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Reduction of Delayed Homologous Recombination by Induction of Radioadaptive Response in RaDR-GFP Mice (Yonezawa Effect): An Old Player With a New Role

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Abstract

Radiotherapy (RT) treats cancer effectively with high doses of ionizing radiation (IR) to killing cancer cells and shrinking tumors while bearing the risk of developing different side effects, including secondary cancer, which is most concerning for long-term health consequences. Genomic instability (GI) is a characteristic of most cancer cells, and IR-induced GI can manifest as delayed homologous recombination (HR). Radioadaptive response (RAR) is capable of reducing genotoxicity, cell transformation, mutation, and carcinogenesis, but the rational evidence describing its contributions to the reduction of radiation risk, in particular, carcinogenesis, remains fragmented. In this work, to investigate the impact of RAR on high-dose, IR-induced GI measured as delayed HR, the frequency of recombinant cells was comparatively studied under RAR-inducible and -uninducible conditions in the nucleated cells in hematopoietic tissues (bone marrow and spleen) using the *Rosa26* Direct Repeat-green fluorescent protein (RaDR-GFP) homozygote mice. Results demonstrated that the frequency of recombinant cells was significantly lower in hematopoietic tissues under RAR-inducible condition. These findings suggest that reduction in delayed HR may be at least a part of the mechanisms underlying decreased carcinogenesis by RAR, and application of RAR would contribute to a more rigorous and scientifically grounded system of radiation protection in RT.

Keywords

adaptive response, ionizing radiation, genomic instability, homologous recombination, rosa26 direct repeat-GFP (RaDR-GFP) mice

Introduction

Radiotherapy (RT) treats cancer effectively using high doses of ionizing radiation (IR) to kill cancer cells and shrink tumors. More than 50% of all patients with cancer need RT as an integral part of treatment at some point of the time. On the other hand, RT bears the risk of developing different side effects including secondary cancer, which is one of the important late side effects causing the great concern for long-term health consequences and impacting on optimal treatment decision-making.[1-4]

Genomic instability (GI), the accumulation of multiple changes to convert a stable genome to an unstable genome, is characterized by varied end points, for example, chromosomal rearrangements and aberrations, amplification of genetic material, micronucleus formation, and gene mutation. The IR is capable of inducing GI in mammalian cells, manifesting as delayed homologous recombination (HR) in vitro and in

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vivo,[5,6] which could be detected in the progeny of an irradiated cell multiple generations after the initial exposure. Arising from many different pathways, including such as IR-induced DNA damage and dysregulation of DNA repair machinery, GI is central to carcinogenesis,[7] which could initiate cancer and augment progression.[8-14] The GI is the driving force responsible for radiocarcinogenesis.[15-17]

Radioadaptive response (RAR) is an evolutionary conserved phenomenon in which exposure to a low dose of IR (priming dose) reduces the biological effects of subsequent higher IR doses (challenge dose). Since the discovery of RAR,[18] it has been demonstrated in a variety of biosystems from simple prokaryotes to higher eukaryotes including mammalian animals with end points such as DNA damage, chromosomal aberrations, cell transformation, cell death, and mutation in vitro and prenatal death, malformation, hematopoietic death, and carcinogenesis in vivo.[19-22] There is a great potential for use of low-dose IR for certain diseases as treatment or prevention and for improved treatment.[23-26] Of note, RAR is demonstrated in the normal cells but cancer cells including cancer stem cells in vitro and in vivo,[27-31] revealing its potential application to improve cancer RT by priming the normal tissues to protect multiple organs from the side effects of RT including the secondary cancer. As a fact, RAR is capable of reducing genotoxicity, cell transformation, mutation, and carcinogenesis,[20,25] while the rational evidence describing its contributions to the reduction in radiation risk, in particular, carcinogenesis, remains fragmented.[32] In addition, as a major concern regarding the application of RAR, it may protect cells initially but predispose surviving cells to increased GI later.

In our previous study, we used the RAR mouse model (Yonezawa Effect) established by Yonezawa and colleagues,[33-35] which could rescue bone marrow death through induced resistance in the hematopoietic system.[36-38] We verified and confirmed this model under the setup in our experimental facility and observed the reduction of high-dose, IR-induced GI measured as frequency of micronucleus erythrocytes in bone marrow cells in surviving mice rescued by RAR.[39] In this work, we investigated the impact of RAR on high-dose, IR-induced GI measured as delayed HR. The frequency of recombinant cells was comparatively studied under RAR-inducible and -uninducible conditions in hematopoietic tissues (bone marrow and spleen) using the Rosa26 Direct Repeat-green fluorescent protein (RaDR-GFP) mice.[40] Significantly decreased frequency of recombinant cells under RAR-inducible condition was found when compared to that under RAR-uninducible condition.

