p53 Mutation in Squamous Cell Carcinomas from Psoriasis Patients Treated with Psoralen + UVA (PUVA)

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Individuals suffering from psoriasis are treated with a combination of psoralen and UVA radiation, commonly referred to as "PUVA" therapy. Epidemiologic studies have shown that PUVA therapy is a risk factor for skin cancer in psoriasis patients. Although PUVA treatment induces skin cancer in laboratory animals, it is unknown whether the increased incidence of skin cancer reported in PUVA-treated psoriasis patients is due to the carcinogenic effects of PUVA or due to other factors such as UVB. Because UV and PUVA induce different types of DNA damage resulting in unique types of *p53* mutation, we investigated whether skin cancers from PUVA-treated psoriasis patients have PUVA-type or UV-type *p53* mutations. Analysis of 17 squamous cell carcinomas (SCCs) from Austrian PUVA-treated patients revealed a total of 25 *p53* mutations in 11 SCCs. A majority of *p53* mutations occurred at 5′TpG sites. Although previous studies have shown that 5′TpA sites are the primary targets for PUVA mutagenesis, substitutions at 5′TpG sites are also quite common. Interestingly, a sizable portion of *p53* mutations detected were C→T or CC→TT transitions, characteristic of UV-induced mutations. Because some psoriasis patients had substantial exposure to UVB before PUVA therapy and because the light sources used in PUVA therapy contained small but significant wavelengths in the UVB region, it is possible that the C→T and CC→TT transitions detected in SCCs from PUVA-treated patients were induced by UVB. Nonetheless, our results indicate that both PUVA and UVB may play a role in the development of skin cancer in Austrian psoriasis patients who undergo PUVA therapy. *Key words: carcinogenesis/mutagenesis/skin cancer.*
J Invest Dermatol 109:238–243, 1997

Psoriasis is a hyper-proliferative chronic skin disorder affecting more than four million people in the United States and an estimated 2% of the worldwide population (Krueger *et al*, 1984). Modern photochemotherapy for psoriasis, known as psoralen plus ultraviolet A (PUVA), was introduced in 1974 and consists of oral or topical administration of psoralen, followed by UVA (320–400 nm) radiation (Haber, 1974; Parrish *et al*, 1974; Honigsmann *et al*, 1987). Although PUVA therapy is highly effective for psoriasis, achieving 80–90% clearance in 8–12 wk (Weinstein and White, 1993), this treatment is usually administered on a long-term basis to prevent recurrence of the disease or to treat new lesions. Several studies have shown, however, that PUVA is a potent mutagen and a carcinogen (Griffin, 1959; Pathak *et al*, 1959; Kripke *et al*, 1982; Ananthaswamy, 1985; Sage *et al*, 1993; Yang *et al*, 1994; Gunther *et al*, 1995; Nataraj *et al*, 1996). In addition, clinical follow-up studies have indicated that PUVA therapy is a risk factor for the development of squamous cell carcinoma (SCC) in humans. Several studies have shown that psoriasis patients who had received extensive PUVA therapy had a 10-fold increase in the incidence of SCC over that in the general population (Stern *et al*, 1979, 1984, 1988; Bruynzeel, 1987; Lindelöf *et al*, 1991; Stern, 1991; Lever and Farr, 1994). Conversely, some European and Japanese PUVA cohort studies did not find any association between PUVA therapy and an increased incidence of SCC (Lindskov, 1983; Tanew *et al*, 1986; Henseler *et al*, 1987; Torinuki and Tagami, 1988). A more in-depth examination of the data in these studies revealed some differences, however. For example, in the United States studies, in which a strong association between PUVA and SCC incidence was noted, doses above 1500 J/cm^2 were administered to a greater percentage of the patients; in contrast, the European studies reported lower UVA exposure levels. There were also major differences between the studies in follow-up time, treatment regimen, cohort size, and skin type analyzed. When all these factors were weighed into the results of the European studies, the results seemed to agree with the original findings of Stern *et al* (reviewed in Studniberg and Weller, 1993).

