



# Reduced High-Dose Radiation-Induced Residual Genotoxic Damage by Induction of Radioadaptive Response and Prophylactic Mild Dietary Restriction in Mice




Author	Bing Wang, Kaoru Tanaka, Takanori Katsube, Kouichi Maruyama, Yasuharu Ninomiya, Guillaume Vares, Cuihua Liu, Hirokazu Hirakawa, Masahiro Murakami, Zeenath Fardous, Nahida Sultana, Kazuko Fujita, Akira Fujimori, Tetsuo Nakajima, Mitsuru Neno
journal or publication title	Dose-Response
volume	19
number	1
page range	1559325820982166
year	2021-02-11
Publisher	SAGE Publications
Rights	(C) 2021 The Author(s).
Author's flag	publisher
URL	<a href="http://id.nii.ac.jp/1394/00001898/">http://id.nii.ac.jp/1394/00001898/</a>

doi: [info:doi/10.1177/1559325820982166](https://doi.org/10.1177/1559325820982166)

# Reduced High-Dose Radiation-Induced Residual Genotoxic Damage by Induction of Radioadaptive Response and Prophylactic Mild Dietary Restriction in Mice

Dose-Response:  
An International Journal  
January-March 2021:1-8  
© The Author(s) 2021  
Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/1559325820982166  
journals.sagepub.com/home/dos



Bing Wang<sup>1</sup> , Kaoru Tanaka<sup>1</sup>, Takanori Katsube<sup>1</sup> , Kouichi Maruyama<sup>1</sup>, Yasuharu Ninomiya<sup>1</sup>, Guillaume Varès<sup>2</sup>, Cuihua Liu<sup>1</sup>, Hirokazu Hirakawa<sup>1</sup>, Masahiro Murakami<sup>1</sup>, Zeenath Fardous<sup>3</sup> , Nahida Sultana<sup>3</sup>, Kazuko Fujita<sup>4</sup>, Akira Fujimori<sup>1</sup>, Tetsuo Nakajima<sup>1</sup>, and Mitsuru Neno<sup>5</sup>

## Abstract

Radioadaptive response (RAR) describes a phenomenon in a variety of in vitro and in vivo systems that a low-dose of priming ionizing radiation (IR) reduces detrimental effects of a subsequent challenge IR at higher doses. Among in vivo investigations, studies using the mouse RAR model (Yonezawa Effect) showed that RAR could significantly extenuate high-dose IR-induced detrimental effects such as decrease of hematopoietic stem cells and progenitor cells, acute radiation hematopoietic syndrome, genotoxicity and genomic instability. Meanwhile, it has been demonstrated that diet intervention has a great impact on health, and dietary restriction shows beneficial effects on numerous diseases in animal models. In this work, by using the mouse RAR model and mild dietary restriction (MDR), we confirmed that combination of RAR and MDR could more efficiently reduce radiogenotoxic damage without significant change of the RAR phenotype. These findings suggested that MDR may share some common pathways with RAR to activate mechanisms consequently resulting in suppression of genotoxicity. As MDR could also increase resistance to chemotherapy and radiotherapy in normal cells, we propose that combination of MDR, RAR, and other cancer treatments (i.e., chemotherapy and radiotherapy) represent a potential strategy to increase the treatment efficacy and prevent IR risk in humans.

## Keywords

adaptive response, dietary restriction, radiogenotoxicity, ionizing radiation, mice

## Introduction

Ionizing radiation (IR) is a carcinogen. It is capable of inducing genotoxicity and genomic instability (GI), in particular, at high doses. Radiation-induced genotoxicity and GI are characterized by varied endpoints such as chromosomal rearrangements and aberrations, micronucleus formation and gene mutation, having a big impact on radiocarcinogenesis.<sup>1,2</sup> GI is central to carcinogenesis<sup>3</sup> and radiation-induced GI is the driving force responsible for radiocarcinogenesis.<sup>1,4,5</sup> As humans are unavoidably exposed to IR at higher doses in some circumstances such as long-term spaceflight mission and radiotherapy, limiting cancer risk from exposure to IR is of great public concern.

A lot of factors could modify IR-induced biological effects including carcinogenesis, such as pre-exposure to IR at low doses, and dietary and life-style related factors. Radioadaptive

<sup>1</sup> National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology, Chiba, Japan

<sup>2</sup> Cell Signal Unit, Okinawa Institute of Science and Technology, Okinawa, Japan

<sup>3</sup> Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Dhaka, People's Republic of Bangladesh

<sup>4</sup> Department of Pathology, School of Medicine, Toho University, Tokyo, Japan

<sup>5</sup> Department of Safety Administration, National Institutes for Quantum and Radiological Science and Technology, Chiba, Japan

Received 25 June 2020; received revised 19 November 2020; accepted 26 November 2020

## Corresponding Authors:

Bing Wang and Mitsuru Neno, National Institutes for Quantum and Radiological Science and Technology, Chiba 263-8555, Japan.

Emails: wang.bing@nirs.go.jp; neno.mitsuru@qst.go.jp



Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (<https://us.sagepub.com/en-us/nam/open-access-at-sage>).