Materials and Methods

Animals

The *Rosa26* Direct Repeat-GFP (RaDR-GFP) mice on the C57BL/6J background[40] were kind gifts from Dr Bevin P. Engelward (Massachusetts Institute of Technology, Cambridge, Massachusetts) via Dr Jac A. Nickoloff (Colorado State University, Fort Collins, Colorado) and maintained in heterozygote background. In the mouse genome, a direct repeat HR substrate is targeted to the ubiquitously expressed Rosa26 locus and HR between 2 truncated enhanced green fluorescent protein (EGFP) expression cassettes can yield a fluorescent signal (Figure 1). Animals bearing homozygote RaDR-GFP background generated as offspring from heterozygote intercrosses were used. They were kept in a specific pathogen-free animal facility until 5-week postpartum and then transferred to a conventional animal facility. C57BL/6J mice aged 5 weeks old were purchased from SLC, Inc (Japan). Animals were kept under a 12-hour light–12-hour dark photoperiod, housed in autoclaved cages with sterilized wood chips, and allowed to standard laboratory chow (MB-1; Funabashi Farm Co, Japan) and acidified water ad libitum. The RaDR-GFP mice were acclimatized to the laboratory conditions 1 week before use. To avoid possible effects from the developmental condition of the animals, 6-week-old mice with a significantly different body weight (more or less than the mean $\pm$ 2 SD) were omitted from this study. Based on the preliminary trials, in the present study, at least 6 mice were used in each experimental point. C57BL/6-Tg (CAG-EGFP) mice aged 8 weeks old were purchased from SLC, Inc (Japan). All experimental protocols (Experimental Animal Research Plan No. 14-1014-2, No. 16-1033 and No. 16-2010-4) involving mice were reviewed and approved by The Institutional Animal Care and Use Committee of the National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology (QST-NIRS), Japan. The experiments were performed in strict accordance with the QST-NIRS *Guidelines for the Care and Use of Laboratory Animals*.

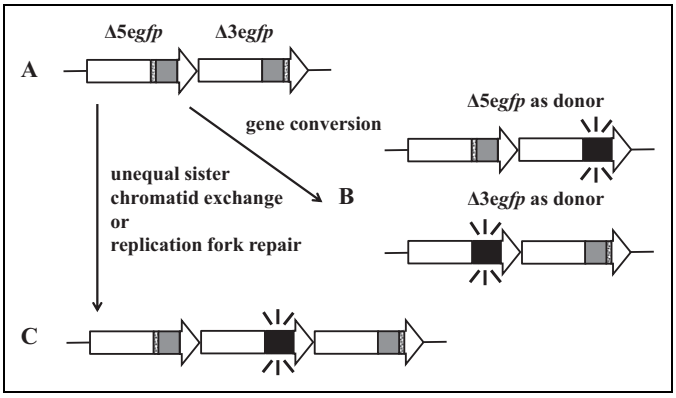

Figure 1. Homologous recombination (HR) in the RaDR-GFP substrate giving rise to fluorescence. The substrate (A) consists of 2 EGFP expression cassettes arranged in tandem (large arrows), one with deletion at 5' (Δ5) end and the other at 3' (Δ3) end of the coding sequences. The cell harboring the full-length EGFP-coding sequences (black bars with glowing markers) after HR events, namely, gene conversion (B) and unequal sister chromatid exchange or replication fork repair (C), gives rise to fluorescence. Dotted bars indicate deletions, gray bars stand for EGFP-coding sequences, and white bars represent the CAG promoter and polyadenylation signal sequences.

Irradiation

X-rays were generated with an X-ray machine (Pantak-320S; Shimadzu, Japan) operated at 200 kVp and 20 mA, using a 0.50 mm Al + 0.50 mm Cu filter. An exposure-rate meter (AE-1321M; Applied Engineering Inc, Japan) with an ionization chamber (C-110, 0.6 mL; JARP, Applied Engineering Inc, Japan) was used for the dosimetry. The dose rate for delivering the priming dose and the challenge dose was at about 0.30 Gy/min and 0.90 Gy/min, respectively. The mice held in acryl containers were exposed to total body irradiation (TBI) at room temperature.

Mouse Model for Radiation-Induced AR

The RAR mouse model for rescue of bone marrow death (Yonezawa Effect) established by Yonezawa and colleagues[34] was adopted, verified, and confirmed in C57BL/6J mice under the experimental conditions in our research facilities. It was applied to the present work using X-rays and RaDR-GFP mice. The timing for delivery of the priming dose and challenge dose was on postnatal age of 6 and 8 weeks of the mice, respectively. A dose of 0.50 Gy was used as the priming dose to verify the existence of RAR in the 30-day survival test and to investigate the frequency of recombinant cells in the nucleated cells in hematopoietic tissues under RAR-inducible and -uninducible conditions. For the challenge dose, based on the preliminary trials, 2 doses, namely, a lethal dose at 6.00 Gy and a sublethal dose at 5.75 Gy, were used in the 30-day survival test. A nonlethal dose at 4.00 Gy was used in the study on the frequency of recombinant cells. The TBI treatment of mice with both the priming dose and the challenge dose was defined as RAR-inducible condition and with the challenge dose alone as RAR-uninducible condition.

