Published in final edited form as:

Radiat Res. 2011 September ; 176(3): 280–290.

Detection of Chromosomal Instability in Bystander Cells after Si490-Ion Irradiation

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Abstract

There is increasing evidence that two of the biological effects associated with low-dose ionizing radiation, genomic instability and bystander responses, may be linked. To verify and validate the link between the two phenomena, the ability of Si490 ions (high-energy particles associated with radiation risk in space) to induce bystander responses and chromosomal instability in human bronchial epithelial (HBEC-3kt) cells was investigated. These studies were conducted at both the population and single cell level in irradiated and nonirradiated bystander cells receiving medium from the irradiated cultures. At the general population level, transfer of medium from silicon-ion (Si490)-irradiated cultures (at doses of 0.073 Gy, 1.2 Gy and 2 Gy) to nonirradiated bystander cells resulted in small increases in the levels of chromosomal aberrations at the first division. Subsequently, single cell clones isolated from irradiated and bystander populations were analyzed for the appearance of *de novo* chromosome-type aberrations after ~50 population doublings using mFISH. Both irradiated and bystander clones demonstrated chromosomal instability (as seen by the *de novo* appearance of translocations and chromosomal fragments), albeit to different degrees, whereas sham-treated controls showed relatively stable chromosomal patterns. The results presented here highlight the importance of nontargeted effects of radiation on chromosomal instability in human epithelial cells and their potential relevance to human health.

Introduction

There is now a large amount of evidence supporting the nontargeted effects of exposure to ionizing radiation, that is, biological consequences that result not from direct traversals of particles through cells but rather occur as secondary events through the transmission of damaged signals from hit cells to neighboring, non-hit cells. This transmission of signaling can be mediated either through gap junctions in confluent, contacting cultures or through soluble mediators in distant, well-separated cells (1, 2). Two phenomena that are important in low-dose radiobiology are genomic instability and bystander responses.

Ionizing radiation-induced genomic instability has been hypothesized to be one of the key events in radiation-induced tumorigenesis. This has been inferred from the delayed appearance of mutations (3–5) and chromosomal aberrations (6–9) several cell generations

postirradiation. Since these changes appear with time, they could not have occurred at the time of irradiation but rather are thought to be a manifestation of genomic instability. In fact, it has been hypothesized that radiation-induced genomic instability is one of the earliest cellular events in the development of tumors after exposure to ionizing radiation (10–12). This was based on observations that differences in radiation-induced tumor susceptibility among different strains of mice correlated with radiation-induced chromosomal instability in mammary epithelial cells from these strains both *in vitro* (9) and *in vivo* (13). Similar correlations between tumor-sensitive strains and induction of chromosomal instability have also been reported for hematopoietic cells (14).

Radiation-induced bystander responses have been extensively documented [reviewed in ref. (1)]. They were first demonstrated after irradiation with very low fluences of α particles, where sister chromatid exchanges were seen in more cells than it was estimated could have been hit by an α particle (15, 16). These non-hit, responding cells were then “bystanders” of either directly hit cells or of energy depositions in extracellular medium. Similar types of experiments have also demonstrated the induction of mutations (17) and specific gene alterations in more cells than were estimated to have been hit by α particles (18). Other studies with similar findings have pointed to extracellular factors as being responsible for these effects, with reactive oxygen species being the most probable candidate (19, 20).

The other method used to study bystander responses has been the transfer of medium from irradiated cells to non-hit cells, which has resulted in enhanced cell death (21, 22), chromosome damage (23) and increased cell proliferation (24) in the nonirradiated populations. It has been suggested that irradiated cells release factors into the medium that result in the observed changes in the recipient cells. More definitive studies on the bystander effect have used a charged-particle microbeam. When only a few cells in a population were irradiated with a microbeam, levels of micronuclei and of apoptosis were found to be much higher than expected, i.e. demonstration of a bystander effect (25, 26). More direct evidence came from studies where irradiated and bystander cells were differentially stained and direct visualization of elevated levels of micronuclei in bystander cells was possible (27). Both mutation induction (28, 29) and oncogenic transformation (30) have been shown to be enhanced in bystander cells after microbeam irradiation of known proportions of cells in a population. These findings suggest that the bystander responses may contribute to radiation-induced tumorigenesis as well.

