Role of Ku80-dependent end-joining in delayed genomic instability in mammalian cells surviving ionizing radiation

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Abstract

Ionizing radiation induces delayed destabilization of the genome in the progenies of surviving cells. This phenomenon, which is called radiation-induced genomic instability, is manifested by delayed induction of radiation effects, such as cell death, chromosome aberration, and mutation in the progeny of cells surviving radiation exposure. Previously, there was a report showing that delayed cell death was absent in Ku80-deficient Chinese hamster ovary (CHO) cells, however, the mechanism of their defect has not been determined. We found that delayed induction of DNA double strand breaks and chromosomal breaks were intact in Ku80-deficient cells surviving X-irradiation, whereas there was no sign for the production of chromosome bridges between divided daughter cells. Moreover, delayed induction of dicentric chromosomes was significantly compromised in those cells compared to the wild-type CHO cells. Reintroduction of the human Ku86 gene complimented the defective DNA repair and recovered delayed induction of dicentric chromosomes and delayed cell death, indicating that defective Ku80-dependent dicentric induction was the cause of the absence of delayed cell death. Since DNA-PKcs-defective cells showed delayed phenotypes, Ku80-dependent illegitimate rejoining is involved in delayed impairment of the integrity of the genome in radiation-survived cells.

Introduction

It is generally accepted that DNA repair pathways are indispensable for the survival of cells exposed to DNA damaging agents, such as ionizing radiation (1-5). However, DNA repair, by itself, may threaten the stability of the genome in the cells surviving DNA damaging agents (6-9). For example, non-homologous end-joining (NHEJ), which is the primary DNA repair pathway functions in G1 phase, is error-prone. It sometimes causes loss or rearrangement of the genetic information through mis-rejoining of DNA double strand breaks. Processing of DNA broken ends by exonucleases and endonucleases also provide another chance to alter DNA sequences. These events result in a loss of heterozygosity as well as gross genome rearrangements. In contrast, homologous recombination is a faithful repair in general, as homologous sister chromatids are used to restore the gap of the genetic information. Although most genome rearrangements are generated directly by the initial radiation exposure (10), recent findings have demonstrated that the integrity of the genome is also endangered eventually, if the cells were survived exposure to DNA damaging agents.

It is well described that ionizing radiation induces delayed effects in the progeny of surviving cells (11-14). This phenomenon is now called radiation-induced genomic instability, which is manifested as the expression of various delayed effects, such as delayed reproductive death or lethal mutation, delayed chromosomal instability, and delayed mutagenesis in the progenies of cells surviving radiation. Radiation-induced genomic instability results in accumulating gene mutations and chromosomal rearrangements, therefore, it has been thought

to play a pivotal role in radiation-induced carcinogenesis (15-18). Because radiation-induced genomic instability is induced in a certain fraction of the progenies stem from a single survived cell, not a single gene mutation but some epigenetic changes may be involved in the initiation of radiation-induced genomic instability. Although oxidative stress and altered chromatin structure have been proposed as the mechanisms of perpetuation of radiation-induced genomic instability (19-24), the mechanism of manifestation has not been fully understood yet. We have shown that delayed unscheduled induction of DNA double strand breaks is involved in the manifestation of delayed phenotypes (25). In fact, our previous study indicated that increased phosphorylated histone H2AX foci, which correspond to DNA double strand breaks, are frequently detected in the progeny of normal human diploid cells surviving X-rays. Moreover, delayed reactivation of p53 in response to DNA damage was manifested in the surviving clones (25). Delayed induction of DNA double strand breaks was also confirmed by delayed induction of chromosomal aberrations (26). Thus, it is evidenced that induction of DNA double strand breaks is induced indirectly in surviving cells from exposure to radiation, suggesting that DNA repair pathways could play roles in protecting the genome of surviving cells from harmful effects of radiation.

