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Decreased DNA Repair but Normal Apoptosis in Ultraviolet-Irradiated Skin of p53-Transgenic Mice

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p53 tumor suppressor plays a vital role in the cellular responses to genotoxic stress. It is believed that p53 regulates the cell cycle by activating the G1 checkpoint after exposure to agents like ionizing radiation, ultraviolet (UV) radiation, or genotoxic chemicals. Recently, it is conjectured that p53 may have additional functions in DNA repair and apoptosis. Previously, we demonstrated that p53-transgenic mice that carry mutant alleles of a p53 gene developed twice as many skin tumors as control mice after UV exposure. To elucidate the molecular mechanisms of mutant p53 in skin cancers, we studied DNA repair efficiency and the rate of apoptosis in murine keratinocytes after UV irradiation. In this report, we show that mutant p53-transgenic mouse skin has reduced repair of UV-induced DNA damage in both in vivo and in vitro radioimmunoassays. In control mice, DNA repair is associated with increased amounts of wild-type p53 protein. Unexpectedly, mutant p53-transgenic mice had slightly increased apoptosis after UV irradiation, suggesting that the wild-type p53 protein in the cells still functions in inducing apoptosis, or that this cell death results from p53-independent mechanisms. These results suggest that mutant p53 interferes with wild-type p53 in the repair of UV-induced DNA damage but not in apoptosis. (Am J Pathol 1996, 148:1113–1123)

The human p53 gene encodes a 393-amino-acid nuclear phosphoprotein with a M_r of 53,000. Current evidence suggests that loss of normal p53 function is associated with cell transformation *in vitro* and development of neoplasms *in vivo* (for review see Refs. 1 and 2). The introduction of an expression vector of the wild-type p53 gene suppresses the growth of human lung cancer cells,[3] human breast cancer cells,[4] and human colorectal carcinoma cells.[5] Conversely, loss of wild-type p53 function through mutation or inactivation leads to cell transformation and tumor formation in experimental systems. The mutant p53 gene, acting in concert with the *ras* oncogene, can cause malignant transformation of primary rat cells in culture.[6] In human tumors, p53 has been shown to be one of the most frequently mutated genes known to date. More than 50% of human malignancies of epithelial, mesenchymal, hematopoietic, lymphoid, and central nervous system origin analyzed thus far were shown to contain an altered p53 gene.[7,8]

Wild-type p53 protein is a crucial protein involved in maintaining genomic stability after genotoxic stress. After a DNA-damaging event, such as ionizing radiation and exposure to actinomycin, the amount of p53 protein in the cells rapidly increases and the cells arrest in G1 phase.[9–12] After the event of ultraviolet (UV)-induced DNA damage, p53 is also increased.[13–15] G1 arrest is possibly achieved by transcriptional regulation of downstream p53 genes.[16] p53 regulates the expression of $p21^{WAF1}$, which is a potent inhibitor of cyclin-dependent kinase (Cdk) activity.[17–19] $p21^{WAF1}$ also directly interferes with DNA synthesis by binding to proliferating

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cell nuclear antigen and blocking its interaction with DNA polymerase.[20] Another p53-regulated gene, GADD45, has been found to suppress cell growth and inhibit progression from G0 to S phase after serum stimulation, although it does not appear to directly interact with Cdk proteins.[21,22] GADD45 also binds to proliferating cell nuclear antigen and has been shown to stimulate nucleotide excision repair *in vitro.*[21] The key function of p53 in regulating the cell cycle is believed to provide time for DNA repair before entry into S phase. Recently, p53 has been found to associate *in vitro* with ERCC3,[23] a nucleotide excision repair protein shown to have a protective effect after ionizing radiation.[24] These data suggest that p53 may directly participate in the DNA repair process. Smith et al[15] showed that the disruption of normal p53 function in human colon carcinoma RKO cells with either the human papillomavirus E6 oncoprotein or a mutant p53 transgene results in reduced repair of UV-induced DNA damage.

Another important role of the p53 protein in maintaining the genomic stability of the cells is to trigger apoptosis after a DNA-damaging event. Apoptosis is a self-protective mechanism by which cells possessing significant DNA damage can be deleted. Recent studies have shown that apoptosis of cultured cells induced by ionizing radiation,[25–27] anticancer drugs,[28] or growth factor deprivation[29,30] is dependent on wild-type p53 function. Furthermore, p53-dependent apoptosis suppresses tumor growth and progression *in vivo*. Symonds et al[31] elegantly showed that choroid plexus tumors induced by inactivation of the pRb family in transgenic mice develop very slowly, owing to p53-mediated apoptosis. Loss of p53 function by either mutation, gene knockout, or binding to SV40 large T antigen represses apoptosis and converts an indolent tumor into a very aggressive one. Using p53 knockout mice, Ziegler et al[32] showed that the rate of apoptosis induced by UV irradiation is significantly higher in the keratinocytes of wild-type p53 mice than p53 knockout mice.