response (RAR) manifests as a decrease of radiosensitivity in which a low-dose of priming IR reduces the detrimental effects of subsequent challenge IR exposure at a higher dose. As an evolutionarily conserved phenomenon, RAR has been demonstrated in a variety of in vitro and in vivo biosystems from simple prokaryotes to higher eukaryotes including mammalian animals.<sup>6</sup> In vivo investigations showed that RAR could reduce challenge dose-induced DNA damage, micronucleus formation, chromosomal aberrations, cell transformation, cell death, hematopoietic death, and carcinogenesis.<sup>6-12</sup> In a series of previous studies using the RAR mouse model (Yonezawa Effect) which could rescue bone marrow death through induced resistance in the hematopoietic system,<sup>13,14</sup> we confirmed that RAR could reduce radiation-induced genotoxicity and GI measured as decreased frequency of micronucleus erythrocytes in bone marrow cells and reduced frequency of delayed homologous recombinant cells in bone marrow nucleated cells and splenocytes.<sup>15,16</sup> On the other hand, dietary and lifestyle-related factors could influence health in many species and play key roles in modulating the risk of developing cancer. As a fact, certain cancers are primarily dependent on dietary habits.<sup>17,18</sup> It has been known since the early work that dietary restriction, i.e. calorie restriction of either calories or macronutrients and fasting, would be a potent intervention against development of cancer including its initiation, progression and metastasis,<sup>19</sup> act synergistically with other treatments,<sup>20</sup> and be capable of decreasing significantly the incidence of both spontaneous and induced neoplasms in experimental carcinogenesis.<sup>19,21-24</sup> For example, on prevention of radiocarcinogenesis, investigations showed that food or caloric restriction decreased dramatically gamma- or X-ray-induced solid tumors and/or leukemias in mice and rats.<sup>25-27</sup> Caloric restriction onset either pre- or post-exposure to X-rays could extend latency of myeloid leukemia, and prevent radiation-induced myeloid leukemia and life shortening in mice.<sup>28-31</sup> Furthermore, post-exposure onset of dietary intervention (calorie restriction) in animal models showed extended lifespan, reduced frequencies of radiocarcinogenesis (late-occurring tumor) and mutations.<sup>32,33</sup> These studies demonstrated that dietary restriction could generally prevent incidence of radiocarcinogenesis, irrespective of calorie restriction onset timing in terms of irradiation, in experimental models.

In this work, for the first time in the research fields of RAR and dietary intervention, the impact from combination of mild dietary restriction (MDR) and induction of RAR on high-dose IR-induced genotoxicity was investigated in a mouse model measured as micronucleus erythrocytes in the bone marrow.

## Materials and Methods

### Animals

Three-week-old C57BL/6 J Mm strain female mice, wean just from breastfeeding, were purchased from SLC, Inc. (Japan). To avoid possible effects from the developmental condition of the animals, any mouse with a significantly different body weight

(more or less than the mean  $\pm$  2 SD) upon arrival was omitted from this study. The selected mice were randomly assigned to 2 experimental groups either allowed free access to a standard laboratory chow MB-1 (Funabashi Farm Co., Japan) or under a MDR. The animals under MDR were given daily (around 9:30 am) 85% of the amount (weight in gram) of the chow consumed by the animals that were allowed to free access to the diet. Ingredients of the diet MB-1 contained 24.2% crude protein, 4.4% crude fat, and 54.4% carbohydrate. The metabolizable energy was 354.0 kcal/100 g. As the mean amount of chow consumed by per mouse allowed free access to the diet was 2.92 g per day, each of the mice under MDR was given daily 2.48 g of the chow. Thus, the weekly metabolizable energy was 72.36 kcal and 61.45 kcal respectively for each of the mice with no diet restriction and under diet restriction. All animals were maintained in a conventional animal facility under a 12 h light-12 h dark photoperiod, housed in autoclaved cages (1 mouse per cage) with sterilized wood chips, and allowed access to acidified water (pH = 3.0  $\pm$  0.2) *ad libitum*.

Animals in each of the experimental groups were further divided into 3 subgroups, namely, the control group (without exposure to radiation), the RAR induction group (receiving both a priming dose and a challenge dose), and the high-dose exposure group (receiving only a challenge dose). Based on our previous studies and preliminary trials, in the present study at least 20 mice were used in each experimental subgroup and the experiment was repeated once. All experimental protocols (Experimental Animal Research Plan No. 09-1049 -1) 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). The experiments were performed in strict accordance with the QST-NIRS Guidelines for the Care and Use of Laboratory Animals.

### Irradiation

X-rays were generated with an X-ray machine (Pantak-320 S, 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-1321 M, 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 Induction of Radioadaptive Response

The mouse model for induction of RAR measured as significant rescue of bone marrow death (Yonezawa Effect) established by Yonezawa and colleagues.<sup>13</sup> In this model there were basically 2 types of combination for the latitude of priming dose and the interval of irradiation timing (between the priming dose and the challenge dose). Both types of combination were

adopted, verified and confirmed in C57BL/6 Jms female mice under the experimental conditions in our research facilities. The type of combination for 0.5 Gy of the priming dose and 2-week interval of irradiation timing was finally applied to the present work. Namely, the timing for delivery of the priming dose and challenge dose was on postnatal ages of 6 and 8 weeks of the mice, respectively. A dose of 0.5 Gy was used as the priming dose to verify the existence of RAR in the 30-day survival test and to investigate the incidence of micronucleus erythrocytes in femur bone marrow. For the challenge dose, a lethal dose at 7.5 Gy was used in the 30-day survival test and a non-lethal dose at 4.0 Gy was used in micronucleus test.

### **Micronucleus Test**

The bone marrow micronucleus test was carried out accordingly.<sup>15</sup> Induction of micronucleus erythrocytes in bone marrow by TBI was used as an index to evaluate both acute radiogenotoxicity and radiation-induced GI depending on early and late timing of measurement after exposure. Mice were sacrificed the following day after the 30-day survival test. Bone marrow smears prepared from both femurs were processed for the enumeration of micronucleated polychromatic erythrocytes (MNPCEs) and micronucleated normochromatic erythrocytes (MNNCEs). The slides were coded to avoid observer bias. The micronuclei were scored using a light microscope at a magnification of 1000 ×. At least 5000 PCEs and 5000 NCEs per mouse were counted and the data for each experimental point were from at least 5 mice.

