Am. J. Hum. Genet. 56:1267–1276, 1995

UV^s Syndrome, a New General Category of Photosensitive Disorder with Defective DNA Repair, Is Distinct from Xeroderma Pigmentosum Variant and Rodent Complementation Group I

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Summary

Previously, we reported two DNA repair–defective siblings who did not belong to any complementation group of xeroderma pigmentosum (XP) or Cockayne syndrome (CS). By surveying other photosensitive patients whose fibroblasts showed similar biochemical phenotypes, we found another nonconsanguineous Japanese patient belonging to the same complementation group as our previous cases. Postreplication repair of the cells derived from these patients was normal, indicating that they cannot be classified as XP variant. Neither transfection nor microinjection of the cells with the human DNA repair gene ERCC1, which is known not to correct any complementation groups of XP or CS, failed to correct the defect of these cells, indicating that they do not belong to the rodent complementation group 1. However, the defect in recovery of RNA synthesis (RRS) after UV irradiation was restored by microinjection of HeLa cell extract. Although clinical manifestations of these patients—such as acute sunburn, dryness, freckling, pigmentation anomalies on sun-exposed skin, and teleangiectasia without neurological abnormalities or tumors—are similar to a mild XP phenotype, cellular characteristics such as UV sensitivity and defective RRS after UV irradiation with normal unscheduled DNA synthesis (UDS) are reminiscent of CS. On the basis of these results, we propose that these patients be included under a general category designated "UV-sensitive" (UV^s) syndrome.

Introduction

There are several genodermatoses that are characterized by photosensitivity of the skin with or without complications. The primary genetic cause of these diseases is defects in DNA repair. Xeroderma pigmentosum (XP) is a rare autosomal recessive disease in which a defect in excision repair of UV-induced DNA damage is found (Cleaver and Kraemer 1989). Cockayne syndrome (CS) is also a rare autosomal recessive disease that involves a deficiency in the ability to repair DNA damage in actively transcribed genes (Venema et al. 1990).

Patients with overlapping manifestations of both XP and CS have been reported in XP groups B (Robbins et al. 1974; Scott et al. 1993), D (Robbins 1991), and G (Vermeulen et al. 1993). These patients have defects in excision repair of UV-induced DNA damage. Recently, we reported two Japanese siblings with mild clinical manifestations but impaired recovery of RNA synthesis (RRS) after UV irradiation (Itoh et al. 1994). This defect in RRS was partially corrected by microinjection of T_4 endonuclease V, indicating that at least pyrimidine dimers are not repaired in these patients. Although mild forms of CS with a late onset have been reported (Kennedy et al. 1980; Miyauchi et al. 1994), our patients did not belong to any complementation group of XP or CS (Itoh et al. 1994). A patient with a similar phenotype to our cases was reported by Fujiwara et al. (1981). In addition, patients with overlapping XP and CS have been reported (Greenhaw et al. 1992). Although a defect in excision repair of UV-induced DNA damage was not detected in these patients with overlapping phenotypes, they exhibited a defect of RRS after UV irradiation, a characteristic feature of CS. Since no complementation analysis was carried out in the studies other than ours, no information concerning the genetic similarities among them is available.

Here, we extended our previous study (Itoh et al. 1994) with regard to the following points: First, we confirmed that the two patients did not belong to the variant group of XP by a postreplication assay and provided evidence that they had no mutations in the human-excision-repair gene ERCC1 (Westerveld et al. 1984) which did not correspond to any complementation groups of XP or CS (Van Duin et al. 1989). Next, to establish a general category of a new syndrome, designated UV-sensitive (UV^s) syndrome, we surveyed other

Received November 30, 1994; accepted for publication March 3, 1995.

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0002-9297/95/5606–0003$02.00

Table 1

Cell Lines Used in This Study

Cell Line	Reference	Source	Remarks
Kps2	Itoh et al. (1994)		
Kps3	Itoh et al. (1994)		
UV^{s}1KO	Fujiwara et al. (1981)		
GM10903	Greenhaw et al. (1992)	Camden cell bank	
GM10905	Greenhaw et al. (1992)	Camden cell bank	
Mps1	Itoh et al. (1994)		Assigned as CS-A in this laboratory
XP2SA	Itoh et al. (1994)	Japanese Cancer Research Resources Bank	Assigned as XP variant
Mori	Itoh et al. (1994)		Normal cell line
Turu	unpublished data		Assigned as a normal cell in this laboratory
W138VA13			Wild-type stable transfectant
CS1MOSV			Stable transfectant of CS1MO (CS-B) cells by SV40 established in this laboratory
Kps3SVY			Stable transfectant of Kps3 cells established in this laboratory
43-3B	Wood and Burki (1982)	Gift from A. Yasui	CHO mutant cell line (rodent complementation group 1)
6L1030[a]	Shiomi et al. (1982); Thompson et al. (1988)	Gift from T. Shiomi	Mouse mutant cell line (rodent complementation group 8).