Although PUVA-treated patients are at an increased risk for developing skin cancer, the etiology of and molecular basis for the increased incidence of SCC in PUVA-treated psoriasis patients are unknown. There could be several explanations for the increased incidence of skin cancer in PUVA-treated patients. First, because PUVA is mutagenic and carcinogenic, skin cancers arising in PUVA-treated patients may be initiated or induced by PUVA. Second, because PUVA treatment is immunosuppressive (Kripke *et al*, 1983; Morison and Kripke, 1984), it may permit the growth of

Manuscript received January 6, 1997; revised April 4, 1997; accepted for publication April 16, 1997.

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Abbreviation: SSCP, single strand conformation polymorphism.

0022-202X/97/$10.50 •

skin cancers induced by other carcinogenic agents. Third, because many psoriasis patients undergo treatment with UVB (290–320 nm) in addition to PUVA (Lindelöf and Sigurgeirsson, 1993), it is possible that UVB is the inducing carcinogen. There is very little evidence, however, to support any of these hypotheses.

The *p53* tumor suppressor gene is a major target for carcinogen-specific mutations, and as such, it may be possible to use this gene as a molecular marker to identify the etiology of skin cancers induced in PUVA-treated patients. Because UV and PUVA induce different types of lesions in DNA, we hypothesized that mutations induced by these agents may also be different. Whereas UV induces primarily cyclobutane-type pyrimidine dimers and pyrimidine (6–4)-pyrimidone photoproducts (Rosenstein and Mitchell, 1987), PUVA induces monofunctional adducts and DNA cross-links (Musajo *et al*, 1967a, 1967b; Cole, 1971; Ben-Hur and Elkind, 1973; Dall'Acqua, 1977; Song and Tapley, 1979; Gasparro *et al*, 1985). Both human skin cancers and UV-induced mouse skin cancers exhibit a high frequency of UV-signature (C→T and CC→TT) mutations (Brash *et al*, 1991; Kress *et al*, 1992; Rady *et al*, 1992; Campbell *et al*, 1993; Dumaz *et al*, 1993; Kanjilal *et al*, 1993; Moles *et al*, 1993a; Sato *et al*, 1993; Ziegler *et al*, 1993, 1994; Kubo *et al*, 1994; Nakazawa *et al*, 1994; Nelson *et al*, 1994; Van der Riet *et al*, 1994; Ren *et al*, 1996). In contrast, PUVA-induced mouse skin cancers contain *p53* mutations at 5′-TA or 5′-TAT sequences that are quite distinct from those found in UV-induced skin cancers (Nataraj *et al*, 1996).

In this study, we analyzed SCCs from PUVA-treated psoriasis patients from Graz, Austria for *p53* mutation to determine whether they harbored PUVA-type or UV-type mutations. The results indicated that *p53* mutations were present in 65% of SCCs analyzed and that the mutational pattern was different from those found in SCCs arising in the general population. Approximately half of the *p53* mutations were located at 5′TpG sites and about one third were UV-signature type, suggesting that both PUVA and UV contributed to the development of skin tumors.

MATERIALS AND METHODS

Patient Characteristics and History of PUVA Treatment The patients were all Caucasians from Graz, Austria and ranged in age from 34 to 58 y at the commencement of PUVA therapy. Seven were male and four were female. Most SCCs examined in this study arose in patients approximately 11–17 y after the first PUVA treatment and 3 mo to 11 y after the last treatment (data not shown). Most patients included in the study had multiple SCCs after PUVA treatment. In addition to 8-methoxy psoralen (8-MOP) and UVA treatment, three patients also received 5-MOP and UVA treatment. Although 5-MOP and 8-MOP have similar photobinding properties, 5-MOP appears to be more cytotoxic *in vitro* (Ashwood-Smith *et al*, 1980). The cumulative 5-MOP/UVA doses used in therapy were not very high, but the total UVA dose for 8-MOP/UVA treatment in some patients was as high as approximately 7000 J/cm^2. The UVA lamps used in PUVA therapy in Europe emit approximately 99.5% of their energy in the UVA region and about 0.5% in the UVB region (Diffey, 1986). Moreover, 9 of 11 patients also had received extensive prior treatment with UVB radiation. It was difficult, however, to determine the total amount of UVB received by these patients because UVB therapy was administered at different dermatology clinics or institutions over a period of 10–15 y before the commencement of PUVA therapy. Therefore, access to such treatment records was difficult.