Enumeration of Recombinant Cells

To comparatively evaluate the homologous recombination frequency in the nucleated cells in the bone marrow and spleen in mice under experimental conditions capable of inducing RAR (receiving both the priming dose of 0.50 Gy X-rays and the challenge dose of 4.00 Gy X-rays) or incapable of inducing RAR (receiving only the challenge dose of 4.00 Gy X-rays), the recombinant cells (GFP-positive cells) in the nucleated bone marrow cells and splenocytes were analyzed by flow cytometry. In brief, the mice were euthanized by CO_2 asphyxiation the following day after the 30-day survival test, and the femurs and spleens were collected. Single-cell suspensions of dissociated bone marrow tissues and spleens in phosphate-buffered saline free from calcium and magnesium ions (PBS (−); Wako Pure Chemical Industries, Ltd, Japan) were filtered through a 40-μm cell strainer (Corning Inc, Corning, New York) after treating with Tris-buffered ammonium chloride for the lysis of red blood cells and washing with PBS (−), and then the nucleated bone marrow cells and splenocytes were fixed with 1% paraformaldehyde phosphate buffer solution (Wako Pure Chemical Industries) at 4°C. Before analysis with flow cytometry, the cells were washed once with PBS (−), resuspended in Opti-MEM (Life Technologies, Carlsbad, California), and filtered through a 35-μm cell strainer (Corning Inc). The cell suspensions were gated using forward and side scatter and analyzed on a BD FACScalibur flow cytometer (Becton Dickinson, Frankloin Lakes, New Jersey) with an excitation laser at 48 nm, emission filters to measure green fluorescence (530 nm), and autofluorescence (580 nm). The cells from C57BL/6-Tg (CAG-EGFP) mice constitutively expressing EGFP and wild-type C57BL/6J mice with no EGFP expression were used, respectively, as a positive control and a negative control for flow cytometry. Cells with significantly higher levels of fluorescence at 530 nm than 580 nm were judged as recombinant cells, namely, RaDR-GFP-positive cells with "green fluorescence." Six to 12 animals were used per experimental point, and for each sample at least 1 million cells were analyzed. The frequency of recombinant cells was expressed as the number of GFP-positive cell parts per million of nucleated bone marrow cells or splenocytes.

Statistical Analysis

Statistical evaluation of the data was done using the χ^2 test for the 30-day survival and Student t test for the recombinant cell frequency. The statistical significance was assigned to $P < .05$.

Results

Validation of RAR in RaDR-GFP Mice Using a Sublethal Dose as the Challenge Dose

Reproducibility of the RAR mouse model (Yonezawa Effect) in RaDR-GFP mice using 30-day survival test as the end point was verified and confirmed. Based on the preliminary trial, no statistically significant gender difference was found in the survival rate in the animals receiving the same treatment; thus, analysis of the data pooled from all animals of both genders in each group was done. Under the RAR-inducible condition, TBI of the animals was performed with a priming dose of 0.50 Gy X-rays at postnatal 6 weeks followed by a challenge dose of 5.75 Gy X-rays at postnatal 8 weeks. Under the RAR-uninducible condition, only the challenge dose of 5.75 Gy X-rays was delivered to the mice at postnatal 8 weeks. Results showed that administration of the priming dose significantly increased the survival rate of the mouse from 29.5% to 98.4% (Figure 2) and clearly indicated that RAR was demonstrated in our experimental setup.

Validation of RAR in RaDR-GFP Mice Using a Lethal Dose as the Challenge Dose

Thirty-day survival test was performed to validate rescue efficacy of RAR against a lethal dose in RaDR-GFP female mice. Under RAR-inducible condition (TBI with a priming dose of 0.50 Gy X-rays at postnatal 6 weeks followed by a challenge

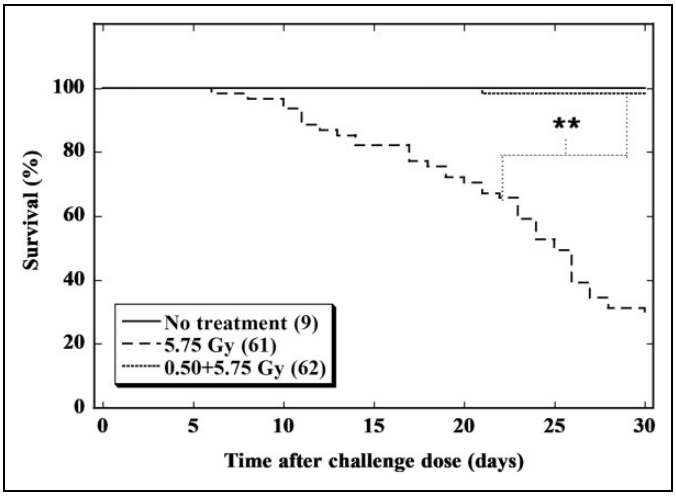

Figure 2. Thirty-day survival test after a challenge dose of 5.75 Gy in RaDR-GFP mice. Effect of a priming dose of 0.50-Gy X-rays on a subsequent challenge dose of 5.75-Gy X-rays on mouse 30-day survival was verified. The solid line denotes the animals (5 female and 4 male) that were untreated with ionizing radiation (IR). The broken line represents the animals (31 female and 30 male) that were irradiated with only the challenge dose at postnatal 8 weeks. The dotted line stands for the animals (31 female and 31 male) that were primed with a dose of 0.50-Gy X-rays at postnatal 6 weeks and then followed by a challenge dose of 5.75-Gy X-rays at postnatal 8 weeks. Two asterisks (**) indicate statistically significant differences ($P < .01$) between the 2 groups that were compared.

