Role of DNA Double-strand Break Repair Genes in Cell Proliferation under Low Dose-rate Irradiation Conditions

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Low dose radiation/Low-dose rate/DNA double-strand breaks (DSBs)/DNA repair.

Radiation-induced DNA double-strand breaks (DSBs) lead to numerous biological effects. To elucidate the molecular mechanisms involved in cellular responses to low dose and low dose-rate radiation, it is informative to clarify the roles of DSB repair related genes. In higher vertebrate cells, there are at least two major DSB repair pathways, namely non-homologous end-joining (NHEJ) and homologous recombination (HR). Here, it is shown that in chicken DT40 cells irradiated with γ-rays at a low dose-rate (2.4 cGy/day), the growth delay in NHEJ-related KU70- and PRKDC (encoding DNA-PKcs)-defective cells were remarkably higher than in cells defective for the HR-related RAD51B and RAD54 genes. DNA-PKcs-defective human M059J cells also showed an obvious growth delay when compared to control M059K cells. RAD54–/–KU70–/– cells demonstrated their highest degree of growth delay after an X-irradiation with a high dose-rate of 0.9 Gy/min. However they showed a lower degree of growth delay than that seen in KU70–/– and PRKDC–/–/– cells exposed to low dose-rate irradiation. These findings indicate that cellular responses to low dose-rate radiation are remarkably different from those to high dose-rate radiation. The fact that both DT40 and mammalian NHEJ-defective cells were highly sensitive to low dose-rate radiation, provide a foundation for the concept that NHEJ-related factors may be useful as molecular markers to predict the sensitivity of humans to low dose-rate radiation.

INTRODUCTION

Ionizing radiation (IR) leads to serious biological consequences: cell death, chromosomal aberrations, and mutations, and there is good evidence that DNA is a principal target for radiation-induced cell lethality.1,2 DNA double-strand breaks (DSBs) are critical and lethal DNA lesions, and as few as one unrepaired DSB is sufficient to lead to cell death.1,2,3 In higher vertebrate cells, DSBs are repaired primarily by two distinct and complementary mechanisms: non-homologous end joining (NHEJ) and homologous recombination (HR).

The repair of DSBs through NHEJ requires DNA-dependent protein kinase (DNA-PK), XRCC4, and DNA ligase IV at the minimum.2–4 DNA-PK is composed of a DNA-binding Ku heterodimer (Ku70 and Ku86) and a catalytic subunit (DNA-PKcs, encoded by the gene PRKDC). DNA-PK is activated to bind to the ends of double-stranded DNA, and activated DNA-PK can phosphorylate XRCC4 and itself in vivo.4,5 DNA ligation is carried out by the XRCC4-DNA ligase IV complex. Artemis,6) which is defective in a variant of human SCID (severe combined immunodeficiency), and an XRCC4-like factor XLF (also named Cernunnos)7) may also be required for NHEJ. Recently ATM (a gene product which is mutated in ataxia-telangiectasia (AT) patients)-Artemis-dependent and 53BP1-dependent NHEJ has been demonstrated as a subpathway for NHEJ.8,9 HR is the predominant pathway for the repair of DSBs during the late S and G2 phases of the cell cycle, and can repair a DSB by using the undamaged sister chromatid as a template.8,10 HR is mediated by the RAD52 epistasis group of proteins, including RAD51 paralogs (such as RAD51B, -C, and -D, and XRCC2 and -3, and DMC1), RAD52, RAD54, and BRCA1 and BRCA2.3,5,11,12 Briefly, following the end processing and the binding of RAD52 to the DNA ends, RAD51 forms a nucleoprotein filament, and a RAD51-BRCA2 complex probably regulates this process. DNA strand exchange generates a joint molecule between
the homologous damaged and undamaged DNA strands in a reaction that is stimulated by the RAD52 and RAD54 proteins. RAD51 paralogs probably act as a functional unit which facilitates the action of RAD51 during HR.11,12)

