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Cockayne Syndrome with Delayed Recovery of RNA Synthesis after Ultraviolet Irradiation but Normal Ultraviolet Survival

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ABSTRACT. We report a girl with Cockayne syndrome (CS) with atypical cellular features. We studied the ultraviolet (UV)-sensitivity of cultured fibroblast cells derived from this case and male CS siblings as positive controls. Cells from this female with CS displayed normal unscheduled DNA synthesis and repair replication capacity. However, the cells also displayed a less depressed level of RNA synthesis after UV irradiation, compared to control CS cells, and showed normal UV survival. This CS case with early onset of abnormalities had more serious clinical manifestations than the control CS siblings. These cytological results suggest that there is considerable clinical and cellular heterogeneity in CS and that cellular sensitivity to UV might not be as essential for the diagnosis of CS as previously thought. (*Pediatr Res* 21: 34–37, 1986)

Abbreviations

UV, ultraviolet
UDS, unscheduled DNA synthesis
CS, Cockayne syndrome
XP, xeroderma pigmentosum
T3, triiodothyronine
T4, thyroxine
TSH, thyroid stimulating hormone
CT, computed tomography
CF, colony formation
TCA, trichloroacetic acid
PBS, phosphate-buffered saline

CS, a rare autosomal recessive disorder, was first described in 1936 (1), and a diagnosis is made clinically on the basis of cachectic dwarfism, deafness, cataracts, retinal pigmentation, photosensitivity, a thickened skull, intracranial calcification, mental deficiency, and characteristic facial features (2, 3). In addition to these findings, cells derived from CS patients have been shown to be highly sensitive to UV-killing, and the detection of high UV-sensitivity has been considered to be essential for the diagnosis of this syndrome (4). Andrews *et al.* (5) have suggested a correlation between the clinical severity of the degenerative neuron system and the cellular DNA repair after UV irradiation in XP, which is another UV-sensitive disease. However, their exact relationship remains controversial in XP and CS. It is therefore important for comparative studies to be performed whenever both clinical and cytological data are available, especially for cases with atypical manifestations or unusual cellular sensitivity to UV. We recently had the opportunity to observe a girl with CS whose fibroblast cells showed normal sensitivity to UV light. We further examined cells from this female with CS and two CS siblings for comparison, as to sensitivity to x-rays, UV-induced UDS, repair replication, and total cellular DNA or RNA synthesis after UV irradiation. The purpose of this study is to clarify whether or not these biochemical defects parallel the severity of the clinical manifestations, particularly that of the neurological deterioration.

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CASE REPORTS

Case 1. K.K. was born without complications after a full-term, noncomplicated gestation with a birth weight of 2.6 kg. There was no family history of consanguinity, congenital disease, mental retardation, or movement disorders. She was a poor feeder from birth, and growth failure persisted. She could support her head at 6 months when delayed acquirement of motor milestones became evident. She sat at 12 months of age, crawled at 24 months, and stood with support at 4 yr of age. She could walk with support until about 6 yr but her gait was broad-based and ataxic. Because of an increasing gait disorder and joint contractures, she remained in bed almost exclusively from 9 yr of age.

On physical examination at 14 yr old, she was small with sunken eyes, a beak-like nose, and a projected maxilla (Fig. 1). Her height was 115 cm, weight 17 kg, and head circumference 44.5 cm. Her mental status was severely retarded and she could not say any meaningful words. She was not unduly sensitive to sunlight. She had small breasts and little pubic hair, and the menarche had not occurred at that time. There were coarse resting tremors in both hands, which increased with intention. Ophthalmological examination revealed retinitis pigmentosa, optic atrophy, and cataracts.

