Published in final edited form as:

Radiat Res. 2012 January ; 177(1): 25–34.

Intrachromosomal Changes and Genomic Instability in Site-Specific Microbeam-Irradiated and Bystander Human-Hamster Hybrid Cells

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Abstract

Exposure to ionizing radiation may induce a heritable genomic instability phenotype that results in a persisting and enhanced genetic and functional change among the progeny of irradiated cells. Since radiation-induced bystander effects have been demonstrated with a variety of biological endpoints under both *in vitro* and *in vivo* conditions, this raises the question whether cytoplasmic irradiation or the radiation-induced bystander effect can also lead to delayed genomic instability. In the present study, we used the Radiological Research Accelerator Facility charged-particle microbeam for precise nuclear or cytoplasmic irradiation. The progeny of irradiated and the bystander human hamster hybrid (A_L) cells were analyzed using multicolor banding (mBAND) to examine persistent chromosomal changes. Our results showed that the numbers of metaphase cells involving changes of human chromosome 11 (including rearrangement, deletion and duplication) were significantly higher than that of the control in the progeny of both nuclear and cytoplasmic targeted cells. These chromosomal changes could also be detected among the progeny of bystander cells. mBAND analyses of clonal isolates from nuclear and cytoplasm irradiations as well as the bystander cell group showed that chromosomal unstable clones were generated. Analyses of clonal stability after long-term culture indicated no significant change in the number of unstable clones for the duration of culture in each irradiated group. These results suggest that genomic instability that is manifested after ionizing radiation exposure is not dependent on direct damage to the cell nucleus.

INTRODUCTION

For many years, the central dogma in radiobiology has been that the nucleus, specifically the DNA, is the principal target for the biological effects of radiation. After irradiation, the initial radiation-induced DNA damage is converted into a mutation or chromosomal aberration during subsequent DNA repair and is expressed by the irradiated cell and its progeny (1). However, in recent years, two phenomena, namely, radiation-induced bystander effects and the cytoplasm irradiation effect (also known as extracellular and extranuclear effects, respectively), have challenged this central dogma (2, 3). This raises the question

whether cytoplasmic irradiation or the bystander effect can also lead to delayed genomic instability.

Radiation-induced genomic instability is typically monitored in the surviving progeny of irradiated cells multiple generations after the initial exposure to ionizing radiation (4). An early report of the delayed effects of radiation exposure demonstrated reduced subcloning efficiencies and a higher frequency of late chromosome aberrations in the progeny of irradiated Chinese hamster V79 cells many generations after X-ray exposure (5). In addition to delayed reproductive death, radiation-induced genomic instability has been reported using a variety of end points, including karyotypic heterogeneity (6, 7), changes in mutation rates (8, 9), gene amplification (10), and micronucleus formation (11) in the progeny of irradiated cells. Genomic instability is a hallmark of cancer. It is well established that an important part of the cancer etiology lies in stepwise accumulation of genetic changes [reviewed in ref. (12)].

Chromosomal rearrangement is the best-characterized end point of radiation-induced genomic instability, and many of the rearrangements described are similar to those found in human cancers (1). Earlier studies on radiation-induced genomic instability, which were performed mainly by Morgan and coworkers, used a human-hamster hybrid cell line that contained human chromosome 4 (13–15). The ability to discriminate this particular chromosome using whole human chromosome fluorescence *in situ* hybridization (FISH) probes enabled them to follow changes in this chromosome over many cellular generations after treatment with ionizing radiation and other agents. Frequent examples of clonal changes involving amplifications, translocation and insertions with clear indications of recombinogenic processes contributing to this class of genomic instability were found (13–15). In human-hamster hybrid A_L cells containing a single copy of human chromosome 11, introduced by Waldren and coworkers, specific mutations in the chromosome can be quantified (16–18). The whole-chromosome FISH probe protocol has also been applied to A_L cells to monitor radiation-induced changes (19). In the present study, we took advantage of recently developed fluorescence-based cytogenetic protocols [multicolor banding (mBAND)] to evaluate intrachromosomal changes in the human chromosome 11 of human-hamster hybrid A_L cells as monitors of genomic instability. The development of multicolor banding FISH techniques (20) allows single chromosomes to be “painted” with a series of colored bands along the axis. Loss or rearrangement of the bands indicates an intrachromosomal aberration such as a pericentric inversion (misrepair of two breaks on different arms of one chromosome), paracentric inversion (misrepair of two breaks on a single chromosome arm), interstitial deletion or translocation (21).

