Induction of p53 Expression in Skin by Radiotherapy and UV Radiation: a Randomized Study

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Background: **p53 protein plays an important role in the response to DNA damage, and radiotherapy can cause radiation dermatitis. p53 and p21 levels increase *in vitro* when DNA is damaged by UVA, UVB, or γ-radiation. To determine whether this response occurs in human skin and predicts the level of radiation dermatitis, we investigated levels of p53 and p21 in skin exposed to different types of radiation as part of a randomized study of women with breast cancer to evaluate topical steroid or emollient cream treatments for radiation dermatitis of their irradiated breast. *Methods:* After surgery but before receiving tangential 5-mV photo-beam radiotherapy (2 Gy and 54 Gy) to the affected breast parenchyma, multiple areas on the backs of 50 women were irradiated with UVA and other areas were irradiated with UVB. Skin biopsy samples were taken from areas of normal unirradiated skin and all irradiated areas, and p53 and p21 were detected immunohistochemically. All statistical tests are two-sided. *Results:* In skin irradiated with UVA or UVB, medians of 4.4% (range = 0%–40.5%) or 45.5% (range = 5.3%–74.6%) p53-positive keratinocytes, respectively, were observed. Radiotherapy produced medians of 31.0% (range = 0%–79.3%) p53-immunoreactive cells after 2 Gy of radiation and 83.2% (range = 37.6%–95.2%) after 54 Gy of radiation. Despite large interindividual differences in p53 response, comparable increases in epidermal p53 response were independent of the type of radiation. A correlation between p53 and p21 was also evident (r_s = .78). In breast skin, there was no association between the p53 response and the degree of erythema (a measure of radiation dermatitis) and no statistically significant difference between treatment arms and p21/p53 responses. *Conclusions:* Individual responses to radiation-induced DNA damage varied widely and may be independent of the type of radiation. The epidermal p53 response does not predict the degree of radiation dermatitis. [J Natl Cancer Inst 2001; 93:128–33]**

p53 and p21, its downstream-effector protein, are sensitive indicators of DNA damage. In normal human epidermis, there are two patterns of p53 immunoreactivity, patch like and diffuse *(1–3).* The patch-like pattern represents a cluster of keratinocytes with a mutated p53 gene *(4–6),* and the diffuse pattern is found in the epidermis after DNA damage has triggered a reactive accumulation of p53 protein *(7,8).* p53 accumulates rapidly in the human epidermis after a single physiologically active dose of UV radiation. Topical sun-protection lotion applied before exposure to UV radiation can, in part, block the epidermal p53 response *(1,9)* and, in animal models, the number of induced p53 mutations are reduced *(10).*

UV radiation generates DNA photoproducts, such as pyrimidine dimers and 6-4 photoproducts *(11,12).* Ionizing irradiation produces double- and single-strand DNA breaks. Cells respond to DNA photoproducts and DNA breaks by accumulation of functionally active p53 protein, a key event in response to cellular stress. Although the initial events and pathways involved are not completely understood, increased protein stability resulting in a prolonged half-life appears to be an important mechanism *(13).* One pathway downstream of p53 is mediated through p21, a general cyclin-dependent kinase inhibitor. The p21 pathway leads to cell cycle arrest at the G_1 restriction point caused by decreased phosphorylation of the retinoblastoma protein *(14).* If DNA damage is more severe, p53 is involved in the pathway leading to apoptotic cell death *(15–18).* The p53 response is thus a normal defense mechanism, protecting an organism from acquiring clones of mutant cells *(19–21). In vitro,* a cellular p53 response has been observed after γ-irradiation *(18,22),* but, to our knowledge, this response has not been studied *in vivo* in human skin. That the cellular p53 response can be observed *in vivo* in animal models has been shown previously *(23–25).*

Unexplained large individual variations of human skin to UV radiation have been observed in the p53 response *(1)* and the DNA repair reaction *(26).* An acute clinical side effect of radiotherapy is radiation dermatitis *(27),* and large differences in the degree of radiation dermatitis have also been noted among individuals after radiotherapy *(28).* However, it has not been determined whether the responses to UV and ionizing radiation are related. We address this issue by measuring changes in the levels of p53 and p21 and assessing erythema in the same patient after exposure to three types of radiation, UVA, UVB, and 5-mV photon-beam radiation. The study also assessed the protective effect of a topically applied steroid on radiation dermatitis, where a major conclusion was that corticosteroid cream reduced acute radiation dermatitis (Bostrom A, Lindman H, Swartling C, Berne B, Bergh J: manuscript submitted for publication). In this study, we investigate interindividual variations in the responses of p53 and p21 to DNA damage; analyze the relationships of p53 and p21 with UVA, UVB, and high-voltage photon-beam irradiation; and determine whether p53 or p21 would be a good marker to predict clinical radiation dermatitis.

