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Increased Hematopoietic Stem Cells/ Hematopoietic Progenitor Cells Measured as Endogenous Spleen Colonies in Radiation-Induced Adaptive Response in Mice (Yonezawa Effect)

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Abstract

The existence of radiation-induced adaptive response (AR) was reported in varied biosystems. In mice, the first in vivo AR model was established using X-rays as both the priming and the challenge doses and rescue of bone marrow death as the end point. The underlying mechanism was due to the priming radiation-induced resistance in the blood-forming tissues. In a series of investigations, we further demonstrated the existence of AR using different types of ionizing radiation (IR) including low linear energy transfer (LET) X-rays and high LET heavy ion. In this article, we validated hematopoietic stem cells/hematopoietic progenitor cells (HSCs/HPCs) measured as endogenous colony-forming units-spleen (CFU-S) under AR inducible and uninducible conditions using combination of different types of IR. We confirmed the consistency of increased CFU-S number change with the AR inducible condition. These findings suggest that AR in mice induced by different types of IR would share at least in part a common underlying mechanism, the priming IR-induced resistance in the blood-forming tissues, which would lead to a protective effect on the HSCs/HPCs and play an important role in rescuing the animals from bone marrow death. These findings provide a new insight into the mechanistic study on AR in vivo.

Keywords

adaptive response, ionizing radiation, heavy ion, colony forming unites-spleen, mice

Introduction

Ionizing radiation (IR) at high doses is detrimental to the exposed organism, and exposure to IR could increase the risk of developing cancers and other health-related problems even including acute radiation syndrome such as bone marrow death. However, biological effects of IR at low dose or low dose rate remain elusive. On the other hand, adaptation to the environmental genotoxic stresses or insults, such as IR, is one of the fundamental characteristics of life. In particular, prior mild stresses can provide some aid to prepare organisms for subsequent more severe stresses.[1] Radiation-induced adaptive response (AR) is a phenomenon that a priming low dose

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of IR induces resistance to a subsequent challenge exposure to IR at higher doses, and its studies could provide important scientific basis for IR risk estimates, protection, and practical applications.[2] Since the editio princeps of AR concept introduced into radiation biology,[3] it has been demonstrated in a variety of in vitro, in utero, and in vivo systems with end points such as DNA damage, chromosomal aberrations, cell transformation, cell death, and mutation in the in vitro experiments and prenatal death, malformation, hematopoietic death, and carcinogenesis.[1,4-6]

High atomic number and energy (HZE) particles such as carbon, oxygen, silicon, and iron are important component sof space radiation, from the solar particle events and the galactic cosmic rays.[7,8] Compared to photon and proton radiation, HZE particles bearing higher energy could cause both acute and long-term damage to bone marrow via increased production of reactive oxygen species, showing stronger detrimental effects (with higher relative biological effectiveness) on the hematopoietic system including decreased peripheral blood counts and reduced hematopoietic stem cells (HSCs) and progenitor cells (HPCs) in laboratory animal models[9-20] and, in addition, raising hematological cancer risk via bone marrow cell reprograming.[21]

The AR mouse model for rescuing bone marrow death established by Yonezawa and colleagues[1,22-24] was repeatedly verified.[25,26] This model was named generally as "Yonezawa Effect" in Japan, which was originally established using low linear energy transfer (LET) X-rays as both the priming and the challenge IR, with the underlying mechanism that induction of radioresistance in blood forming tissues by the priming IR rescued the bone marrow death caused by the challenge high dose. In a series of investigations in our laboratory, we first verified and confirmed the existence of AR in mice using low-LET X-rays to deliver both the priming and the challenge IR and the 30-day survival test to estimate the efficacy for rescuing bone marrow death. Then, we further demonstrated the existence of AR using low-LET X-rays as the priming IR and high-LET heavy ion IR (carbon, neon, and silicon particles) as the challenge IR. Recently, we showed that, in this model, AR could be induced by high-LET heavy ion IR (carbon particles) as the priming IR and low-LET X-rays or high-LET heavy ion IR (carbon and neon particles) as the challenge IR.[20-29] In the present work, we validated the HSCs//HPCs measured as endogenous colony-forming units-spleen (CFU-S) under AR inducible and uninducible conditions using combination of low-LET X-rays and high-LET heavy ion IR as priming and challenge IR. By verifying the number of CFU-S under different conditions, 12 days after the animals received the challenge IR, the present investigation was aimed to study the recovery of HSCs/HPCs in this AR mouse model. We confirmed the consistency of CFU-S number with the AR induction conditions: significantly increased number of CFU-S under AR inducible conditions and no markedly increased number of CFU-S under AR uninducible conditions.

Materials and Methods

Animals

Five-week-old C57BL/6J Jms strain female mice were purchased from SLC, Inc (Japan) and maintained in a conventional animal facility under a 12 hour light–12 hour dark photoperiod. The animals were housed in autoclaved aluminum cages with sterilized wood chips and allowed to access standard laboratory chow (MB-1; Funabashi Farm Co, Japan) and acidified water ad libitum. The animals were acclimatized to the laboratory conditions for 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 + 2 SD) were omitted from this study. Based on our preliminary trials, in the present study, at least 6 mice were used in each experimental point. All experimental protocols involving mice were reviewed and approved by The Institutional Animal Care and Use Committee of the National Institute of Radiological Sciences (NIRS). The experiments were performed in strict accordance with the NIRS *Guidelines for the Care and Use of Laboratory Animals*.

Irradiation

For low-LET IR, 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. For high-LET heavy ion IR, the monoenergetic ion beam of carbon, neon, and iron particles was generated and accelerated by a synchrotron, the Heavy Ion Medical Accelerator in Chiba at NIRS, Japan. The beam energy was 290 MeV/nucleon, 400 MeV/nucleon, and 500 MeV/nucleon for carbon, neon, and iron particles, corresponding to an average LET value of about 15 keV/μm, 30 keV/μm, and 200 keV/μm, respectively. The dose rate was at about 0.10 Gy/min and 2.00 Gy/min for delivery of the priming dose and the challenge dose, respectively. The mice held in acryl containers were exposed to total body irradiation at room temperature.