Previously, Chang and Little reported that radiation-induced genomic instability was abrogated in xrs5 cells, which are NHEJ-deficient Chinese hamster cells defective in Ku80 protein (27). It was found that delayed reproductive death was not observed in these cells, however, the reason of the absence of delayed reproductive death in xrs5 cells has not been

elucidated yet. We have hypothesized that defective NHEJ in xrs5 cells decreases the chance of mis-rejoining of the broken ends, which result in the formation of dicentric chromosomes involved in division halt. Therefore, we examined delayed chromosomal instability in two NHEJ-defective cells, xrs5 and xrs6 cells, and compared the frequency with the wild-type CHO cells. Firstly, we found that delayed induction of DNA double strand breaks in those cells, determined by DNA repair foci formation, was similar. Furthermore, delayed induction of chromatid breaks showed no defect in Ku80-deficient cells. However, delayed induction of dicentric chromosomes was significantly compromised in both xrs5 and xrs6 cells. These results demonstrate that Ku80-dependent mechanism is involved in delayed induction of dicentric chromosomes, and that dicentric chromosomes caused by the mis-rejoining of broken ends are associated with the induction of delayed cell death through the inhibition of cell division. This conclusion was confirmed by the experiment, in which the reintroduction of human Ku80 gene into xrs5 cells restored both delayed dicentric formation and delayed reproductive death. Furthermore, delayed induction of dicentric chromosomes were observed in cells defective in the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs). In summary, our present study clearly indicates that the progenies of surviving cells induce delayed DNA damage several generations after the initial insult, and DNA repair capacity is an important determinant for the integrity of the genome in the cells surviving radiation exposure.

Experimental procedure

Cell culture and irradiation.

Chinese hamster ovary (CHO) cells, XRCC1-defective EM-9 cells, and Ku80-defective xrs5 and xrs6 cells were cultured in αMEM supplemented with 10% fetal bovine serum (FBS) (TRACE Bioscience PTY Ltd., Australia). Severe combined immunodeficiency (*Scid*) mouse (C.B-17 *scid/scid*) and isogenic wild-type mouse (C.B-17 +/+) embryonic fibroblasts were cultured in DMEM supplemented with 10% FBS (28). Embryonic fibroblasts from C3H/He wild-type mouse and $Atm^{-/-}$ knockout mouse were cultured in DMEM supplemented with 10% FBS (29). The human KU86 gene were introduced into xrs5 cells by electroporation, and the cells were cultured in αMEM containing 200 μg/ml of G418 and 10% fetal bovine serum (TRACE Bioscience PTY Ltd., Australia). Exponentially growing cells were irradiated with X-rays from an X-ray generator at 150 kVp and 5 mA with a 0.1-mm copper (SOFTEX M-150WE, Softex, Osaka). The dose rate was 0.44 Gy/min. Dose rates were determined with an ionization chamber.

Cell survival

Cell survival was determined by colony formation assay. Cells cultured in T25 flasks were irradiated with various doses of X-rays, collected by trypsinization, counted the cell number, and appropriate numbers of cells, which give 10^2 surviving cells, were seeded into at least three 100-mm dishes. The cultures were incubated in a CO_2 incubator for 10 days before

fixation with ethanol. The colonies were stained with Giemsa's solution, and those consisting more than 50 cells were counted.

Analysis of delayed effects

Cells cultured in T25 flasks were irradiated with various doses of X-rays. CHO cells were irradiated with 8 and 10 Gy of X-rays, while xrs5 and xrs6 cells were irradiated with 2 and 4 Gy. EM-9 cells were irradiated with 6 and 8 Gy of X-rays. After irradiation, the irradiated cells and the control cells were collected by trypsinization, and appropriate numbers of cells, which give 10^2 surviving cells, were seeded into ten 100-mm dishes. Ten days after irradiation, the primary colonies formed in ten independent dishes were collected by trypsinization. The number of cells was counted, and total population doubling levels (PDLs) were calculated by using the total numbers of cells in ten dishes divided by the number of colonies formed in ten dishes. They were used as the cells at 15 to 20 population doublings. Rest of the cells was used for the secondary colony formation, and 10^2 cells were re-inoculated into another ten 100-mm dishes. After 10 days, all the colonies formed in ten dishes were collected by trypsinization, counted the number of cells, and total population doubling levels were calculated. They were used as the cells at 30 to 35 population doublings after irradiation.

Detection of giant cells an delayed chromosomal bridge formation

Exponentially growing cells were plated onto 22 x 22 mm coverslips, and incubated for 24

hours before fixation with methanol. Then, cells were stained with 5% Giemsa's solution. The cells, which occupied an area in the colony several times greater than the rest of the cells, were considered to be giant cells as described previously. Chromosomal bridges, which were detected between two dividing daughter nuclei in the anaphase cells, were counted.