It appears that p53 is involved with Bcl-2/Bax in the process of inducing apoptosis. Bcl-2 protein is thought to prevent most types of apoptotic cell death (reviewed by Reed[33]), whereas Bax protein (a homologous protein to Bcl-2) heterodimerizes with Bcl-2 and promotes apoptosis.[34] Previous studies of non-Hodgkin's lymphomas and breast cancers have demonstrated an inverse relationship between p53 mutation and Bcl-2 expression.[35] p53 protein has been found to be a regulator of Bcl-2 and Bax in gene expression both *in vitro* and *in vivo.*[36,37] Wild-type p53-triggered apoptosis is inhibited by Bcl-2 in proliferating lymphoid cells,[38] and in a v-*myc*-induced T cell lymphoma line[39] as well as in myeloblastic leukemia cells.[36,40]

We previously reported that transgenic mice that carry multiple copies of a mutant p53 allele are predisposed to UV-induced squamous cell carcinomas.[41] To investigate the possible mechanisms of the increased frequency of UV-induced skin tumors in p53-transgenic mice, we examined the DNA repair efficiency and the rate of apoptosis in the skin of p53-transgenic mice after UV irradiation.

Materials and Methods

Mice

p53-transgenic mice were kindly provided by Dr. A. Bernstein. These mice contain a mutant p53 genomic fragment that was originally cloned from the Friend cell line CB7.[42] The mutant p53 gene has a mutation at residue 193 (Arg to Pro).[24] This mutant protein, unlike the wild-type p53 protein, complements Ras in the transformation of rat primary cells.[43] The transgenic p53 mice were mated with normal CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA). Fifty percent of the offspring were transgenic, and fifty percent were normal mice. To distinguish the p53-transgenic mice from non-transgenic littermates, DNA extracted from mouse tail biopsies by the proteinase K/sodium dodecyl sulfate method[44] were subjected to slot-blot analysis using an *Eco*RI-*Hin*dIII fragment as a probe.[43] The normal CD-1 littermates were used as controls.

Isolation and Culture of Murine Keratinocytes

Mice at the age of 6 to 8 weeks were sacrificed and the tails were dissected. The tail was disinfected in 2.5% betadine for 1 minute and 70% ethanol for 1 minute followed by two rinses in phosphate-buffered saline (PBS). The skin of the tail was then separated from the tailbone with a scalpel blade and dissected into smaller pieces of 2 to 3 cm^2. The tail-skin biopsies were incubated in Dulbecco's modified minimal essential medium (DMEM) containing 0.25% dispase at 4°C overnight. The epidermal layer was separated from the dermis layer with a pair of forceps. The epidermal layer was trypsinized twice for 3 minutes each at 37°C. The keratinocyte suspension was collected and transferred to a tube containing DMEM supplemented with 10% fetal bovine serum, and centrifuged at 1000 rpm for 5 minutes. The cell pellet was resuspended and incubated at 37°C in DMEM/

Hams F12 medium containing fetal bovine serum treated with 8% chelax (Bio-Rad Laboratories, Richmond, CA), 4 mmol/L glutamine, 1 μg/ml hydrocortisone, 10 ng/ml epidermal growth factor, 10 ng/ml cholera toxin, 0.5% bovine pituitary extract, and 1.3 mmol/L $CaCl_2$. After 24 hours, the cells were washed with PBS twice and incubated with a similar DMEM/Hams F12 medium containing 0.05 mmol/L $CaCl_2$ and 0.05 μCi/ml [^{14}C]thymidine (51.5 mCi/mmol) and allowed to grow for 2 days. The cells were washed twice with PBS before UVB irradiation. Cells were either harvested immediately after UV irradiation or replenished with fresh, prewarmed medium (0.05 mmol/L $CaCl_2$) and allowed to repair DNA for various times.

UV Exposure

For UVB irradiation, a bank of four FS40 sunlamps (Westinghouse, Bloomfield, NJ) was used. The intensity of the UV light was measured by an IL 700 radiometer fitted with a WN 320 filter and an A127 quartz diffuser (International Light, Newburyport, MA). Before UVB irradiation, the hair on the dorsal surface of the mice was shaved using an Oster electric clipper with a number 40 blade. The mice were then exposed to 5000 J/m^2 UVB. The mice were sacrificed at specific time points after UVB irradiation, and the dorsal skin was dissected for DNA extraction and immunohistochemical analysis. UV irradiation of cultured keratinocytes was carried out with the same lamps. The cells received 250 J/m^2 UVB.

DNA Extraction

The fat on the skin biopsies was scraped away with a scalpel blade. The skin biopsies or cultured keratinocytes were incubated in a lysis buffer containing 100 mmol/L $NaCl_2$, 20 mmol/L EDTA, 50 mmol/L Tris-HCl (pH 8.0), 0.5% sodium dodecyl sulfate, and 1 mg/ml proteinase K (GIBCO BRL, Gaithersburg, MD) at 37°C overnight. DNA was extracted successively with phenol and phenol/chloroform (1:1) and by ethanol precipitation. RNA was removed by digestion with pancreatic RNAse (Sigma Chemical Co., St. Louis, MO). The DNA concentration was measured with a Lambda 3 UV/VIS spectrophotometer (Perkin-Elmer Corp., Norwalk, CT).