[a] 6L1030 cells are US31-fused with LTA (X-irradiated normal mouse fibroblast line) (T. Shiomi, T. Itoh, M. Yamaizumi, M. Wakasugi, T. Matsunaga, and O. Nikaido, unpublished data).

patients with clinical and cellular characteristics similar to our cases and found another nonconsanguineous patient reported by Fujiwara et al. (1981) to belong to the same genetic group.

Material and Methods

Cell Lines and Media

The cells used in this study are listed in table 1. All cells were cultured in Dulbecco's modified Eagle's MEM (DMEM; Flow laboratories) supplemented with 10% FCS (Gibco) and antibiotics (penicillin G [100 U/ml], streptomycin [100 μg/ml]) in a humidified 5% CO_2 incubator.

Postreplication Repair Assay

The increase in size of newly synthesized DNA after UV irradiation (postreplication repair) was analyzed by the sedimentation velocity method as described previously (Kaufmann and Cleaver 1981; Lehmann 1981). Cells were irradiated with UV light (254 nm) at a dose of 5 J/m^2, returned to fresh medium, and incubated for 60 min before being labeled for 30 min with 0.37 MBq/ml (10 μCi/ml) of [^{3}H] thymidine. Cells were harvested and irradiated with 20 Gy of X-rays on ice. A suspension of cells was layered on the top of 5 ml of 5%–20% (w/v) alkaline sucrose gradients with 0.1 M NaOH, and centrifuged at 50,000 rpm for 90 min at 4°C with an RPS55T rotor (Hitachi). After centrifugation, drop fractions were collected onto Whatman grade 17 paper strips, and the acid-insoluble radioactivities were counted in a liquid scintillation counter.

ERCC1 Expression

The human ERCC1 coding sequence was isolated from mRNA prepared from HeLa cells by RT-PCR using the primers 5′-AGGCTCAATACTGGAGATGGA-CCCTGGGAAGGAC-AAAG (5′ end of gene) and 5′-AGGTCTTAGGCTCGAGTCAGGG-TACTTTCAAG-AAGGGCTC (3′ end of gene). The amplified fragment was cloned into the expression vector pcDNAI/Neo (Invitrogen) via the *Xho*I restriction site (pcDNA-ERCC1). The direction of the insert was determined by cutting the plasmid with *Bgl*II.

DNA Transfection and Selection of Transformants

One day before DNA transfection $5 \times 10^5 - 1 \times 10^6$ recipient cells (43-3B, 6L1030, and Kps3SVY) were seeded into 100-mm dishes. Transfection was carried out using the calcium-phosphate precipitation method (mammalian transfection kit; Stratagene). The pcDNA-ERCC1 was linearized with *Sac*II and *Sca*I to remove the

replication origin of SV40 and was collected by ethanol precipitation. Twenty milligrams of linearized plasmid DNA was added to each dish, and 1–2 d after transfection, selection of transformants was started by adding G418 to the medium. The concentration of G418 was dependent on the transfected cell lines, ranging from 400 μg/ml to 800 μg/ml. The selection medium was replaced with fresh medium every 3–5 d. After 2–3 d (43-3B, 6L1030) or 4–6 wk (Kps3SVY), colonies were isolated and grown into mass cultures.

UV Survival Assay

Appropriate numbers of cells were inoculated onto 60-mm dishes and left to attach for 10 h. Subsequently, cells were rinsed with phosphate-buffered saline and exposed to UV light (254 nm) at a fluence rate of 0.7 J/m^2/s. Primary cell lines, such as GM10903, GM10905, Mori, and Kps3 cells, were subsequently incubated for 14 d. Transformed cell lines, such as 43-3B, 6L1030, and Kps3SVY cells, were incubated in nonselective medium for 10 d (43-3B, 6L1030) or 3–4 wk (Kps3SVY), colonies were fixed with 80% methanol and stained with Giemsa. For each dose, three dishes were used for transformants and four dishes were used for primary cells. The relative survival was plotted versus the UV dose on semilogarithmic paper.

Preparation of HeLa Cell Extract

HeLa cell extract was prepared as described previously (Itoh et al. 1994). About 0.5 ml of HeLa cell pellet was lysed by homogenization and the addition of ammonium sulfate. The lysate was ultracentrifuged, and the supernatant was precipitated with ammonium sulfate (20%–50% saturation). The precipitate collected by microcentrifugation was redissolved in buffer A (0.1 M KCl; 0.2 mM EDTA; 4 mg leupeptin/ml; 1 mg pepstatin/ml; 0.5 mM DTT; 0.5 mM phenylmethylsulfonyl fluoride; 10% glycerol; 0.01% NIKOL BL-8SY [octaethyleneglycol mono-n-dodecyl ether; NIKKO Chemicals]; 0.01% NP40 [Nonidet P-40]; and 20 mM Tris-HCl, pH 7.9), and then the solution was dialyzed against the same buffer. After dialysis, the precipitate was collected by microcentrifugation and redissolved in buffer A′ (0.4 M KCl buffer A). The protein concentration of cell extract was in the range of 10–15 mg/ml.