Mouse Model for Radiation-Induced AR

The AR mouse model for rescue of bone marrow death and study on increase in the number of CFU-S established by Yonezawa and colleagues[24,30] was adopted, verified, and confirmed under the experimental conditions in our research facilities and finally applied to a series of our investigations using both low-LET X-rays and high-LET particles. The timing for delivery of the priming dose and challenge dose was on postnatal ages of 6 and 8 weeks of the mice, respectively. The present work was a part of the investigations focusing on the recovery of HSCs/HPCs under AR. The AR inducible and uninducible

Table 1. Efficacy for Induction of AR by Combination of Different Types of IR in Mice.

Dose, Gy		Thirty-day Survival, %		
Priming IR	Challenge IR	Challenge IR	Priming + Challenge IR	AR Induction
X-rays (0.50)	X-rays (7.50)	16.7	83.3	Yes
X-rays (0.50)	C (6.50)	15.0	66.7	Yes
X-rays (0.50)	Fe (6.00)	23.3	26.7	No
C (0.45)	X-rays (7.50)	16.7	73.3	Yes
Fe (0.45)	X-rays (7.50)	16.7	0.0	No
C (0.45)	C (6.50)	10.0	36.7	Yes
C (0.45)	Ne (5.50)	21.6	70.0	Yes
C (0.45)	Fe (5.75)	80.0	90.0	No

Abbreviations: AR, adaptive response; IR, ionizing radiation.

conditions obtained in our previous studies for rescue of bone marrow death[19-27] are summarized (Table 1). In Table 1, the "Yes" for AR induction meant a successful AR induction as judged by a significant increase in the animal survival after receiving the priming radiation prior to the challenge radiation in the 30-day survival test, and the "No" for no significant increase in the survival was induced in the presence of the priming radiation. To look for suitable experimental conditions, especially both the challenge dose altitude of each type of IR and the timing that makes the CFU-S more distinctly observable, 3 to 4 sublethal doses for the challenge IR of each type in each combination of the priming and the challenge exposure were validated in preliminary trials, and the doses listed in Table 1 were finally used. In brief, the combination exposures were (1) the priming dose was 0.50 Gy for low-LET X-rays and 0.45 Gy for high-LET carbon or iron particles; (2) the challenge dose for X-rays was 5.00 Gy in the combined exposure to priming X-rays and 4.75 Gy to priming carbon or iron particles; (3) the challenge dose for carbon particles was 5.00 Gy and 5.25 Gy following the priming IR from X-rays and carbon particles, respectively; and (4) the challenge dose was 5.00 Gy and 5.5 Gy for neon particles and iron particles, respectively.

Enumeration of Endogenous CFU-S

To evaluate the number of HSCs/HPCs in vivo in mice under experimental conditions capable or incapable of inducing AR, the method established by Till and McCulloch,[31] bearing advantages over the exogenous transplant system, including simplicity and rapidity, avoiding in vitro cell manipulation,[32,33] was adopted and applied to the present study. In brief, the mice were killed 11, 12, or 13 days after receiving the challenge dose. The spleens were removed, weighed, and then fixed in Bouin fixative (having a mixing ratio 15:5:1 of saturated picric acid, formaldehyde, and glacial acetic acid; purchased from Wako Pure Chemical Industries, Ltd, Japan) for 24 hours. The macroscopic nodules or colonies on the surface of the organ were counted as endogenous CFU-S using a stereoscopic microscope (Nikon SMZ-10; Nikon Instech Co, Ltd, Japan) at 10× magnification. Timing of spleen sample collection was according to that the numeration of endogenous CFU-S formed 11 days or later after irradiation provides a measure of viable pluripotent HSCs/HPCs,[31,34] and longer intervals were avoided due to the colonies fused together, preventing scoring of individual colonies. Based on the ease for clear distinction of individual colonies, data obtained on 12 days after the challenge IR were used to judge the consistency of CFU-S number change and AR induction. Six to 12 animals were used per experimental point.

Statistical Analysis

Statistical evaluation of the data was done using the Student *t* test, and the statistical significance was assigned to $P < .05$.

Results

Verification of the Radiation-Induced AR Mouse Model Using CFU-S as the End Point

Reproducibility of the radiation-induced AR mouse model (Yonezawa Effect) using CFU-S as the end point[30] was verified under the experimental setup in the present study. Under the AR inducible conditions, the animals were total body irradiated with a priming dose of 0.50 Gy X-rays at postnatal 6 weeks followed by a challenge dose of 5.00 Gy X-rays at postnatal 8=weeks. Under the AR uninducible conditions, the animals were total body irradiated with only a challenge dose of 5.00 Gy X-rays at postnatal 8 weeks. The number of CFU-S was measured on 11, 12, and 13 days after the challenge IR. Results showed that the priming dose markedly increased the mean number of CFU-S from 3.2 to 11.8, 4.0 to 36.2, and 5.6 to 50.2 on the days 11, 12, and 13, respectively, after the challenge IR (Figure 1). Results clearly indicated that AR was induced with efficient reliability and reproducibility in our experimental setup using the number of CFU-S as the end point. Serving also as a positive control, the verification work was performed in parallel to the following investigations using combination of different types of IR.

Validation of CFU-S in AR Induced by Priming IR From Low-LET X-Rays and Challenge IR From High-LET Particles

The CFU-S assay was performed to validate whether significant increase in the number of CFU-S occurred in the animals under AR inducible condition (exposure of a priming dose of 0.50 Gy X-rays at postnatal 6 weeks followed by a challenge dose of 5.00 Gy carbon particles at postnatal 8 weeks) and the AR uninducible condition (the animals irradiated with only the challenge dose; the animals irradiated with a priming dose of 0.50 Gy X-rays at postnatal 6 weeks followed by a challenge dose of 5.50 Gy iron ions). The mean number of CFU-S was significantly increased from 7.8 to 15.0, 15.9 to 28.6, and 24.3 to 44.0, respectively, on 11, 12, and 13 days after the challenge