Detection of delayed chromosomal aberrations

Exponentially growing cells were treated with 0.033 μg/ml colcemid (GIBCO, Grand Island, NY) for 1 hour, and mitotic cells were collected. They were treated with 0.075 M potassium chloride for 20 min, fixed in ice-cold Carnoy’s fixative (methanol:acetic acid, 3:1) for 30 min, and spread on slide glasses using an air-drying method. After staining with 3% Giemsa's solution, chromosome aberrations were classified as previously described (Savage, 1976). Three independent experiments were performed, and total 200 metaphases were counted per each sample.

Detection of delayed DNA damage

Delayed induction of DNA double strand breaks were determined by 53BP1 foci. The cells cultured on coverslips were fixed with 4% formaldehyde, permeabilized with 0.5% Triton X-100, and then were washed extensively with phosphate buffered saline (PBS). The primary antibody, anti-53BP1 antibody (Bethyl laboratories Inc, TX), were diluted in 100 μl of TBS-DT (20 mM Tris-HCl, 137 mM NaCl, pH7.6, containing 50 mg/ml skim milk and 0.1%

Tween-20), and applied on the cover slips. The samples were incubated for 2 hours in a humidified CO_2 incubator at 37°C. The primary antibody was washed with PBS, and Alexa594-labelled anti-rabbit IgG antibodies (Molecular Probes, Inc., OR) were added. The cover slips were incubated for 1 hour in a humidified CO_2 incubator at 37°C. They were then washed with PBS and counterstained with 0.1 mg/ml of DAPI. The samples were examined with an Olympus fluorescence microscope AX80 (Olympus, Tokyo). Digital images were captured by a Quantix 1400 camera (Photometrics, AZ), and the images were analyzed by IPLab Spectrum analysis software (Signal Analytics Corporation, VA).

Immunoblotting and detection

Exponentially growing cells were lysed in lysis buffer (50 mM Tris-HCl (pH7.2), 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, and 0.1% SDS) containing 1 mM 4-(2-aminoethyl)-benzensulfonyl fluoride hydrochloride. The cell lysate was sheared through 28 G needle 5 times and cleared by centrifugation at 15,000 rpm for 10 min at 4°C, and then supernatant was used as total cellular protein. Total protein concentration was determined by the BCA protein assay (Pierce, Rockford, IL). Protein samples (16 μg) were electrophoresed on SDS-polyacrylamide gel and were electrophoretically transferred to a polyvinyl difluoride membrane in a transfer buffer (100 mM Tris, 192 mM glycine). After overnight incubation with blocking solution (10% skim milk), the membrane was incubated with anti-Ku80 monoclonal antibody (clone 111, KAMIYA Biomedical Co.) or anti-XRCC1 polyclonal

antibody (NOVUS Biologicals), a biotinylated anti-mouse IgG antibody, and streptavidine-alkaline phosphatase. The bands were visualized after addition of nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate as a substrate.

Data analysis

A Student's *t* test was used to evaluate significant difference between the control and irradiated cells. *P* values of less than 0.05 were considered significant difference.

Results

Exponentially growing CHO, XRCC1-defective EM-9 cells, and Ku80-deficient xrs5 and xrs6 cells were exposed to various doses of X-irradiation. Both xrs5 and xrs6 cells show significant reduction of cell survival as compared to CHO cells (Figure 1). In order to compare delayed induction of genomic instability at the same survival levels, 8 Gy and 10 Gy of X-rays were irradiated to CHO, while 2 and 4 Gy of X-rays and 6 and 8 Gy of X-rays were exposed to xrs5 and xrs6 cells, and EM-9 cells, respectively. To compliment the defect in xrs5 cells, the human Ku86 gene was introduced by electroporation. As shown in Figure 2, the introduced cells expressed significant amount of human KU80 protein, and as a result, radiation sensitivity was also complimented (Figure 1).

Manifestation of radiation-induced genomic instability was determined by the expression of the delayed effects. As shown in Table 1, delayed reproductive death was completely absent in both xrs5 and xrs6 cells, whereas CHO and EM-9 cells showed decreased cloning efficiency. Induction of giant cells and delayed chromosomal bridges were also abrogated in xrs5 and xrs6 cells (Tables 1 and 2). In order to confirm that defective induction of these delayed phenotypes are caused by the simple Ku80-deficiency, the human Ku86 gene was introduced into xrs5 cells. It was confirmed that complementation of the defective Ku80 function in xrs5 cells simultaneously restored delayed reproductive death, giant cell formation and delayed chromosomal bridge formation to the levels observed in CHO cells. Thus, the results clearly indicated that Ku80-dependent rejoining is involved in

manifesting delayed phenotypes in the progenies of X-ray-surviving cells.