Microinjection Assay

Microinjection assay was performed as described elsewhere (Yamaizumi et al. 1986), with some modifications. Kps3 cells were seeded on coverslips and incubated until they became subconfluent. HeLa cell extract or ERCC1 mRNA prepared from pcDNA-ERCC1 using a mCAP mRNA capping kit (Stratagene) was microinjected into the cytoplasm of either Kps3 or 43-3B cells with glass needles. After incubation for 3–4 h (HeLa cell extract) or 12–16 h (ERCC1 mRNA), cells were irradiated with UV light (254 nm) at a dose of 15 J/m^2. Subsequently, RRS was measured as described elsewhere (Itoh et al. 1994). In brief, after UV irradiation, the cells were incubated for 23 h in the culture medium, labeled for 1 h with [^{3}H] uridine (100 μCi/ml), and fixed. The coverslips were then mounted on glass slides, dipped in Kodak NTB-3 emulsion, and exposed for 24 h at 4°C. Grains above the nuclei of UV-irradiated cells were counted under a microscope.

Cell-Fusion Complementation Test

Cell fusion was performed on a small scale by the method described elsewhere (Itoh et al. 1994). In brief, 20 μl aliquots ($1–2 \times 10^4$ cells) of cell suspensions fused with Sendai virus were plated on the center of coverslips (18 mm × 18 mm) in 30-mm dishes and 20 μl ($1–2 \times 10^4$ cells) of parental cells was plated on each side of the fused cells. Cells were incubated for 20 h and then irradiated with UV light at a dose of 15 J/m^2. Then, RRS was measured as described above.

Results

Sedimentation of DNA through Alkaline Sucrose Gradient

To exclude the possibility of a defect in postreplication repair, we analyzed the size of newly synthesized DNA on subsequent post-UV incubation of Kps3 cells. In normal cells (Turu), DNA molecules synthesized after UV irradiation at a dose of 5 J/m^2 were not different from those in unirradiated cells (fig. 1*A*). In contrast, the sizes of DNA synthesized after UV irradiation in XP variant cells (XP2SA) were smaller than those in unirradiated cells (fig. 1*B*). Such sedimentation patterns are typical for the XP variant. Since under the same conditions, Kps3 cells exhibited the same pattern as normal cells (fig. 1C), we excluded the possibility that Kps3 cells belonged to the XP variant.

UV Survival of Kps3SVY Cells Transfected with the ERCC1 Gene

To date, no human complementation group corresponding to the rodent complementation group 1 has been reported. To investigate whether Kps3 cells are different from rodent group 1, UV sensitivity of Kps3SVY cells (a cell line of the new complementation group transformed by SV40) transfected with the human DNA repair gene ERCC1 was determined by measuring colony-forming ability after UV irradiation. We amplified the ERCC1 gene by RT-PCR and inserted the PCR fragment into the eukaryotic expression vector pcDNAI/Neo (pcDNA-ERCC1). Although transformants of 6L1030 cells (rodent complementation group 8) with ERCC1 were still sensitive to UV irradiation, those of 43-3B cells (rodent complementation group 1) became resistant to UV, indicating that the amplified cDNA was active (fig. 2*A*). However, all of the Kps3SVY cells