Patients and Methods

This study was a part of a double-blind randomized study that also evaluated the effects of a potent topical corticosteroid application (mometasone furoate) and placebo (emollient cream) on radiation dermatitis. The patients received oral and written information before inclusion in the study.

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See "Notes" following "References."

Patients

Fifty women with breast cancer between the ages of 47 and 77 years (mean = 59.5 years) entered the study, which was approved by the ethical committee with jurisdiction for Uppsala University Hospital, Sweden. Patients with known cutaneous or severe systemic disease were excluded. All patients had been treated by sector resection of a morphologically verified invasive breast cancer without lymph node metastasis. No concomitant systemic anticancer treatment was administrated. No patient had recently been in the sun or in a tanning bed before entering the study. All patients were Caucasian and had the following skin types, according to Fitzpatrick *(29):* 32 patients had skin type 3, 15 had skin type 2, and three had skin type 4. Patients were instructed to apply the "blinded" cream to the irradiated breast twice a week from the start of the radiotherapy until they had received a total radiation dose of 24 Gy and then to apply it once a day until 3 weeks after radiotherapy was completed.

Irradiation

Skin on the back of all patients was UV irradiated. UVB was administered to nine areas (each 7 mm in diameter) in a stepwise fashion (300–1500 J/m^2) with a monochromator (Applied Photophysics, Leatherhead, Surrey, U.K.) emitting a narrow band at 313 nm (±4–6 nm) through a 1-m liquid light guide with a 7-mm aperture at an irradiance of 15–17 mW/cm^2. UVA was administered with an UVASUN lamp (Muthzas Co., Munich, Germany) equipped with a UVA filter (330–450 nm) emitting with an irradiance of 86 mW/cm^2. UVA was delivered to four areas of skin, each 50 × 50 mm. Each area received a dose of 10, 20, 40, or 80 J/cm^2. Radiotherapy was started after the UV tests were completed. All patients received tangential 5-mV photon-beam radiotherapy to the breast parenchyma, at 2 Gy per visit from a Philips SL 75–5 linear accelerator (Philips Inc., Crawley, U.K.) as described previously *(30),* for 5 days per week until a total dose of 54 Gy was reached.

Evaluation of Erythema and Skin Sampling

Degree of UV-induced erythema was determined from readings 24 hours after irradiation. The following scale was used: 0 = no erythema; 1 = just perceptible erythema (the minimal erythema dose); 2 = mild erythema; 3 = marked erythema; 4 = marked erythema and slight edema; 5 = marked erythema and strong edema; and 6 = bullous reaction. In addition, the degree of pigmentation after UVA irradiation was assessed. For UVB, the test sites that most closely corresponded to a site receiving twice the minimal erythema dose were subjected to skin biopsy 24 hours after irradiation. For UVA, biopsy sites received 40 or 80 J/cm^2 (14 patients received 40 J/cm^2 because of a previous medical history of high sensitivity to UV radiation). Biopsy samples of normal nonirradiated and irradiated back skin were taken at the same time, 24 hours after UV irradiation.

Three breast skin biopsy samples were taken 1 cm below the areola of the photon-beam-irradiated breast. The first sample was taken before the start of radiotherapy, the second was taken 20–30 hours after the first 2-Gy dose of photon-beam radiation, and the third was taken immediately after radiotherapy was completed (total radiation dose = 54 or 56 Gy). Erythema was evaluated, with a reflectance spectrophotometer, four times during radiotherapy (after 24, 34, 44, and 54 Gy) and 3 weeks after treatment was completed. The mean of these data, including data from the five irradiated areas of the breast, was calculated for each patient and used as an objective erythema score. Reciprocal areas from the nonirradiated breast were used as normal control site.

