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*Advances in Brief*

# Defective Repair of DNA Double-Strand Breaks and Chromosome Damage in Fibroblasts from a Radiosensitive Leukemia Patient

**Christophe Badie, George Iliakis, Nicolas Foray, Ghazi Alsbeih, Gabriel E. Pantellias, Ryuichi Okayasu, Nge Cheong, Nicola S. Russell, Adrian C. Begg, Colin F. Arlett,[1] and Edmond P. Malaise**

*Institute Gustave-Roussy, 94805 Villejuif, France [C. B., N. F., G. A., E. P. M.]; Thomas Jefferson University, Philadelphia, Pennsylvania 19107 [G. I., G. E. P., R. O., N. C.]; Department of Experimental Therapy, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, the Netherlands [N. S. R., A. C. B.]; and Medical Research Council, Cell Mutation Unit, University of Sussex, Brighton, BN1 9RR, United Kingdom [C. F. A.]*

## Abstract

**A radiation-sensitive fibroblast culture (180BR) established from an acute lymphoblastic leukemia patient who died following radiotherapy is defective in the repair of radiation-induced DNA double-strand breaks. The cells also show a reduced capacity to repair interphase chromosome damage visualized by means of premature chromosome condensation and metaphase chromosome aberrations measured by fluorescence *in situ* hybridization on chromosome 4. This case represents the first example in humans where hypersensitivity to ionizing radiation can be ascribed directly to a defect in DNA and chromosome repair, and the defect may underlie the cancerous phenotype observed.**

## Introduction

The pre-eminence of the DNA dsb[2] as the lesion of major impact for cell killing induced by ionizing radiation is widely accepted (1) and is supported by extensive studies in rodent cells showing a correlation between defects in dsb rejoining and cell lethality (2). Radiosensitive XR-1 and xrs-6 mutants of Chinese hamster cells are defective in both V(D)J rejoining and dsb repair. Cells from immunocompromised SCID mice (SCID/St and SC3T3/W) show hypersensitivity to the lethal and the chromosome breaking (clastogenic) action of ionizing radiation and are also defective both in dsb rejoining and V(D)J recombination (3). These three rodent cell lines represent distinct complementation groups (4). Very recently (5) the gene defective in xrs-6 cells has been shown to encode Ku 80, a subunit of the Ku protein, which binds to free double-stranded DNA ends, thereby implicating Ku in the processing of both damage-specific and V(D)J-induced site-specific dsb. We report here DNA repair studies on a fibroblast culture (180BR) established from an acute lymphoblastic leukemia patient (6). This patient was not overtly immunocompromised and had no clinical features consistent with the diagnosis of AT (7). However, during the course of radiotherapy treatment the patient proved to be radiation sensitive and died from severe radiation morbidity. While the cellular radiosensitivity was in the same range as in AT for both dividing and plateau phase cells, 180BR cells could be distinguished from AT by their normal level of radiation-induced inhibition of DNA synthesis (8). Our studies now reveal that 180BR cells are defective in the repair of both dsb and chromosome damage.

## Materials and Methods

Fibroblast cultures of 180BR and 1BR.3 (normal) were established in the laboratory of C. F. A., and normal HF19 cells were a generous gift from Dr. Roger Cox. The HF19 and 1BR.3 normal cell lines have proved to have very similar radiobiological characteristics in a comparative study of cell survival and the induction and repair of dsb carried out in the laboratory of E. P. M. (data not shown). dsb repair was measured by pulse-field gel electrophoresis. Cells were labeled with 0.01 $\mu Ci \cdot ml^{-1}$ [$^3$H]dThd (6.7 $Ci \cdot mmol^{-1}$; Amersham) for 72 h, and were subsequently incubated in nonradioactive medium for 8 days to achieve a plateau phase of growth. They were irradiated at 4°C using a $^{137}$Cs source at a dose rate of 1.45 $Gy \cdot min^{-1}$. Cells were assayed for DNA dsb at different times after irradiation. For this purpose, they were trypsinized, embedded in 0.5% low melting point agarose, and blocks were cut containing approximately $2 \times 10^5$ cells. Cells in blocks were incubated in lysis solution (0.5 M EDTA, 2% Sarcosyl, 1 $mg \cdot ml^{-1}$ proteinase K in 0.5 M EDTA, pH 8.0) at 0°C for 1 h followed by 45 h at 50°C. After lysis, cells were rinsed twice in TE buffer (10 mM Tris, 1 mM EDTA; pH 8.0) and incubated for 1 h with 0.1 $mg \cdot ml^{-1}$ RNase at 37°C. DNA was separated by electrophoresis in 0.7% agarose gels (Seakem GTG-FMC) in 0.5X TBE buffer (1X TBE: 90 mM Tris base, 90 mM boric acid, 2.5 mM EDTA). Electrophoresis was performed using a CHEF DRII (Bio-Rad) apparatus at 14°C. The migration conditions ensured separation of the three chromosomes of *Schizosaccharomyces pombe* (3.5, 4.68, and 5.7 megabase pairs) and included a ramping to 5400 s at 2700 s for a total of 72 h (24 h at 35 V followed by 48 h at 50 V), then 24 h at 60 V with a constant pulse time of 1800 s. Following electrophoresis, gels were stained with ethidium bromide, photographed, and cut into strips.

