Sister Chromatid Exchange-Related Characteristics of Excision Repair-Proficient Xeroderma Pigmentosum Cells

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A high incidence of skin cancer characterizes patients with xeroderma pigmentosum (XP). XP patients have hereditary defects in repair mechanisms of ultraviolet light (UV)-induced damage to DNA. Progress in elucidating the pathogenesis of cutaneous cancers can be expected by analysis of the biologic defects of cultured cells from XP patients. Such information may also contribute, at least in part, to an understanding of carcinogenesis in general. *J Invest Dermatol 92: 289S–292S, 1989*

We previously reported the clinical and cell biologic characteristics of XP in Northeast District of Japan [1]. Table I summarizes the results of that study. In 26 cases examined, seven cases (27%) had UDS (unscheduled DNA synthesis) levels of 80% or more of normal. We refer to these cases as "UDS-proficient XP" in this report. A relatively high prevalence of UDS-proficient XP is a characteristic of the Japanese population, as Takebe pointed out in his recent review [2]. UDS-proficient XP patients are mild to moderate in clinical expressions, and the time of occurrence of skin cancer in their life is generally later than that in UDS-deficient XP patients (mean 32.6 years; range 19–54 years vs 13.8 years in our study). Despite a later time of appearance, the incidence of skin cancers (basal cell carcinoma, squamous cell carcinoma, or both) in UDS-proficient XP patients (five in seven cases) was not less than that in UDS-deficient XP patients (nine in 19) in this study. This is again consistent with results in the whole of Japan [2].

A high incidence of skin cancer in XP patients has been related to their deficiency in excision repair of damaged DNA. For XP patients classified in one of the nine complementation groups, each has more or less deficiency for excision repair. What then is the defect in UDS-proficient or excision repair-proficient XP?

UDS-proficient XP cells are characterized, first, by a normal activity of excision repair, as reflected in a normal level of UDS. Second, they are slightly more sensitive to killing by UV and this sensitivity is highly augmented by caffeine, as shown in Table II. Third, UDS after UV irradiation decreases less rapidly than in normal cells during the post-irradiation incubation (Fig 1) (about a 50% increase in cumulative thymidine incorporation after the same dose of UV exposure). This finding is consistent with the delayed ligation reported in UDS-proficient XP cells [3]. Incompleteness of ligation is again exaggerated by caffeine. As a fourth difference, we will present here the characteristics of excision-proficient XP cells with respect to UV-induced sister chromatid exchange (SCE) and the effect of caffeine on SCE induction.

SCE is a reciprocal recombination between two identical double helical DNA chains contained, respectively, in a pair of sister chromatids. The recombination occurs at an apparently homologous site in DNA resulting in the exchange in sister chromatids. By differential staining of the sister chromatids, metaphase chromosomes assume a "harlequin appearance," and the frequency of SCE is quantitatable. Results are usually expressed as a mean frequency after scoring for more than 30 metaphase cells. When cells are treated with mutagens, including UV, the frequency of SCE increases in a dose-dependent fashion. In this paper, the induced SCE is expressed as the increase in the frequency of SCE/cell above that in untreated, control cells.

The conventional method of differential staining of sister chromatids is as follows: Cells, usually in culture, are incubated for two rounds of cell cycles in the presence of an appropriate concentration of 5-bromodeoxyuridine (BrdUrd; 10 μM in this study). Of the resulting two double stranded DNA chains dispensed into a pair of sister chromatids, at the second metaphase, one contains two polynucleotide chains in which dTMP is substituted with 5-Br-dUMP; another double helix in another sister chromatid contains only one strand substituted. A sister chromatid containing the singly substituted DNA is stained darker by "fluorescence plus Giemsa" staining [4].

As shown in Table III, the sensitivity of UDS-proficient XP cells to SCE induction by UV is normal or slightly more than normal, and caffeine augments UV-induced SCE considerably. In four UDS-proficient XP lymphoblastoid cell lines examined, three (XPL5, XPL19 and XPL20) responded in this way, while the fourth (XPL18) did not clearly respond to caffeine for unknown reason(s) [5]. These findings significantly contrast those in normal cell lines and those in UDS-deficient cell lines, which are highly sensitive to UV in SCE induction but respond to caffeine in the normal range.

The SCE-augmenting effect of caffeine, typically seen in UDS-proficient XP cells, was further analyzed with XPL19 cells. When UV-irradiated cells were kept growth-arrested in a spent medium that was able to support cell viability but not proliferation, UV-induced and caffeine-augmented SCE level were stable for at least 2 d.