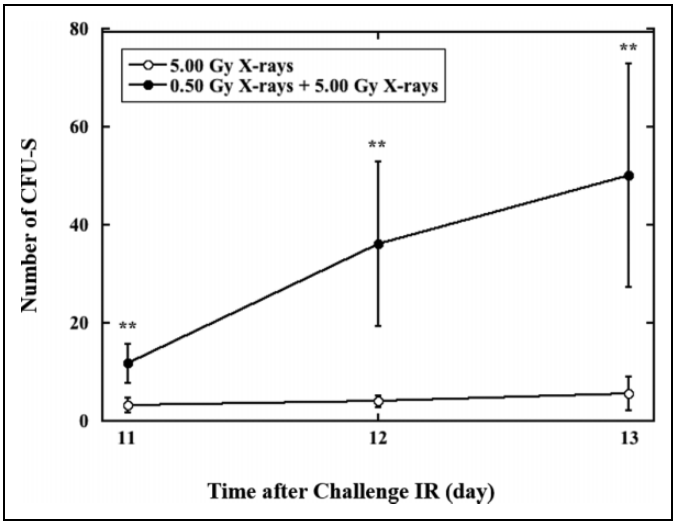

Figure 1. Verification and confirmation of adaptive response in mice (Yonezawa Effect) induced by low-LET X-rays as both priming and challenge ionizing radiation (IR) using colony-forming units-spleen (CFU-S) as the end point. Effect of a priming dose of 0.50 Gy X-rays on a subsequent challenge dose of 5.00 Gy X-rays on the number of CFU-S was verified. Under the adaptive response (AR) inducible condition, the animals were total body irradiated with a priming dose of 0.50 Gy X-rays at postnatal 6 weeks and then followed by a challenge dose of 5.00 Gy X-rays at postnatal 8 weeks (closed circles with solid line). Under the AR uninducible condition, the animals were total body irradiated with only the challenge dose (open circles with solid line). The number of CFU-S was measured on the days 11, 12, and 13 after the challenge IR. Data of each experimental point were from 6 to 12 mice. Two asterisks (**) indicate statistically significant differences ($P < .01$) between the 2 groups that were compared.

IR (Figure 2A). On the other hand, no increased number of CFU-S was observed in the animals receiving both the priming X-rays and the challenge iron IR when compared to the animals receiving only the challenge iron IR (Figure 2B). On the 11th day after the challenge iron IR, the mean number of CFU-S was even markedly higher in the animals receiving only the challenge iron IR when compared to the animals receiving both the priming X-rays and the challenge iron IR (Figure 2B), indicating an additive effect on reducing CFU-S from the combined exposure. These results clearly showed that under AR inducible condition when the priming IR was from low LET X-rays and the challenge IR was from high LET carbon particles, increased number of CFU-S was confirmed. On the other hand, under AR uninducible condition, when the priming IR was from low-LET X-rays and the challenge IR was from high-LET iron particles, no increased number of CFU-S was confirmed.

Validation of CFU-S in AR Induced by Priming IR From High-LET Particles and Challenge IR From Low-LET X-Rays

The CFU-S assay was performed to validate whether significant increase in the number of CFU-S occurred under AR inducible condition (ie, the animals irradiated with a priming dose of 0.45 Gy carbon ions at postnatal 6 weeks followed by a challenge dose of 4.75 Gy X-rays at postnatal 8 weeks vs the animals receiving only the challenge dose of 4.75 Gy X-rays at postnatal 8 weeks) and the AR uninducible condition (ie, the animals irradiated with 0.45 Gy iron ions at postnatal 6 weeks followed by a challenge dose of 4.75 Gy X-rays at postnatal 8 weeks vs the animals receiving only the challenge dose of 4.75 Gy X-rays at postnatal 8 weeks). On the 11th day after the challenge IR, difference in the mean number of CFU-S was not statistically significant between the animals irradiated with a priming dose of 0.45 Gy carbon particles followed by a challenge dose of 4.75 Gy X-rays and the animals receiving only the challenge dose of 4.75 Gy X-rays (Figure 3A). The mean number of CFU-S was significantly increased from 6.7 to 22.3 and 4.2 to 32.5, respectively, on 12 and 13 days after the challenge IR (Figure 3A). On the other hand, no increased mean number of CFU-S was observed in the animals receiving both the priming dose from iron particles followed by the challenge dose from X-rays and the animals irradiated with only the challenge dose from X-rays (Figure 3B). These results clearly showed that under AR inducible condition when the priming IR was from high-LET carbon particles and the challenge IR was from low-LET X-rays, increased mean number of CFU-S was confirmed. Under AR uninducible condition, when the priming IR was from high LET iron particles and the challenge IR was from low LET X-rays, no increased mean number of CFU-S was observed.

Validation of CFU-S in AR Induced by High-LET Particles as Both Priming IR and Challenge IR

The CFU-S assay was performed to validate whether significant increase in the number of CFU-S occurred under AR inducible condition (ie, the animals irradiated with a priming dose of 0.45 Gy carbon particles at postnatal 6 weeks followed by a challenge dose of 5.25 Gy carbon particles or 5.00 Gy neon particles at postnatal 8 weeks vs the animals irradiated with only the challenge dose) and the AR uninducible condition (ie, the animals irradiated with a priming dose of 0.45 Gy carbon particles at postnatal 6 weeks followed by a challenge dose of 5.50 Gy iron particles at postnatal 8 weeks). On 11, 12, and 13 days after the challenge IR, the mean number of CFU-S was significantly increased from 2.6 to 9.2, 7.0 to 22.4, and 15.5 to 43.0 for the animals receiving the combined exposure to carbon particles when compared to those receiving only the challenge IR from carbon particles (Figure 4A); the mean number of CFU-S was from 1.3 to 2.8, 4.0 to 8.6, and 4.7 to 10.1 for the animals exposed to the priming IR from carbon particles followed by the challenge IR from neon particles, and the increase was statistically significant on the 12th day (Figure 4B). On the other hand, no increased mean number of CFU-S was observed in the animals receiving both the priming dose from carbon particles followed by the challenge dose from iron particles when compared to those receiving only the challenge dose from iron particles (Figure 4C). These results clearly showed that under AR inducible condition when the priming

