Sister Chromatid Exchange Induced by X-Irradiation of Retinoblastoma Lymphocytes

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Lymphocyte cultures were employed to assess the degree of spontaneous and induced chromosomal fragility in retinoblastoma. Sister chromatid exchange (SCEs) were scored in metaphases. Three unilateral, three bilateral, eleven family members and controls were studied. Retinoblastoma (RB) lymphocytes did not exhibit increased spontaneous fragility. X-irradiation (25–200 rad) did not significantly increase SCE in unilateral retinoblastoma lymphocytes when compared with controls (P > 0.50). However, bilaterally affected subjects and three unaffected relatives demonstrated a statistically significant increase in SCE (P < 0.01). In conclusion, hereditary retinoblastoma lymphocytes appear more radiosensitive than sporadic retinoblastoma, perhaps, reflecting the increased second malignancies in germinal mutation retinoblastoma. In addition, the analysis of radiation-induced SCE in peripheral blood lymphocytes of RB patients and family members may provide a valuable tool increasing the accuracy of genetic counseling for this disorder. Additional studies of RB patients and families are needed to assess the relevance of this approach to genetic counseling. Invest Ophthalmol Vis Sci 25:698–702, 1984

This study was undertaken in order to assess the degree of spontaneous and induced sister chromatid exchanges in retinoblastoma patients and family members as compared with controls. Recent studies have suggested that retinoblastoma patients and family members are at higher risk for the development of second nonocular malignancies, the most prevalent of which is osteogenic sarcoma of the femur.1–7 Since many of these tumors occur outside the field of radiation therapy1,4,8,9,10 and in patients who never received radiation at all,2,5,11,12 we sought to determine if there exists an independent variable such as increased sister chromatid exchange, which might account for this occurrence. This study seeks to determine the potential applicability of the phenomenon of sister chromatid exchange (SCE) to the analysis of lymphocytes of retinoblastoma (RB) patients and family members.

It has been demonstrated that mammalian chromatin substituted with Bromodeoxyuridine (BrdU) can be differentially stained in cytological preparations, utilizing the fluorescent dye Hoechst 33258 followed by Giemsa.13–16 After two mitotic divisions in the presence of BrdU, the chromosomes contain one chromatid in which the DNA is unifiliarily substituted while the DNA of the second chromatid is bifiliarily substituted, the fully substituted chromatid being less intensely stained than the half-substituted one. This procedure provides a technique of high resolution for detecting exchange points between sister chromatids.

This study examined some of the characteristics of the SCE’s detectable in retinoblastoma lymphocytes exposed to several dosages of x-irradiation. A comparison was made between the number of SCE’s per cell in unilaterally affected retinoblastoma patients, those bilaterally affected, family members, and controls.

Materials and Methods

The population studied (Tables 1, 2) consisted of six patients; three unilaterally affected and three bilaterally affected, ranging in age from 4–40 years. For the purposes of this study, all bilateral retinoblastoma patients were presumed to be carriers of a germinal gene mutation, hence, hereditary; whereas, those unilaterally affected were considered representative of a sporadic mutation. Eleven family members were studied. None were found to have retinoblastoma. In addition, no evidence could be found for regressed RB lesions such as retinomas or retinocytomas in the population studied. To date, none of the subjects nor family members has been affected by sarcomatous tumors. Thirteen controls ranging in ages from 6–48 years were studied. Cultures of peripheral blood lymphocytes were established for the unilateral RB patients, those bilat-
erally affected, family members, and controls. Informed consent was obtained from all those studied.

Whole blood from all donors was grown in synthetic medium (GIBCO RPMI, media series 1640) to which BrdU (5 μg/ml, Sigma) and gentamycin (final concentration 0.1 μg/ml, Schering Diagnostics) were added upon initiation of the cultures. All cultures containing BrdU were kept in the dark to avoid photolysis of BrdU containing DNA. Because of the ability of BrdU to inhibit the enzyme ribonucleotide reductase, d-cyt (1 × 10⁻⁴ M, Calbiochem) was added to the cultures.

After being stimulated to divide by phytohemaglutinin, the lymphocytes were grown in culture for 72 hr. Cultures were harvested according to standard procedure after a 2-hr exposure to Colcemid (0.8 Mg/ml), treated with a hypotonic solution (1:1 KCl:Na citrate 0.4 g %) and fixed in 3:1 methanol:glacial acetic acid. The slides were air-dried and stained first with Hoechst 33258 (0.5 μg/ml) and then in 3% Giemsa solution (pH 6.8). In this way, SCE's could be detected by light microscopic examination of the preparations.