Skin Biopsy Samples and Immunohistochemistry

The protocol above resulted in six biopsy samples (3-mm in diameter) per patient. Ten of these samples were technically insufficient, leaving a total of 290 interpretable punch biopsy samples. The 3-mm punch biopsy samples were immediately fixed in neutral-buffered formalin. After 24–48 hours, the tissue was embedded in paraffin and 4-μm-thick sections were cut. Immunohistochemistry was performed essentially as described previously *(31).* Briefly, sections were cooked in a 750-W microwave oven (two 5-minute periods in 10 m*M* citric buffer [pH 6.0]) and stained with avidin–biotin–coupled immunoperoxidase. An automated standardized procedure (Ventana Medical Systems Inc, Tucson, AZ) was used with primary antibodies D-07 (code M7001; dilution 1 : 200; DAKO A/S, Glostrup, Denmark), which reacts with both wild-type and mutant human p53 protein, and WAF-1 (Ab-1, dilution 1 : 40; Oncogene Research Products from CALBIOCHEM, Cambridge, MA), which reacts with human p21 protein. Sections were counterstained with Mayer's hematoxylin.

Scoring of p53 and p21 Immunoreactivity

Sections of immunostained skin were evaluated under the microscope by one person (Dr. Christina Nyberg, University Hospital, Uppsala, Sweden). All epidermal keratinocyte nuclei from four randomly chosen high-power fields, covering more than 50% of the total epidermal length in each biopsy sample, were evaluated and counted. In anti-p53-stained sections, the difference between nonimmunoreactive and immunoreactive keratinocyte nuclei was clear-cut, and cells were scored as positive or negative. Anti-p21-stained sections showed gradients of reactivity, rendering binary assessment impossible. Consequently, the proportion of immunohistochemically positive cells was estimated on the following scale of 1–4: 1 = less than 25% immunoreactive keratinocytes; 2 = 25% to 50% immunoreactive keratinocytes; 3 = 50% to 75% immunoreactive keratinocytes; and 4 = more than 75% immunoreactive keratinocytes. This modified scale was motivated by a diffuse reaction pattern for the p21 antibody. The intensity of the p21 immunoreaction was determined as weak, moderate, or strong.

Methods for Statistical Analysis

This study did not have the statistical power to investigate possible differences in local and systemic relapse frequencies. It would, of course, have been ideal to have a larger study population to be able to investigate whether the p53 activation pattern in the normal skin of a cancer patient is associated with the p53 response status in the same patient's tumor cells.

Nonparametric methods were used for the statistical analysis. The Spearman's rank correlation test was used to calculate potential correlations. The corresponding correlation coefficient is denoted r_s in the text. Because multiple correlation analyses were performed, only P values of less than .01 were considered to be statistically significant. For comparison of two groups, the Mann–Whitney U test was used. All statistical tests are two-sided.

Results

The average number of cells counted per anti-p53-stained biopsy sample was 528. Skin type did not influence p53 immunoreactivity in nonirradiated or irradiated skin. Table 1 shows a summary of the mean percentage of p53-positive cells and p21 score. Fig. 1 shows p53 immunoreactivity in the following six groups: 1) normal back skin, 2) normal breast skin, 3) UVA-irradiated back skin, 4) UVB-irradiated back skin, 5) 2 Gy of photon-beam-irradiated breast skin, and 6) 54 Gy of photon-beam-irradiated breast skin. Table 2 shows correlations in p53 immunoreactivity between the different groups.

Nonirradiated Skin

In general, breast skin was slightly thinner than back skin. The number of keratinocytes, however, did not differ statistically significantly between back (mean = 618 keratinocytes; range = 427–936 keratinocytes) and breast (mean = 583 keratinocytes; range = 384–938 keratinocytes) skin. A few scattered p53-positive keratinocytes were present in 41 of the 50 biopsy samples from normal breast (Fig. 2, a) and 43 of the 49 from back skin (Fig. 2, b). p53-positive keratinocytes were 2.3% of cells (median = 1.2%; range = 0%–31.1%) in breast skin and 1.8% (median = 1.3%; range = 0%–8.9%) in back skin. In one patient (No. 30), 31.1% of nuclei of the unirradiated breast skin were p53 positive. Only a few keratinocytes showed weak p21 staining.

