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G₂-Phase Chromosomal Radiosensitivity of Primary Fibroblasts from Hereditary Retinoblastoma Family Members and Some Apparently Normal Controls

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Abstract

We previously described an enhanced sensitivity for cell killing and γ -H2AX focus induction after both high-dose-rate and continuous low-dose-rate γ irradiation in 14 primary fibroblast strains derived from hereditary-type retinoblastoma family members (both affected *RBI*^{+/-} probands and unaffected *RBI*^{+/+} parents). Here we present G₂-phase chromosomal radiosensitivity assay data for primary fibroblasts derived from these RB family members and five Coriell cell bank controls (four apparently normal individuals and one bilateral RB patient). The RB family members and two normal Coriell strains had significantly higher (~1.5-fold, $P < 0.05$) chromatid-type aberration frequencies in the first postirradiation mitosis after doses of 50 cGy and 1 Gy of ¹³⁷Cs γ radiation compared to the remaining Coriell strains. The induction of chromatid-type aberrations by high-dose-rate G₂-phase γ irradiation is significantly correlated to the proliferative ability of these cells exposed to continuous low-dose-rate γ irradiation (reported in Wilson *et al.*, *Radiat. Res.* 169, 483–494, 2008). Our results suggest that these moderately radiosensitive individuals may harbor hypomorphic genetic variants in genomic maintenance and/or DNA repair genes or may carry epigenetic changes involving genes that more broadly modulate such systems, including G₂-phase-specific DNA damage responses.

Introduction

Individual genetic variation in cellular DNA damage response pathways can influence DNA damage signaling thresholds, rates of DNA repair, and *in vitro* radiosensitivity after exposure to low-dose and low-dose-rate ionizing radiation (1). Such genetic variants presumably play an important role in determining an individual's predisposition to spontaneous and DNA-damaging agent-induced cancers (2–5), and sensitivity to the genotoxic effects of mutagens has been shown to be highly heritable in studies examining monozygotic and dizygotic twins

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and first-degree relatives (6–10). Significantly reduced DNA damage signaling and repair capacity has been documented *in vitro* for several cancer predisposition and chromosomal instability syndromes, including ataxia telangiectasia (AT), Nijmegen breakage syndrome (NBS), and LIG4 syndrome (11–13), while a broad spectrum of radiation responses has also been observed for cells derived from apparently normal individuals (1,14–17).

The induction of chromosomal aberrations after exposure to radiation or other genotoxic agents (both exogenous and endogenous) is mediated through the misrepair or lack of repair of DNA double-strand breaks (DSBs), which subsequently determines (to a great extent) the proliferative and carcinogenic potential of surviving cells (18–21). Several cytogenetic assays have been developed to measure radiation-induced chromosomal aberrations during different phases of the cell cycle for use as potential radiation and cancer biomarkers (22–25). One assay, the G₂ chromosomal radiosensitivity assay, has been developed to measure the response of cells (typically lymphocytes or fibroblasts) to irradiation in G₂ phase (26–29). This G₂ assay measures the yield of chromatid-type aberrations (mostly chromatid gaps and breaks) at the first postirradiation mitosis in cells irradiated hours previously during the G₂ phase. The repair of radiation-induced DSBs in G₂-phase mammalian cells occurs primarily through non-homologous end joining (NHEJ) and homologous recombinational repair (HRR) (20). The relative contribution of NHEJ and HRR to the repair of radiation-induced DSBs in G₂-phase human cells has not been fully established, although it is apparent that both repair pathways contribute to the repair of double-stranded DNA damage induced by radiation (19,30–32).

The G₂ assay has been applied to a number of radiation sensitivity and cancer predisposition syndromes, including AT, Bloom syndrome, dysplastic nevus syndrome, familial polyposis, Fanconi anemia, Gardner syndrome, Li-Fraumeni syndrome, Wilms tumor and xeroderma pigmentosum, as well as to patients with prostate, head and neck, breast and other types of cancer (27–29,33–42). The high frequencies of chromatid-type aberrations observed in cells derived from these patients have been attributed to deficiencies in DNA repair or related signaling pathways (27–29,43,44). However, the G₂ assay has demonstrated only moderate correlation to the degree of *in vivo* radiosensitivity observed in the clinic (40–42). G₂ hypersensitivity has been studied as a potential marker of heritable low-penetrance predisposition to cancer. Roberts *et al.* demonstrated G₂-phase hypersensitivity in 23 of 37 first-degree relatives of radiosensitive breast cancer patients compared to only one of 15 first-degree relatives of breast cancer patients with normal radiosensitivity (9). A recent large-scale examination of G₂ chromosomal radiosensitivity and postirradiation apoptotic responses of peripheral blood lymphocytes from 211 untreated breast cancer patients and 170 matched controls did not reveal any significant differences between the two groups for either end point, although it was suggested that both cases and controls with high familial risk of breast cancer were more radiosensitive (40).

