Normal Cytotoxic Response of Skin Fibroblasts from Patients with Li-Fraumeni Familial Cancer Syndrome to DNA-damaging Agents in Vitro


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ABSTRACT

Skin fibroblasts from patients with the Li-Fraumeni familial cancer syndrome have been reported to show abnormalities in their response to X-irradiation. We have examined the response of fibroblasts from affected and nonaffected individuals in three families to treatment with four DNA-damaging agents: X-rays, UV light, N-methyl-N’-nitro-N-nitrosoguanidine, and mitomycin C. Test cells along with additional cell bank control strains were received coded and blinded. The same strains were studied on 2 or 3 separate occasions; each of these groups was coded differently. The cytotoxic effects of the four agents were examined by a colony formation assay. Sensitivity to the induction of chromosomal aberrations by X-rays was also measured. In all cases, the response of cells from affected individuals did not differ significantly from that of cells from unaffected (not at risk) family members nor of cell bank controls. The response of somatic cells from members of Li-Fraumeni cancer families to DNA-damaging agents does not appear to be a fruitful approach to the detection of at-risk individuals.

INTRODUCTION

There has been considerable recent interest in the hypothesis that gene carriers for certain cancer-prone syndromes may be detected by the response of their somatic cells in vitro to DNA-damaging agents. The ability to identify individuals at increased risk for certain types of cancer has obvious implications for genetic counseling and in screening for the early detection and treatment of cancer. In addition, the association of hypersensitivity to DNA-damaging agents with enhanced susceptibility to cancer should aid in the elucidation of cellular and molecular mechanisms for these disorders.

In 1979, Blattner et al. (1) described a cancer-prone family which was ascertained through siblings with childhood cancer and which included a diversity of tumor types with multiple cases of soft tissue sarcomas, brain tumors, breast cancers, and leukemias in family members. A high frequency of second malignant neoplasms including spontaneous and radiation-related second tumors was observed. Study of γ-radiation cell survival in fibroblasts revealed a relative resistance to cell killing in affected and at-risk family members but not in their spouses or other controls (2). The clinical features of that family, including the range of tumor types, young age at onset, and occurrence of multiple primary tumors, was consistent with the familial cancer syndrome described by Li and Fraumeni (3–5).

In the present investigation, we have examined the response of skin fibroblast cell strains isolated from affected and nonaffected individuals in three additional families with the Li-Fraumeni syndrome to the cytotoxic effects of X-rays, UV light, mitomycin C, and MNNG. These agents were chosen as representative four classes of DNA-damaging agents. We also studied X-ray induced chromosomal aberrations in these individuals. The response of cells from affected and at-risk individuals did not differ significantly from that of cells from unaffected cases or cell bank controls studied in parallel experiments.

MATERIALS AND METHODS

Patient Ascertainment. Patients were identified from a survey of 3-year survivors of childhood soft tissue sarcoma treated at the University of Texas, M. D. Anderson Hospital, with diagnosis before 1976. Information on family history and current status of the patients was obtained by telephone interview from 159 of 163 eligible patients or their next of kin. All reported cancers in patients or their first or second-degree relatives were confirmed by medical records and/or death certificates.

The incidence and distribution of cancer in relatives of the 159 sarcoma patients were investigated by segregation analysis, as performed under a unified version of the mixed model (6, 7). This analysis revealed a low but significant degree of familial clustering of cancer. The most likely explanation of the data overall was provided by an autosomal dominant gene model as contrasted with a multifactorial model. Contrasting the likelihood of observing each pedigree under the autosomal dominant, sporadic, or chance model and multifactorial model revealed that the familial clustering of cancer was highly significant in 11 kindreds. In 9 of these kindreds, the distribution of cancer in relatives favored an autosomal dominant mode of transmission. The individual cancer sites in these kindreds were similar to those observed in the Li-Fraumeni syndrome, including tumors of soft tissue, breast, bone, and brain. From the 9 kindreds in which the cancer pattern suggested an autosomal dominant gene, we selected 3 for this study in which the proband and other relatives also had second malignant neoplasms suggestive of increased susceptibility to radiation-induced neoplasia. Pedigrees of these 3 kindreds are shown in Fig. 1.

