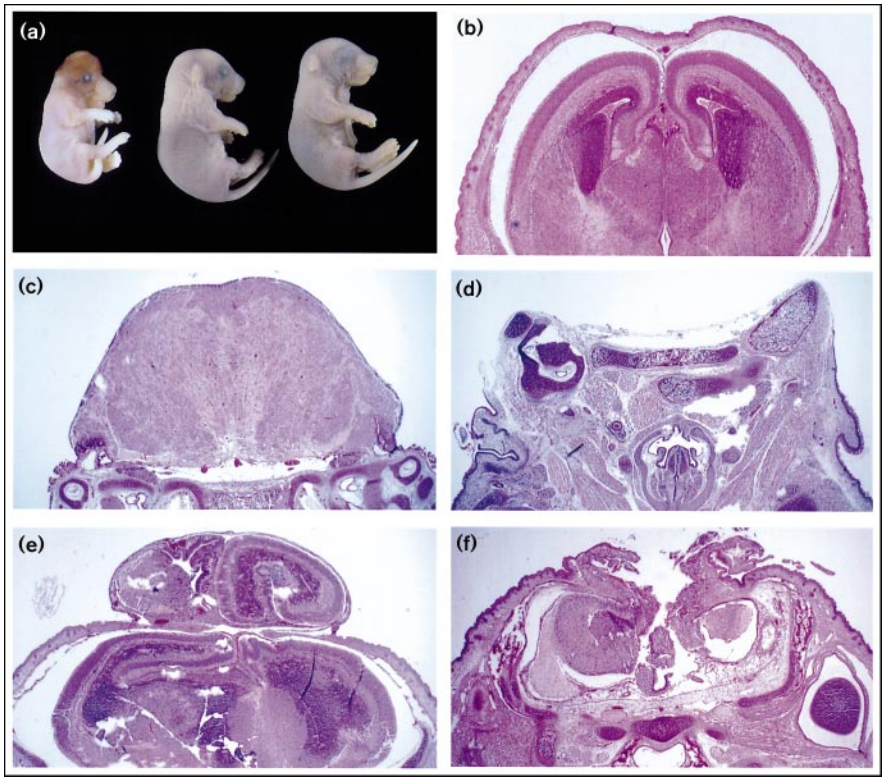
Table 1

Inheritance of *p53* alleles among *XPC* wild-type, heterozygous and homozygous mutant progeny from crosses of *p53* heterozygous mice.

	$XPC^{+/+}$	$XPC^{+/-}$	$XPC^{-/-}$
$p53^{+/+}$	36/132 (27 %)*	61/206 (30 %)	64/234 (27 %)
	18/18†	24/37	35/29
$p53^{+/-}$	74/132 (56 %)	99/206 (48 %)	129/234 (55 %)
	36/38	51/48	68/61
$p53^{-/-}$	22/132 (17 %)	46/206 (22 %)	41/234 (18 %)
	17/5	35/11	32/9

*Inheritance of *p53* alleles is expressed as a percentage of the total number of progeny for each genotypic group of *XPC*. †Ratio of males to females. *XPC* mutant female mice of hybrid background (50 % 129/Sv and 50 % C57Bl/6) were crossed with *p53* heterozygous male mice of the 129/Sv strain background (purchased from The Jackson Induced Mutant Resource). Progeny identified as heterozygous at both loci were intercrossed generating all nine possible combinations of *XPC* and *p53* genotypes. The resulting hybrid strain background of 75 % 129/Sv and 25 % C57Bl/6 was maintained in all subsequent crosses. Mutant *XPC* and *p53* alleles were distinguished from wild-type alleles by Southern-blot analysis.