All irradiations were performed on cells that had completed at least one round of replication in BrdU. X-ray were produced by a cobalt-60 teletherapy unit at 1.2 MeV. The irradiation rate was 125 rads/min. Doses of 25, 50, 100, and 200 rads were administered.

Cytogenetic analysis was performed on 50 stained metaphases from each culture and SCE's scored. Sister chromatid exchanges are defined as reciprocal exchanges between sister chromatids, ie, a lightly stained area on one chromatid must correspond to a reciprocally darkly stained region on the sister chromatid. The following criteria were adhered to when scoring SCE's. The exchange of stain between sister chromatids was reciprocal in all SCEs scored. A terminal reciprocal exchange was scored as one SCE. Exchanges of stain occurring at the centromere were included as one SCE provided there were no obvious twists of the chromatids.

Results

Two metaphase preparations containing SCE's are shown in Figure 1.

The results obtained are summarized in Figure 2. An analysis of the experimental data, shows that x-irradiation did not significantly increase the rate of SCE in unilateral retinoblastoma lymphocytes when compared with controls (P > 0.50). In addition, there was no statistically significant difference in spontaneous SCE level between the unilaterally affected patients, those bilaterally affected, their family members and controls (P > 0.50). However, bilaterally affected subjects and three unaffected relatives (see Table 2) demonstrated a two- to three-fold increase in radiation-induced SCE that was dose dependent (P < 0.01).

Table 1. Population studied: patients

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>History</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>10</td>
<td>M</td>
<td>Bilateral RB-dx at 2 years OS* enucleated RT to OD†—3500 Rads</td>
</tr>
<tr>
<td>Patient 2</td>
<td>16</td>
<td>F</td>
<td>Bilateral RB-dx at 6 months OS enucleated RT to OD—3500 Rads</td>
</tr>
<tr>
<td>Patient 3</td>
<td>40</td>
<td>M</td>
<td>Bilateral RB-dx at 11 months OS enucleated RT to OD—4500 Rads</td>
</tr>
<tr>
<td>Patient 4</td>
<td>4</td>
<td>M</td>
<td>Unilateral RB-dx at 2 years Unifocal OD enucleated</td>
</tr>
<tr>
<td>Patient 5</td>
<td>13</td>
<td>M</td>
<td>Unilateral—RB involving 80% of retina of Unifocal vs Multifocal Dx at 2.5 years—OD enucleated</td>
</tr>
<tr>
<td>Patient 6</td>
<td>11</td>
<td>F</td>
<td>Unilateral RB—Unifocal Dx at 6 months OS enucleated at 14 months</td>
</tr>
</tbody>
</table>

* OS = left eye; † OD = right eye.

Discussion

The use of radiosensitivity and of spontaneous or induced SCE as indicators of an underlying defect in DNA replication/repair is controversial. This is apparent from a review of the relevant literature that contains studies leading to conflicting conclusions.

Knight et al, in a previous study employing mixed lymphocyte cultures to assess the degree of spontaneous chromosomal fragility in RB patients and families, found that there was no statistically significant differ-

Table 2. Population studied: family members

<table>
<thead>
<tr>
<th>Family</th>
<th>Relationship to patient</th>
<th>Age (yr)</th>
<th>Pertinent history</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>mother</td>
<td>29</td>
<td>unaffected</td>
</tr>
<tr>
<td>1</td>
<td>father</td>
<td>32</td>
<td>unaffected*</td>
</tr>
<tr>
<td>1</td>
<td>paternal uncle</td>
<td>37</td>
<td>unaffected*</td>
</tr>
<tr>
<td>2</td>
<td>mother</td>
<td>39</td>
<td>unaffected*</td>
</tr>
<tr>
<td>2</td>
<td>father</td>
<td>51</td>
<td>died of metastatic lung cancer</td>
</tr>
<tr>
<td>3</td>
<td>daughter</td>
<td>5.5 mos</td>
<td>unaffected</td>
</tr>
<tr>
<td>4</td>
<td>sister</td>
<td>11</td>
<td>unaffected</td>
</tr>
<tr>
<td>4</td>
<td>mother</td>
<td>37</td>
<td>unaffected</td>
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<tr>
<td>4</td>
<td>father</td>
<td>41</td>
<td>unaffected</td>
</tr>
<tr>
<td>5</td>
<td>father</td>
<td>48</td>
<td>unaffected</td>
</tr>
<tr>
<td>6</td>
<td>father</td>
<td>44</td>
<td>unaffected</td>
</tr>
</tbody>
</table>

* Unaffected relatives with increased SCE after exposure to x-irradiation.
ence in the occurrence of chromosomal breaks or translocations as compared with controls. These results may be interpreted as compatible with those obtained in this study; i.e., there was no statistically significant increase in sister chromatid exchange occurring spontaneously.