The samples were placed in scintillation vials, agarose was melted, and radioactivity was measured by scintillation counting. The fraction of DNA migrating out of the well was calculated from the equation:

$$\%\ \text{of fraction of activity released} = 100 \times \frac{\text{migrated cpm}}{\text{migrated cpm+well cpm}}$$

The data were fitted using the formula: unrepaired dsb $(t) = e^{-\ln 2 \cdot t / T(t)}$ with $T(t) = c_0 + c_1 \cdot (1 - e^{-c_2 t})$. This last formula describes the repair half-time $T(t)$ as a parameter which is time dependent. At time $t = 0$, $T(t) = c_0$, $T(t)$ increases up to a maximum value of $c_0 + c_1$.

Chromosome damage was measured by PCC in interphase cells or by FISH analysis of metaphase spreads.

Premature chromosome condensation involves the fusion of a mitotic and an interphase cell, resulting in the condensation of interphase chromatin into distinct chromosomes which can then be analyzed for breakage. Briefly, density-inhibited cultures were given 6.0 Gy using a $^{137}$Cs source at a dose rate of 1.45 $Gy \cdot min^{-1}$. Cultures were then returned to 37°C for various lengths of time before being trypsinized and fused with mitotic Chinese hamster ovary cells to induce PCC. The fusion mixture contained approximately $8 \times 10^5$ Chinese hamster ovary cells along with $5 \times 10^5$ HF19 or 180BR cells. After centrifugation at $200 \times g$ for 5 min the supernatant was discarded, and the pellet was resuspended in 0.7 ml ice-cold serum-free medium containing 100 hemagglutination units of UV-inactivated Sendai virus. This suspension was kept in ice for 15 min to allow for virus attachment. Subsequently, Colcemid was added at a final concentration of $10^{-6}$ M and samples were transferred to 37°C for 70 min; in that time cell fusion and induction of PCC were completed. After the incubation period for chromosome condensation, samples were treated in hypotonic (0.075 M) KCl solution, fixed in methanol:acetic acid (3:1), and stained with 2% Giemsa. The analysis of PCC fragments was by means of light microscopy.

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[1] To whom requests for reprints should be addressed.

[2] The abbreviations used are: dsb, double-strand break; AT, ataxia telangiectasia; PCC, premature chromosome condensation; FISH, fluorescence *in situ* hybridization.

For FISH analysis metaphase spreads were prepared and hybridized to a whole chromosome probe of chromosome 4 using the method described by Pinkel *et al.* (9), with some modifications. The probe for chromosome 4 (obtained from Dr. J. Gray, University of San Francisco) was labeled with biotin-dUTP by nick translation. Denatured metaphase preparations were hybridized with 10 μl of a mixture consisting of 50% formamide, 10% dextran sulfate, 2X SCC (pH 7), 1 μg Cot-1 DNA (GIBCO-BRL), and 40 ng biotinylated probe DNA (chromosome 4), which had been denatured at 70°C for 5 min and at 37°C for 1 h. Hybridization was allowed to take place overnight at 37°C in a moist chamber. After washing, staining of the biotinylated probe was accomplished by incubating the preparations with avidin-fluorescein (Vector-Laboratories, Burlingame CA), 5 μg/ml in TN buffer (0.1 M Tris, 0.15 M sodium chloride) + 0.5% blocking reagent for 20 min in the dark. Amplification of the signal was obtained by incubation with goat anti-avidin (5 μg/ml) in TN buffer + 0.5% blocking reagent followed by incubation with avidin-fluorescein. The slides were washed once in TNT buffer (0.1 M Tris, 0.15 M sodium chloride, 0.5% Tween 80) before application of 7.5 μl of a fluorescence antifade solution containing 0.2 μg/ml propidium iodide or 4',6-diamidine-2-phenylindole dihydrochloride (Boehringer Mannheim, Mannheim, Germany) to counterstain the total DNA.

