p53 Mutations in Human Aggressive and Nonaggressive Basal and Squamous Cell Carcinomas

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ABSTRACT

Purpose: The purpose is to investigate whether aggressive basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) differ from nonaggressive BCC and SCC with respect to the p53 mutation spectrum and whether specific mutations can serve as prognostic indicators of tumor aggressiveness.

Experimental Design: We analyzed 342 tissues from patients with aggressive and nonaggressive BCCs and SCCs for p53 mutations by single-strand conformation polymorphism and nucleotide sequencing.

Results: p53 mutations were detected in 33 of 50 aggressive BCCs (66%), 37 of 98 nonaggressive BCCs (38%), 28 of 80 aggressive SCCs (35%), 28 of 56 nonaggressive SCCs (50%), and 3 of 29 samples of sun-exposed skin (10%). About 71% of the p53 mutations detected in aggressive and nonaggressive BCCs and SCCs were UV signature mutations. The frequency of CC to TT mutations in aggressive and nonaggressive BCCs and SCCs were UV signature mutations. The frequency of CC to TT mutations in aggressive (36%) and nonaggressive SCCs (39%) was 2-fold higher than in aggressive (18%) and nonaggressive (14%) BCCs. In contrast, aggressive BCCs had a higher frequency (24%) of transversions than nonaggressive BCCs (8%) and aggressive (14%) and nonaggressive (11%) SCCs did.

Conclusions: Our results indicate that UV radiation is responsible for the induction of p53 mutations and perhaps for the initiation of both aggressive and nonaggressive BCCs and SCCs. Although some differences in p53 mutation frequency, types of mutation, and hot spots were seen between aggressive and nonaggressive BCCs and SCCs, these factors do not constitute as clear-cut diagnostic or prognostic indicators of tumor aggressiveness. Tumor aggressiveness may be attributable to other genetic changes or events that occur during tumor progression.

INTRODUCTION

NMSCs are the most frequent types of human cancer. In the United States, ~1,000,000 patients are diagnosed annually with NMSC, with the skin of the head and neck as the most frequent sites of origin (1–3). These tumors frequently develop on the nose, ear, and forehead as a result of chronic sun exposure (1–3). Additionally, as lifetime sun exposure through recreational and occupational activities increases and the atmospheric ozone decreases, the incidence of skin cancer is increasing, and the age of patients at initial presentation is decreasing. Although the mortality rate for cutaneous malignancy is only 5–10% per year, the morbidity from treatment of NMSC of the head and neck in terms of cosmetic deformity, loss of function, medical costs, and adverse psychological effects is tremendous (4–6).

The risk for developing BCC and SCC is attributable not only to cumulative sun exposure but also to geographic location, climate, race, and other as yet undetermined genetic factors. Most of these cancer patients are easily and successfully treated when the cancers are detected early. However, a significant number of head and neck skin cancers are very aggressive and can invade regional structures such as cartilage, muscle, bone, and regional nerves and metastasize to lymph nodes. All of these factors adversely affect prognosis (6). These aggressive skin tumors have high rates of recurrence (47.2%) and metastasis (34.8%) and are associated with a marked decrease in survival (4). Because early diagnosis is still difficult, clinical management of patients with aggressive head and neck skin tumors has not improved dramatically. The lack of progress in the identification and treatment of these cancers illustrates the need for molecular studies of the genetic changes associated with aggressive behavior. At present, there is no information on the genetic alterations associated with the development of aggressive skin cancers, and it is not clear whether the aggressive tumors are a biologically distinct subset of skin cancers. Studies during the past decade have shown that mutations in the p53 gene are implicated in many processes related to the etiology and development of NMSC of the head and neck (7–12). Developing an accurate profile of the frequency and spectrum of these mutations may reveal information that would be useful in the diagnosis of aggressive skin cancers. In addition, there is evidence

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3 The abbreviations used are: NMSC, nonmelanoma skin cancer; BCC, basal cell carcinoma; SCC, squamous cell carcinoma; SSCP, single-strand conformational polymorphism.
that the mutation profile of a given tumor may indicate its resistance or response to certain chemotherapies or radiotherapies (13–15).