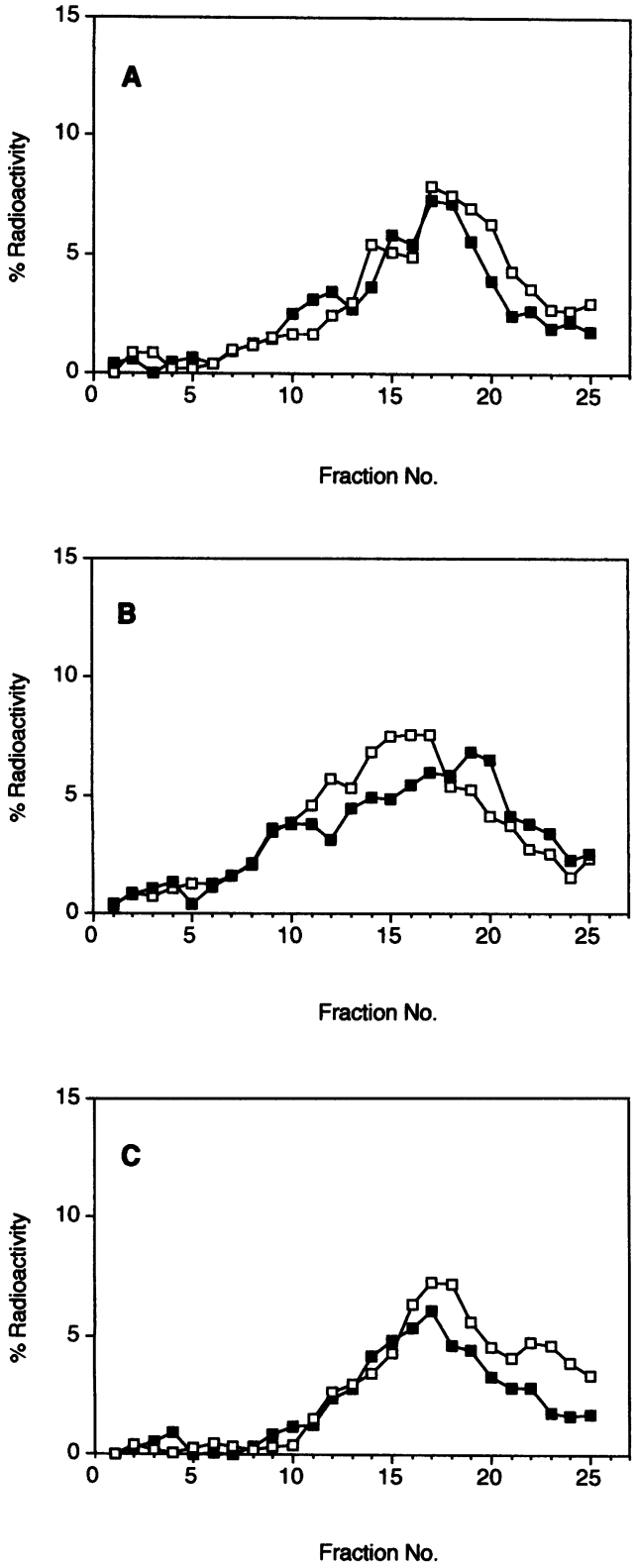

Figure 1 Alkaline sucrose gradient profiles of normal (Turu) (*A*), XP variant (XP2SA) (*B*), and Kps3 (*C*) cells. Cells were irradiated with UV at a dose of 5 J/m^2, grown for 1 h, and incubated for 30 min with [^{3}H] thymidine (0.37 MBq/ml). Centrifugation was performed for 1.5 h at 50,000 rpm. Unblackened squares (□) indicate unirradiated cells (control); and blackened squares (■) indicate UV-irradiated cells.

transfected with the pcDNA-ERCC1 (designated Kps3SVY-ERCC1 cells) were sensitive to UV irradiation (fig. 2*C*).

Microinjection of ERCC1 mRNA and HeLa Cell Extract into Kps3 Cells

To exclude the possibility that the failure of transfection of the ERCC1 gene to correct the defect in Kps3 cells was due to the transformation efficiency of human cells being lower than that of rodent cells, we microinjected mRNA transcribed in vitro from the ERCC1 gene into Kps3 cells. Although RRS in 43-3B cells was restored to a normal level with this mRNA, RRS after UV irradiation was not restored in Kps3 cells (data not shown). These results indicate that Kps3 cells do not belong to the rodent complementation group 1. The defect in RRS after UV irradiation of Kps3 cells was corrected to an almost normal level by microinjection with HeLa cell extract (fig. 3), suggesting that the defect in RRS of Kps3 cells is caused by abnormality or absence of an unknown repair factor(s).

Survival, UDS, and RRS after UV Irradiation of Cells with Similar Characteristics

The reported biochemical characteristics of GU (GM10905), AU (GM10903) and UV^{s}1KO cells were similar to those of our cases. We compared the survival of Kps3 cells following UV irradiation with those of GU and AU under our assay conditions (fig. 4). The UV sensitivity of UV^{s}1KO cells was almost the same as that of Kps3 cells (Fujiwara et al. 1981). Thus, all of Kps2, Kps3, GM10903, GM10905, and UV^{s}1KO cells had similar UV sensitivities, comparable to that of CS cells (fig. 4 and unpublished data). Furthermore, they exhibited a normal level of UDS and a reduced level of RRS after UV irradiation, consistent with the cellular characteristics of CS.

Cell-Fusion Complementation Test

To determine whether GM10903, GM10905, and UV^{s}1KO cells belong to the same complementation group as Kps3 cells, we performed complementation analysis based on cell fusion. As this method provides both positive and negative controls on the same coverslip (see Material and Methods), we could easily judge the results from changes in the grain number of the fused cells. Some examples of complementation analysis showing the correction of RRS in heterokaryons between Kps3 and GM10905, and those between UV^{s}1KO and Mps1 (CS group A) are shown in figures 5*A* and 5*B*, respectively. However, fusions between Kps3 and UV^{s}1KO did not show corrected RRS after UV irradiation (fig. 5*C*). To confirm the fidelity of the inspection, we counted the grains in the fused cells, and the results are summarized in table 2. The defect in Kps3 cells was complemented by GM10903 and GM10905 cells but