Measurements of G₂-phase chromosomal radiosensitivity in retinoblastoma (RB) patients and first-degree family members were reported by Chaum *et al.* (45) and in two reports by Sanford *et al.* (26,46) using the radiomimetic agent bleomycin and radiation, respectively. The spontaneous and bleomycin-induced aberration frequencies in bilateral (hereditary-type) and unilateral (sporadic) RB lymphocytes reported in the study of Chaum *et al.* (45) did not differ significantly from those of normal control lymphocytes. Similarly, a retinoblastoma tumor cell line examined by Darroudi *et al.* (47) did not demonstrate an increased aberration induction after X irradiation in G₂ phase compared to normal untransformed fibroblasts. On the other hand, the first study of Sanford *et al.* reported a mean frequency of 3.2 chromatid breaks/cell (range 1.0–5.1) for eight bilateral (including one familial unilateral) RB fibroblast strains and 4.1 breaks/cell for a sporadic unilateral RB fibroblast strain compared to 0.4 breaks/cell (range 0.2–2.0) for 29 normal fibroblast strains after an X-ray dose of 53 cGy (26). In the second study of Sanford *et al.*, the authors reported mean frequencies of 2.2 breaks/cell (range 1.3–3.2) for

13 additional bilateral RB fibroblast strains, 0.7 breaks/cell (range 0.4–2.1) for six additional unilateral RB fibroblast strains, and 0.4 breaks/cell (range 0.3–0.5) for eight additional normal fibroblast strains after 53 cGy (46). Some of the unaffected first-degree relatives displayed high aberration frequencies (≥ 1.1 breaks per cell) similar to those of the bilateral RB patients. In a later study by Scott *et al.*, evaluation of the same collection of fibroblast strains demonstrated higher aberration frequencies in the normal strains (average of ~ 2.2 breaks/cell, range 1.1–3.3), different kinetics of repair, and more interexperimental variability, with 12 of the 53 normal strains (23%) being designated as moderately radiosensitive (33).

We previously described an enhanced sensitivity for cell killing and γ -H2AX focus induction after both high-dose-rate and continuous low-dose-rate γ irradiation in primary fibroblast strains derived from 14 hereditary-type (bilateral) RB family members for both the affected *RBI*^{+/-} probands and the unaffected *RBI*^{+/+} parents (1,48,49). In the present study, we present data on the G₂-phase chromosomal radiosensitivity of fibroblasts derived from these RB family members and five Coriell controls (four normal strains and one *RBI*^{+/-} strain) after acute doses of 50 cGy and 1 Gy high-dose-rate ¹³⁷Cs γ radiation. The relative radiosensitivity of the Coriell control strains examined in this study was established by previous survival and cytogenetic assays performed in this laboratory² (1,49,50).

Materials and Methods

Cell Culture and Irradiations

The protocol employed in this study was modified slightly from the standardized National Cancer Institute (NCI) and Paterson Institute for Cancer Research (PICR) G₂ chromosomal radiosensitivity assay protocol (28). The fibroblast strains used in this study (identified in Tables 1 and 2) have been described previously (1,48–50). All cultures were grown in α -MEM (GIBCO/Invitrogen) supplemented with 15% FBS, 100 μ g/ml streptomycin sulfate, 100 U/ml penicillin G (all Sigma), and 1 $\times$ GlutaMAXTM-I (L-alanyl-L-glutamine, Gibco/Invitrogen) in a 37°C incubator with 95% air/5% CO₂. A single lot of FBS was used in all experiments to reduce experimental variability. Cultures were routinely passaged prior to contact inhibition and maintained in exponential growth for a minimum of 5 days until sufficient cell numbers were available for experimental analysis. Cultures were prepared for the G₂ assay by replacing the culture medium with fresh medium 24 h prior to irradiation. Asynchronous cultures were irradiated at 37°C with 0 (sham) or 50 cGy or 1 Gy of 662 keV γ rays supplied by a J. L. Shepherd and Associates Mark I-68A cesium-137 beam irradiator at a dose rate of ~ 2.5 Gy/min.

