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 radioresponse. 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 AT3 (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, 13, 31); 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 tuberculosis (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, 13, 31), conferred an altered susceptibility to an environmental agent such as radiation. This report describes our assessment of the 60Co γ-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)].

MATERIALS AND METHODS

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° in humidified 5% CO2 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 Mycoplasma contamination by the Hoechst staining method (4) and a double-label method measuring the incorporation of [6-3H]thymidine; D0, dose in rads reducing survival to 10%; D10, dose in rads reducing survival by 63% in the exponential region (i.e., inverse of the slope of exponential region).
FdUrd, rinsed, and covered with Hanks' balanced salt solution; (b) γ-irradiated in a Gammatron 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/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 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°C 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°C 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 Dₐ₀ 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 D10 values than the mean D10 values 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 D10 values after oxic irradiation reflects a reduced shoulder region in the survival curves without a significant change in the D0's. This can be interpreted as the reduced capacity of cell strains to accumulate sublethal damage in their DNA. The D10 parameter responds to both changes in the survival curve shoulder and changes in the exponential part of the curve (from which a D0 value is determined) and can therefore be a more sensitive parameter than D0 for assessing the radiosensitivity of a given human skin fibroblast strain.

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 members showed normal in vitro sensitivity. Further confirmation of this culture in each of the 4 experiments 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).

### Table 1

Parameters (calculated for each strain by least-squares linear regression analysis of pooled data) of oxic γ-ray survival curves for fibroblast strains from members of AML family

<table>
<thead>
<tr>
<th>Strain</th>
<th>Clinical description</th>
<th>No. of experiments</th>
<th>Passage range</th>
<th>PE0(%)</th>
<th>n</th>
<th>D0</th>
<th>D10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controlc</td>
<td>Normals</td>
<td>19</td>
<td>7–29</td>
<td>30 ± 7</td>
<td></td>
<td>2.0 ± 0.5</td>
<td>138 ± 13</td>
</tr>
<tr>
<td>GM38</td>
<td>Normal</td>
<td>6</td>
<td>13–23</td>
<td>33 ± 17</td>
<td></td>
<td>2.2 ± 0.6</td>
<td>132 ± 9</td>
</tr>
<tr>
<td>W38</td>
<td>Normal</td>
<td>6</td>
<td>29</td>
<td>35</td>
<td></td>
<td>1.8 ± 0.6</td>
<td>142 ± 12</td>
</tr>
<tr>
<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>
</tr>
<tr>
<td>1461T</td>
<td>Normal</td>
<td>6</td>
<td>13–18</td>
<td>21 ± 7</td>
<td></td>
<td>2.5 ± 0.7</td>
<td>130 ± 8</td>
</tr>
<tr>
<td>3151T</td>
<td>Normal</td>
<td>4</td>
<td>7, 12</td>
<td>36 ± 14</td>
<td></td>
<td>2.2 ± 0.4</td>
<td>126 ± 5</td>
</tr>
<tr>
<td>AT2BE</td>
<td>AT</td>
<td>2</td>
<td>13–17</td>
<td>6 ± 0</td>
<td></td>
<td>1.0 ± 0.3</td>
<td>73 ± 5</td>
</tr>
<tr>
<td>2649T</td>
<td>Carcinoma</td>
<td>4</td>
<td>10–18</td>
<td>17 ± 14</td>
<td></td>
<td>1.3 ± 0.2</td>
<td>117 ± 5</td>
</tr>
<tr>
<td>2650T</td>
<td>AML</td>
<td>5</td>
<td>6–23</td>
<td>45 ± 14</td>
<td></td>
<td>2.3 ± 0.7</td>
<td>136 ± 11</td>
</tr>
<tr>
<td>409T</td>
<td>AML</td>
<td>6</td>
<td>11–24</td>
<td>25 ± 7</td>
<td></td>
<td>1.6 ± 0.3</td>
<td>129 ± 6</td>
</tr>
<tr>
<td>2642T</td>
<td>AML</td>
<td>4</td>
<td>12–15</td>
<td>9 ± 2</td>
<td></td>
<td>0.6 ± 0.2</td>
<td>166 ± 17</td>
</tr>
<tr>
<td>2647T</td>
<td>Normal</td>
<td>2</td>
<td>11–15</td>
<td>37 ± 1</td>
<td></td>
<td>3.8 ± 2.4</td>
<td>114 ± 14</td>
</tr>
<tr>
<td>2648T</td>
<td>Normal</td>
<td>4</td>
<td>9–20</td>
<td>37 ± 9</td>
<td></td>
<td>1.9 ± 0.3</td>
<td>136 ± 6</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 D10 or D0 values for the experimental strain and the normal controls (mean values or GM38) differed significantly (p < 0.05).
- f The radioresistance of a strain was compared to that for normal controls using the standard error of difference test (17).

### Table 2

Parameters of hypoxic γ-ray survival curves

Cells from monolayer cultures were handled as for the oxic γ-ray survival experiments (see "Materials and Methods") except that cell suspensions were flushed with nitrogen for 15 min immediately prior to and during the irradiation. Parameters and abbreviations are defined in Table 1.

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of experiments</th>
<th>Passage PE (%)</th>
<th>n</th>
<th>D0</th>
<th>D10</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM38c</td>
<td>4</td>
<td>15–23</td>
<td>18 ± 8</td>
<td>1.7 ± 0.3</td>
<td>195 ± 9</td>
</tr>
<tr>
<td>AT2BE</td>
<td>5</td>
<td>6–20</td>
<td>3 ± 2</td>
<td>1.1 ± 0.2</td>
<td>89 ± 3</td>
</tr>
<tr>
<td>409T</td>
<td>5</td>
<td>12–18</td>
<td>30 ± 9</td>
<td>1.7 ± 0.6</td>
<td>157 ± 12</td>
</tr>
<tr>
<td>2642T</td>
<td>2</td>
<td>14–19</td>
<td>12 ± 7</td>
<td>1.6 ± 0.8</td>
<td>160 ± 17</td>
</tr>
<tr>
<td>2649T</td>
<td>3</td>
<td>15–21</td>
<td>7 ± 4</td>
<td>2.0 ± 0.7</td>
<td>138 ± 9</td>
</tr>
<tr>
<td>2650T</td>
<td>2</td>
<td>13–19</td>
<td>51 ± 12</td>
<td>2.1 ± 0.4</td>
<td>182 ± 9</td>
</tr>
</tbody>
</table>

- a Average survival response of 4 normal control strains as reported previously (24).
- b Mean ± S.E.
- c Normal strain used as matched control in this set of experiments.
- d Instances where D10 or D0 values for the experimental strain and the normal controls (mean values or GM38) differed significantly (p < 0.05).
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 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 y-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 y-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 y-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.
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.

ACKNOWLEDGMENTS

We wish to acknowledge the helpful comments offered by Dr. J. D. Childs, Dr. N. E. Gentner, and Dr. A. K. Myers in the preparation of this manuscript; the technical assistance of P. A. Knight and A. K. Anderson; and the helpful discussions with B. P. Smith and Dr. P. J. Smith.

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 radioresponse to be normal (D0 of 396 ± 9 rads compared to 416 ± 18 rads for controls). This suggests that the “leukemia (AG3778) which derived from a maternal aunt with breast cancer and a family history of leukemia. In: J. J. Mulvihill, R. W. Miller, and J. F. Fraumeni, Jr. (eds.), Genetics of Human Cancer, pp. 263–268. New York: Raven Press, 1977.


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