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Advances in Brief

Induction of Cancer, Actinic Keratosis, and Specific *p53* Mutations by UVB Light in Human Skin Maintained in Severe Combined Immunodeficient Mice[1]

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Abstract

To study the mechanism and risk of human skin cancer from solar light, we exposed human skin transplanted to severe combined immunodeficient mice to daily doses of UVB for periods of approximately 2 years. We have succeeded for the first time in inducing cancer and solar (actinic) keratosis in human skin by UVB. Of 18 normal skins exposed to doses of 7.3×10^5 to 1.8×10^6 J/m^2, 14 actinic keratoses (77.8%) and 3 squamous cell carcinomas (16.7%) developed, whereas neither actinic keratosis nor cancer was observed in 15 human skins not exposed to UVB. Each human skin showed a different susceptibility, and skins sensitive for actinic keratosis were also sensitive for cancer induction. Among *p53* mutations at various sites, mutation at codon 242 (C TGC → C CGC; Cys → Arg) was specifically observed in both skin cancers and actinic keratoses. Furthermore, double or triple mutations were induced in all UVB-induced skin cancers and in three of eight actinic keratoses. Most of the mutations (17 of 20) occurred at dipyrimidine sites.

Introduction

Solar light has continuously influenced the development, metabolism, and so on of all organisms on the earth. For human beings, it is beneficial in general, but a part of solar light, UV radiation, can be harmful. Recently, the depletion of stratosphenic ozone and the consequent increase in environmental levels of genotoxic UVB (290–320 nm) in sunlight have lead to fear of a rise in the frequency of skin cancer in human populations (1, 2). In the present study, we maintained human skins in improved SCID[3] mice (3–6), which we irradiated daily with UVB for a long period (~2 years) to confirm the direct link between UVB and the development of human skin cancer, solar (actinic) keratosis, and molecular changes in *p53* and *ras* genes.

Materials and Methods

Human Skin. Human skins resected by total mammectomy of five breast cancer patients, phimectomy of four phimosis patients, and surgical operation of two actinic keratosis patients with skin grafting at the Surgery 1 of Osaka University Hospital, Urological Clinics of Osaka Police Hospital, and Tondabayashi Hospital and Dermatology of Kobe University Hospital were used with their permission for heterotransplantation to SCID mice. Only the human skins free of mycoplasma, human hepatitis B and C antigens/antibodies, adult T-cell leukemia, HIV, and Wasserman reaction were accepted into the SPF room of the barrier section of the Institute of Experimental Animal Sciences, Osaka University. Normal skin at the breast and clavicular areas and foreskin of the penis are clothed and have less chance to be exposed to solar light than unclothed areas of the body.

SCID Mice. Inbred C.B17-*scid/scid* mice (F_{25-30}) showing undetectable IgG and IgM (<1 μg/ml; Refs. 3–5) and wild-type C.B17-+/+ ($F_{38+24-25}$) were used for experiments. C.B17-*scid*/+ male and female mice were provided by Dr. M. J. Bosma, Institute of Cancer Research, Philadelphia, in 1986, and then C.B17-*scid/scid* homozygotes showing undetectable serum IgG and IgM (<1 μg/ml) were maintained by selective brother-sister inbreeding by T. N. to diminish leaky and leukemic SCID mice (4, 5). Mice were maintained in the complete barrier condition, lit from 4:00 a.m. to 6:00 p.m., at 23 ± 1°C and 50–70% humidity with autoclaved mouse diet CRF-1 (Charles River Japan, Kanagawa, Japan) and acidified, chlorinated, and filtrated (by MILLIPORE) water (3–5). All animal experiments were carried out in the barrier section of the Institute of Experimental Animal Sciences following the Osaka University Guidelines for Animal Experimentation.

