



## Original Contribution

# Expression of p53 Tumor Suppressor Protein in Sun-exposed Skin and Associations with Sunscreen Use and Time Spent Outdoors: A Community-based Study

Jolieke C. van der Pols<sup>1,2</sup>, Chunxia Xu<sup>2</sup>, Glen M. Boyle<sup>2</sup>, Peter G. Parsons<sup>2</sup>, David C. Whiteman<sup>2</sup>, and Adele C. Green<sup>2</sup>

<sup>1</sup> School of Population Health, University of Queensland, Herston, Australia.

<sup>2</sup> Queensland Institute of Medical Research, Herston, Australia.

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The *p53* gene is a tumor suppressor gene that is commonly mutated in skin cancer and sun-exposed skin, and this can be detected through immunohistochemical expression of the p53 protein. The authors hypothesized that time spent outdoors is associated with p53 protein expression in human skin and that sunscreen use counteracts the association. In 1996, they investigated this in a community-based cross-sectional study in Australia. Detailed information about skin type, time spent outdoors, and sunscreen use was collected from 139 residents of a subtropical township who also provided a skin biopsy from the back of the hand for measurement of p53 expression. Increasing time spent outdoors was positively associated with immunoreactivity in the whole epidermis and in the basal layer of the epidermis. After adjustment for confounders, p53 immunoreactivity was twice as high for people who used sunscreen 1 or 2 days per week as for those who used sunscreen daily (whole epidermis: ratio estimate = 2.0, 95% confidence interval: 1.1, 3.6; basal layer: ratio estimate = 1.7, 95% confidence interval: 0.9, 3.1). The authors conclude that p53 immunoreactivity in the skin is a marker of exposure to ultraviolet light in the past 6 months, but this may be mitigated by regular application of sunscreen.

immunohistochemistry; suncreening agents; tumor suppressor protein p53; ultraviolet rays

Abbreviation: UV, ultraviolet.

The p53 protein is a central component of the mechanisms that protect skin cells from malignant transformation (1). When activated by cellular stresses such as DNA damage or oncogenic signals, this transcription-factor protein induces cell-cycle arrest and apoptosis to prevent potentially cancerous cells from progressing to cancer (2). The most common cause of loss of normal (“wild-type”) p53 function is mutations within the *p53* gene, which leads to the expression of mutant p53 protein (3) and indicates a reduced capacity to trigger programmed cell death. Recent studies have shown that mutant p53 protein may directly affect gene expression and down-regulate the function of other proteins in the p53 pathway (3).

Exposure to ultraviolet (UV) light is the principal environmental cause of skin cancer (4). High doses of UV radiation to the epidermis cause mutations in the *p53* gene. These mutations are present in more than 90 percent of human squamous cell carcinomas, approximately 50 percent of human basal cell carcinomas, and 20 percent of melanomas (5–7). Mutation of the *p53* gene and increased expression of p53 protein in normal skin are thought to be early events in the formation of basal cell and squamous cell carcinoma of the skin (8, 9).

Studies have shown that expression of the p53 protein can be induced by experimental UV exposure (10–12) and that p53 expression is more common in skin samples taken from

chronically sun-exposed body sites compared with rarely exposed sites (13, 14). Experimental studies have shown that sunscreen use can reduce DNA damage following UV exposure and attenuate the epidermal expression of p53 (15, 16). A small, controlled study in Swedish human volunteers showed that use of a broad-spectrum sunscreen can reduce expression of p53 in the skin (17). To our knowledge, no studies have yet reported the extent to which amount of time spent outdoors and sunscreen use by free-living, nonrestricted adults are associated with levels of p53 expression. Information on these associations would help investigators to better understand the biologic effects of different patterns of sun exposure at the population level. Sun avoidance and use of sunscreen are recommended in high-risk populations (18), but to date, the extent to which these behaviors are associated with p53 expression in the community is unknown.