Figure 1

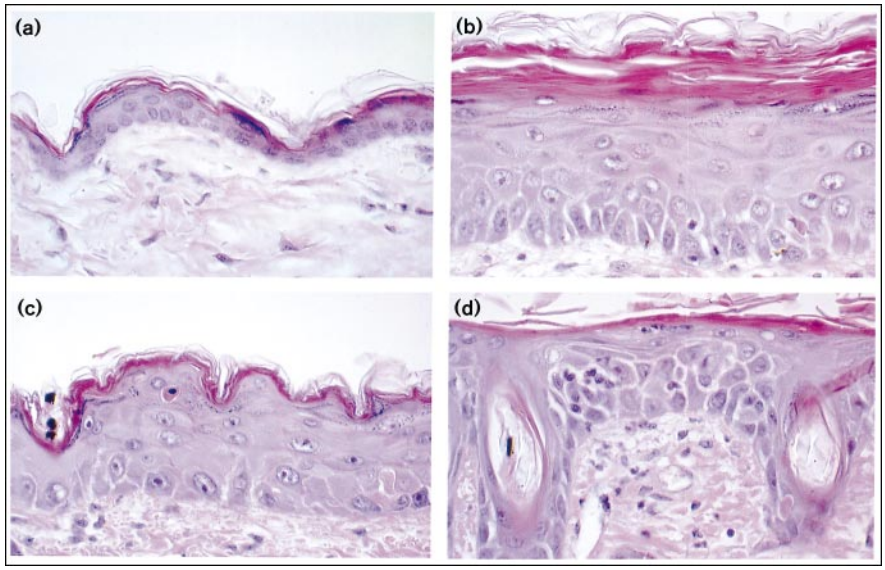


Central nervous system abnormalities observed in day 16.5 *XPC*$^{-/-}$ *p53*$^{-/-}$ embryos. (a) Gross appearance of (from left to right) *XPC*$^{-/-}$ *p53*$^{-/-}$, *XPC*$^{-/-}$ *p53*$^{+/-}$ and *XPC*$^{-/-}$ *p53*$^{+/+}$ embryos. The *XPC*$^{-/-}$ *p53*$^{-/-}$ embryo is exencephalic. (b–f) coronal sections of the cranial area of *XPC*$^{-/-}$ *p53*$^{-/-}$ embryos. (b) Histological section of an unaffected mouse embryo demonstrating an intact cranial vault and normal symmetrical cerebral hemispheres. (c) Histological section showing typical exencephaly in a female *XPC*$^{-/-}$ *p53*$^{-/-}$ animal. The brain parenchyma is dysplastic, with no evidence of normal hemisphere development. (d) Histological section of a female *XPC*$^{-/-}$ *p53*$^{-/-}$ animal showing a complete absence of brain parenchyma, a condition resembling anencephaly in humans. (e) Histological section showing an encephalocele in a female *XPC*$^{-/-}$ *p53*$^{-/-}$ embryo. Note the intact membrane surrounding the extruded neural tissue and the disorganization of the extracranial and intracranial parenchyma. (f) Histological section showing a protrusion of a small amount of neural tissue through a tiny opening in a male *XPC*$^{-/-}$ *p53*$^{-/-}$ embryo. This condition resembles human meroanencephaly, a variant of anencephaly. Magnification of embryo sections was 16×. Crosses of *XPC*$^{-/-}$ *p53*$^{+/-}$ females with *XPC*$^{-/-}$ *p53*$^{+/-}$ males were established to examine 13.5–18.5 day embryos for developmental abnormalities. The sex of embryos was determined by Southern analysis of yolk sac genomic DNA using a hybridization probe consisting of a 300 bp *Bgl*II–*Pst*I fragment from the Y chromosome-specific *sry* gene [15]. Embryos were fixed in 10 % buffered formalin, paraffin embedded, sectioned and stained with hematoxylin and eosin.