There have been some attempts to examine the link between the two phenomena and to determine whether bystander cells are also genomically unstable. Re-examination of initial data of chromosomal instability in hematopoietic stem cells (6) has suggested that the instability could be derived from nonirradiated cells. Others have used a shielding grid to alter the ratio of irradiated to nonirradiated cells and have shown that changing the ratio of hit to non-hit cells altered cell survival but not the number of clones expressing instability (31), which indicates that a bystander effect may be responsible, at least in part, for the expression of instability. Subsequent data have shown that chromosomal instability can be induced in bystander cells *in vivo* (32). Using a coculturing protocol, we have since reported on the ability of α particles to induce chromatid-type aberrations in bystander human fibroblasts (BJ1-htert) (33). In addition, when examined over 25 population doublings, the bystander populations had significantly higher frequencies of chromatid aberrations (two- to threefold higher) compared to controls.

Here we present data from experiments designed to investigate the ability of heavy ions to induce chromosomal aberrations in bystander cells immediately after irradiation and to determine whether chromosomal instability is seen in the irradiated and bystander cells as a function of time in culture.

The silicon ions used in these studies are one of several high-energy particles that are seen in space radiation (others include iron and carbon) and that present significant risk to space exploration. The ability of these high-energy particles to induce either genomic instability (34–36) or bystander responses (37–40) has been examined independently. However, to our knowledge, the studies reported here are the first to examine the link between these phenomena as a consequence of high-energy particle irradiation.

Materials and Methods

Cell Culture and Irradiation

h-TERT-immortalized human bronchial epithelial cells (HBEC-3kt) were received as a kind gift from Dr. Jerry Shay of the University of Texas Southwestern Medical Center in Dallas, TX. These cells were maintained in serum-free medium (keratinocyte-SFM supplemented with EGF and bovine pituitary extract, Invitrogen/Gibco) on collagen-coated flasks (Sigma). Confluent cultures in T-25 flasks were irradiated with 119 keV/ μ m silicon ions (490 MeV/nucleon) accelerated by the Heavy Ion Medical Accelerator in Chiba (HIMAC) at the National Institute of Radiological Sciences (NIRS) Chiba, Japan. The details of the HIMAC beam-delivery system, physical characteristics, biological irradiation procedures, and dosimetry have been described elsewhere (41, 42). The LET value was obtained by adjusting the thickness of the Lucite absorbers in front of the cell samples. Cells were irradiated with low fluences (50% of the cells remained untraversed by a particle), intermediate fluences and high fluences, which corresponded to 0.073 Gy, 1.2 Gy and 2 Gy, respectively. At the lowest fluence, where 50% of cells are unhit, ~35% of cells would receive one particle hit, ~12% two particle hits, and ~3% three particle hits. At 1.2 Gy and 2 Gy, 0.001% and 0.0001% of the cells remained unhit, respectively.

Sham-irradiated and irradiated flasks were returned to the incubator for 1 h, after which medium was removed, passed through a 0.2- μ m filter, and applied to nonirradiated cultures. One hour later, cells were trypsinized and reseeded onto 100-mm dishes for survival and single cell clone isolation or into T-25 flasks for chromosomal analyses at 24 h postirradiation.

Between 12 and 24 single cell clones were isolated from each population 14 days postirradiation. These clones were frozen down and five or six clones from each treatment population were randomly selected for expansion and further analyses.

Preparation of G₂ Prematurely Condensed Chromosomes and Giemsa Analyses

Control, bystander and irradiated populations were assayed for chromosomal aberrations at the first cell division postirradiation, i.e. at 24–27 h postirradiation. In addition, single cell clones were analyzed at ~45–50 population doublings postirradiation.