To determine whether non-nuclear irradiation or the bystander effect can induce genomic instability, we combined a cell site-specific microbeam [which can carry out precise nuclear or cytoplasmic irradiation and which produced bystander cells and their progeny (22)], together with the chromosome analysis methodology (mBAND). Our results suggest that genomic instability after ionizing radiation exposure is not dependent on direct damage to the cell nucleus.

MATERIALS AND METHODS

Cell Culture and Irradiation Conditions

Human-hamster hybrid A_L cells containing a standard set of Chinese hamster ovary-K1 (CHO-K1) chromosomes plus a single copy of human chromosome 11 have been widely used in mutation assays in previous studies by our group (3, 23–26). The advantage of this cell line for mutation assays is that human chromosome 11 encodes the cell surface antigen CD59 that renders A_L cells sensitive to killing by a specific monoclonal antibody E7.1. In

the presence of complement, only cells mutant at the locus survive (17). Cells were maintained in Ham's F-12 medium supplemented with 8% heat-inactivated FBS, 100 mg/ml penicillin-streptomycin, and 2×10^{-4} M glycine at 37°C in a humidified 95% air/5% CO₂ incubator. The population doubling time is about 16 h for this A_L cell line.

Approximately 200 exponentially growing A_L cells in 2 µl volume were plated into dishes in medium containing 1 mM dibutyryl cAMP for 16–24 h to enhance cell spreading for irradiation (3). The special microbeam dishes are constructed by drilling a ¼-inch hole in the center of 60-mm-diameter non-tissue-culture dishes (24). A 3.8-µm-thick polypropylene film is applied using epoxy over the bottom of the hole, creating a miniwell that is then coated with Cel-Tak (BD Biosciences, Bedford, MA) to ensure cell attachment. The nuclei of attached cells were stained with a 100 nM solution of Hoechst 33342 dye 45 min before microbeam irradiation. The stained cells were targeted at the nuclear centroid in each cell with two α particles (nuclear irradiation), in the cytoplasm with eight particles (cytoplasmic irradiation), or in 20% of cell nuclei with 30 particles (bystander irradiation). Based on our previous studies (3, 27), the doses to either the nucleus or cytoplasm induced significant mutations at the CD59 locus of A_L cells. Also, in bystander irradiation, the dose of 30 particles is so high that targeted cells die and only the progeny of bystander cells survive (23). Similarly handled sham-irradiated cells were used as controls. The relevant dose of two particles to the nucleus and eight particles to the cytoplasm of one A_L cell is about 0.24 and 0.96 Gy, respectively (27). Cells were trypsinized 1 h after irradiation and pooled in a T-25 flask from each group or replated into 100-mm petri dishes at an appropriate density to facilitate selection of individual cell colonies. Metaphase cells from the pooled cell population from each group were collected 10 days after microbeam irradiation for cytogenetic analyses.

Chromosomal Changes in Short- and Long-Term Clonally Expanded Populations after Irradiation

Fifteen individual cell colonies for each group from one independent experiment were picked 7 days after irradiation and expanded for another 10 days, after which time the clonal population was divided in half. One half was cultured overnight, and metaphase cells were collected the following day for analysis of chromosomal instability (first turn analysis). The other half was stored at –80°C. Some of these frozen clones were later thawed and cultured for 40 days after irradiation before collecting metaphase chromosomes for second turn analysis.

Cytogenetic Analyses by mBAND Assay

1. Preparation of slides—The Calyculin A premature chromosome condensation (PCC) technique was used to collect the metaphase cells as described (28). Briefly, 1 day before the indicated time, the cells from four groups and each individual cell clone population were subcultured overnight. The cells were treated with Calyculin A (50 nM in DMSO) for 30 min in a CO₂ incubator at 37°C. After treatment with Calyculin A, cells detached spontaneously or were easy to detach from culture flasks. Prematurely condensed chromosome samples were prepared according to a conventional cytogenetic procedure. Briefly, cells were treated with a 75 mM KCl solution for 15 min at 37°C and fixed in an ice-cold 3:1 mixture of methanol:acetic acid for 30 min; this was repeated two or three times. After fixation the cells were either processed immediately or frozen at –20°C.