were *p53*$^{+/-}$ and 22 (17 %) were *p53*$^{-/-}$. Similarly, among 206 *XPC*$^{+/-}$ mice, 61 (30 %) were also *p53*$^{+/+}$, 99 (48 %) were *p53*$^{+/-}$ and 46 (22 %) were *p53*$^{-/-}$. Finally, among 234 *XPC*$^{-/-}$ mice, 64 (27 %) were also *p53*$^{+/+}$, 129 (55 %) were *p53*$^{+/-}$ and 41 (18 %) were *p53*$^{-/-}$. The slight but consistent deviation from strict Mendelian segregation of *p53* alleles apparently derives from a selective loss of females among *p53*$^{-/-}$ progeny (Table 1). Loss of female *p53*$^{-/-}$ mice has been noted previously and results principally from neural tube defects (primarily exencephaly) in the developing embryo [12,13]. A recent study identified exencephaly in 20 of 106 (19 %) *p53*$^{-/-}$ female embryos [13]. Many of these embryos also exhibited a variety of craniofacial malformations. The same study identified a single exencephalic animal among 128 *p53*$^{-/-}$ male embryos. We observed neural tube defects in 7 of 13 (54 %) *XPC*$^{-/-}$ *p53*$^{-/-}$ mutant female embryos and in 1 of 14 *XPC*$^{-/-}$ *p53*$^{-/-}$ mutant male embryos. Histological examination of four abnormal *XPC*$^{-/-}$ *p53*$^{-/-}$ embryos revealed a variable spectrum of neural tube abnormalities. One embryo showed exencephaly with a complete absence of membranes surrounding extruded brain tissue (Fig. 1a,c). A second embryo demonstrated absence of the cranial vault and a complete lack of neural tissue (Fig. 1d), a condition resembling anencephaly in humans [14]. The third embryo suffered an encephalocele, a condition in which a portion of highly disorganized neural tissue was exposed but was surrounded by an intact membrane (Fig. 1e). The intracranial neural tissue in this embryo was also grossly disorganized. In a fourth embryo, there was a small opening in the cranium through which a small amount of disorganized neural tissue was extruded (Fig. 1f). This state is strikingly similar to a variant of anencephaly in humans, termed meroanencephaly [14]. Interestingly, this embryo was a male, as determined by hybridization of yolk sac DNA to a chromosome Y-specific probe [15]. Deficiency in p53 protein is clearly implicated in developmental abnormalities [12,13], possibly by altering normal apoptosis and cell-cycle regulation. Further detailed studies are in progress to examine the consequence of defects in the *XPC* gene, and in NER in general, on the genesis of these developmental abnormalities.

The consequences of acute skin exposure to UV-B light were examined in adult male mice representing all nine genotypic combinations of *XPC* and *p53*. In one experiment, the shaved dorsal skin of two male adult mice of each genotype was exposed to 1.2 kJ m^{-2} of UV-B radiation per day for 7 days. In a second experiment, two other males of each genotype were exposed to 2.1 kJ m^{-2} of UV-B radiation per day for 7 days. Histological examination of skin