Cultures were treated with Calyculin A (50 nM) for 30 min. Cells were then collected and centrifuged and the pellet was resuspended in a small amount of medium (200 μ l). Cells were then treated with 0.075 M KCl for 11 min at 37°C. One milliliter of fixative (methanol:acetic acid 3:1) was added to the suspension before the cells were pelleted and resuspended in fixative. Cells were dropped onto clean slides and examined under a phase-contrast microscope for optimum separation of the chromosomes. Slides containing G₂ prematurely condensed chromosomes were stained for 10 min with 5% Giemsa in Sorensen's buffer (10 mM phosphate, 10 mM sodium citrate) and scored under light microscopy as detailed earlier (8).

Analyses of Single Cell Clones

Si3—These clones were derived from populations irradiated with 0.073 Gy. The medium was left on for 2 h, after which cultures were harvested and seeded for the isolation of colonies.

Si4—These clones were also derived from populations irradiated with 0.073 Gy, but the medium was changed 1 h postirradiation, and 2 h postirradiation cultures were harvested and seeded at low densities to isolate single cell clones.

Si8—These clones were isolated from populations that received 2 Gy. The medium was replaced 1 h postirradiation with fresh medium and cells were reseeded 1 h later to develop single cell colonies.

Si12—These clones were derived from nonirradiated populations that were treated with conditioned medium (from the population irradiated with 2 Gy) for 1 h prior to seeding cells for colony formation.

mFISH Analysis

G₂ prematurely condensed chromosomes were hybridized with the 24XCyte mFISH probe kit (MetaSystems, Altussheim, Germany) following the protocol of the manufacturer. Chromosomes were counterstained with DAPI/antifade-solution (MetaSystems). G₂ prematurely condensed chromosome spreads were viewed under an Olympus BX61 microscope (Olympus, Tokyo, Japan) equipped with six filter sets specific for the applied fluorochromes. Chromosome images were captured with a charge coupled device camera and the coordinates of the cells were recorded. Karyotyping was performed with the ISIS software (MetaSystems). A minimum of 25 cells were analyzed for each sample.

Determination of Instability by mFISH

A clone was determined to be unstable using criteria described previously (43) if at least 5% of the cells were aberrant and if the clone was made up of at least three different subpopulations. Although these criteria were described for a system only when the analysis was confined to a fraction of the diploid chromosome complement, we adopted the same criteria in the present study where we analyzed the entire chromosome complement by mFISH. The cell line, HBEC-3kt, employed in this study exhibited a spontaneous stable 16;5 translocation in all the cells. For this reason, wherever applicable, a cell with this 16;5 translocation will be referred to as normal. Only translocations and chromosomal fragments were used to determine instability. Chromosomal losses (and much less frequently, gains) were not factored into the assessment of instability.

Statistical Analysis

For chromosomal aberrations at the first division postirradiation, the frequencies in the irradiated populations at different doses were compared to those of the control population using the χ^2 test. Similar analyses were applied to compare the aberration frequencies between the bystander and control populations.

Clustered logistic regression was used to compare the chance of having chromosomal aberrations between the Si3 clones and control clones and between Si4 clones and controls. Since cells within an individual clone are likely to be correlated, clones were included into the model as the clustering factor.

Results

Chromosomal Aberrations at the First Cell Division Postirradiation

To determine whether irradiation with low fluences of Si490 ions could induce chromosomal aberrations in bystander cells either in the same population or via factors released into medium, cells were irradiated with 0.073 Gy (50% of cells presumably were not traversed by a particle), 1.2 Gy (all cells hit with a mean of 11 particles), or 2 Gy. One hour postirradiation, medium from irradiated flasks was transferred to nonirradiated cultures, and irradiated and bystander populations were assayed for chromosomal aberrations 24 h postirradiation. The results are shown in Figs. 1 and 2.

