Association of \textit{in Vitro} Radiosensitivity and Cancer in a Family with Acute Myelogenous Leukemia\textsuperscript{1}

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\textbf{ABSTRACT}

The \(\gamma\)-ray sensitivity of skin fibroblasts from six members of a cancer family was investigated using a colony-forming assay. Fibroblasts from the three members with cancer (two sisters with acute myelogenous leukemia and the mother with cervical carcinoma) showed a significant (\(p < 0.05\)) increase in radiosensitivity, while three members without cancer (the father and two sons) showed a normal radioresponse. The possibility that the increased \(\gamma\)-ray sensitivity was due to defective DNA repair was investigated using assays for DNA repair replication, single-strand break rejoining, and removal of enzyme-sensitive sites in \(\gamma\)-irradiated DNA. Results of these assays indicate that the kinetics of enzymatic repair of radiogenic DNA damage in general, and the rejoining of single-strand scissions and excision repair of base and sugar radioproducts in particular, were the same in the cell lines from the sensitive and clinically normal family members.

\textbf{INTRODUCTION}

The excessive occurrence of cancer is a feature of over 200 different human single-gene diseases (18), including those which exhibit chromosomal instability and radiation sensitivity (9, 20). Striking \textit{in vitro} radiosensitivity has been a feature of all skin fibroblasts and lymphoblast cultures assayed from individuals with the cancer-prone disorder AT\textsuperscript{3} (21, 32). In addition, fibroblasts or lymphoblasts from individuals with any of several other genetic disorders that show cancer proneness, neurodegeneration, or both features frequently display significant increases in radiosensitivity; e.g., hereditary retinoblastoma (33); Friedreich's ataxia (10); Huntington's disease (1, 11). Since ionizing radiation is known to be leukemogenic (14, 15), we were prompted to investigate whether the host factor, which segregated in one AML family described previously (12, 15), conferred an altered susceptibility to an environmental agent such as radiation. This report describes our assessment of the \(^{60}\text{Co}\ \gamma\)-ray sensitivity and DNA repair capacity of fibroblast cells from the available members of this family [both parents and 4 of their 6 children including the proband (Chart 1)].

\textbf{MATERIALS AND METHODS}

\textbf{Cell Lines.} GM 38, GM 43, and WI38 from clinically normal individuals were obtained from the Institute for Medical Research, Camden, N. J., and AT2BE (CRL 1343), a radiosensitive strain from a patient with AT (22, 25), was obtained from the American Type Culture Collection, Rockville, Md. Cultures from the 6 members of the AML cancer family (cf. Chart 1) and 1461T and 3151T from clinically normal individuals were supplied by Meloy Laboratories, Springfield, Va. All fibroblasts were grown as monolayer cultures in Ham's F-12 medium (7) fortified with 15\% (v/v) heat-inactivated fetal calf serum, 1 mm glutamine, penicillin (100 IU/ml), and streptomycin (100 IU/ml), and incubated at 37\°C in humidified 5\% CO\textsubscript{2} in air. Culture supplies were purchased from Microbiological Associates, Inc., Walkersville, Md. or Grand Island Biological Co., Burlington, Ontario, Canada. All cultures were routinely checked for \textit{Mycoplasma} contamination by the Hoechst staining method (4) and a double-label method measuring the incorporation of [6-\textsuperscript{3}H]uracil and [\(\text{U-}\text{\textsuperscript{14}}\text{C}\)]uridine (28, 29). These radioactive compounds were obtained from New England Nuclear, Lachine, Quebec, Canada.

\textbf{\(\gamma\)-Ray Sensitivity.} To measure the radiosensitivity of the skin fibroblast cultures, samples of cells in suspension (1 to 2 \xspace \times \xspace 10\textsuperscript{5}/ml) were exposed at 4\°C to graded doses of \(^{60}\text{Co}\ \gamma\)-rays from a Gammabeam 150c (Atomic Energy of Canada Limited, Ottawa, Ontario, Canada) at a dose rate of 70 to 76 rads/min. Feeder cells (5) were exposed at 4\°C to \(^{60}\text{Co}\ \gamma\)-radiation (5 kilorads) in a Gammacell 220 (Atomic Energy of Canada Limited) at a dose rate of 15.9 to 17.7 kilorads/min prior to seeding with experimental cells to give 8 to 8 \xspace \times \xspace 10\textsuperscript{5} total cells/100-mm-diameter tissue culture plate. Experimental cultures were incubated for 18 to 24 days with twice-weekly changes of medium before fixing the resulting colonies 5 to 10 min with Bouin's fixative (27) and staining 5 min with a 0.04\% (w/v) aqueous solution of crystal violet (Fisher Scientific Co., Mississauga, Ontario, Canada). Colonies composed of 100 or more cells were enumerated.

