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UV and skin cancer: Specific p53 gene mutation in normal skin as a biologically relevant exposure measurement

(DNA damage/p53 mutation/carcinogenesis)

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ABSTRACT **Many human skin tumors contain mutated p53 genes that probably result from UV exposure. To investigate the link between UV exposure and p53 gene mutation, we developed two methods to detect presumptive UV-specific p53 gene mutations in UV-exposed normal skin. The methods are based on mutant allele-specific PCRs and ligase chain reactions and designed to detect CC to TT mutations at codons 245 and 247/248, using 10 μg of DNA samples. These specific mutations in the p53 gene have been reported in skin tumors. CC to TT mutations in the p53 gene were detected in cultured human skin cells only after UV irradiation, and the mutation frequency increased with increasing UV dose. Seventeen of 23 samples of normal skin from sun-exposed sites (74%) on Australian skin cancer patients contained CC to TT mutations in one or both of codons 245 and 247/248 of the p53 gene, and only 1 of 20 samples from non-sun-exposed sites (5%) harbored the mutation. None of 15 biopsies of normal skin from non-sun-exposed or intermittently exposed sites on volunteers living in France carried such mutations. Our results suggest that specific p53 gene mutations associated with human skin cancer are induced in normal skin by solar UV radiation. Measurement of these mutations may be useful as a biologically relevant measure of UV exposure in humans and as a possible predictor of risk for skin cancer.**

One of the key problems in conducting informative epidemiological studies on cancer is the lack of accurate methods for measuring exposure to suspected carcinogens in individuals. Mechanism-based exposure measurement methods—e.g., quantification of DNA adducts in target or surrogate cells—are being developed to overcome this problem (1). While most such assays can estimate "recent" or "current" exposure, they cannot measure cumulative exposure of target cells, which may be the most relevant measure in cancer epidemiology. To overcome this difficulty, the measurement of mutation frequency—e.g., of the *HPRT* gene—has been proposed (2). We have proposed that carcinogen-specific mutation patterns in cancer-related genes—i.e., oncogenes and tumor suppressor genes—could be developed as biologically relevant measures of exposure to environmental carcinogens (3).

Molecular analysis of tumors has revealed various changes in oncogenes and tumor suppressor genes. Since most cancers are considered to result from exposure to environmental carcinogens, it is reasonable to assume that some or most critical genetic changes are induced by such risk factors (3–5). Animal tumors induced by specific carcinogens support this assumption: mouse skin and liver tumors and rat mammary tumors induced by 7,12-dimethylbenz[*a*]anthracene (DMBA) often contain an A to T mutation at codon 61 of the Ha-*ras* gene, while those induced by *N*-methyl-*N*-nitrosourea (MNU) or *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) harbor G to A mutations at codon 12 of the same gene (reviewed in refs. 3 and 5). These findings can be interpreted in accordance with known mechanisms of action of the carcinogens used: DMBA is considered to form a major adduct with adenine (6), while MNU and MNNG produce O^6-methylguanine, which is a promutagenic lesion for G to A transition (7). There are other examples in which carcinogen-specific genetic footprints can be found in animal tumors (reviewed in refs. 3 and 5).

A few examples suggest that critical genetic changes in human tumors may also be determined by exposure to major risk factors. For example, six samples of liver hemangiosarcoma from patients who worked in vinyl chloride plants were analyzed for the *RAS* mutation; five showed a G to A transition at codon 13 of the *KRAS* gene (8). This mutation is consistent with the type of vinyl chloride–DNA adducts and mutation spectra found in bacteria (9), and vinyl chloride is a major etiological factor for this extremely rare cancer in humans (10). Other examples include studies which showed that liver tumors from areas with high exposure to aflatoxin B1 contained a much higher prevalence of G to T transversion in the p53 gene than similar tumors from low-exposure areas (11, 12). Another example has come from the study of Brash *et al.* (13), who found that p53 mutation spectra in squamous cell carcinomas of the skin were quite different from those found in internal tumors and were consistent with the occurrence of C to T or CC to TT transitions at sites of dipyrimidine photoproducts (14, 15). Similar results have been obtained in basal cell carcinomas (16). A very recent study by Kress *et al.* (17) has shown that UVB-induced mouse skin carcinomas also contain UV-specific mutations in the p53 gene, including CC to TT tandem base mutations.