Radioimmunoassay (RIA)

The radioimmunoassay was performed as described.[45] The antisera used were raised against DNA that was irradiated with UV light (UVC). The specificity of the RIA has been verified by the following assays: photoreactivation *in vivo*[46] and *in vitro*,[47] digestion with T4 endonuclease V,[48] and UVB photoisomerization.[49]

Slot-Western Analysis of Cyclobutane Dimers

One microgram of DNA was suspended in 50 μl of 6X standard saline citrate and filtered onto a nitrocellulose membrane through a slot-blot apparatus. The membrane was then incubated *in vacuo* at 80°C for 30 minutes. The membrane was blocked with 5% milk at 4°C overnight, hybridized with purified IgG specific for cyclobutane dimer antibody (1 μg/ml) in PBS containing 0.5% Tween 80 at room temperature for 1 hour, and then hybridized with horseradish-peroxidase-conjugated goat anti-rabbit secondary antibody (1:10,000 dilution) for 1 hour at room temperature. The signals were detected with the ECL-Western chemiluminescence detection kit (Amersham, Arlington Heights, IL).

Immunohistochemistry

The skin biopsies were formalin fixed and paraffin embedded. Six-micron sections were prepared and mounted onto saline-coated slides. Sections were then slowly boiled for 12.5 minutes in the microwave oven in a citrate buffer. Sections were stained for p53 using a polyclonal antibody CM1 (Dimension Laboratories, Missisauga, Ontario), which reacts with both wild-type and mutant forms of p53. A sensitive streptavidin peroxidase method was used as described previously.[50] For detection of Bcl-2 expression, a rabbit polyclonal antibody specific for murine Bcl-2 was used.[51] In some cases, the antiserum was preabsorbed with competing Bcl-2 peptide to confirm the specificity of the immunostaining results.

In Situ *Detection of Apoptosis*

After exposure to 5000 J/m^2 UVB, the skin biopsies from the shaved dorsal area were dissected and fixed with 10% formalin. Sections were stained in a routine fashion with hematoxylin and eosin. The method used to identify apoptotic cells has previously been published.[52] Briefly, 400 consecutive keratinocytes were examined for the presence of cells with apoptotic features. Counts are expressed as the number of apoptotic cells per 400 epithelial keratinocytes and were performed in duplicate. The crite-

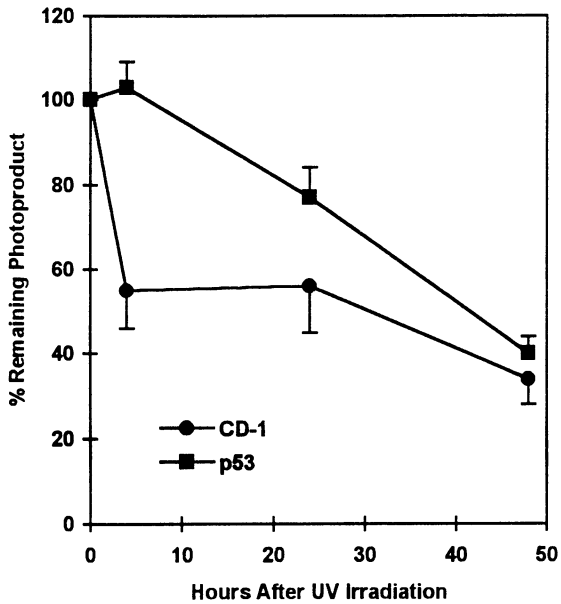

Figure 1. *RIA of cyclobutane dimers in p53-transgenic and CD-1 mouse skin after 5000 J/m² UVB irradiation. Assay was performed at 0, 4, 24, and 48 hours after UV irradiation. Values are the mean ± SD for six mice.*

ria used to identify apoptotic cells were 1) cells with remarkable condensation of chromatin and cytoplasm, 2) cytoplasmic fragments containing condensed chromatin, and 3) intra- and extracellular chromatin fragments with a diameter of ≤2 μm.

Apoptosis Detected by Gel Fragmentation

DNA samples from skin biopsies were fractionated on 1.5% agarose gels in 1X Tris-acetate/EDTA electrophoresis buffer and stained with ethidium bromide. The gel was photographed under UV light with Polaroid 55 film.

Results

Reduced DNA Repair in p53-Transgenic Mice

The p53-transgenic mice were exposed to 5000 J/m² UVB irradiation. Skin biopsies were obtained from shaved dorsal areas, and DNA was extracted. The amount of cyclobutane dimers in the skin cells was monitored by RIA at 0, 4, 24, and 48 hours after UV irradiation. An antibody specific for cyclobutane dimers[45] was used in this assay. Nontransgenic littermates were used as controls. Figure 1 showed that the repair rate of cyclobutane dimers was decreased in p53-transgenic mice (n = 6) at 4 hours (CD-1, 55 ± 9, *versus* p53, 103 ± 6; $P = 0.0002$) and