It is possible that delayed phenotypes are caused by Ku80-dependent mis-rejoining of delayed DNA double strand breaks. Mis-rejoining of DNA breaks and delayed induction of DNA double strand breaks were examined by chromosome analysis and 53BP1 foci analysis, respectively. As shown in Figure 3, delayed induction of DNA double strand breaks was measured by 53BP1 foci in cells at 30-35 PDL post irradiation. While the frequency of 53BP1 foci in the control CHO cells was approximately 0.14 ± 0.07, it was increased to 0.41 ± 0.19 in surviving cells. The frequency of 53BP1 foci in the unirradiated xrs5 cells were relatively higher (0.18 ± 0.08) compared to the CHO cells, and it was about 0.51 ± 0.27 in the surviving cells. Thus, it is indicated that delayed DNA damage was induced similarly in both CHO and xrs5 cells. Delayed chromosomal instability was examined at 15-20 PDL post irradiation (Table 3) and 30-35 PDL post irradiation (Table 4). We found that induction of chromatid breaks in xrs5 and xrs6 cells was comparable to CHO and EM9 cells. Interestingly, delayed formation of dicentric chromosomes was defective in xrs5 and xrs6 cells, while the control levels of dicentric chromosome were comparable among those cells. To confirm whether defective induction of dicentric chromosomes is related to Ku80-deficiency, delayed chromosomal instability was examined in the complimented xrs5 cells. We observed that delayed induction of dicentric chromosomes was increased to the level observed in CHO cells. Similar results were obtained in both cells at 15-20 PDL and 30-35 PDL post irradiation. Delayed chromosomal instability was also examined in cells derived from

DNA-PKcs-defective *Scid* mouse and ATM-knockout mouse (Table 5). Cells were irradiated with equivalent 10% survival doses and delayed induction of dicentric chromosomes was analyzed 30-35 PDL post-irradiation. As shown in Table 5, the frequency of dicentric chromosomes in the unirradiated *Scid* and ATM-knockout cells was slightly higher than that in the wild-type cells, and increased dicentric frequencies in surviving cells were observed in both cases.

Discussion

It is well established that ionizing radiation induces delayed destabilization of the genome in the progeny of cells surviving ionizing radiation. Previously, several studies have demonstrated that delayed DNA double strand breaks are induced several generations after the initial insult (25, 30, 31), which has been proven by examining the delayed induction of foci of DNA damage checkpoint factors, such as phosphorylated histone H2AX. While phosphorylated histone H2AX foci are frequently used as biochemical markers for DNA double strand breaks, the foci of other DNA damage checkpoint factors, such as phosphorylated ATM foci and 53BP1 foci, are colocalized with phosphorylated histone H2AX foci (32-37), and they could also be used as alternative markers for DNA damage (38). In the present study, we confirmed that the frequency of 53BP1 foci was higher in the progenies of surviving cells compared to unirradiated cells. Thus, it is quite reasonable to think that delayed induction of DNA double strand breaks in the progeny of surviving cells associated with pleiotropic manifestation of radiation-induced genomic instability.

Radiation-induced genomic instability has been reported commonly in various cell systems including human and rodent cells (11-14). However, Chang and Little demonstrated that delayed reproductive death, one characteristic manifestation of radiation-induced genomic instability, was not observed in Ku80-deficient xrs5 cells (27). The authors suggested that the cellular processing of DNA double strand breaks during repair must play a role in delayed reproductive death. Here, we found that not only delayed cell death but also