2. mBAND hybridization—The samples were dropped onto cleaned slides, air-dried and aged overnight before hybridization. The slides were pretreated for 90 s with pepsin to digest cytoplasmic proteins and then rinsed in 1× PBS. Each slide was postfixed for 10 min using 1% formaldehyde in 1× PBS + 50 mM MgCl₂, slides were rinsed in 1× PBS for 2 × 5

min, followed by 30 min in 2× SSC at 75°C for denaturation of chromosomes and allowed to cool to room temperature. After the transfer of slides to 0.1× SSC at room temperature for 1 min, chromosomes were denatured again in 0.07 N NaOH at room temperature for 1 min, followed by precooled incubation in 0.1× SSC and 2× SSC at 4°C for 1 min each. The samples were dehydrated sequentially in 70% and 100% ethanol for 3 min each and then air-dried. The denatured probe cocktail (Metasystems, Germany) was pipetted onto the denatured chromosomes, overlaid with a cover slip and sealed with rubber cement, and incubated in a humidified chamber at 37°C for a minimum of 2 days. Posthybridization washing steps with 1× SSC at 75°C for 5 min was followed by washing with 4× SSCT (4× SSC containing 0.05% Tween-20) at room temperature for 5 min. Blocking of nonspecific targets with provided milk buffer for 30 min, followed by application of biotin-labeled probes (Metasystems, Germany) with Cy5 for 30 min at 37°C. Slides were washed for 2 × 5 min in 4× SSCT, air-dried, mounted using DAPI/antifade (Metasystems, Germany), and overlaid with 24 × 60-mm² cover slips. Slides were stored at 4°C or –20°C prior to analysis.

Microscope analysis was performed using a Zeiss Axioplan 2 imaging fluorescence microscope (Zeiss, Germany) with a motorized stage and filter sets for FITC, Spectrum Orange, Texas Red, Cy5, DEAC and DAPI. At least 100 images were auto-captured, processed and analyzed using Isis imaging software (Metasystems, Germany).

Statistical Analysis

Significance of the data on the differences in the results for fractions of aberrant cells obtained from the irradiated and the control groups was determined using Student's *t* test. Significance of the distribution of human chromosome changes was determined using the χ^2 test.

RESULTS

MBAND Profile of Human Chromosome 11

Single and partial overlap of the five fluorochromes results in a continuous ratio of color along the length of chromosome 11. The imaging software assigns “pseudocolors” to sections of the chromosome with a similar color ratio to produce a reproducible pattern that is largely independent of chromosome length. Figure 1a and b shows the labeling scheme and the band profile of normal human chromosome 11. Cells were chosen for analysis only if at least 18 chromosomes were present in each spread. All suspected aberrations involving human chromosome 11 were confirmed by observation of the original fluorochrome pattern. Figure 1c–h shows band profiles of specific chromosomal rearrangements involving human chromosome 11 such as paracentric and pericentric duplication or inversion, insertion, interstitial or terminal deletion, and translocation.

Changes Involving Human Chromosome 11 in the Progeny of the Pooled Cell Population after Nuclear or Cytoplasmic Irradiation and in Bystander Cells

Progeny of surviving cells after direct nuclear or cytoplasm irradiation and nuclear irradiation-associated bystander cells were harvested 10 days after irradiation (approximately 15 population doublings) for cytogenetic (mBAND) analysis. The chromosomal changes involving human chromosome 11 rearrangements (e.g., translocation, duplication, paracentric or pericentric inversion, insertion, interstitial or terminal deletion) and whole human chromosome 11 deletion or duplication were observed in some aberrant metaphase cells. The distribution of those changes in analyzed metaphase spreads is shown in Table 1. Our mBAND experiments showed that deletion was the main aberration in the progeny of the irradiated pooled cell populations. The second highest intrachromosomal aberration was translocations. Insertions and inversions were seldom found in the progeny of

the pooled cell population. Nuclear irradiation induced more interstitial deletions than the other two kinds of irradiation. In contrast, cytoplasmic and bystander irradiations induced more centromeric deletions.