A peripheral blood sample was obtained from each proband for karyotyping. No constitutional karyotypic abnormalities were observed. Skin biopsies were obtained as described below from probands of 3 different families and from affected, unaffected, and at-risk family members of 1 of the kindreds. By selecting some of the controls from unaffected branches of the family we were able to collect case and control samples at the same time and to minimize variation attributable to sample processing. As segregation analysis revealed that there was no evidence for non-Mendelian transmission of cancer predisposition or for residual familial aggregation of cancer in addition to that attributable to the autosomal dominant gene, spouses and their relatives represented ideal controls. In addition, other controls were derived from unaffected members of families ascertained for other genetic studies and from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository at the Coriell Institute for Medical Research.

Fibroblast Cell Strains. The cell strains studied along with pertinent clinical information are described in Table 1. Skin biopsies were obtained in the Genetics Clinic at the University of Texas M. D. Anderson Hospital in Houston or by local physicians according to the same protocol. The biopsy specimens were coded and sent immediately to the Coriell Institute for Medical Research in Camden, NJ, where fibroblast cell strains were established and X-ray-induced chromosome aberrations were studied. The cell strains were sent to the Harvard School of Public Health where the cytotoxicity assays were carried out. In 2 additional series of experiments, the cell strains were recoded at

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: MNNG, N-methyl-N’-nitro-N-nitrosoguanidine; EBSS, Earle’s balanced salt solution.
the Institute for Medical Research and sent to Harvard along with 4 similarly coded cell bank controls from the National Institute of General Medical Sciences Repository. All cell strains were regularly tested for Mycoplasma infection and found to be negative.

Cell Culture and Survival Assay. The cells were maintained in Eagle's minimal essential medium (GIBCO) with Earle's salt solution supplemented with 15% fetal bovine serum (Armour), 900 mg/l D-glucose, 0.66 mg/l sodium pyruvate, and 50 µg/ml gentamicin. Except where indicated, the same serum lot was used in all experiments. The cells were maintained at 37°C in a humidified environment of 5% CO2/95% air with regular medium changes. They were subcultivated at a 1:4 dilution when they became confluent. Cells to be used for experiments were suspended by exposure for 5 min to 0.25% trypsin in calcium and magnesium-free EBSS 3-4 days after subculture when they were in active growth. The cells were suspended in complete medium with serum, counted, and reseeded at low density into 3 replicate 100-mm Petri dishes (Costar) in appropriate numbers such that 30–70 viable colonies resulted per dish when the cloning efficiency and toxicity of the particular treatment were taken into consideration. In most experiments, dishes were seeded at 2 different cell densities for each dose level. The cell number never exceeded 40,000 per dish. The cells were treated with radiation or drugs 18–20 h after seeding and then returned to the incubator for 14–21 days to allow for colony formation. The colonies were then stained and those containing more than 50 viable-appearing cells scored as survivors. Surviving fractions were calculated, and the mean survival for each dose level pooled from 1-9 separate experiments was fitted to a straight line by linear regression analysis. The extrapolation number (α), the D<sub>0</sub> (inverse of the slope or dose necessary to reduce survival to 37% on the linear portion of curve), and the D<sub>10</sub> (initial dose necessary to reduce survival to 10%) were derived from the resultant survival.

Treatment Procedures for Survival Assay. Exposure to all agents was initiated 18–20 h after seeding at low density. X-irradiation was carried out either with a 100-kV Philips Industrial Generator operating at 10 mA and yielding a dose rate of 78 rads per min to the cells or with a GE MaxiMax X-ray Generator operating at 220 kV and 15 mA and yielding 80 rads per min to the cells. UV light irradiation was derived from a bank of 5 G8T5 GE germicidal lamps emitting primary 254 nm light that were housed in a specially constructed radiation chamber (8). Prior to irradiation, the medium was removed from each culture and the cells were rinsed with EBSS. Immediately following exposure at a dose rate of 0.28 J/m²/sec, the cultures were overlayed with fresh medium containing serum and returned to the incubator for colony formation.