delayed induction of giant cells and chromosome bridge were absent in xrs5 cells. Furthermore, other Ku80-deficient cell line, xrs6 (39), also revealed deficiency in the induction of those delayed phenotypes, while EM9 cells, defective in single-strand break repair, showed no apparent defect in delayed genomic instability induction. Thus, it was confirmed that DNA double-strand break repair process is involved in manifestation of delayed phenotypes. One possible explanation of the defective induction of some delayed phenotypes was that error-free DNA repair, such as homologous recombination, reduced the incidence of transmissible damage in the absence of error-prone NHEJ repair. If so, delayed induction of DNA double strand breaks was lower in Ku80-defective cells than the control CHO cells. Therefore, we checked whether delayed DNA damage was less frequent in xrs5 cells. The results clearly indicated that it was not the case, as we detected normal level of delayed induction of DNA damage in xrs5 cells. We also found that delayed chromatid breaks were similarly induced between Ku80-deficient cells and the wild-type cells (Table 3 and 4). Thus, even without Ku80-dependent repair, genomic instability by itself could be induced in the progenies of surviving cells. Then, the second possibility was that defective NHEJ in xrs5 cells decreased the chance of mis-rejoining of the broken ends that occurred many generations after the initial insult. In fact, delayed induction of chromosome bridges between two daughter cells was significantly reduce in xrs5 and xrs6 cells (Table 2). Furthermore, delayed induction of dicentric chromosomes was completely absent in both xrs5 and xrs6 cells (Table 3 and 4). Although several studies have reported that chromosome breakages are more

frequent in ku80-deficient cells (40, 41), the frequency of dicentric chromosome was relatively low considering the frequency of chromosome breaks (40). These results supported our conclusion that the formation of dicentric chromosome caused by delayed DNA damage was compromised in Ku80-deficient cells. Although a back-up NHEJ may undertake mis-rejoining of broken ends in the absence of Ku-dependent NHEJ (42), it is highly likely that a major pathway of illegitimate rejoining the DNA breaks is Ku80-dependent process (43, 44). We also confirmed that radiation-induced genomic instability was manifested in cells derived from DNA-PKcs-defective *Scid* mouse. Moreover, delayed dicentric formation was normally detected in *Scid* cells. Therefore, DNA-PK-independent rejoining, which was suggested previously (45), is involved in delayed dicentric formation. Recently, it has been postulated that XRCC4/DNA Ligase IV-dependent but DNA-PKcs-independent rejoining needs Ku80/70 complex (46). Thus, it is highly possible that Ku80-dependent mis-rejoining is involved in delayed generation of dicentric chromosomes, by which chromosome bridges is generated. Such mis-rejoining inhibits segregation of two daughter cells, which results in delayed induction of giant cells as well as delayed reproductive death.

It should be mentioned that delayed induction of chromatid breaks, observed in both surviving CHO and NHEJ-defective cells, could be a source of initiating ATM-dependent DNA damage checkpoint, which results in the execution of delayed cell death. However, defective p53 function in Chinese hamster cells incapacitates induction of irreversible cells cycle arrest and apoptosis in damaged cells (47, 48). Thus, although DNA breaks are

associated with cells death in normal cells with the wild-type p53 function, it could not be a major cause of delayed cell death in Chinese hamster cells. Furthermore, it is well known that the broken ends are protected by telomere healing in rodent cells (49, 50), which enables DNA damage checkpoint activation. Thus, although delayed chromatid breaks were induced in Ku80-deficient cells, they might not be involved in delayed reproductive death in Chinese hamster cells.

It should be very interesting to know the consequence of cells harboring such chromatid breaks. Previously, it was reported that ionizing radiation induced genomic instability in the progeny of surviving CHO cells, which resulted in a heritable mutator phenotyps. For example, mutation frequency at the HPRT locus in surviving clones was persistently higher than the unirradiated progenies (51). It was expected that such chromatid breaks caused large deletions at the HPRT locus, however, multiplex PCR analysis revealed that point mutations are the predominant type of genetic alterations in the mutants (data not shown). Because cells with micronuclei were frequently observed among the surviving cells, persistent chromatid breaks, which are not involved in delayed cell death under the p53-dysfunctional condition, may result in a loss of genetic materials. It has been proved that ionizing radiation induces delayed genomic instability, which accumulates genetic alterations including gene mutations, loss of heterozygosity, and chromosome rearrangements, concurrently with delayed reproductive death (11-14). As Ku80-deficiency compromised delayed cell death through the formation of dicentric chromosomes, it is likely that cells with

defective DNA repair capacity are more susceptible to carcinogenesis induced by DNA damaging agents (52).

Our present study demonstrated that DNA repair pathway is an important determinant of cellular response to ionizing radiation not only in the immediate response but also in cells surviving radiation exposure. Survived cells induced DNA double strand breaks many generations after the initial insult. Although the mechanism of delayed DNA damage induction has to be determined, delayed dicentric formation indicated that delayed DNA damage was induced in G1 phase. Such delayed DNA damage could be repaired by NHEJ repair, but it also provided a chance to engender mis-rejoining. These results should bring a new insight into how DNA repair protects the integrity of the genome from the insults of DNA damaging agents.