As expected, in the directly irradiated populations, there was an increase in the percentage of cells with chromosomal aberrations as a function of radiation dose (Fig. 1). While only 16% of the cells had chromosomal aberrations in the control population, frequencies in the irradiated populations ranged from 45% at the lowest dose to 80% and 100% at 1.2 Gy and 2 Gy, respectively. The differences between the irradiated populations and controls were found to be highly significant ($P < 0.0001$ for 1.2 Gy and 2 Gy, $P = 0.0035$ for 0.073 Gy).

In addition, there was an elevation in the percentage of cells with aberrations in the bystander populations that received medium from irradiated populations (Fig. 2). Transferring medium from control flasks had no effect (about 16% of the cells had aberrations in both populations), but medium from irradiated flasks resulted in a higher frequency of cells with chromosomal aberrations. This induction appeared to reach a plateau, with an increase between 0.073 Gy (22% of the cells with aberrations) and 1.2 Gy (30% of the cells) but no difference thereafter. While the frequencies of chromosomal aberrations in bystander populations were not found to be significantly different from those of controls by the χ^2 test (data not shown), they were consistently higher than the yields of aberrations in the control populations.

Chromosomal Aberrations in Single Cell Clones that Survived Exposure to 0.073 Gy

After chromosomal aberrations in irradiated and bystander populations were examined at 24 h postirradiation, single cell clones were analyzed for the presence of chromatid and chromosome-type aberrations about 50 population doublings postirradiation by Giemsa staining.

As can be seen in Fig. 3, between 6 and 11% of the cells in five different control clones had chromatid-type aberrations. Between 20 and 33% of the cells in the Si4 clones (clones that were derived from irradiated populations whose medium was removed 1 h postirradiation and transferred to nonirradiated, bystander populations; fresh medium was supplemented after 1 h) had chromatid aberrations (~3 times that of controls). All of these aberrations were of the simple type, including gaps and breaks. In contrast, only 12–20% of the cells in the Si3 clones (clones that were derived from populations that were irradiated with 0.073 Gy but the medium was not removed until the cells were harvested and seeded onto 100-mm dishes 2 h postirradiation) had chromatid aberrations (~2 times that of controls). When analyzed for chromosome-type aberrations (Fig. 4), both Si3 and Si4 clones had higher frequencies of cells with aberrations (10–18% and 22–27%, respectively) compared to controls (5–8% cells with aberrations). Again, Si4 clones had on average higher yields of aberrations than Si3 clones.

According to the logistic regression, the odds ratio for having chromosome aberrations between Si3 clones and controls was 2.44 ($P < 0.0001$), and that between Si4 clones and controls was as high as 4.97 ($P < 0.0001$). On the other hand, the odds ratio for having chromatid-type aberrations between Si3 clones and controls was 3.0 ($P < 0.0001$), and that

between Si4 clones and controls was as high as 4.38 ($P < 0.0001$). Based on these statistical analyses, both Si3 and Si4 clones were much more likely to develop both chromatid-type and chromosome-type aberrations in comparison to the controls, and the differences were highly significant.

mFISH Analyses of Single Cell Clones Isolated after Exposure to 0.073 Gy

The Giemsa analyses described above indicate that single cell clones from populations irradiated with 0.073 Gy had elevated yields of both chromatid-type and chromosome-type aberrations at about 50 population doublings postirradiation. Since Giemsa analyses can only detect gross chromosomal changes, mFISH was conducted on five clones to determine whether more subtle changes such as translocations were also present in these clones.

