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Research Article

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Carrier Detection in Xeroderma Pigmentosum

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Abstract

We were able to detect clinically normal carriers of xeroderma pigmentosum (XP) genes with coded samples of either peripheral blood lymphocytes or skin fibroblasts, using a cytogenetic assay shown previously to detect individuals with cancer-prone genetic disorders. Metaphase cells of phytohemagglutinin-stimulated T-lymphocytes from eight individuals who are obligate heterozygotes for XP were compared with those from nine normal controls at 1.3, 2.3, and 3.3 h after x-irradiation (58 R) during the G_2 phase of the cell cycle. Lymphocytes from the XP heterozygotes had twofold higher frequencies of chromatid breaks or chromatid gaps than normal ($P < 10^{-5}$) when fixed at 2.3 or 3.3 h after irradiation. Lymphocytes from six XP homozygotes had frequencies of breaks and gaps threefold higher than normal. Skin fibroblasts from an additional obligate XP heterozygote, when fixed ~ 2 h after x-irradiation (68 R), had a twofold higher frequency of chromatid breaks and a fourfold higher frequency of gaps than fibroblasts from a normal control. This frequency of aberrations in cells from the XP heterozygote was approximately half that observed in the XP homozygote. The elevated frequencies of chromatid breaks and gaps after G_2 phase x-irradiation may provide the basis of a test for identifying carriers of the XP gene(s) within known XP families. (*J. Clin. Invest.* 1990. 85:135–138.) chromatid damage • DNA repair • xeroderma pigmentosum • x-irradiation

Introduction

Xeroderma pigmentosum (XP)[1] is a rare autosomal-recessive, progressive, degenerative disease associated with sun sensitivity, cutaneous pigmentation, neoplasia, abnormal DNA repair, and, in some patients, neurologic degeneration (1). XP heterozygotes are clinically normal individuals who cannot be recognized by any simple, reliable test known to date. However, their identification is important for genetic counseling, epidemiologic studies, and cancer control.

In previous studies (2–4), we found that clinically normal obligate carriers of genes for ataxia-telangiectasia and hereditary cutaneous malignant melanoma could be detected by their cellular responses to x-irradiation (100 R) during the G_2 phase of the cell cycle. Skin fibroblasts or stimulated peripheral blood lymphocytes, fixed 2–3 h after x-irradiation showed higher than normal frequencies of chromatid breaks and gaps. This abnormal response also characterized cells from individuals genetically predisposed to a high risk of cancer including XP patients (5, 6), as well as malignant tumor cells of diverse histopathology and tissue origin (7).

In the present study, we compared cytogenetic responses to G_2 phase x-irradiation of peripheral blood lymphocytes or skin fibroblasts from XP patients, obligate XP heterozygotes (their parents), and normal controls. The magnitude of the difference in radiation-induced chromatid damage between cells from XP heterozygotes and normal controls suggests that this response can provide the basis of an assay for detecting carriers of the gene(s) in XP families.

Methods

The age and sex of individual donors of peripheral blood or skin fibroblasts are indicated in Tables I and II. Procedures of culture, irradiation, and processing of cells for chromosome analysis for skin fibroblasts have been described. In the earlier study (8), 100 R was the dose in air equivalent to 68 R in the Leighton tube at the growth surface. For the peripheral blood assay, freshly drawn blood, 3.5 ml, was added to a T-25 flask containing 35 ml RPMI 1640 medium with 15% FBS, 0.29 mg/ml additional glutamine, 10 U/ml heparin, and 1% (vol/vol) PHA (HA 15, Burroughs-Wellcome, Research Triangle Park, NC). The medium was equilibrated with 10% CO_2 in air to adjust pH, and warmed (37°C) before addition of blood sample. The culture was incubated upright for 72 h before x-irradiation. For irradiation, cells were distributed to seven pyrex centrifuge tubes (15 ml), centrifuged at 150 *g*, and the pellets x-irradiated (58 R) as described (4). In the earlier study, 100 R was the dose in air equivalent to 58 R in the centrifuge tube at the pellet. The pellets were then resuspended in 5 ml of culture medium lacking heparin and PHA, and two cultures each incubated for 0.5, 1.5, and 2.5 h; one additional unirradiated control was harvested at 2.5 h. Cultures were treated with colcemid (0.1 μg/ml) for 1 h before fixation, except those harvested at 0.5 h, which received colcemid immediately after irradiation. In Table I, the "hours post irradiation" 1.3, 2.3, and 3.3 include handling and chromosome-processing time up to fixation (2 min medium addition, two centrifugations 9 min each, 20 min hypotonic incubation, and an additional 10 min for solution replacements) (Fig. 1). Cells were processed in suspension essentially as described (9), and drops of cell suspension were added to dry glass slides for staining and analysis. 50 or 100 metaphase cells were examined per variable.