Induction of Chromosomal Aberrations. Radiation-induced chromosomal aberrations were examined following irradiation of confluent fibroblast cultures with X-rays generated by a Varian Clinac linear accelerator operated at 4 MeV, yielding a dose rate to the cells of 300 rads/min. Cultures were grown and maintained as for the survival experiments. Replicate cultures were irradiated with 100, 200, or 400 rads, and suspensions of 10<sup>6</sup> irradiated cells were subcultured into appropriately labeled 75-cm² flasks (Lux). Cultures were incubated for 24 h and then were refed and reincubated for 20 h, at which time 0.25 µg Velban (GIBCO) was added to each flask to arrest cycling cells in mitosis. Metaphases were collected over the next 4 h for a total incubation period of 48 h. Cultures were then harvested and fixed using a method previously described by Nichols et al. (9). Slides were prepared, dried on a 56°C hotplate, and stained in 4% Giemsa (Harleco) for 5–7 min. Nonirradiated control cultures were handled in an identical fashion.

Metaphases were chosen as appropriate for scoring on the basis of quality in spreading, staining, and general morphology. Only cells with 44–46 chromosomes were scored. For each of the 8 cell strains from familial cancer syndrome cases, 100 cells were scored at doses of 0, 100, and 200 rads; 50 cells were scored at the 400-rad dose and at all doses for the 2 control cell strains. Aberrations scored consisted of chromatid breaks, fragments, triradials, quadriradials, chromosome breaks,acentric fragments, dicentrics, polycentrics, rings, and abnormal chromatid gaps, isocho- chromatid gaps, prematurely divided medium group chromosomes, and pulverized chromosomes were recorded but not scored as aberrations.

RESULTS

Three series of experiments were carried out to determine the cytotoxic effects of X-rays in skin fibroblast cell strains from cases and controls. The first was performed by J. Nove during the second half of 1984 when the cells were grown in Reheis serum (Armour Pharmaceutical Corp) lot 63206 and irradiated at 37°C with the Philips X-ray Generator. The second series was performed by W. Dahlberg in 1986; the cells were grown in Reheis serum lot 67604 and irradiated at ambient temperature with the GE MaxiMax Generator. In the first experiments, the cells were received coded with the University of Texas M. D. Anderson Hospital numbers shown in Table 1, whereas the second group was received coded with the Institute for Medical Research numbers WN 10 through WN 24 as shown in Table 2. A third smaller series involving 4 cell strains with the Institute for Medical Research code numbers WN 41
Table 1: Cell strains and clinical characteristics of patients and controls