It is well known that mutations in the p53 gene are linked to many types of cancers (16–18). When p53 is mutated, it may lose its ability to function normally as a shepherd of the DNA repair process or its ability to induce programmed cell death in damaged cells (10, 19, 20). Several studies have shown that human BCCs and SCCs harbor unique mutations in the p53 tumor suppressor gene that are not commonly found in other human cancers (7–12). The presence of C to T and CC to TT transitions at dipyrimidine sequences, termed UV signature mutations, provides strong evidence that UV radiation in sunlight is responsible for the induction of most NMSC (7–12). In addition, the finding that p53 mutations are present in actinic keratosis, which may be a precursor to SCC, as well as in sun-damaged human skin (10, 11, 21–28), suggests that p53 mutations arise early during the development of NMSC. This hypothesis is supported by laboratory studies demonstrating that clones of keratinocytes with mutant p53 protein (29) and p53 mutations can be detected in UV-irradiated mouse skin well before the appearance of skin tumors (30, 31).

Because the etiology and molecular alterations associated with the pathogenesis of aggressive skin cancers are unknown, we sought to identify molecular alterations in the p53 tumor suppressor gene in aggressive and nonaggressive BCCs and SCCs. To identify parameters that may serve as diagnostic and prognostic indicators of aggressive skin cancers, we determined the relationship between molecular alterations and clinical, pathologic, and epidemiological factors. Having such information could enable physicians to detect potentially aggressive skin cancers at earlier stages of disease progression and help identify patients who are appropriate candidates for novel therapeutic approaches. Understanding the nature of genomic alterations would also increase our knowledge of the relative importance of UV radiation and other carcinogens in the etiology of the disease.

**PATIENTS AND METHODS**

**Patients and Tumors.** The subjects included in this study were patients enrolled in the NMSC registry at The University of Texas M. D. Anderson Cancer Center for whom tissue was available. This registry includes all patients with biopsy-confirmed SCC or BCC of the head and neck. We collected clinical, demographic, and epidemiological data, as well as tissue samples (tumor and adjacent normal tissue) from each patient. The medical records of all patients were reviewed. The institutional review board approved the study. The participating physicians obtained informed consent from each patient. The tumors were classified as clinically aggressive if they exceeded 2 cm in one surface dimension, invaded muscle, bone, or cartilage, or were metastatic to lymph nodes or distant sites. We analyzed frozen tissues or formalin-fixed, paraffin-embedded tumor tissues from patients with NMSC, and one pathologist (A. E. N.) verified the pathologic diagnosis.

**Evaluation of Carcinogen Exposure.** Detailed information on suspected risk factors for skin cancer, including demographics, skin type according to the Fitzpatrick score, history of sunburns, occupational and recreational sun exposure, previous history of cancer, family history of cancer among first-degree relatives, tobacco use, and exposure to chemicals was ascertained by conducting standardized interviews with the patients. The χ² test and t test were used to compare samples with different types of mutations and epidemiological variables. Sun exposure was computed based on exposed hours spent in the sun for occupational and recreational activities. Skin color was originally reported on a scale of 1–10 and then dichotomized as light (≤5) and dark (>5). Agricultural carcinogen exposure was derived from the patients’ recounting of exposures to pesticides, herbicides, or fertilizers. Petrochemical exposure included exposure to cutting oils, asphalt, benzene, gasoline, mineral oils, creosote, dry-cleaning agents, acrylic and oil-based paints, varnish, or paraffin waxes.

**DNA Extraction.** DNA was extracted from frozen tissues by the phenol-chloroform method. However, DNA from paraffin-embedded sections was extracted by two different methods. The first was the sonic method (32), with minor modifications. Five to 10 μm sections were deparaffinized with xylene, suspended in 100–200 μl of lysis buffer (50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM MgCl₂, 0.01% gelatin, 0.5% Tween 20, and 0.5 mg/ml proteinase K), and sonicated with sterile glass beads (Sigma Chemical Co., St. Louis, MO) at 35°C for 30 min with a Branson Model 2200 sonicator (Branson Ultrasonics, Danbury, CT). The samples were then boiled for 15 min and centrifuged for 1 min. The resulting supernatant was stored at ~20°C until used. In the second method, after deparaffinization, samples were washed twice with 100 and 70% ethanol, dried in a heating block, and suspended in Release-IT reagent (CPG, Lincoln Park, NJ). The tissues were ground with disposable pestles and lysed directly in amplification tubes in a thermal cycler, according to the manufacturer’s instructions. The lysed samples were stored at −20°C. Each sample was screened for p53 mutations by both methods.