Cytogenetic Preparations

After irradiation, the cultures were returned to the 37°C incubator and 0.1 μ g/ml colcemid (Gibco/Invitrogen) was added 30 min later. Ninety minutes postirradiation, the cultures were collected and processed by standard cytogenetic techniques (51). Briefly, the culture medium was transferred to conical tubes to ensure collection of any detached mitotic cells and the remaining adherent cells were detached with 0.25% trypsin/EDTA (Gibco/Invitrogen) in Ca²⁺/Mg²⁺-free PBS (Dulbecco's PBS-A). The single cell suspensions were centrifuged at 300g. After the supernatant was aspirated, the cell pellet was gently broken up, resuspended in 8 ml of 37°C 75 mM KCl hypotonic buffer, and incubated for 7 min at 37°C, after which 2 ml of freshly prepared 3:1 methanol:acetic acid (Carnoy's fixative) was added. The suspensions were incubated for 2 min at room temperature and centrifuged at 200g. After the supernatant was aspirated, the cell suspensions were then fixed by the dropwise addition of fresh fixative. After an additional centrifugation, the fixation process was repeated twice (for a total of three

²P. F. Wilson, Genetic factors affecting radiation sensitivity and genomic stability. Ph.D. dissertation, Colorado State University, 2006.

fixative rinses). Cell suspensions were dropped onto clean, wet microscope slides, air-dried and desiccated for 24 h at 37°C. Slides were stained with a 10% Giemsa solution (Gurr® R66, BDH Chemicals, Ltd.) prepared in a pH 6.8-buffered solution (Sorenson's buffer), washed vigorously in McIlvaine's rinsing buffer (approximately 18 mM citric acid/16 mM disodium phosphate prepared in distilled water) and distilled water, and air-dried. The slides were sealed with Cytoseal™ 60 mounting medium (Microm International) and a glass cover slip (VWR).

Chromosome Aberration Scoring

A total of 150–250 diploid metaphase spreads per dose were examined for each fibroblast strain in two or three independent experiments using a Nikon Microphot microscope equipped with a 100× oil-immersion objective and 2× optivar lens (strains GM04505 and GM08447 were examined in one experiment only). Only metaphase spreads with good chromosome morphology with minimal overlap and cytoplasmic interference were scored. The modal chromosome number for all strains was 46 and ranged from 45–47. The classification scheme used in this study for scoring chromosomal (chromatid-type) aberrations was similar to that described by Savage (52), Sanford and coworkers (26,46), and Scott and coworkers (9,29, 33–35). Chromatid gaps were defined as fully discontinuous chromatid fragments detached at a distance less than the width of the chromatid arm. Chromatid breaks were defined as fully discontinuous chromatid fragments that either were displaced at a distance greater than the width of the chromatid arm or were no longer aligned with the axis of the chromatid arm. Small achromatic chromatid fragments that did not appear to be completely severed from the chromatid arm were not scored. While some subjectivity in the scoring criteria for gaps and breaks exists among different research groups (53–55), the sum of chromatid gaps and breaks minimizes variation in scoring and interlaboratory comparisons. In this case, a single individual (PFW) scored the metaphase spreads on coded slides so the aberration scoring criteria were applied consistently. Symmetrical and asymmetrical chromatid-type exchanges were observed at much lower frequencies and were also scored.

Data and Statistical Analyses

The standard errors of the mean (SEM) reported in Tables 1 and 2 were calculated by combining the standard deviations of the aberration frequencies for two or three independent experiments by the square root of the sum-of-the-squares method and dividing this value by the square root of the number of experiments conducted per strain. Statistically significant differences between the aberration frequencies (chromatid breaks and total chromatid-type aberrations) for individual strains or groups of strains were tested using Student's *t* tests for two independent sample distributions (SigmaPlot, Systat Software, Inc.). Correlations between aberration frequencies and the continuous low-dose-rate survival parameters reported in ref. (1) were assessed using the Pearson product-moment correlation coefficient fit by the least-squares method (SigmaPlot).