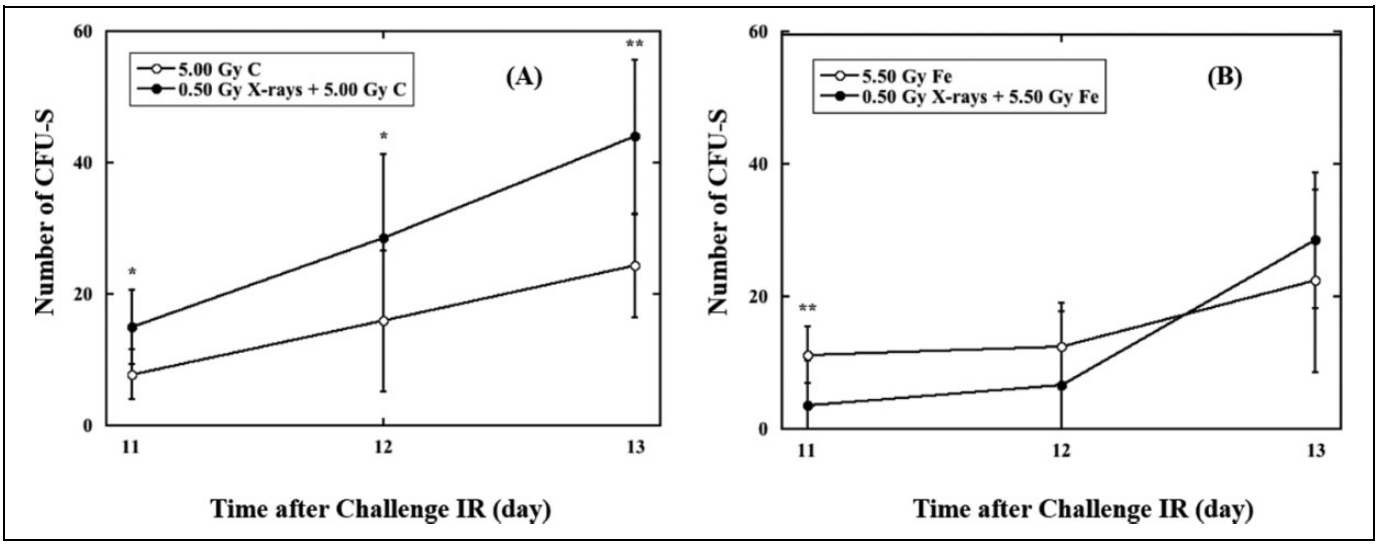

Figure 2. Validation of adaptive response (AR) in mice (Yonezawa Effect) induced by low LET X-rays as the priming ionizing radiation (IR) and high LET particles as the challenge IR using colony forming units-spleen (CFU-S) as the end point. Effect of a priming dose of 0.50 Gy X-rays on a subsequent challenge dose from carbon ions (A) or iron ions (B) on the number of CFU-S was verified. Under the AR inducible condition, the animals were total body irradiated with a priming dose of 0.50 Gy X-rays at postnatal 6 weeks, and then followed by a challenge dose of 5.00 Gy carbon ions (A) at postnatal 8 weeks. Under the AR uninducible condition, (1) the animals were total body irradiated with a priming dose of 0.50 Gy X-rays at postnatal 6 weeks, and then followed by a challenge dose of 5.50 Gy iron ions (B), and (2) the animals were only total body irradiated with the challenge dose. Closed circles with solid line stand for the groups receiving both the low dose and the high dose at postnatal 6 weeks and 8 weeks, respectively. Open circles with solid line stand for the groups receiving only the challenge dose at postnatal 8 weeks. The number of CFU-S was measured on the days 11, 12, and 13 after the challenge IR. Data of each experimental point were from 6 to 12 mice. One asterisk (*) stands of statistically significant differences ($P < .05$) between the 2 groups that were compared. Two asterisks (**) indicate statistically significant differences ($P < .01$) between the 2 groups that were compared. C stands for carbon and Fe stands for iron.