<table>
<thead>
<tr>
<th>Cell strain</th>
<th>Clinical status</th>
<th>Family</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>172S</td>
<td>Affected</td>
<td>STS032</td>
<td>26</td>
<td>F</td>
<td>Rhabdomyosarcoma, age 3; malignant fibrous histiocytoma, age 16</td>
</tr>
<tr>
<td>174S</td>
<td>Affected</td>
<td>STS032</td>
<td>28</td>
<td>F</td>
<td>Rhabdomyosarcoma, age 3; malignant cystosarcoma phylloides of breast, age 20</td>
</tr>
<tr>
<td>041S</td>
<td>Affected</td>
<td>STS045</td>
<td>22</td>
<td>F</td>
<td>Embryonal rhabdomyosarcoma, age 2; osteosarcoma, age 14, arising in irradiated field</td>
</tr>
<tr>
<td>0875</td>
<td>Affected</td>
<td>STS204</td>
<td>24</td>
<td>M</td>
<td>Embryonal rhabdomyosarcoma, age 5; osteosarcoma, age 21</td>
</tr>
<tr>
<td>171S</td>
<td>At risk</td>
<td>STS032</td>
<td>26</td>
<td>M</td>
<td>Fraternal twin of 172S</td>
</tr>
<tr>
<td>170S</td>
<td>Nonaffected</td>
<td>STS032</td>
<td>52</td>
<td>F</td>
<td>Mother of 171S, 172S, and 174S</td>
</tr>
<tr>
<td>173S</td>
<td>Nonaffected</td>
<td>STS032</td>
<td>49</td>
<td>M</td>
<td>Sibling of 170S</td>
</tr>
<tr>
<td>175S</td>
<td>Nonaffected</td>
<td>STS032</td>
<td>78</td>
<td>F</td>
<td>Mother of 170S</td>
</tr>
<tr>
<td>GM1650</td>
<td>Cell bank control</td>
<td></td>
<td>37</td>
<td>F</td>
<td>Apparently normal</td>
</tr>
<tr>
<td>GM1652</td>
<td>Cell bank control</td>
<td></td>
<td>11</td>
<td>F</td>
<td>Apparently normal daughter of GM1650</td>
</tr>
<tr>
<td>GM495</td>
<td>Cell bank control</td>
<td></td>
<td>29</td>
<td>M</td>
<td>Clinically normal parent of child with low growth hormone level</td>
</tr>
<tr>
<td>GM2674</td>
<td>Cell bank control</td>
<td></td>
<td>29</td>
<td>F</td>
<td>Apparently normal</td>
</tr>
</tbody>
</table>

Table 2: Results of X-ray survival experiments

<table>
<thead>
<tr>
<th>Cell strain</th>
<th>Mean survival parameters (rads)</th>
<th>Cloning efficiency (%)</th>
<th>D10</th>
<th>D20</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDAH code</td>
<td>IMR code</td>
<td>Clinical status</td>
<td>Passage level</td>
<td>2-8</td>
</tr>
<tr>
<td>172S</td>
<td>WN14</td>
<td>Affected</td>
<td>7-8</td>
<td>29-54</td>
</tr>
<tr>
<td>174S</td>
<td>WN15</td>
<td>Affected</td>
<td>6-7</td>
<td>26-37</td>
</tr>
<tr>
<td>041S</td>
<td>WN20</td>
<td>Affected</td>
<td>4-6</td>
<td>37-46</td>
</tr>
<tr>
<td>0875</td>
<td>WN22</td>
<td>Affected</td>
<td>5-7</td>
<td>21-36</td>
</tr>
<tr>
<td>171S</td>
<td>WN10</td>
<td>At risk</td>
<td>6-7</td>
<td>34-55</td>
</tr>
<tr>
<td>170S</td>
<td>WN12</td>
<td>Nonaffected</td>
<td>6-10</td>
<td>15-40</td>
</tr>
<tr>
<td>173S</td>
<td>WN17</td>
<td>Nonaffected</td>
<td>7-10</td>
<td>1.2-3.1</td>
</tr>
<tr>
<td>175S</td>
<td>WN19</td>
<td>Nonaffected</td>
<td>6-8</td>
<td>4-10</td>
</tr>
<tr>
<td>WN11, 18, 22, 43</td>
<td>Control (GM 1650)</td>
<td>13-16</td>
<td>3-7</td>
<td>125</td>
</tr>
<tr>
<td>WN42</td>
<td>Control (GM 652)</td>
<td>14</td>
<td>11-18</td>
<td>147</td>
</tr>
<tr>
<td>WN16, 21</td>
<td>Control (GM 495)</td>
<td>13-14</td>
<td>4-10</td>
<td>84</td>
</tr>
<tr>
<td>WN13, 24</td>
<td>Control (GM 2674)</td>
<td>14-16</td>
<td>4-11</td>
<td>116</td>
</tr>
</tbody>
</table>

Results are mean of 3 or 4 separate experiments for M. D. Anderson Hospital strains (individual data are shown in Figs. 1 and 2) and 2 to 8 experiments for cell bank controls (data are pooled from separately coded samples).