While a possible link between carcinogen exposure and genetic changes found in tumors may exist as described above, it does not provide a means for biologically relevant exposure estimation, nor does it prove their causal relationship, unless evidence is provided that a given carcinogen indeed induces the critical gene mutations found in tumors. In other words, it is necessary to detect such critical gene mutations after carcinogen exposure but before tumor occurrence (3). Unlike other mutation assays, which rely on phenotypic selections of mutants, cancer-related gene mutation assay has to be based on genotype analysis since phenotypic expression of these genes is carcinogenesis *per se*. We and others (18–20) have recently developed quantitative and sensitive methods to detect specific *RAS* gene mutations after carcinogen exposure but before cell trans-

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Abbreviations: AS-PCR, allele-specific PCR; AS-LCR, allele-specific ligase chain reaction.

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formation or tumor appearance. These studies with model systems indicated that genotype-based mutation assays of cancer-related genes can be developed as a biologically relevant method of measuring exposure to carcinogens.

To develop and validate such a method in humans, we chose UV radiation as the model carcinogen. This model provided us with two important advantages: UV causes skin cancer (21) and UV-specific p53 gene mutations have been found in human (13, 16, 22, 23) and mouse (17, 24) skin cancers. In fact, a UV-specific CC to TT tandem base mutation in the p53 gene has so far been found mainly in skin cancers, while an oxygen radical may also induce the mutation (25). Here we report development of two sensitive methods to detect a UV-specific mutation (CC to TT) in the p53 gene by using UV-exposed human skin cell cultures and their application in normal human skin.

MATERIALS AND METHODS

Cell Culture and UV Irradiation *in Vitro*. Normal human keratinocytes from adult donors were isolated as described (26) and were grown in serum-free keratinocyte medium