Because the lot of FBS can affect repair of DNA damage (8), serum lots were screened for optimal repair with cells from a known normal control. Optimal repair for normal control blood lymphocytes was ≤ 60 aberrations (gaps + breaks)/100 metaphase cells at 2–3 h postirradiation and ≤ 40/100 cells for skin fibroblasts. Because jetting of a cell suspension through a syringe needle can be damaging to cells, the blood was drawn slowly into the syringe, the needle removed, and the blood allowed to flow from the syringe barrel into the culture vessel of warmed (37°C) culture medium. During and after x-irradiation, cultures were maintained at 37°C. Both cultures and medium were protected from light of < 500 nm wavelength by use of wraps and yellow light filters to prevent the generation of photoproducts that can damage DNA and yield chromatid aberrations (10).

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1. *Abbreviation used in this paper:* XP, xeroderma pigmentosum.

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Table I. Chromatid Damage in Lymphocytes of Normal, XP Heterozygous, and XP Individuals after G_2 Phase X-Irradiation (58 R)

Donor designation	Age/sex	Chromatid breaks/gaps average/ 100 metaphase cells* Hours postirradiation 1.3	2.3	3.3
Normal				
12094	30/M	62/56	18/14	10/6
11894	32/M	73/42	21/17	10/8
11927	34/F	82/64	16/18	6/6
12014	36/M	64/60	22/18	8/8
11965	47/M	86/48	18/22	12/12
12018	50/M	84/72	28/22	8/8
11861	57/M	68/62	24/18	10/8
11866	70/F	62/62	22/16	10/8
11860	72/M	76/58	25/12	10/10
Mean (SE)		73±3/58±3	22±1/18±1	9±1/8±1
XP hetero.				
12092[a]	29/F	56/54	46/46	24/26
11808[a‡]	31/M	66/28	36/33	24/23
12335[c]	37/F	80/68	52/54	26/22
11723[b]	43/M	66/36	29/39	17/19
11792[b]	43/F	73/33	43/32	28/16
11784	43/F	63/52	45/34	24/22
12382[d‡]	50/F	78/50	50/34	22/8
12383[d]	53/M	82/54	54/32	28/14
Mean (SE)		70±3/47±5	44±3/38±3	24±1/19±2
XP				
11819	5/F	62/77	59/44	31/22
12386[a]	10/M	72/82	62/64	26/26
12004[b]	13/F	57/62	47/38	27/25
11857[c]	16/M	74/64	65/31	25/17
12128[d§]	20/F	72/64	48/22	42/4
11844	29/M	81/72	98/93	56/29
Mean (SE)		70±4/70±3	63±8/49±11	34±5/20±4

* Spontaneous incidence, breaks/gaps average (range) per 100 cells:

$$\text{Normal } \frac{0.3\ (0\text{–}2)}{0.6\ (0\text{–}4)};\ \text{XP heterozygotes } \frac{0.2\ (0\text{–}2)}{0};\ \text{XP } \frac{0}{0}.$$

[a–d] Heterozygous parents and their homozygous children have the same superscript.
‡ 12382 with breast cancer and 11808 with basal cell carcinoma of the skin.
§ XP-A (XP 12BE).

Selection and coding of blood donors or skin fibroblast cultures were made by Dr. Kraemer and Mr. Jones. All experiments were carried out in the In Vitro Carcinogenesis Section of the National Cancer Institute (NCI). The coded preparations were analyzed at Howard University and decoded at NCI only after the data had been tabulated. Aberrations scored as chromatid breaks showed distinct dislocation of the broken segment. Gaps showed chromatid discontinuities longer than the chromatid width with no displacement of the segment distal to the lesion. Statistical comparisons of data were based on the *t* test after taking a square root transformation of the aberration frequencies. Two-sided *P* values are reported.