Maintenance of Human Skin in SCID Mice. Human skins were cut into 1–1.5-cm elliptic pieces in a 0.9% NaCl solution containing high concentrations of antibiotics (50,000 units/ml penicillin G, 25 mg/ml panipenem, 25 mg/ml betamipron, and 50 mg/ml streptomycin sulfate). Thirty-three normal skins (17 from the breast, 14 from the foreskin of the penis, and 2 from the clavicular area of an actinic keratosis patient) and 3 lesional skins on the face of actinic keratosis patients were placed and fixed by autoclips to the back of SCID mice, from which the same size of the mouse skins were removed. The mice were anesthetized with 0.77% tribromoethanol (Aldrich Chemical Co., Milwaukee, WI). When mice died or were dying, transplanted human skins were removed and transferred to other SCID mice by the same procedure.

UVB Exposure. Two weeks after transplantation, about half of the transplanted human skins from each donor were exposed daily to UVB at the intensity of 2.8 J/m^2/s, and the other half were unirradiated (Table 1). The source of UVB used was a bank of four fluorescent sunlamps of wavelengths 280–360 nm (main peak, 313 nm; Toshiba FL 20S•E, Toshiba, Tokyo, Japan). Wavelengths shorter than 288 nm were completely cut off by a Kodacel cellulose triacetate sheet K6808 (Eastman Kodak, Rochester, NY) to simulate solar UV radiation on the surface of the earth. Dose intensity was measured by an UV Radiometer UV103 (UVB filter, 313 nm; Macam, Livingston, Scotland). Daily UVB doses were 8000 J/m^2 on Monday, Wednesday, and Saturday, and 2000 J/m^2 on Tuesday, Thursday, Friday, and Sunday. The minimum erythema dose for Japanese by sunlamp (UVB) is approximately 300–1500 J/m^2, depending on skin type (7). This corresponds to 12–60 min of exposure to solar light at noon in midsummer in the Osaka area.

Detection of *p53* and *ras* Mutations. Mutations of *p53* and K-*ras* genes were examined for the original and transplanted human skins by PCR and single-strand conformational polymorphism (without radioisotopes) direct sequencing method from serial thin sections of neutral formalin-fixed, paraffin-embedded, biopsied specimens (5, 8). The following PCR primers were used for the amplification of the *p53* gene. Exon 5 was divided into two regions. Primer pairs were: 5′-TCTGTCTCCTTCCTCTTCCTA-3′ and 5′-CATGTGCTGTGACTGCTTGT-3′ and 5′-TGTGCAGCTGTGGGTTGATTC-3′ and 5′-CAGCCCTGTCGTCTCTCCAG-3′ for exon 5; 5′-TTGCTCTTAGGTCTGGCCCC-3′ and 5′-CAGACCTCAGGCGGCTCATA-3′ for exon 6; 5′-TAGGTTGGCTCTGACTGTACC-3′ and 5′-TGACCTGGAGTCTTCCAGTGT-3′ for exon 7; and 5′-AGTGGTAATCTACTGGGACGG-3′ and 5′-ACCTCGCTTAGTGCTCCCTG-3′ for exon 8. The primer pair for exon 1 of K-*ras* gene was 5′-CATGTTCTAATATAGTCACA-3′ and 5′-CTCTATTGTTGGATCATATTCGTCC-3′. Details of experimental conditions for DNA amplification, single-strand conformational polymorphism, and direct sequencing are given in the previous reports (5,

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[3] The abbreviations used are: SCID, severe combined immunodeficient; SCC, squamous cell carcinoma.