Results

Tables 1 and 2 report the mean spontaneous and radiation-induced chromatid-type aberration frequencies ($\pm$ SEM) after irradiation in G₂ phase for the five Coriell control and 14 RB family member primary fibroblast strains examined in this study. The radiation-induced aberration frequencies in Tables 1 and 2 have been corrected for the spontaneous (0 cGy) levels of aberrations (chromatid-type exchanges were not observed in unirradiated cultures). Significant differences ($P < 0.05$ by Student's *t* test) in aberration frequencies between individual strains and the average for the three Coriell strains with normal radiosensitivity (AG01521, GM02149 and GM06419) are denoted with asterisks. The mean spontaneous chromatid break frequency was 0.12 per cell (range 0.07–0.17 per cell) and the mean spontaneous total chromatid-type aberration frequency was 0.4 per cell (range 0.3–0.6 per cell) for the 19 strains examined and

did not differ significantly among them. There were also no significant differences in the radiation-induced chromatid-type exchange frequencies among these strains (despite an approximately twofold increase observed in five of the 14 RB family member strains after 1 Gy).

The mean radiation-induced chromatid break and total chromatid-type aberration frequencies ($\pm$ SD) for groups of these strains are summarized in Table 3 (weighted per unit dose and averaged for the data for 50 cGy and 1 Gy). The strains are grouped by *RB1* genotype for the RB family members and by radiosensitivity group for the Coriell controls. The average radiation-induced chromatid break and total chromatid-type aberration frequencies for the 14 RB family member strains and the two radiosensitive Coriell strains were approximately 37–55% and 26–39% higher, respectively, than those measured in the three Coriell strains with normal radiosensitivity. Separating the RB family members by *RB1* genotype, the average radiation-induced chromatid break and total aberration frequencies were 44% and 34% higher, respectively, for the *RB1*^{+/+} strains and 37% and 26% higher, respectively, for the *RB1*^{+/-} strains compared to controls. The unaffected RB family member fibroblast strains had slightly higher aberration frequencies compared to the affected RB family member strains, although this difference was not statistically significant. The two radiosensitive Coriell strains and all of the RB family member fibroblast strains, except the affected proband MF-14R in RB family IV, had significantly higher radiation-induced chromatid-type aberration frequencies than the three Coriell strains with normal radiosensitivity. A histogram of total chromatid-type aberration frequencies averaged for the data for 50 cGy and 1 Gy is shown in Fig 1.

In Fig. 2A, the total chromatid-type aberration frequencies (spontaneous plus radiation-induced) measured for the doses in this study are plotted as a function of the dose rates required to reduce relative survival to 10% or 1% during continuous low-dose-rate irradiation (1). The low-dose-rate survival assay described in ref. (1) measures the proliferative capacity (colony-forming ability) of cells exposed continuously to low-dose-rate (0.5–8.4 cGy/h) ¹³⁷Cs γ radiation for 2 weeks and provides a means to accentuate minor differences in radiosensitivity observed after acute, high-dose-rate exposures. The continuous-irradiation dose rates required for 10% and 1% relative survival are significantly correlated with the G₂ assay aberration frequencies ($R^2 = 0.33$ – 0.39 for 50 cGy, $R^2 = 0.62$ – 0.64 for 1 Gy, $P < 10^{-8}$). No significant correlation between the spontaneous (0 cGy) aberration frequencies and survival after continuous low-dose-rate irradiation was observed ($R^2 = 0.05$). In Fig. 2B, the 19 fibroblast strains are identified individually using the same legend symbols from Fig. 1; this arrangement of strains is very similar for the data sets plotted in Fig. 2A. This correlation between high-dose-rate G₂-phase chromosomal radiosensitivity and the capacity of cells to proliferate under continuous low-dose-rate irradiation was not entirely unexpected. Previous studies from this laboratory reported that the inability of cells to proliferate during continuous low-dose-rate irradiation at higher dose rates (i.e., those approaching the proliferation-limiting low dose rate) correlated with cell cycle redistribution and checkpoint-induced arrest in the radiosensitive G₂ phase of the cell cycle² (56,57).