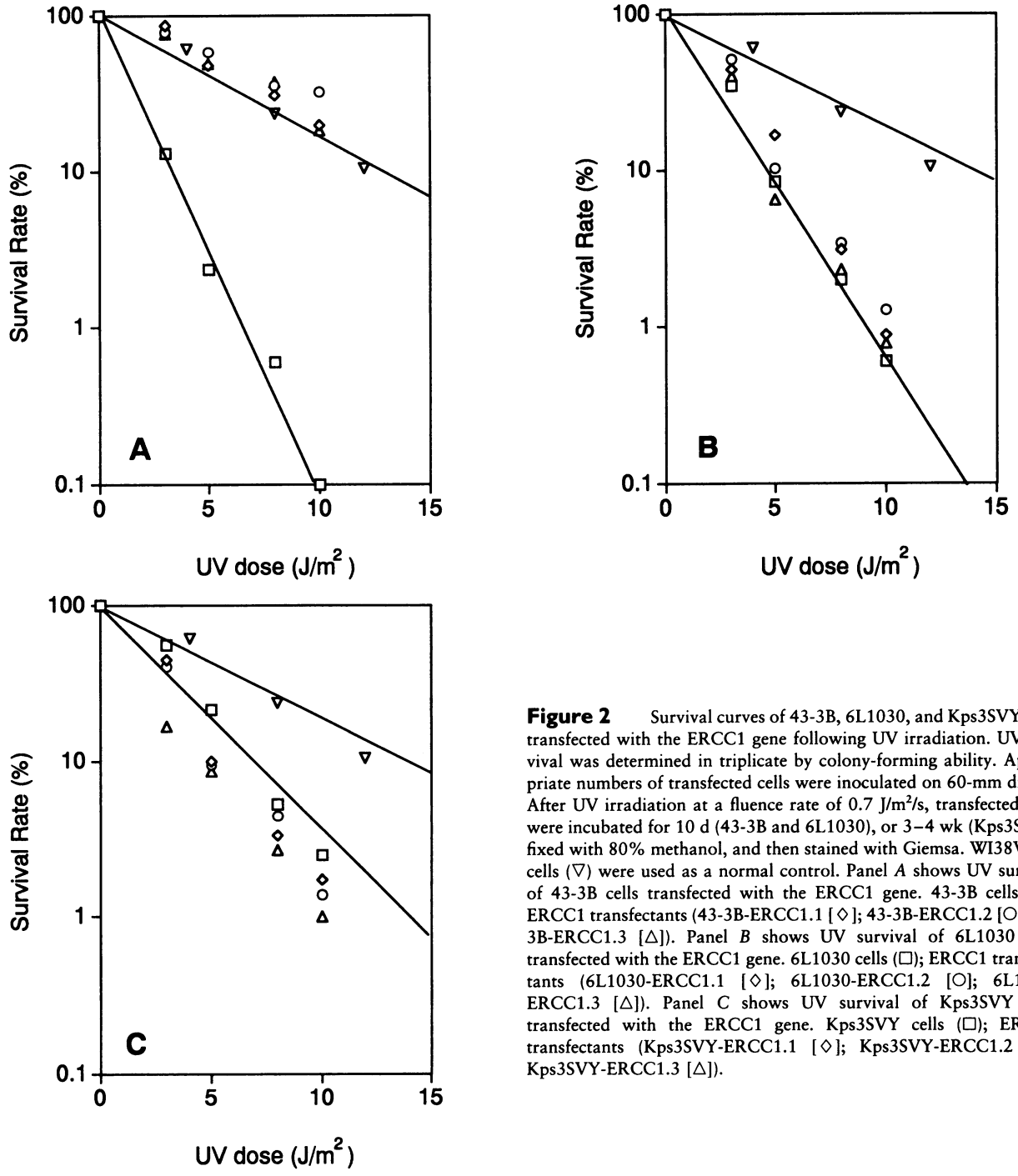

Figure 2 Survival curves of 43-3B, 6L1030, and Kps3SVY cells transfected with the ERCC1 gene following UV irradiation. UV survival was determined in triplicate by colony-forming ability. Appropriate numbers of transfected cells were inoculated on 60-mm dishes. After UV irradiation at a fluence rate of 0.7 J/m^2/s, transfected cells were incubated for 10 d (43-3B and 6L1030), or 3–4 wk (Kps3SVY), fixed with 80% methanol, and then stained with Giemsa. WI38VA13 cells (▽) were used as a normal control. Panel *A* shows UV survival of 43-3B cells transfected with the ERCC1 gene. 43-3B cells (□); ERCC1 transfectants (43-3B-ERCC1.1 [◇]; 43-3B-ERCC1.2 [○]; 43-3B-ERCC1.3 [△]). Panel *B* shows UV survival of 6L1030 cells transfected with the ERCC1 gene. 6L1030 cells (□); ERCC1 transfectants (6L1030-ERCC1.1 [◇]; 6L1030-ERCC1.2 [○]; 6L1030-ERCC1.3 [△]). Panel *C* shows UV survival of Kps3SVY cells transfected with the ERCC1 gene. Kps3SVY cells (□); ERCC1 transfectants (Kps3SVY-ERCC1.1 [◇]; Kps3SVY-ERCC1.2 [○]; Kps3SVY-ERCC1.3 [△]).

not by UV^{s}1KO cells (table 2). These results indicate that the defect in Kps3 cells belongs to the same complementation group as that of UV^{s}1KO cells, but those of GM10903 and GM10905 cells belong to a different group.