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Figure legends

Figure 1. Survival curves of CHO cells and Ku80-deficient cells. ○, CHO cells; ●, EM9 cells; ◆, xrs5 cells; ▲, xrs6 cells, ◇, xrs5 cells with the human KU86 gene.

Figure 2. Expression of Ku80 and XRCC1 in CHO and mutants. Total proteins were extracted from exponentially growing cells. Samples (16 μg) were subjected to western blot analysis probed with anti-Ku80 and XRCC1 antibodies. To check the expression of exogenous human Ku80 protein, total proteins from normal human diploid (NHD) cells were used.

Figure 3. Delayed DNA damage induction in cells 30-35 PDL after irradiation.

Delayed induction of DNA double strand breaks was examined by 53BP1 foci formation. Both control and X-irradiated cells were fixed and stained with anti-53BP1 antibody as described in *Materials and Methods*.

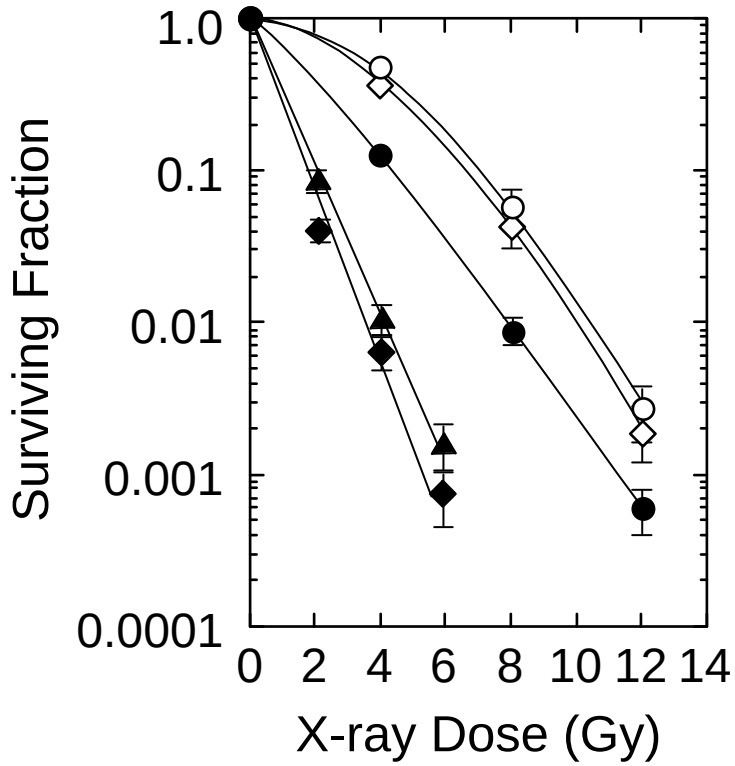

Figure 1. K. Suzuki et al.

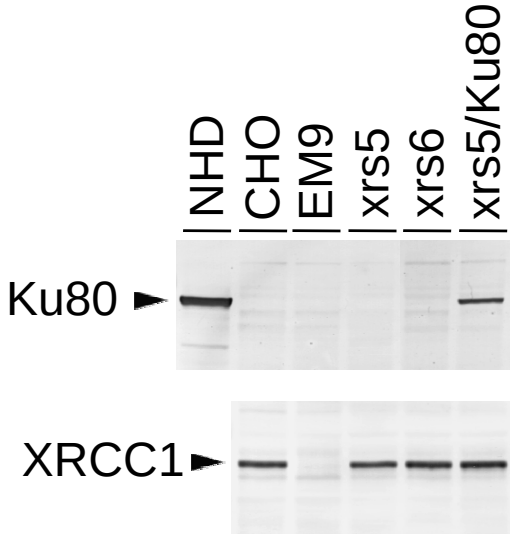

Figure 2. K. Suzuki et al.