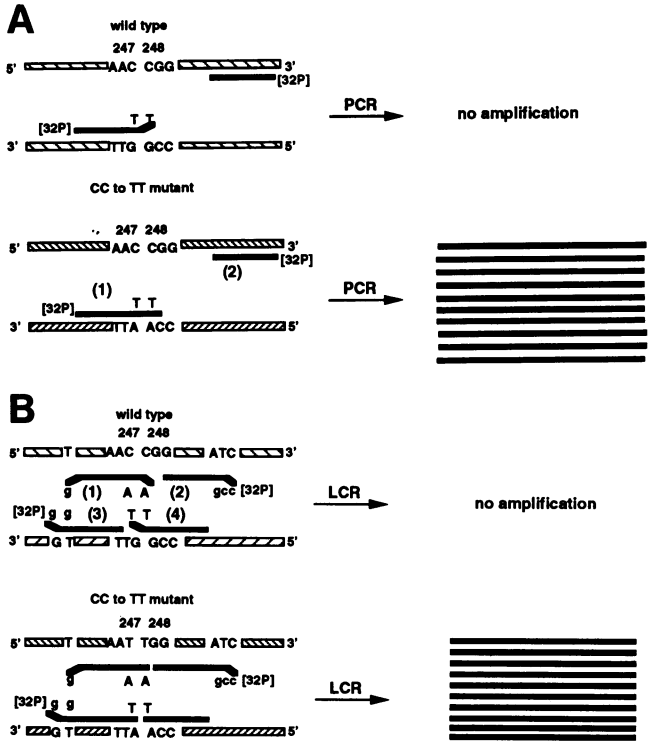

FIG. 1. Schematic representation of strategies for detection of CC to TT mutations at codons 247/248 of p53 by AS-PCR (*A*) and AS-LCR (*B*) methods. These methods are designed to amplify only the CC to TT mutant alleles. The same strategies were used for detection of codon 245 mutation. (*A*) Amprimers for detection of mutation by AS-PCR: (1) for codon 245, 5'-AAC AGT TCC TGC ATG GGC AA-3' (t_m = 60.2°C); (1) for codons 247/248, 5'-C TGC ATG GGC GGC ATG AAT T-3' (t_m = 55.3°C); (2) for both codons, 5'-CAA GTG GCT CCT GAC CTG GA-3' (t_m = 55.8°C). (*B*) Amprimers used for AS-LCR for codon 245: (1), 5'-TT GCC CAT GCA GGA ACT GTT ACA C cg-3' (t_m = 66.2°C); (2), ^{32}P-labeled 5'-cgg GAT GGG CCT CCG GTT CAT G-3' (t_m = 66.1°C); (3), ^{32}P-labeled 5'-cgg G TGT AAC AGT TCC TGC ATG GGC-3' (t_m = 68.3°C); (4), 5'-AAC ATG AAC CGG AGG CCC ATC CTC c-3' (t_m = 67.3°C). Amprimers used for AS-LCR for codons 247/248: (1), 5'-A ATT CAT GCC GCC CAT GCA GGA g-3' (t_m = 67.1°C); (2), ^{32}P-labeled 5'-cgg GAT GGT GAG GAT GGG CCT CC-3' (t_m = 67.9°C); (3), ^{32}P-labeled 5'-gg GT TCC TGC ATG GGC GGC ATG AA-3' (t_m = 66.0°C); (4), 5'-T TGG AGG CCC ATC CTC ACC ATC ATC-3' (t_m = 65°C).

(GIBCO) with 1.2 mM $CaCl_2$ at 32°C in a 90% air/10% CO_2 humidified incubator. Cells at passage 2 were exposed to UVB radiation from a BLE-8T312 lamp (Spectronics, Westbury, NY) (peak emission at 312 nm). Total cellular DNA was prepared as described (18).

Skin Biopsy. Three- to 5-mm-diameter, full thickness biopsies of normal skin were taken from 26 skin cancer patients in Australia and from 17 normal volunteers in France. Paired punch biopsies of normal skin were obtained from 17 of the Australian skin cancer patients, one from a sun-exposed site (shoulder) and the other from a non-sun-exposed site (buttock); none of these biopsies was immediately adjacent to a skin cancer. Biopsies were immediately frozen in liquid nitrogen and kept at −20°C to −80°C until use.

Detection of CC to TT Mutation of the p53 Gene in Normal Skin Cells. Two methods were developed, both designed to amplify only mutant alleles, with 10 μg of DNA samples. We chose mutations at codons 245 and 247/248 since both have been found in skin cancers (13).

Mutant Allele-Specific PCR (AS-PCR). This is a modified nested PCR in which the entire exon 7 of the p53 gene was initially amplified and then the mutant allele was selectively amplified by using mutant allele-specific oligonucleotides (Fig. 1). For the first PCR, the "amprimers" used were 5'-A CTG GCC TCA TCT TGG GCC T-3' and 5'-TGT GCA GGG TGG CAA GTG GC-3' (27). Each PCR mixture (100 μl) consisted of 10 μg of genomic DNA, 1× PCR buffer (Boehringer Mannheim), 400 μM each dNTP, and 2 units of *Taq* polymerase and amprimers. Amplification was initiated by denaturation at 96°C for 2 min and by the hot start method (70°C) (28), followed by 40 cycles of 96°C, 1 min; 60°C, 1 min; 72°C, 30 sec. The amplified exon 7 of the p53 gene was purified by spin column chromatography (Microcon; Amicon) and lyophilized, and it served as the template for the second PCR. The mutant AS-PCR assay was performed in the reaction mixture (100 μl), which consisted of the amplified p53 exon 7 DNA, 1× PCR buffer, 400 μM each dNTP, 2 units of *Taq* polymerase, and 200 μM each 5'-^{32}P-end-labeled amprimer; amprimer sequences are given in Fig. 1. PCR was performed by initially denaturing DNA at 96°C for 2 min and by the hot start method at 75°C, followed by 35 cycles of 94°C, 30 sec; 59°C for codon 245 mutation and 54°C for codons 247/248, 1 min; 72°C, 30 sec. Amplified DNAs were lyophilized and subjected to 10% PAGE. Amplified CC to TT mutations were detected by autoradiography.