The prepared slides were viewed under a fluorescence microscope (Minolta) under blue light excitation (380–490 nm) to visualize the fluorescence probe and green light (465–550 nm) or UV light (330–380 nm) for visualization, respectively, of the propidium iodide or 4',6-diamidine-2-phenylindole dihydrochloride counterstains. The slides were coded before viewing and scored "blind" by one observer. For each dose point, 100 metaphases were scored as either normal or aberrant.

## Results

The initial yields of DNA dsb measured by a pulse-field gel electrophoresis technique immediately after irradiation at 4°C are very similar in 180BR and in control, normal, HF19 cells (data not shown). Repair kinetics confirm that there is very little rejoining of dsb in 180BR in contrast to the control cell line (Fig. 1). We have also assessed the dose-dependent induction and repair kinetics of interphase chromosome breaks (Fig. 2) as measured by PCC (10, 11). The dose effect relationships are straight lines (data not shown). For 180BR, the initial yield is 6.7 breaks per cell per Gy and for HF19 it is 5.3, a value which has been reported elsewhere (12). The difference

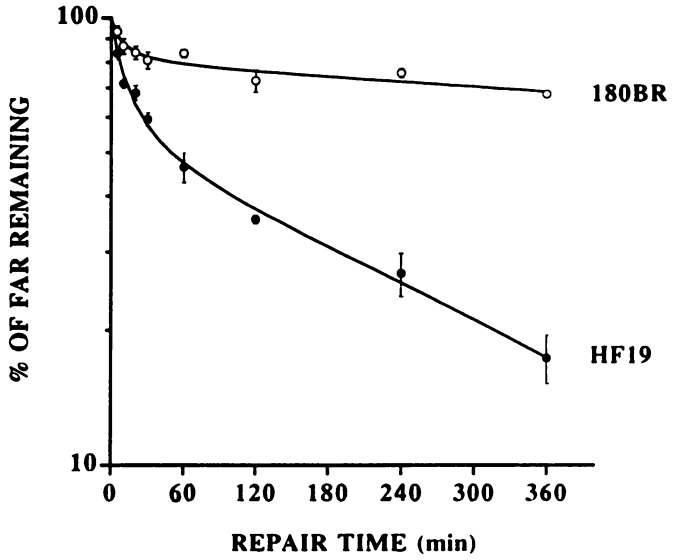


Fig. 1. The repair of dsbs. The fraction of activity released (*FAR*) as a function of postirradiation incubation time interval at 37°C. Cells in plateau phase were given 30 Gy. The relative proportion of unrejoined breaks (initial damage, 100%) was plotted against the postirradiation incubation time interval at 37°C. Each point represents the mean of the data from at least three independent irradiation experiments. ○, 180BR cells; ●, normal control HF19 cells. *Errors bars*, mean value ± SE.

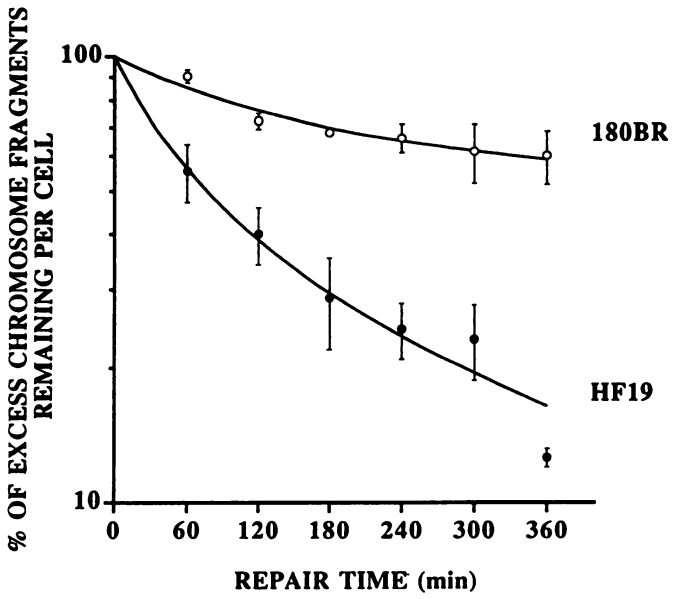


Fig. 2. Repair kinetics of interphase chromosome damage after exposure of plateau phase cells to 6 Gy as visualized and analyzed using the PCC technique. Data are presented as mean values from three experiments ± SE. In each experiment, at least 30 cells were scored per time point. ○, 180BR cells; ●, control normal HF19 cells.