Accumulating evidence indicates that the biological effects of low dose and low dose-rate radiation are different from those of high dose and high dose-rate radiation.13–15) To elucidate the fundamental molecular mechanisms of cellular responses to low dose and low dose-rate radiation, it is essential to clarify the role of DSB repair-related genes. Chronic low dose-rate irradiation is considered to be a form of multi-fraction irradiation. Thus recovery from DNA damage under low dose-rate irradiation conditions is assumed to result from the repair of sub-lethal damage (SLD). In the study of a split dose recovery using chicken DT40 cells and their NHEJ and HR mutants, SLD recovery was suggested due to DSB repair mediated by HR but not NHEJ.16,17) However, Kreder et al.18) showed that rodent Ku80 and DNA-PKcs mutants as well as HR mutants showed the same cell survival after a pulsed low dose-rate (average 1 Gy/h) and high dose-rate (3.3 Gy/min) irradiation, suggesting that SLD recovery by low dose-rate irradiation may be different from that by split-dose irradiation. Here it is shown that the growth delay in NHEJ-related KU70- and PRKDC-defective DT40 cells irradiated with γ-rays at 2.4 cGy/day were remarkably higher than that observed in HR-related RAD51B- and RAD54-defective cells. In addition RAD54- KU70- cells showed the highest degree of growth delay among the mutants examined after high dose-rate X-irradiation, while they showed lower than KU70-defective cells under low dose-rate irradiation.

MATERIALS AND METHODS

Cells

Chicken B lymphocyte DT40 cells,19,20) and KU70-21) PRKDC- cells,22) RAD54-21) RAD51B-13) and RAD54- KU70-21) cells which were generated from DT40 cells were obtained from the RIKEN BioResource Center (Ibaraki, Japan). These cells were cultured in alpha-MEM medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS, SAFC Biosciences), 1% chicken serum, penicillin, streptomycin and 10 μM β-mercaptoethanol (Invitrogen).

Human glioblastoma DNA-PKcs-defective M059J cells and M059K cells (which have a normal expression of DNA-PKcs)23,24) were obtained from the ATCC, and were cultured in D-MEM/F-12 1:1 mixture medium (Invitrogen) supplemented with 10% FBS, penicillin and streptomycin.

Low dose-rate irradiation

Chronic low dose-rate irradiations were delivered in the long-term low dose-rate irradiation facility in the CRIEPI (Tokyo, Japan) at a 370 GBq137Cs γ-ray source. Exponentially growing cells were irradiated at 37°C in 95% air −5% CO2 (Hirasawa, Tokyo, Japan). The dose rate was 2.4 cGy/day, and the dose rate was determined with a photoluminescence glass dosimeter.25) Unirradiated cells were cultured under identical conditions.

DT40 cells, and mutants established from DT40 cells growing in suspension were counted in triplicate using a Coulter Counter (model Z1, Beckman Coulter), and were diluted three times a week to maintain optimum growth conditions.

Exponentially growing M059J and M059K cells were harvested by trypsinization and resuspended in fresh medium. Cells were counted, diluted and plated twice a week to maintain optimum growth conditions.

High dose-rate irradiation

Cells were exposed to high dose-rate X-rays from an X-ray machine (MBR-320R, Hitachi Medical, Tokyo, Japan) operated at 250 kV and at 10 mA with 1 mm aluminum and 0.5 mm copper filters at room temperature. The dose rate was 0.9 Gy/min. After irradiation, cells were counted in triplicate every 24 h.

Cell survival assays

Clonogenic survival was determined with a colony formation assay. DT40 cells and the derived mutant cell lines were counted and then plated in alpha-MEM medium containing 1.5% methylcellulose (Sigma-Aldrich), 15% FBS, 1.5% chicken serum and 10 μM β-mercaptoethanol. After incubation for 9 days, colonies were counted. The colony-forming efficiency of unirradiated DT40 wild-type, KU70- PRKDC- cells, RAD54- and RAD51B- cells were 49%, 45%, 62%, 47%, 62% and 64%, respectively. Surviving fractions were normalized to those for unirradiated controls.

M059J and M059K cells were harvested by trypsination and resuspended in fresh medium. Cell were counted, diluted and plated on 60 mm tissue culture dishes. After incubation for two weeks, colonies were stained with crystal violet and colonies consisting of more than 50 cells were counted. The colony-forming efficiency of unirradiated M059J and M059K cells were 24% and 8.4%, respectively.