Karyotypes of one of these clones, Si4-C4, are presented in Fig. 5. In 18% of cells, fragments of chromosomes 9 and X were detected (panels A and B). Other fragments observed came from chromosome 3 (seen in 29% of cells) and chromosome 1 (in 8% of cells) (panel D). Twenty-four percent of the cells also had translocations between various chromosomes (panels C and E). Not a single cell analyzed in this clonal population had a “normal” karyotype.

mFISH painted G₂ prematurely condensed chromosomes from clone Si4-A1 are presented in Fig. 6. One particular translocation, t(15;14), was frequently observed (65% of cells, panels A–D), often with a t(X;5) translocation (24% of 65% cells, panel C) or some other translocation (22% of 65%, panel A) or chromosome fragments (12%, panels B and D). Significantly, 6% of the cells analyzed had a normal karyotype (panel E).

mFISH analyses of the five clones isolated after 0.073 Gy are summarized in Table 1. By definition, all five clones were chromosomally unstable, in that all of them had more than 5% aberrant cells and three or more subpopulations. Out of the five, three clones had varying extent of normal karyotypes (ranging from 6 to 25% of the cells analyzed in a particular clone) while the other two clones had 100% abnormal karyotypes.

mFISH Analyses of Irradiated and Bystander Single Cell Clones after Exposure to 2 Gy Si490 Ions

While it is true that in populations irradiated with 0.073 Gy, 50% of the cells were not traversed by a particle, it is not possible to definitively determine which of the above assayed clones were derived from irradiated cells and which were bystander clones. Therefore, as a more definitive approach, mFISH analyses of five clones isolated from the 2-Gy irradiated population (known irradiated) and five clones from the population that received medium from the 2-Gy flask (known bystanders) were performed.

mFISH karyotypes from clone Si8-B2 (irradiated with 2 Gy) are presented in Fig. 7. A trio of translocations, t(17;3), t(5;17) and t(3;5), was detected in all the cells analyzed. These translocations probably arose from a complex interchange at the time of irradiation. In addition, t(12;6) was present in 60% of cells. Cells that did not have this particular aberration had only one copy of chromosome 6 (e.g. panel B). Additional translocations and fragments were also observed (e.g. panels A and E).

The analyses of five clones isolated after irradiation with 2 Gy are summarized in Table 2. Using the standard criteria for defining the basis for chromosome instability, all the clones were deemed to be unstable. It appears that the degree of instability is variable, with some clones demonstrating higher numbers of subpopulations than others.

A representative sample of five different clones isolated from population that received conditioned medium from 2-Gy irradiated culture were examined to define the karyotypes in bystander cells. As shown in Fig. 8, 36% of the cells analyzed in one of the clones, Si12-C1, had a normal karyotype (panel A). The other 64% of cells showed a variety of translocations including t(2;4) in 20% of cells (panel B), translocations involving chromosome 1 (32% of cells, panels C and E) and translocations involving chromosome 7 (16% of cells, panels D and E). In one instance, chromosome 1 and chromosome 7 translocations were seen in the same cell (panel E).

The results for five bystander clones are presented in Table 3. Of the five clones, only two of them met our definition of chromosomal instability, i.e., had more than three subpopulations that made up at least 5% of the karyotypes analyzed (Si12-B4 and Si12-C1).

From the mFISH data of all the clones analyzed, there appears to be no correlation between the number of subpopulations and the number of aberrant cells in any given individual clone.

Discussion

The data presented here indicate that high-energy silicon ions can induce bystander responses and that both irradiated and bystander cells can exhibit chromosomal instability as observed by the appearance of *de novo* aberrations that appear many cell generations postirradiation.

Our finding of increases of approximately twofold in chromosomal aberrations in bystander populations (populations that received medium from irradiated populations) at the first division postirradiation is similar to that observed previously (33). In that study bystander populations were observed to have approximately twofold higher frequencies of aberrations than controls after α -particle irradiation. Furthermore, similar to the data presented here, it was found that the degree of induction of aberrations in the bystander cells was independent of the dose delivered.