Photon-Beam Radiotherapy

Compared with nonirradiated skin, no changes were detected by light microscopy in skin biopsy samples taken after the patient had received 2 Gy of photon-beam radiation. However, severe morphologic changes, similar to what has been described previously *(32),* were observed (Fig. 2, d) in biopsy samples taken after radiotherapy was completed (total dose = 54 Gy). The epidermis in these

Table 1. p53- and p21-immunoreactive cells in normal and irradiated skin*

	Value (95% CI)	
Nonirradiated skin		
Back skin		
% p53 positive	1.8 (1.3 to 2.3)	
p21 score	0.7 (0.6 to 0.9)	
Breast skin		
% p53 positive	2.3 (1.0 to 3.5)	
p21 score	0.6 (0.5 to 0.7)	
Irradiated skin		
UVA, 68.8 J/cm^2 on back skin		
% p53 positive	7.4 (5.2 to 9.6)	
p21 score	0.9 (0.8 to 1.0)	
UVB, 628 J/m^2 on back skin		
% p53 positive	45.2 (40.3 to 50.1)	
p21 score	2.6 (2.2 to 2.9)	
	Emollient treated Value (95% CI)	***Topical steroid*** Value (95% CI)
Treated skin		
Photon beam, 2 Gy on breast skin		
% p53 positive	31.6 (21.8 to 41.4)	28.5 (19 to 38)
p21 score	1.1 (0.8 to 1.3)	1.0 (0.9 to 1.1)
Photon beam, 54 Gy on breast skin		
% p53 positive	77.0 (70.7 to 83.3)	83.3 (78.8 to 87.8)
p21 score	3.3 (2.9 to 3.7)	3.5 (3.1 to 3.8)
Erythema score (breast skin)	7.5 (6.7 to 8.3)	5.4 (4.8 to 6.0)

*For normal and irradiated skin, the mean percentage (including 95% confidence interval [CI]) of p53-immunoreactive cells is shown. The mean score (including 95% CI) for the number of p21-positive cells is also shown (scores: 1 = <25%; 2 = 25%–50%; 3 = >50%–75%; and 4 = >75% immunoreactive keratinocytes). The location for the skin biopsy sample and the mean administered UVA and UVB dose are shown. Mean values for breast skin treated with photon-beam radiation are split in the two treatment groups (topical steroid cream and emollient cream). There was a marked interindividual heterogeneity in the number of p53-positive cells in the various irradiated and nonirradiated samples. Erythema scores were lower in patients treated with topical steroids than in those treated with only moisturizer, but no statistically significant difference was observed in p53 immunoreactivity after completed radiotherapy (54 Gy) in these groups. No statistically significant difference was found between use of topical steroids and moisturizer with respect to the p53/p21 response of epidermal keratinocytes to high-voltage photon-beam radiation (2 Gy, $P = .59$; 54 Gy, $P = .23$). All statistical tests were two-sided. A supplementary table containing an overview of the data from the 50 patients in this study can be found as an on-line supplement on the Journal's web site <http://www.jnci.oupjournals.org>.

samples was thin, containing only one to three layers of keratinocytes, and the keratinocytes were enlarged with cytologic atypia. The dermis had dilated capillaries and a partly perivascular chronic inflammatory infiltrate. Immunohistochemically, 30.1% (median = 31.0%; range = 0%–79.3%) of the keratinocytes were positive for p53 after 2 Gy of irradiation (Fig. 2, c). There were fewer than 25% p21-positive cells (score = 1.1), and the intensity of p21 staining was weak. The most widespread, intense, and uniform staining for p53 and p21 was observed in skin that had received 54 Gy of irradiation. After radiotherapy was completed, 80.1% (median = 83.2%; range = 37.6%–95.2%) of keratinocytes were p53 positive (Fig. 2, d). In most cases, 75% or more of the keratinocytes (score = 3.4) showed a moderate-to-strong intensity of p21 staining. Objective measurements of erythema varied between scores of 2.3 and 11.2 (mean score = 6.4).

UVA Irradiation

No morphologic changes were found in skin after UVA irradiation compared with nonirradiated skin. In UVA-irradiated skin, 7.4% (median = 4.4%; range = 0%–40.5%) of the keratinocytes overexpressed p53 (Fig. 2, e). p53 immunoreactivity did not differ statistically significantly between patients irradiated with 40 J/cm^2 and patients irradiated with 80