Mutant Allele-Specific Ligase Chain Reaction (AS-LCR). The LCR method (29) was designed with four primers to amplify only CC to TT mutations in the p53 gene (Fig. 1). Amprimers used are shown in Fig. 1. The primers were designed and selected by use of the OLIGO primer analysis program version 4.0 (MedProbe, Oslo). The LCR was performed with 20 units of Ampligase (Epicentre Technologies, Madison, WI). After initial denaturation at 96°C for 2 min and hot start at 75°C, the reaction was continued for 35 cycles at 96°C, 3 min; 60°C, 1 min; 72°C, 30 sec. Amplified DNAs were analyzed for the presence of the mutations as described for the PCR method.

Each experiment contained at least two control samples (nonexposed cultured skin cells) to detect possible PCR (LCR) cross-contamination. Since no control showed mutation bands, we concluded that cross-contamination occurs only very rarely.

RESULTS

Induction of p53 CC to TT Mutations in Cultured Human Keratinocytes by UV Radiation: Detection by AS-PCR and AS-LCR Methods. To examine whether UV-specific mutations in the p53 gene can be induced and detected in human skin cells, we exposed early passages of human keratinocyte

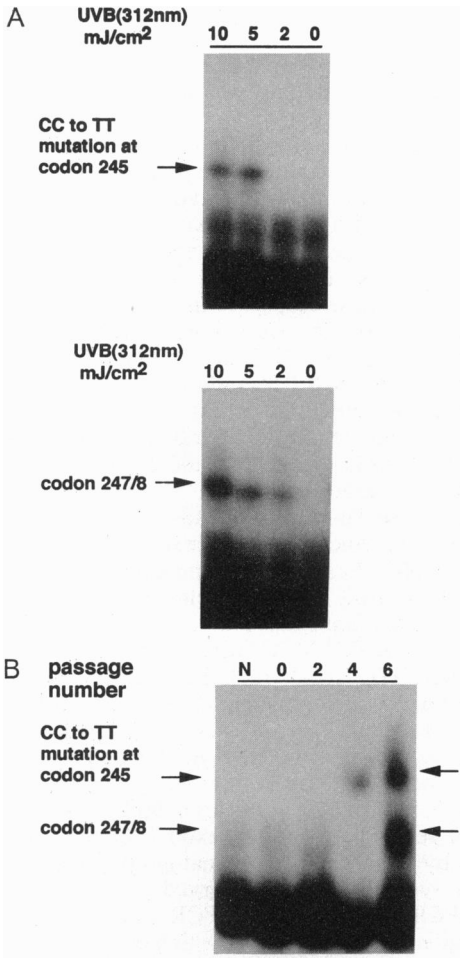

FIG. 2. Detection of UVB-induced p53 CC to TT mutations at codons 245 and 247/248 in cultured human normal keratinocytes by AS-PCR and AS-LCR. (*A*) AS-PCR analysis: secondary cultures were exposed to different doses of UVB (0, 2, 5, 10 mJ/m^2) and DNA was isolated after four passages. (*B*) AS-LCR analysis: human keratinocytes were exposed to UVB (10 mJ/m^2) at second passage. DNA was isolated after different passage numbers and subjected to AS-LCR. The CC to TT mutation-specific LCR was carried out for codons 245 and 247/248 separately, but the products were electrophoresed in the same lanes. Lane N, control cells without UVB exposure after two to four passages.

cultures to UVB and examined for the presence of CC to TT mutations at codon 245 and codons 247/248 of the p53 gene as described above and in Fig. 1. All experiments were performed from samples taken from two individuals and similar results were obtained.