Normal laboratory test results included those for complete blood counts, serum electrolytes, growth hormone, T3, T4, TSH, plasma cortisol, serum copper and ceruloplasmin, urine copper, heavy metals, amino acids, urinary arylsulfatase A, and chromosome studies. Skull radiograms showed thickening of the calvarium. Cranial CT revealed marked enlargement of the entire ventricular system with cortical atrophy but no intracranial calcification. At 16 yr of age she died of aspiration pneumonia at home but an autopsy could not be performed.

Cases 2 and 3. K.Ki and T.Ki. were male siblings with typical CS cases and were not related to case 1. They were described previously (6). In brief, they were considered normal for the 1st yr of life, but delayed acquirement of motor milestones became

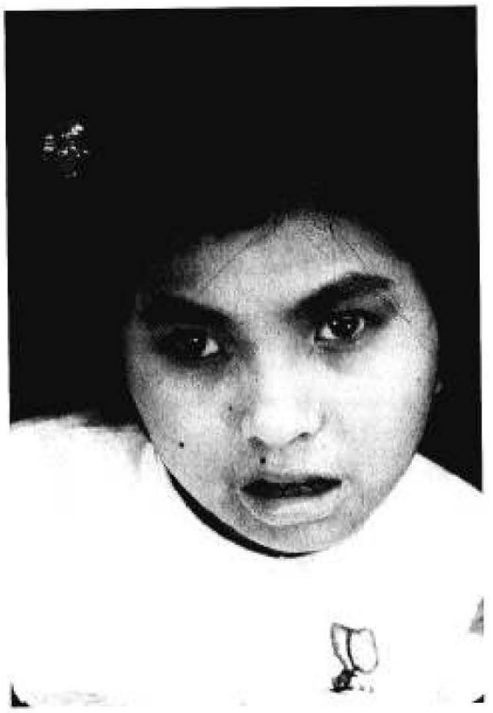

Fig. 1. Facial appearance of case 1.

evident from then onward. Both showed the characteristic features of microcephaly, a short stature, mental retardation, retinitis pigmentosa, photosensitive skin, and deafness. CT scanning revealed calcification in the bilateral basal ganglia. Their faces were similar in appearance to that of case 1. Using standard G- and R-banding techniques, peripheral blood lymphocytes revealed normal karyotypes in these siblings as in case 1. Cell strains derived from these cases were included in this cytological study for comparison with case 1.

METHODS

Cell culture. Normal cell strains (AK and YO) (7) and the three CS cell lines (CCS1, 2, and 3) from cases 1, 2, and 3, respectively, were included in this study along with two excision-defective XP cell lines (XP24TO) (7) and (XP1KY) (8) and one ataxia telangiectasia cell line (GM2052) (9). XP24TO and XP1KY fibroblasts were provided by Y. Suzuki, the Department of Biochemistry, Tokyo Medical and Dental University, Tokyo, Japan and H. Takebe, Radiation Biology Center, Kyoto University, Kyoto, Japan, respectively. GM2052 cells were obtained from the Institute of Medical Research, Camden, NJ. All cell lines were grown in Dulbecco's modified Eagle's medium (Grand Island Biological Co., Grand Island, NY) supplemented with 10% fetal calf serum (Flow Laboratories, Rockville, MD), 100 μg streptomycin/ml, and 100 U penicillin G/ml at 37° C in a humidified atmosphere containing 5% CO_2. At the time of the experiment, the fibroblasts were between the 3rd and 12th passage levels *in vitro*.

Cellular sensitivities to UV and x-rays. UV and x-ray sensitivity tests involving CF assays were performed essentially as previously described (9, 10). Cells, 1.5×10^3, of each fibroblast cell strain were seeded in a 100-mm culture dish (Nunc, Roskilde, Denmark) followed by UV or x-ray irradiation. The survival percentage ratios (counts of colonies in a test dish/counts of colonies in a control dish) were determined.