Figure 2 shows the fraction of aberrant cells with alterations to human chromosome 11 after irradiation. Compared to control, 14.5% of aberrant cells were induced 10 days after two α particles targeted to the nuclei, which was significantly higher than in the control cells (6.8%). There were 12.1% cells with aberrations in the bystander cells after 20% of the cell nuclei were targeted with 30 α particles, which was also significantly higher than in the controls. In this case, the targeted cells were killed, so the cell progeny were from the untargeted bystander cells. The results also showed that 9.7% of the aberrant cells were induced 10 days after cytoplasmic irradiation by eight α particles using a site-specific microbeam irradiation, which was significantly different from the control (6.8%). The data suggest that irradiation of the cytoplasm may induce genomic instability in the cell progeny. The above results indicate that genomic instability after ionizing radiation exposure is not dependent on direct damage to the cell nucleus.

Chromosomal Instability Induced in Clones after Nuclear Irradiation and Cytoplasm Irradiation and in the Bystander Cells

Investigation of chromosomal instability in clones of irradiated cells is another important end point to directly characterize the extent of the induced genomic instability. A minimum of 100 metaphases from 15 colonies were scored for rearrangements in human chromosome 11. Chromosomally unstable clones were defined as any clone derived from a single cell that showed at least three distinct metaphase subpopulations involving rearrangements of human chromosome 11 in which the rearrangements account for a minimum of 5% of the total metaphases scored, as described by Limoli *et al.* (29) and Marder and Morgan (13).

According to the above definition, there was no chromosomally unstable clone in the control group (Table 2), although we observed five clones showing two rearrangements in human chromosome 11 and two clones showing a higher ratio of rearranged metaphase spreads. In colony C7, 96.5% of metaphase spreads showed paracentric inversion plus interstitial deletion. In colony C10, 95.6% of metaphase spreads were duplication of p-arm subpopulation.

In the nuclear irradiation group, 91.2% of metaphase spreads were normal, with no rearrangement involving human chromosome 11 in clone N2 (Table 3). However, three rearranged populations were observed, consisting of 2.9% (interstitial deletions), 3.9% (reciprocal translocations), and 2.0% (terminal deletions). In clone N12, four rearranged subpopulations were found. Ninety-two percent of metaphase cells analyzed in clone N12 showed the q-arm deletion, and the remaining rearranged subpopulations included interstitial deletions, nonreciprocal translocations and paracentric duplications. Four types of rearranged subpopulations were also observed in clone N14, and the amount of rearranged metaphase cells was 6.4%. Four types of rearranged subpopulations were found in the clone N8; however, the metaphase cells involving human chromosome 11 rearrangement accounted for only 4.6%. Thus only three chromosomally unstable colonies were found within clonal isolates from the nuclear irradiation group.

Analysis of 15 clonal isolates from the bystander cells showed three unstable clones [Clones B5, B6 and B10 (Table 4)]. Three rearranged subpopulations, consisting of paracentric inversions (Fig. 1f, 93%), centromeric deletions (1%), and terminal deletions (1%), were observed in clone B5. A total of 7.2% of metaphase cells involving human chromosome 11 rearrangements occurred in clone B6, consisting of q-arm deletion subpopulation (0.9%), p-arm translocation plus single q-arm subpopulation (0.9%), q-arm translocation

subpopulation (2.7%), and centromeric deletion subpopulation (2.7%). The maximum number of types of subpopulations were found in Clone B10 among the 60 clonal isolates. The rearranged subpopulation included q-arm translocation (80.4%), partial p-arm translocation (5.9%), p-arm translocation (2.9%), paracentric duplication of p-arm (3.9%), terminal deletion (2.9%), piece of centromere (1%), and centromeric deletion (1%).

Table 5 shows the three unstable clones (Cy8, Cy9 and Cy13) that were detected in 15 clonal isolates from the cytoplasmic irradiation group. The main rearranged subpopulation in Clone Cy8 was q-arm deletion plus two insertions with the end part of q-arm to two hamster chromosomes (89.8%). The rest of the subpopulations were duplication of p-arm (3.7%), reciprocal translocation (0.9%), and translocation of p-arm terminal (0.9%). Clone Cy9 had three rearranged subpopulations [centromeric deletion (4.8%), non-reciprocal translocation (1.9%), and interstitial deletion (0.9%)].