### **Physiological Endpoints**

Physiological conditions were comparatively studied in mice that were allowed free access to the diet and being under MDR. The assessments included evaluating changes in body mass and main organ/tissue weights, and measurements of peripheral hemogram and bone marrow cellularity (number of nucleated cells). Body weight gain was monitored weekly from onset of MDR at postnatal age 4 weeks to the end of experiment at postnatal age 13 weeks. Main organ and intra-abdominal fat weights, peripheral hemogram, and femur bone marrow cellularity were measured at the end of the experiment. For analysis of hemogram, peripheral blood was collected with a heparinized syringe in vacutainer blood collection tubes containing EDTA (Venoject II, Terumo Co., Japan), then samples were immediately subjected to a differential blood cell count and hemoglobin concentration measurement using a blood cell differential automatic analyzer (SYSMEX K-4500, Sysmex Corporation, Japan). The data for each experimental subgroup were from at least 5 mice.

### **Statistical Analysis**

Statistical evaluation of the data was done using the Chi-squared test for the 30-day survival and micronucleus test, and

Student's *t*-test for the other endpoints. The statistical significance was assigned to  $P < 0.05$ .

## **Results**

### **Validation of Physiological Effects From Mild Dietary Restriction**

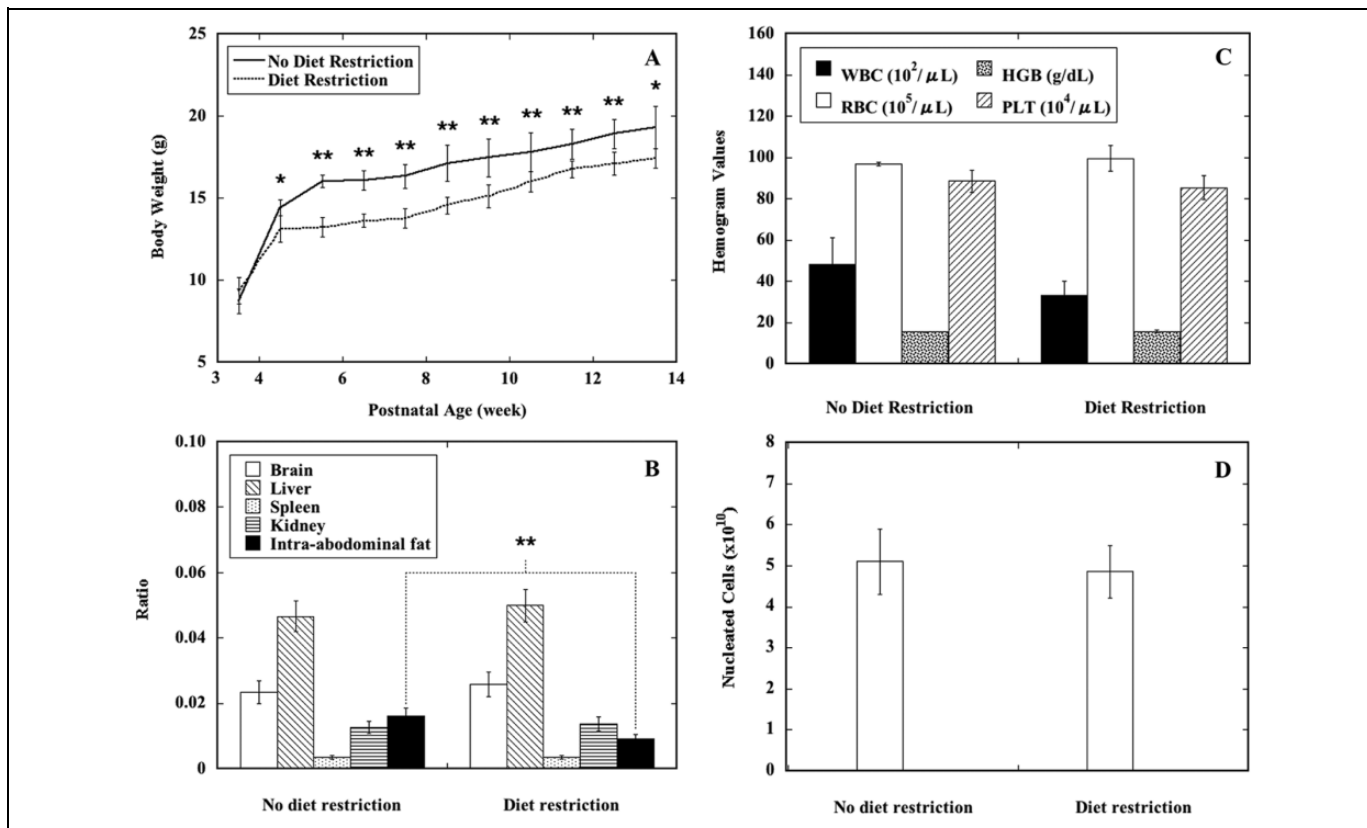
Physiological effects were assessed by evaluating changes in body mass and main organ/tissue weights, peripheral hemogram, and bone marrow cellularity. Body mass measurements of animals under MDR pointed to a general significantly lower body weight gain 1 week after onset of MDR until the end of the experiment compared to that of animals allowed free access to the diet (Figure 1A). No significant changes in the weight of brain, liver, spleen, and kidney except for a markedly decreased intra-abdominal fat weight were observed in mice being under MDR (Figure 1B). No changes were observed in peripheral hemogram measured as cell count and hemoglobin concentration (Figure 1C) and bone marrow cellularity measured as number of nucleated cells (Figure 1D). In addition, all mice being under MDR looked normal and healthy throughout the whole monitoring period. Results indicated that there is no significant detrimental physiological effect from MDR on mice during the whole period of diet regimen in our experimental setup.

