Association of in Vitro Radiosensitivity and Cancer in a Family with Acute Myelogenous Leukemia

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ABSTRACT

The γ-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 radioreponse. The possibility that the increased γ-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 γ-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.

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 in vitro radiosensitivity has been a feature of all skin fibroblasts and lymphoblast cultures assayed from individuals with the cancer-prone disorder AT (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 displayed significant increases in radiosensitivity; e.g., hereditary retinoblastoma (33); Friedreich’s ataxia (10); Huntington’s disease (1, 16, 21); and tuberous sclerosis (3). Extensive surveys for such radiosensitivity in numerous human cell lines were presented recently (2, 21, 26, 34).

Additional evidence that an individual’s genetic constitution can play a significant role in the development of neoplasms comes from the clustering of cancers in some families (6, 8, 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, 16, 21), and tuberous sclerosis (3). Extensive surveys for such radiosensitivity in numerous human cell lines were presented recently (2, 21, 26, 34).

γ-Ray Sensitivity. To measure the radiosensitivity of the skin fibroblast cultures, samples of cells in suspension (1 to 2 × 10^6/ml) were exposed at 4° to graded doses of 60Co γ-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° to 60Co γ-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 6 to 8 × 10^4 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., Missisauga, Ontario, Canada). Colonies composed of 100 or more cells were enumerated.

γ-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 de novo DNA synthesis. Unlabeled cultures of 2 to 4 × 10^5 attached cells were (a) incubated for 2 hr in F-12 medium (supplemented with 10% dialyzed fetal calf serum) containing 6.5 μM BrdUrd and 1 μM
Radiosensitivity in AML Cancer Family

FdUrd, rinsed, and covered with Hanks' balanced salt solution; (b) γ-irradiated in a Gammacell 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]dTd per ml [specific activity, 50 to 55 Ci/mmol; 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 Nal 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]dTd-labeled, γ-irradiated (50 kilorads, Nγ) cultures were incubated for up to 2 hr at 37° and lysed, and their DNA's were coextracted with lysed [14C]dTd-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 D₀ 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)
The differences in the survival response of the 3 strains from the 2 strains 2649T and 409T also showed significantly lower GM 38 and 2650T (Table 2; Chart 3). The survival curves for with cancer (409T, 2642T, and 2649T) was found by irradiating oxic conditions, previously used to detect AT heterozygotes (24). From the D10 values, each of the 3 strains again showed increased radiosensitivity when compared to strains AT2BE AT and may possibly represent the presence of 2 subpopulations, one more sensitive than the other; the biphasic nature of the survival curves was absent in experiments with hypoxic irradiation (Table 2).

The oxic survival data showed complete concordance between the development of cancer in family members and a decrease in D10 values for the corresponding fibroblast strains; as well, fibroblasts from each of the clinically normal family members showed normal in vitro sensitivity. Further confirmation of the increased γ-ray sensitivity in strains from individuals with cancer (409T, 2642T, and 2649T) was found by irradiating under hypoxic conditions, previously used to detect AT heterozygotes (24). From the D10 values, each of the 3 strains again showed increased radiosensitivity when compared to strains GM 38 and 2650T (Table 2; Chart 3). The survival curves for the 2 strains 2649T and 409T also showed significantly lower D0 values under the hypoxic conditions.