Mutations of the *p53* gene can occur in cells that are located in either the outer layers or the basal layer of the epidermis (19). It remains unclear whether the location of p53-damaged cells within the epidermis is associated with skin cancer risk, but mutations in the more differentiated outer layers of the skin are thought to be less detrimental than mutations in the stem-cell-rich basal layer of the epidermis. The thickness of the epidermis and skin color are known to attenuate the amount of UV light that reaches the basal layer of the epidermis (20), and these factors may modify the distribution of p53 expression in the upper and basal layers of the epidermis (12, 21, 22).

Therefore, we hypothesized that recent time spent outdoors (i.e., in the previous 6 months) would be associated with p53 protein expression in human skin and that sunscreen use would counteract the association. We investigated this in the current study by utilizing biopsies of sun-exposed skin that had been provided by participants in a population-based randomized trial assessing skin cancer prevention. We also hypothesized that p53 protein expression in the basal layer of the epidermis would be differentially associated with these and other factors associated with skin cancer risk. On the basis of available evidence, we expected that persons who spent more time outdoors would show higher expression of p53 than persons who tended to stay indoors, and that use of sunscreen would be associated with lower p53 expression.

## MATERIALS AND METHODS

### The Nambour Skin Cancer Prevention Trial

Study participants were randomly selected from the Nambour Skin Cancer Prevention Trial, which was carried out in Australia between 1992 and 1996. Full details of the trial have been reported elsewhere (23, 24). In summary, the trial participants were randomly chosen residents of Nambour, a township in southeastern Queensland, Australia (latitude 26°S). In a two-by-two factorial design, participants were randomized among four treatment groups: 1) daily application of a broad-spectrum, water-resistant Sun Protection Factor 16 sunscreen to the head, neck, arms, and hands, plus beta-carotene supplementation; 2) daily use of sunscreen plus placebo tablets; 3) beta-carotene only; or

4) placebo only. Participants not assigned to daily application of sunscreen were asked to continue application of sunscreen at their usual, discretionary rate.

Participants received full skin examinations by dermatologists in 1992 (baseline), 1994, and 1996 (cessation of the trial). All skin cancers clinically diagnosed were examined histologically by a single dermatopathologist. General practitioner notifications and regular questionnaires were used for reporting of skin cancer treated between these skin examinations. When notification of a newly treated skin lesion was received, that person's medical records were examined; pathology reports were obtained whenever available. Only histologically confirmed skin cancers were considered here.

At the final clinic visit of the trial in 1996, 162 randomly selected participants were invited to provide a 2-mm punch biopsy from the back of the left hand. The biopsy sample was fixed in formalin and stored in paraffin. During this visit, participants also completed a questionnaire that asked about smoking behavior and posed the following questions: "During the last 6 months, how much time did you spend outdoors during the whole day (from sunrise to sunset) on a typical weekday/weekend day?"; "How many days per week do you apply sunscreen to your hands?"; and "Overall, have your occupational activities been mainly: outdoors/both outdoors and indoors/indoors?". History of skin cancer before the trial, skin color, propensity to sunburn, and outdoor/indoor nature of leisure activities were recorded on the baseline questionnaire.

All participants provided written informed consent, and the Queensland Institute of Medical Research Ethics Committee approved the study.

### Immunohistochemical staining

Using immunohistochemistry, mutant p53 protein in skin tissue sections is made visible microscopically through binding with a color-labeled, protein-specific antibody. Wild-type p53 protein has a very short half-life (5–20 minutes) and is not readily detectable by immunohistochemistry, while the mutated *p53* gene encodes a protein with a substantially prolonged half-life (5–24 hours) as indicated by p53 immunoreactivity (25–27).