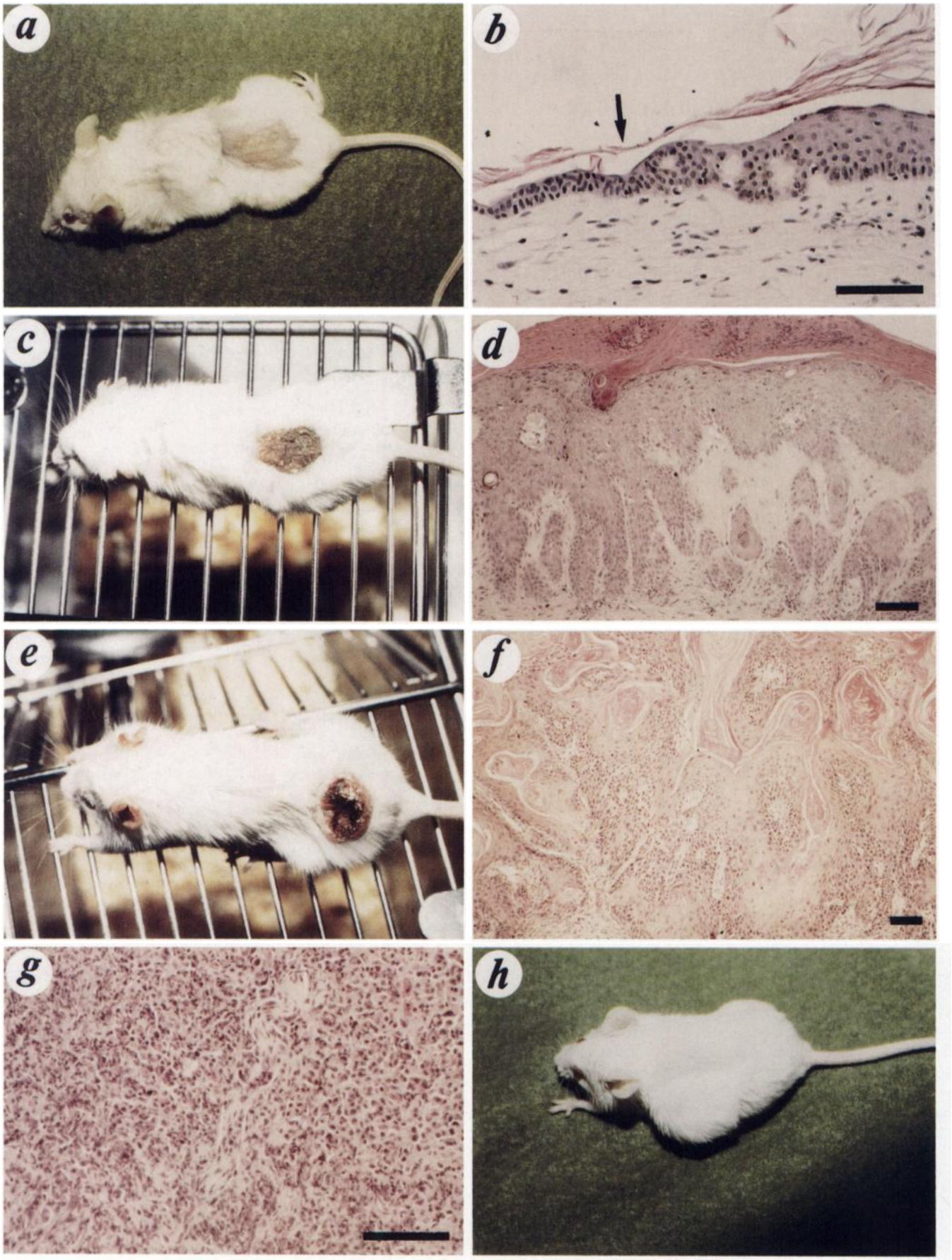

Fig. 1. Macroscopic and microscopic views of transplanted human skin with or without UVB irradiation. H&E staining. *Scale bars*, 100 μm. *a*, transplanted human skin showing pale yellow color, in contrast to the albino mouse skin where the hair is removed, 247 days after transplantation of the foreskin (MT) to SCID mice. *b*, histological view of human skin (MT) transplanted to SCID mice. Thin mouse *(left)* and thick human *(right)* skin. *Arrow*, border, distinguishable by the brown pigments in basal cell layer in the human skin. *c*,

Table 1 *Actinic keratosis and cancer induction by UVB in the human skin maintained in SCID mice*