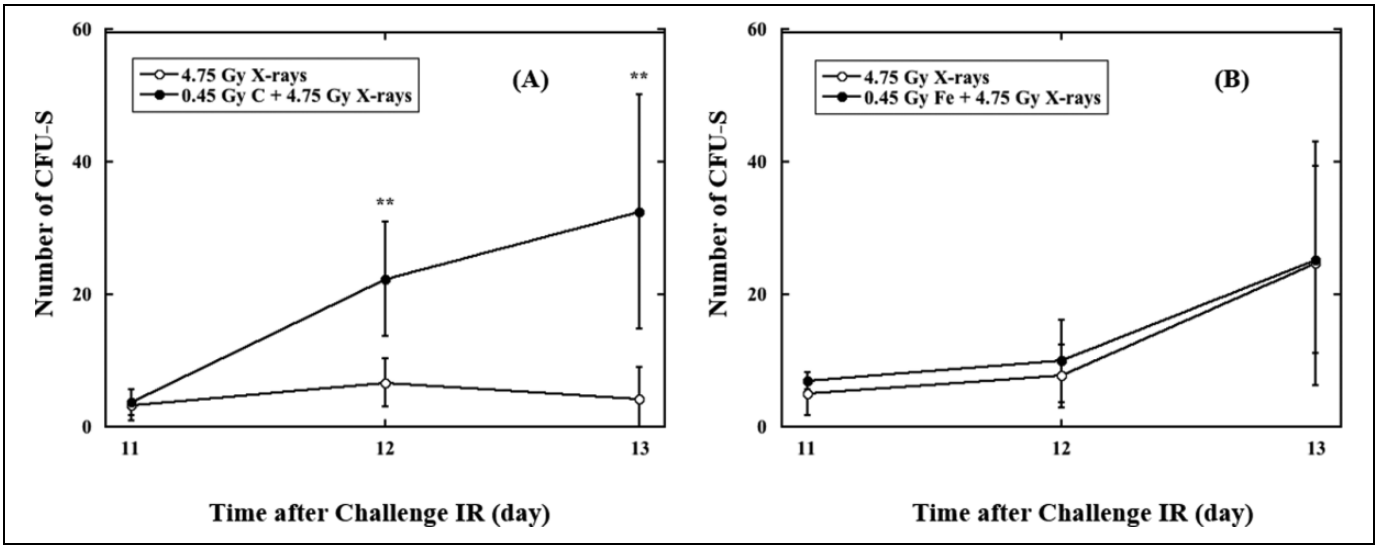

Figure 3. Validation of adaptive response in mice (Yonezawa Effect) induced by high-LET particles as the priming ionizing radiation (IR) and low-LET X-rays as the challenge IR using colony forming units-spleen (CFU-S) as the end point. Effect of a priming dose of 0.45 Gy carbon ions (A) or 0.45 Gy iron ions (B) on a subsequent challenge dose of 4.75 Gy from X-rays on the number of CFU-S was verified. Under the adaptive response (AR) inducible condition, the animals were total body irradiated with a priming dose of 0.45 Gy carbon ions at postnatal 6 weeks, and then followed by a challenge dose of 4.75 Gy X-rays at postnatal 8 weeks. Under the AR uninducible condition, the animals were total body irradiated with 0.45 Gy iron ions at postnatal 6 weeks, and then followed by a challenge dose of 4.75 Gy X-rays at postnatal 8 weeks. Closed circles with solid line stand for the groups receiving both the priming dose and the challenge dose at postnatal 6 weeks and postnatal 8 weeks, respectively. Open circles with solid line stand for the groups receiving only the challenge dose at postnatal 8 weeks. The number of CFU-S was measured on the days 11, 12, and 13 after the challenge IR. Data of each experimental point were from 6 to 12 mice. Two asterisks (**) indicate statistically significant differences ($P < .01$) between the 2 groups that were compared. C stands for carbon and Fe stands for iron.

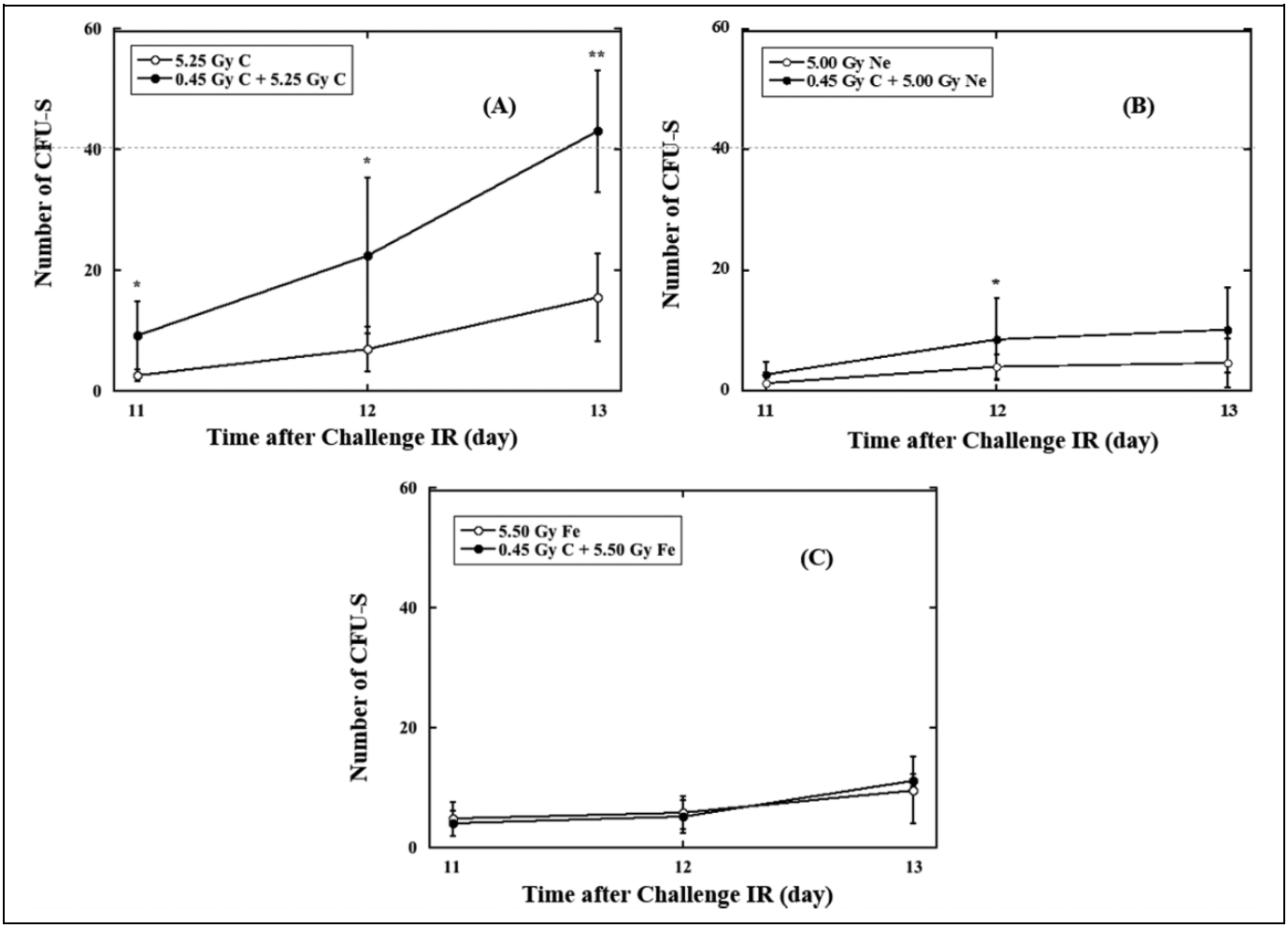

Figure 4. Validation of adaptive response in mice (Yonezawa Effect) induced by high-LET particles as both the priming ionizing radiation (IR) and the challenge IR using colony-forming units-spleen (CFU-S) as the end point. Effect of a priming dose of 0.45 Gy carbon ions on a subsequent challenge dose from carbon ions (A), neon ions (B), or iron ions (C) on the number of CFU-S was verified. Under the adaptive response (AR) inducible condition, the animals were total body irradiated with a priming dose of 0.45 Gy carbon ions at postnatal 6 weeks, and then followed by a challenge dose of 5.25 Gy carbon ions or of 5.00 Gy neon ions at postnatal 8 weeks. Under the AR uninducible condition, (1) the animals were total body irradiated with a priming dose of 0.45 Gy carbon ions at postnatal 6 weeks, and then followed by a challenge dose of 5.50 Gy iron ions, and (2) the animals were only total body irradiated with the challenge dose. Closed circles with solid line stand for the groups receiving both the priming dose and the challenge dose at postnatal 6 weeks and postnatal 8 weeks, respectively. Open circles with solid line stand for the groups receiving only the challenge dose at postnatal 8 weeks. The number of CFU-S was measured on the days 11, 12, and 13 after the challenge IR. Data of each experimental point were from 6 to 12 mice. One asterisk (*) stands for statistically significant differences ($P < .05$) between the 2 groups that were compared. Two asterisks (**) indicate statistically significant differences ($P < .01$) between the 2 groups that were compared. C stands for carbon, Ne stands for neon, and Fe stands for iron.