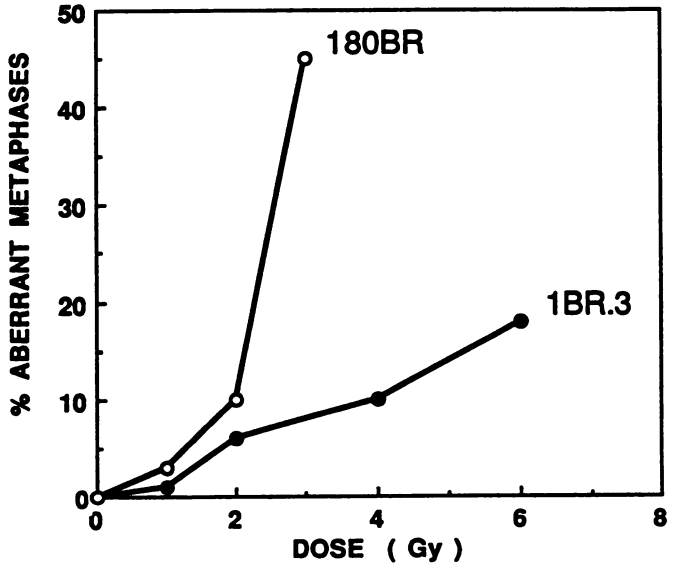


Fig. 3. Relationship between the percentage of aberrant metaphases and radiation dose. ○, 180BR cells; ●, 1BR.3 cells.

in the initial part of the repair kinetic curves for DNA dsb may explain the small difference observed in initial damage seen with the PCC. For both cell lines, the striking similarity between the repair rates for the dsb and chromosome fragments implies that these two phenomena are correlated. A similar correlation has already been described in rodent lymphoblastoid cell lines (13). The susceptibility of 180BR cells to the induction of radiation damage expressed in metaphase chromosomes and measured by FISH (9) on chromosome 4 is displayed in Fig. 3. As anticipated on the basis of the PCC results, 180BR cells show a greater sensitivity to the induction of chromosome breakage.

## Discussion

The present case represents the first example in humans where hypersensitivity to ionizing radiation can be ascribed directly to a

defect in repair, here specifically the repair of dsb and interphase chromosome breaks. In support of the PCC data, which are a predicted consequence of the dsb repair defect, it is of interest that the fibroblast culture also shows an elevated frequency of induced metaphase chromosome aberrations (acentric fragments and translocations) measured by FISH analysis on chromosome 4 48 h after irradiation. It remains a speculation as to whether the leukemia observed in this individual is a direct consequence of this repair defect. The susceptibility to chromosome damage revealed by the PCC and metaphase studies could provide a mechanism to generate genomic instability (14) whatever the initial source of damage.

Hypersensitivity to the lethal action of ionizing radiation has previously been reported for a number of human cell cultures (15, 16). However, attempts to relate this hypersensitivity to molecular defects in DNA repair have been disappointing even with AT, a cancer-prone syndrome with associated neuropathology and immune deficiency (7) which displays consistent cellular radiohypersensitivity (17, 18) and which has been under intensive investigation for over 20 years.

The present culture (180BR) provides an important resource which may enable us to isolate directly from human material a gene involved in the repair of ionizing radiation damage and possibly predisposing to leukemia. However, the primary fibroblast culture has, so far, proved intractable to immortalization in two of our laboratories and, unfortunately, no blood-derived material is available to generate a lymphoblastoid cell line. It is not yet known if 180BR cells have any defects in V(D)J rejoining. We consider this unlikely, however, since the patient showed no obvious immune deficiencies. Similarly, it is not yet known if the cells have defects in binding to free double-stranded DNA ends as has been shown in the xrs-6 radiation sensitive, dsb repair defective Chinese hamster ovary cells (5).

A possible human analogue of these rodent cell lines is seen in a patient suffering from a severe combined immunodeficiency syndrome without the commonly associated defects in adenosine deaminase activity. The human SCID patient is defective in V(D)J rejoining and both fibroblasts and granulocyte-macrophage-colony-forming units are hypersensitive to the lethal action of ionizing radiation (19). No data have been published on the repair of dsb in such cells.

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