Total cellular DNA and RNA syntheses. Total cellular DNA synthesis was measured by a pulse-labeling method, as previously described (10). Briefly, logarithmically growing cells were cultured with 0.02 μCi/ml [2-^{14}C]dThd (50 mCi/mmol, New England Nuclear, Boston, MA) for 24 h. At 20 h after seeding of the prelabeled cells, the cells were irradiated with UV and then pulse-labeled with 5 μCi/ml [methyl-^{3}H]thymidine([^{3}H]dThd) (62 Ci/mmol, New England Nuclear) for 10 min, followed by counting of the radioactivity in the TCA-insoluble cell materials. Total cellular RNA synthesis was also measured, using 5 μCi/ml [^{3}H] uridine (30 Ci/mmol, New England Nuclear) instead of the [^{3}H] dThd used for DNA synthesis.

Unscheduled DNA synthesis. The technique of Robbins *et al.* (11) was mainly used to measure UDS. Cells were plated in single chamber Lab-Tek slides (Miles Scientific, Inc.) at a density of 5×10^4 cells per slide. After 24 h, [^{3}H]dThd (20Ci/mmol, New England Nuclear) was added to the growth medium (5 μCi/ml), and the incubation was continued for another 30 min. The cultures were washed with PBS (8.0 g NaCl, 0.2 g KCl, 1.15 g Na_2HPO_4 and KH_2PO_4 in 1 liter distilled H_2O) and then irradiated with UV. After washing twice with PBS, serum-free growth medium containing [^{3}H]dThd(5 μCi/ml) was added for 120 min. The slides were then coded, washed, fixed, and autographed.

Studies on repair replication. The UV-induced DNA repair replication was measured as previously described (10). Briefly, 1×10^6 cells, mock-irradiated and irradiated at 15 and 30 J/m^2, were incubated for 3.5 h with 10 μCi/ml [^{3}H]dThd (20Ci/mmol, New England Nuclear) as the repair label. DNA was extracted from the cells, and then the parental density DNA fractions were isolated from neutral CsCl gradients, followed by centrifugation in alkaline (pH 12.5) CsCl gradients. The ratio of ^{3}H radioactivity to DNA was calculated by collecting the fractions of the parental DNA peaks and counting.

RESULTS

Sensitivity to UV and x-rays. The ability of CCS1 fibroblasts to form colonies after UV irradiation was very similar to that of normal controls, but distinct from those of the more UV-sensitive CCS2, CCS3, and XP24TO fibroblasts (Fig. 2). As to the colony-

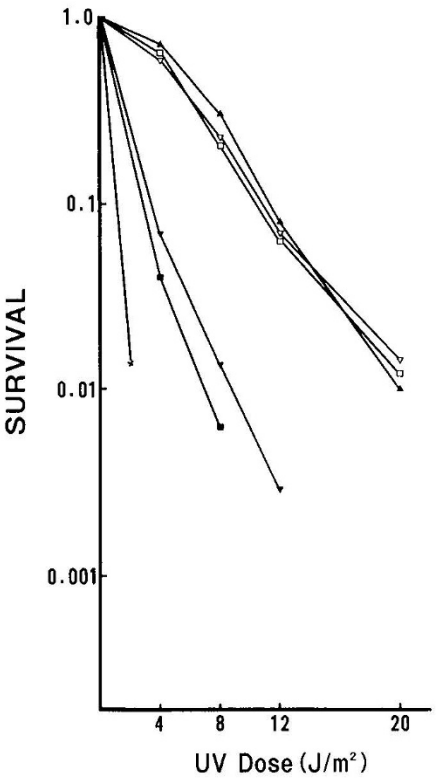

Fig. 2. UV survival curves for cells irradiated in the exponential growth phase. Fibroblasts from normal donors, AK (□), YO (▽); index cases, CCS1 (▲), CCS2 (■), CCS3 (▼); and XP24TO (x).

forming ability after x-ray irradiation, the CCS1 strain, like the other two CS strains, showed no hypersensitivity in comparison to that of GM2052 cells (Fig. 3).