RESULTS

Figure 1 shows survival curves for chicken B lymphocyte DT40 cells and KU70- PRKDC- RAD54-, RAD51B- and RAD54- KU70- cells used in this study after irradiation with X-rays at a high dose-rate (0.9 Gy/min). The cell survival curves obtained with this system were almost identical to those shown in previous reports.11,19–22) KU70- and PRKDC- cells showed a biphasic type cell survival curve, and the surviving fractions of these mutants were lower than those of DT40 wild-type, RAD54- and RAD51B- cells in the low dose region. After irradiation with 0.5 Gy, the
surviving fraction of KU70–/– and PRKDC–/–/– cells were 0.650 ± 0.022, respectively.

Supplemental Fig. 1 shows the cell proliferation rate for DT40 wild-type, KU70–/–, PRKDC–/–/–, RAD54–/–, RAD51B–/– and RAD54–/–KU70–/– cells irradiated with 137Cs γ-rays at a low dose-rate (2.4 cGy/day). Exponentially growing cells in suspension were irradiated in a CO2 incubator kept at 37°C. Unirradiated cells were cultured in the same incubator but outside of the γ-ray field. Good cell growth conditions were achieved under experimental conditions. Since the growth rate was different among the various cell lines used to compare the degree of low dose-rate irradiation growth delay, the ratio of the cell number in irradiated cultures to that in unirradiated cultures was calculated using the results in Supplemental Fig. 1 (Fig. 2). Growth delay was observed in all of the cell lines under chronic low dose-rate irradiation. The ratio was 0.69 ± 0.09 in wild-type cells irradiated with γ-rays at 0.9 Gy/min for 28 days. The growth delay in KU70–/– cells was the highest among the mutant strains with a ratio of 0.042 ± 0.006. PRKDC–/–/– cells also showed a higher degree of growth delay (0.15 ± 0.03). In contrast, RAD54–/– and RAD51B–/– cells did not show the remarkable growth delays. The ratio was 0.53 ± 0.17 and 0.42 ± 0.05 in RAD54–/– and RAD51B–/– cells, respectively. Interestingly, the growth delay in RAD54–/–KU70–/– cells was lower than in KU70–/– cells, but higher than that of RAD54–/– cells: the ratio was 0.27 ± 0.03 after a 28 day irradiation.

Previous reports and these results (Fig. 1) indicate that RAD54–/–KU70–/– cells were most sensitive to an acute irradiation among the mutant cell lines. To assess growth delay induced by an acute high dose-rate irradiation, wild-type DT40 cells and the mutant cells were irradiated with 0.67 or 2 Gy of X-rays at 0.9 Gy/min. After irradiation, the number of cells was counted at 24 h intervals. Fig. 3 and Supplemental Fig. 2 show the cell proliferation rate and the ratio of number of cells in irradiated cultures to that in unirradiated cultures, respectively. 0.67 Gy is equivalent to the total dose for a 28 day irradiation at the low dose-rate of 2.4 cGy/day. After a high dose-rate X-irradiation, RAD54–/– KU70–/– cells showed a remarkably higher degree of growth delay among the mutants (Fig. 3A and 3B). The growth delay in KU70–/– and PRKDC–/–/– cells was higher than that in RAD54–/– and RAD51B–/– cells after 0.67 Gy of X-irradiation (Fig. 3A). Expectedly from Fig. 1, KU70–/–, RAD54–/–, and RAD51B–/– cells showed almost similar ratio of cell number after 2 Gy of X-irradiation, although that of PRKDC–/–/– cells was slightly higher.

Figure 4 shows survival curves for human glioblastoma DNA-PKcs-defective M059J and control M059K cells (which have a normal expression of DNA-PKcs) after irradiation with X-rays at a high dose-rate (0.9 Gy/min). As previously reported, M059J cells were more sensitive than M059K cells to X-rays. Finally, to determine the importance of DNA-PK in human cells, the effect of the chronic low dose-rate irradiation at 2.4 cGy/day on cell proliferation was observed in M059J and M059K cells. Predictably growth delay was observed in M059J cells exposed to low dose-rate irradiation (Fig. 5 and Supplemental Fig. 3).
DISCUSSION

After high dose-rate X-irradiation at 0.9 Gy/min, RAD54+/− KU70+/− cells showed the highest degree of growth delay among the mutants examined (Fig. 3), while they showed lower under chronic low dose-rate irradiation at 2.4 cGy/day than KU70−/− cells (Fig. 2). One possibility is that the biological effect of chronic low dose-rate irradiation in KU70+/− cells may be enhanced by HR. Asynchronous KU70+/− cells showed a biphasic pattern of radiation sensitivity to high...
dose-rate radiation (Fig. 1). *KU70*−/+ cells are sensitive to IR in G1 to early S phase but are resistant in late S-G2 phase, suggesting that Ku competes with HR in late S-G2 phase. More efficient HR in *KU70*−/+ cells may cause the enhancement of growth delay under chronic low dose-rate irradiation.