### **Validation of Radioadaptive Response Induction in Mice Under Mild Diet Restriction**

Reproducibility of the RAR mouse model (Yonezawa Effect) was verified in mice allowed free access to the diet (Figure 2A) and being under MDR (Figure 2B) using 30-day survival test. For mice allowed free access to the diet, the survival rate was 16.7% and 80.0% respectively for animals receiving a challenge dose of 7.5 Gy at postnatal 8 weeks and animals receiving both a priming dose of 0.5 Gy at postnatal 6 weeks and a challenge dose of 7.5 Gy at postnatal 8 weeks. For mice being under MDR, the survival rate was 13.3% and 76.7% respectively for animals receiving a challenge dose of 7.5 Gy and animals receiving both a priming dose of 0.5 Gy and a challenge dose of 7.5 Gy. Results demonstrated that the priming dose could successfully induce a RAR in mice regardless of the diet regimen. Furthermore, neither marked differences in survival rate after exposure to the challenge dose alone, nor significant differences in efficacy for induction of RAR were observed regardless of the diet regimen. These results clearly demonstrated that there is no significant modifying effect from MDR on responses to the high challenge dose alone and induction of RAR measured as the survival rate in 30-day survival test.

### **Validation of Micronucleus Incidence in Bone Marrow Erythrocytes**

Incidence of MNPCEs (Figure 3A) and MNNCEs (Figure 3B) in bone marrow erythrocytes was assessed in mice allowed free



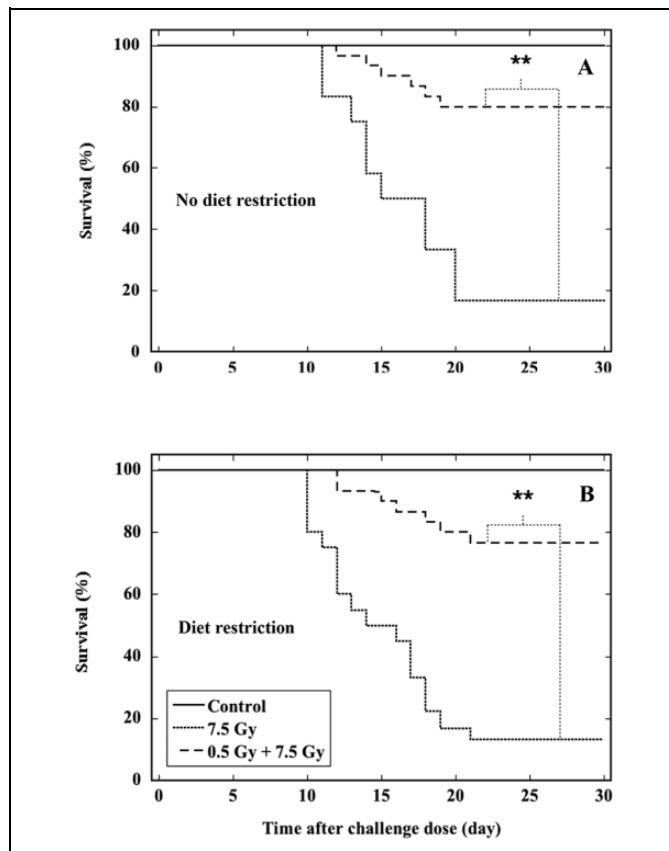
**Figure 1.** Physiological effects from diet restriction on mice. Body weight gain (A), ratio of organ or tissue weight to the body weight (B), peripheral hemogram (C), and bone marrow cellularity (D) were measured to comparatively study the effect. Body weight (g) is presented as mean  $\pm$  SD (A). The solid line and dotted line stand for mice having free access to the diet and being under diet restriction, respectively. The ratio of organ or tissue weight (g) to the body weight (g) measured at postnatal age 13 weeks is presented as mean  $\pm$  SD (B). The open bar, striped bar, dotted bar, transversely striated bar, and solid bar stand for brain, liver, spleen, kidney, and intra-abdominal fat, respectively. Peripheral hemogram was measured at postnatal age 13 weeks (C). Cell count or hemoglobin concentration is presented as mean  $\pm$  SD. The solid bar, open bar, dotted bar and striped bar stands for white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), and blood platelet count (PLT), respectively. Bone marrow cellularity was measured as total cell count of nucleated cells in 2 femurs per mouse (D). One (\*) and 2 asterisks (\*\*\*) respectively indicate statistically significant differences at  $P < 0.05$  and  $P < 0.01$  between the 2 groups that were compared.

access to the diet and being under MDR. In general, regardless of the diet regimen, exposure of animals to the challenge dose (4.0 Gy) alone induced a markedly increased incidence of MNPCEs and MNNCEs, and induction of RAR by the priming dose (0.5 Gy) significantly reduced the incidence of MNPCEs and MNNCEs caused by the subsequent challenge dose. While for animals receiving the challenge dose, incidence of MNPCEs and MNNCEs was respectively  $5.5 \pm 1.1$  and  $4.0 \pm 1.2$  per mille (‰) in mice that were allowed free access to the diet. Incidence of MNPCEs and MNNCEs was respectively  $3.1 \pm 1.1$  and  $3.0 \pm 1.0$  ‰ in mice under MDR. On the other hand, for animals receiving both the priming dose and the challenge dose, incidence of MNPCEs and MNNCEs was respectively  $1.8 \pm 0.5$  and  $1.8 \pm 0.5$  ‰ in mice that were allowed free access to the diet. Incidence of MNPCEs and MNNCEs was respectively  $1.0 \pm 0.3$  and  $0.9 \pm 0.4$  ‰ in mice under MDR. Of note, in animals receiving both the priming dose and the challenge dose, incidences of both MNPCEs and MNNCEs in mice under MDR were significantly lower

than that in mice that were allowed free access to the diet. Results clearly demonstrated that induction of RAR could relieve radiogenotoxicity caused by the high challenge dose, and increased efficacy for reduction of radiogenotoxicity could be further achieved in combination with MDR.