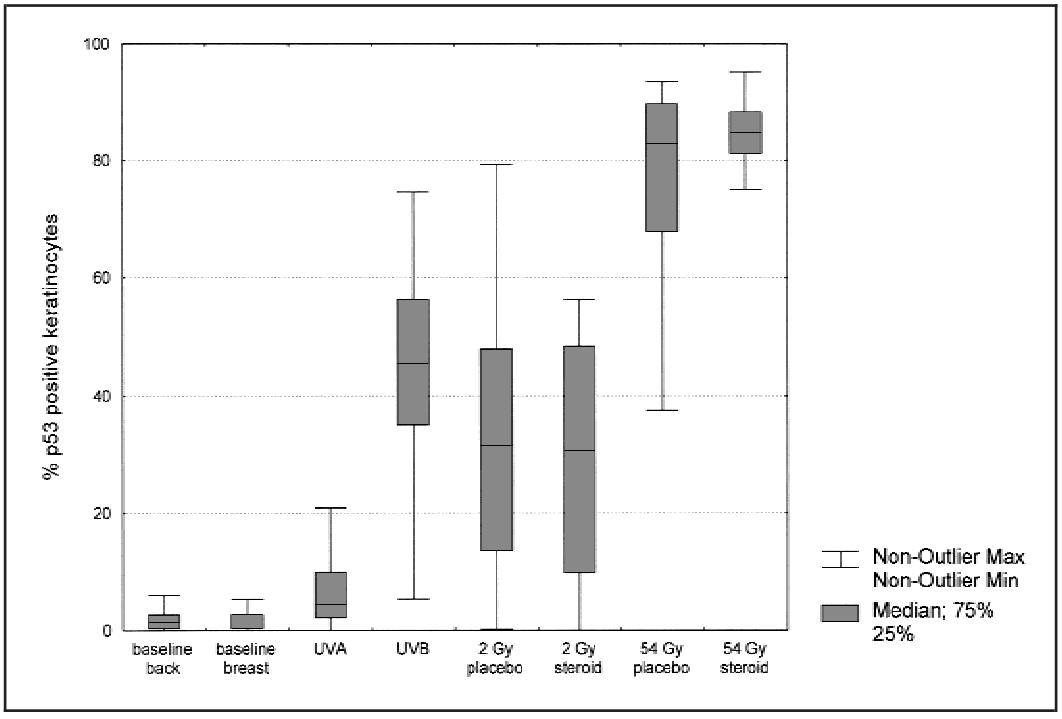

Fig. 1. p53 immunoreactivity in the following six groups: normal back skin, normal breast skin, and UVA-, UVB-, 2 Gy of photon-beam-, and 54 Gy of photon-beam-irradiated skin. **Bars** represent the range within the different groups as indicated. The **box plots** for 2 Gy and 54 Gy of radiation have been split by topical treatment.

Table 2. Correlations

*a. Correlations before treatment**

Subjects (n = 50)	Variable 1, irradiation	Variable 1, sample	Variable 2	Correlation, r_s	*P*
All	Baseline	Back p53% versus	Breast p53%	.58	<.001
All	Baseline	Back p53% versus	UVA p53%	.37	.011
All	Baseline	Back p53% versus	UVB p53%	.40	.005
All	Baseline	Breast p53% versus	2 Gy p53%	.47	.001
All	UVA	Back p53% versus	UVB p53%	.44	.002
All	UVA	Back p53% versus	2 Gy p53%	.50	<.001
All	UVB	Back p53% versus	2 Gy p53%	.61	<.001

b. Correlations after placebo treatment†

Subjects (n = 25)	Variable 1, irradiation	Variable 1, sample	Variable 2	Correlation, r_s	*P*
Placebo	Baseline	Breast p53% versus	54 Gy p53%	.28	.2
Placebo	Baseline	Breast p53% versus	Erythema	.34	.09
Placebo	UVA	Back p53% versus	54 Gy p53%	.21	.3
Placebo	UVA	Back p53% versus	Erythema	.09	.7
Placebo	UVB	Back p53% versus	54 Gy p53%	.40	.05
Placebo	UVB	Back p53% versus	Erythema	.20	.3
Placebo	2 Gy	Breast p53% versus	54 Gy p53%	.46	.03
Placebo	2 Gy	Breast p53% versus	Erythema	−.0025	1.0
Placebo	54 Gy	Breast p53% versus	Erythema	−.17	.4

c. Correlations after cortisone treatment†

Subjects (n = 24)	Variable 1, irradiation	Variable 1, sample	Variable 2	Correlation, r_s	*P*
Cortisone	Baseline	Breast p53% versus	54 Gy p53%	.021	.9
Cortisone	Baseline	Breast p53% versus	Erythema	−.14	.5
Cortisone	UVA	Back p53% versus	54 Gy p53%	.18	.4
Cortisone	UVA	Back p53% versus	Erythema	−.33	.1
Cortisone	UVB	Back p53% versus	54 Gy p53%	−.06	.8
Cortisone	UVB	Back p53% versus	Erythema	−.52	.008
Cortisone	2 Gy	Breast p53% versus	54 Gy p53%	.16	.5
Cortisone	2 Gy	Breast p53% versus	Erythema	−.038	.9
Cortisone	54 Gy	Breast p53% versus	Erythema	−.16	.4