The yields of aberrations observed in bystander cells in this study are similar to those reported for the induction of micronuclei in bystander cells by several investigators. Using a charged-particle microbeam, Prise *et al.* reported that after irradiation of four cells with five α particles each, ~2.5% of about 3,000 cells showed micronuclei compared to 1% of the control cells showing micronuclei (26). The fraction of cells expressing micronuclei remained relatively constant for increased numbers of α particles (up to 15 particles) delivered to the same number of cells. We have previously reported that after microbeam irradiation, bystander human fibroblasts had incidences of micronuclei ranging between 1.3- and 1.65-fold higher than controls, with no clear increase with increasing numbers of α particles delivered to hit cells (27).

High-energy ions have previously been demonstrated to induce genomic instability in a range of experimental systems. HZE iron and gold particles have been shown to be capable of inducing delayed chromosomal aberrations in a human-hamster cell line (35). Martins *et al.* used neon, argon and lead ions to induce dicentrics in human fibroblasts at 20–25 passages postirradiation (36). Carbon ions have been shown to induce *de novo* chromosome aberrations in human lymphocytes up to day 36 postirradiation (34). Therefore, there is good evidence of the ability of high-energy particles to induce instability in hit cells. While the level of instability in the single cell clones in this study was somewhat higher than that reported previously, it should be noted that the method used analyses all the chromosomes of a cell as opposed to fractions of the genome in earlier reports. Therefore, it is reasonable that analyzing the entire genome would detect alterations not previously detected by more

restricted methods. It remains possible that the htert transformation (required for long-term culture to conduct these kinds of experiments) is also contributing to a somewhat higher frequency of instability. The presence of normal karyotypes in some clones derived from the population irradiated with 0.073 Gy suggests that these clones might have arisen from bystander cells and not hit cells. First, given the low fluences used, only a fraction of the cells would be hit. Furthermore, only a fraction of those cells that were hit would survive and go on to give rise to colonies, thereby increasing the likelihood of more bystander clones being represented in the sampling. In addition, the probability of a cell maintaining a normal karyotype after being hit with a silicon particle at 490 MeV/nucleon would be low. Therefore, it is plausible (but not conclusive) that clones with normal karyotypes, such as clone Si4-A1, were derived from bystander cells. However, the converse is not true; at 0.073 Gy it is not possible to determine whether a clone without any normal karyotypes arose from an irradiated or a bystander cell.

Since chromosomal translocations are transmissible, nonlethal aberrations that can be passed on to daughter cells, a sequence of events could be proposed based on the relatively frequencies of aberrations in clone Si4-A1. The presence of normal karyotypes would suggest that the original cell had a normal karyotype at least until the first cell division. The presence of t(15;14) in 65% of the cells suggests that this translocation occurred relatively early in the growth of the clone and that t(X;5) and other translocations occurred at subsequent times. Alternatively, it is possible that the t(15;14) translocation conferred a growth advantage that resulted in the outgrowth of the subclone with that particular translocation and other translocations occurred during the expansion of the subclone. In any event, the presence of normal karyotypes is a strong indication that none of the translocations detected in the clone were present at the first division but rather occurred *de novo* as a function of time in culture – the definition of genomic instability.

A similar scenario could be put forward for the pattern of translocations detected in Si8-B2. Since this clone was isolated from a population irradiated with 2 Gy, it is probable that the initial irradiation created the complex exchange involving chromosomes 3, 5 and 17. It is also possible that the 12;6 translocation was produced at the same time and that this particular translocation was lost in a subpopulation (since if these cells never had the translocation, they would have two normal copies of chromosome 12, which was never the case).

It should be noted that in the control populations at the first cell division postirradiation, ~16% of the cells analyzed demonstrated chromosomal aberrations, while this frequency was reduced to between 5–8% in control clones after 50 population doublings. This may be due to inherent differences in the analyses of populations and clones derived from single cells.

Several investigators have identified potential signaling molecules that may play key roles in the development of bystander responses. A model has previously been proposed that implicated COX-2 as a central protein in expression of bystander responses (1). It has been demonstrated that ionizing radiation can induce a host of cytokines that in turn can induce COX-2 and iNOS via MAP kinases and NF- κ B (44, 45). These two genes regulate the production of ROS and NO, respectively. As stated earlier, both ROS and NO have previously been thought to be involved in the propagation of bystander responses. The link between the cellular signaling discussed above and the development of chromosomal aberrations in nonirradiated bystander cells remains to be elucidated.