Fig. 2. X-ray survival curves for skin fibroblast cell strains isolated from 4 affected individuals with the Li-Fraumeni familial cancer syndrome. Patients in A and B are from the same family. Three separate survival experiments were carried out for each strain; O, data from the first series of experiments; •, data from the second series (see text). Cloning efficiencies and survival curve parameters are shown in Table 2. A, 172S; B, 174S; C, 041S; D, 0875.
X-RAY SENSITIVITY OF LI-FRAUMENI SYNDROME CELLS

Table 3 Survival of cells following exposure to other DNA-damaging agents

<table>
<thead>
<tr>
<th>Cell strain (MDAH code)</th>
<th>UV light (J/m²)</th>
<th>Mitomycin C (µg/ml)</th>
<th>MNNG (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D₀</td>
<td>D₁₀</td>
<td>D₀</td>
</tr>
<tr>
<td>Cell strain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>172S</td>
<td>1.79</td>
<td>5.2</td>
<td>0.15</td>
</tr>
<tr>
<td>174S</td>
<td>1.90</td>
<td>5.3</td>
<td>0.21</td>
</tr>
<tr>
<td>041S</td>
<td>1.82</td>
<td>5.1</td>
<td>0.10</td>
</tr>
<tr>
<td>087S</td>
<td>1.72</td>
<td>4.8</td>
<td>0.15</td>
</tr>
<tr>
<td>171S</td>
<td>1.96</td>
<td>5.8</td>
<td>0.17</td>
</tr>
<tr>
<td>170S</td>
<td>1.87</td>
<td>5.1</td>
<td>0.12</td>
</tr>
<tr>
<td>173S</td>
<td>1.55</td>
<td>4.6</td>
<td>ND</td>
</tr>
<tr>
<td>175S</td>
<td>1.80</td>
<td>5.6</td>
<td>0.09</td>
</tr>
</tbody>
</table>

- Results are from 1 or 2 separate experiments. Passage levels ranged from 3 to 8. All drug exposures were for 1 h.
- MDAH, M. D. Anderson Hospital.
- ND, not done (cloning efficiency too low).

Table 4 Percentage of cells with radiation-induced chromosomal aberrations for individual cell strains

<table>
<thead>
<tr>
<th>Cell strain</th>
<th>Designation</th>
<th>Dose of X-ray (rads)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>GM1650</td>
<td>Control</td>
<td>6</td>
</tr>
<tr>
<td>GM2674</td>
<td>Control</td>
<td>6</td>
</tr>
<tr>
<td>170S</td>
<td>Not affected</td>
<td>5</td>
</tr>
<tr>
<td>173S</td>
<td>Not affected</td>
<td>4</td>
</tr>
<tr>
<td>175S</td>
<td>Not affected</td>
<td>2</td>
</tr>
<tr>
<td>171S</td>
<td>At risk</td>
<td>5</td>
</tr>
<tr>
<td>172S</td>
<td>Affected</td>
<td>30</td>
</tr>
<tr>
<td>174S</td>
<td>Affected</td>
<td>6</td>
</tr>
<tr>
<td>041S</td>
<td>Affected</td>
<td>2</td>
</tr>
<tr>
<td>087S</td>
<td>Affected</td>
<td>1</td>
</tr>
</tbody>
</table>

- Culture not scored owing to contamination.

Chromosomal aberration frequencies for the individual cell strains are shown in Table 4. For each patient a linear relationship between the arcsin-transformed proportion of the aberrant cells and dose was fitted, and the effect of the radiation was measured by the slope of the fitted line. Cells resistant to the chromosomal effects of radiation should show a smaller slope, while sensitive cells will have a larger slope. The slope derived from combining the cell bank controls with the nonaffected individuals (family controls) did not differ significantly (P > 0.05) from that of the affected individuals. Cell line 172S showed a base-line aberration frequency before irradiation of 30%, with a 10- to 20-fold excess of chromatid and chromosome type abnormalities. This patient received at least 1 cycle of vincristine, actinomycin D, and cyclophosphamide i.v. along with 5000 rads irradiation to the left knee in 1974. The source of the cell line was a biopsy taken from the right forearm in 1983. It is not uncommon for chromosomal aberrations to persist in fibroblasts for many years from therapy.⁴