Discussion

The G₂-phase chromosomal radiosensitivity assay data presented here clearly support the assignment of a phenotype of moderate *in vitro* radiosensitivity in cells derived from these RB family members, as we reported previously using other radiobiological assays (1,48,49). G₂-phase irradiation of the three Coriell strains with normal radiosensitivity yielded mean values of ~ 3.2 chromatid breaks and ~ 7.3 total chromatid-type aberrations per cell/Gy. The RB family and the two radiosensitive Coriell strains had ~ 30 – 50% higher radiation-induced aberration frequencies, having mean values of 4.5–4.9 chromatid breaks and 9.5–10.1 total chromatid-type aberrations/cell/Gy, respectively. Interestingly, GM06419, a bilateral *RB1*^{+/-} strain from

the Coriell cell bank, demonstrated the lowest radiation-induced aberration frequencies of all the strains examined (~ 2.8 chromatid breaks and ~ 7.1 total chromatid-type aberrations/cell/Gy). A similar radioresistant response for GM06419 in the G₂ assay was reported by the group of Sanford (26,46) and was the only *RBI*^{+/-} strain examined in common with this study. These results provide further evidence that *RBI* mutation alone is not directly correlated with radiosensitivity.

The chromatid break frequencies in the three Coriell strains with normal radiosensitivity measured in this study are roughly four times higher than those reported for a large collection of apparently normal strains in the two reports by Sanford *et al.* (~ 3.2 breaks/cell/Gy for the current study compared to ~ 0.8 breaks/cell/Gy reported by Sanford *et al.*) (26,46). Scott *et al.* (33) re-evaluated the same collection of normal strains used by Sanford and coworkers and reported a higher average number of breaks (~ 2.2 breaks/cell/Gy) and a broader range (1.1–3.3 breaks/cell/Gy) for 53 normal strains. Their data are more comparable to this study and others (17). Despite these differences recorded for the normal strains, the radiation-induced aberration frequencies measured for the affected RB patients in this study (~ 4.3 breaks/cell/Gy, range 3.3–5.2) are consistent with the average of 4.4–6.4 breaks/cell/Gy reported by Sanford *et al.* and Scott *et al.* There is some subjectivity regarding the scoring of chromatid “gaps” and “breaks” and even differences in the criteria adopted for distinguishing them, depending on the particular laboratory (53–55). While this could underlie some discrepancies in comparisons of G₂ assay results, the reason for the differences in aberration yields reported for the normal strains but not the RB patient strains in this study compared to the studies of Sanford *et al.* is not immediately obvious.

Recently, this laboratory developed an extension of the G₂ chromosomal radiosensitivity assay in which γ -H2AX foci are detected immunocytochemically and measured on cytocentrifuged metaphase chromosome spreads (49). Phosphorylation of histone variant H2AX on serine 139 at sites of DSBs by the DNA-PK, ATM and ATR protein kinases produces cytologically visible γ -H2AX foci and provides a sensitive measure of DSB induction and repair at low radiation doses (58,59). It should be noted that G₂-phase/mitotic cells have 4N DNA content and twice as many radiation-induced γ -H2AX foci per unit dose compared to G₀/G₁-phase cells (defined as having 2N DNA content), and approximately 20–50% of the initial radiation-induced DSBs are repaired in the 90 min required to perform the G₂ assay (60,61). Using the same irradiation and mitotic collection schedule used in this study, after a dose of 50 cGy, the unaffected RB parents had a mean of 29 radiation-induced γ -H2AX foci/cell (range of 20–40), the radiosensitive Coriell strains GM04505 and GM08447 both had 31 radiation-induced γ -H2AX foci/cell, and the group of Coriell controls with normal radiosensitivity had a mean of 20 radiation-induced γ -H2AX foci/cell (range of 14–26). Thus, for this DNA damage end point, the relative radiosensitivities were qualitatively similar to the results reported in this study. Not unexpectedly, the frequencies of radiation-induced γ -H2AX foci and chromatid-type aberrations after irradiation in G₂ phase in these strains are significantly correlated ($P < 10^{-8}$).

In conclusion, this study supports the utility of the G₂ chromosomal radiosensitivity assay as a means to assess the relative cellular radiosensitivity of cells compared to a group of validated “normally responding” controls and is predictive of relative cellular radiosensitivity in the continuous low-dose-rate irradiation colony formation assay we described previously (1). The significantly higher frequencies of chromatid-type aberrations in the unaffected parents of the RB families after irradiation in G₂ phase compared to the Coriell control strains suggests that low-penetrance or hypomorphic mutations may affect the integrity of DSB repair (either NHEJ or HRR) or associated cell cycle checkpoint pathways in these cells and possibly underlie their radiosensitivity phenotype. Alternatively, epigenetic changes in genes that could more broadly affect genome maintenance, including the capacity to process radiation-induced DNA damage,

may also underlie the mild hypersensitivities we have observed (Liu *et al.*, unpublished results). If such mild genomic maintenance defects likewise affect germline mutation rates, such genetic polymorphisms or epigenetic changes could be important underlying factors in determining the frequency of genetic conditions predisposing to cancer, such as heterozygosity for *RB1* or other tumor suppressor genes relevant to the etiology of childhood cancers.