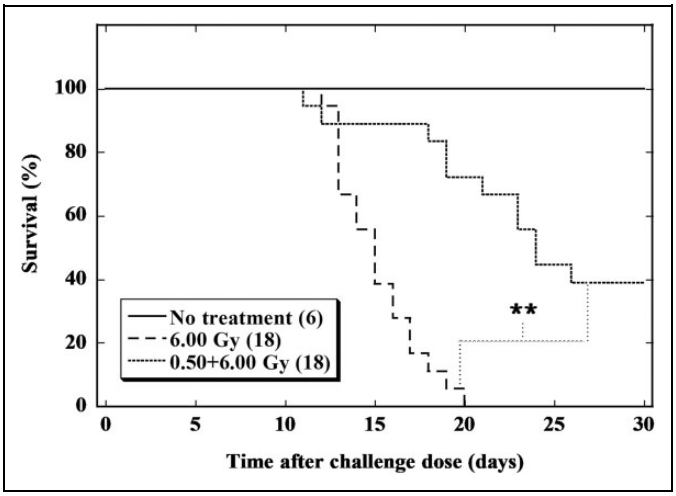

Figure 3. Thirty-day survival test after a challenge dose of 6.00 Gy in male RaDR-GFP mice. Effect of a priming dose of 0.50-Gy X-rays on a subsequent challenge dose of 6.00-Gy X-rays on mouse 30-day survival was verified. The solid line betokens the mice (6 animals) that were untreated with IR. The broken line signifies the mice (18 animals) that were irradiated with only the challenge dose at postnatal 8 weeks. The dotted line indicates the mice (18 animals) that were primed with a dose of 0.50-Gy X-rays at postnatal 6 weeks, and then followed by a challenge dose of 6.00-Gy X-rays at postnatal 8 weeks. Two asterisks (**) indicate statistically significant differences ($P < .01$) between the 2 groups that were compared.

dose of 6.00 Gy X-rays at postnatal 8 weeks), the survival rate was 38.9%. On the other hand, under RAR-uninducible condition (TBI with only the challenge dose of 6.00 Gy X-rays at postnatal 8 weeks), all the animals died within 20 days after exposure. The increase in the survival rate was statistically significant (Figure 3). The results clearly showed that under RAR inducible condition, still more than one-third of the animals could survive from a lethal dose indicating the high efficacy of RAR for rescuing the animals from high dose-induced bone marrow death.

Validation of the Frequency of Recombinant Cells in the Nucleated Bone Marrow Cells in RaDR-GFP Mice

Frequency of recombinant cells measured as GFP-positive cells and expressed in number of per million of nucleated bone marrow cells was flow cytometrically studied in each experimental group in male RaDR-GFP mice (Figure 4). "Control group" was sham-treated with IR, "0.50-Gy group" was irradiated with only a priming dose of 0.50 Gy X-rays at postnatal 6 weeks. Animals in the "4.00-Gy group" were irradiated with only a challenge dose of 4.00 Gy X-rays at postnatal 8 weeks. Animals in the "0.50+4.00-Gy group" were primed with a dose of 0.50 Gy X-rays at postnatal 6 weeks and then followed by a challenge dose of 4.00 Gy X-rays at postnatal 8 weeks. The number of GFP-positive cells per million of nucleated bone marrow cells in the "control group", the "0.50-Gy group", the "4.00-Gy group", and the "0.50+4.00-Gy" group were

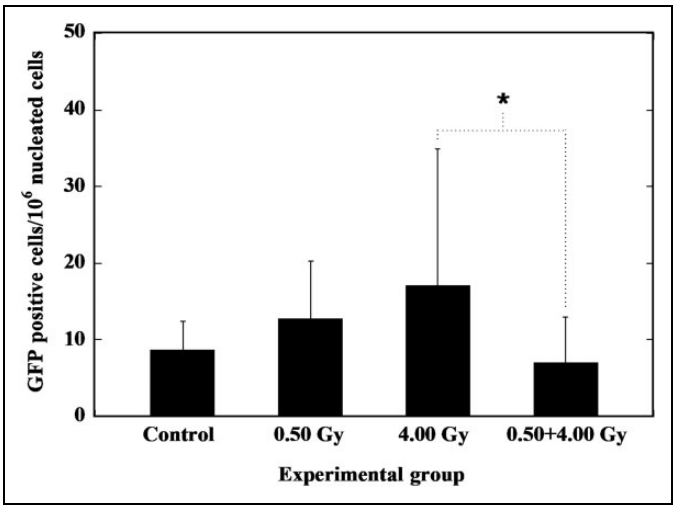

Figure 4. Number of GFP-positive cell parts per million of nucleated bone marrow cells in male RaDR-GFP mice. Validation of GFP-positive cell appearance in number parts per million nucleated bone marrow cells among animals in different experimental groups. Animals in the "control group" were untreated with ionizing radiation (IR). Animals in the "0.50-Gy group" were irradiated with only a priming dose of 0.50-Gy X-rays at postnatal 8 weeks. Animals in the "4.00-Gy group" were irradiated with only a challenge dose of 4.00-Gy X-rays at postnatal 8 weeks. Animals in the "0.50+4.00-Gy group" were primed with a dose of 0.50-Gy X-rays at postnatal 6 weeks, and then followed by a challenge dose of 4.00-Gy X-rays at postnatal 8 weeks. The number of animals in the control group, the 0.50-Gy group, the 4.00-Gy group, and the 0.50+4.00-Gy group were 8, 8, 11, and 11, respectively. One asterisk (*) symbolizes statistically significant differences ($P < .05$) between the 2 groups that were compared.