Total cellular DNA and RNA syntheses. Eight J/m^2 produced the initial depression in DNA and RNA syntheses in all cells studied (Figs. 4 and 5). The levels of [^{3}H]dThd incorporation into CCS1 and normal cells were elevated 2 h after UV irradiation and were significantly different from that of CCS2 and CCS3 cells 2, 6, and 10 h after UV irradiation. [^{3}H]uridine incorporation into CCS1 cells, as in the cases of CCS2 and CCS3 cells, was more decreased than that into control cells during the first

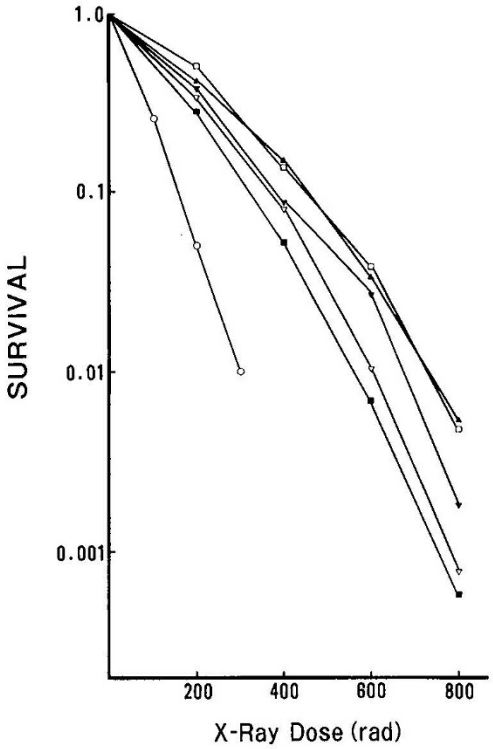

Fig. 3. X-ray survival curves for cells irradiated in the exponential growth phase. AK (□), YO (▽), CCS1 (▲), CCS2 (■), CCS3 (▼), and GM2052 (○).

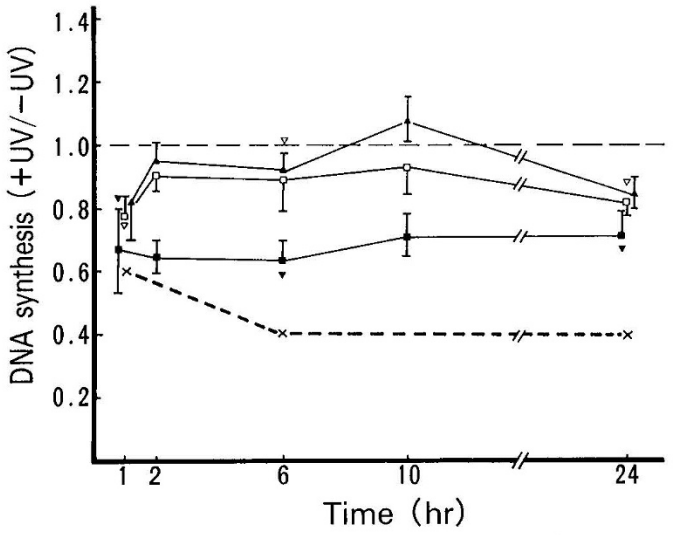

Fig. 4. Total cellular DNA synthesis activity after 8 J/m^2 UV irradiation. Cells (5 × 10^4) prelabeled with [^{14}C]dThd were seeded in a 35-mm dish, irradiated, and then pulse-labeled with [^{3}H]dThd. AK (□), YO (▽), CCS1 (▲), CCS2 (■), CCS3 (▼), and XP24TO (x), *Bars*, SE.