Expression of the p53 protein in the paraffin-embedded skin sections was investigated using the DO-7 monoclonal antibody (Novocastra Laboratories, Newcastle upon Tyne, United Kingdom) at a 1:50 dilution. The immunohistochemical staining method, using microwave antigen retrieval, has been described previously (7). All slides were counterstained with hematoxylin and examined at 400× magnification by one of the authors (C. X.), who was blind to subject identity. For each subject, a negative control section was examined to monitor staining quality based on the level of nonspecific background staining. The intensity of the counter-stain and maintenance of tissue architecture were also considered for staining quality. Sections considered of poor quality were discarded or replaced with spare slides cut from the same tissue block. Only epidermal cells in which the nucleus was stained brownish-red, including keratinocytes and melanocytes, were interpreted as p53-positive cells. The numbers of epidermal cells, basal cells (epidermal cells attached to the

dermal-epidermal junction), and p53-positive cells in the whole section were counted. Cells in three randomly selected sections were counted twice for reproducibility analysis.

We assessed p53 immunoreactivity in two ways: 1) by the proportion (percentage) of p53-positive cells in the whole epidermis (the number of p53-positive cells in the whole section divided by the total number of epidermal cells in the whole section) and 2) by the proportion (percentage) of p53-positive cells in the basal layer (the number of p53-positive cells in the basal layer divided by the total number of basal cells in the whole section). We also created a variable that indicated the pattern of p53 distribution, called the "basal p53 proportion." This was calculated as the ratio of the number of p53-positive cells in the basal layer to the number of p53-positive cells in the whole epidermis  $\times$  100 (percent), where a low percentage indicates that a large number of p53-positive cells are confined to the upper epidermis and a high percentage indicates that p53-positive cells predominate in the basal layer. The thickness of the epidermis was measured as the ratio of the total number of epidermal cells in the whole section to the number of basal cells in the section.

### Data analysis

We first assessed whether general subject characteristics were associated with p53 immunoreactivity. These characteristics were selected because they may be associated with sun exposure patterns or sensitivity to UV damage in the skin. We then investigated whether solar UV exposure patterns, reported by the participants as the amount of time spent outdoors, was associated with p53 immunoreactivity.

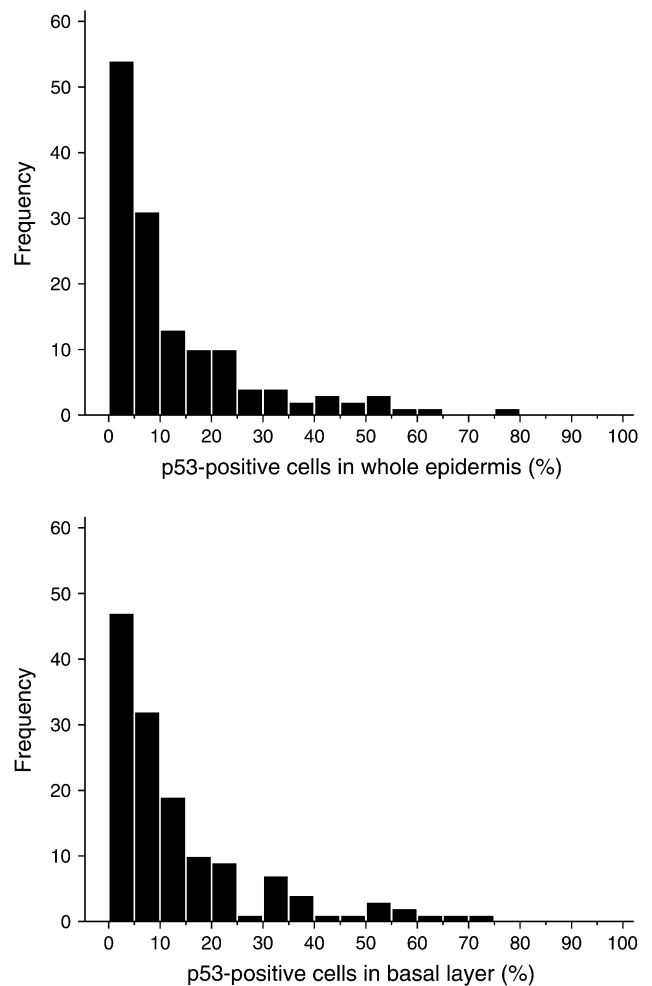
Subsequently, we investigated whether reported use of sunscreen was associated with p53 immunoreactivity. All trial participants reported frequency of sunscreen use on different body parts, including the back of the hand (from which the biopsies were taken). Thus, we investigated whether the reported frequency of sunscreen use on the back of the hand was associated with p53 immunoreactivity independently of skin type and color, amount of time spent outdoors, and other predictors of p53 immunoreactivity.