Fig. 2 shows the CC to TT mutation at codons 245 and 247/248 in the p53 gene was detectable with the PCR or LCR assay only in UVB-exposed keratinocytes. The mutations increased in a UV dose-dependent manner (Fig. 2*A*) and were not detectable until after four passages of cells following UV irradiation (Fig. 2*B*). It may be that a few passages are necessary to fix the mutations or that cells with the p53 mutations had a selective growth advantage and became detectable after a few passages. The mutations at codons 247/248 were more frequently induced than those at codon 245. At passage 4, the mutation was detectable only when >3 μg of DNA (equivalent to $\approx 5 \times 10^5$ cells) was assayed, suggesting that the frequency of this mutation in the exposed cell population is at least 1 in 5×10^5 cells (data not shown). Under these conditions, no mutation was observed in any passages of cells without UV exposure (Fig. 2). These results

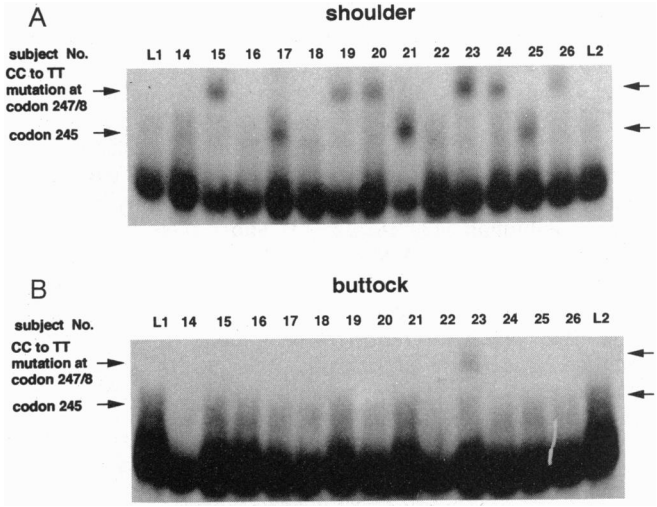

FIG. 3. Detection of CC to TT mutations in the p53 gene in biopsies of normal skin from sun-exposed and non-sun-exposed sites in the same individuals. Two punch biopsies (one from shoulder and the other from buttock) of normal skin were obtained from each of 16 Australian skin clinic patients. DNA was isolated and subjected to AS-LCR assay to detect the CC to TT mutations at codons 245 and 247/248 of the p53 gene as described in Figs. 1 and 2. Lanes L1 and L2, control DNA isolated from skin biopsies removed from two volunteers living in Lyon, France; information on each subject is given in Table 1.

indicate that both methods are sensitive to detect UV-induced CC to TT mutations of the p53 gene.

Detection of UV-Specific (CC to TT) Mutations by PCR and LCR in Sun-Exposed and Non-Sun-Exposed Normal Skin Biopsies from the Same Individuals. To examine a possible correlation between sun exposure and the presence of UV-specific p53 gene mutations in human skin, we collected normal skin biopsies from sun-exposed (shoulder) and non-sun-exposed (buttock) sites on the same Australian skin clinic patients. All except two had a primary diagnosis of skin cancer. Both types of biopsies were collected from 17 patients (subjects A10–A26) and all were taken from sites distant from the skin lesions.