The Variation of Chromosomal Instability after Short- and Long-Term Culture in Clonal Isolates after Radiation Exposure

The above analyses indicated that chromosomally unstable clones occurred after directed nuclear and cytoplasm irradiation as well as in bystander cells. However, the extent of chromosomal stability in both stable and unstable clones observed in the first round is uncertain with increasing culture time. Representative clonal isolates with higher or lower amounts of aberrant subpopulations in each group were chosen for culture for 40 days after irradiation for second-round analysis.

Table 6 shows that no chromosomally unstable clone was observed again in the control group after a longer time in culture. The original number of subpopulations for clone C15, one aberrant subpopulation (49.5% of rearranged metaphase cells) identified at the first round of analysis, was found to increase to five, and the fraction of rearranged cells decreased dramatically to 4.5% after the intervening 22-day period. In the nuclear irradiation group, the amount of the unstable clones did not change. However, both unstable clones became stable. Two of the stable clones, which had a higher ratios of rearranged metaphase cells, became unstable. However, clones B6 and B10, which were the progeny of the bystander cells, continuously showed unstable features after longer-term culture. In both, the number of the subpopulations increased (Table 6). For the cytoplasmic irradiation, one unstable clone remained unstable (Cy8) and two unstable clones became stable (Cy9 and Cy13). A stable clone (Cy7) became unstable after longer-term culture.

DISCUSSION

The development of the mBAND technique (20) allows for the identification of intrachromosomal exchanges, such as inversions, deletions or transpositions within a single painted chromosome, as well as interchromosomal exchanges or insertions, and translocations to unpainted chromosomes. The human-hamster hybrid A_L cells line has been used to study the radiation-induced bystander effect in our group for a decade (23–26). The PCR assay indicated that larger gene fragments of deletions or mutations in human chromosome 11 such as the APO-A1, CAT, WT and PTH genes were induced after nuclear or cytoplasmic irradiation (23), which demonstrated certain chromosomal rearrangements in human chromosome 11. Using the human chromosome as the basis for chromosomal instability analysis has shown that it is an elegant and efficient system (13).

The mBAND profile of the clone involving APO-A1 gene mutation, confirmed by PCR (23), showed q-arm deletion or nonreciprocal translocation of p-arm in the current mBAND experiments (Fig. 1c). The clone involving CAT, WT and/or PTH gene mutation checked by PCR generally showed reciprocal translocation. However, the profile of the mBAND

pseudocolor was normal in the mutant involving small gene fragment (e.g. CD59 gene) mutation that was previously confirmed by PCR. Thus different assays have advantages and disadvantages. Mutation assays can identify the small gene fragment mutation/deletion. mBAND can identify not only large gene fragment deletions but also intrachromosomal rearrangements (such as insertions or inversions).

Investigation of chromosomal instability among the clones allowed us to directly characterize the extent of induced genomic instability. It also helped us to determine whether chromosomal instability in cells in a population was mainly from the accumulation of a few clones in which the fraction of cells with chromosome aberrations is high or the result of a large number of clones in which the fraction of cells with chromosome aberrations are moderate. Table 5 indicates that the fractions of rearranged metaphase cells in the unstable clones (Cy9 and Cy13) were not all higher. Some stable clones also showed a higher ratio of rearranged metaphase cells (e.g., C7 and C10). The instability occurred soon after irradiation in some surviving colonies (e.g., N12 and B10) and persisted in subclones of this original survivor for several generations. In other surviving colonies (e.g., N4 and N11), genomic instability was not manifested for at least 27 generations and began to appear only after long-term culture (Table 6). The presence of chromosomal damage itself, however, was not always indicative of continuing instability. For example, clone B9 showed a q-arm deletion but was stable in the first-round analysis, was maintained in many generations, and showed no other type of chromosomal rearrangement in the second-round analysis. In contrast, some subpopulations in some unstable clones disappeared after longer-term culture, and those unstable clones became the stable clones. The variation analyses of clonal stability indicated that there were no significant changes in the number of unstable clones for the duration of culture in each irradiated group. This result is consistent with the report of Ponnaiya *et al.* indicating that chromosomal instability is a dynamic process and that the overall stability of a clone may change with time in culture after cellular irradiation (30). This observation may also explain the phenomenon of the relative fluctuation in radiation-induced genomic instability. The disappearance of some subpopulations may be due to the occurrence of apoptosis in the rearranged cells. The initial damage to cells after irradiation determines the increased fractions of chromosomal aberrations in cell populations since there is no significant change in the chromosomal stability of the clone over the time in culture.