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FIG. 1.

Histogram of mean radiation-induced chromatid-type aberrations/cell/Gy (averaged for 50 cGy and 1 Gy) for the 19 primary fibroblast strains examined in this study using the G_2 chromosomal radiosensitivity assay.

FIG. 2.

Panel A: Plot of the dose rates (in cGy/h) required to reduce relative survival to 10% (filled symbols, heavier dashed line) and 1% (open symbols, lighter dashed line) in the continuous low-dose-rate irradiation colony formation assay (1) compared to the total chromatin-type aberration frequencies (spontaneous plus radiation-induced) measured after 0, 50 cGy and 1 Gy high-dose-rate irradiation in G_2 phase in this study. The low-dose-rate colony formation assay described in ref. (1) measures the proliferative capacity of cells continuously exposed to low-dose-rate (0.5–8.4 cGy/h) ^{137}Cs γ radiation for 2 weeks. Panel B: Identification of the individual fibroblast strains from panel A using the 50 cGy/1% low-dose-rate survival data set and the same legend symbols from Fig. 1; this arrangement of strains is repeated for each of the G_2 assay data sets for 50 cGy and 1 Gy.

TABLE 1
G₂ Assay Chromatid-Type Aberration Frequencies in the *RBI*^{+/+} and *RBI*^{+/-} Coriell Control Fibroblast Strains

Spontaneous and radiation-induced chromatid-type aberrations/cell ^b (mean ± SEM)						
Fibroblast strain	Sex	Age	<i>RBI</i> genotype	No. of experiments ^a	Dose	
						Chromatid breaks Chromatid gaps Chromatid exchanges Total aberrations
AG01521	M	3 days	+/+	3	0	0.16 ± 0.09 0.30 ± 0.10 0 0.46 ± 0.13
					50 cGy	1.57 ± 0.28 1.66 ± 0.27 0.01 ± 0.01 3.23 ± 0.39
GM02149	F	54 years	+/+	3	1 Gy	3.37 ± 0.38 3.37 ± 0.34 0.02 ± 0.02 6.76 ± 0.51
					0	0.09 ± 0.06 0.26 ± 0.09 0 0.35 ± 0.11
GM04505 (radiosensitive)	F	20 years	+/+	1	50 cGy	1.80 ± 0.26 2.62 ± 0.25 0.01 ± 0.01 4.43 ± 0.36
					1 Gy	3.26 ± 0.36 4.15 ± 0.30 0.02 ± 0.01 7.43 ± 0.47
					0	0.07 ± 0.03 0.23 ± 0.05 0 0.30 ± 0.06
					50 cGy	2.57 ± 0.23* 3.16 ± 0.15 0 5.73 ± 0.27*
GM06419	M	1 year	+/-	3	1 Gy	5.71 ± 0.34* 4.75 ± 0.19 0.02 10.48 ± 0.39*
					0	0.08 ± 0.06 0.19 ± 0.08 0 0.27 ± 0.10
					50 cGy	1.32 ± 0.30 2.16 ± 0.28 0 3.48 ± 0.41
GM08447 (radiosensitive)	F	2 days	+/+	1	1 Gy	2.91 ± 0.41 4.31 ± 0.38 0.01 ± 0.01 7.23 ± 0.56
					0	0.10 ± 0.05 0.24 ± 0.05 0 0.34 ± 0.07
					50 cGy	2.06 ± 0.16* 2.87 ± 0.14 0.01 4.94 ± 0.21*
					1 Gy	4.55 ± 0.26* 4.11 ± 0.14 0 8.66 ± 0.30*

Note.

* indicates statistically significant difference ($P < 0.05$ using Student's *t* test) from the average response of the three Coriell strains with normal radiosensitivity (AG01521, GM02149, GM06419).

^a Approximately 150–250 metaphase spreads were scored per dose in two or three independent experiments.

^b Radiation-induced chromosomal aberration frequencies are background-corrected.