More CC to TT mutations were detected by both analyses in sun-exposed than in non-sun-exposed parts of the skin (Fig. 3; Table 1). Thirteen of 17 sun-exposed skin biopsies showed CC to TT mutations in at least one assay and in at least one of two codons tested—6 at codon 245 and 10 at codons 247/248. Only 1 of 17 non-sun-exposed skin biopsies had the CC to TT mutation; this mutation was found at codons 247/248 when assayed by AS-LCR but was not found in two trials of AS-PCR. The difference in prevalence of the mutation detected by either method in either codon between sun-exposed (13/17) and non-sun-exposed (1/12) sites was statistically highly significant ($P = 0.005$); when at least one assay gave a positive response, we judged that the sample had a mutation. While most samples gave consistent responses when assayed by PCR and LCR methods, or twice by the same method, some samples gave inconsistent results. This has been expected; since presumably very few cells with the p53 gene mutation are present in each biopsy sample, the mutant cells cannot be equally distributed to each assay tube.

Since 10 μg of DNA corresponding to 1.5×10^6 cells was used for each assay, we concluded that at least 1 in 1.5×10^6 cells contained these specific mutations in positive biopsy samples.

Detection of CC to TT p53 Mutation in Human Normal Skin Biopsies from France and Australia. Normal skin biopsies from 9 Australian skin cancer patients (subjects A1–A9 in Table 2) and from 17 volunteers living in France (subjects

Table 1. Detection of CC to TT mutations in sun-exposed and non-sun-exposed normal skin on the same Australian skin clinic patients

					CC to TT mutation in the p53 gene			
			Skin lesion		Shoulder		Buttock	
Subject	Sex	Age, yr	Type	Site	Codon 245	Codons 247/248	Codon 245	Codons 247/248
A10	F	56	BCC	R. nasolabial fold	P−	P−	L−	P−,L−
A11	M	62	BCC	L. nose	P−	P+	L−	P−,L−
A12	F	80	BCC	Behind r. ear	P+	P−	L−	P−,L−
A13	F	73	BCC	R. inner canthus	P−,L−	P+	L−	P−,L−
A14	M	83	BCC	Scapula	P−,L−	P−,L−	L−	P−,L−
A15	M	73	BCC	Scapula	P+,L−	P+,L+	L−	P−,L−
A16	M	58	SW	Ear	L−	P−,L−	L−	P−,L−
A17	M	49	BCC	Scapula	L+	P−,L−	L−	P−,L−
A18	M	59	BCC	Scapula	L−	P+,L−	L−	P−,L−
A19	M	44	BCC	Loin	L−	P+,L+	L−	P−,L−
A20	M	71	BCC	Scapula	L−	P+,L+	L−	P−,L−
A21	M	71	BCC	Temple	L+	P−,L−	L−	P−,L−
A22	M	66	AK	Neck	L−	P−,L−	L−	P−,L−
A23	M	70	SCC	Forehead	P+,L−	P+,P+,L+	P−,L−	P−,P−,L+
A24	M	54	BCC	Nose	P−,L+	P+,P−,L+	P−,L−	P−,P−,L−
A25	M	54	BCC	Upper arm	P+,L+	P+,P−,L−	P−,L−	P−,P−,L−
A26	M	47	BCC	Loin	L−	P+,L+	L−	P−,L−

SW, seborrheic wart; AK, actinic keratosis; BCC, basal cell carcinoma; SCC, squamous cell carcinoma; R. (or r.), right; L., left. The following detection methods were used: P, allele-specific PCR; L, allele-specific LCR.