*__a:__ Nonparametric correlations of p53 immunoreactivity before radiotherapy and after 2 Gy of 5 mV photon-beam radiotherapy to the breast. Data from all 50 patients were used for this calculation. Both Spearman's rank coefficient of correlation (r_s) and the corresponding *P* value are shown; all statistical tests are two-sided. In all studied groups, there was a statistically significant correlation between p53 and p21 immunoreactivity. When we compared all skin samples (n = 290), a very good correlation between p53 and p21 was seen (r_s = .78; *P*<.001). There was a strong association between the number of p21-immunoreactive cells and the intensity of the p21 reaction. Within-individual p53 immunoreactivity correlated irrespective of whether samples were from nonirradiated, UVA-irradiated, UVB-irradiated, or 2-Gy-irradiated skin. In nonirradiated skin, the number of p53-positive back skin cells correlated with the number of p53-positive cells in breast skin (r_s = .58; *P*<.001). There was also a positive correlation among nonirradiated skin and skin irradiated with UVA, UVB, and 2-Gy photon-beam radiation. There was a clear and statistically significant correlation between p53 immunoreactivity in UVA-irradiated skin and UVB-irradiated skin (r_s = .44; *P* = .002) and between UVA-irradiated skin and skin that had received 2 Gy of photon-beam irradiation (r_s = .50; *P*<.001). The strongest correlation was between p53 immunoreactivity in UVB-irradiated skin and 2-Gy photon-beam irradiated skin (r_s = .61; *P*<.001). In samples taken from skin irradiated with 54 Gy of photon-beam irradiation, p53 and p21 immunoreactivities were consistently stronger and were not statistically significantly correlated with immunoreactivities in other samples.

†__b__ and __c:__ Nonparametric correlations for both breast erythema and p53 immunoreactivity after radiotherapy was completed in the group of 25 emollient-treated patients and in the topical steroid group of 24 patients. Analyzing the steroid-treated groups and non-steroid-treated patient groups separately could only identify a weak correlation in the p53 reactions after 54 Gy of photon-beam radiation compared with after 2 Gy of radiation and UVB in the non-steroid-treated group. The average objective erythema of the breast showed no correlation to p53 or p21 immunoreactivity in any calculation, except for the negative correlation to UVB in the steroid-treated group. Because the group of emollient-treated patients showed an opposite but very weak correlation, the analysis of UVB versus erythema for all patients did not reveal any correlation (r_s = −.18; *P* = .2).

J/cm^2. Fewer than 25% of keratinocytes were p21 immunoreactive (mean score = 0.9), and the intensity of p21 staining was weak.

UVB Irradiation

The administered dose of UVB (300–1500 J/m^2) depended on the patient's sensitivity to UVB (i.e., the minimal erythema dose), which did not correlate directly with the patient's skin type (r_s = .21). There was no clear correlation between the physical UVB dose and the number of p53-positive cells observed (r_s = .28). Morphologic changes (i.e., epidermal edema, sunburned cells, and dermal inflammation) were observed in UVB-irradiated skin compared with nonirradiated skin, 45.2% (median = 45.5%; range = 5.3%–74.6%) of keratinocytes were p53 positive (Fig. 2, f), and increased p21 expression with a moderate-to-strong intensity was found in approximately 50% of the cells (score = 2.6).

We have not, in our initial aims, analyzed the topographic distribution of p53 immunoreactivity in different layers of the epidermis. However, the five cases with strongest p53 reaction to UVA and to 2 Gy, as well as the five with the weakest reaction, were reviewed to examine eventual differences in expression patterns. We found that UVA resulted in p53 immunoreactivity within all epidermal layers with relatively more positivity within the basal cell layer when compared with UVB. The expression pattern for 2 Gy and UVA showed a similar pattern, i.e., positivity within all nucleated levels of epidermis with a tendency to a relative increase in basal cells. In skin irradiated with 54 Gy, the epidermis was severely altered without differences in p53 immunoreactivity in basal or suprabasal cells.