Similar case was observed in the cells treated with DNA topoisomerase II (Top2) inhibitors, VP16 and ICRF–193. Adachi et al. (2003) have reported that *KU70*−/+ cells are extremely sensitive to Top2 inhibitors compared with wild-type or *RAD54*−/+ cells. *RAD54*−/+ *KU70*−/+ cells were less sensitive than *KU70*−/+ cells, suggesting that the cytotoxicity of Top2 inhibitor is enhanced by HR. On the other hand, Adachi et al. (2004) have reported that *RAD54*−/+ cells were sensitive to DNA topoisomerase I (Top1) inhibitor, camptothecin (CPT), but *KU70*−/+ cells were more resistant than wild-type cells. In addition, *RAD54*−/+ *KU70*−/+ cells were more resistant to CPT than *RAD54*−/+ cells and, less pronouncedly, wild-type cells. Their reports suggested that the role of NHEJ and HR are very differently depending on the nature of DNA damage. Although it may be irrelevant to the comparison of the DNA damage by the enzyme and IR, the quality of DNA damage itself may be different between chronic low dose-rate and acute high dose-rate radiation.

The other possibility is the activation of a backup DSB repair pathway in *RAD54*−/+ *KU70*−/+ cells irradiated with chronic low dose-rate radiation. Recently, ATM/Artemis- and 53BP1-dependent NHEJ pathways have been identified. 53BP1 plays a role in a pathway distinct from the Ku-dependent and Artemis-dependent NHEJ pathways, but requires DNA ligase IV. In addition, a backup NHEJ mechanism (B-NHEJ), involving DNA Ligase III and PARP–1, have also been identified. LIG4−/− *RAD54*−/+ mouse embryonic fibroblasts (MEF) repair DSBs as efficiently as LIG4−/+ MEFs suggesting that the increased repair efficiency in G2 phase relies on an enhanced function of the B-NHEJ pathway. These backup DSB repair pathways may be enhanced in *RAD54*−/+ *KU70*−/+ cells and rescue them from spontaneous and/or low dose-rate radiation-induced DNA damage. More studies are required to define the mechanisms active in ATM/Artemis-, 53BP1- and/or ligase III/PARP-1-defective mutants.

Chronic low dose-rate irradiation is considered to be a form of multi-fraction irradiation. Thus recovery from DNA damage under low dose-rate irradiation conditions is assumed to result from the repair of sub-lethal damage (SLD). In the study of a split dose recovery using DT40 cells and their NHEJ and HR mutants, SLD recovery was suggested due to DSB repair mediated by HR but not NHEJ. On the other hand, Kreder et al. showed that rodent Ku80 and DNA-PKcs mutants as well as the HR mutant showed the same cell survival after a pulsed low dose-rate (average 1 Gy/h) and high dose-rate (3.3 Gy/min) irradiation. In this study, the growth delay in NHEJ-defective cells irradiated with γ-rays at a low dose-rate of 2.4 cGy/day was higher than that of HR-defective cells (Fig. 2 and 5), although DT40 cells are known to have high levels of HR activity. These results suggest that NHEJ becomes more important for the cellular response to lower dose-rate irradiation. Two possible factors to explain this can be suggested. One is the fluctuation in activity of the two DSB repair pathways, NHEJ and HR, during cell cycle. NHEJ is accomplished without the need for extensive homology between damaged DNA ends. *RAD54*−/+ cells showed a relatively constant sensitivity to ionizing radiation during cell cycle. In addition, DNA-PK activity does not substantially change during the cell cycle. Thus, NHEJ is a relatively stable DSB repair pathway during the cell cycle. However, HR is efficient in the late S and G2 phases of the cell cycle because of the requirement for sister chromatids as repair templates. The repair of DSBs by HR is down-regulated during the G0, G1 and early S phases. DSBs are induced infrequently during chronic low dose-rate irradiation when compared to acute high dose-rate irradiation. Taking the cell cycle distribution into consideration, NHEJ is thought to be a predominant pathway for the repair of DSBs induced at a low frequency.