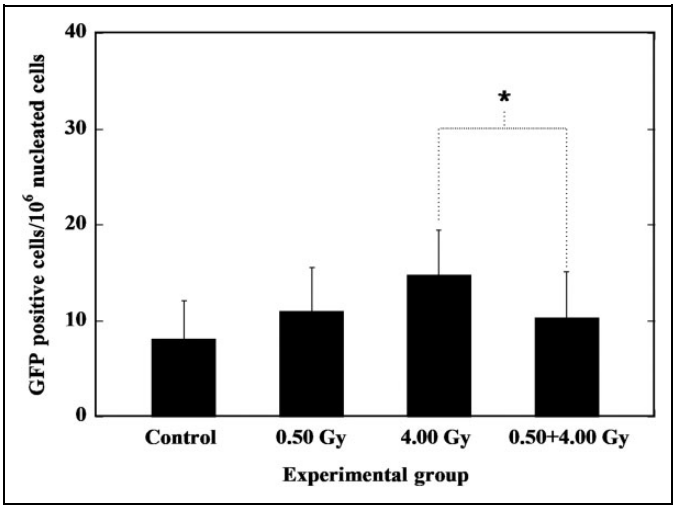

Figure 5. Number of GFP-positive cell parts per million splenocytes in male RaDR-GFP mice. Validation of GFP-positive cell appearance in number parts per million splenocytes among animals in different experimental groups. Either the naming of each experimental group or the number of animals used was the same as those described in the legend of Figure 4. One asterisk (*) symbolizes statistically significant differences ($P < .05$) between the 2 groups that were compared.

8.60 ± 3.80, 12.7 ± 7.50, 17.0 ± 17.9, and 7.00 ± 6.00, respectively. There was a statistically marked increase in the frequency of recombinant cells for the "4.00-Gy group" compared to the "control group." On the other hand, there was a statistically significant decrease in the frequency of recombinant cells in the "0.50 + 4.00-Gy group" compared to the "4.00-Gy group." There was no statistical difference in the frequency of recombinant cells between the "control group" and the "0.50+4.00-Gy group". These results clearly showed that increased frequency of recombinant cells was observed under AR-uninducible condition, decreased frequency of recombinant cells was confirmed under AR-inducible condition, and induction of RAR could markedly reduce the frequency of recombinant cells in the "0.50 + 4.00-Gy group" to the background level, namely, the "Control group".

Validation of the Frequency of Recombinant Cells in Splenocytes in RaDR-GFP Mice

Flow cytometry was performed to validate whether significant decrease in the frequency of recombinant cells occurred under RAR-inducible condition or the RAR-uninducible condition. Naming of each experimental group was the same as those mentioned earlier. The number of GFP-positive cells per million of splenocytes in the "control group", the "0.50-Gy group," the "4.00-Gy group," and the "0.50+4.00-Gy" group were 8.10 ± 4.00, 11.0 ± 4.50, 14.8 ± 4.70, and 10.3 ± 4.80, respectively (Figure 5). There was a statistically significant increase in the frequency of recombinant cells for the "4.00-Gy group" compared to the "control group." On the other hand, there was a statistically significant decrease in the frequency of recombinant cells in the "0.50+4.00-Gy group" compared to the "4.00-Gy group." There was no statistical difference in the frequency of recombinant cells between the "control group" and the "0.50+4.00-Gy group." These results clearly showed that increased frequency of recombinant cells was observed under RAR-uninducible condition, decreased frequency of recombinant cells was confirmed under RAR-inducible condition, and induction of RAR could significantly reduce the frequency of recombinant cells in the "0.50+4.00-Gy group" to a low level similar to that in the "0.50-Gy group."

Discussion

Radiotherapy, as a critical and inseparable component of comprehensive treatment, is one of the more cost-effective cancer therapeutic modalities on a global scale. On the other hand, IR protection is a critical issue in many fields including cancer RT, where IR is a double-edged sword having a well-established role in the cancer treatment while bearing the risk of inducing secondary cancer. Despite important scientific and technological advances in radiation delivery, RT still induces irreversible side effects on the normal tissues surrounding the tumor, thus making the tolerance dose of the normal tissue a major dose-limiting factor and also resulting in the increased secondary cancer risk. Research and development of the intervention strategies to protect the normal tissues from the detrimental effects of radiation exposure at high doses remain a significant unmet medical need and an attractive goal as well.

The maintenance of genomic stability by DNA repair mechanisms is essential for cellular integrity to prevent errors during DNA replication.[41] Unrepaired DNA damage and acquired genomic alteration due to genotoxic stress and carcinogen insults such as IR could generate GI. The GI could provide the cell a shorter cell cycle and/or an advantage of bypassing intracellular and immunological control systems, cause an accumulation of chromosomal aberrations and mutations, and give cancerous cells an advantage in both growth and malignant transformation,[12] being responsible for various clinical phenotypes including carcinogenesis.[42] As one of the critical DNA repair pathways, HR is usually error free, but it could sometimes result in cancer-promoting mutations. In fact, IR could directly induce HR,[43,44] where it may mainly function by preventing misrepaired DNA double-strand breaks and broken replication forks to decrease the mutation load of the cells and thus reduce GI and carcinogenesis. On the other hand, IR-induced GI played a critical role in carcinogenesis[45] and increased delayed HR is one of the phenomena of IR-induced GI.[5,6]