Fig. 1A, B. Metaphase preparations showing SCEs (representative SCEs indicated by arrows).
Cziezel et al.\(^{21}\) studied twelve retinoblastoma patients and found increased chromosomal aberrations, aneuploidy, and chromatid-type aberrations. The authors interpreted this data as reflecting increased fragility in somatic chromosomes of RB patients comparable with that present in patients with Fanconi's anemia, Bloom's syndrome, ataxia telangiectasia, and xeroderma pigmentosum, all of which are associated with an increased incidence of malignant disease.\(^{22}\) In addition, Chaganti et al. have reported that the frequency of SCE is markedly increased in Bloom’s syndrome, a recessively inherited disorder associated with a high incidence of chromatid breakage and malignant disease.\(^{23}\) It has been reported that SCEs occur during DNA synthesis in the presence of damaged DNA.\(^{24-26}\) This hypothesis may provide an explanation for the increase in SCE in cells exposed to exogenous agents such as ultraviolet (UV) light,\(^{27,28}\) the antibiotic 14 miotomycin, and other chemical mutagens,\(^{29}\) as well as Bloom’s syndrome.\(^{23}\)

Weichselbaum et al.\(^{30-32}\) have reported that skin fibroblasts from patients with germinal retinoblastoma appear to be more sensitive to the effects of x-rays than do fibroblasts from patients with sporadic RB or normal controls. The sensitivity was intermediate between that observed in Louis Bar Syndrome (ataxia telangiectasia) and normal controls. These results are supported by our findings of increased SCE induced by x-irradiation of hereditary retinoblastoma lymphocytes.

Ejima et al.\(^{33}\) studied the radiosensitivity of diploid fibroblast strains derived from fourteen retinoblastoma patients and five nonretinoblastoma patients with constitutional anomalies involving chromosome 13. Their findings did not show increased radiosensitivity in RB fibroblasts. Moreover, several of the RB cell lines tended to exhibit a degree of radioresistance. Cox and Masson\(^{34}\) in a similar quantitative study, on cell survival of human diploid fibroblasts exposed to ionizing radiation, expressed caution when interpreting the results obtained—especially when the cell survival was intermediate between normal controls and ataxia telangiectasia patients. These authors felt that the broad distribution of radiosensitivities of normal subjects as well as the intermediate increase in x-ray sensitivity noted in RB fibroblasts makes it difficult to distinguish the RB group from the upper limits of normal radiosensitivity. Arlett and Harcourt\(^{35}\) assayed γ-ray sensitivity for cell killing in 54 human cell strains including normals, ataxia telangiectasia patients, and RB patients. They found that cell strains from hereditary RB patients are more radiosensitive than those of the sporadic form, which could not be distinguished from controls. Their findings are in agreement with those reported by Little\(^{36}\) and also can be correlated with the results obtained in this study.

According to the somatic mutation theory of cancer, a somatic mutation in an already predisposed individual may lead to carcinogenesis.\(^{37,38}\) This may provide an explanation for the increased incidence of nonocular malignancies in hereditary retinoblastoma patients and family members.

In conclusion, it appears that hereditary retinoblastoma lymphocytes are more radiosensitive than sporadic RB lymphocytes perhaps reflecting the in-
increased susceptibility to malignancies in germlinal mutation RB patients and families. Although additional conclusions derived from this study would be premature at this time, it is tempting to speculate that the analysis of sister chromatid exchange induced by irradiation of retinoblastoma lymphocytes potentially may provide us with a marker for the detection of the RB gene carrier state, thus, offering a clinically useful tool in genetic counseling for this disorder. This technique may allow us to identify carriers of autosomal dominant retinoblastoma. Additional studies of larger patient populations are needed to elucidate this point.

Key words: retinoblastoma, sister chromatid exchange, x-irradiation, genetic counseling

References