L1–L17) were analyzed for the presence of CC to TT mutations in the p53 gene by AS-PCR. Four samples, all from sun-exposed sites of Australian skin cancer patients, contained the mutation at codons 247/248 and one of them also showed the mutation at codon 245 (Fig. 4). When the same samples were subjected to AS-LCR, the same results were obtained for codon 245 for all samples, whereas two samples (subjects A3 and A6) showed different responses as compared to the PCR (Table 2). Altogether, 5 of 6 (83%) biopsies of sun-exposed sites in Australians showed the mutation in one or the other assay. Three biopsies of non-sun-exposed sites from Australian cancer patients (subjects A7–A9) and 17 biopsies from volunteers living in Lyon, France (subjects L1–L17), gave negative responses (Table 2). Thus, all positive biopsies were taken from sun-exposed sites of the skin from Australians, confirming a good association between CC to TT mutation of the p53 gene and sun exposure history. While no samples from volunteers living in France were positive, the comparison of these results with those obtained from Australians is hindered by the fact that the former were young normal individuals, whereas the latter were older skin cancer patients. The difference could be due to the extent of sun exposure but might also be due to age or poorer DNA repair among people predisposed to skin cancer (30).

Table 2. Summary of detection of CC to TT mutations in normal skin adjacent to or distant from the skin cancer in 9 Australian skin cancer patients (A1–A9) and from 17 volunteers living in Lyon (L1–L17)

					Mutation	
	Sex	Cancer		Biopsy	Codon	Codons
Subject	(age, yr)	Type	Site	site	245	247/248
A1	M(51)	BCC	Back	Back	P−,L−	P+,L+
A2	M(69)	BCC	Back	Back	P+,L+	P+,L+
A3	M(64)	BCC	Tmp	Tmp	P−,L−	P−,L+
A4	M(62)	BCC/SCC	Neck	Neck	P−,L−	P+,L+
A5	F(61)	BCC	Clav	Clav	P−,L−	P−,L−
A6	F(41)	SCC	Back	Back	P−,L−	P+,L−
A7	M(63)	BCC	R. ear	Abd	P−	P−
A8	F(57)	BCC	L. tmp	Btk	P−	P−
A9	M(67)	BCC	R. tmp	Hip	P−	P−
L1,L2	M (33, 35)	None	—	Arm	P−,L−	P−,L−
L3–L17	M/F (1–55)	None	—	Multi	P−	P−

R., right; L., left; Tmp, temple; Clav, clavicle; Abd, abdomen; Btk, buttock; Arm, forearm; Multi, foreskin, leg, abdomen, face, and forearm; BCC, basal cell carcinoma; SCC, squamous cell carcinoma. The following detection methods were used: P, allele-specific PCR; L, allele-specific LCR.

DISCUSSION

We have developed methods to detect CC to TT tandem base mutations at codons 245 and 247/248 of the p53 gene in apparently normal human skin cells. The presumed mutant bands at codons 245 and 247/248 were reamplified, cloned, and sequenced. We confirmed that they were derived from the correct regions of the p53 gene (data not shown). We observed dose-dependent induction of CC to TT mutations by UVB in cultured human skin cells and analysis of normal skin biopsies showed a higher prevalence of these mutations in skin from sun-exposed than from non-sun-exposed sites. It may be, therefore, that measurement of these mutations in normal skin will be a useful measure of biologically relevant UV exposure and predictor of risk of UV-induced skin cancer. It is important to emphasize that the p53 gene mutations detected represent the outcome of a series of

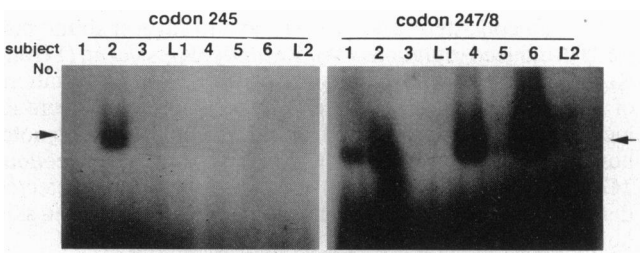

FIG. 4. Detection of CC to TT mutations in codons 245 (*Left*) and 247/248 (*Right*) of the p53 gene in normal skin biopsies by AS-PCR. Six normal skin biopsies from Australian skin cancer patients and two from normal volunteers living in France were collected and their total DNA was analyzed for the presence of a CC to TT mutation at codon 245 (*Left*) and at codons 247/248 (*Right*) of the p53 gene by AS-PCR. Biopsy sites and other information on each subject are listed in Table 2.