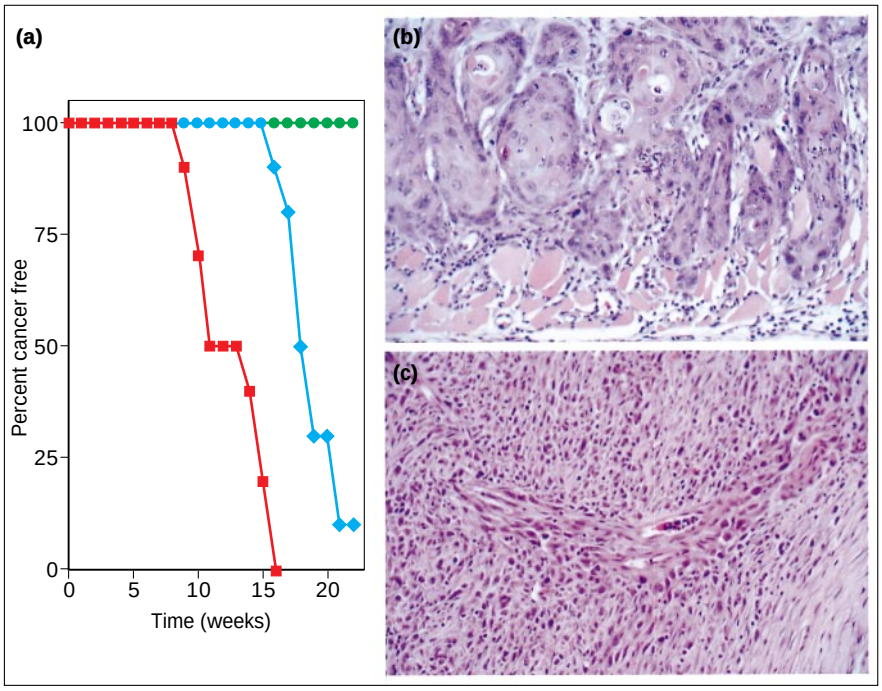
Figure 2



Hypersensitivity to acute exposure of UV-B radiation 24 h after seven daily doses (2.1 kJ m^{-2}). **(a)** Histological section of the dorsal skin of an irradiated wild-type mouse. Note the mild hyperkeratosis and epidermal hyperplasia with absence of dysplasia. **(b)** Histological section of the dorsal skin of an irradiated *XPC*$^{-/-}$ *p53*$^{+/+}$ mouse. Note the marked increase in keratosis and epidermal hyperplasia. **(c)** Histological section of the dorsal skin of a UV-irradiated *XPC*$^{-/-}$ *p53*$^{+/-}$ mouse. Note the additional presence of nuclear pleomorphism, dyskeratosis and delayed nuclear maturation, indicative of dysplasia; a premalignant state. **(d)** Histological section of the dorsal skin of a UV-irradiated *XPC*$^{-/-}$ *p53*$^{-/-}$ mouse demonstrating more pronounced dysplasia. (Magnification (255×) is the same in all panels.) The dorsal surface of the mice was shaved and irradiated with UV-B light (peak 310 nm, filtered through Kodacel sheeting; Eastman Kodak) using two FS15T8 lamps (Light Sources, Inc.) at a fluence of 5 J m^{-2} s^{-1} for 7 min (2.1 kJ m^{-2}) or 4 min (1.2 kJ m^{-2}) once a day for 7 days. Mice were sacrificed 24 h after the last day of irradiation and the dorsal skin was dissected and fixed in 10 % buffered formalin for 24 h. Skin biopsies were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin.

biopsies was carried out in a blinded fashion. Sections from all *XPC*$^{-/-}$ *p53*$^{+/+}$ mice showed increased sensitivity to irradiation with UV-B light, as shown by pronounced hyperkeratosis and epidermal hyperplasia (Fig. 2b). This response was not observed in similarly irradiated mice that were wild-type or heterozygous for the *XPC* gene, regardless of the *p53* genotype (Fig. 2a). The acute response to UV-B light was further aggravated as a function of the p53 genotype in *XPC*$^{-/-}$ mice. In addition to pronounced hyperkeratosis and epidermal hyperplasia, sections of the skin of *XPC*$^{-/-}$ *p53*$^{+/-}$ animals showed epidermal dysplasia, as shown by nuclear pleomorphism and the presence of

Figure 3



Accelerated skin cancer in *XPC*$^{-/-}$ *p53*$^{+/-}$ mice. **(a)** Plot comparing latency for the development of skin cancer in *XPC*$^{-/-}$ *p53*$^{+/-}$ (red squares) and *XPC*$^{-/-}$ *p53*$^{+/+}$ (blue diamonds) mice. No skin cancers were detected in any of the four other genotypic groups tested (*XPC*$^{+/+}$ *p53*$^{+/+}$, *XPC*$^{+/-}$ *p53*$^{+/+}$, *XPC*$^{+/+}$ *p53*$^{+/-}$ and *XPC*$^{+/-}$ *p53*$^{+/-}$ mice) (green circles). **(b)** Histology of a moderately differentiated squamous cell carcinoma (grade 2) from a *XPC*$^{-/-}$ *p53*$^{+/+}$ mouse. Note the incompletely keratinized centers. **(c)** Histology of a poorly differentiated squamous cell carcinoma (grade 4) from a *XPC*$^{-/-}$ *p53*$^{+/-}$ mouse. No evidence of keratinization is present. Tumor cells are atypical, spindle shaped and have few recognizable intercellular bridges. Magnification of tumor sections was 160×. Six groups of mice consisting of 10 animals (5 males and 5 females) per group were treated with 1.2 kJ m^{-2} of UV-B each day for up to 22 weeks. The dorsal skin was shaved weekly or as required. Mice were examined weekly for hypersensitivity to UV radiation and skin cancer. UV treatments were stopped in *XPC*$^{-/-}$ *p53*$^{+/-}$ mice at 16 weeks (100 % incidence of skin cancer). Treatments were continued in the five other groups of mice until 22 weeks. Mice were sacrificed and autopsied and tissue was processed for histological examination as described in Fig. 2.

mitotic figures (Fig. 2c). Dysplasia was more pronounced in sections from $XPC^{-/-}$ $p53^{-/-}$ animals (Fig. 2d). In summary, solar keratosis following acute skin exposure to UV-B radiation was observed in $XPC^{-/-}$ $p53^{+/+}$ animals and worsened progressively in $XPC^{-/-}$ $p53^{+/-}$ or $XPC^{-/-}$ $p53^{-/-}$ mice.