TABLE 2
G₂ Assay Chromatid-Type Aberration Frequencies in the *RB1*^{+/+} and *RB1*^{+/-} Retinoblastoma Family Member Fibroblast Strains

Spontaneous and radiation-induced chromatid-type aberrations/cell ^b (mean ± SEM)						
Fibroblast (RB family)	Sex	Age	<i>RB1</i> genotype	No. of experiments ^a	Dose	
						Chromatid breaks Chromatid gaps Chromatid exchanges Total aberrations
MF-2M (I)	F	33 years	+/+	3	0	0.09 ± 0.06 0.22 ± 0.09 0 0.31 ± 0.11
					50 cGy	2.43 ± 0.30* 2.99 ± 0.32 0.01 ± 0.01 5.43 ± 0.44*
MF-3R (I)	F	14 mos.	+/-	2	1 Gy	4.83 ± 0.48* 5.05 ± 0.35 0.02 ± 0.02 9.90 ± 0.59*
					0	0.11 ± 0.06 0.26 ± 0.07 0 0.37 ± 0.09
MF-4F (I)	M	33 years	+/+	2	50 cGy	1.93 ± 0.22* 2.51 ± 0.17 0.01 ± 0.01 4.45 ± 0.28*
					1 Gy	4.26 ± 0.35* 4.97 ± 0.29 0.04 ± 0.04 9.27 ± 0.46*
MF-6F (II)	M	24 years	+/+	2	0	0.08 ± 0.05 0.24 ± 0.07 0 0.32 ± 0.09
					50 cGy	1.66 ± 0.24 2.40 ± 0.19 0.01 ± 0.01 4.07 ± 0.34
MF-7R (II)	M	14 mos.	+/-	3	1 Gy	3.80 ± 0.33* 4.67 ± 0.26 0.03 ± 0.01 8.47 ± 0.42*
					0	0.12 ± 0.07 0.45 ± 0.12 0 0.57 ± 0.14*
MF-10R (III)	M	4 mos.	+/-	2	50 cGy	2.22 ± 0.28* 2.45 ± 0.25 0 4.67 ± 0.38*
					1 Gy	4.99 ± 0.49* 4.92 ± 0.36 0.01 ± 0.01 9.92 ± 0.61*
MF-11F (III)	M	41 years	+/-	2	0	0.12 ± 0.09 0.35 ± 0.12 0 0.47 ± 0.15
					50 cGy	1.88 ± 0.30* 2.43 ± 0.29 0.01 ± 0.01 4.32 ± 0.42*
MF-12M (III)	F	36 years	+/+	3	1 Gy	4.60 ± 0.48* 4.09 ± 0.39 0.01 ± 0.01 8.70 ± 0.62*
					0	0.08 ± 0.05 0.31 ± 0.09 0 0.39 ± 0.10
MF-11F (III)	M	41 years	+/-	2	50 cGy	2.64 ± 0.26* 2.29 ± 0.19 0.01 ± 0.01 4.94 ± 0.32*
					1 Gy	5.07 ± 0.37* 4.75 ± 0.30 0.03 ± 0.02 9.85 ± 0.52*
MF-12M (III)	F	36 years	+/+	3	0	0.15 ± 0.08 0.27 ± 0.09 0 0.42 ± 0.12
					50 cGy	2.64 ± 0.34* 3.37 ± 0.30 0.02 ± 0.02 6.03 ± 0.45*
MF-12M (III)	F	36 years	+/+	3	1 Gy	4.58 ± 0.59* 5.07 ± 0.44 0.01 ± 0.01 9.66 ± 0.74*
					0	0.10 ± 0.07 0.35 ± 0.10 0 0.45 ± 0.12
MF-12M (III)	F	36 years	+/+	3	50 cGy	2.56 ± 0.31* 2.79 ± 0.24 0.02 ± 0.01 5.37 ± 0.39*
					1 Gy	5.53 ± 0.48* 4.80 ± 0.33 0.01 ± 0.01 10.34 ± 0.58*