Human skin	Period (days)	UVB dose (J/m^2)	Keratosis	Cancer		
			Incidence (%)	Incidence (%)	Origin	Histology
Normal skin from breast cancer patients (YI, 53y; ST, 44y; TF, 45y; TH, 46y; TN, 23y)						
Unirradiated	~745	0	0/8 (0.0)	0/8 (0.0)		
Irradiated	~620	~1,890,000	7/9 (77.8)[a]	1/9 (11.1)	Human	SCC[b]
Normal skin from phimosis patients (HO, 20y; NT, 55y; HY, 24y; MT, 21y)						
Unirradiated	~831	0	0/6 (0.0)	0/6 (0.0)		
Irradiated	~592	~1,754,000	6/8 (75.0)[a]	1/8 (12.5)	Human	USC[c,d]
Normal skin from actinic keratosis patient (YT, 83y, female)						
Unirradiated	366	0	0/1 (0.0)	0/1 (0.0)		
Irradiated	278	730,000	1/1 (100)	1/1 (100)	Human	SCC[e]
Keratosis skin from actinic keratosis patients (YT, 83y; SBT, 77y, male)						
Irradiated	~416	~1,434,000		1/3 (33.3)	Human	SCC[f]

[a] $P < 0.01$ *vs.* unirradiated controls by Fisher's exact test.
[b] Cancer was determined at a UVB dose of 1,836,000 J/m^2 (ST).
[c] USC, undifferentiated human skin cancer.
[d] Cancer was determined at a UVB dose of 1,368,000 J/m^2 (NT).
[e] Cancer was determined at a UVB dose of 730,000 J/m^2 (YT).
[f] SCC (YT, 632,000 J/m^2), and adenoma of the sweat gland was also induced in another transplanted keratotic skin (YT, 1,008,000 J/m^2).

8). The single-stranded DNA generated by asymmetric PCR was sequenced from both directions using ABI PRISM 310 Genetic Analyzer (Perkin–Elmer, Foster City, CA).

Results and Discussion

Induction of Actinic Keratosis and Cancer in Human Skin. Transplanted human skins were well maintained in SCID mice and identified both macroscopically and microscopically by the pale yellow color and pigments of the human skin against the albino mouse skin (Fig. 1*a* and *b*). After 2×10^5 J/m^2 of UVB irradiation, the human skins became irregularly thick and brownish, whereas no changes were observed in the mouse skin, simply because the mouse skin was covered by hair (Fig. 1*c*). Biopsies were made at the time of transfer of each human skin, and microscopic examination revealed that significantly high incidence of actinic keratosis was induced in the UVB irradiated human skin (Fig. 1*d*), whereas no actinic keratoses were observed in unirradiated human skins (Table 1). Although histological changes were not detected in the mouse skin around the transplanted site because of the hair, skin erosion and necrosis were induced in the ears of all UVB-irradiated mice.

After the long continued UVB irradiation (7.3×10^5 to 18.4×10^5 J/m^2) to the normal human skin, three ulcerated skin tumors developed sequentially in the brownish (keratosis) human skin and grew rapidly (Fig. 1*e*). Two were well-differentiated SCCs from the normal skins of the breast cancer patient (ST) and the actinic keratosis patient (YT), and the other was an undifferentiated skin cancer from the foreskin (NT; Fig. 1 *f* and *g*). These skin tumors grew in SCID mice but not in wild-type C.B17-+/+ mice (Fig. 1 *h*). Furthermore, tumor and human tissue DNA were amplified by the primers of human *p53* and K-*ras* genes, whereas mouse DNA was not. These confirmed immunologically and genetically that induced skin tumors were malignant and originated in the human skin. This is the first successful experimental induction of cancer and precancerous lesions in human tissue by UVB (6). Chemical treatment to human skin (9) and internal organs (6) with or without radiation rarely induced human tumors, but almost all induced tumors originated from surrounding mouse tissues. Chemical carcinogens or promoters act on mouse tissues in addition to human tissues, whereas UVB exposure affects only human skin because of the hair in mouse skin.

Fig. 2 shows the time of pathohistological diagnosis of actinic keratosis and cancer in the UVB-irradiated human skin from each donor. The time of diagnosis for actinic keratosis was widely distributed, suggesting the different sensitivity of each individual donor to UVB (1). Skins developing cancer also showed early occurrence of actinic keratosis. Although only one skin piece was tested, both actinic keratosis and cancer developed earlier in the normal skin of an 83-year-old actinic keratosis patient (YT). This may also suggest the different genetic susceptibility of human skins to solar light (1, 2) or photo- or chronological aging.