Mice that are homozygous mutant in the *XPA* and *XPC* genes have a significant predisposition to UV radiation-induced skin cancer [3–5]. We treated six genotypic groups of mice (10 animals per group, 5 males and 5 females) comprising $XPC^{+/+}$, $XPC^{+/-}$ and $XPC^{-/-}$ animals that were either $p53^{+/+}$ or $p53^{+/-}$ with 1.2 kJ m^{-2} of UV-B radiation per day for up to 22 weeks. Within 2 weeks of treatment, all $XPC^{-/-}$ $p53^{+/+}$ and $XPC^{-/-}$ $p53^{+/-}$ mice showed marked atrophy of the ears and a hypersensitivity response in the shaved region of skin. The response consisted of erythema followed by scaling and ulceration of the skin, which was subsequently surrounded by adherent hyperkeratotic lesions. The size of the affected area and the severity of the response was more pronounced in $XPC^{-/-}$ $p53^{+/-}$ than in $XPC^{-/-}$ $p53^{+/+}$ mice. After 8 weeks of treatment, corneal opacity was observed in the $XPC^{-/-}$ $p53^{+/-}$ mice and later in some $XPC^{-/-}$ $p53^{+/+}$ animals. None of the other four groups ($XPC^{+/+}$ $p53^{+/+}$, $XPC^{+/-}$ $p53^{+/+}$, $XPC^{+/+}$ $p53^{+/-}$ and $XPC^{+/-}$ $p53^{+/-}$) demonstrated hypersensitivity to UV radiation. Skin tumors appeared in the $XPC^{-/-}$ $p53^{+/-}$ mice between 8 and 9 weeks of treatment (Fig. 3a). At 16 weeks, all 10 such animals had skin cancer and exposure was discontinued. In contrast, the first skin tumors in $XPC^{-/-}$ $p53^{+/+}$ mice only appeared at 16 weeks (Fig. 3a) and it took 22 weeks for 9 of the 10 mice in this group to develop skin cancer (Fig. 3a). No tumors were detected in any of the other four groups of mice (Fig. 3a).

Skin tumors were examined histologically and graded in a blinded fashion. Tumors from the $XPC^{-/-}$ $p53^{+/+}$ mice ranged from carcinoma *in situ*, to well differentiated squamous cell carcinoma (grade 1), to moderately differentiated squamous cell carcinoma (grade 2) (Fig. 3b). Tumors from $XPC^{-/-}$ $p53^{+/-}$ mice were more aggressive, ranging from well differentiated squamous cell carcinoma (grade 1), to poorly differentiated squamous cell carcinoma (grade 4) (Fig. 3c). Sections also showed focal areas of hyperplasia, hyperkeratosis and mild to severe dysplasia. Hyperplasia and dysplasia in corneal epithelium correlated with animals in which corneal opacity was observed. In summary, $XPC^{-/-}$ $p53^{+/-}$ mice demonstrated an aggravated response to UV-B radiation and a significantly shorter latency in the development of skin cancer than $XPC^{-/-}$ $p53^{+/+}$ mice. High-grade squamous cell carcinoma has not been previously documented in UV radiation-induced skin tumors in *XPA* or *XPC* mutant mice. This feature apparently correlates with the *p53* heterozygous status. Chronic exposure of $p53^{-/-}$ mice that are also $XPC^{+/+}$, $XPC^{+/-}$ or $XPC^{-/-}$ is in progress. Preliminary results indicate an even more extreme cancer predisposition in $XPC^{-/-}$ $p53^{-/-}$ mice.

Our studies document an interaction of the *XPC* and *p53* genes with respect to cellular responses to acute and chronic exposure to UV-B radiation. Additionally, there are suggestions of a synergistic interaction between these two genes with respect to normal embryogenesis. Future studies will focus on these interactions, with a particular emphasis of the role of the *XPC* and *p53* genes in the pathogenesis of environmental carcinogenesis.

Acknowledgements

We thank our laboratory colleagues for discussions and for critical review of the manuscript. Studies supported by grant CA44247-11 from the USPHS to E.C.F. and by postdoctoral fellowships from the American Cancer Society (D.L.C.) and Friends of the Center for Human Nutrition, University of Texas Southwestern Medical Center (L.B.M.).

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