IR was from high-LET carbon particles and the challenge IR was from high LET carbon or neon particles, increased mean number of CFU-S was confirmed. Under AR uninducible condition, when the priming IR was from high LET carbon particles and the challenge IR was from high LET iron particles, no increased mean number of CFU-S was observed.

Discussion

A better understanding of AR and other nontargeted effects is needed to understand to which extent application of low-dose IR might be beneficial to humans.[35] To date, investigations using the AR mouse model (Yonezawa Effect) have obtained many substantial achievements in the study of radiation-induced AR at whole body level. In a series of comprehensive investigations, Yonezawa and colleagues verified the existence of AR under a variety of experimental conditions (ie, doses of priming and challenge IR, intervals between priming and challenge IR, and age and strain of the animals).[23] These efforts helped this AR mouse model to lay a cornerstone for in vivo AR research. Of note, based on the priming dose and the interval between priming and challenge exposures and the timing for delivery of the priming dose, 2 different phenotypes of AR were observed, involving different mechanisms; the first

phenotype was induced 2 weeks after a 0.3 to 0.5 Gy priming IR, which was due to *Trp53*-dependent radioresistance in blood-forming tissues,[26,36] and the second phenotype was observed 2 months after a 0.05 to 0.1 Gy priming exposure and the AR resulted from the interaction between blood-forming tissue and the central nervous system.[37,38] As rescue of bone marrow death is the basic criteria for judgment of a successful induction of AR in mice under Yonezawa Effect, studying the recovery of HSCs/HPCs is of critical significance from the point of view of mechanism research, the model for the first phenotype was applied to the present work to validate the HSCs/HPCs measured as endogenous CFU-S under AR inducible and uninducible conditions using combination of low-LET X-rays and high-LET heavy ion IR as priming and challenge IR. It is known that bone marrow, as the site in the body where self-renewal and differentiation of HSCs to mature blood cells mainly occurs, is extremely radiosensitive. Exposure to IR at high doses could devastate bone marrow leading to bone marrow death. In addition, hematopoietic capability is the most critical factor preventing radiation-induced bone marrow death. Even sublethal doses of IR could cause a decrease in hematopoietic cells and a deficit to bone marrow hematopoietic microenvironment. Ionizing radiation-induced decline in total bone marrow hematopoietic cells is accompanied with elevated adipocytes into the marrow cavity, leading consequently to the inhibition of bone marrow microenvironment recovery and hematopoiesis.[39] As the number of endogenous CFU-S could reflect both the number of HSCs/HPCs[32,40] and the environment for hematopoiesis,[41] endogenous CFU-S assay is capable of evaluating the hematopoietic capability.

In this AR mouse model, previous studies showed that successful induction of AR by priming low-LET X-rays or γ-rays was regardless of the dose rate,[42] and mechanistic study suggested that priming IR-induced decreased p53, Bax, and apoptosis positive cell accumulation in the spleen might favor the recovery of hematopoietic function from challenge IR-induced acute injury, manifesting as stimulated recovery of spleen weight and endogenous CFU-S, contributing to a decrease in bone marrow death.[43] Studies on the protective effects induced by low doses of low-LET IR indicated that the underlying mechanisms included enhanced antioxidative capacities, increased cellular DNA repair capacity leading to such as reduced initial DNA damage in AR in mice in vivo,[44,45] and reduced cell death and mutations in vitro.[5,46] These induced responses were tightly conserved throughout evolution, being basic responses critical to life.[4] On the other hand, as high-LET IR from heavy particles induced biological effects qualitatively different from those induced by low-LET IR from photons,[47] for example, high-LET IR induced more clustered DNA damage and higher rates of residual chromatin breaks,[48] cellular radiosensitivity correlated with the frequency of residual chromatin breaks,[49] and the recovery ratio of the potentially lethal damage depended on the quality of IR.[50] In the present work, increased endogenous CFU-S was observed in the animals under AR inducible conditions, being consistently well with the 30-day survival results: Under AR inducible conditions for rescue of bone marrow death, AR manifested as significantly increased number of HSCs/HPCs could be induced regardless the priming low dose of IR was from low-LET X-rays or certain high-LET heavy ions such as carbon particles. These results indicate that induction of AR may protect the ability of animals to hinder the decline in the total HSCs/HPCs through possibly inducing radioresistance in the hematopoietic tissue and maintaining the hematopoietic microenvironment. These findings suggest that induction of AR by low-LET and certain high-LET heavy ions may share at least some mechanisms in common. In fact, mechanistic study in vitro in cultured human fibroblasts showed that gene expression profiles following low-LET γ-rays and decays of high-LET like ^{125}I shared the majority of genes in common, indicating that both types of IR elicited similar signal transduction pathways,[51] and the extent of DNA clustered damage may not be the major factor modulating gene expression after exposure.[52] Low doses of low-LET X-rays were effective in reducing chromosomal aberrations and mutation frequency induced by high-LET IR.[2,53] These findings suggest that the biological defense mechanisms induced by prior low doses from either low-LET IR or high-LET IR may be considered as effective countermeasures, being sufficient enough against the damages caused by subsequent higher challenge doses from either low-LET or high-LET IR. On the other hand, it is noticed that heavy ions with higher atomic number and higher energy (ie, iron particles) failed to induce AR regardless of its use as priming or challenge IR. These findings also suggest that induction of AR may depend on the quality of IR at least to a certain extent. Is there a threshold for the atomic number and higher energy of the heavy ions to induce AR in this mouse model? More questions remain to be answered through further research on especially the underlying molecular mechanisms.