DNA Extraction DNA was extracted from paraffin-embedded sections using the method of Heller *et al* (1991), with minor modifications. Five- to 10-μm sections were deparaffinized by xylene, suspended in 100–200 μl of lysis buffer (50 nM KCl, 10 mM Tris, pH 8.3, 1.5 mM $MgCl_2$, 0.01% gelatin, 0.5% Tween 20, and 0.5 mg proteinase K per ml), and sonicated with sterile glass beads (Sigma Chemical Co., St. Louis, MO) at 45°C for 5–10 min using a Branson Model 2200 sonicating water bath (Branson Ultrasonics, Danbury, CT). The samples were then boiled for 10 min and spun for 20 s. The resulting supernatant was stored at −20°C until used.

Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP) Analysis Exons 5–8 of the *p53* gene were amplified separately using upstream and downstream primers specific for each exon (Pierceall *et al*, 1991). Five microliters of the lysates were used as templates in a 50-μl solution containing 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 1.5 mM $MgCl_2$; 0.001% gelatin; 150 μM each of dATP, dGTP, dCTP, and dGTP; 2.5 μCi of [α-^{32}P]dCTP; upstream and downstream primers (10 μM each); and 5 U AmpliTaq (PE Xpress, Foster City, CA). PCR was performed for two cycles at 94°C (1 min), 65°C (2 min), and 72°C (2.5 min); five cycles at 94°C (1 min), 60°C (2 min), and 72°C (2.5 min); 30 cycles at 94°C (1 min), 55°C (2 min), and 72°C (2.5 min); and finally one step at 72°C for 15 min. SSCP analysis was performed using a mutation detection enhancement ultra–high-resolution gel (AT Biochem, Malvern, PA). In this analysis, 3 μl of the PCR product was mixed with 7 μl of sequencing stop solution, heated to 94°C for 5 min, quick-cooled on ice, and loaded onto a 0.25× mutation detection enhancement gel. The gel was then run at 6 W for 14–18 h. Extreme precaution was taken to prevent contamination of PCR reactions, including the use of blank PCR controls without DNA templates and inclusion of human placental DNA in every PCR reaction. PCR-SSCP analysis was performed twice for each sample.

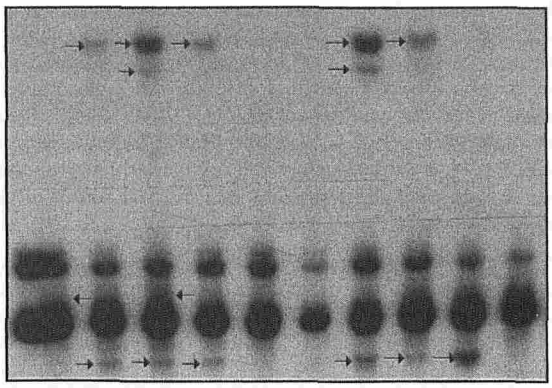

Figure 1. Detection of *p53* mutation by PCR-SSCP analysis in SCCs from PUVA-treated psoriasis patients. PCR-SSCP analysis of genomic DNAs corresponding to exon 7 reveals aberrant bands (→) in some tumors (A1, I4, I2, D2, K2, I3, and H1), suggesting that these tumors may harbor mutations. In contrast, DNAs from human placenta (P) and tumors D1, G1, and K1 do not show aberrant bands, which indicates that these samples may not have *p53* mutations at exon 7.