The RAR is well established in varied biosystems, being capable of reducing the risk of late adverse effects of IR exposures, in particular, reduction in late genomic toxicity/GI and carcinogenesis. Although it still remains uncertain and in a vague proposition as to whether RAR will have any utility in establishing risks of IR to humans,[20] keen interest has been shown to RAR.[22,25] As approaches directing toward reducing IR-induced GI are in hope of controlling the initiation and

progress of the secondary cancer in RT, induction of RAR, as a promising radiation countermeasure, is expected to be applied for clinical trials such as priming the normal tissue with low dose of IR before performing radiotherapy. A better understanding of RAR is needed to know to which extent low-dose IR might be beneficial in humans.[46] The RAR could induce protective effects through mechanisms critical to life[21] such as reduction in cell death, chromosomal aberrations, mutations, GI, and malignant transformation *in vitro*,[22,47] and enhancement of antioxidative capacities, increase in DNA repair capacity, and increase in radioresistance in the hematopoietic tissue in vivo.[38,48,49]

To gain insight into the genomic stability in vivo after treatment with RAR-inducible and -uninducible conditions, using the RAR mouse model (Yonezawa Effect), we confirmed that RAR could reduce late genomic toxicity/GI with 2 end points at different levels: in our previous study, reduction of micronuclei in the erythrocytes in bone marrow in the surviving mice rescued by RAR,[39] and in the present work, reduction in delayed HR induced by a challenge high dose in the mice treated under RAR-inducible condition. These results suggest at least in part the mechanisms underlying RAR-induced decrease in carcinogenesis. These findings also imply the potential for applying RAR to protect normal tissues from IR during RT.

The mouse RAR model (Yonezawa Effect) is a well-designed system in investigating the late health effect in vivo such as GI and carcinogenesis. There are 2 different phenotypes of RAR in this model that involved different mechanisms: The first phenotype could be induced 2 weeks after a 0.30 to 0.50 Gy priming IR with the involvement of the induced radioresistance in blood-forming tissues, and the second phenotype could be induced 2 months after a 0.05 to 0.10 Gy priming exposure with the interaction between blood-forming tissue and the central nervous system. Interestingly, partial exposure to priming IR of trunk or head is also effective for induction of RAR. The model for the first phenotype was applied to the present study. Compared to most of the existing models,[50] applying RaDR-GFP mice to this system further adds technology advantages to the mechanistic research, for example, the approach to quantification of recombinant cells in vivo opens new doors to RAR studies, in particular, giving rise to more opportunity for understanding the underlying molecular mechanisms linking to RAR-induced reduction of carcinogenesis. In addition to that, the assay of recombinant cells has the advantage over time and labor-intensive cytogenetic analysis, as it is capable of detecting and quantifying recombinant cells in situ within an intact organ[40,51]; thus, this system could be used to study the clonal expansion of a single cell as well as to reveal the location and cell types that have undergone HR. For example, in mouse spleen as subpopulations of splenocytes responded differently to IR at low dose and high dose,[52] this system makes it possible to study the susceptibility of different cell types to IR-induced GI under RAR-inducible and -uninducible conditions. By using this system, it is expected to reveal the relationship among different end points for GI leading to a breakthrough in understanding of the molecular mechanisms underlying RAR, for example, the micronuclei formation reposted in our previous work[39] and delayed hyper-recombination observed in the present study.

To modify the radioresistance of both the cancer cells and the surrounding normal tissues, triggering the mechanisms to selectively increase the radioresistance of the normal tissues and sensitize the cancer cells is of great importance. Approaches such as development of radioprotective agents, pharmaceutical gene regulating, and intervening dietary habit, psychological stress and life style[13] were proposed, but the efficacy of these approaches is still largely uncertain. For example, for development of radioprotective agents, in addition to the lack of tissue-specific protection by most of the agents, even for amifostine, which was reported being capable of mitigating IR-induced delayed GI,[53] its selective cytoprotective activity was only proved in patients with head and neck squamous cell carcinoma undergoing RT[54]; predisposition of the surviving cells to increased GI is still of great concern. The personalized cancer RT with other therapeutic modalities from integrated research involving physics, radiobiology, and clinical studies, tailored to the particular patient and type of cancer, must be developed. On the other hand, research and development of these therapeutic modalities are relatively time consuming and costly, depending also on the advancement of technology. Of note, as RAR can selectively protect normal cells from the side effects of IR at higher dose, its combination with RT would consequently make cancer RT a selective treatment for cancer. In this regard, clinical application of RAR would generally benefit cancer RT from the point of view of increasing the maximum tolerated dose, reducing the risk of secondary cancer in addition to being the simpler, more convenient, cost effective, and less time consuming.

Taken together, our results demonstrate that induction of RAR could relieve RI-induced GI in the hematopoietic system and suggest that reduction in delayed HR may be at least a part of the mechanisms underlying decreased carcinogenesis by RAR. Results bring new knowledge to the characterization of the "Yonezawa Effect" by providing a new insight into the mechanistic study in the RAR mouse model. These findings indicate that application of RAR would improve and revolutionize clinical medicine via contributing to a more rigorous and scientifically grounded system of radiation protection in RT.