DISCUSSION

Since the initial report (10) that skin fibroblasts derived from patients with Xeroderma pigmentosum are highly sensitive to the cytotoxic effects of UV light in vitro, there has been considerable interest in the hypothesis that somatic cells from other patients with a genetic predisposition to spontaneous or induced cancer might show abnormalities in their response to DNA-damaging agents. Such a phenomenon would not only allow the development of in vitro assays for gene carriers or at-risk individuals in a family, but also should aid in our understanding of mechanisms of cancer susceptibility. This hypothesis was given further impetus by the observations that fibroblasts from patients with ataxia telangiectasia were highly sensitive to cell

⁴ W. W. Nichols, unpublished observations.
killing by X-rays (11), whereas those from Fanconi anemia patients were sensitive to DNA cross-linking agents (12).

These differences in sensitivity to X-ray and chemically induced cell killing have often been correlated with an increased sensitivity to spontaneous and induced chromosome abnormalities. Thus, increased spontaneous chromosome abnormalities have been observed in the classic chromosome fragility syndromes ataxia telangiectasia, Bloom’s syndrome, and Fanconi’s anemia (13). All 3 have been reported to have increased frequencies of chromosomal aberrations induced by X-irradiation (13), and Fanconi’s anemia also has an increased chromosomal response to DNA cross-linking agents (14, 15). Patients with xeroderma pigmentosum have a normal incidence of spontaneous chromosomal aberrations and no increased response to X-irradiation, but they show an increased incidence of induced chromosome aberrations in responses to treatment with UV light and chemicals such as 4-nitroquinoline-1-oxide that involve long chain repair mechanisms (16, 17). The results with most other disorders, however, have proven disappointing and in some cases difficult to interpret. This owes largely to the fact that the degrees of sensitivity reported have been small and the findings difficult to reproduce among laboratories.

Weichselbaum and coworkers (18), for example, reported that cell strains from some patients with hereditary retinoblastoma showed a small but significant increase in their sensitivity to the cytotoxic effect of X-rays as compared with cell strains isolated from normal individuals or those with the sporadic form of the disease. Hereditary retinoblastoma patients are at increased risk for the induction of tumors, particularly osteogenic sarcomas, by radiation. The results of Weichselbaum et al. (18) have been confirmed in several laboratories (19–21) but not in others (22–24). In general, the discrepancy appears to arise because of a lower or broader range of sensitivities of normal control cell strains observed in some laboratories. Several investigators have reported that fibroblasts from ataxia telangiectasia heterozygotes are also characterized by a small but significantly increased hypersensitivity to the cytotoxic effects of X-rays (25, 26). However, others have reported that the response of A-T heterozygous cells does not differ significantly from that of a broad range of controls (27). Similarly, abnormalities in the response of cells from individuals with other cancer-prone disorders have proven to be small and/or difficult to reproduce (19, 28–31). Most of these studies have been carried out with cells derived from isolated patients with these various disorders, rather than from large families including affected, at-risk, and clearly unaffected individuals.

Recently, Bech-Hansen and coworkers (2) examined the X-ray sensitivity in vitro of skin fibroblasts isolated from various members of a family with the Li-Fraumeni familial cancer syndrome. They observed that cells from affected individuals were consistently more resistant to the cytotoxic effects of X-irradiation in vitro than were cells from nonaffected individuals. This type of study has the advantage that cell strains obtained under similar conditions from members of the same family can be compared in a “blinded” fashion in a single series of experiments. This protocol avoids the problems inherent in comparing results obtained from single individuals in various families, which may have been established and maintained under different conditions, to those of cell bank or historical controls.

