An *In Vitro* Investigation of X-ray Sensitivity in Fibroblasts from Patients with Retinoblastoma

Ralph Weichselbaum,* Daniel M. Albert,† J. Robert Cassady,‡ and John D. Little§

In vitro x-ray survival experiments were performed on fibroblast strains derived from nine patients with sporadic unilateral retinoblastoma, 26 patients with hereditary retinoblastoma, and six normal controls. The x-ray sensitivity of the strains derived from the sporadic retinoblastoma patients and normal controls did not significantly differ from one another. The fibroblast strains derived from patients with hereditary retinoblastoma were significantly more radiosensitive to killing by x-rays as measured by clonogenic survival than either the sporadic strains or the strains derived from normal controls. We hypothesize that the increased in vitro radiosensitivity observed in some hereditary retinoblastoma strains is a reflection of an as yet uncharacterized defect in DNA or DNA replication postirradiation. Invest Ophthal Vis Sci 24:958–961, 1983

Retinoblastoma is a malignant eye tumor found almost exclusively in young children and occurs at a frequency of one per 18,000 births. Approximately 60% of all cases arise sporadically and are characterized by tumors that are usually unilateral and unilateral.1 In contrast, hereditary retinoblastoma is an autosomal dominant and comprises approximately 40% of all patients. Tumors are frequently bilateral and multifocal.5 Hereditary retinoblastoma patients are diagnosed at an earlier age than those with sporadic retinoblastoma (10 vs 24.2 months)1,2 and have an increased incidence of spontaneous second tumors (usually osteosarcoma). These individuals also have an elevated incidence of radiation-induced second tumors, when compared to that observed in patients with other childhood malignancies so treated.3–5 D-deletion retinoblastoma represents a third form of the disease and is associated with other characteristics of the D-deletion syndrome.6,7

Several genetic disorders associated with a predisposition to malignancy have now been identified in which cells are hypersensitive to killing by DNA-damaging agents. Conditions for which an increased cellular sensitivity as determined by clonogenic survival has been reported include xeroderma pigmentosum, ataxia telangiectasia, Cockayne syndrome, and Fanconi’s anemia.8–10 It is of interest that cells derived from patients with certain degenerative diseases not associated with a high incidence of malignancy (eg,
Table 1. In vitro X-ray sensitivity of diploid fibroblast strains from patient with sporadic and hereditary retinoblastoma

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
<th>Clinical classification</th>
<th>X-ray sensitivity (mean Do in rads)</th>
<th>Group Do (± 1 SDM)</th>
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* Published case reports. Abbreviations: MEEI, Massachusetts Eye and Ear Infirmary; HSPH, Harvard School of Public Health; IMR, Institute for Medical Research.

Huntington’s disease) also are hypersensitive to killing by x-rays.11

We report x-ray survival parameters on fibroblast strains derived from patients with hereditary and sporadic retinoblastoma. Hereditary retinoblastoma as a model for spontaneous and induced tumors is enhanced by the absence of abnormalities in the immune system that are associated with other clinical conditions that exhibit in vitro sensitivity to cytotoxic agents.9,12

Materials and Methods

Fibroblasts from patients with retinoblastoma and normal controls were established from punch biopsies or conjunctival snips. The medium routinely used to culture cells is Eagle’s MEM (Gibco) supplemented with 15% fetal calf serum (from Microbiological Associates), 900 mg/l of D-glucose, 0.66 mg/l of sodium pyruvate, and 50 μg/ml of gentamycin. Most cell strains derived by this method become senescent at
and of the survival curve. Do and $f_i$ are derived from the ordinate and is a measure of the shoulder region.

In radiation survival experiments, cells from exponentially growing stock cultures are removed with trypsin and plated at low density into 10 cm diameter culture dishes. Eighteen hours after plating, dishes were irradiated with a GE Maximar x-ray generator operating at 220 Kv and 15 mA, yielding a dose rate of 80 rad/min. Following irradiation, dishes were returned to incubator at 37°C with an atmosphere of 95% air and 5% CO$_2$. Fourteen to 21 days later dishes were fixed and stained with 0.75% methylene blue and examined under a low power dissecting microscope. Only colonies of 50 or more cells are scored as survivors. Cloning efficiency ranges from less than 1% to 44%. No correlation was observed between cloning efficiency and radiosensitivity. The calculated survival parameters were the Do and $f_i$. The Do is the inverse of the slope of the straight line portion of the x-ray survival curve and a measure of the radiosensitivity. The $f_i$ is the back extrapolate of the slope to the ordinate and is a measure of the shoulder region and of the survival curve. Do and $f_i$ are derived from least squared linear regression analysis of points above 100 rad. A typical radiation survival curve is shown in Figure 1.

Results

Table 1 summarizes results of in vitro x-ray survival experiments performed on fibroblast strains derived from nine patients with sporadic unilateral retinoblastoma, 26 patients with hereditary retinoblastoma (as determined by family history or diagnosis of bilateral tumors), and six normal controls. The mean Dos (the x-ray sensitivity) of the strains derived from the sporadic retinoblastoma patients and normal controls did not significantly differ from one another. They were $D_0 = 147 \pm 5$, respectively (one standard deviation of the mean). The fibroblast strains derived from patients with hereditary retinoblastoma were significantly more radiosensitive to killing by x-rays as measured by clonogenic survival ($D_0 = 113 \pm 3$) than either the sporadic strains or the strains derived from normal controls. In all cell lines extrapolation numbers are less than two and no significant difference was noted in this parameter between normal or retinoblastoma cell strains.

Discussion

In several previous reports we suggested that fibroblasts derived from patients with hereditary retinoblastoma strains is a reflection of an as yet uncharacterized defect in DNA repair or DNA replication post-irradiation. Such a defect may be present in all the somatic cells of an afflicted individual. Thus, these patients may be hypermutable and predisposed to developing cancer. This is consistent with Knudson's two "hit" hypothesis and also consistent with the observed incidence of spontaneous and radiation-induced tumors seen among patients with hereditary retinoblastoma.

We hypothesize that the increased in vitro radiosensitivity observed in some hereditary retinoblastoma strains is a reflection of an as yet uncharacterized defect in DNA repair or DNA replication post-irradiation. Such a defect may be present in all the somatic cells of an afflicted individual. Thus, these patients may be hypermutable and predisposed to developing cancer. This is consistent with Knudson's two "hit" hypothesis and also consistent with the observed incidence of spontaneous and radiation-induced tumors seen among patients with hereditary retinoblastoma.

As can be seen in Table 1, there is heterogeneity of radiosensitivity within the 26 fibroblast strains of hereditary retinoblastoma examined in our laboratory. This heterogeneity of response to a cytotoxic agent may be similar to the variation in in vitro response to UV light seen in the various complementation groups of xeroderma pigmentosum as well as the xeroderma pigmentosum variant. Thus, in vitro radiosensitivity studies as a prenatal or antenatal test to identify patients with retinoblastoma does not appear feasible at this time.

We are currently preparing to embark on a prospective trial to attempt to identify patients with hereditary retinoblastoma or patients at high risk for second tumors by studying x-ray survival experiments, as well as by prophase banding of chromosomes derived from fibroblasts and lymphocytes of affected individuals. We are also studying the sensitivity of these cells to x-ray-induced mutagenesis as well as attempting to correlate DNA repair at the cellular, chromosomal and molecular levels.

Key words: x-ray sensitivity, retinoblastoma, sporadic retinoblastoma, hereditary retinoblastoma, DNA repair

References