There have been several studies that failed to observe chromosomal instability after both *in vitro* and *in vivo* exposure to ionizing radiation. The phenomenon of radiation-induced karyotypic instability has been studied extensively in the clonal descendants of irradiated murine (6, 31), hamster-human hybrid (13–15, 29) and human cells (4, 9). However, this phenotype has not been observed in some experimental settings (32–34) or in epidemiological studies (35–38). It was argued by Dugan and Bedford (34) that starting with cells that are tumor-derived or immortalized or are in some way abnormal in the processing of radiation damage may already provide the kind of precondition that may be necessary for radiation-induced instability. However, the kinds of karyotypic aberrations that are used in the criteria to classify the unstable clone subpopulation may need to be addressed. In addition, the kind of statistical test that is used to indicate the significance may be important (39).

How ionizing radiation induces this delayed chromosomal instability is not well understood. Exposure of cells to ionizing radiation results in a variety of DNA lesions, including DNA base alterations, DNA-DNA and DNA-protein crosslinks, and single- and double-strand DNA breaks (40). There is substantial evidence that the DNA double-strand break is the primary lesion involved in chromosomal rearrangements. On average, 40 double-strand breaks are induced by per Gy of X radiation (41). Some DNA double-strand breaks are

rapidly rejoined after radiation exposure (42), and the misrepaired damage is likely to contribute to the delayed chromosome instability observed. In addition, Mothersill *et al.* (43) showed that effects of genomic instability can be induced in surviving progeny by a wide range of genotoxic agents and other substances that cause oxidative stress. However, genomic instability was not induced by agents that induce unrepairable DNA damage (cisplatin) or by agents that poison the cell by preventing cytoskeletal assembly/disassembly (vincristine or the taxol derivative docetaxel). Limoli *et al.* (15), who conducted experiments with four different endonucleases and examined over 300 clones, failed to find any evidence of induced chromosomal instability. However, they found instability in their system using bleomycin, neocarzinostatin and ionizing radiation. Both authors agreed that DNA breaks without the generalized oxidative stress did not induce the phenotypic expression of persistent instability. The reverse situation of only oxidative stress without DNA damage, however, could not be tested since radical damage would induce damage to DNA (43).

Although the cytoplasm will be exposed prior to the α particle entering the nucleus and upon exiting when the nucleus is exposed, the biological effects induced by cytoplasmic exposure were significantly less than the nuclear exposure to same number of particles (3, 27). There is evidence that cytoplasm is another biological target after exposure to ionizing radiation (3). DMSO, a scavenger of ROS, significantly suppressed the induction of CD59 mutation induced by targeted cytoplasmic irradiation in A_L cells. It is possible that exposure of cellular cytoplasm to ionizing radiation results in a variety of lesions involving oxidation in cellular membrane lipoproteins and dysfunction in mitochondria (44). In this process, higher concentrations of ROS would be induced and some signaling factors would be activated. Higher concentrations of ROS and activated signaling factors could lead to the damage of DNA in cytoplasm-targeted cells. This could also be the case in bystander cells. Cells with chromosomal damage will undergo DNA repair or die by apoptotic or necrotic processes. Continuous free radicals and DNA-damaging molecules derived from dying cells elevate the level of chromosomal aberrations in the next generation of the cells. Taken together, our results show for the first time that genomic instability could arise in the progeny of both cytoplasm-irradiated and nuclear-irradiated cells as well as in the bystander cells. The extent of induced genomic instability after irradiation may be decided mainly by the initial radiation damage events.

Acknowledgments

We appreciate the RARAF staff of Columbia University for the cell irradiation for all experiments and Dr. Jarah Meador for the critical reading of the paper. This work was supported by NIH grants P01CA49062 and P41RR-11623 to CRG and the One hundred talent program from CAS (Y063020BRO) and 973 program from the Department of Chinese Science (2010CB834201) to BH.

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FIG. 1.

Labeling scheme (panel a) and profile (panel b; left: band of pseudocolor, right: five colors along the axis of chromosome) of normal human chromosome 11 mBAND as well as several specific rearrangements (panel c: deletion plus translocation; panel d: p-arm fourfold inversion duplication; panel e: q-arm isochromosomal duplication; panel f: paracentric inversion; panel g: pericentric inversion; panel h: p to q-arm duplication; panel i: amplification) observed in the progeny of irradiated cells.