The present study was therefore initiated to determine whether resistance to ionizing radiation in vitro is a general characteristic of cells from affected individuals with the Li-Fraumeni syndrome. We examined 6 cell strains from 1 family representing affected, nonaffected, and at-risk individuals, as well as cells from single affected individuals in 2 other families. As can be seen in Table 2, the cytotoxic response of cells from the affected individuals as a group was not significantly different from that of the nonaffected individuals, of the coded cell bank controls received simultaneously and examined in the same experiments, or of the normal control strains studied in other experiments in our laboratory over the past 2 years. There were also no detectable between-group differences in the sensitivity of these cell strains to the induction of chromosomal aberrations by X-rays (Table 4). Furthermore, as can be seen in Table 3, there was no systematic difference between the response of cells from affected and nonaffected individuals to 3 other DNA-damaging agents including UV light, the DNA cross-linking agent mitomycin C, and the monofunctional alkylating agent MNNG.

While we have not examined the specific cell lines found to be radiation resistant by Bech-Hansen et al. (2), the methods used in that study are generally comparable to those used here. Our failure to find radiation resistance under similar experimental conditions in cells from patients with similar clinical characteristics may be explained in at least 3 possible ways. First, there may be heterogeneity among the kindreds described as having the Li-Fraumeni syndrome. At present there is no consistent genotypic or phenotypic marker to identify individuals who carry this putative cancer-predisposing gene. Classification of kindreds is based largely on clinical characteristics. The families in this study were selected from a systematically ascertained series because there was evidence for segregation of an autosomal dominant cancer-predisposing gene, and there was evidence for possible susceptibility to radiation-induced second malignant neoplasms. Therefore, the failure to find evidence for an abnormal response to radiation in these selected patients suggests that “radiation resistance” is not the genetic mechanism undergoing the clinical characteristics of the familial syndrome of sarcoma, breast cancer, other diverse neoplasms, and frequent second malignant neoplasms.

Second, radiation resistance may or may not be attributable to a single gene and may be independent of the cancer-predisposing gene. Hence, increased resistance to X-irradiation may not be a general characteristic of cells from patients with the Li-Fraumeni syndrome but may occur in certain families unrelated to cancer proneness. This conclusion is reminiscent of the findings with Gardner’s syndrome: skin fibroblasts from affected members of 1 family were found to be slightly but significantly sensitive to killing by DNA-damaging agents in vitro (28), where affected individuals from 2 other families were normal in their response (29).

Third, differences in cultural and experimental conditions such as the particular serum lot used in our experiments may have masked the relatively small effect observed by Bech-Hansen and coworkers (2). This latter conclusion is reminiscent of the conflicting results reported for hereditary retinoblastoma and A-T heterozygotes described above. Although, as can be seen in Figs. 2 and 3, we found remarkable agreement in most cases between experiments carried out by different investigators at different points in time, unexplained variation in X-ray sensitivity does sometimes occur such as that we observed for multiple experiments with strain 170S from an unaffected patient (Fig. 3B) and with cell bank control strain GM 1650. The variation we have observed in cell sensitivity among strains from apparently normal individuals is not unlike that previously reported by Cox and Masson (32).

Our results indicate that a familial cancer predisposition resembling that described by Li and Fraumeni is not consis-
amently associated with an abnormal response of somatic cells to DNA-damaging agents in vitro. Given the wide range of normal variation, the unknown genetic control of this response, the difficulty in reproducing small differences in patient populations, and the conflicting results observed previously in ataxia telangiectasia heterozygotes and hereditary retinoblastoma patients, such studies do not appear to be a very fruitful approach to the detection of at-risk individuals in most families with genetic disorders predisposing to cancer. Furthermore, it seems unlikely that this approach will elucidate mechanisms of cancer proneness in general. More definitive genetic markers should be sought.

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REFERENCES

Normal Cytotoxic Response of Skin Fibroblasts from Patients with Li-Fraumeni Familial Cancer Syndrome to DNA-damaging Agents in Vitro


Cancer Res 1987;47:4229-4234.

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