The other factor to consider is the difference in the efficiency of NHEJ versus HR. On other word, NHEJ may be more efficient than HR for the repair of low numbers of DSBs. As shown in Fig. 1, the surviving fractions of *KU70*−/+ and *PRKDC*−/− cells were lower than those of *RAD54*−/+ and *RAD51B*−/+ cells in the low dose region (< 1–2 Gy). The growth delay of *KU70*−/+ and *PRKDC*−/− cells irradiated with 0.67 Gy of X-rays was remarkably higher than that of *RAD54*−/+ and *RAD51B*−/+ cells (Fig. 3A). This interpretation is supported an estimate showing that NHEJ occurs at a minimum of 3.3–fold more frequently than HR. In addition, it has been suggested that Ku80 regulates the most accurate chromosomal DSB repair events, and that a Ku-independent mechanism uses microhomology-mediated repair which frequently leads to inaccurate DSB repair events.

In conclusion, cellular responses to chronic low dose-rate irradiation are distinctively different from responses to high dose-rate radiation, even in the process of DSB repair. Thus, in the estimation of the risks from low dose-rate radiation, results obtained using low dose-rate radiation are extremely important. The fact that both DT40 and mammalian NHEJ-defective cells were highly sensitive to low dose-rate radiotherapy, provide a foundation for the concept of using NHEJ-related factors as molecular markers to predict the sensitivity of humans to low dose-rate radiation.

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SUPPLEMENTAL FIGURES

Supplemental Fig. 1. Proliferation of wild-type (A), KU70\(^{-/-}\) (B), PRKDC\(^{-/-}\) (C), RAD54\(^{-/-}\) (D), RAD51B\(^{-/-}\) (E) and RAD54\(^{-/-}\) KU70\(^{-/-}\) (F) DT40 cells irradiated with \(\gamma\)-rays at a low dose-rate (2.4 cGy/day) (●), and unirradiated controls (○). The initial number of cells present was defined as 1. Insets indicate the results from 21 to 28 days irradiation. Error bars represent standard errors of the mean (SEM), which were obtained from three to six independent experiments.

Supplemental Fig. 2. Proliferation of wild-type (A), KU70\(^{-/-}\) (B), PRKDC\(^{-/-}\) (C), RAD54\(^{-/-}\) (D), RAD51B\(^{-/-}\) (E) and RAD54\(^{-/-}\) KU70\(^{-/-}\) (F) DT40 cells irradiated with 0.67 Gy (●) or 2 Gy (◆) of X-rays at a high dose-rate (0.9 Gy/min), and of unirradiated control cells (○). The initial number of cells present was defined as 1. Error bars represent standard errors of the mean (SEM), which were obtained from three independent experiments.
Supplemental Fig. 3. Proliferation of M059K (A) and M059J (B) cells irradiated with γ-rays at a low dose-rate (2.4 cGy/day) (●), and unirradiated controls (○). The initial number of cells present was defined as 1. Insets indicate results from 42 to 56 days irradiation. Error bars represent standard deviations (SD), which were obtained from two independent experiments.

REFERENCES

1. Hall, E. J. (1994a) DNA strand breaks and chromosomal aberrations. In: Radiobiology for the Radiologist, pp. 15–27, Lipincott, Philadelphia.

2. Khanna, K. K. and Jackson, S. P. (2001) DNA double-strand breaks: signaling, repair and the cancer connection. Nat. Genet. 27: 247–254.

3. Van Gent, D. C., Hoeijmakers, J. H. J. and Kanaar, R. (2001) Chromosomal stability and the DNA double-stranded break connection. Nat. Rev. Genet. 2: 196–206.

4. Lees-Miller, S. P. and Meek, K. (2003) Repair of DNA double strand breaks by non-homologous end joining. Biochimie 85: 1161–1173.