Group differences were tested using analysis of variance for an unbalanced design (PROC GLM). Data on the p53 immunoreactivity outcome variables and the thickness of the epidermis were  $\log_e$ -transformed to achieve a normal distribution.

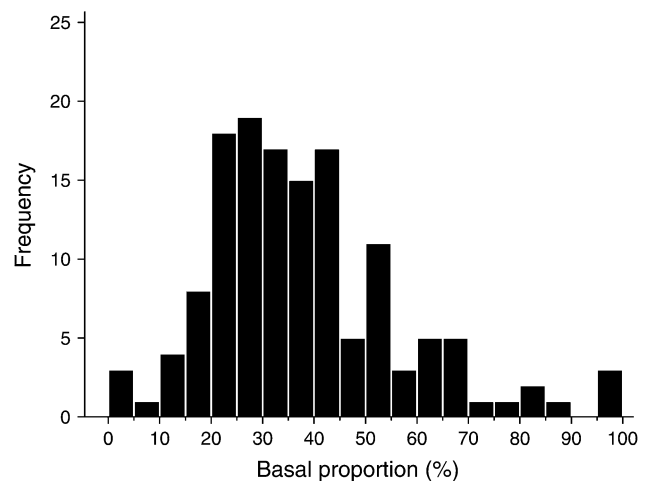
All tests were two-sided, and a significance level of  $p < 0.05$  was used. All analyses were carried out using the Statistical Analysis System, version 8.02 (SAS Institute, Inc., Cary, North Carolina).

### RESULTS

Of the 162 invited participants, 144 (89 percent) provided a skin sample by punch biopsy. Immunohistochemical staining of p53 was successfully carried out in 139 (97 percent) of these samples. The distribution of p53 immunoreactivity was skewed, with most biopsies showing low-to-medium proportions of p53-positive cells (figures 1 and 2); 85



**FIGURE 1.** Proportion of p53-positive cells (%) in dorsal hand epidermis, Nambour Skin Cancer Prevention Trial, Australia, 1996.



**FIGURE 2.** Proportion of all p53-positive cells (%) in the basal layer of the epidermis, Nambour Skin Cancer Prevention Trial, Australia, 1996.

**TABLE 1. General subject characteristics (median and 25th and 75th percentiles) by p53 immunoreactivity of the skin, Nambour Skin Cancer Prevention Trial, Australia, 1996**