Nucleotide Sequencing Wild-type and shifted (mutant) bands were cut out of the dried SSCP gels, and the DNA was eluted by incubating at 80°C for 30 min in 200 μl of Tris-ethylenediamine tetraacetic acid. The eluted DNA was then reamplified by PCR, subcloned into pCR II vectors, and transformed according to the manufacturer's instructions in the TA cloning kit (Invitrogen Corporation, San Diego, CA). Five to eight colonies from each sample were sequenced bidirectionally using M13(rev) and M13(-40) primers with Sequenase version 2.0 (USB, Cleveland, OH). In some cases, genomic DNA from SSCP-positive tumors was amplified by PCR and sequenced to confirm the mutation. In addition, to rule out PCR-generated mutations, we analyzed human placental DNA simultaneously in every PCR and sequencing reaction.

RESULTS

SCCs From PUVA-Treated Patients Display p53 Mutation by PCR-SSCP Analysis A total of 17 SCCs from 11 PUVA-treated psoriasis patients were analyzed for *p53* mutations by PCR-SSCP analysis in exons 5–8. About 90% of all mutations reported in all cancers occur between exons 5 and 8 of the *p53* gene (Greenblatt *et al*, 1994). Eleven of 17 (65%) SCCs examined had an altered SSCP pattern: 8 of 17 (47%) exhibited mutations in exon 7, and 4 of 17 (23.5%) had mutations in exon 8. In addition, 1 tumor had a mutation in exon 5 and another tumor had one in exon 6 (data not shown). The representative SSCP profile of exon 7 shown in **Fig 1** reveals both wild-type and shifted bands in 7 tumors but only wild-type bands, identical to those present in human placental DNA, in 3 other tumors. The greater intensity of wild-type bands relative to mutant bands could reflect the possibility that only one *p53* allele is mutated and the other is wild-type, that contaminating normal cells are present in tumor tissues, or both. Similarly, in another SSCP gel, only 1 of 7 SCCs revealed aberrant bands at exon 7 (data not shown). In addition, 3 tumors had mutations in

more than one exon. For example, tumor I3 had mutations in exons 5 and 7, tumor A1 in exons 6 and 7, and tumor D2 in exons 7 and 8 (data not shown). Because PCR-SSCP was performed for each exon separately, it was impossible to determine whether multiple mutations in different exons occurred in the same *p53* allele or in different alleles. Nonetheless, a second PCR-SSCP analysis of all 17 SCCs revealed that although the band intensities varied slightly, the band pattern for each exon was identical to that found in the first PCR-SSCP analysis.

SCCs From PUVA-Treated Psoriasis Patients Contain Both PUVA-Type and UV-Type Mutations Aberrantly shifted and wild-type bands from dried SSCP gels were cut out, reconstituted, reamplified, and sequenced to identify the nature of the *p53* mutation. By this analysis, we detected a total of 24 missense mutations and 1 deletion in 11 of 17 SCCs **(Table I)**. Interestingly, tumor J2 had a 90-bp deletion (codons 269 to 299) in exon 8. Analogous to mutant bands, apparently normal-looking SSCP bands from several SCCs and human placental DNA were also subcloned and sequenced. In all cases, however, only the wild-type sequences were detected. More important, repeat analysis of genomic DNA from several tumors using PCR followed by subcloning and sequencing revealed *p53* mutations that were identical to those found in the first analysis. Nonetheless, substitutions at thymine bases (12 of 25; 48%) represented a significant portion of the *p53* mutational spectra in PUVA-treated SCCs **(Table I)**. Of the 12 mutations, 8 were T→C transitions, 3 were T→A transversions, and 1 was a T→G transversion.