UV–cell interactions, including the extent of UV exposure, immunological status, fidelity of DNA polymerization, and genetic background of individuals such as skin types and difference in DNA repair capacities (30, 31).

While our study clearly shows that UV-specific p53 gene mutations can be detected in UV-exposed skin cells *in vitro* and *in vivo*, and our methods can detect mutated cells in a normal cell population, they may not provide a quantitative guide to UV exposure. Since we used the p53 gene as the target and mutated p53 genes may provide a growth advantage, a cell population with p53 mutations may increase relative to those with wild-type p53 genes. In fact, we observed these mutations in UV-exposed cultured cells only after several passages, suggesting that the mutated cells might be selectively recruited in a DNA-damaged cell population. In addition, we detected CC to TT mutations at codon 245 or 247/248 in at least 1 per 1.5×10^6 cells from skin biopsy samples. This is an extremely high mutation frequency and suggests that selective clonal expansion of mutant cells may have occurred *in vivo*. However, it is important to emphasize that we measured "cumulative" mutations in populations of human skin cells that had been exposed to the sun for decades. If a nonlethal, UV-induced mutation occurs in stem cell populations in the skin such as basal keratinocytes or hair follicle cells, all subsequent cell divisions will give rise to mutated progeny cells. Continuing exposure to the sun would be expected to give rise to further mutations and increase the prevalence of mutated cells among all basal cells. If, in addition, the mutated cells have a growth advantage (e.g., due to mutation of the p53 gene) the prevalence of mutated cells would increase further. Furthermore, if we assume that a series of genetic alterations is necessary for skin carcinogenesis, as in colon carcinogenesis, the mutation frequency of each critical gene must be higher than we generally assume (32). Thus, the mutation frequency we observed in the p53 gene in normal skin of 41- to 80-year-old subjects ($\approx 10^{-6}$ per base) may be reasonable. Ziegler *et al.* (23) recently reported that 45% of the point mutations of the p53 gene in basal cell carcinomas are accompanied by a second point mutation on the other allele. Since these mutations are UV-like, the results also suggest a high mutation frequency induced by UV (23).

The CC sequence at codons 247/248 was more frequently mutated to TT than that at codon 245 in both UV-exposed cultured human skin cells and in skin biopsies. The cytosine residue in the CC sequence at codons 247/248 is known to be methylated (13) and deamination of the 5-methylcytosine may result in a C to T single base mutation (33). This characteristic, however, does not explain the high prevalence of CC to TT tandem base mutations at codons 247/248. Another difference between the two CC sequences is location on the transcribing strand at codons 247/248 and on the nontranscribing strand at codon 245. It has been shown that strand bias exists for repair efficiency of UV-induced DNA damage and replication fidelity, but no consistent data are available to suggest strand bias for UV-induced mutations (34). Recently, Kress *et al.* (17) and Kanjilal *et al.* (24) showed that C to T or CC to TT mutations of the p53 gene in UV-induced mouse skin tumors were all located on the nontranscribed strand. It is interesting to note, however, that the C to T or CC to TT mutations at codons 247/248 (transcribing strand) are more frequently detected than those at codon 245 (nontranscribing strand) in human skin tumors (13, 15, 16).

Skin cancer is one of the commonest human cancers and its incidence is increasing (35). UVB irradiance at the surface of the earth is almost certainly increasing as a result of depletion of stratospheric ozone; this trend will lead to increases in skin cancer incidence and other health effects of UV radiation (36). Further development of the methods described in this paper will assist in accurate quantitative measurement of the relationship between UV exposure and skin cancer incidence and will, therefore, improve our capacity to predict the effects of and respond appropriately to UV irradiance changes.

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