## Discussions

Increased opportunity for exposure to IR due to such as cancer radiotherapy and long-term space mission is associated with an increasing risk of health effects in humans, in particular, carcinogenesis. Reducing health risk from unavoidable IR exposure of normal tissue in medical treatment and spaceflight crew is of great concern and significance. To modify the radioresistance of normal tissues and cancer cells whenever possible, triggering the mechanisms to selectively increase the radioresistance of the normal tissues and sensitize the cancer cells is of great importance. Many approaches such as development of radioprotective agents, pharmaceutical gene regulation, and intervening dietary



**Figure 2.** Induction of RAR in mice having free access to the diet and being under diet restriction. Induction of RAR (Yonezawa Effect) by a priming dose of 0.5 Gy X-rays at postnatal 6 weeks followed by a subsequent challenge dose of 7.5 Gy X-rays at postnatal 8 weeks was verified in mice having free access to the diet (A) and being under diet restriction (B). The solid line, broken line, and dotted line stand for the mice receiving no TBI (control), the mice receiving the challenge dose alone (7.5 Gy), and mice receiving both the priming dose and the challenge dose (0.5 Gy + 7.5 Gy), respectively. Two asterisks (\*\*\*) indicate statistically significant differences ( $P < 0.01$ ) between the 2 groups that were compared.

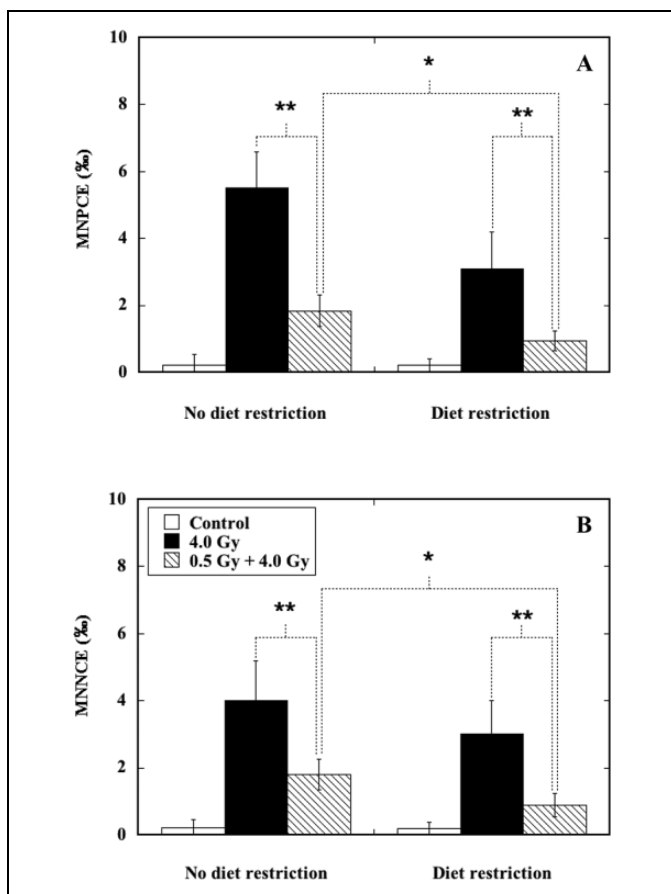
habit, psychological stress and life style were proposed.<sup>34</sup> When compared to the research and development of these therapeutic modalities, intervention of life-style factors is less time-consuming and with a low cost, which neither depends on the advancement of technology. Notably, dietary restriction, as one of the mostly used intervention of life-style factors, showed a great promise as a modifier to prevent incidence of radiation-induced myeloid leukemia in mice, irrespective of calorie restriction onset timing in terms of irradiation.<sup>28-31</sup> It is rational that initiatives to reduce the health risk from IR exposure should focus on the tenets of appropriate combination of different approaches. To date, there is no study on the efficacy of combination of induction of RAR and dietary restriction for reduction of radiogenotoxicity and IR-induced GI.

The mouse RAR model (Yonezawa Effect) is a well-designed system in investigating the late health effect *in vivo* such as IR-induced GI. In a series of our previous

investigations, we demonstrated that induction of RAR could relieve IR-induced GI measured as micronucleus formation and delayed homologous recombination in the hematopoietic system.<sup>15,16</sup> Recently, we also verified the modifying effects from dietary factors on the biological response to IR exposure of mice. Our studies confirmed that there existed an interaction between life-style factors (i.e., high fat diet, low fat diet and alcohol drinking) and IR, which could lead to altered responses to IR in mice.<sup>35-37</sup> In the present work, combination of MDR and RAR was further verified in the mouse RAR model. Results indicated that MDR would not influence the phenotype of RAR measured as 30-day survival, and combined treatment with MDR and RAR could achieve a higher efficacy to relieve radiogenotoxicity and radiation-induced GI measured as reduced micronucleus frequency in the erythrocytes in the bone marrow.

It is known that normal cells and tumor cells from both mice and humans respond differently to induction of RAR. For example, in normal cells RAR could be induced measured as increased cell survival and decreased micronucleus frequency in the cultured cells *in vitro*, and increased cell proliferation and reduced apoptosis *in vivo*. On the other hand, higher induction of apoptosis along with high expression of pro-apoptotic gene Bax and lower expression of anti-apoptotic gene Bcl-2 was induced in tumor cells in tumor-bearing mice under RAR induction condition.<sup>38-40</sup> These works demonstrated clearly that RAR could be induced in normal cells but not in tumor cells under both *in vitro* and *in vivo* conditions. Interestingly, diet intervention also has the potential to differently change the response of normal tissue and tumors, namely, to alleviate genotoxicity of normal tissue and enhance cytotoxicity of cancer cells. Thus, it is expected that innovative use of diet intervention as a novel therapeutic option would bring an additional clinical benefit in the treatment of cancer patients. These studies suggested a very important clinic-relevant phenomenon and implied the potential for application of RAR to protect normal tissues without diminishing the efficacy of tumor radiotherapy.<sup>40</sup> Collectively, both induction of RAR and appropriate dietary restriction could selectively protect normal cells from the side effects of IR at higher doses and sensitize cancer cells to IR. In this regards, clinically combined application of prophylactic MDR and RAR before cancer radiotherapy would have a great potential for benefiting cancer patients from the point of view of increasing the maximum tolerated dose and reducing the risk of secondary cancer.