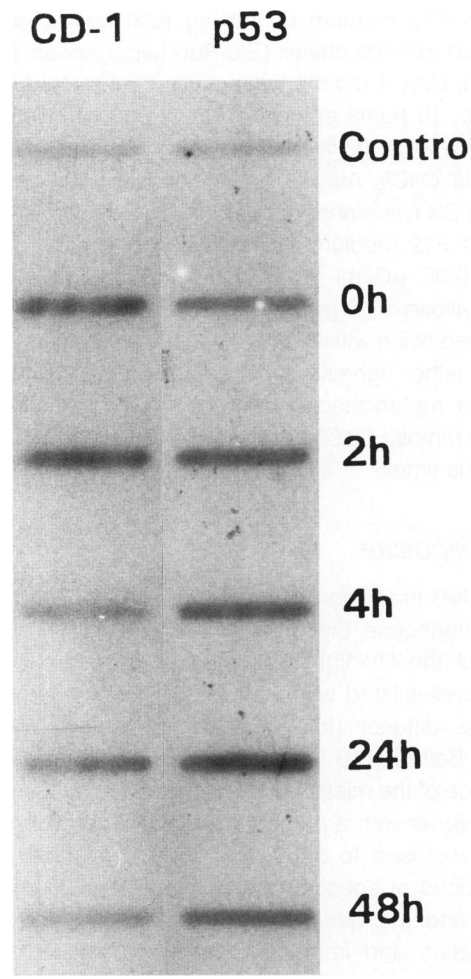

Figure 2. *Slot-Western analysis of cyclobutane dimers in p53-transgenic and CD-1 mouse skin after 5000 J/m² UVB irradiation. Skin biopsies were obtained at 0, 2, 4, 24, and 48 hours after UV irradiation. Skin biopsies without UV irradiation were used as controls.*

24 hours (CD-1, 56 ± 11, *versus* p53, 77 ± 7; $P = 0.001$) after UV irradiation.

To confirm the findings of reduced DNA repair in p53-transgenic mice, 5 μg of DNA was filtered onto nitrocellulose filters through a slot-blot apparatus. The filters were then subjected to Western analysis using the cyclobutane dimer IgG and goat anti-rabbit IgG. This is a direct binding assay as opposed to the competitive binding assay (RIA) used above. Figure 2 showed that, as expected, the cyclobutane dimers were instantly increased after UV irradiation, peaked at 2 hours, and reduced at 4, 24, and 48 hours after UV irradiation in normal CD-1 mice. The amount of cyclobutane dimers remained high in p53-transgenic mice after UV irradiation.

To eliminate the possibility that the differences in DNA repair between CD-1 and p53-transgenic mice were not due to the differences in the skin responses, such as hyperplasia, to UV irradiation, we

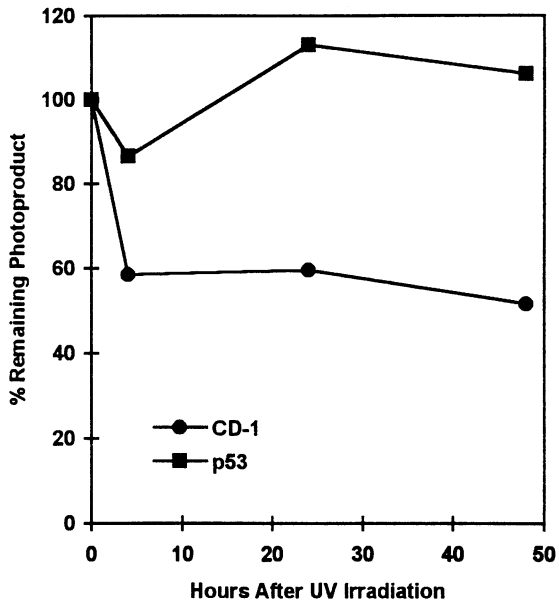

Figure 3. *RIA of cyclobutane dimers in cultured keratinocytes from p53-transgenic and CD-1 control mice.*

isolated mouse keratinocytes and examined the cyclobutane repair rates after UV irradiation *in vitro*. The cells were labeled with [^{14}C]thymidine for 2 days and then exposed to 250 J/m^2 UVB. Cells were harvested at 0, 4, 24, and 48 hours after UV irradiation, and DNA was extracted from the cells. Equal amounts of [^{14}C]labeled DNA were used for RIA. Figure 3 showed that in normal CD-1 mice the amount of cyclobutane dimers was reduced at 4, 24, and 48 hours to 50 to 60% compared with the amount of dimers at 0 hours after UV irradiation, whereas it remained virtually unchanged in p53-transgenic mice.

p53 Expression after UV Irradiation

Several reports have demonstrated that UV irradiation induces wild-type p53 expression in cell lines and in normal human skin.[13–15] We examined whether a similar increase of wild-type p53 protein is induced in murine skin cells. Figure 4 shows that, after exposure to 5000 J/m^2 UVB, the wild-type p53 protein in CD-1 mouse skin was remarkably increased at 4 hours, peaked at 48 hours, and returned to basal levels at 72 hours. The increase of p53 protein levels was mainly observed in epithelial keratinocytes. A striking increase in p53 protein was also observed in hair follicle epithelium, with a similar time course. Another response to UV irradiation is the increased thickness of the layers of keratinocytes