Discussion

Elsewhere, we reported two siblings (Kps2 and Kps3) who showed no clinical manifestations except for slight cutaneous photosensitivity and cutaneous pigmentation but had biochemical characteristics of CS (Itoh et al. 1994). Cell-fusion complementation analysis revealed that the repair defect in Kps3 cells was complemented by XP-A, B, D, F, and G cells and CS-A and B cells. Microinjection of Kps3 cell extract corrected UDS in XP-C and XP-E cells. The following three observations suggest that they cannot be classified as XP variant: (1) UV sensitivity of Kps3 cells was not enhanced in the

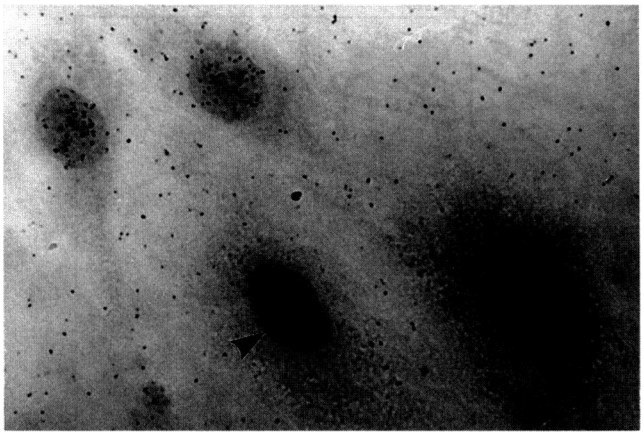

Figure 3 Correction of RRS in Kps3 cells microinjected with HeLa cell extract. HeLa cell extract was microinjected into the cytoplasm of Kps3 cells, which were then incubated for 4 h, irradiated with UV (15 J/m^2), incubated for a further 23 h and then labeled with [^{3}H] uridine for 1 h. The arrowheads show microinjected Kps3cells.

presence of caffeine (Itoh et al. 1994); (2) none of the seven independent XP variant cells showed impaired RRS after UV irradiation (data not shown); (3) in UV-^{s}1KO cells, molecular-weight increase of pulse-chased

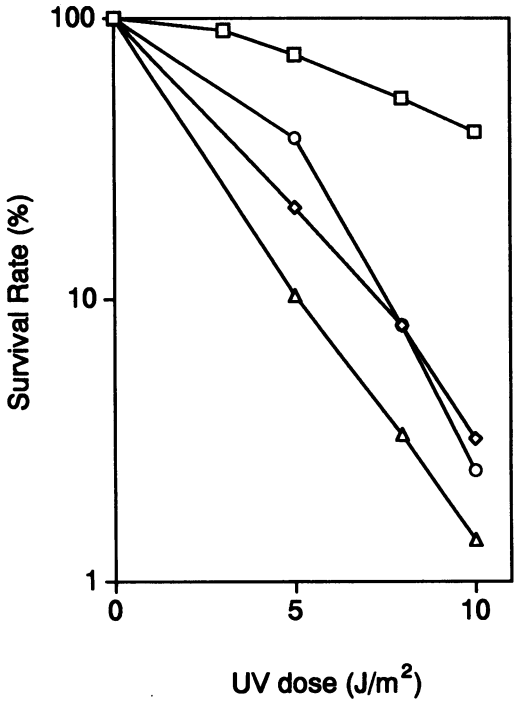

Figure 4 UV survival curves of GM10903, GM10905 and Kps3 cells. Appropriate numbers of cells were inoculated onto 60-mm dishes. After UV-irradiation at a fluence rate of 0.7 J/m^2/s, cells were incubated for 14 d, fixed with 80% methanol, and stained with Giemsa. Each point represents an average of four dishes. Mori cells (□) were used as a normal control. GM10903 cells (○); GM10905 cells (◇); Kps3 cells (△).