It should be pointed out that appropriate dietary restriction without malnutrition is critical for induction of beneficial health effects. For examples, moderate food restriction could suppress aging rate.<sup>41</sup> MDR in the present work could enhance the efficacy of RAR-induced radiogenotoxicity, and in addition, with a concomitant decrease in body weight gain and intra-peritoneal fat weight in mice. Conversely, severe dietary restriction or malnutrition due to unbalanced diet could increase sensitivity of normal tissues to radiogenotoxicity, radiation-induced bone marrow death and life shortening in mice.<sup>36,42</sup>



**Figure 3.** Incidence of MNPCEs and MNNCEs induced by TBI in femur bone marrow erythrocytes (RBC) in mice having free access to the diet and being under diet restriction. Incidence as the number of MNPCEs per 1000 PCEs (A) or of MNNCEs per 1000 NCEs (B) is presented as mean  $\pm$  SD. The open bar, solid bar, and striped bar stand for samples from mice receiving no TBI (control), the mice receiving a challenge dose alone (4.0 Gy), and mice receiving both a priming dose and a challenge dose (0.5 Gy + 4.0 Gy), respectively. One (\*) and 2 asterisks (\*\*), respectively indicate statistically significant differences at  $P < 0.05$  and  $P < 0.01$  between the 2 groups that were compared.

A complete understanding of the underlying mechanisms is imperative in reducing health risk due to IR exposure in humans. Protection against radiocarcinogenesis could be achieved by different mechanisms (i.e., free radical scavenging, caloric restriction, anti-inflammation, and humoral factors), and the efficacy could be enhanced by targeting multiple mechanisms at the same time.<sup>43</sup> For dietary intervention in the animal models, it is firmly established that the effect depends on many variables, such as diet composition, feeding regimen, age of onset and genetics, in addition to the calories provided in the diet.<sup>44</sup> For caloric restriction, the anticancer and genomic effects could be established more rapidly in mitotic tissues. Acute caloric restriction showed reduced detrimental effects and aging alterations in the organs in both animal models and in humans, as possibly the highly conserved mechanisms for health enhancement.<sup>45,46</sup>

MDR may share some common pathways with RAR to activate mechanisms consequently resulting in suppression of

genotoxicity. IR could cause both acute tissue damage and late effects including long-term or residual bone marrow injury. As a carcinogen, IR poses a significant cancer risk in part by induction of GI, for example, development of leukemia in hematopoietic system. Exposure of mice to a sublethal dose of TBI could induce a persistent increase in ROS production in hematopoietic stem cells. Studies showed that increased the generation and accumulation of mitochondrial ROS was via overexpression of miR-22 or nicotinamide adenine dinucleotide phosphate oxidase and led to induction of hematopoietic GI.<sup>47,48</sup> Exposure of mice to TBI could induce persistent oxidative stress in hematopoietic stem cells at least in part via up-regulation of nicotinamide adenine dinucleotide phosphate oxidase and associated with sustained increases in oxidative DNA damage, DNA double-strand breaks, and inhibition of clonogenic function.<sup>47,49</sup> On the other hand, a majority of studies in animal models and humans showed that dietary restriction-induced reduced mutations and radiocarcinogenesis are underlying mechanisms of increased antioxidant glutathione level, improved redox state, decreased mitochondrial ROS production, reduced oxidative stress and damage, and decreased chronic inflammation, leading to an overall reduction of steady-state oxidative damage to macromolecules including proteins, lipids and DNA in animals.<sup>23,32,33,50-54</sup> Meanwhile, RAR is also known as through mechanisms of increased antioxidant capability, decreased ROS production, reduced oxidative stress and damage, increased DNA repair and decreased chronic inflammation.<sup>7-12</sup>

As diet and metabolism link to cancer, for cancer treatment, it was demonstrated that dietary restriction could simultaneously target many signal pathways that were targeted by anticancer drugs<sup>20</sup> and cancer radiotherapy. For example, in mouse model, investigation showed that calorie restriction could augment efficacy of radiotherapy in breast cancer.<sup>55</sup> In humans, study showed that caloric restriction coupled with radiotherapy could decrease metastatic burden in triple negative breast cancer.<sup>56</sup> These studies indicated that diet intervention could be used in conjunction with chemotherapy and radiotherapy to render cancer cells more susceptible to treatment, which could lead to new cancer therapies using the metabolism-involved mechanisms. Dietary restriction could increase DNA repair in mice.<sup>57</sup> Equally interesting and important, increasing convincing evidence in animal models showed that post-exposure onset of calorie restriction could also reduce frequencies of radiocarcinogenesis. Pre-exposure caloric restriction could prevent the initiation of direct genotoxic leukemogenesis, and post-exposure could improve mitochondrial function, reduce production of reactive oxygen species, and relieve indirect and epigenetic leukemogenesis.<sup>28-33,58</sup>

In summary, the present work demonstrated that combined treatment with RAR and MDR could more efficiently relieve high-dose IR-induced genotoxicity in mice. These findings indicated that diet intervention could be a potential modifiable factor for reducing risk from IR exposure and provide a clear direction for initiating such studies prior to clinical implementation in humans. As a potentially promising strategy for reducing long-term IR health risk from cancer radiotherapy, combination of RAR induction and lifestyle management (i.e., MDR) could maximize medical utilization of IR, via

increasing the organ tolerance dose and reducing radiogenotoxicity, radiation-induced GI and radiocarcinogenesis.