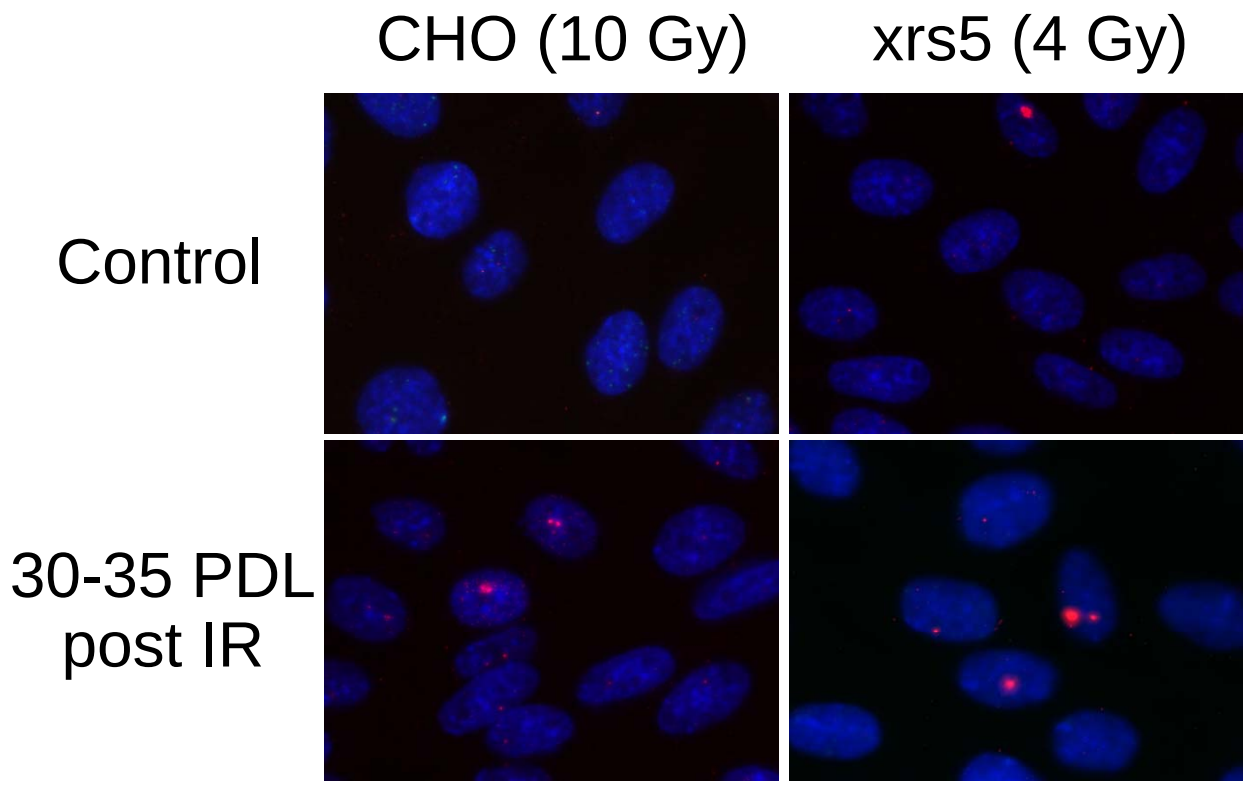

Figure 3. K. Suzuki et al.

Table 1 Cloning efficiency and frequency of giant cells at 30-35 PDL post irradiation.

Cells	Cloning efficiencies (%)	% colonies with giant cells
CHO		
0 Gy	99.8 ± 12.6	7.0 ± 3.5
8 Gy	75.8 ± 11.6	15.1 ± 5.7
10 Gy	78.0 ± 10.2	21.4 ± 8.3
EM9		
0 Gy	79.0 ± 12.3	3.9 ± 1.4
6 Gy	70.5 ± 3.1	9.8 ± 3.6
8 Gy	65.5 ± 8.4	13.7 ± 3.5
xrs5		
0 Gy	63.0 ± 15.4	4.2 ± 2.5
2 Gy	61.0 ± 2.8	4.5 ± 1.9
4 Gy	64.0 ± 8.0	4.7 ± 3.1
xrs6		
0 Gy	57.5 ± 2.7	5.0 ± 3.3
2 Gy	54.3 ± 6.8	4.8 ± 3.5
4 Gy	58.3 ± 6.2	4.2 ± 3.9
xrs5/Ku80		
0 Gy	89.7 ± 10.4	6.5 ± 3.3
8 Gy	69.1 ± 9.6	12.0 ± 7.5
10 Gy	65.8 ± 11.4	17.8 ± 6.4

Table 2 Delayed chromosome bridge formation in mitotic cells at 30-35 PDL post irradiation