5. Matsumoto, Y., Suzuki, N., Namba, N., Umeda, N., Ma, X., J., Morita, A., Tomita, M., Enomoto, A., Serizawa, S., Hirano, K., Sakai, K., Yasuda, H. and Hosoi, Y. (2000) Cleavage and phosphorylation of XRCC4 protein induced by X-irradiation. FEBS Lett. 478: 67–71.

6. Mosshous, D., Callebaut, I., de Chasseval, R., Corneo, B., Cavazzana-Calvo, M., Le Deist, F., Tezcan, I., Sanal, O., Bertrand, Y., Philippe, N., Fischer, A. and de Villartay, J. P. (2001) Artemis, a novel DNA double-strand break repair/V(D)J recombination protein, is mutated in human sever combined immune deficiency. Cell 105: 177–186.

7. Ahnesorg, P., Smith, P. and Jackson, S. P. (2006) XLF interacts with the XRCC4-DNA ligase IV complex to promote DNA nonhomologous end-joining. Cell 124: 301–313.

8. Riballo, E., Kühne, M., Rief, N., Doherty, A., Smith, G. C. M., Recio, M.-J., Reis, C., Dahlin, K., Fricke, A., Krempler, A., Parker, A. R., Jackson, S. P., Gennery, A., Jeggo, P. A. and Löbrich, M. (2004) A pathway of double-strand break rejoining dependent upon ATM, Artemis, and proteins locating to γ-H2AX foci. Mol. Cell 16: 715–724.

9. Iwabuchi, K., Hashimoto, M., Matsui, T., Kurihara, T., Shimizu, H., Adachi, N., Ishiai, M., Yamamoto, K., Tauchi, H., Takata, M., Koyama, H. and Date, T. (2006) 53BP1 contributes to survival of cells irradiated with X-ray during G1 without Ku70 or Artemis. Genes Cells 11: 935–948.

10. Sonoda, E., Hochegger, H., Saberi, A., Taniguchi, Y. and Takeda, S. (2006) Differential usage of non-homologous end-joining and homologous recombination in double strand break repair. DNA Repair 5: 1021–1029.

11. Takata, M., Sasaki, M. S., Sonoda, E., Fukushima, T., Morrison, C., Albala, J. S., Swagemakers, S. M. A., Kanaar, R., Thompson, L. H. and Takeda, S. (2000) The Rad51 paralog Rad51B promotes homologous recombinational repair. Mol. Cell. Biol. 20: 6476–6482.

12. Takata, M., Sasaki, M. S., Tachiiri, S., Fukushima, T., Sonoda, E., Schild, D. S., Thompson, L. H. and Takeda, S. (2001) Chromosome instability and defective recombinational repair in knockout mutants of the five Rad51 paralogs. Mol. Cell. Biol. 21: 2858–2866.

13. Marples, B., Wouters, B. G., Collis, S. J., Chalmers, A. J. and Joiner, M. C. (2004) Low-dose hyper-radiosensitivity: a consequence of ineffective cell cycle arrest of radiation-damaged G2-phase cells. Radiat. Res. 161: 247–255.

14. Matsumoto, H., Hamada, N., Takahashi, A., Kobayashi, Y. and Ohtani, T. (2007) Vanguards of paradigm shift in radiation biology: radiation-induced adaptive and bystander responses. J. Radiat. Res. 48: 97–106.

15. Koana, T., Okada, M. O., Ogura, K., Tsujimura, H. and Sakai, K. (2007) Reduction of background mutations by low-dose X irradiation of Droso phila spermatocytes at a low dose rate. Radiat. Res. 167: 217–221.

16. Utsumi, H., Tano, K., Takata, M., Takeda, S. and Elkind, M. M. (2001) Requirement for repair of DNA double-strand breaks by homologous recombination in split-dose recovery. Radiat. Res. 155: 680–686.

17. Hao, B. S. S., Tanou, K., Takeda, S. and Utsumi, H. (2007) Split dose recovery studies using homologous recombination deficient gene knockout chicken B lymphocyte cells. J. Radiat. Res. 48: 77–85.
18. Kreder, N. C., ten Cate, R., Rodermond, H. M., van Bree, C., Franken, N. A. P., Zdzienicka, M. Z. and Haveman, J. (2004) Cellular response to pulsed low-dose rate irradiation in X-ray sensitive hamster mutant cell lines. J. Radiat. Res. 45: 385–391.