### Acknowledgments

The authors thank Ms. Hiromi Arai (QST-NIRS, Japan) for performing X-irradiations. The expert technical assistance and administrative support of Mr. Sadao Hirobe, Ms. Mikiko Nakajima, Ms. Hiromi Arai, Ms. Nobuko Tsutsumi, Ms. Hiromi Utsumi and Ms. Yasuko Morimoto (QST-NIRS, Japan), are gratefully acknowledged. This paper is dedicated to the memory of the late Dr. William Francis Morgan (Pacific Northwest National Laboratory, USA) for his encouragement and comments on this work. Thanks are also due to the anonymous peer reviewers for providing the constructive comments that strengthened the presentation of this work.

### Declaration of Conflicting Interests


The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.


### Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was partially supported by the National Institute of Radiological Sciences, National Institutes for Quantum and Radiological Science and Technology, Japan, and JSPS KAKENHI 25340041, Japan. Ms. Zeenath Fardous and Ms. Nahida Sultana were fellows of the Nuclear Researchers Exchange Programme (in 2018 and 2019, respectively), which was supported by the Ministry of Education, Culture, Sport, Sciences and Technology (MEXT), Japan, and the Nuclear Safety Research Association, Japan. References

### ORCID iDs

Bing Wang  <https://orcid.org/0000-0002-7180-639X>

Takanori Katsube  <https://orcid.org/0000-0002-6374-4272>

Zeenath Fardous  <https://orcid.org/0000-0003-3522-5624>

### References

- Morgan WF, Day JP, Kaplan MI, McGhee EM, Limoli CL. Genomic instability induced by ionizing radiation. *Radiat Res.* 1996; 146(3):247-258.
- Huang L, Snyder AR, Morgan WF. Radiation-induced genomic instability and its implications for radiation carcinogenesis. *Oncogene.* 2003;22(37):5848-5854.
- Schmitt MW, Prindle MJ, Loeb LA. Implications of genetic heterogeneity in cancer. *Ann N Y Acad Sci.* 2012;1267:110-116.
- 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.
- Smith LE, Nagar S, Kim GJ, Morgan WF. Radiation-induced genomic instability: radiation quality and dose response. *Health Phys.* 2003;85(1):23-29.
- 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.* United Nations. 1994:185-272.
- Olivieri G, Bodycote J, Wolff S. Adaptive response of human lymphocytes to low concentrations of radioactive thymidine. *Science.* 1984;223(4636):594-597.
- Wolff S. The adaptive response in radiobiology: evolving insights and implications. *Environ Health Perspect.* 1998;106(suppl 1): 277-283.
- Mitchel RE. Low doses of radiation are protective in vitro and in vivo: evolutionary origins. *Dose Response.* 2006;4(2):75-90.
- Varès G, Wang B, Tanaka K, Nakajima T, Neno M, Hayata I. Radiation-induced adaptive response with reference to evidence and significance: a review. *Indian J Radiat Res.* 2006;3(1):16-34.
- Tapio S, Jacob V. Radioadaptive response revisited. *Radiat Environ Biophys.* 2007;46(1):1-12.
- Neno M, Wang B, Varès G. In vivo radioadaptive response: a review of studies relevant to radiation-induced cancer risk. *Human Exp Toxicol.* 2015;34(3):272-283.
- 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.
- Yonezawa M. Induction of radio-resistance by low dose X-irradiation [in Japanese]. *Yakugaku Zasshi.* 2006;126(10): 833-840.
- 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;54:45-51.
- Liu C, Hirakawa H, Tanaka K, et al. Reduction of delayed homologous recombination by induction of radioadaptive response in RaDR-GFP mice (Yonezawa Effect): an old player with a new role. *Dose Response.* 2019;17(1):1559325819833840.
- De Pergola G, Silvestris F. Obesity as a major risk factor for cancer. *J Obes.* 2013;2013:291546.
- Kerr J, Anderson C, Lippman SM. Physical activity, sedentary behaviour, diet, and cancer: an update and emerging new evidence. *Lancet Oncol.* 2017;18(8):e457-e471.
- Lv M, Zhu X, Wang H, Wang F, Guan W. Roles of caloric restriction, ketogenic diet and intermittent fasting during initiation, progression and metastasis of cancer in animal models: a systematic review and meta-analysis. *PLoS One.* 2014;9(12):e115147.
- Klement RJ, Fink MK. Dietary and pharmacological modification of the insulin/IGF-1 system: exploiting the full repertoire against cancer. *Oncogenesis.* 2016;5:e193.
- Sivertsen I., Hastings WH. A preliminary report on the influence of food and function on the incidence of mammary gland tumor in "A" stock albino mice. *Minnesota Med.* 1938;21:873-875.
- Fontana L, Partridge L, Longo VD. Dietary restriction, growth factors and aging: from yeast to humans. *Science.* 2010; 328(5976):321-326.
- Hursting SD, Dunlap SM, Ford NA, Hursting MJ, Lashinger LM. Calorie restriction and cancer prevention: a mechanistic perspective. *Cancer Metab.* 2013;1(1):10.
- Nencioni A, Caffa I, Cortellino S, Longo VD. Fasting and cancer: molecular mechanisms and clinical application. *Nat Rev Cancer.* 2018;18(11):707-719.
- Gross L, Dreyfuss Y. Reduction in the incidence of radiation-induced tumors in rats after restriction of food intake. *Proc Natl Acad Sci U S A.* 1984;81(23):7596-7598.