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Declaration of Conflicting Interests

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References

1. Hall EJ. Intensity-modulated radiation therapy, protons, and the risk of second cancers. *Int J Radiat Oncol Biol Phys*. 2006;65(1):1-7.
2. Friedman DL, Whitton J, Leisenring W, et al. Subsequent neoplasms in 5-year survivors of childhood cancer: the Childhood Cancer Survivor Study. *J Natl Cancer Inst*. 2010;102(14): 1083-1095.
3. Berrington de Gonzalez A, Gilbert E, Curtis R, et al. Second solid cancers after radiation therapy: a systematic review of the epidemiologic studies of the radiation dose-response relationship. *Int J Radiat Oncol Biol Phys*. 2013;86(2):224-233.
4. Dracham CB, Shankar A, Madan R. Radiation induced secondary malignancies: a review article. *Radiat Oncol J*. 2018;36(2):85-94.
5. Huang L, Grim S, Smith LE, et al. Ionizing radiation induces delayed hyperrecombination in Mammalian cells. *Mol Cell Biol*. 2004;24(11):5060-5068.
6. Allen CP, Hirakawa H, Nakajima NI, et al. Low- and high-LET ionizing radiation induces delayed homologous recombination that persists for two weeks before resolving. *Radiat Res*. 2017; 188(1):82-93.
7. Schmitt MW, Prindle MJ, Loeb LA. Implications of genetic heterogeneity in cancer. *Ann N Y Acad Sci*. 2012;1267:110-116.
8. Charames GS, Bapat B. Genomic instability and cancer. *Curr Mol Med*. 2003;3(7):589-596.
9. Komarova NL. Genomic instability in cancer: biological and mathematical approaches. *Cell Cycle*. 2004;3(8):1081-1085.
10. Negrini S, Gorgoulis VG, Halazonetis TD. Genomic instability–an evolving hallmark of cancer. *Nat Rev Mol Cell Biol*. 2010; 11(3):220-228.
11. Michael WS, Marc JP, Lawrence AL. Implications of genetic heterogeneity in cancer. *Ann N Y Acad Sci*. 2012;1267:110-116.
12. Yao Y, Dai W. Genomic instability and cancer. *J Carcinog Mutagen*. 2014;5:1000165.
13. Ferguson LR, Chen H, Collins AR, et al. Genomic instability in human cancer: Molecular insights and opportunities for therapeutic attack and prevention through diet and nutrition. *Semin Cancer Biol*. 2015;(suppl 35): S5-S24.
14. Bakhoum SF, Landau DA. Chromosomal instability as a driver of tumor heterogeneity and evolution. *Cold Spring Harb Perspect Med*. 2017;7(6):a029611.
15. Morgan WF, Day JP, Kaplan MI, McGhee EM, Limoli CL. Genomic instability induced by ionizing radiation. *Radiat Res*. 1996; 146(3):247-258.
16. Limoli CL, Ponnaiya B, Corcoran JJ, et al. Genomic instability induced by high and low LET ionizing radiation. *Adv Space Res*. 2000;25(10):2107-2117.
17. Smith LE, Nagar S, Kim GJ, Morgan WF. Radiation-induced genomic instability: radiation quality and dose response. *Health Phys*. 2003;85(1):23-29.
18. Olivieri G, Bodycote J, Wolff S. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. *Science*. 1984;223(4636):594-597.
19. United Nations Scientific Committee on the Effects of Atomic Radiation. Adaptive response to radiation in cells and organisms. In: United Nations Scientific Committee on the effects of Atomic Radiation, United Nations, *Sources and Effects of Ionizing Radiation: UNSCEAR Report to the General Assembly with Scientific Annexes*. New York, NY; 1994:185-272.
20. Wolff S. The adaptive response in radiobiology: evolving insights and implications. *Environ Health Perspect*. 1998;106(suppl 1): 277-283.
21. Mitchel RE. Low doses of radiation are protective in vitro and in vivo: evolutionary origins. *Dose Response*. 2006;4(2):75-90.
22. Varès G, Wang B, Tanaka K, Nakajima T, Nenoi M, Hayata I. Radiation-induced adaptive response with reference to evidence and significance: a review. *Indian J Radiat Res*. 2006;3(1):16-34.
23. Scott BR. Radiation-hormesis phenotypes, the related mechanisms and implications for disease prevention and therapy. *J Cell Commun Signal*. 2014;8(4):341-352.
24. Scott BR. Small radiation doses enhance natural barriers to cancer. *J Am Phys Surg*. 2017;22(4):105-110.
25. Nenoi M, Wang B, Vares G. In vivo radioadaptive response: a review of studies relevant to radiation-induced cancer risk. *Human Exp Toxicol*. 2015;34(3):272-283.
26. Cui J, Yang G, Pan Z, et al. Hormetic response to low-dose radiation: focus on the immune system and its clinical implications. *Int J Mol Sci*. 2017;18(2):280.
27. Jiang H, Li W, Li X, Cai L, Wang G. Low-dose radiation induces adaptive response in normal cells, but not in tumor cells: in vitro and in vivo studies. *J Radiat Res*. 2008;49(3):219-230.
28. Liang X, Gu J, Yu D, et al. Low-dose radiation induces cell proliferation in human embryonic lung fibroblasts but not in lung cancer cells: importance of ERK1/2 and AKT signaling pathways. *Dose Response*. 2016;14(1). doi: 10.1177/1559325815622174.
29. Yang G, Yu D, Li W, et al. Distinct biological effects of low-dose radiation on normal and cancerous human lung cells are mediated by ATM signaling. *Oncotarget*. 2016;7(44):71856-71872.
30. Yang G, Li W, Jiang H, et al. Low-dose radiation may be a novel approach to enhance the effectiveness of cancer therapeutics. *Int J Cancer*. 2016;139(10):2157-2168.
31. Zhao X, Cui JW, Hu JH, Gao SJ, Liu XL. Effects of low-dose radiation on adaptive response in colon cancer stem cells. *Clin Transl Oncol*. 2017;19(7):907-914.
32. Takahashi A, Ohnishi T. Molecular mechanisms involved in adaptive responses to radiation, UV light, and heat. *J Radiat Res*. 2009;50(5):385-393.