The priming dose used in the present work was higher than 100 mGy which was extensively used in the field of so-called low-dose research. It is known that in the experimental study on AR induction or hormesis, the low doses used in the in vivo systems are often relatively higher than that used in the in vitro systems, and the altitude of the dose seems to be dependent of the biosystems. On the other hand, when thinking about the clinical application of induction of AR or hormesis for the treatment of patients with cancer to protect the normal tissue from being damaged by radiotherapy at high doses, 0.50 Gy could be considered as a low dose. More importantly, application of AR or hormesis should be more practical and acceptable for most of the patients via research and development of medication based on the molecular mechanisms underlying induction of AR or hormesis rather than application via exposure of the patient to priming low dose of radiation.

Taken together, results (Table 2) in the present study showed that under AR inducible conditions, regardless of the quality of IR (ie, low LET and high LET, photons and particles) for the combination of the priming dose and the challenge dose, the priming IR could induce an increased number of HSCs/HPCs as measured by the number of endogenous CFU-S, which may contribute to the rescue of bone marrow death.

Table 2. Measurement of CFU-S under AR Inducible and Uninducible Conditions in Mice.

AR Inducible Conditions	Dose, Gy		Consistency of CFU-S Increase with AR Induction Conditions
	Priming IR	Challenge IR	
Yes	X-rays (0.50)	X-rays (5.00)	Yes
Yes	X-rays (0.50)	C (5.00)	Yes
No	X-rays (0.50)	Fe (5.50)	Yes
Yes	C (0.45)	X-rays (4.75)	Yes
No	Fe (0.45)	X-rays (4.75)	Yes
Yes	C (0.45)	C (5.25)	Yes
Yes	C (0.45)	Ne (5.00)	Yes
No	C (0.45)	Fe (5.50)	Yes

Abbreviations: AR, adaptive response; CFU-S, colony forming units-spleen; IR, ionizing radiation.

Results indicated the significance and possible application of AR to the reduction in acute radiation syndrome induced by high dose from either low- or high-LET IR. These findings bring new knowledge to the characterization of the Yonezawa Effect by providing a new insight into the mechanistic study on the hematopoietic system in the AR mouse model in vivo.

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Authors' Note

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References

1. Takahashi A, Ohnishi T. Molecular mechanisms involved in adaptive responses to radiation, UV light, and heat. *J Radiat Res*. 2009;50(5):385-393.
2. 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 ed. *Sources and Effects of Ionizing Radiation: UNSCEAR Report to the General Assembly with Scientific Annexes*. New York,NY: United Nations; 1994:185-272.
3. Olivieri G, Bodycote J, Wolff S. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. *Science*. 1984;223(4636):594-597.
4. Mitchel RE. Low doses of radiation are protective in vitro and in vivo: evolutionary origins. *Dose Response*. 2006;4(2):75-90.
5. 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.
6. 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.
7. Hellweg CE, Baumstark-Khan C. Getting ready for the manned mission to Mars: the astronauts' risk from space radiation. *Naturwissenschaften*. 2007;94(7):517-526.
8. Reitz G. Characteristic of the radiation field in low Earth orbit and in deep space. *Z Med Phys*. 2008;18(4):233-243.
9. Phillips TL, Ross GY, Goldstein LS, Ainsworth J, Alpen E. In vivo radiobiology of heavy ions. *Int J Radiat Oncol Biol Phys*. 1982;8(12):2121-2125.
10. Hagan MP, Holahan EV, Ainsworth EJ. Effects of heavy ions on cycling stem cells. *Adv Space Res*. 1986;6(11):201-211.
11. Ainsworth EJ, Afzal SM, Crouse DA, Hanson WR, Fry RJ. Tissue responses to low protracted doses of high LET radiations or photons: early and late damage relevant to radio-protective countermeasures. *Adv Space Res*. 1989;9(10):299-313.
12. Datta K, Suman S, Trani D, et al. Accelerated hematopoietic toxicity by high energy ^{56}Fe radiation. *Int J Radiat Biol*. 2012; 88(3):213-222.
13. Suman S, Datta K, Trani D, Laiakis EC, Strawn SJ, Fornace AJ Jr. Relative biological effectiveness of ^{12}C and ^{28}Si radiation in C57BL/6J mice. *Radiat Environ Biophys*. 2012;51(3):303-309.