**PCR-SSCP Analysis.** PCR-SSCP analysis of DNA was performed with intronic primers for exons 4–9 of the p53 gene. All primers were synthesized by Genosys Technologies, Inc. (The Woodlands, TX). The 25-μl reaction mixtures contained 6 μl of genomic DNA, 200 nm of forward and reverse primers, 0.75 units of TaqDNA polymerase (Promega, Madison, WI), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.001% gelatin, 75 μM each dATP, dCTP, dGTP, and dTTP, and 2.5 μCi of [α³²P]dCTP. This was overlaid with mineral oil (Sigma Chemical Co.). PCR was performed in a DNA thermal cycler (Perkin-Elmer, Foster City, CA) for 42 cycles as follows, unless otherwise indicated. The DNA was first denatured at 94°C for 4 min, then two cycles of denaturation at 94°C for 1 min, primer annealing at 65°C for 1 min, and sequence extension at 72°C for 1 min was performed. The next five cycles consisted of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. Finally, a 15-min extension at 72°C was performed to complete the extension. To perform SSCP, 3 μl of PCR product was added to 7 μl of STOP dye, heated at 94°C for 5 min, and cooled on ice before loading of the gels. The gels consisted of 5% 2× Mutation Detection Enhancement gel solution (FMC Bioproducts, Rockland, ME), 10% glycerol, 0.6× Tris-borate EDTA, 10% ammonium persulfate, and N,N,N′,N′′-tetramethylxyl-
enendiamine. The gels were run at 6 W for 16 h, dried, and exposed to autoradiographic film, which was developed to visualize the bands. Extra measures were taken to ensure the validity of the results: negative controls containing double-distilled water instead of DNA were always used to confirm lack of contamination, and human placental DNA was included in every PCR run as a normal control.

Nucleotide Sequencing. The shifted bands were cut out of the dried SSCP gels, and the DNA was eluted with 100 μl of Tris-EDTA buffer at 80°C for 30 min. The DNA was reamplified by PCR using the primers and conditions described above, and the PCR product was enzymatically treated with shrimp alkaline phosphatase and exonuclease I for 15 min at 37°C and then treated for 15 min at 80°C. The samples were directly sequenced with the Thermo Sequenase-radiolabeled terminator cycle sequencing kit (Amersham, Cleveland, OH) according to the manufacturer’s instructions. We also excised from the gel and sequenced DNA bands from non-sun-exposed skin and human to rule out any PCR-generated artifacts.

RESULTS

A total of 342 tissues (50 aggressive BCCs, 98 nonaggressive BCCs, 80 aggressive SCCs, 56 nonaggressive SCCs, 29 sun-exposed skin samples, and 29 nonsun-exposed skin samples) was screened for p53 mutations by PCR-SSCP analysis of exons 4–9, the region where >95% of p53 mutations have been reported (33). Because of the large sample size, we used SSCP analysis to rapidly screen for p53 mutations and then reamplified samples exhibiting abnormal bands and directly sequenced the PCR products. This analysis revealed p53 mutations in 33 of 50 aggressive BCCs, 37 of 98 nonaggressive BCCs, 28 of 80 aggressive SCCs, and 28 of 56 nonaggressive SCCs (Table 1). In contrast, none of 29 nonaggressive BCC samples and 3 of 29 sun-exposed skin samples had SCCs (Table 1). In contrast, none of 29 non-sun-exposed skin BCCs, 28 of 80 aggressive SCCs, and 28 of 56 nonaggressive samples was screened for.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of mutations/no. of samples analyzed</th>
<th>Mutation frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggressive BCC</td>
<td>33/50</td>
<td>66</td>
</tr>
<tr>
<td>Nonaggressive BCC</td>
<td>37/98</td>
<td>38</td>
</tr>
<tr>
<td>Aggressive SCC</td>
<td>28/80</td>
<td>35</td>
</tr>
<tr>
<td>Nonaggressive SCC</td>
<td>28/56</td>
<td>50</td>
</tr>
<tr>
<td>Sun-exposed skin</td>
<td>3/29</td>
<td>10</td>
</tr>
<tr>
<td>Non-sun-exposed skin</td>
<td>0/29</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of mutations/no. of samples analyzed</th>
<th>Mutation frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-aggressive SCC</td>
<td>28/56</td>
<td>50</td>
</tr>
<tr>
<td>Aggressive SCC</td>
<td>28/80</td>
<td>35</td>
</tr>
</tbody>
</table>