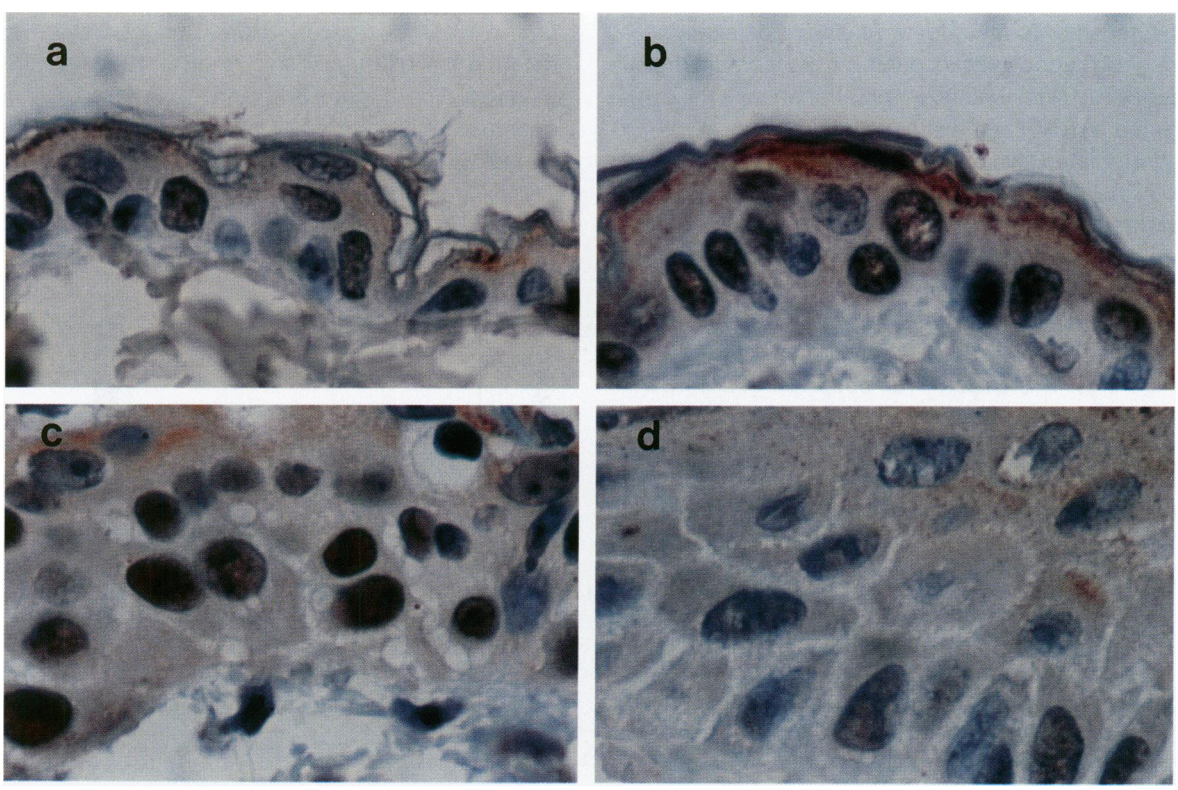

Figure 4. *Increased p53 expression in murine keratinocytes after UV irradiation.* a: *No UV.* b *to* d: *5000 J/m^2 UVB, at 4, 48, and 72 hours, respectively. Magnification, ×1000.*

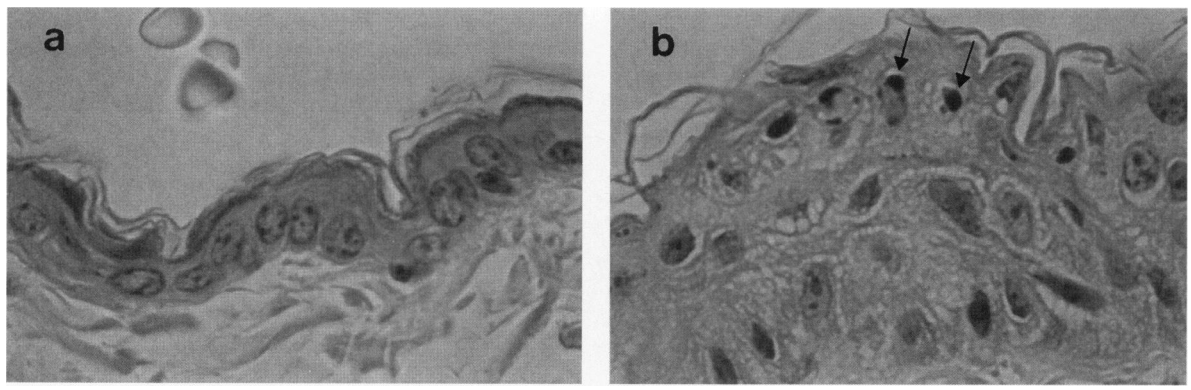

Figure 5. *In situ detection of apoptotic cells in the epithelium of p53-transgenic mice 48 hours after UV irradiation.* **a:** *No UV.* **b:** *5000 J/m².* **Arrows** *indicate apoptotic cells. Magnification, ×1000.*

in the epithelium. Normal murine epithelium has only one or two layers of keratinocytes (Figure 4a). At 48 hours after 5000 J/m² UV irradiation, the epithelium has three to four layers of keratinocytes. The increased layers of keratinocytes after UV irradiation were also observed in the p53-transgenic mice.