In terms of sequence context for base substitutions at thymine, only one arose at 5′-TpA, a known site of frequent PUVA-induced mutation (Sage *et al*, 1993; Yang *at al*, 1994). Two base substitutions occurred at 5′-TpT sequences, whereas 9 of 12 (80%) occurred at 5′-TpG sites **(Table I)**. The representative nucleotide sequence data shown in **Fig 2** reveal that two tumors, D2 and A1, contained base substitutions at thymine in exon 7 at 5′-TpG and 5′-TpA sites, respectively. Several mutations (8 of 25; 32%) had the

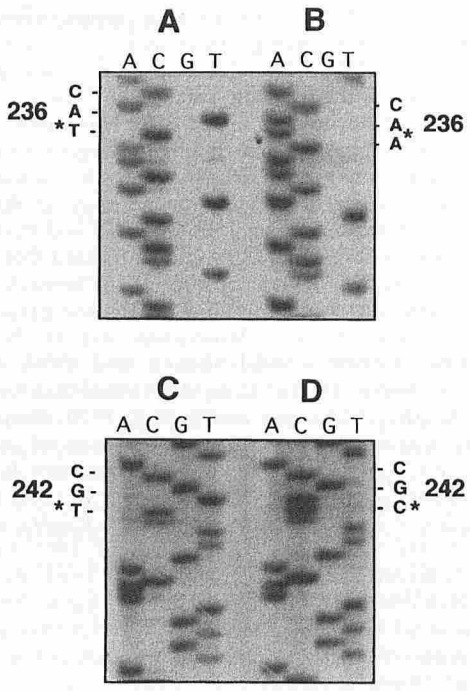

Figure 2. Presence of missense mutations in *p53* exon 7 in two SCCs. Nucleotide sequencing of PCR products from DNA isolated from dried SSCP gels corresponding to normal bands from human placental DNA *(A,C)* and aberrant bands from two tumor DNAs *(B,D)* reveals wild-type sequences in human placental DNA and mutant sequences in tumor DNAs. *(B)* tumor A1 contains a T→A transversion in codon 236 at a 5′-TpA site, which results in substitution of a tyrosine codon (TAC) by a codon for asparagine (AAC). *(D)* tumor D2 contains a T→C transition in codon 242 at a 5′-TpG site, which results in substitution of a cysteine codon (TGC) by a codon for arginine (CGC). The 5′→3′ sequence is read from bottom to top. Wild-type and corresponding mutated bases are shown (*).

Table I. Types of *p53* Mutations Detected in SCCs from PUVA-Treated Patients

Tumor	Exon	Codon	Base Change	Surrounding Sequence (5′→3′)[a]	Amino Acid Change
I3	5	135	G→T	TTT**G**CC	Cys→Phe
	5	139	T→A	CT**T**GGG	Lys→stop
	5	168	G→T	TGT**G**CT	His→Tyr
G1	7	227	C→T	GCT**C**TG	Ser→Phe
H1	7	240	T→G	AG**T**TCC	Ser→Arg
	7	242	T→C	CC**T**GCA	Cys→Arg
I4	7	233	T→C	AG**T**GGA	His→Arg
	7	242	T→C	CC**T**GCA	Cys→Arg
K2	7	242	T→C	CC**T**GCA	Cys→Arg
D2	7	242	T→C	CC**T**GCA	Cys→Arg
	7	246	G→T	CAT**G**AC	Met→Ile
I3	7	235	T→C	GT**T**GTA	Asn→Asp
A1	7	236	T→A	AC**T**ACA	Tyr→Asn
	7	238	T→C	TG**T**GTA	Cys→Arg
K1	7	258	T→A	TCT**T**CC	Glu→Val
D2	8	289	C→T	AAT**C**TC	Leu→Phe
	8	293–294	CC→TT	CT**CC**CC	Glu→Lys
	8	294–295	CC→TT	AG**CC**TC	Pro→Phe
C1	8	278	C→T	TCT**C**CT	Pro→Ser
J1	8	275	T→C	TT**T**GTG	Cys→Ala
	8	276	G→C	TGT**G**CC	Ala→Pro
	8	289	C→T	AAT**C**TC	Leu→Phe
	8	293–294	CC→TT	CT**CC**CC	Glu→Lys
	8	294–295	CC→TT	AG**CC**TC	Pro→Phe
J2	8	269–299	Deletion		

[a] The sequence is shown 5′→3′ in the strand (transcribed or nontranscribed) that has the thymine or the dipyrimidine bases. Mutated bases are shown in bold. 5′TpG, 5′TpA, 5′TpT, and dipyrimidine sequences are underlined.