Similar to what is known for UV radiation, ionizing radiation did not lead to p53 immunoreactivity in mesenchymal cells of the dermis. Only rare p53-positive dermal cells were seen after 54 Gy of radiation.

Discussion

The major finding in this study was the close correlation between p53 expression after photon-beam radiotherapy and UV irradiation, especially with UVB irradiation (r_s = .61). The large number of patients (50 patients) and multiple biopsy sites (six sites) also permitted an overview of interindividual variations in normal skin and sensitivity to different sources of radiation. The long-term goal with this project was to gain insight into the differences in radiation sensitivity in normal tissues so that therapies can be tailored specifically for each individual and also to understand the value of topical steroid for protection of radiation dermatitis.

After UV radiation-induced DNA

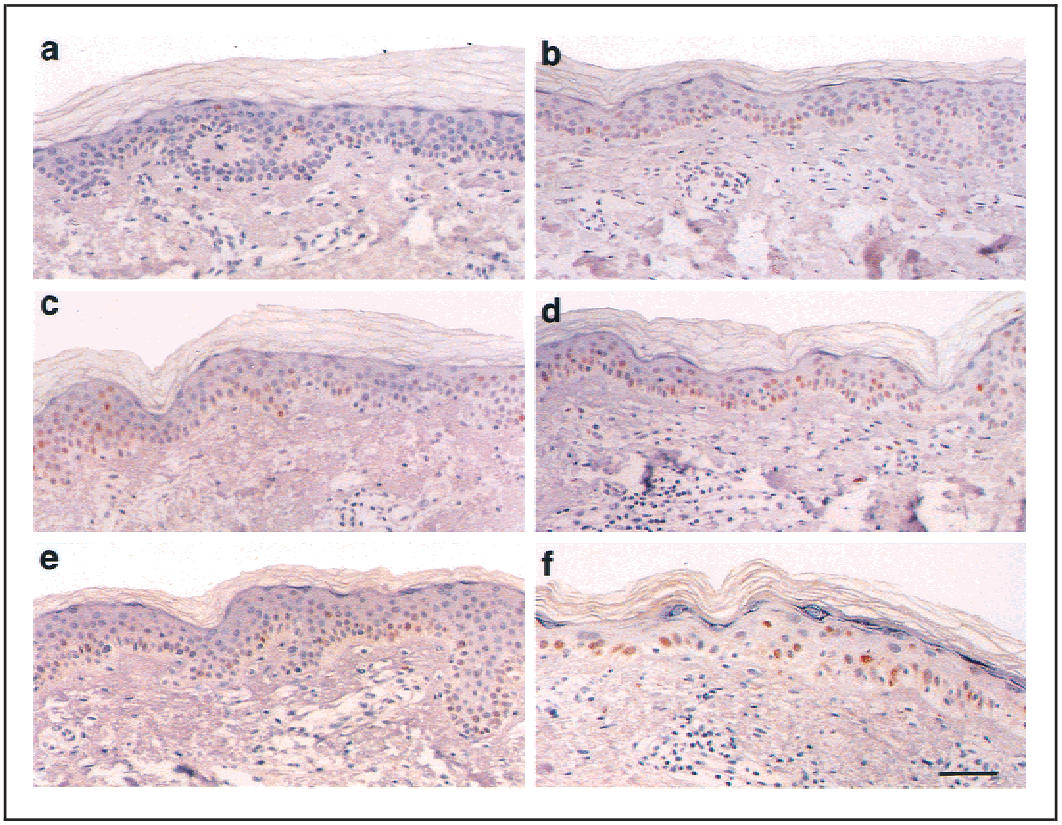

Fig. 2. Immunohistochemically stained sections of skin, displaying the various layers of the epidermis and the upper part of underlying dermis. The p53 antibody from one patient who showed a strong p53 response was used. Immunoreactive keratinocyte nuclei are **brown,** and nonimmunoreactive nuclei are stained **blue** with hematoxylin. Normal, nonirradiated breast skin (**a**) and back skin (**b**) have a few scattered keratinocytes with various levels of p53 immunoreactivity. Twenty-four hours after 2 Gy of high-voltage photon-beam irradiation, an increased number of p53 immunoreactive cells are seen in the epidermis (**c**). After radiotherapy was completed (54 Gy), the epidermis is severely damaged, and keratinocytes show strong p53 immunoreactivity (**d**). Twenty-four hours after UV irradiation, epidermal keratinocytes show an elevated p53 response (**e**). UVA = 80 J/cm^2. (**f**) UVB = 600 J/m^2. **Scale bar** for all panels = 120 μm.