FIG. 2.

Induced abnormal cells involving human chromosome 11 changes in the progeny of pooled survival cells 10 days after nuclear irradiation (two α particles targeted to the cellular nuclear centroid of each cell; Nuclei IR) and cytoplasm irradiation (eight α particles targeted to the cytoplasm of each cell; Cytoplasm IR) as well as bystander irradiation (20% of cell nuclei with 30 particles; Bystander). Here the changes include the rearrangements involving human chromosome 11 (translocation, paracentric or pericentric inversion, insertion, interstitial or terminal deletion, and duplication of partial fragment of human chromosome 11) and whole human chromosome 11 deletion or duplication.

TABLE 1
Distribution of Changes Involving Human Chromosome 11 in Analyzed Metaphase Spreads of Progeny of Irradiated and Control Cells

Group	Total no. of spreads	Changes within chromosome 11													Fraction of total changes		
		Deletion			Translocation			Duplication		Total changes		Whole chromosome change					
		terminal	interstitial	centromeric	p-arm	q-arm	p-arm	q-arm	reciprocal	insertion	paracentric	pericentric	Total changes	disomy		deletion	Total changes
Control	1015	1	3	2		17	13			3			39	3	27	30	0.068
Nuclear irradiation	441	1	8** (1)		1	21** (7)	8 (6)	2	1		6** (1)		48** (17)	2 (1)	14 (12)	16 (13)	0.145
Bystander irradiation	421	0		6** (1)		18** (7)	7 (5)	1		3 (1)			35** (16)		16 (11)	16 (12)	0.121
Cytoplasm irradiation	1175	4 (1)	4 (3)	10* (2)	1	31* (20)	16 (15)	1		2	3 (3)		72** (45)	5 (3)	37 (31)	42 (35)	0.097

Notes.
Numbers in parentheses indicates the expected aberration metaphase spreads according to the rule of Fisher's exact test.
* $P < 0.01$
** $P < 0.001$ (χ^2 test).

TABLE 2

Metaphase Populations with Rearranged Human Chromosome 11 in Colony Isolates from Control Group Cells

Clone	% aberrant cells ^a	% cells involving human chromosome 11 rearrangement ^b	No. of populations with rearrangements ^c
C1	2.3	0.8	1
C2	1.9	0.0	0
C3	2.5	0.0	0
C4	0.8	0.8	1
C5	2.6	0.9	1
C6	1.9	0.9	1
C7	98.0	96.6	2
C8	7.0	2.6	2
C9	6.6	1.9	2
C10	97.3	96.5	2
C11	4.0	2.0	1
C12	6.6	0.0	0
C13	7.9	2.0	2
C14	2.8	1.8	1
C15	52.5	49.5	1

^a Cells showing rearrangements, whole chromosome duplications, or deletion involving human chromosome 11.

^b Cells showing insertions, reciprocal and nonreciprocal translocations involving human chromosome 11, and partial deletions and duplications within human chromosome 11.

^c A rearranged population consisted of one or more metaphase cells showing the same rearrangement(s) of human chromosome 11.

TABLE 3

Metaphase Populations with Rearranged Human Chromosome 11 in Colony Isolates from Nuclear Irradiated Cells

Clone	% abnormal cells ^a	% cells involving human chromosome 11 rearrangement ^b	No. of populations with rearrangements ^c
N1	2.4	1.6	1
N2	13.2	8.8	3 ←
N3	4.4	0.0	0
N4	100.0	98.0	1
N5	17.6	14.3	2
N6	9.8	6.3	2
N7	2.9	2.0	2
N8	8.3	4.6	4
N9	0.0	0.0	0
N10	8.7	6.8	1
N11	100.0	98.2	1
N12	98.2	96.5	4 ←
N13	4.8	1.0	1
N14	12.7	6.4	4 ←
N15	2.9	1.0	1

Note. Arrow indicates the unstable clone depending on the definition described by Limoli.

^aCells showing rearrangements, whole chromosome duplications, or deletion involving human chromosome 11.

^bCells showing insertions, reciprocal and nonreciprocal translocations involving human chromosome 11, and partial deletions and duplications within human chromosome 11.