UV signature (C→T or CC→TT transition) at dipyrimidine sites, primarily in exon 8 **(Table I)**. Multiple mutations were found in 8 of 12 tumors, some of which had base substitutions at thymine as well as C→T or CC→TT transitions. In addition, we detected a few silent mutations. For example, tumor A1 had an altered SSCP band in exon 6 that subsequent sequence analysis revealed to be a silent mutation (data not shown). Not all *p53* mutations present in tumors contribute to tumor development, as some missense as well as silent mutations may arise after initiation because the population of cells is repeatedly exposed to PUVA or UVB, or both, during tumor progression.

SCCs From PUVA-Treated Psoriasis Patients Exhibit Hotspots for *p53* Mutation Analogous to *p53* mutation hotspots in PUVA-induced murine skin cancers (Nataraj *et al*, 1996), four human SCCs from PUVA-treated psoriasis patients contained identical T→C transitions (predicting a Cys→Arg substitution) at codon 242 **(Table I; Fig 3)**. Interestingly, codon 242 is not frequently mutated in other types of human cancer, including skin cancer (Ziegler *et al*, 1993; Greenblatt *et al*, 1994). In addition, two tumors had identical CC→TT transitions at codons 293–294 and 294–295 and a C→T transition at codon 289 **(Table I)**.

DISCUSSION

Psoriasis patients as a group do not deviate from the general population with regard to the prevalence of internal malignancies (Lindelöf *et al*, 1990), and *p53* mutations have not been detected in psoriatic lesions (Moles *et al*, 1993b). One study reported that 46% of non-melanoma skin cancers from PUVA-treated psoriasis patients were immunopositive for *p53* protein expression (Proby *et al*, 1993). Because *p53* immunostaining is not a good indicator of the

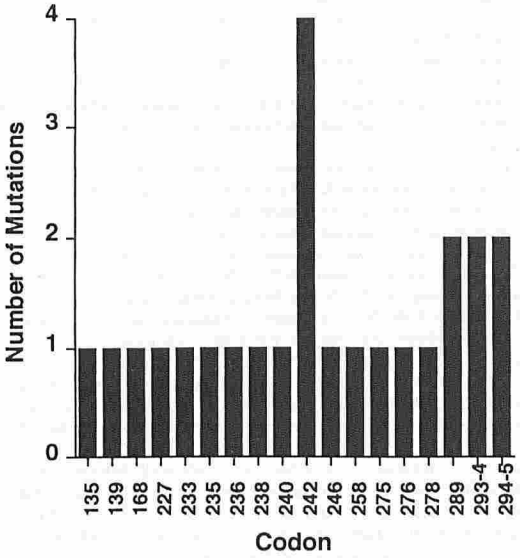

Figure 3. SCCs arising in PUVA-treated patients display hotspots for *p53* mutation. The numbers of *p53* mutations at various codons detected in SCCs were obtained from **Table I** and plotted on a graph. Codon 242 is a hotspot for *p53* mutation, followed by codons 289, 293–294, and 294–295.

presence or absence of *p53* gene mutations (Kubo *et al*, 1994), however, it provides no information about the etiology of these cancers, as genetic analysis might. In the current study, we analyzed skin cancers arising in PUVA-treated psoriasis patients from Austria for *p53* mutation to determine whether these skin cancers have a PUVA etiology or a UV etiology. Our *p53* mutation analysis revealed a total of 24 missense mutations and 1 deletion in 11 of 17 SCCs from psoriasis patients who had undergone PUVA therapy. Interestingly, 16 of 25 *p53* mutations occurred in conserved domains II (amino acids 117–142), IV (amino acids 234–258), and V (amino acids 270–286). Almost all of the PUVA-type mutations (at 5′-TpG, 5′-TpT, and 5′-TpA sites) were in these conserved domains. It is noteworthy that both codon 242 (a hotspot for *p53* mutation in SCCs from PUVA-treated psoriasis patients) and codon 238 code for cysteine, which represent two of the four binding sites for the tetrahedrally bound Zn^{2+} atom essential for the core structure. Mutations at these sites and in other conserved domains are predicted to destabilize the p53 protein (Cho *et al*, 1994). Based on the crystal structure of the p53 core domain, it appears that the majority of the PUVA-type mutations affect the p53 structure more than the UV-type mutations (C→T and CC→TT) at codons 289, 293–294, and 294–295, which are outside these domains.