Knowledge of defective DNA repair in xeroderma pigmentosum and AT (20) prompted us to investigate whether the strains which displayed increased radiosensitivity and which derived 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 radiation, 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(%)</th>
<th>n</th>
<th>D10</th>
<th>D0</th>
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<tr>
<td>Control</td>
<td>Normals</td>
<td>19</td>
<td>7-29</td>
<td>30 ± 7</td>
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<td>2.0 ± 0.5</td>
<td>138 ± 13</td>
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<td>6</td>
<td>13-23</td>
<td>33 ± 17</td>
<td></td>
<td>2.2 ± 0.6</td>
<td>132 ± 9</td>
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<td>WI38</td>
<td>Normal</td>
<td>1</td>
<td>29</td>
<td>35</td>
<td></td>
<td>1.8 ± 0.6</td>
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<td>GM43</td>
<td>Normal</td>
<td>2</td>
<td>11,15</td>
<td>23 ± 2</td>
<td></td>
<td>1.1 ± 0.1</td>
<td>158 ± 6</td>
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<td>1461T</td>
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<td>13-18</td>
<td>21 ± 7</td>
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<td>2.5 ± 0.7</td>
<td>130 ± 8</td>
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<td>3151T</td>
<td>Normal</td>
<td>4</td>
<td>7,12</td>
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<td></td>
<td>2.2 ± 0.4</td>
<td>126 ± 5</td>
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<td>AT2BE</td>
<td>AT</td>
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<td>13-17</td>
<td>6 ± 0</td>
<td></td>
<td>1.0 ± 0.3</td>
<td>73 ± 5</td>
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<td>2649T</td>
<td>Carcinoma</td>
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<td>10-18</td>
<td>17 ± 14</td>
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<td>1.3 ± 0.2</td>
<td>117 ± 5</td>
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<td>2650T</td>
<td>Normal</td>
<td>5</td>
<td>6-23</td>
<td>45 ± 14</td>
<td></td>
<td>2.3 ± 0.7</td>
<td>136 ± 11</td>
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<td>409T</td>
<td>AML</td>
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<td>11-24</td>
<td>25 ± 7</td>
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<td>1.6 ± 0.3</td>
<td>129 ± 6</td>
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<tr>
<td>2642T</td>
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<td>166 ± 17</td>
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<tr>
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<td>9-20</td>
<td>37 ± 9</td>
<td></td>
<td>1.9 ± 0.3</td>
<td>136 ± 6</td>
</tr>
</tbody>
</table>

- **n**: Number of times strains were subcultured (1:2 dilutions) before use in survival experiments.
- **PE**: Plating efficiency; n, intercept on the ordinate obtained by extrapolation of exponential region of the curve.
- **Average survival response of the 5 normal control strains based on least-squares linear regression analysis of pooled survival.
- **Mean ± S.E.
- **Instances where D10 or D0 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).**

### Table 2

| Strain  | No. of experiments | Passage | PE (%) | n | D10 |  | D0    |
|---------|-------------------|---------|--------|---|-----|--|--|------|
| Control | 24                | 13-48   | 1.8 ± 0.3 | 206 ± 9 | 597 ± 17 |
| GM38    | 4                 | 15-23   | 20 ± 8  | 1.7 ± 0.3 | 195 ± 9  | 548 ± 16 |
| AT2BE   | 5                 | 6-20    | 3 ± 2   | 1.1 ± 0.2 | 89 ± 6  | 206 ± 1   |
| 409T    | 5                 | 12-18   | 30 ± 9  | 1.7 ± 0.6 | 157 ± 12 | 411 ± 31 |
| 2642T   | 2                 | 14-19   | 12 ± 7  | 1.6 ± 0.8 | 160 ± 17 | 444 ± 47 |
| 2649T   | 3                 | 15-21   | 7 ± 4   | 2.0 ± 0.7 | 138 ± 9  | 413 ± 29 |
| 2650T   | 3                 | 13-19   | 51 ± 12 | 2.1 ± 0.4 | 182 ± 9  | 560 ± 19 |

- **n**: Average survival response of 4 normal control strains as reported previously (24).
- **Mean ± S.E.
- **Normal strain used as matched control in this set of experiments.”

Parameters and abbreviations are defined in Table 1.
Radiosensitivity in AML Cancer Family

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 radio sensitive. 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 D10 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 (D10 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 γ-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 γ-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 γ-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.
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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 be radiosensitive could be explained by the presence of some putative DNA repair defect which leads to the persistence of a small number of strand openings (undetected by the in vitro enzymatic assay; Chart 5). Such strand openings could provide the opportunity for an increased incorporation of viral DNA and in turn increase the chance for host cell transformation (19).

Further investigation in vitro of this family may help to understand some of the genetic factors that determine cancer proneness in humans. The study reported here does not provide direct evidence for a defect in DNA repair in the strains 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 "leukemia factor" was not present and therefore not a contributing influence to sensitivity to gamma irradiation of skin fibroblasts in Friedreich's ataxia. Lancet, 2: 474–475, 1979.

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