Table II. Chromatid Damage in Skin Fibroblasts of Normal, XP Heterozygous, and XP Individuals Fixed 2 h after G_2 Phase X-Irradiation (68 R)

Donor	Age/sex	Chromatid damage per 100 metaphase* cells Breaks	Gaps
Normal			
GM 3652	24/M	20	20
XP hetero.			
KR 4052	57/M	43	78
XP			
KR 4050	30/M	70	155

* Spontaneous incidence breaks/gaps:

$$\text{Normal } \frac{0}{1};\ \text{XP hetero. } \frac{0}{1};\ \text{XP } \frac{0}{1}.$$

Results

Exposure of human cells to x-rays during G_2 phase of the cell cycle results in chromatid breaks and gaps in the first postirradiation metaphase cells. The frequencies of breaks and gaps in stimulated blood lymphocytes fixed at 1.3, 2.3, and 3.3 h after x-irradiation (58 R) are summarized in Table I. Cells from nine clinically normal healthy donors were compared with those from six XP patients and eight XP obligate heterozygous carriers of the XP gene (parents of XP patients). At 1.3 h postirradiation, after postirradiation frequencies of chromatid breaks in cells from normal donors, XP heterozygotes, and XP patients did not differ significantly; frequencies of gaps were not significantly different between cells from normal donors and XP heterozygotes, but XP cells had significantly more gaps than those from normal or XP heterozygotes ($P = 0.023$, 0.004, respectively). At 2.3 and 3.3 h postirradiation cells from XP heterozygotes had at least twofold higher frequencies of chromatid breaks or gaps than cells from normal donors (*P*

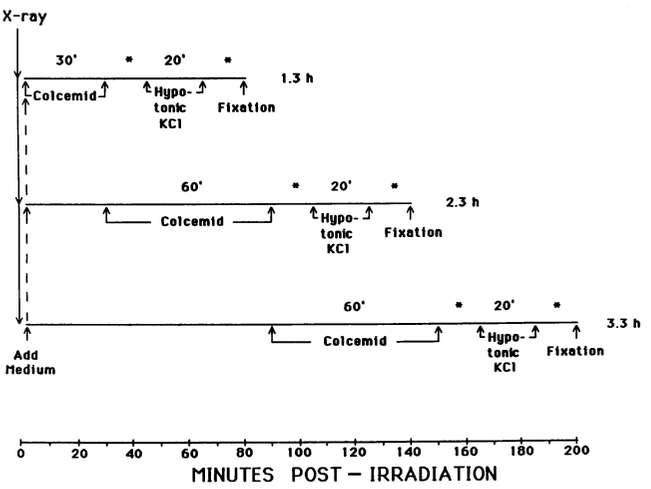

Figure 1. Protocol for processing blood lymphocyte cultures during the irradiation–fixation interval.

$< 10^{-5}$). Cells from the six XP homozygotes had aberration frequencies approximately threefold higher than normal ($P < 10^{-6}$), except for gaps in cells from XP patient of complementation group A (XP-A). The low level of chromatid gaps in the XP-A patient was also observed in fibroblasts from this patient and has been discussed elsewhere (5, 11). The abnormally high frequency of breaks observed here in lymphocytes from this patient was not observed in the fibroblast assay.

Table II compares the frequencies of chromatid breaks and gaps in coded cultures of skin fibroblasts from a normal donor, an XP heterozygote and his son, an XP patient, 2 h after x-irradiation (68 R) during G_2. Skin fibroblasts from the father had a twofold higher frequency of chromatid breaks and approximately fourfold higher frequency of gaps than cells from the normal control. This frequency of aberrations in cells from the heterozygote was approximately half that seen in the homozygous patient.

Discussion

Although cells from XP patients are known to be deficient in the repair of UV-induced pyrimidine dimers (12), they also appear to be deficient in the repair of DNA damage induced by fluorescent light (10, 13), alkylating agents (14, 15), and in some cases ionizing radiation (5, 16–18). Unlike XP patients, carriers of XP gene(s) are clinically normal, but show a small excess incidence of skin cancer in some families (19). Further, XP heterozygotes are reported to show certain developmental disabilities common among the homozygotes (20). Their detection in the human population is important for genetic counseling, epidemiologic studies, and cancer control because they carry genes for cancer-proneness.