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Abbreviations:
MMC: mitomycin C
SCE: sister chromatid exchange
UDS: unscheduled DNA synthesis
UV: ultraviolet light
XP: xeroderma pigmentosum

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Table I. Cancer Incidence and UDS Level of Cell Lines from XP patients

UDS-Deficient XP			UDS-Proficient XP		
Patients	UDS (% of Normal)	Skin Cancer	Patient	UDS (% of Normal)	Skin Cancer
XP-1 to XP-26 except those in right column (19 cases)	65% 2% in 9 cases	9 in 19 cases (13.6 ± 5.4y)[a,b]	XP-5	80%	+ (20y)[a]
			XP-10	100%	+ (≦54y)
			XP-18	83%	−
			XP-19	97%	−
			XP-20	89%	+ (19y)
			XP-22	85%	+ (32y)
			XP-25	83%	+ (38y)
					5 in 7 cases

[a] Age at diagnosis of cancer.
[b] Mean ± SD of 9 cases.

Table II. UV Sensitivities of Human Lymphoblastoid Cell Lines and D_{37} After Post-irradiation Incubation in the Absence and Presence of Caffeine (1 mM)

		D_{37} (J/m²)[b]		
Cell Lines[a]	UDS (% of Normal)	− Caffeine (A)	+ Caffeine (B)	A/B
NL3	. . .	16.9	13.3	1.3
NL9	. . .	16.8		
XPL18	83	18.2		
XPL19	97	13[c]	3.9	3.3
XPL20	89	15[c]	4.3	3.5
XPL15[d]	2	1.67	1.68	1.0
XPL17	2	2.3		

[a] NL and XPL denote the normal and XP lymphoblastoid cell lines, respectively.
[b] 37% (1/e) survival dose or mean lethal dose.
[c] Estimated by extrapolation.
[d] Complementation group A.

Caffeine was effective in proliferating cells but not in growth-arrested cells (Fig 2).

After confirming that the presence of caffeine in only one of two cell cycles was enough to observe SCE augmentation (although the increment was reduced to about a half), UV-irradiated cells were treated with caffeine for different periods in the second cell cycle. As shown in Fig 3, the presence of caffeine in the S phase was essential for the effect, but its presence in neither G_1- nor G_2-phase was required. From these results, we may speculate that the augmentation by caffeine of UV-induced SCE is due to lesions remaining after removal of UV-induced pyrimidine dimers, for which UDS-proficient XP cells are proficient.

The possibility that caffeine augmented UV-induced SCE by acting synergistically with BrdUrd was ruled out by an experiment in which caffeine was present in the first or second cell cycle and BrdUrd was present in two cell cycles or only in the first one. This

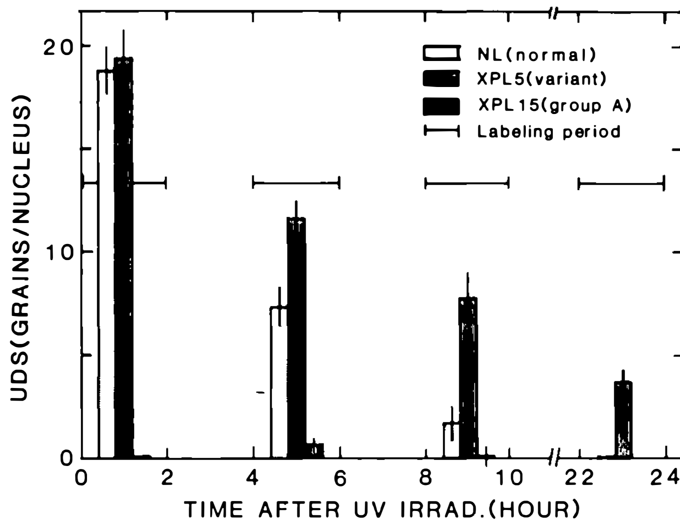

Figure 1. UDS of UV-irradiated human lymphoblastoid cells. NL1, XPL5, and XPL15 are cell lines from a normal donor, a UDS-proficient (variant), and a UDS-deficient (group A) XP patient, respectively. Immediately after exposure to 12.6 J/m² UV light of 254 nm, cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 2 mM L-glutamine. Cells were labeled with ^{3}H-dThd (10 μCi/ml) for the period indicated *(horizontal bars)*. Autoradiographic grain count was scored for about 150 interphase cells for each value (mean ± SE).