Spontaneous and radiation-induced chromatid-type aberrations/cell ^b (mean ± SEM)									
Fibroblast (RB family)	Sex	Age	RB1 genotype	No. of experiments ^a	Dose	Chromatid breaks	Chromatid gaps	Chromatid exchanges	Total aberrations
MF-13M (IV)	F	23 years	+/+	3	0	0.17 ± 0.10	0.33 ± 0.12	0	0.50 ± 0.16*
					50 cGy	2.52 ± 0.35*	2.82 ± 0.28	0.01 ± 0.01	5.35 ± 0.45*
MF-14R (IV)	F	4 mos.	+/-	3	1 Gy	5.09 ± 0.51*	5.27 ± 0.37	0.01 ± 0.01	10.37 ± 0.63*
					0	0.18 ± 0.17	0.32 ± 0.11	0	0.50 ± 0.20*
MF-15F (IV)	M	24 years	+/+	3	50 cGy	1.53 ± 0.29	2.19 ± 0.28	0.01 ± 0.01	3.73 ± 0.40
					1 Gy	3.63 ± 0.47*	4.42 ± 0.38	0.01 ± 0.01	8.06 ± 0.60*
MF-16M (V)	F	35 years	+/+	3	0	0.15 ± 0.10	0.36 ± 0.12	0	0.51 ± 0.16*
					50 cGy	2.05 ± 0.35*	2.59 ± 0.33	0.02 ± 0.01	4.66 ± 0.48*
MF-17R (V)	F	2 years	+/-	3	1 Gy	4.63 ± 0.56*	4.90 ± 0.39	0.01 ± 0.01	9.54 ± 0.68*
					0	0.16 ± 0.09	0.33 ± 0.11	0	0.49 ± 0.14*
MF-18F (V)	M	45 years	+/+	3	50 cGy	2.09 ± 0.29*	2.62 ± 0.25	0.03 ± 0.02	4.74 ± 0.38*
					1 Gy	4.12 ± 0.46*	4.35 ± 0.34	0.04 ± 0.01	8.51 ± 0.57*
MF-17R (V)	F	2 years	+/-	3	0	0.11 ± 0.08	0.21 ± 0.09	0	0.32 ± 0.12
					50 cGy	2.09 ± 0.30*	2.51 ± 0.27	0.01 ± 0.01	4.61 ± 0.40*
MF-18F (V)	M	45 years	+/+	3	1 Gy	4.35 ± 0.46*	4.32 ± 0.33	0.04 ± 0.02	8.71 ± 0.57*
					0	0.07 ± 0.06	0.20 ± 0.08	0	0.27 ± 0.10
MF-18F (V)	M	45 years	+/+	3	50 cGy	2.11 ± 0.33*	2.85 ± 0.27	0.01 ± 0.01	4.97 ± 0.43*
					1 Gy	4.37 ± 0.47*	5.65 ± 0.35	0.01 ± 0.01	10.03 ± 0.59*

Note.

* indicates statistically significant difference ($P < 0.05$ using Student's t test) from the average response of the three Coriell strains with normal radiosensitivity (AG01521, GM02149, GM06419).

^a Approximately 150–250 metaphase spreads were scored per dose in two or three independent experiments.

^b Radiation-induced chromosomal aberration frequencies are background-corrected.

TABLE 3
Summary of G₂ Chromosomal Radiosensitivity Assay Data for the *RBI*^{+/+} and *RBI*^{+/-} Retinoblastoma Family Member and Coriell Control Fibroblast Strains

Group	No. of fibroblast strains	Radiation-induced chromatid breaks/cell/Gy (mean ± SD, range)	Radiation-induced chromatid aberrations/cell/Gy (mean ± SD, range)
<i>RBI</i> ^{+/+} retinoblastoma family members	8	4.54 ± 0.56* (3.56–5.33)	9.73 ± 0.80* (8.31–10.54)
<i>RBI</i> ^{+/-} retinoblastoma family members	6	4.33 ± 0.65* (3.35–5.18)	9.20 ± 1.06* (7.76–10.86)
All retinoblastoma family members	14	4.45 ± 0.59* (3.35–5.33)	9.50 ± 0.92* (7.76–10.86)
<i>RBI</i> ^{+/+} and <i>RBI</i> ^{+/-} Coriell controls with normal radiosensitivity	3	3.15 ± 0.34 (2.78–3.43)	7.28 ± 0.78 (6.61–8.15)
Radiosensitive <i>RBI</i> ^{+/+} Coriell controls	2	4.88 ± 0.77* (4.34–5.43)	10.12 ± 1.20* (9.27–10.97)

Note.

* indicates statistically significant difference ($P < 0.05$ using Student's *t* test) from the average response of the three Coriell strains with normal radiosensitivity (AG01521, GM02149, GM06419).