Cells	% cells with chromosome bridge
CHO	
0 Gy	2.6 ± 1.5
8 Gy	9.0 ± 4.1
10 Gy	15.3 ± 6.7
EM9	
0 Gy	3.8 ± 2.1
6 Gy	6.4 ± 3.4
8 Gy	16.0 ± 6.0
xrs5	
0 Gy	8.1 ± 4.4
2 Gy	8.2 ± 3.9
4 Gy	7.3 ± 3.1
xrs6	
0 Gy	13.0 ± 7.8
2 Gy	10.2 ± 7.4
4 Gy	9.3 ± 6.8
xrs5/Ku80	
0 Gy	4.8 ± 2.8
8 Gy	8.2 ± 7.3
10 Gy	14.3 ± 8.6

Table 3 Delayed chromosomal instability in surviving cells at 15-20 PDL post irradiation

Cells	No. of cells with aberrations (%)			No. cells counted
	Dicentrics	Chromatid gaps/breaks	Others [a]	
CHO				
0 Gy	3 (0.4)	24 (3.0)	1 (0.1)	801
8 Gy	37 (4.4)	64 (7.6)	5 (0.6)	841
10 Gy	38 (4.6)	93 (11.2)	10 (1.2)	832
EM9				
0 Gy	9 (1.1)	30 (3.6)	2 (0.2)	841
6 Gy	30 (3.5)	62 (7.3)	15 (1.8)	850
8 Gy	35 (4.1)	82 (9.6)	17 (2.0)	857
xrs5				
0 Gy	8 (1.0)	65 (8.1)	2 (0.3)	800
2 Gy	9 (1.1)	119 (14.8)	11 (1.4)	805
4 Gy	13 (1.6)	133 (16.5)	8 (1.0)	808
xrs6				
0 Gy	11 (1.4)	64 (8.0)	5 (0.6)	802
2 Gy	16 (2.0)	101 (12.6)	12 (1.5)	801
4 Gy	17 (2.1)	98 (12.0)	14 (1.7)	818
xrs5/Ku80				
0 Gy	7 (0.8)	26 (3.1)	4 (0.5)	827
8 Gy	28 (3.1)	74 (8.3)	8 (0.9)	891
10 Gy	31 (3.5)	85 (9.7)	9 (1.0)	876

[a] Rings, fragments and chromatid exchanges.

Table 4 Delayed chromosomal instability in surviving cells at 30-35 PDL post irradiation

Cells	No. of cells with aberrations (%)			No. cells counted
	Dicentrics	Chromatid gaps/breaks	Others [a]	
CHO				
0 Gy	4 (0.5)	20 (2.4)	0	828
8 Gy	44 (5.3)	60 (7.2)	4 (0.5)	832
10 Gy	39 (4.6)	112 (13.2)	12 (1.4)	848
EM9				
0 Gy	9 (1.1)	31 (3.7)	6 (0.7)	840
6 Gy	52 (6.1)	88 (10.3)	12 (1.4)	856
8 Gy	49 (5.7)	124 (14.5)	24 (2.8)	858
xrs5				
0 Gy	8 (1.0)	66 (7.0)	8 (1.0)	800
2 Gy	4 (0.5)	92 (11.3)	8 (1.0)	816
4 Gy	12 (1.3)	96 (10.4)	8 (0.9)	920
xrs6				
0 Gy	12 (1.5)	56 (6.9)	4 (0.5)	812
2 Gy	16 (1.7)	132 (14.3)	8 (0.9)	921
4 Gy	16 (1.9)	116 (14.1)	4 (0.5)	824
xrs5/Ku80				
0 Gy	6 (0.7)	26 (3.1)	3 (0.4)	849
8 Gy	32 (3.7)	71 (8.2)	5 (0.6)	868
10 Gy	41 (4.6)	96 (10.7)	7 (0.8)	897

[a] Rings, fragments and chromatid exchanges.

Table 5 Delayed chromosomal instability in surviving cells at 30-35 PDL post irradiation

Cells	No. of cells with Dicentrics (%)	No. cells counted
CB17/wt		
0 Gy	8 (2.2)	352
6 Gy	45 (14.4)	313
CB17/SCID		
0 Gy	21 (6.3)	331
2 Gy	62 (17.4)	356
C3H/He $ATM^{+/+}$		
0 Gy	6 (1.6)	376
6 Gy	59 (15.4)	382
C3H/He $ATM^{-/-}$		
0 Gy	54 (15.7)	345
3 Gy	115 (32.0)	359