33. Yonezawa M, Misonoh J, Hosokawa Y. Acquired radioresistance after small dose X-irradiation in mice. *J Radiat Res*. 1990;31(3): 256-262.
34. Yonezawa M, Misonoh J, Hosokawa Y. Two types of X-ray-induced radioresistance in mice: Presence of 4 dose ranges with distinct biological effects. *Mutat Res*. 1996;358(2):237-243.
35. Yonezawa M. Induction of radio-resistance by low dose X-irradiation. *Yakugaku Zasshi (in Japanese)*. 2006;126(10):833-840.
36. Nose M, Wang B, Itsukaichi H, et al. Rescue of lethally irradiated mice from hematopoietic death by pre-exposure to 0.5 Gy X rays without recovery from peripheral blood cell depletion and its modification by OK432. *Radiat Res*. 2001;156(2):195-204.
37. Otsuka K, Koana T, Tomita M, Ogata H, Tauchi H. Rapid myeloid recovery as a possible mechanism of whole-body radioadaptive response. *Radiat Res*. 2008;170(3):307-315.
38. Wang B, Tanaka K, Ninomiya Y, et al. Increased hematopoietic stem cells/hematopoietic progenitor cells measured as endogenous spleen colonies in radiation-induced adaptive response in mice (Yonezawa Effect). *Dose Response*. 2018; 16(3). doi: 10.1177/1559325818790152.
39. Wang B, Tanaka K, Ninomiya Y, et al. Relieved residual damage in the hematopoietic system of mice rescued by radiation-induced adaptive response (Yonezawa Effect). *J Radiat Res*. 2013;54(1): 45-51.
40. Sukup-Jackson MR, Kiraly O, Kay JE, et al. Rosa26-GFP direct repeat (RaDR-GFP) mice reveal tissue- and age-dependence of homologous recombination in mammals in vivo. *PLoS Genet*. 2014;10(6):e1004299.
41. Kansikas M, Nyström M, Peltomäki P. Disorders of DNA repair mechanisms and their clinical significance. *Duodecim*. 2017; 133(3):259-265.
42. Rancoule C, Vallard A, Guy JB, et al. Impairment of DNA damage response and cancer. *Bull Cancer*. 2017;104(11):962-970.
43. Benjamin MB, Little JB. X rays induce interallelic homologous recombination at the human thymidine kinase gene. *Mol Cell Biol*. 1992;12(6):2730-2738.
44. Cao J, DePrimo SE, Stringer JR. Cell cycle dependence of radiation-induced homologous recombination in cultured monkey cells. *Mutat Res*. 1997;374(2):233-243.
45. Huang L, Snyder AR, Morgan WF. Radiation-induced genomic instability and its implications for radiation carcinogenesis. *Oncogene*. 2003;22(37):5848-5854.
46. Tapio S, Jacob V. Radioadaptive response revisited. *Radiat Environ Biophys*. 2007;46(1):1-12.
47. Varès G, Wang B, Tanaka K, Kakimoto A, Eguchi-Kasai K, Nenoi M. Mutagenic adaptive response to high-LET radiation in human lymphoblastoid cells exposed to X-rays. *Mutat Res*. 2011;706(1-2):46-52.
48. Otsuka K, Koana T, Tauchi H, Sakai K. Activation of antioxidative enzymes induced by low-dose-rate whole-body gamma irradiation: adaptive response in terms of initial DNA damage. *Radiat Res*. 2006;166(3):474-478.
49. Phan N, De Lisio M, Parise G, Boreham DR. Biological effects and adaptive response from single and repeated computed tomography scans in reticulocytes and bone marrow of C57BL/6 mice. *Radiat Res*. 2012;177(2):164-175.
50. Tang FR, Loke WK, Khoo BC. Low-dose or low-dose-rate ionizing radiation-induced bioeffects in animal models. *J Radiat Res*. 2017;58(2):165-182.
51. Kimoto T, Kay JE, Li N, Engelward BP. Recombinant cells in the lung increase with age via de novo recombination events and clonal expansion. *Environ Mol Mutagen*. 2017;58(3):135-145.
52. Bogdándi EN, Balogh A, Felgyinszki N, et al. Effects of low-dose radiation on the immune system of mice after total-body irradiation. *Radiat Res*. 2010;174(4):480-489.
53. Dziegielewski J, Baulch JE, Goetz W, et al. WR-1065, the active metabolite of amifostine, mitigates radiation-induced delayed genomic instability. *Free Radic Biol Med*. 2008;45(12): 1674-1681.
54. Bourhis J, Rosine D. Radioprotective effect of amifostine in patients with head and neck squamous cell carcinoma. *Semin Oncol*. 2002;29(6 suppl 19):61-62.