Several approaches have been used in efforts to distinguish heterozygous XP individuals from normal. These include: (*a*) direct measures of DNA repair after ultraviolet (UV) irradiation (21–26); (*b*) DNA repair synthesis after UV irradiation in heterokaryons containing one heterozygous and one or more XP homozygous cell nuclei (27); (*c*) host–cell reactivation of UV- or γ-irradiated adenovirus type 2, and host–cell reactivation of chemically treated or UV-irradiated herpes simplex virus-1 (28–30); (*d*) induction of plasminogen activator by UV light (31); (*e*) NAD conversion to poly (ADP-ribose) synthesis after UV irradiation (32). Diminished repair of DNA damage and enhanced production of plasminogen activator after UV irradiation have been reported only in some XP heterozygotes, particularly parents of XP-A patients. Giannelli and Pawsey (27), using heterokaryons between fibroblasts from XP patients and fibroblasts from normal or heterozygous subjects, noted that normal cells contain at least three- to fourfold the amount of "XP enzyme" necessary for maximal unscheduled DNA synthesis (27). A test was devised to dilute the XP-enzyme to rate-limiting levels by producing heteropolykaryons containing one heterozygous and one or more homozygous XP nuclei. In such heteropolykaryons, nuclei from heterozygotes could be detected by their reduced enzyme levels. In tests for host–cell reactivation of chemically treated or UV-irradiated adenovirus-2 or herpes simplex-1, skin fibroblasts from XP heterozygotes could not be distinguished from those of normal individuals (28, 30). However, a reduced capacity to repair UV or γ-irradiated adenovirus-2 as measured by formation of viral structural antigens was observed in fibroblasts from four XP heterozygotes representing three complementation groups (29). Treatment of cells with DNA-damaging agents stimulates the conversion of NAD to the chromosomal polymer poly (ADP-ribose) which, in turn, rapidly depletes the cellular NAD pool. Fibroblasts from three XP heterozygotes of groups A, C, and D could be distinguished from normal by their drastic lowering of NAD levels after UV or chemical (*N*-methyl-*N'*-*nitro*-*N*-nitrosoguanidine) treatment (32). None of the above approaches has provided a simple, precise, and reproducible test for identifying carriers of the XP gene(s).

The major finding of the present study is that both blood lymphocytes and skin fibroblasts from obligate carriers of the XP gene(s), like those from XP patients, show a higher than normal extent of chromatid damage 2–3 h after x-irradiation (58 and 68 R, respectively). In blood lymphocytes from XP heterozygotes and normal individuals, the extent of chromatid damage was equivalent in metaphase cells collected by colcemid for 0.5 h immediately after x-irradiation and fixed by 1.3 h. This observation indicates that the cells from XP heterozygotes are not more radiosensitive than those from normal individuals. In metaphase cells collected by colcemid from 0.5 to 1.5 h or from 1.5 to 2.5 h and fixed by 2.3 and 3.3 h postirradiation, respectively, frequencies of chromatid breaks and gaps decreased precipitously in cells from normal individuals, presumably from efficient repair of the radiation-induced DNA damage. However, in metaphase cells from XP heterozygotes and XP patients, collected during the same intervals, chromatid damage persisted at significantly higher levels, presumably from inefficient or unbalanced DNA repair (5, 11). In skin fibroblasts from XP heterozygotes and homozygotes, the extent of chromatid damage in metaphase cells collected by colcemid from 0.5 to 1.5 h postirradiation and fixed at $\sim$ 2 h was significantly higher than normal; the level in the heterozygote was intermediate between that of the normal and XP individual.

As mentioned earlier, carriers of XP genes are clinically normal individuals who cannot be detected by any simple reproducible test known to date. The twofold higher than normal level of chromatid damage at 2–3 h postirradiation observed here in cells from obligate carriers of XP genes can provide the basis of a test for the detection of XP carriers. Although this same high level of chromatid damage has also been reported in cells from individuals with diverse cancer-prone genes (3–5), this assay would be useful in identifying carriers within known XP families.

We presented evidence that persistence of chromatid damage after x-irradiation during G_2 phase results from deficient or unbalanced DNA repair (5, 8). The prevalence of this phenotype in so many different cancer-prone genetic conditions suggests that a number of genes may be involved. DNA repair after initial strand incision is a multienzymatic process controlled by a substantial number of genes, mutations in any one of which could lead to unbalanced or deficient DNA repair manifesting as enhanced chromatid damage. Furthermore, such a repair deficiency in cycling cells would lead to genetic instability that may be essential for all neoplastic development (8, 33).

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