14. Miousse IR, Shao L, Chang J, et al. Exposure to low-dose ^{56}Fe-ion radiation induces long-term epigenetic alterations in mouse bone marrow hematopoietic progenitor and stem cells. *Radiat Res*. 2014;182(1):92-101.
15. Chang J, Feng W, Wang Y, et al. Whole-body proton irradiation causes long-term damage to hematopoietic stem cells in mice. *Radiat Res*. 2015;183(2):240-248.
16. Boerma M, Sridharan V, Mao XW, et al. Effects of ionizing radiation on the heart. *Mutat Res*. 2016;770(PtB):319-327.
17. Chang J, Luo Y, Wang Y, et al. Low doses of oxygen ion irradiation cause acute damage to hematopoietic cells in mice. *PLoS One*. 2016;11(7): e0158097.
18. Wang Y, Chang J, Li X, et al. Low doses of oxygen ion irradiation cause long-term damage to bone marrow hematopoietic progenitor and stem cells in mice. *PLoS One*. 2017;12(12): e0189466.
19. Chang J, Wang Y, Pathak R, et al. Whole body proton irradiation causes acute damage to bone marrow hematopoietic progenitor and stem cells in mice. *Int J Radiat Biol*. 2017;93(12):1312-1320.
20. Chang J, Feng W, Wang Y, et al. ^{28}Si total body irradiation injures bone marrow hematopoietic stem cells via induction of cellular apoptosis. *Life Sci Space Res*. 2017;13:39-44.
21. Muralidharan S, Sasi SP, Zuriaga MA, et al. Ionizing particle radiation as a modulator of endogenous bone marrow cell reprogramming: implications for hematological cancers. *Front Oncol*. 2015;5:231.
22. Yonezawa M, Misonoh J, Hosokawa Y. Acquired radioresistance after small dose X-irradiation in mice. *J Radiat Res*. 1990;31(3): 256-262.
23. Yonezawa M. Induction of radio-resistance by low dose X-irradiation. *Yakugaku Zasshi (In Japanese)*. 2006;126(10):833-840.
24. 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.
25. 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.
26. 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.
27. Wang B, Tanaka K, Varès G, et al. X-rays-induced radioresistance against high LET irradiations from accelerated heavy ions in mice. *Radiat Res*. 2010;174(4):532-536.
28. Wang B, Tanaka K, Ninomiya Y, et al. X-ray-induced radioresistance against high-LET radiations from accelerated neon-ion beams in mice. In: Nenoi M ed. *Current Topics in Ionizing Radiation Research*. Rijeka, Croatia: Intech-Open Access; 2012: 199-214.
29. Tanaka K, Wang B, Ninomiya Y, et al. Mechanistic study on heavy-ion-induced adaptive response *in vivo* in mice. Paper presented at: The 57th annual meeting of the Japan Radiation Research Society; October 2, 2014. Japan: Kagoshima-shi, Kagoshima Prefecture.
30. Yonezawa M, Horie K, Kondo H, Kubo K. Increase in endogenous spleen colonies without recovery of blood cell counts in radioadaptive survival response in C57BL/6 mice. *Radiat Res*. 2004;161(2):161-167.
31. Till JE, McCulloch EA. A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat Res*. 1961; 14:213-222.
32. Marsh JC, Boggs DR, Bishop CR, Chervenick PA, Cartwright GE, Wintrobe MM. Factors influencing hematopoietic spleen colony formation in irradiated mice. I. The normal pattern of endogenous colony formation. *J Exp Med*. 1967;126(5):833-849.
33. Inoue T, Hirabayashi Y, Mitsui H, et al. Survival of spleen colony-forming units (CFU-S) of irradiated bone marrow cells in mice: evidence for the existence of a radioresistant subfraction. *Exp Hematol*. 1995;23(12):1296-300.
34. Magli MC, Iscover NN, Odartchenko N. Transient nature of early haematopoietic spleen colonies. *Nature* 1982;295(5849):527-529.
35. Tapio S, Jacob V. Radioadaptive response revisited. *Radiat Environ Biophys*. 2007;46(1):1-12.
36. Horie K, Kubo K, Yonezawa M. p53 dependency of radio-adaptive responses in endogenous spleen colonies and peripheral blood-cell counts in C57BL mice. *J Radiat Res*. 2002;43(4):353-360.
37. Yonezawa M. Radioadaptive survival response in mice. In: Yamada T, Mothersill C, Michael BD, Potten CS eds. *Biological Effects of Low Dose Radiation*. Amsterdam, Netherlands: Elsevier Sciences; 2000:93-99.
38. Misonoh J, Yonezawa M. Dose ranges for radioadaptive response in mice on the viewpoint of aquired radio-resistance after low dose irradiation. In: British Nuclear Energy Society, ed. *Health Effects of Low Dose Radiation: Challenges of the 21st Century*. London, England: Thomas Telford Services; 1997:169-174.
39. Green DE, Rubin CT. Consequences of irradiation on bone and marrow phenotypes, and its relation to disruption of hematopoietic precursors. *Bone*. 2014;63:87-94.
40. Necas E, Znojil V. Bone marrow response to single small doses of irradiation: implications for stem cell functional organization. *Exp Hematol*. 1988;16(10):871-875.
41. Hiraoka A, Yamagishi M, Ohkubo T, Kamamoto T, Yoshida Y, Uchino H. Effect of a streptococcal preparation, OK-432, on hematopoietic spleen colony formation in irradiated mice. *Cancer Res*. 1981;41(7):2954-2958.
42. Shiraishi K, Tachibana A, Yonezawa M, Kodama S. Adaptive response of bone marrow stem cells induced by low-dose rate irradiation in C57BL/6 mice. *Int Congress Series*. 2005;1276:264-265.
43. Yonezawa M, Takahashi A, Ohnishi K, Misonoh J, Ohnishi T. Suppression of X-ray-induced apoptosis by low dose pre-irradiation in the spleen of C57BL/6 mice. *Int Congress Series*. 2002;1236:471-475.
44. 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.
45. 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.
46. 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.

47. Held KD. Effects of low fluences of radiations found in space on cellular systems. *Int J Radiat Biol*. 2009;85(5):379-390.
48. Suzuki M, Kase Y, Nakano T, Kanai T, Ando K. Residual chromatin breaks as biodosimetry for cell killing by carbon ions. *Adv Space Res*. 1998;22(12):1663-1671.
49. Suzuki M, Kase Y, Nakano T, Kanai T, Ando K. Correlation between cell killing and residual chromatin breaks measured by PCC in six human cell lines irradiated with different radiation types. *Int J Radiat Biol*. 2000;76(9):1189-1196.
50. Suzuki M, Kase Y, Nakano T, Kanai T, Ando K. Change in radiosensitivity with fractionated-dose irradiation of carbon-ion beams in five different human cell lines. *Int J Radiat Oncol Biol Phys*. 2000;48(1):251-258.
51. Sokolov M, Panyutin IG, Neumann R. Genome-wide gene expression changes in normal human fibroblasts in response to low-LET gamma-radiation and high-LET-like ^{125}IUdR exposures. *Radiat Prot Dosimetry*. 2006;122(1-4):195-201.
52. Sokolov M, Smirnova NA, Camerini-Otero RD, Neumann RD, Panyutin IG. Microarray analysis of differentially expressed genes after exposure of normal human fibroblasts to ionizing radiation from an external source and from DNA-incorporated iodine-125 radionuclide. *Gene*. 2006;382:47-56.
53. Wolff S, Jostes R, Cross FT, Hui TE, Afzal V, Wiencke JK. Adaptive response of human lymphocytes for the repair of radon-induced chromosomal damage. *Mutat Res*. 1991; 250(1-2):299-306.