UV-Induced Apoptosis

The role of p53 in apoptosis has attracted much attention in recent years. p53 has been shown to be crucial in inducing apoptosis,[53,54] which is a self-protecting mechanism to conserve the integrity of the genome. To investigate whether a defect in p53-mediated apoptosis accounts for the increased incidence of skin tumors in p53-transgenic mice induced by UVB, we examined the apoptosis rate in UV-irradiated skin of p53-transgenic mice compared with that of normal CD-1 mice. The apoptotic cells with remarkable condensation of chromatin and cytoplasm were easily identified under the microscope (Figure 5). The frequency of apoptotic cells increased significantly at 24 hours, peaked at 48 hours, and dropped at 72 hours after UV irradiation (Figure 6). This pattern was observed in both CD-1 and p53-transgenic mice. The frequency of apoptotic cells was slightly higher in p53-transgenic mice than in CD-1 mice, but there was no statistical difference between any two groups (4 hours, $P = 0.27$; 24 hours, $P = 0.28$; 48 hours, $P = 0.20$; 72 hours, $P = 0.58$).

UV-induced apoptosis was also examined by gel electrophoresis of genomic DNA isolated from keratinocytes. The DNA of apoptotic cells is often digested by endogenous endonucleases, which cleave internucleosomal DNA to form a ladder of oligonucleosome fragments.[14,55,56] DNA was extracted from UV-irradiated skin biopsies, and the integrity of the DNA was analyzed on agarose gels. Fragmented DNA in the usual oligonucleosomal pattern was detected in increased amounts at 24 and 48 hours after UV irradiation (Figure 7). There was no striking difference between two groups except for slightly more DNA fragmentation in the p53-transgenic mouse skin at 48 hours, which is consistent with the above *in situ* assay.

Bcl-2 plays an important role in apoptosis.[33] Studies have shown that p53 interacts with Bcl-2 in inducing apoptosis.[36–40,57,58] The expression of Bcl-2 in UV-irradiated skin was examined by immunohistochemistry. There was no difference between untreated CD-1 and p53-transgenic mice (Figure 8, a and b), but decreased expression of Bcl-2 was noted in the p53-transgenic mice at 48 hours after UV irradiation (Figure 8, c and d). A less obvious decrease of Bcl-2 expression was also seen in the control CD-1 mice after UV irradiation.

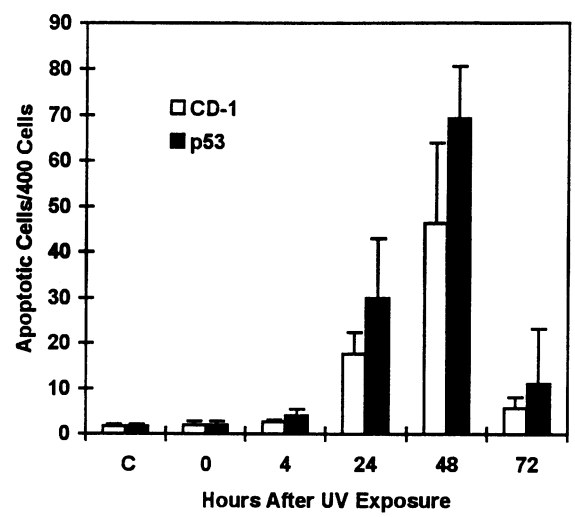

Figure 6. *In situ detection of apoptosis in p53-transgenic and CD-1 keratinocytes. Skin biopsies were obtained at 0, 4, 24, 48, and 72 hours after 5000J/m² UVB irradiation. Skin biopsies without UV irradiation were used as controls (C). The determination of apoptotic cells was described in Material and Methods.*

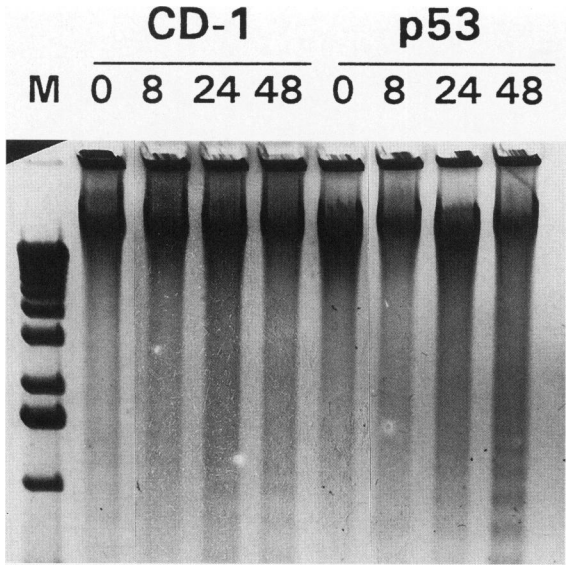

Figure 7. *Gel analysis of DNA integrity of murine skin after UV irradiation. Both p53-transgenic and CD-1 mice were exposed to 5000 J/m² UVB. Skin biopsies were obtained at 0, 8, 24, and 48 hours after UV irradiation. DNA was extracted and 10 μg of DNA from each sample were subjected to gel electrophoresis analysis on 1.5% agarose gel. M, 1-kb ladder marker (GIBCO BRL).*

Discussion

Many studies have indicated that the p53 gene is mutated in squamous cell carcinomas.[59–61] The mutations are usually UV-specific C→T or CC→TT transitions.[60,62] Alterations of the p53 gene through either point mutations, deletions, or rearrangements result in either no expression of wild-type p53 or overexpression of mutant p53 protein and can contribute to tumor development. The key function of p53 is believed to be regulation of the cell cycle by inhibiting the onset of S phase. When a cell is exposed to a DNA-damaging agent, p53 protein is rapidly increased to arrest the cell in G1 phase to allow DNA repair to occur before progressing to S phase.[9,10] Conversely, cells with no wild-type p53 protein or containing mutant p53 protein cannot undergo cell cycle arrest when exposed to DNA-damaging agents[63,64] and thus fail to repair damaged DNA before DNA replication.