After UVB irradiation to the transplanted lesional skin (face) of the actinic keratosis patients, SCC and adenoma of the sweat gland developed microscopically at relatively low doses in two keratosis lesions of a patient (YT; Table 1 and Fig. 2).

Mutations of *p53* and *ras* Genes. Because the involvement of the *p53* tumor suppressor gene has been reported in SCC and actinic keratosis in human skin (10–14) and also UVB-induced skin cancer in mice (15), UVB-irradiated and unirradiated skin pieces from eight donors (Table 2) were examined for *p53* and K-*ras* mutations. Although mutations of the K-*ras* oncogene were detected in none of the UVB-induced skin cancers and actinic keratoses, a variety of mutations were detected in exons 5, 6, 7, and 8 of the *p53* gene at codons 138, 145, 149, 155, 160, 162, 167, 176, 178, 199, 206, 239, 242, 247, 273, and 282, showing higher frequencies at codons 242 and 273, as in the case of human lung cancer (16, 17). However, these mutations showed no significant links to UVB irradiation except for T:A → C:G transition at codon 242 (C TGC → C CGC; Cys → Arg). As shown in Table 2, *p53* mutations at codon 242 were significantly induced in all UVB-induced skin cancers and in seven of eight UVB-irradiated human skin pieces from six donors that developed actinic keratoses, except one skin (one piece) with mutation at codon 247. Three of seven UVB-induced actinic keratoses had additional (double) mutations at codons 155, 199, and 239. Although one donor (two pieces) with a mutation at codon 242 remained in the histologically normal range 177 and 179 days after transplantation (276,000 and 280,000 J/m^2, respectively), it progressed to actinic keratosis with early invasion (Fig. 1*d*) 592 days after transplantation (1,754,000 J/m^2). Furthermore, all UVB-induced skin cancers possessed additional (double or triple) mutations at codon 160 (ST), at codons 178 and 206 (NT), and at codon 179 (YT; Table 2).

Similar results were obtained in actinic keratosis lesions on the face

actinic keratosis of human foreskin (MT) after UVB irradiation (614,000 J/m^2), showing brown, thick and irregular surface. Erosion and necrosis are induced at the mouse ear. *d*, histology of UVB-induced actinic keratosis with early invasion (HO; 1,754,000 J/m^2). Mutation at codon 242 was detected in this lesion. However, transplantation of this lesion to SCID mice did not form malignant tumors. *e*, ulcerated skin tumor developed in the UVB-irradiated human skin. Normal human skin at the clavicular area of an actinic keratosis patient (YT) received 730,000 J/m^2 of UVB. *f*, well-differentiated SCC (YT). Double mutations at codons 179 and 242 were detected in this lesion. *g*, undifferentiated skin cancer developed in the transplanted foreskin (NT) after UVB irradiation (1,368,000 J/m^2). Examination of surface makers revealed that S-100 and vimentin were weakly positive, but keratin, epithelial membrane antigen, and HMB-45 were negative, suggesting undifferentiated SCC or amelanotic malignant melanoma. *h*, tumor growth after s.c. transplantation of UVB-induced skin cancer into the SCID mouse. Skin cancer did not grow in wild-type C.B17-+/+ mice, indicating that cancer derived from human skin but not from mouse skin.

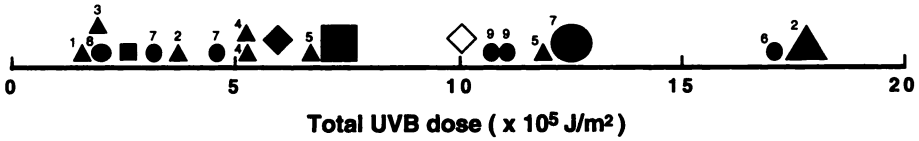

Fig. 2. Time of pathohistological diagnosis of actinic keratosis and cancer developed in UVB-irradiated human skins. Normal human skins at the breast and clavicular areas from five breast cancer patients (▲; *1*, YI; *2*, ST; *3*, TF; *4*, TH; *5*, TN) and an actinic keratosis patient (■; YT), and foreskins from 4 phimosis patients (●; *6*, HO; *7*, NT; *8*, HY; *9*, MT). Larger symbols indicate skin cancer. SCC (◆) and adenoma of the sweat gland (◇) developed in the transplanted lesional skin (face) of an actinic keratosis patient (YT) after UVB irradiation. Duplicated numbers on the symbols indicate two skin pieces from the same donor.