\textbf{\(\gamma\)-Ray-induced DNA Repair Replication.} This parameter of DNA repair is used as a gross measure of the repair activity in damaged DNA. The labeling regime was designed to allow the incorporation of exogenous nucleotides into DNA during the repair of radioproducts while inhibiting \textit{de novo} DNA synthesis. Unlabeled cultures of 2 to 4 \xspace \times \xspace 10\textsuperscript{5} attached cells were (a) incubated for 2 hr in F-12 medium (supplemented with 10\% dialyzed fetal calf serum) containing 6.5 \mu\text{M} BrdUrd and 1 \mu\text{M}...
FdUrd, rinsed, and covered with Hanks’ balanced salt solution; (b) γ-irradiated in a Gammarcell 220 either with (hypoxia) or without (oxia) nitrogen (99.98% pure, <10 ppm O2; Air Products, Brampton, Ontario, Canada), flushing (15 min) prior to and during irradiation; (c) then incubated for 2 hr with 10 μCi [methyl-3H]dThd per ml [specific activity, 50 to 55 Ci/μmol; Amersham/Searle, Oakville, Ontario, Canada or New England Nuclear (Canada)] in F-12 medium containing 6.5 μM BrdUrd, 1 μM FdUrd, and 1 mM hydroxyurea; and finally (d) incubated for 1 hr in F-12 medium containing 6.5 μM BrdUrd and 1 μM FdUrd. Hydroxyurea, BrdUrd, and FdUrd were purchased from Calbiochem-Behring Corp., La Jolla, Calif. The extent of repair replication occurring during the 2-hr postirradiation labeling period was determined by equilibrium centrifugation of the radioactive and density-labeled DNA in NaI gradients as described previously (22). The magnitude of repair replication has been expressed as dpm per μg DNA based on the six 300-μl-peak fractions.

Enzymatic Assay. The number of strand breaks and base defects in γ-ray-damaged DNA was assessed using a method described previously (22, 23). In brief, [3H]dThd-labeled, γ-irradiated (50 kilorads, N2) cultures were incubated for up to 2 hr at 37° and lysed, and their DNA’s were coextracted with lysed [14C]dThd-labeled unirradiated cells of the same subcultures. The various DNA samples were incubated at 37° with or without a Micrococcus luteus protein extract containing strand-incising activity (endonucleases and DNA glycosylases) toward γ-ray-induced DNA sites. The number of single-strand breaks and extract-sensitive sites was determined by velocity centrifugation in alkaline sucrose gradients.

RESULTS

Fibroblast survival was monitored after γ-irradiation. The results of colony-forming assays after oxic γ-irradiation for a normal control (GM 38), a sensitive control (AT2BE), and the 6 experimental cell strains are presented in Chart 2. The parameters for these oxic γ-ray survival curves are summarized in Table 1. In comparing the survival curves (Chart 2a) and the D10 values (Table 1), fibroblasts from the 3 clinically normal members of the family (2650T from the father and 2647T and 2648T from the dizygotic male twins), it is apparent that their radiosensitivity is similar to that of the clinically normal strains tested during the period of this study. Fibroblasts from the mother (2649T) and the leukemic daughters (409T and 2642T)
were significantly more sensitive based on their $D_{10}$ values than the mean $D_{10}$ value for the normals, though clearly much less sensitive than an AT homozygote (AT2BE) (Table 1; Chart 2). The differences in the survival response of the 3 strains from the females in the family presumably reflect inherent differences of the strains. The decrease in $D_{10}$ values after oxic irradiation reflects a reduced shoulder region in the survival curves without a significant change in the $D_0$'s. The survival curves for the 2 strains 2642T and 2649T also showed significantly lower $D_{0}$ and $D_{10}$ (Table 2; Chart 3). The survival curves for fibroblast strains from members of AML family from cancer-bearing individuals in this family had impaired ability to repair γ-ray damage to their DNA. To measure repair capacity, we first studied DNA repair replication in acutely irradiated (50 kilorads) cultures (Chart 4). Under oxic irradiation, all strains except the sensitive control strain (AT2BE) showed levels of DNA repair replication similar to that of the normal strain (GM 38) irrespective of the different γ-ray sensitivity established in the colony-forming assay. Cells irradiated under hypoxic conditions showed reduced levels of repair replication in each case, consistent with the effect of oxygen reported previously (24); oxygen enhancement effects of 1.5- to 2.2-fold were observed. However, the level of repair in the strains

### Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Clinical description</th>
<th>No. of experiments</th>
<th>Passage range</th>
<th>$PE^b$(%)</th>
<th>$n^b$</th>
<th>$D_0$</th>
<th>$D_{10}$</th>
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<tbody>
<tr>
<td>Control</td>
<td>Normals</td>
<td>19</td>
<td>7-29</td>
<td>30 ± 7$^a$</td>
<td>2.0 ± 0.5</td>
<td>138 ± 13</td>
<td>402 ± 15</td>
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<tr>
<td>GM38</td>
<td>Normal</td>
<td>6</td>
<td>13-23</td>
<td>33 ± 17</td>
<td>2.2 ± 0.6</td>
<td>132 ± 9</td>
<td>407 ± 15</td>
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<td>W38</td>
<td>Normal</td>
<td>1</td>
<td>29</td>
<td>35</td>
<td>1.8 ± 0.6</td>
<td>142 ± 12</td>
<td>411 ± 19</td>
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<tr>
<td>GM43</td>
<td>Normal</td>
<td>2</td>
<td>11, 15</td>
<td>23 ± 2</td>
<td>1.1 ± 0.1</td>
<td>158 ± 6</td>
<td>382 ± 9</td>
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<td>1461T</td>
<td>Normal</td>
<td>6</td>
<td>13-18</td>
<td>21 ± 7</td>
<td>2.5 ± 0.7</td>
<td>130 ± 8</td>
<td>417 ± 17</td>
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<tr>
<td>3151T</td>
<td>Normal</td>
<td>4</td>
<td>7, 12</td>
<td>36 ± 14</td>
<td>2.2 ± 0.4</td>
<td>126 ± 5</td>
<td>391 ± 10</td>
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<tr>
<td>AT2BE</td>
<td>AT</td>
<td>2</td>
<td>13-17</td>
<td>6 ± 0</td>
<td>1.0 ± 0.3</td>
<td>73 ± 5$^a$</td>
<td>169 ± 9$^a$</td>
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<tr>
<td>2649T</td>
<td>Carcinoma</td>
<td>4</td>
<td>10-18</td>
<td>17 ± 14</td>
<td>1.3 ± 0.2</td>
<td>117 ± 5</td>
<td>301 ± 1$^e$</td>
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<td>2650T</td>
<td>Normal</td>
<td>5</td>
<td>6-23</td>
<td>45 ± 14</td>
<td>2.3 ± 0.7</td>
<td>136 ± 11</td>
<td>425 ± 19</td>
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<td>409T</td>
<td>AML</td>
<td>6</td>
<td>11-24</td>
<td>25 ± 7</td>
<td>1.6 ± 0.3</td>
<td>129 ± 6</td>
<td>360 ± 13$^a$</td>
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<tr>
<td>2642T</td>
<td>AML</td>
<td>4</td>
<td>12-15</td>
<td>9 ± 2</td>
<td>0.6 ± 0.2$^a$</td>
<td>166 ± 17</td>
<td>290 ± 31$^a$</td>
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<td>2647T</td>
<td>Normal</td>
<td>2</td>
<td>11-15</td>
<td>37 ± 1</td>
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<td>114 ± 14</td>
<td>417 ± 30</td>
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<td>2648T</td>
<td>Normal</td>
<td>4</td>
<td>9-20</td>
<td>37 ± 9</td>
<td>1.9 ± 0.3</td>
<td>136 ± 6</td>
<td>400 ± 10</td>
</tr>
</tbody>
</table>

$^a$ Number of times strains were subcultured (1:2 dilutions) before use in survival experiments.

$^b$ PE, plating efficiency; $n$, intercept on the ordinate obtained by extrapolation of exponential region of the curve.

$^c$ Average survival response of the 5 normal control strains based on least-squares linear regression analysis of pooled survival.

$^d$ Mean ± S.E.

$^e$ Instances where $D_{10}$ or $D_0$ values for the experimental strain and the normal controls (mean values or GM38) differed significantly ($p < 0.05$). The radiosensitivity of a strain was compared to that for normal controls using the standard error of difference test (17).