A comparison of type of mutation by histology and aggressiveness showed that nonaggressive SCCs had a higher but significantly higher frequency of tandem CC to TT mutations than nonaggressive BCCs (39 versus 14%; P = 0.02). There was a higher but nonstatistically significant difference between the number of CC to TT mutations in the aggressive SCCs as compared with the aggressive BCCs (36 versus 18%; P = 0.12). Transversions among aggressive BCCs were more common than in nonaggressive BCCs (24 versus 8%), although this difference was not statistically significant (P = 0.06). Although most of the NMSCs had a single p53 mutation, several tumors had multiple mutations, however, these differences were not statistically significant. In particular, aggressive BCCs had more multiple mutations than nonaggressive BCCs did (43 versus 21%; P = 0.06; data not shown). Similar results were found when BCCs with multiple mutations were compared with both aggressive SCCs (43 versus 26%) and nonaggressive SCCs (43 versus 20%; data not shown). The presence of multiple p53 mutations in the same tumor has been well documented in a number of human cancers, including NMSC (23, 34–36).

Because p53 mutations in human BCCs and SCCs are reported to occur at certain hot spot codons (11, 12), we analyzed the p53 mutation data to determine whether there was a predilection for mutations at certain hot spots in aggressive and nonaggressive BCCs and SCCs. Fig. 1 shows that codons 241 and 248 were the major hot spots for p53 mutations in aggressive and nonaggressive BCCs, and no significant differences were seen between these two tumor types. In addition to codons 241 and 248, a second p53 mutation hot spot occurred at codons 250 and 258 in nonaggressive BCCs but not in aggressive BCCs. In contrast, codons 177, 179, and 241 were the major hot spots in aggressive SCCs but not in nonaggressive SCCs (Fig. 1).

In general, the p53 mutations detected in aggressive and nonaggressive BCCs and SCCs were predominantly C to T and CC to TT transitions at dipyrimidine sequences. This suggests that these mutations were induced by the UV radiation present in sunlight and that tumor aggressiveness may not be related to different patterns of sunlight exposure in our patient population. We analyzed other potential risk factors associated with skin cancer in the group of patients described in Table 3.

Table 3 describes the general characteristics of the 65 NMSC patients included in this study. The mean age at registration was 65, and the range was 38–87. Most of the patients were males (88%), and 64 were white. More than two-thirds (69%) reported a first-degree relative with cancer, and 39% reported at least one relative with skin cancer (nonmelanoma or melanoma). A history of previous NMSC was reported by 60% of the patients and a history of a solid tumor or hematopoietic cancer by 12%. Most patients had light skin (72%; 1–5 on a scale of 1–10), and 79% were either former smokers or current smokers. Average lifetime sun exposure was 854 weeks and varied greatly.

When we compared putative risk factors between patients with only one type of mutation such as those with C to T mutations and patients with CC to TT mutations, we found that patients with CC to TT mutations were more likely to report having light skin color (≤5 versus >5 in a scale of 1–10; P = 0.01) and a higher, but nonstatistically significant, average lifetime sun exposure (1142 versus 804 h, t test = −1.19). How-
ever, there was no association between p53 mutation frequency and light skin color or higher average lifetime sun exposure. In addition, there was no correlation with tumor aggressiveness in these individuals. Also, no differences in chemical exposures or smoking were found. When we compared risk factors between patients with CC to TT mutations and transversions, no significant differences were found.

**DISCUSSION**

Because improvements in the management of aggressive and metastatic BCC and SCC of the head and neck have not occurred, early diagnosis is essential for improving mortality and morbidity in patients with this disease. As there are no specific markers available for distinguishing highly aggressive BCC and SCC from nonaggressive BCC and SCC, it is difficult to tell *a priori* which patients are at risk for developing biologically aggressive skin cancer. Identifying specific genetic markers that are associated with aggressive BCC and SCC may result in earlier diagnosis of biologically aggressive skin cancers of the head and neck.