Thirteen of 25 mutations (52%) occurred at 5′-TpG sites, 2 of 25 at 5′-TpT sites, and only 1 at a 5′TpA site. Contrary to this finding, other studies have shown that 5′-TpA sites are the primary targets for PUVA-induced mutagenesis (Sage *et al*, 1993; Yang *et al*, 1994; Chiou and Yang, 1995; Nataraj *et al*, 1996) and photoadduct formation (Boyer *et al*, 1987; Sage and Moustacchi, 1987; Sage and Bredberg, 1991), although base substitutions and mutations also occur at 5′-TpG and 5′-TpT sites (Miolo *et al*, 1989; Sage, 1993; Yang *et al*, 1994; Chiou and Yang, 1995). It is not clear why 5′-TpG and not 5′-TpA sequences are the preferred targets for mutations in this population of tumors. It is possible that processing of psoralen adducts *in vivo* in humans might be different and produce different *p53* mutational spectra than in rodents or cells in culture. In addition, differences in site specificity between *in vitro* and *in vivo* studies could reflect the fact that very high doses of PUVA, which do not mimic conditions in the clinic, were used in most *in vitro* mutagenesis studies. This notion is supported by the finding that when murine fibroblasts containing *supF* DNA were treated with low, clinically relevant doses of PUVA (5 μM 8-MOP + 0.1 J/cm^2 UVA), only 19% of the mutations occurred at 5′-TpA sites after PUVA treatment (Gunther *et al*, 1995). More important, a greater number (12 of 42; 29%) of mutations occurred at 5′-TpG sites.

It is noteworthy that 32% of the *p53* mutations we detected in SCCs from Austrian PUVA-treated patients were UV-signature mutations (C→T or CC→TT transitions) at dipyrimidine sites. There could be several explanations why UV-signature mutations were present in SCCs from some PUVA-treated psoriasis patients. First, most psoriasis patients in Austria receive UVB therapy before PUVA therapy. In fact, in our cohort, 9 of 11 patients had prior treatment with UVB. Strikingly, all 4 tumors harboring UV-signature mutations (C1, D2, G1, and J1) arose in patients who were treated previously with UVB radiation. Second, because exposure to sunlight was thought to be beneficial for psoriasis, the patients were encouraged to get ample exposure to sunlight. Third, the PUVA lamps used in psoriasis therapy in Austria emit about 0.5% of the total energy in the UVB region (below 320 nm). Fourth, because PUVA exposure also generates reactive oxygen species (Carraro and Pathak, 1991; Potapenko, 1991), which are known to induce CC→TT transitions (Reid and Loeb, 1993), it is possible that the CC→TT mutations found in some SCCs from PUVA-treated patients were induced by reactive oxygen intermediates.