(YT and SBT), one of which (YT) had already possessed a transition at codon 242 without experimental UVB exposure. After UVB irradiation (604,000 J/m^2), mutation at codon 242 was also induced in another keratosis skin (SBT; Table 2). Later, these progressed to SCC and adenoma of the sweat gland, and additional (double) mutations were detected at codons 206 and 282 in those lesions, respectively. Altogether, 17 of 20 mutations (85.0%) detected in UVB-induced actinic keratoses and tumors occurred at dipyrimidine sites.

Codon 242, a highly conserved site in exon 7 of the *p53* gene, is located in the most common region of somatic (tumor specimen) and germ-line *p53* mutation in patients with Li-Fraumeni cancer syndrome (18, 19). Mutations at codon 242 were reported in lung cancer, intracranial ependymoma, and hepatocellular carcinoma in humans (16, 17, 20). However, all of those in the previous reports (16, 17, 20) occurred at G of the TGC, whereas all mutations at codon 242 detected in spontaneous and UVB-induced skin cancers and keratoses occurred at a dipyrimidine site, which is the suspected target sequence for UVB mutagenesis. Although mutation at codon 242 has been specifically observed in UVB-induced actinic keratosis and cancer of human skin, *p53* mutations reported in the patients are nearly random (10–14). Additional mutations were detected in all UVB-induced skin cancers and some actinic keratoses in the present study. UVB-induced mutation at codon 242 seems essential and initial to cancer and keratosis susceptibility, but combinations with additional mutations in the *p53* gene and others may be required for malignant transformation.

Table 2 *Specific p53 mutations in UVB-irradiated human skin developing actinic keratosis and cancer*

		Codon 242
	No. of donors (no. of skin pieces)	C TGC → C CGC (Cys → Arg)
Normal human skin		
Unirradiated (ST, HO, NT, HY, MT, YT)	6 (14)	0 (0)
UVB-induced actinic keratosis (ST, TH, HO[a], NT, HY, MT)	6 (8)	5 (7)[b]
UVB induced skin cancer (ST, NT, YT)	3 (3)	3 (3)[c]
Keratosis skin on the face of actinic keratosis patients		
Unirradiated (YT, SBT)	2 (2)	1 (1)[d]
UVB irradiated (YT, SBT)	2 (3)	2 (3)
UVB induced tumors (YT)	1 (2)	1 (2)[e]

[a] Biopsied skin pieces after 276,000 (177 days) and 280,000 (179 days) J/m^2 of UVB irradiation were histologically normal but possessed a mutation at codon 242. These progressed to actinic keratoses with early invasion (Fig. 1*d*) 592 days after transplantation (UVB dose, 1,754,000 J/m^2).

[b] $P < 0.01$ *vs.* unirradiated controls by Fisher's exact test. One skin had a C:G → T:A transition at codon 247 (AAC C→AAT C; Asn → Asn; ST), and three of seven skin pieces with mutation at codon 242 had additional mutations at codons 155 (C ACC → C GCC; Thr → Ala; HO), 199 (CGA → GAA; Gly → Glu; HY), and 239 (AAC→AGC; Asn→Ser HY).

[c] $P < 0.05$ *vs.* unirradiated controls by Fisher's exact test. Additional (double or triple) mutations at codon 160 (ATG G → ATA G; Met → Ile; ST), at codons 178 (CAC → CGC; His →Arg) and 206 (T TTG → T CTG; Leu → Leu; NT), and at codon 179 (CAT → CGT; His → Arg; YT).

[d] YT.

[e] Double mutations at codons 206 and 242 and at codons 242 and 282 (CGG → CAG; Arg → Gln) in the microscopic lesions of SCC and adenoma of the sweat gland, respectively.

Not only for cancer and environmental researches, long-maintained human skin and other organs will allow us to study physiological and pathological response and interaction of normal human tissues.

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