The p53 suppressor gene is a major target for carcinogen-specific mutations (16–18). Alterations of the p53 gene have been implicated in the pathogenesis of human NMSC (7–12). The presence of UV-signature (C to T and CC to TT) mutations in human NMSC provides strong evidence that the UV radiation present in sunlight is responsible for the induction of these skin cancers. Laboratory studies have also shown that UV-induced mouse skin cancers frequently harbor C to T and CC to TT mutations in the *p53* gene (37–39). Interestingly, multiple exogenous carcinogens have been linked to specific mutational spectra in the *p53* gene. For example, persistent exposure to tobacco smoke has been linked to G to T transversions characteristic of exposure to carcinogens from tobacco smoke in lung cancer patients (17, 18, 40) or to hydrogen radicals generated, for example, by γ-radiation (41). Our studies have also shown that human SCC arising in psoriasis patients treated with long-term psoralen and UVA therapy harbor both UV-signature mutations and psoralen and UVA-signature *p53* mutations (42). However, skin cancers induced in mice by psoralen and UVA radiation contain unique *p53* mutations at DNA cross-linking sites and do not have UV-signature mutations (43).

Because the specific mechanism for UV-induced mutations is well known, it seems particularly clear from the results of this study that UV exposure plays a role in the pathogenesis of both aggressive and nonaggressive BCC and SCC. That C to T and CC to TT mutations were the predominant types of *p53* mutations in aggressive and nonaggressive BCCs and SCCs suggests that those mutations were most likely induced by UV radiation. Although the overall frequency and type of UV-signature *p53* mutations were similar in aggressive and nonaggressive BCCs and SCCs, subtle differences in the frequencies of CC to TT transitions at dipyrimidine sites and transversions were noticed between aggressive and nonaggressive skin cancers. For example, there were twice as many CC to TT transitions in SCCs as in BCCs. Similarly, transversions were more common in aggressive BCCs than in nonaggressive BCCs and aggressive and nonaggressive SCCs. One possible explanation for the increased frequency of transversions in aggressive BCC’s is that some of these BCCs were unrelated to UV exposure. However, this was not the case because all of the BCCs, with the exception of one, a nonaggressive BCC, originated on sun-exposed sites. Nonetheless, aggressive and nonaggressive BCCs and SCCs had different *p53* mutation hot spots. Although more mutations occurred at codons 241 and 248 in BCCs, there were no differences between aggressive and nonaggressive BCCs. However, aggressive BCCs had the second highest number of *p53* mutations at codons 250 and 258, and nonaggressive BCCs did not have any mutations at these two codons. Analogous to aggressive BCCs, aggressive SCCs also had more mutations at codons 177, 179, and 241 than did nonaggressive SCCs. Thus there appears to be some correlation between tumor aggressiveness and CC to TT mutations, transversions, and hot spot codons. Whether these differences are useful in the diagnosis or prognosis of aggressive BCCs and SCCs remains to be established.

We failed to detect *p53* mutations in about half of the tumor tissues analyzed. One reason for this may be that we analyzed only exons 4–9 of *p53* gene; it is quite possible that mutations could also occur outside these exons. Therefore, it is possible that there could be some differences between aggressive and nonaggressive BCCs and SCCs in terms of *p53* mutations outside of exons 4–9. This possibility remains to be tested.

Another reason may be that some of the samples could have contained large amounts of normal cell contamination, which can mask detection of mutations by SSCP and direct sequencing, particularly if only one *p53* allele is mutated in tumor cells. This is further complicated by the fact that we did not use microdissected tumor tissues of fixed or frozen tumor specimens for *p53* mutation analysis. Instead, we analyzed DNA extracted from whole tissue sections for *p53* mutations. Also, some paraffin-embedded sections used in the analysis did not contain tumor cells with *p53* mutation because of intratumoral heterogeneity. Thus, some or all of these variables could affect interpretation of the data. Nonetheless, while most NMSCs had a single *p53* mutation, several tumors harbored multiple *p53* mu-