Subject characteristic	No. of subjects	% p53-positive cells		Basal p53 proportion (%)†
		Whole epidermis	Basal layer of the epidermis	
<b>Sex</b>				
Male	78	8** (4, 22)‡	10** (5, 22)	35 (25, 46)
Female	61	5** (2, 13)	7** (2, 11)	36 (24, 48)
<b>Age group (years)</b>				
30–39	73	7 (3, 21)	9 (4, 20)	36 (25, 43)
40–49	18	9 (4, 13)	9 (5, 20)	37 (29, 52)
50–59	22	11 (3, 25)	10 (5, 28)	30 (18, 45)
60–69	19	6 (1, 11)	6 (2, 12)	33 (20, 64)
≥70	7	4 (3, 9)	4 (4, 8)	35 (31, 44)
<b>Skin color</b>				
Fair	80	9 (4, 20)	9 (4, 21)	36 (25, 50)
Medium	52	6 (3, 15)	8 (4, 13)	35 (25, 44)
Olive	7	3 (3, 16)	6 (2, 12)	21 (18, 64)
<b>Skin type</b>				
Always burns	34	7 (2, 17)	7 (4, 17)	37 (31, 50)
Burns, then tans	96	7 (3, 16)	9 (4, 17)	35 (23, 47)
Only tans	9	9 (4, 34)	9 (6, 25)	27 (20, 31)
<b>Cigarette smoking status (by sex)</b>				
<b>Males</b>				
Current smoker	15	6 (3, 40)	9 (3, 50)	32 (25, 43)
Former smoker	30	11 (6, 24)	13 (7, 33)	36 (25, 50)
Never smoker	33	7 (4, 21)	9 (4, 20)	35 (25, 46)
<b>Females</b>				
Current smoker	5	24* (10, 26)	25* (17, 32)	41 (29, 42)
Former smoker	13	4* (3, 7)	5* (4, 10)	36 (32, 50)
Never smoker	43	5* (2, 13)	6* (2, 11)	33 (23, 48)
<b>Skin cancer history</b>				
Did not have skin cancer before or during the trial	111	7 (3, 19)	9 (4, 20)	35 (25, 48)
Had skin cancer before or during the trial§	28	7 (3, 13)	8 (4, 15)	35 (23, 44)

\*  $p < 0.05$ ; \*\* $p < 0.01$  (for group differences).

† Reflects the percentage of all p53-positive cells that are located in the basal layer.

‡ Numbers in parentheses, 25th and 75th percentiles.

§ Self-reported history of any type of skin cancer treated by a doctor at any time before the trial.

persons (61 percent) had more than 5 percent p53-positive cells in the whole epidermis, and 31 persons (22 percent) had more than 20 percent; 91 persons (65 percent) had more than 5 percent p53-positive cells in the basal layer, and 31 (22 percent) had more than 20 percent. The intraclass correlation coefficient for the sections that were counted twice for reproducibility analysis was 0.98.

Univariate analysis showed that males had significantly higher levels of p53 immunoreactivity than females in both the whole epidermis and the basal layer (table 1). None of

the other selected general subject characteristics appeared to be statistically associated with p53 immunoreactivity in univariate analysis. We repeated univariate analyses of all variables in table 1 after stratifying for sex and observed that current female smokers had much higher p53 immunoreactivity than female non- or ex-smokers (table 1). Therefore, subsequent analyses of UV exposure indicators and sunscreen use were adjusted for sex and smoking status. The remaining stratified analyses did not show group differences, and those results are not presented.

**TABLE 2. Indicators of ultraviolet light exposure (median and 25th and 75th percentiles) by p53 immunoreactivity of the skin, Nambour Skin Cancer Prevention Trial, Australia, 1996**

Exposure indicator	No. of subjects	% p53-positive cells		Basal p53 proportion (%)†
		Whole epidermis	Basal layer of the epidermis	
No. of hours spent outdoors on a typical weekday during the past 6 months				
≤1	53	7** (3, 13)‡	8* (4, 12)	35 (26, 46)
2–4	50	5** (2, 12)	7* (2, 12)	35 (23, 54)
5–8	30	11** (6, 36)	16* (6, 34)	34 (28, 43)
9–12	5	22** (9, 42)	24* (14, 50)	37 (34, 41)
No. of hours spent outdoors on a typical weekend day during the past 6 months				
≤1	21	4** (2, 13)	5** (3, 11)	33 (26, 45)
2–4	84	6** (3, 14)	8** (3, 14)	36 (23, 49)
5–8	32	16** (6, 30)	17** (7, 39)	34 (27, 41)
9–12	1	9**	14**	54
Location of occupational activities (overall)				
Mainly outdoors	38	8 (4, 16)	9 (4, 17)	36 (25, 43)
Both indoors and outdoors	42	10 (3, 29)	14 (5, 31)	30 (23, 42)
Mainly indoors	58	5 (3, 13)	7 (4, 11)	39 (26, 48)
Location of sports or leisure activities (overall)§				
Mainly outdoors	36	8 (4, 16)	9 (4, 18)	34 (23, 43)
Both indoors and outdoors	41	9 (4, 21)	12 (5, 25)	37 (26, 48)
Mainly indoors	62	5 (3, 13)	7 (3, 11)	36 (28, 50)

\*  $p < 0.05$ ; \*\* $p \leq 0.01$  (for sex- and smoking status-adjusted group differences).