The small intercept ($n$) value for 2642T was characteristic of this culture in each of the 4 experiments.
DISCUSSION

Our assessment of radiosensitivity in 6 strains which were derived from members of a "leukemia" family showed the strains from clinically normal individuals to have normal radiosensitivity and the strains from members with cancer (or in the case of 2642T, who later developed cancer) to be radiosensitive. The strain from the mother (2649T) showed the most striking sensitivity while the strain from the proband was clearly not as sensitive (Table 1; Chart 2). Although the designation of a normal radioresponse by nature is somewhat arbitrary rather than absolute, our assignment of increased sensitivity in 3 members of this family holds whether we use the mean $D_{10}$ value for either unrelated normal controls or clinically normal members of the family (i.e., 2647T, 2648T, and 2650T) for comparison. We entertain the possibility that the increased radiosensitivity is the expression of the "leukemogenic" factor being transmitted through the maternal side of this family; the variability in its in vitro expression may be due to modifying factors.

The complete concordance between the presence of cancers in vivo and cellular radiosensitivity ($D_{10}$ values) in vitro is noteworthy and could be considered as support for the interaction of hereditary and environmental factors in the development of cancer in members of this family. We believe it to be fortuitous that the strains available from this family derived only from affected females and clinically normal males. The cancer proneness is clearly not restricted to females in the family; 2 older sons developed AML (Chart 1).

Aside from the marked in vitro radiosensitivity observed in all AT homozygotes, moderate but significant increases in the level of $\gamma$-ray sensitivity have been associated with several genetic recessive disorders [Friedreich's ataxia (10), Rothmund-Thomson's syndrome (30), and AT heterozygotes (24)] and dominant disorders [hereditary retinoblastoma (33), Huntington's disease (1, 14, 21), and tuberous sclerosis (21), several of which confer an increased cancer risk. The putative cancer factor transmitted in the present family shows a dominant mode of inheritance with incomplete penetrance among maternal relatives of the proband (409T) (31). Evidence for defective DNA repair of $\gamma$-ray-induced damage has so far only been presented for 2 recessive traits: AT (25) and Rothmund-Thomson's syndrome (30). Our assays for DNA repair capacity from the 5 family members tested was again the same as for the normal control strain.

Using an in vitro enzymatic assay, we also investigated the time-dependent repair of $\gamma$-ray-induced single-strand breaks and $M. luteus$ extract-sensitive sites in the DNA from several strains of this family (such sites are presumed to contain radiation-damaged base or sugar moieties). Both the initial yield and the subsequent rate of disappearance of single-strand breaks (Chart 5a) and extract-sensitive sites (Chart 5b) were similar in all strains tested; only in the repair-deficient control strain (AT2BE) was site removal abnormally slow (25) (Chart 5b). Thus, the DNA repair capacity, as far as these assays can tell us, is normal in strains from this family.

![Chart 3](image3.png)

Chart 3. Colony-forming ability after $^{60}$Co $\gamma$-irradiation in nitrogen of the 3 cancer-affected family members, the father, and a normal and a radiosensitive control.

![Chart 4](image4.png)

Chart 4. Levels of repair replication induced in DNA of controls (GM38 and AT2BE) and AML family strains by 50 kilorads of $^{60}$Co $\gamma$-irradiation under oxygen and hypoxia. Open bars, level of repair replication for oxic radiation; cross-hatched bars, level for hypoxic radiation. The magnitude of each bar is the mean of 2 to 6 determinations (each corrected for background).

![Chart 5](image5.png)

Chart 5. Time-dependent disappearance of $\gamma$-ray-induced DNA damage in control cultures and 3 family members. a, single-strand breaks; b, $M. luteus$ extract-sensitive sites. Points, means of 3 or more determinations. The average number of single-strand breaks immediately after irradiation was $0.98 \pm 0.15$ per $10^7$ daltons of DNA while the average number of extract-sensitive sites was $1.06 \pm 0.07$ per $10^7$ daltons of DNA.
when applied to cells from members of the present family demonstrated no significant differences from normal control strains. How extensively defective DNA repair figures in the moderately increased in vitro radiosensitivity now described by several laboratories for various genetic disorders and in fibroblasts from the family studied by us is not clear. New assays capable of detecting a defined range of DNA lesions should clarify this situation.

The earlier observation of increased transformation by simian virus 40 (31) of strains from the individuals which we report to several laboratories for various genetic disorders and in fibroblasts from this family, but our results do suggest a correlation between cancer proneness in vivo and enhanced radiosensitivity in vitro. Should this relationship hold true for other cancer families, in vitro radiosensitivity may have predictive value in determining high-risk members in such families.

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Note Added in Proof

We recently assessed the γ-ray sensitivity of a fibroblast strain (AG3778) which derived from a maternal aunt with breast cancer and found its radiosresponsive to be normal (D₀ of 396 ± 9 rads compared to 416 ± 18 rads for controls). This suggests that the "leukemia factor" was not present and therefore not a contributing influence to the development of cancer in this individual.

REFERENCES

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