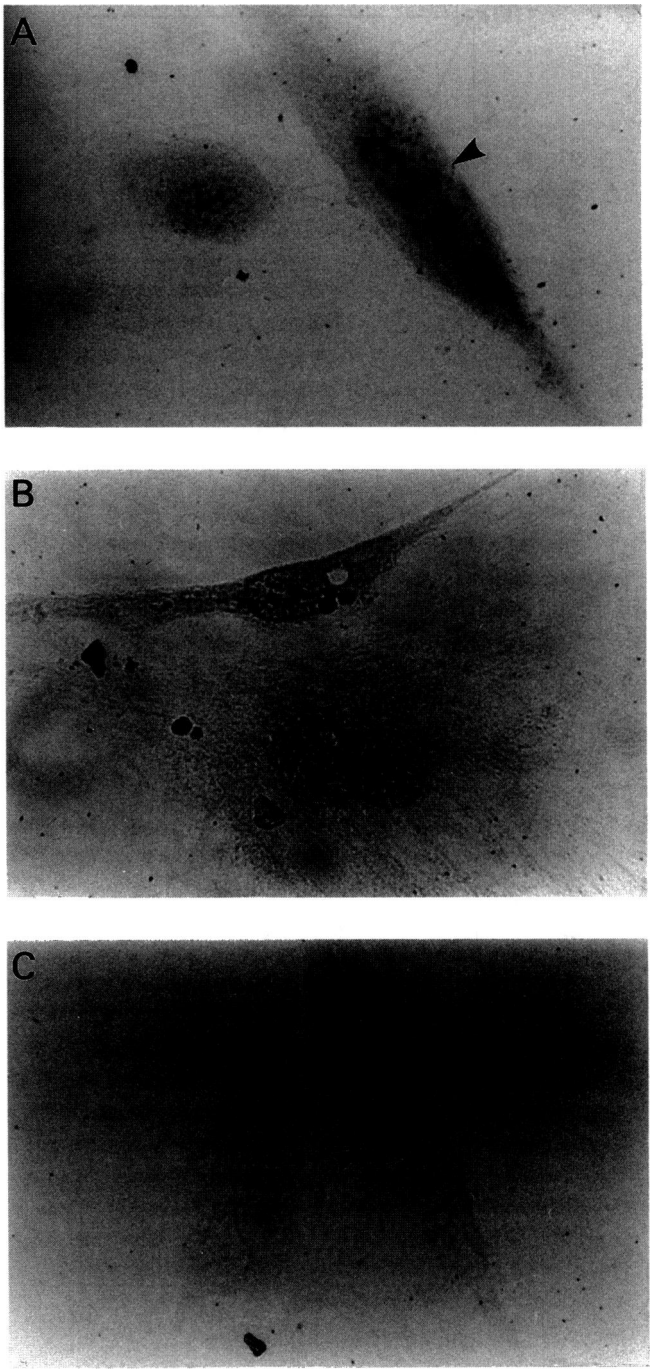

Figure 5 RRS after UV-irradiation in fused cells. The procedure for complementation analysis is described in Material and Methods. Arrowheads show fused cells between GM10905 and Kps3 (*A*), between UV^{s}1KO and Mps1 (*B*), and between UV^{s}1KO and Kps3 (*C*).

DNA was normal after UV irradiation (Fujiwara et al. 1981). Furthermore, we showed that Kps3 cells had a normal pattern of postreplication repair in this study. All of these results indicate that these cells do not belong to any complementation groups of CS or XP, including XP variant.

Trichothiodystrophy (TTD) is another very rare

Table 2

Complementation of RRS by Cell Fusion

Fused Cells	Cell type	RRS (grains/nucleus ± SEM)[a]	Complementation
Kps2/Kps3[b]	Heterokaryons	14.7 ± 1.5	–
	Kps2	16.8 ± 2.2	
	Kps3	10.7 ± 1.1	
GM10903/Kps3	Heterokaryons	51.2 ± 3.0	+
	GM10903	14.6 ± 2.6	
	Kps3	12.1 ± .15	
GM10905/Kps3	Heterokaryons	23.6 ± 3.9	+
	GM10905	4.7 ± 0.7	
	Kps3	8.2 ± 1.1	
GM10903/GM10905	Heterokaryons	9.2 ± 1.3	–
	GM10903	10.5 ± 2.0	
	GM10905	3.6 ± 0.5	
UV^{s}1KO/Kps3	Heterokaryons	31.0 ± 1.8	–
	UV^{s}1KO	19.4 ± 3.1	
	Kps3	35.2 ± 2.3	
UV^{s}1KO/Mps1 (CS-A)	Heterokaryons	50.1 ± 3.2	+
	UV^{s}1KO	16.4 ± 3.4	
	Mps1	23.9 ± 2.7	
UV^{s}1KO/CS1MOSV (CS-B)	Heterokaryons	63.2 ± 5.6	+
	UV^{s}1KO	8.1 ± 1.2	
	CS1MOSV	19.1 ± 2.1	

[a] Data are mean ± SEM of 20 determinants.
[b] Itoh et al. (1994).

genetic disorder, in which patients show sulfur-deficient brittle hair associated with mental and physical retardation, ichthyosis, unusual face, and, in many but not all patients, photosensitivity. Cells from photosensitive patients with TTD are deficient in excision repair of UV-induced DNA damage (Stefanini et al. 1986, 1992; Lehmann et al. 1988; Broughton et al. 1990). Cell fusion analyses have shown that the repair deficiency of this group overlaps XP-D (Stefanini et al. 1986, 1992, 1993*a*; Lehmann et al. 1988). Recently, a new nucleotide-excision-repair gene associated with TTD has been reported (Stefanini et al. 1993*b*). All these TTD patients exhibit the characteristic clinical manifestations described above, and cells from these patients have reduced UDS. These characteristics are quite different from those of UV^s patients, suggesting that UV^s syndrome and TTD are different syndromes.