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**Table 2** Type and frequency of *p53* mutations in aggressive and nonaggressive BCC and SCC

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Transitions at dipyrimidine sites</th>
<th>Transitions at nondipyrimidine sites</th>
<th>Transversions</th>
<th>Frameshifts</th>
<th>Silent mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CC to TT</td>
<td>C to T</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aggressive BCC</td>
<td>6/33 (18)</td>
<td>14/33 (42)</td>
<td>2/33 (6)</td>
<td>8/33 (24)</td>
<td>1/33 (3)</td>
</tr>
<tr>
<td>Nonaggressive BCC</td>
<td>5/37 (14)</td>
<td>24/37 (65)</td>
<td>2/37 (5)</td>
<td>3/37 (8)</td>
<td>0/37 (0)</td>
</tr>
<tr>
<td>Aggressive SCC</td>
<td>10/28 (36)</td>
<td>10/28 (36)</td>
<td>1/28 (4)</td>
<td>4/28 (14)</td>
<td>0/28 (0)</td>
</tr>
<tr>
<td>Nonaggressive SCC</td>
<td>11/28 (39)</td>
<td>10/28 (36)</td>
<td>3/28 (11)</td>
<td>3/28 (11)</td>
<td>1/28 (4)</td>
</tr>
</tbody>
</table>

*a* Number with mutation/number examined (%).

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mutations. The presence of multiple $p53$ mutations has also been reported in human and UV-induced mouse skin cancers and head and neck cancers (23, 34, 38). It is possible that mutant $p53$ alleles with single-base changes were targets of secondary and tertiary mutational events, perhaps because of repeated exposure to sunlight. Alternatively, multiple mutations may arise independently in different clonal subpopulations during continued sunlight exposure or from endogenous sources during tumor development. This possibility is supported by the finding that primary tumor masses often consist of clones of cells that differ in karyotype, surface receptors, growth rate, and metastatic ability (44). The presence of multiple mutations in the same tumor is consistent with our earlier observation that suggests field cancerization (23). Field cancerization is postulated to result from prolonged exposure to carcinogens, and consequently, entire regions of tissues or organs become genomically unstable and predisposed to aberrant growth (45–47).

The finding that both aggressive and nonaggressive BCCs and SCCs contained UV-signature $p53$ mutations at similar frequencies suggests that $p53$ mutations may play a role in tumor initiation but not in tumor progression. In this regard, several studies have shown that mutations in the $p53$ gene preceded the appearance of skin cancer. For example, $p53$ mutations have been detected in sun-exposed skin from normal skin cancer patients and can serve as an indicator of prior sun exposure in humans (10, 11, 21–28). Our studies showed that noncancerous skin adjacent to skin cancers harbors $p53$ mutations different from those present in the skin cancers (23). In addition, UV-specific $p53$ mutations were found in actinic keratoses (10, 22, 25, 26), and normal skin flanking the actinic keratoses also contains a small number of $p53$ mutations (10, 25, 26), which suggests that actinic keratoses are clonal proliferations of cells containing $p53$ mutations. Berg et al. (29) analyzed UVB-irradiated mouse skin for the presence of cells expressing mutant $p53$ protein and found several clusters of cells in the epidermis that reacted with an antibody specific for mutant $p53$ protein after 17 or 30 daily UVB exposures, which would cause skin tumors ~80 or 30 weeks, respectively. Such clusters expressing the mutant $p53$ protein persisted in the skin for at least 56 days after UVB irradiation. Similarly, Jonason et al. (25) demonstrated that whole mount preparations of human skin contained clonal patches of $p53$-mutated keratinocytes. Such patches were larger and more frequent in sun-exposed skin than in sun-protected skin. It is estimated that these clonal patches represent as much as 4% of the epidermis. These results indicate that $p53$ mutations are causally related to UVB exposure and that they arise very early during skin cancer development.

Although our data on a small number of patients revealed associations with only skin color and sun exposure, it is possible that the etiology of skin cancer is indeed affected by multiple factors and a complex pattern of exposure history, which includes UV radiation and perhaps other carcinogens. At the very least, our data suggest that UV radiation plays a substantial role in the induction of $p53$ mutations and initiation of skin cancer. It is possible that other genetic changes or events that occur during tumor progression may contribute to tumor aggressiveness.

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