TABLE 4

Metaphase Populations with Rearranged Human Chromosome 11 in Colony Isolates from the Bystander Cells

Clone	% abnormal cells ^a	% cells involving human chromosome 11 rearrangement ^b	No. of populations with rearrangements ^c
B1	1.2	1.2	2
B2	1.0	1.0	1
B3	4.1	0.8	1
B4	0.8	0.8	1
B5	99.0	95.0	3 ←
B6	8.8	7.1	4 ←
B7	2.6	0.0	0
B8	3.3	0.0	0
B9	96.2	88.6	1
B10	98.0	98.0	7 ←
B11	6.1	2.6	3
B12	0.0	0.0	0
B13	2.7	0.9	1
B14	1.8	0.0	0
B15	2.8	0.9	1

Note. Arrow indicates the unstable clone depending on the definition described by Limoli.

^aCells showing rearrangements, whole chromosome duplications, or deletion involving human chromosome 11.

^bCells showing insertions, reciprocal and nonreciprocal translocations involving human chromosome 11, and partial deletions and duplications within human chromosome 11.

^cA rearranged population consisted of one or more metaphase cells showing the same rearrangement(s) of human chromosome 11.

TABLE 5

Metaphase Populations with Rearranged Human Chromosome 11 in Colony Isolates from Cytoplasmic Irradiated Cells

Clone	% abnormal cells ^a	% cells involving human chromosome 11 rearrangement ^b	No. of populations with rearrangements ^c
Cy1	1.8	1.8	1
Cy2	4.0	0.8	2
Cy3	4.7	0.9	1
Cy4	2.7	1.8	2
Cy5	3.6	0.0	0
Cy6	3.4	0.0	0
Cy7	4.0	2.0	1
Cy8	98.1	95.3	4 ←
Cy9	8.6	7.6	3 ←
Cy10	6.7	3.8	4
Cy11	1.0	0.0	0
Cy12	12.1	4.7	4
Cy13	8.6	8.6	6 ←
Cy14	6.7	1.9	2
Cy15	2.8	0.0	0

Note. Arrow indicates the unstable clone depending on the definition described by Limoli.

^aCells showing rearrangements, whole chromosome duplications, or deletion involving human chromosome 11.

^bCells showing insertions, reciprocal and nonreciprocal translocations involving human chromosome 11 and partial deletions and duplications within human chromosome 11.

^cA rearranged population consisted of one or more metaphase cells showing the same rearrangement(s) of human chromosome 11.

TABLE 6

Variation of Chromosomal Instability Induced in Cell Clones after α -Particle Irradiation

Clone	Short-term culture (~27 population doublings)		Long-term culture (~60 population doublings)	
	% cells involving human chromosome 11 rearrangement ^a	No. of populations with rearrangements ^b	% cells involving human chromosome 11 rearrangement ^a	No. of populations with rearrangements ^b
C12	0.0	0	0.0	0
C6	0.9	1	2.6	3
C15	49.5	1	4.5	5
C13	2.0	2	0.0	0
C7	96.6	2	97.0	1
C10	96.5	2	90.0	2
N10	6.8	1	1.0	1
N4	98.0	1	96.0	3 ←
N11	98.2	1	99.0	5 ←
N5	14.3	2	27.2	2
N8	4.6	4	0.0	0
N14	6.4	4 ←	2.0	1
N12	96.5	4 ←	99.0	1
B7	0.0	0	0.0	0
B8	0.0	0	1.9	1
B4	0.8	1	3.8	3
B9	88.6	1	96.0	1
B6	7.1	4 ←	10.0	6 ←
B10	98.0	7 ←	94.0	14 ←
Cy7	2.0	1	19.0	5 ←
Cy14	1.9	2	0.0	0
Cy9	7.6	3 ←	0.0	0
Cy10	3.8	4	1.0	1
Cy8	95.3	5 ←	100.0	5 ←
Cy13	8.6	6 ←	2.9	2

Note. Arrow indicates the unstable clone depending on the definition described by Limoli.

^aCells showing deletions, duplications, insertions, and reciprocal and nonreciprocal translocations involving human chromosome 11.

^bA rearranged population consisted of one or more metaphase cells showing the same rearrangement(s) involving human chromosome 11.