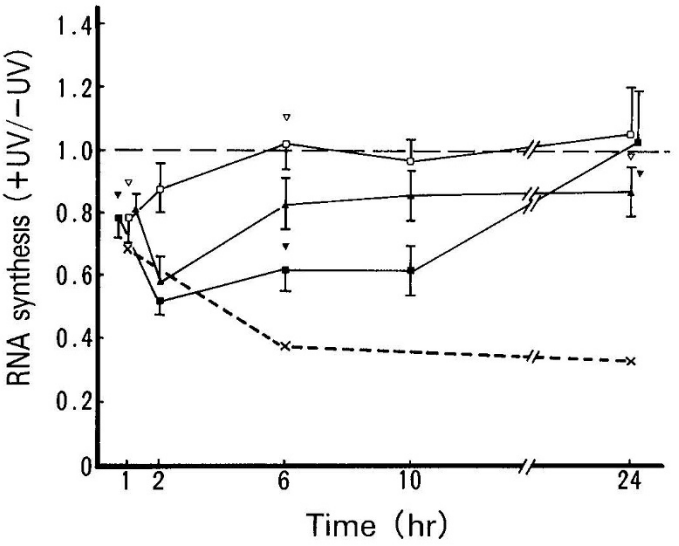

Fig. 5. Total cellular RNA synthesis activity after 8 J/m^2 UV irradiation. Cells (5 × 10^4) prelabeled with [^{14}C]dThd were seeded in a 35-mm dish, irradiated, and then pulse-labeled with [^{3}H]uridine. AK (□), YO (▽), CCS1 (▲), CCS2 (■), CCS3 (▼), and XP24TO (x), *Bars*, SE.

2 h after UV irradiation (8 J/m^2). However, recovery of RNA synthesis in CCS1 cells after UV irradiation was intermediate between that of normal controls and that of CCS 2 and CCS 3 cells. In these three CS strains, recovery of DNA and RNA syntheses was seen 24 h after irradiation when both nucleic acid syntheses in the XP24TO strain had still not recovered.

UDS. The UDS level after UV irradiation in CCS1 cells was similar to those in the control, CCS2 and CCS3 cells, in contrast with the low level in XP24TO cells (Fig. 6).

Repair replication. Quantitative measurement of the amounts of repair replication indicated that repair-proficient cells of AK, CCS1, CCS2, and CCS3 revealed high levels of repair replication synthesis, particularly at high dose of UV (30 J/m^2) in comparison to cells of two XP strains.

DISCUSSION

The clinical symptoms of the three described cases agreed with major criteria of Sugarman *et al.* (2) except for the lack of intracranial calcification in case 1. We further observed a typical facial features, ankylosis, optic atrophy, hypogonadism, and deterioration of the clinical course in case 1. Growth and development are normal during the 1st yr in typical CS, as in cases 2 and 3, but in case 1 these disorders became manifest soon after birth and neurological deterioration progressed more rapidly. However, CS cases with early onset of manifestations have also been reported (12). The diagnosis of CS in case 1 is based primarily on the clinical findings as well as in cases 2 and 3 since no consistent biochemical abnormality has been discovered in CS.

Ordinarily, fibroblasts derived from CS cases are extremely sensitive to UV but not to x-rays (4, 13), as judged by their colony-forming ability. However, there have been some reports of the cross-sensitivity of CS fibroblast strains to UV and x-rays (14, 15). A certain CS strain (16) was shown to have the UV sensitivity that was intermediate between those in the published survival curves for normal and CS cells. These results suggest the broad spectrum of the sensitivity of CS cell strains to UV, but there has been no report of cell strains which had the same level of sensitivity to UV killing as normal cells. On the other hand, normal sensitivity to UV has been noticed in some fibroblasts derived from some cases of xeroderma pigmentosum (17), another UV-sensitive disease. Therefore, it may not be surprising if a CS cell line with normal UV survival is found.