19. Baba, T. W., Giroir, B. P. and Humphries, E. H. (1985) Cell lines derived from avian lymphomas exhibit two distinct phenotypes. Virology 144: 139–151.

20. Buerstedde, J. M., Reynaud, C. A., Humphries, E. H., Olson, W., Ewert, D. L. and Weill, J. C. (1990) Light chain gene conversion continues at high rate in an ALV-induced cell line. EMBO J. 9: 921–927.

21. Takata, M., Sasaki, M. S., Sonoda, E., Morrison, C., Hashimoto, M., Usami, H., Yamaguchi-Iwai, Y., Shinohara, A. and Takeda, S. (1998) Homologous recombination and non-homologous end-joining pathways of DNA double-strand break repair have overlapping roles in the maintenance of chromosomal integrity in vertebrate cells. EMBO J. 17: 5497–5508.

22. Fukushima, T., Takata, M., Morrison, C., Araki, R., Fujimori, A., Abe, M., Tatsumi, K., Jasins, M., Dhar, P. K., Sonoda, E., Chiba, T. and Takeda, S. (2001) Genetic analysis of the DNA-dependent protein kinase reveals an inhibitory role of Ku in late S-G2 phase DNA double-strand break repair. J. Biol. Chem. 276: 44413–44418.

23. Allalunis-Turner, M. J., Barron, G. M., Day, R. S., III, Dobler, K. D. and Mirzayans, R. (1993) Isolation of two cell lines from a human malignant glioma specimen differing in sensitivity to radiation and chemotherapeutic drugs. Radiat. Res. 134: 349–354.

24. Lees-Miller, S. P., Godbout, R., Chan, D. W., Weinfeld, M., Day III, R. S., Barron, G. M. and Allalunis-Turner, J. (1995) Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line. Science 267: 1183–1185.

25. Hoshi, Y., Nomura, T., Oda, T., Iwasaki, T., Fujita, K., Ishikawa, T., Kato, A., Ikegami, T., Sakai, K., Tanooka, H. and Yamada, T. (2000) Application of a newly developed photoluminescence glass dosimeter for measuring the absorbed dose in individual mice exposed to low-dose rate 137Cs γ-rays. J. Radiat. Res. 41: 129–137.

26. Adachi, N., Suzuki, H., Iiizumi, S. and Koyama, H. (2003) Hypersensitivity of nonhomologous DNA end-joining mutants to VP-16 and ICRF-193. J. Biol. Chem. 278: 35897–35902.

27. Adachi, N., So, S. and Koyama, H. (2004) Loss of nonhomologous end joining confers camptothecin resistance in DT40 cells. J. Biol. Chem. 279: 37343–37348.

28. WU, W., Wang, M., Wu, W., Singh, S. K., Mussfeldt, T. and Iliakis, G. (2008) Repair of radiation induced DNA double strand breaks by backup NHEJ is enhanced in G2. DNA Repair 7: 329–338.

29. Buerstedde, J. M. and Takeda, S. (1991) Increased ratio of targeted to random integration after transfection of chicken B cell lines. Cell 67: 179–188.

30. Takeda, S., Masteller, E. L., Thompson, C. B. and Buerstedde, J. M. (1992) RAG-2 expression is not essential for chicken immunoglobulin gene conversion. Proc. Natl. Acad. Sci. USA 89: 4023–4027.

31. Bezzubova, O. Y. and Buerstedde, J. M. (1994) Gene conversion in the chicken immunoglobulin locus: a paradigm of homologous recombination in higher eukaryotes. Experientia 50: 270–276.

32. Yoshida, M., Hosoi, Y., Miyachi, H., Ishii, N., Matsumoto, Y., Enomoto, A., Nakagawa, K., Yamada, S., Suzuki, N. and Ono, T. (2002) Roles of DNA-dependent protein kinase and ATM in cell-cycle-dependent radiation sensitivity in human cell. Int. J. Radiat. Biol. 78: 503–512.

33. Guirouilh-Barbat, J., Huck, S., Bertrand, P., Pirizio, L., Desmaze, C., Sabatier, L. and Lopez, B. S. (2004) Impact of the Ku80 pathway on NHEJ-induced genome rearrangements in mammalian cells. Mol. Cell 14: 611–623.

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