In conclusion, the data presented here provide evidence that irradiation with high-energy particles can induce chromosomal aberrations in bystander cells and that these bystander

cells can go on to manifest genomic instability. Further, these bystander responses can be induced either in cells that are in contact with each other or via medium-borne factors. Finally, these data strengthen the link between the two phenomena that may be significant to radiation-induced carcinogenesis, especially at low doses of ionizing radiation.

Acknowledgments

The experiments presented here were conducted at NIRS under the Space Radiation Research Unit of the NIRS International Open Laboratory. Additional analyses of experiments conducted at NIRS were performed at Columbia University. Work presented here was supported by funding from the NIRS and NIH grant P01-CA49062-18 and R01-ES 012888.

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FIG. 1. Chromosomal aberrations detected in irradiated populations 24 h after Si490-ion irradiation ($\pm$ SE). *Observed frequencies are significantly different from expected values as determined by the χ^2 test ($P < 0.001$).

FIG. 2.

Chromosomal aberrations observed in bystander populations (recipients of medium from control and irradiated flasks) 24 h postirradiation ($\pm$ SE). **Observed frequencies are significantly different from expected values as determined by the χ^2 test ($P < 0.05$).

FIG. 3.

Percentage of cells with chromatid-type aberration in single cell clones isolated from populations irradiated with 0.073 Gy Si490 ions. Chromosomal analyses by Giemsa staining were conducted at ~50 population doublings. Lines indicate the range of control values (percentage of cells with chromatid aberrations in five different control clones).

FIG. 4.

Percentage of cells with chromosome-type aberrations in single cell clones isolated from populations irradiated with 0.073 Gy Si490 ions as assayed by Giemsa staining. Lines indicate the range of control values (the percentage of cells with chromosome-type aberrations in five different control clones).

FIG. 5.
mFISH analyses of G₂ prematurely condensed chromosomes from clone Si4-C4. This clone was isolated from the population that received 0.073 Gy Si490 ions.

FIG. 6. mFISH analyses of G₂ prematurely condensed chromosomes from clone Si4-A1. This clone was isolated from the population that received 0.073 Gy Si490 ions.

FIG. 7. mFISH analyses of G₂ prematurely condensed chromosomes from clone Si8-B2. This clone was isolated from the population that received 2 Gy Si490 ions.

FIG. 8. mFISH analyses of G₂ prematurely condensed chromosomes from clone Si12-C1. This clone was isolated from a bystander population that received medium from a flask irradiated with 2 Gy Si490 ions.

Table 1
Summary of mFISH Analyses of Single Cell Clones Isolated from Populations irradiated with 0.073 Gy Si490 Ions

Clone	Normal karyotype (%)	Aberrant karyotype (%)	No. of subpopulations
Si4-A1	6	94	9
Si4-A2	25	75	4
Si4-B2	19	81	3
Si4-C3	0	100	4
Si4-C4	0	100	12

Table 2
Summary of mFISH Analyses of Single Cell Clones Isolated from Populations Irradiated with 2 Gy Si490 Ions

Clone	Normal karyotype (%)	Aberrant karyotype (%)	No. of subpopulations
Si8-A2	0	100	4
Si8-B2	0	100	7
Si8-C2	0	100	12
Si8-C3	0	100	5
Si8-C4	0	100	8

Table 3
Summary of mFISH Analyses of Single Cell Clones Isolated from Bystander Populations
that Received Medium from Cells Irradiated with 2 Gy Si490 Ions

Clone	Normal karyotype (%)	Aberrant karyotype (%)	No. of subpopulations
Si12-A2	100	0	1
Si12-B4	56	44	4
Si12-C1	36	64	6
Si12-C2	94	6	2
Si12-C4	80	20	2