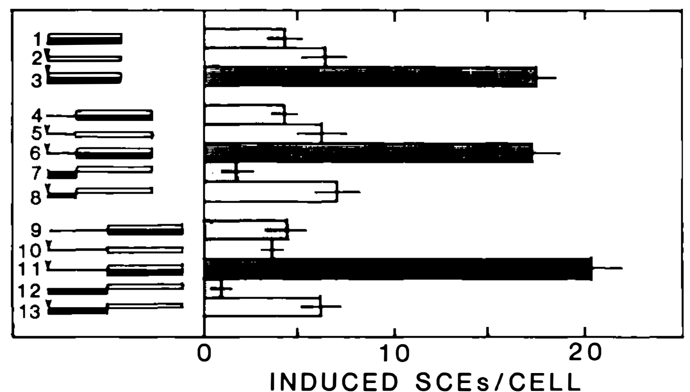

Figure 2. Persistence of caffeine effect in UV-irradiated and then growth-arrested XPL19 cells. *1*: cells were incubated in the growth medium (cf. Fig 1) for two cell cycles in the presence of 10 μM BrdUrd *(open box)* and 1.0 mM caffeine *(filled box)*; *2,3*: cells were irradiated with 2 J/m² UV *(arrowhead)* and incubated with BrdUrd and caffeine as indicated; *4–8, 9–13:* unirradiated or irradiated cells were incubated for 24 and 48 h, respectively, in spent medium (growth arresting condition), then cultured for two cell cycles in the growth medium. BrdUrd and caffeine were present as indicated.

Table III. Augmentation of UV-Induced SCE by Caffeine[a]

Cell Line	UV (J/m²)	Induced SCE (SCE's/Cell) − Caffeine (A)	+ Caffeine (B)	B/A
Normal				
NL1	2	1.9	3.2	1.7
	5	4.2	8.6	2.0
NL3	2	2.5	6.3	2.5
	5	3.9	6.8	1.7
UDS-proficient XP				
XPL19	2	2.3	13.0	5.7
XPL20	2	4.2	19.2	4.6
UDS-deficient XP				
XPL15	1	16.8	31.9	1.9

[a] 1 mM caffeine was present with BrdUrd for two cell cycles immediately after UV irradiation.
Caffeine at this concentration induced 1.5 ± 1.1 SCE's/cell without UV in all cell lines, which is not considered in calculation of B/A ratios.

Table IV. Persistence of Induced SCE Level after Chemical Treatment of Normal Cells[a]

Post-Treatment Incubation (48 h)	SCE/Cell (Mean ± SE, and Range) 1 μM MMC[b]		2 μM 4NQO[c]	
−	−	7.4 ± 0.6 (3–16)	−	7.2 ± 0.4 (2–12)
−	+	56.5 ± 2.8 (28–89)	+	29.3 ± 1.1 (15–40)
In SPENT medium	+	56.8 ± 2.4 (31–89)	+	30.8 ± 1.8 (13–46)
In FRESH medium	+	10.0 ± 2.0 (2–74)	+	14.8 ± 2.1 (4–46)

[a] NL3 cells were treated with chemicals for 2 h and then incubated in the indicated media before medium change to the fresh medium containing BrdUrd.
[b] Mitomycin C.
[c] 4-nitroquinoline 1-oxide.

protocol also allows differential staining. All of these combinations showed that the presence and absence of BrdUrd in culture medium or in DNA incorporated as a substituent in the first cell cycle did not influence the results.

A fact possibly related to the persistence of UV-induced, SCE-forming lesions has been found in normal cells [6]. Cells of NL3, a lymphoblastoid cell line from a normal donor, were treated with mitomycin C (MMC) and then incubated in the spent medium for 2 days before addition of BrdUrd in fresh medium for differential staining. During a 2-d incubation, lesions to be expressed as SCE did not diminish under the growth-arrested conditions, while they did diminish to nearly normal levels under growing conditions (Table IV). The same was true in 4NQO-treated cells. A change in distribution of SCE frequencies in MMC-treated cells (Fig 4*A*) suggests that the reduction of MMC-induced SCE to a background level in growth medium is not due to the death of severely damaged cells and the prevalence of undamaged cells during the period of 48 h, but rather to repair, because no cell with a background SCE frequency was found immediately after MMC treatment. Unaffected cells, if a few existed, could not prevail in the cell population at 48 h

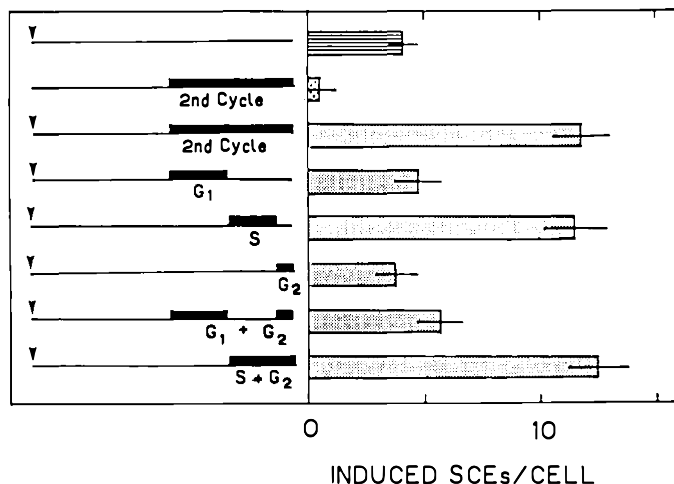

Figure 3. Sensitive phase in cell cycle to post-UV caffeine treatment. XPL19 cells were irradiated wih 2 J/m² UV *(arrowhead)* and cultured in growth medium (cf. Fig 1) contaning BrdUrd for two cell cycles (60 h). In the second cell cycle 1.5 mM caffeine was added as indicated by *filled boxes.* Phases in cell cycle indicated were confirmed by addition of ³H-thymidine in the same time schedules with caffeine and counting of labeled mitotic cells on metaphase preparations for SCE scoring.