† Reflects the percentage of all p53-positive cells that are located in the basal layer.

‡ Numbers in parentheses, 25th and 75th percentiles.

§ Data were collected in 1992.

The p53 immunoreactivity of both the whole epidermis and the basal layer was significantly higher among people who had spent more time outdoors on weekdays and weekend days during the past 6 months, both univariately and after adjustment for sex and smoking status (table 2).

In univariate analysis, the proportion of p53 protein in the basal layer was not significantly associated with any of the indicators of UV exposure or general subject characteristics. Epidermal thickness showed an inverse association with basal p53 proportion, but this was not significant ( $p = 0.08$ ; data not shown).

Univariate comparisons of reported use of sunscreen on the hands showed no significant group differences for any of the outcome variables (table 3). In a multivariable model allowing for confounders (time spent outdoors on weekdays and weekends in the past 6 months, sex, smoking status, skin type, and skin color), use of sunscreen on the hands was significantly associated with lower p53 immunoreactivity in the whole epidermis ( $p = 0.03$ ,  $R^2 = 0.31$ ) and in the basal layer ( $p = 0.04$ ,  $R^2 = 0.28$ ). Participants who used sunscreen more regularly showed lower p53 immunoreactivity. The multivariable-adjusted mean p53 immunoreactivity in sub-

jects who applied sunscreen on their hands 1 or 2 days per week was twice that in subjects who applied sunscreen on their hands daily in both the total epidermis (ratio estimate = 2.0, 95 percent confidence interval: 1.1, 3.6) and the basal layer (ratio estimate = 1.7, 95 percent confidence interval: 0.9, 3.1).

Reported use of sunscreen on the hands was not associated with basal p53 proportion after allowing for other predictors in the multivariable model.

## DISCUSSION

To our knowledge, this study is the first to report that use of sunscreen is independently associated with the expression of p53 protein in human skin in a community-based setting. Our data show that daily use of sunscreen is associated with half the amount of epidermal p53 immunoreactivity as irregular use of sunscreen. While the cancer-preventive effects of sunscreen use are known (24, 28), this study provides novel evidence that p53 immunoreactivity, a biologic marker of UV damage, is associated with use of sunscreen in the community.

**TABLE 3. Sunscreen use (median and 25th and 75th percentiles) by p53 immunoreactivity of the skin, Nambour Skin Cancer Prevention Trial, Australia, 1996**

Sunscreen use on hands (no. of days per week)	No. of subjects	% p53-positive cells		Basal p53 proportion (%)†	Adjusted mean % p53-positive cells			
		Whole epidermis	Basal layer of the epidermis		Whole epidermis‡		Basal layer§	
					%	95% CI¶	%	95% CI
Never	21	6* (3, 14)#	9* (4, 11)	39 (27, 50)	12	6, 22	11	6, 22
1–2	44	12* (4, 24)	11* (4, 27)	35 (26, 44)	19	11, 34	18	10, 32
3–4	21	7* (5, 16)	9* (7, 24)	37 (28, 44)	14	8, 25	14	6, 25
5–6	13	5* (2, 10)	7* (1, 10)	33 (29, 39)	7	3, 13	6	3, 12
7	22	6* (3, 16)	7* (4, 24)	34 (21, 50)	10	5, 17	11	6, 19

\*  $p \leq 0.01$  (for multivariable-adjusted group differences).

† Reflects the percentage of all p53-positive cells that are located in the basal layer.