Using RIA, we examined the DNA repair efficiency in the skin of p53-transgenic mice that carry both wild-type and a mutant p53 gene. We elected to use this model because it closely mimics the real-life situation in which mutations of the p53 gene originate in one of the alleles. Our data indicate that the DNA repair induced by UV irradiation is reduced in the skin of transgenic mice (Figures 1 to 3). One-half of the damaged DNA was repaired by 4 hours in the normal mice, but it remained unrepaired in the p53-transgenic mice. DNA repair was associated with an increase of wild-type p53 protein in normal mice (Figure 4). The wild-type p53 protein in the skin of p53-transgenic mice may also increase after UV irradiation, but the mutant p53 protein may diminish the wild-type p53 function.

Our data support the findings of Smith et al[15] that nucleotide excision repair is reduced in cells carrying a mutant p53. In their study, the authors transfected RKO colon carcinoma cell lines, which carry either wild-type p53 or mutant p53, with UV-damaged CAT plasmid DNA. Transient CAT gene expression in cells carrying mutant p53 was 2.5- to 3.6-fold less than cells carrying wild-type p53 at 72 hours after transfection. The exact mechanism by which p53 modulates DNA repair is still unclear. p53 may regulate GADD45, which is turned on in cells exposed to stimuli that arrest cell growth and produce genotoxic stress.[65] Immunodepletion of GADD45 from cellular extracts resulted in reduced nucleotide excision repair activity in an *in vitro* assay, whereas addition of recombinant GADD45 increased nucleotide excision repair activity.[21] GADD45 also associates with proliferating cell nuclear antigen, which is required for nucleotide excision repair *in vitro.*[66] p53 may directly participate in the repair process as p53 protein has been recently found to bind to ERCC3[23] and other excision repair enzymes including XPD, XPB, and CSB.[67] Furthermore, p53 was found to bind to single-strand DNA lesions.[68,69]

Our results differ from findings in p53 knockout mice. Ishizaki et al[70] reported that UV-induced thymidine dimers and (6–4) photoproducts were removed at a similar rate in p53-deficient and normal embryonic fibroblasts. The reason for the discrepancy in DNA repair between p53-deficient mice and our p53-transgenic mice, which carry alleles of mutant p53 gene, is unclear. It may be due to different responses to UV-induced DNA damage in keratinocytes and embryonic fibroblasts. Alternatively, reduced nucleotide excision repair may be a manifestation of a gain of function of the mutant p53 protein. Gain of function of the mutant p53 is a crucial feature in carcinogenesis. Sun et al[71] demonstrated that, in the heterozygous state in which both wild-type and mutated p53 gene were expressed, the mutant p53 dominates the wild-type p53 in controlling transcriptional activity, in governing cell growth, and in progressing tumor promotion-sensitive phenotype. This dominant negative effect was seen in a 1:1 ratio and was more striking at a ratio of 1:3 (wild-type:mutant).

A reduced DNA repair rate in the presence of the mutant p53 explains the increased skin tumor incidence in the p53-transgenic mice after UV irradia-