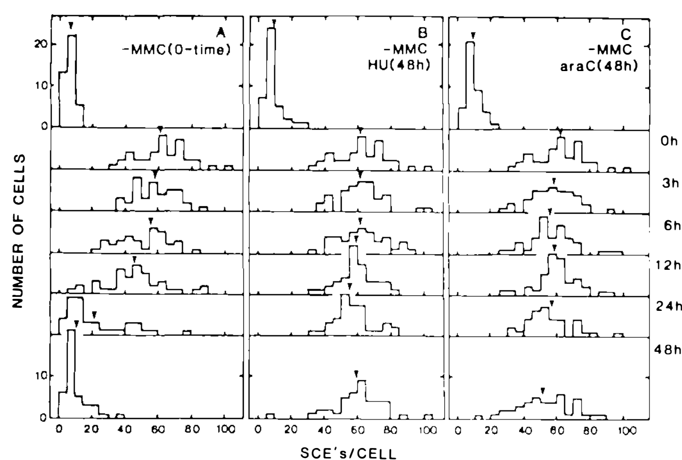

Figure 4. Distribution of SCE frequencies in NL3 cells. Cells were treated with 1 μM mitomycin C (MMC) for 2 h. Immediately thereafter (0-time) and after incubation in the growth medium *(A)*, or in that containing 1 mM hydroxyurea *(B)* or 0.1 mM arabinosyl cytosine (araC) for periods indicated on the *right-hand side,* cells were cultured in the presence of 10 μM BrdUrd for two cell cycles for differential staining of sister chromatids. *Top panels* are those without MMC treatment. *Arrowheads* are the mean frequency of SCE's.

(about 2 cell cycles). Inhibitors of DNA replication were similarly effective in maintaining a high level of SCE frequency (Fig 4*B*,*C*).

Lesions induced in DNA by UV or certain chemicals may be modified by cellular activity such as excision repair, but lesions causing SCE must be persistent for at least 2 d, especially under growth-arrested conditions. For UDS-proficient XP cells, this persistence was still observed under growing conditions, although to a lesser degree. The lesion as an immediate cause of SCE is removed during cell cycle, presumably in the S phase in normal cells as well as UDS-proficient XP cells, but in the latter the process may be slow and further delayed in the presence of caffeine in the S phase.

A known defect in DNA metabolism of UDS-proficient XP cells is in the so-called "post-replication repair" or, more correctly, "the replication of damaged DNA with gap formation" or "discontinuous DNA synthesis with gap formation" [7] followed by a gap filling step. If the second step is sensitive to caffeine in UDS-proficient XP cells, as seen in inhibition by caffeine of ligation of newly synthesized DNA strands after UV-irradiation [3,7], the gaps or nicks remaining after removal of lesions may also last long in the presence of caffeine, which results in augmented SCE. In the case of UDS-deficient XP cells, post-UV DNA replication would leave many gaps resulting in many SCEs, but caffeine would not effectively inhibit the sealing of gaps as shown in Table III. This may be consistent with the fact that the molecular size of newly synthesized DNA in classical XP cells in the presence of caffeine after UV-irradiation is intermediate between those in normal and UDS-proficient XP cells [7].

Thus it is likely that pyrimidine dimers or chemical adducts on DNA are removed by excision repair in normal and excision-proficient XP cells, but gaps are not fully filled up under growth-arrested conditions. The remaining gaps or nicks are the direct cause of SCE.

This simple explanation may contradict a generally accepted fact that in mutagen-treated normal cells the size distribution of the alkali-denatured DNA shifts to low molecular weight as a result of excision repair, but then recovers gradually to the normal distribution. It should be noted, however, that the frequency of SCE as the double strand reciprocal recombination of DNA is on the order of 1 per 10^8 bp compared with DNA size of the order of 10^4 nucleotides in size analysis in alkaline solution.

An alternative but related possibility is a difference in modulating chromatin structure between normal and excision-proficient XP cells. This may be an important avenue to explore, but before that we need many technical improvements and fundamental knowledge of the structure and dynamics of chromatin.

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