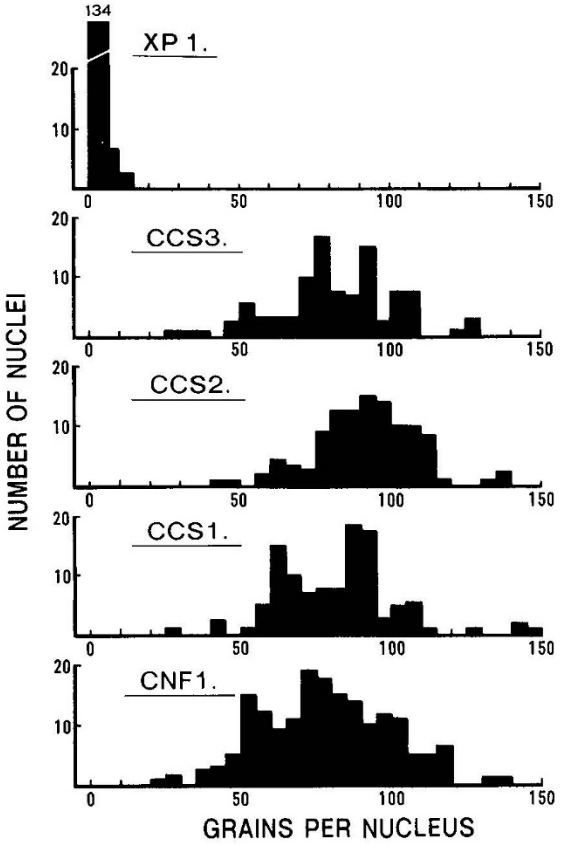

Fig. 6. Histograms of autographic data on [^{3}H]dThd incorporation into nuclei of fibroblasts after 20 J/m^2 UV irradiation.

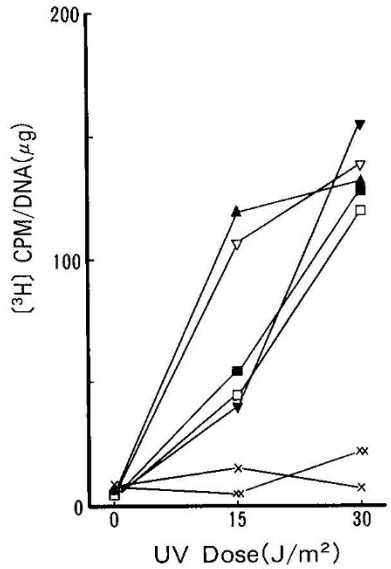

Fig. 7. Repair replication analysis on alkaline CsCl gradients of the parental density DNA from AK (□), YO (▽), CCS1 (▲), CCS2 (■), CCS3 (▼) and XP24TO (x) and XP1KY (xx).

In general, the cellular level of nucleic acid synthesis after UV irradiation is a more sensitive indicator of UV-induced damage; for example, the above mentioned XP variant cells showed a slow recovery of DNA synthesis after UV irradiation (18). Mayne *et al.* (19) reported that the DNA or RNA synthesis capability of CS cells was also depressed after UV irradiation but that the level of depression was variable. They further suggested the possibility that there was some correlation between the level of nucleic acid synthesis and cell survival after UV irradiation in CS (19). In fact, we found that the level of total cellular DNA or RNA after UV irradiation was less depressed in CCS1 cells.

The molecular repair defect in CS has not been clarified although some complementation groups have been demonstrated (20, 21) (Fig. 7). The *in vitro* heterogeneity among CS cell strains might be due to a genetic difference at the loci responsible for this disease (21), and might reflect the broad spectrum of UV sensitivity shown by the ability of CF or nucleic acid synthesis after UV irradiation.

On the other hand, CS cases show a wide range of clinical severity and some CS cases with clinically atypical features have been reported (22, 23). Schmickel *et al.* (4) suggested that the cellular defects in CS might be ones in the repair of UV-induced damage. But we found that the level of UV sensitivity or the DNA repair defect did not parallel the severity of the clinical manifestations in case 1. Therefore, it seems valid to state that there is considerable clinical and cellular heterogeneity in CS as there is in XP.

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