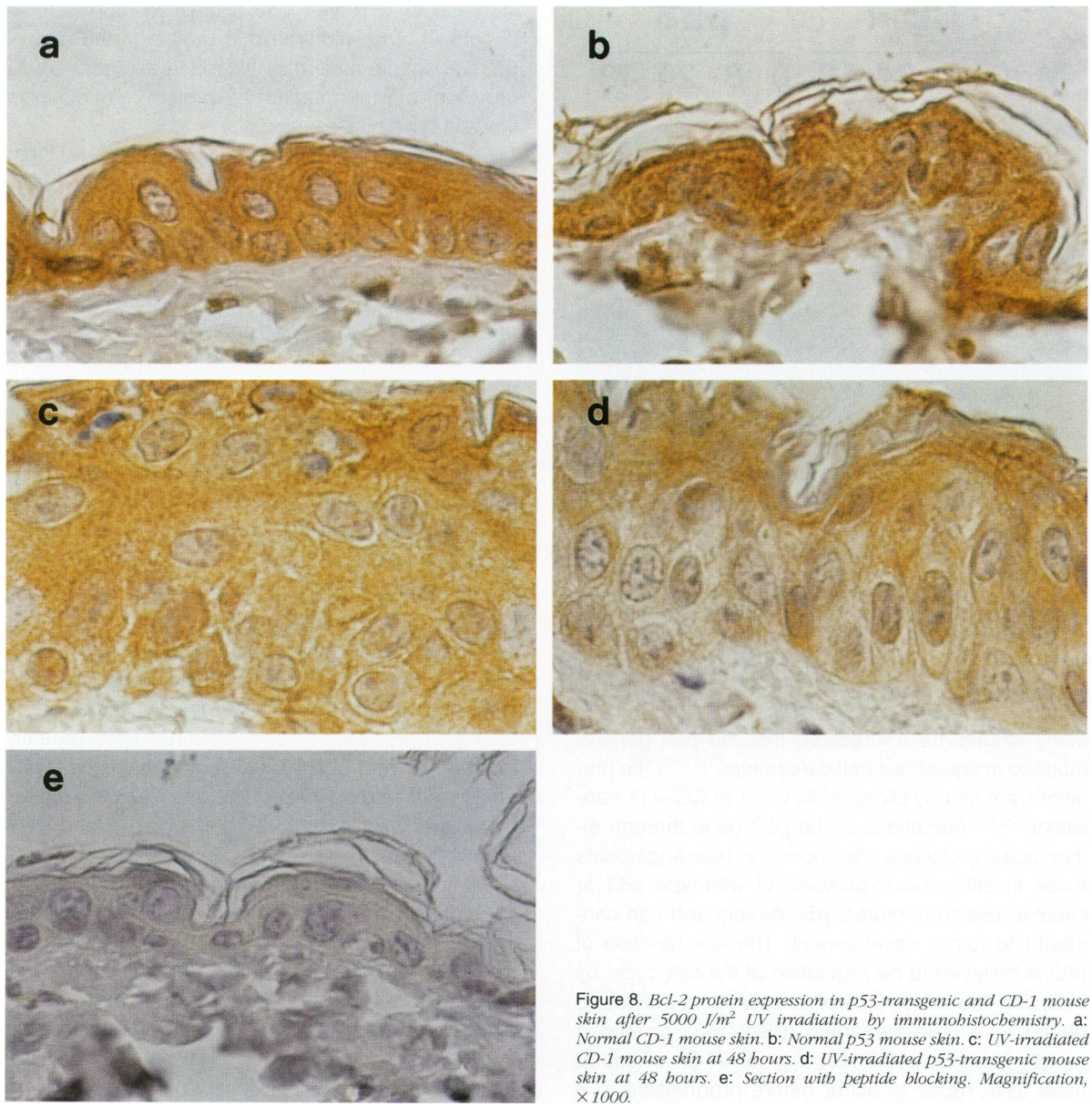

Figure 8. *Bcl-2 protein expression in p53-transgenic and CD-1 mouse skin after 5000 J/m² UV irradiation by immunohistochemistry.* a: *Normal CD-1 mouse skin.* b: *Normal p53 mouse skin.* c: *UV-irradiated CD-1 mouse skin at 48 hours.* d: *UV-irradiated p53-transgenic mouse skin at 48 hours.* e: *Section with peptide blocking. Magnification, ×1000.*

tion.[41] The exact ratio of the wild-type and mutant p53 protein in the keratinocytes exposed to UV under conditions that block DNA repair is unclear. However, it is known that the transgenic mouse contains 10 to 20 copies of the mutant p53 gene and that the majority of the p53 proteins in these animals is the mutant type.[41,43]

Another role of p53 in maintaining cellular genomic stability is implicated in inducing apoptosis. p53 was first shown to induce apoptosis in cells of hematopoietic origin. Using p53-deficient mice, it was shown that $p53^{-/-}$ thymocytes,[25,26,28] bone marrow cells,[28] and intestinal epithelial cells[25,72] were resistant to ionizing radiation-induced apoptosis. Ziegler et al[32] demonstrated that UV-induced apoptosis in keratinocytes is also dependent on wild-type p53 function. However, some studies have shown that apoptosis can occur in response to DNA damage independent of p53 function. Strasser et al[38] reported, for example, that the T lymphoblasts from $p53^{-/-}$ mice were just as sensitive as those from $p53^{+/+}$ mice to γ-radiation, indicating that p53 is not the only inducer of apoptosis in response to this insult. Moreover, Merlo et al[73] reported that starvation-induced apoptosis is p53 independent. We observed no significant differences in UV-induced apoptosis of keratinocytes in normal and p53-transgenic mice that carry both wild-type and mutant p53

(Figure 6). The kinetics of programmed cell death are similar in normal and p53-transgenic mice. Our data differ from the findings by Ziegler et al,[32] who showed that keratinocytes in the p53-deficient mice had significantly less apoptosis after UV irradiation, and suggest that the wild-type p53 protein in the mutant p53 mice may still function to induce apoptosis. Decreased Bcl-2 protein levels after UV irradiation (Figure 8) suggest that Bcl-2 plays an important role in UV-induced apoptosis of keratinocytes. Our data support the findings by Gillardon et al[74] that Bcl-2 mRNA levels were significantly reduced 48 hours after a single dose of UV irradiation and those of Zhan et al,[75] who observed reduction in Bcl-2 mRNA after x-ray irradiation of a human AML cell line.

In summary, keratinocytes from p53-transgenic mice that carry a mutant allele of the p53 gene have reduced DNA repair in response to UV exposure but similar apoptosis rates compared with normal mice. The reduced DNA repair in p53-transgenic mice may be responsible for the increased incidence of skin tumors after UV irradiation.

Acknowledgments

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