Nonetheless, our results indicate that the *p53* mutation spectra seen in SCCs from Austrian PUVA-treated psoriasis patients are intrinsically different from those seen in SCCs from the general (nonpsoriatic) population **(Fig 4)**. For instance, 16 of 25 (64%) *p53* mutations detected in SCCs from PUVA-treated patients were located at putative sites of psoralen photoaddition (5′-TpA, 5′-TpG, or 5′-TpT) *versus* only 22% at the same regions in SCCs from the general population (chi-square test for PUVA subgroup; $p = 0.0017$). Interestingly, about 32% of *p53* mutations in SCCs from PUVA-treated patients had the UV-signature (C→T and CC→TT), which is comparable to the 43% frequency in SCCs arising in the general population (chi-square test for UV subgroup; $p = 0.416$). In addition to these differences, SCCs from PUVA-treated psoriasis patients exhibited hotspots for *p53* mutations at codon 242 and, to a lesser extent, at codons 289, 293–294, and 294–295 **(Fig 3)**. This could be attributed to the inefficient repair of PUVA- and/or UV-induced photoproducts at these sites. In support of this notion, Tornaletti and Pfeiffer (1994) demonstrated that highly mutated sites in *p53* are regions of slow DNA repair of UV lesions.

The *p53* mutations detected in SCCs from PUVA-treated psoriasis patients cannot be attributed to the persistence of DNA damage

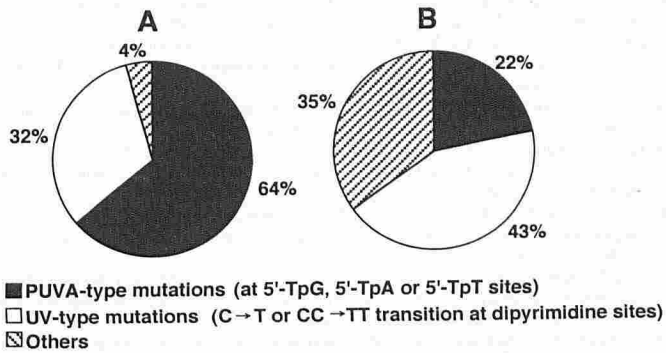

Figure 4. The *p53* mutation spectra of human SCCs from PUVA-treated psoriasis patients differ from those reported in SCCs from the general population. *(A) p53* mutation data for SCCs from PUVA-treated patients were obtained from **Table I** (n = 25). *(B) p53* mutation data for human SCCs from the general population were pooled from four studies (Brash *et al*, 1991; Pierceall *et al*, 1991; Moles *et al*, 1993a; Nelson *et al*, 1994). A total of 28 mutations were included in this analysis.

in skin tumors that gave rise to mutations during PCR because most SCCs that had mutations arose in patients 3 mo to 11 y after the last PUVA treatment, and it is unlikely that the DNA damage persists for such a long time. Several studies have shown that a majority of PUVA- and UVB-induced DNA lesions in eukaryotic cells are repaired in 48–72 h (Vos and Hanawalt, 1987; Zarebska *et al*, 1987). Moreover, our previous studies have shown that PUVA-exposed mouse skin adjacent to the tumor did not have *p53* mutations (Nataraj *et al*, 1996). In addition, we can rule out the possibility that the mutations found in SCCs were due to PCR artifacts because we detected identical mutations by two separate PCR and sequencing analyses of genomic DNA from some tumors, and PCR-SSCP and nucleotide sequencing of human placental DNA did not reveal *p53* mutations in exons 5–8.

Finally, the identification of carcinogens responsible for the induction of human cancers, based on the mutational fingerprint, is quite complex. Some patients in this study also had prior treatment with arsenic and methotrexate, although there are no conclusive data linking these compounds with *p53* mutations. Complicating factors notwithstanding, our study shows that both PUVA and UV may be responsible for the induction of skin cancer in PUVA-treated psoriasis patients in Austria. Because the PUVA therapy regimen used in treating psoriasis patients in the United States is different from that used in Austria, it remains to be determined whether SCCs from PUVA-treated patients in the United States contain the PUVA or UV type of *p53* mutation, or both. Such studies are currently under way.

We thank Susan Loughlin for technical assistance, Dr. Renate Schollnast for help in the collection of clinical data, Dr. Mary-Ann Gregurich for statistical analysis of our data, and Jude Richard for editorial comments. This work was supported by National Cancer Institute Grant R01-CA67174 to H.N.A. A.J.N. was supported by a predoctoral fellowship from the R. E. "Bob" Smith Foundation.

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