damage *(1,8),* p53 and p21 proteins have been detected in higher than normal levels in the epidermis, and we have demonstrated individual differences in the expression of p53 protein after exposure to sunlight *(9).* Interindividual differences in the radiosensitivity of human skin have been detected with clinical end points *(28),* and the effects of ionizing radiation on human skin have also been studied, in part *(33).* However, to our knowledge, the expression of p53 and p21 during and after radiotherapy has not been analyzed.

We observed that, after 2 Gy of photon-beam radiation, the p53 response in epidermis showed the same large interindividual variation as UV-irradiated skin. Aside from skin repeatedly subjected to 5-mV photon-beam irradiation (total dose = 54 Gy), where most (80%) of the keratinocytes were p53 positive, UVB irradiation provoked the strongest response; approximately half (45%) of the epidermal cells were strongly immunoreactive for p53. In addition, we observed that only 2 Gy of photon-beam irradiation caused a substantial p53 response (30%) and that UVA irradiation only caused a relatively mild response (7%). The p53 responses induced by different types of radiation might be caused by variations in DNA damage associated with wavelength-related differences in absorption. Alternatively, different types of radiation could trigger different p53 activation pathways, in a manner independent of the degree of DNA damage.

It should be noted that a patient with a strong reaction to UVA will tend to react strongly to UVB and photon-beam (2 Gy) irradiation and vice versa. Although the correlation is strongest between UVB and photon-beam (2 Gy) irradiation (r_s = .61), there appears to be a general intrinsic variation in the general p53 responsiveness. The molecular reasons for this interindividual variation are unclear; however, large interindividual differences have also been shown in repair of cyclobutane pyrimidine dimers and 6-4 photoproducts in human skin exposed to solar-simulating radiation *(26).* There does not appear to be a simple association of skin type and p53 or p21 response. All patients received the same dose of radiotherapy (2 Gy), and the p53 response did not correlate with skin type (r_s = .12). The doses of UVA and UVB radiation that were given were determined because of different clinical responses and did not clearly correlate with the intensity of the p53 response (UVA r_s = .11; UVB r_s = .28). Samples from UVB-irradiated skin were taken from areas subjected to double the minimal erythema dose, which resulted in a "just perceptible erythema" *(2).* UV radiation causes thymine dimers and 6-4 photoproducts in DNA; thus, DNA has been proposed as the chromophore for erythema *(34).* However, there is no simple association between the UV radiation-induced increased levels of p53 in epidermis and clinical erythema *(35).* Skin irradiated with a total of 54 Gy was severely damaged and had strong p53 and p21 responses. Eventually, individual variations are probably masked by the severe damage caused by large amounts of ionizing radiation. The p21 response, in general, mirrored the p53 response, in agreement with the hypothesis that, after DNA damage, p53 induces p21. In general, when there was a strong p53 response (UVB and especially 54 Gy of photon-beam radiation), there was a strong p21 response. However, skin that had received only 2 Gy of photon-beam radiation had a weaker p21 response than would be expected from the p53 response. The explanation for this observation is unclear.

In this study, we show that a few scattered p53-positive cells (approximately 2%) are present in normal, previously sun-exposed back skin and nonexposed breast skin. Although some patients may have an early history of sun exposure to the breasts, we assume that breast skin of most women in the age group studied (47–77 years old) has not been exten-

sively exposed to the sun. The p53-positive cells have not been characterized. These cells could represent single cells that have acquired a p53 mutation, not sufficient to cause clonal expansion, which is so commonly found in chronically sun-exposed skin *(4,5,36)*. The use of laser microdissection, gene amplification, and DNA sequencing of single cells *(37)* would be an important tool to specifically analyze these cells.

In summary, large interindividual differences were observed in the radiation-induced p53 response in human skin that is independent of the type of radiation used. The p53 response did not reflect the individual radiation erythema. The biologic importance of being a weak or a strong p53 responder is not known. The mechanisms regulating the increase in p53 *in vivo* in response to cellular damage and the role of p53-positive cells in normal nonirradiated skin need additional study.

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