Fujiwara et al. (1981) and Cleaver et al. (1992, 1993) reported patients with phenotypes similar to our cases but performed no complementation group assignment. In this study, we performed complementation analysis between some of these cells and found that the defect in UV^{s}1KO cells belong to the same complementation group as that of Kps3 cells but that GM10903, and GM10905 cells showed defects belonging to a different complementation group. Thus, these results indicate that the patients Kps2, Kps3, and UV^{s}1KO belong to the same group independent of well-known UV-sensitive diseases such as XP or CS or possibly TTD, despite the lack of consanguinity. Clinical manifestations of these patients are summarized in table 3. They had only mild photosensitivity and no neurological abnormalities. As common clinical manifestations, acute sunburn, photosensitivity, dryness, freckles, pigment anomalies, and teleangiectasia were recognized. From their clinical manifestations and biochemical characteristics, we propose that this new DNA repair–defective complementation group should be included under the general category of UV^s syndrome. We presume that the frequency of this syndrome is as low as that of CS from the incidence among the cases encountered in the two laboratories (M. Yamaizumi and Y. Fujiwara, unpublished data). However, this frequency might be an underestimation since clinicians may often overlook UV^s patients because of their mild clinical manifestations.

At present, 11 nucleotide-excision-repair-deficient

Table 3

Clinical Features of XP, CS, and UV^s Syndrome

Clinical Features	XP	CS	Kps2/Kps3	UV^{s}1KO
Skin:				
Acute sunburn	+	+	+	+
Skin tumors	+	−	−	−
Teleangiectasia	+	+	+	+
Freckling	+	−	+	+
Eye:				
Pigmentary retinopathy	−	+	−	−
Pupillary abnormalities	−	+	−	−
Neurological findings:				
Cerebellar signs	+	+	−	−
Decrease in intelligence quotient	+	+	−	−
Sensorineural hearing loss	+	+	−	−
Calcification of the basal ganglia	−	+	−	−
Polyneuropathy	+	+	−	−
Microcephaly	+	+	−	−
Demyelinating neuropathy	−	+	−	−
Primary neuronal degeneration	+	−	−	−
Deep tendon reflexes				
Hyporeflexia/areflexia	+	−	−	−
Hyper-reflexia	−	+	−	−
Hydrocephalus	+	+	−	−
Others:				
Immature sexual development	+	+	−	−
Cachectic dwarfism	−	+	−	−
Thickening of skull	−	+	−	−
Kyphoscoliosis	−	+	−	−
Pectus carinatum	−	+	−	−
Osteoporosis	−	+	−	−
Hydrocephalus	+	+	−	−

NOTE.—In XP, some complementation groups do not show neurological involvements.

complementation groups have been identified in rodent cells. These include the excision-repair cross-complementing (ERCC) 2, 3, 5, and 6 genes which correct XP group D, B, G, and CS group B cells, respectively (Weeda et al. 1990; Flejter et al. 1992; Troelstra et al. 1992; O'Donovan and Wood 1993; Scherly et al. 1993). Recently, it was suggested that ERCC4 is equivalent to XP-F (Biggerstaff et al. 1993). Rodent complementation group 1 (ERCC1) shows severe UV sensitivity (fig. 1*A*; Westerveld et al. 1984), and mice homozygous for defective ERCC1 alleles are runted at birth and die from liver failure before weaning (McWhir et al. 1993). Thus, the phenotype of this group is quite different from those of UV^s syndrome, and it is reasonable that patients with UV^s syndrome do not have mutations in ERCC1. Therefore, the gene complementing UV^s syndrome is not included in the ERCC 1, 2, 3, 4, 5, or 6 genes, but it is still possible that the defect in UV^s patients corresponds to one of the remaining ERCC groups.

Recently, it has been reported that XPB/ERCC3 (Schaeffer et al. 1993) and XPD/ERCC2 (Drapkin et al. 1994; Schaeffer et al. 1994) are components of transcription factor TFIIH, with which XPC protein is associated (Drapkin et al. 1994). CSB protein is a DNA helicase assumed to be involved in repair of actively transcribed genes (Troelstra et al. 1992). The defect of UV^s syndrome in RRS after UV irradiation was corrected by microinjection of HeLa cell extract. The putative factor defective in UV^s syndrome might function like these transcription-coupled repair factors suggested by the defect in RRS after UV irradiation, despite the normal level of UDS. Although UV^s patients exhibit almost the same level of UV sensitivity as those with CS, clinical manifestations of UV^s patients are very mild. At least two explanations for this observation are possible: (1) the factor is expressed either after some stage of development or in restricted organs such as the skin; or (2) the defect in RRS after UV irradiation is slowly corrected. Further experiments will be necessary to clarify this. To date, all UV^s patients identified have been young and have not developed skin cancer. It is of interest whether patients with UV^s syndrome will develop cancer(s) at later stages, as has been observed in those with XP variant.

Acknowledgments

We thank Dr. Akira Yasui and Dr. Tadahiro Shiomi for providing the 43-3B and 6L1030 cells, respectively. We are grateful to Dr. Mituo Ikenaga for technical advice and to Mrs. Yuka Itoh for preparation of the manuscript. This work was supported by grants from the Ministry of Education, Science and Culture of Japan and the Okukubo Memorial Fund for Medical Research at the Kumamoto University School of Medicine.

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