‡ Multivariable-adjusted mean percentage of p53 positivity in the whole epidermis, adjusted for skin type and color, time spent outdoors (weekdays and weekend days), sex, and smoking status.

§ Multivariable-adjusted mean percentage of p53 positivity in the basal layer.

¶ CI, confidence interval.

# Numbers in parentheses, 25th and 75th percentiles.

Our study showed that lower levels of epidermal p53 expression were associated with more regular sunscreen use, but we were unable to identify whether this was due to prevention of mutations in the *p53* gene and reduced expression of mutant p53 or a reduction in normal, UV-induced up-regulation of p53. The results illustrate the potentially beneficial biologic effects of sunscreen use among community members at high risk of skin cancer, be it through prevention of the cellular stresses that cause induction of p53 expression or through protection against UV-induced mutation.

More frequent use of sunscreen was associated with lower p53 immunoreactivity in both the whole epidermis and the basal epidermis. Thus, it appears that use of a broad-spectrum sunscreen with a Sun Protection Factor of 15+ in this population attenuated p53 expression throughout the epidermis, not just in the upper or lower layers, since the basal proportion was unaffected. In particular, UVA (UV with a wavelength of 320–<400 nm) is able to penetrate into the deeper layers of the epidermis, and this appears to be reflected in the localization of mutational changes within squamous tumors, as shown by Agar et al. (21). In their study, UVA fingerprint mutations of the *p53* gene in eight biopsied squamous cell carcinomas and eight actinic keratoses were predominantly found in the basal layer. Our community-based study findings complement those from Agar et al.'s tumor series (21). Together these studies illustrate the importance of protecting the skin from damaging UV radiation through the use of broad-spectrum sunscreens, particularly those attenuating the effects of UVA as well as UVB (UV with a wavelength of 290–<320 nm).

We found epidermal p53 immunoreactivity to be closely associated with the amount of time spent outdoors in the preceding 6 months, confirming the view that p53 immunoreactivity is a marker of chronic UV exposure. We did not collect data on UV exposure in the days immediately preceding collection of the biopsy or on patterns of UV exposure more than 6 months before data collection. It is possible that time spent outdoors in the days immediately before

biopsy may have been an additional predictor of p53 immunoreactivity in these subjects. However, our study is in agreement with previous studies that have shown increased p53 expression in chronically sun-exposed skin (13, 14). Thus, besides an acute, transient response following UV exposure (10, 15), there appears to be a chronic effect of UV light on p53 expression in exposed skin, despite the process of continuous epidermal renewal. This was also shown by a study of Swedish volunteers in which p53-mutated and -overexpressing keratinocytes were found in the epidermis of chronically sun-exposed skin that had been protected from UV radiation for up to 2 months (17, 19).

We determined that the significantly higher p53 immunoreactivity in current female smokers as compared with female non- and ex-smokers was due to differential patterns of time spent outdoors, since the association between sex and p53 immunoreactivity disappeared after we allowed for time spent outdoors (data not shown). The association between UV exposure and p53 immunoreactivity in the skin is not known to vary by sex. Lung cancers in current smokers show more frequent mutations in the *p53* gene than do lung cancers in former and never smokers (29). To our knowledge, the effect of smoking on p53 status in the skin has not been reported to date.

The rates of p53 positivity in this study are consistent with those reported by other investigators. Einspahr et al. (13) found a mean 12.1 percent (standard deviation, 14.4) p53 positivity in normal skin adjacent to solar keratoses on the dorsal skin of the forearms and hands. In our study, we found a mean 12.8 percent (standard deviation, 14.7) p53 positivity in the whole epidermis.

We conclude that p53 immunoreactivity in the skin is a marker of UV exposure during the past 6 months. Sunscreen use in the community is associated with reduced p53 immunoreactivity of the skin. Longitudinal investigation would provide more insights into the temporal relations between sunscreen use and levels of epidermal p53 expression in the community.

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Conflict of interest: none declared.

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