26. Gross L, Dreyfuss Y. Inhibition of the development of radiation-induced leukemia in mice by reduction of food intake. *Proc Natl Acad Sci U S A*. 1986;83(20):7928-7931.
27. Gross L. Inhibition of the development of tumors or leukemia in mice and rats after reduction of food intake. Possible implications for humans. *Cancer*. 1988;62(8):1463-1465.
28. Yoshida K, Inoue T, Nojima K, Hirabayashi Y, Sado T. Caloric restriction reduces the incidence of myeloid leukemia induced by a single whole-body radiation in mice. *Proc Natl Acad Sci U S A*. 1997;94(6):2615-2619.
29. Yoshida K, Inoue T, Hirabayashi Y, Matsumura T, Nemoto K, Sado T. Radiation-induced myeloid leukemia in mice under calorie restriction. *Leukemia*. 1997;11(suppl 3):410-412.
30. Yoshida K, Hirabayashi Y, Inoue T. Caloric restriction reduces the incidence of radiation-induced myeloid leukaemia. *IARC Sci Publ*. 2002;156:553-555.
31. Yoshida K, Hirabayashi Y, Watanabe F, Sado T, Inoue T. Caloric restriction prevents radiation-induced myeloid leukemia in C3H/HeMs mice and inversely increases incidence of tumor-free death: implications in changes in number of hemopoietic progenitor cells. *Exp Hematol*. 2006;34(3):274-283.
32. Shang Y, Kakinuma S, Yamauchi K, et al. Cancer prevention by adult-onset calorie restriction after infant exposure to ionizing radiation in B6C3F1 male mice. *Int J Cancer*. 2014;135(5):1038-1047.
33. Kakomi S, Nakayama T, Shang Y, et al. The effects of short-term calorie restriction on mutations in the spleen cells of infant-irradiated mice. *J Radiat Res*. 2020;61(2):187-196.
34. 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;35(suppl):S5-S24.
35. Varès G, Wang B, Ishii-Ohba H, Nenoï M, Nakajima T. Diet-induced obesity modulates epigenetic responses to ionizing radiation in mice. *PLoS One*. 2014;9(8):e106277.
36. Wang B, Tanaka K, Katsube T, Maruyama K, Ninomiya Y, Nenoï M. Dietary modification of mouse responses to total-body-irradiation. In: Nenoï M, ed. *Evolution of Ionizing Radiation Research*. Intech-Open Access Publisher; 2015:63-87.
37. Nakajima T, Varès G, Wang B, Nenoï M. Chronic intake of Japanese Sake mediates radiation-induced metabolic alterations in mouse liver. *PLoS One*. 2016;11(1):e0146730.
38. 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.
39. Yu H, Liu N, Wang H, Shang Q, Jiang P, Zhang Y. Different responses of tumor and normal cells to low-dose radiation. *Contemp Oncol*. 2013;17(4):356-362.
40. 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):1559325815622174.
41. Skulachev MV, Severin FF, Skulachev VP. Aging as an evolvability-increasing program which can be switched off by organism to mobilize additional resources for survival. *Curr Aging Sci*. 2015;8(1):95-109.
42. Yamauchi K, Ono T, Ayabe Y, et al. Life-shortening effect of chronic low-dose-rate irradiation in calorie-restricted mice. *Radiat Res*. 2019;192(4):451-455.
43. Oliai C, Yang LX. Radioprotectants to reduce the risk of radiation-induced carcinogenesis. *Int J Radiat Biol*. 2014;90(3):203-213.
44. Vaughan KL, Kaiser T, Peadar R, Anson RM, de Cabo R, Mattison JA. Caloric restriction study design limitations in rodent and nonhuman primate studies. *J Gerontol A Biol Sci Med Sci*. 2017;73(1):48-53.
45. Spindler SR. Rapid and reversible induction of the longevity, anticancer and genomic effects of caloric restriction. *Mech Ageing Dev*. 2005;126(9):960-966.
46. Spindler SR, Dhahbi JM. Conserved and tissue-specific genetic and physiologic responses to caloric restriction and altered IGF1 signaling in mitotic and postmitotic tissues. *Annu Rev Nutr*. 2007;27:193-217.
47. Pazhanisamy SK, Li H, Wang Y, Batinic-Haberle I, Zhou D. NADPH oxidase inhibition attenuates total body irradiation-induced haematopoietic genomic instability. *Mutagenesis*. 2011;26(3):431-435.
48. Liu Z, Li T, Zhu F, Deng S, Li X, He Y. Regulatory roles of miR-22/Redd1-mediated mitochondrial ROS and cellular autophagy in ionizing radiation-induced BMSC injury. *Cell Death Dis*. 2019;10:227.
49. Wang Y, Liu L, Pazhanisamy SK, Li H, Meng A, Zhou D. Total body irradiation causes residual bone marrow injury by induction of persistent oxidative stress in murine hematopoietic stem cells. *Radic Biol Med*. 2010;48(2):348-356.
50. Gredilla R, Barja G. Minireview: the role of oxidative stress in relation to caloric restriction and longevity. *Endocrinol*. 2005;146(9):3713-3717.
51. Pamplona R, Barja G. Mitochondrial oxidative stress, aging and caloric restriction: the protein and methionine connection. *Biochim Biophys Acta*. 2006;1757(5-6):496-508.
52. Kowaltowski AJ. Caloric restriction and redox state: does this diet increase or decrease oxidant production? *Redox Rep*. 2011;16(6):237-241.
53. Walsh ME, Shi Y, Remmen HV. The effects of dietary restriction on oxidative stress in rodents. *Free Radic Biol Med*. 2014;66:88-99.
54. Redman LM, Smith SR, Burton JH, Martin CK, Il'yasova D, Ravussin E. Metabolic slowing and reduced oxidative damage with sustained caloric restriction support the rate of living and oxidative damage theories of aging. *Cell Metab*. 2018;27(4):805-815.e4.
55. Saleh AD, Simone BA, Palazzo J, et al. Caloric restriction augments radiation efficacy in breast cancer. *Cell Cycle*. 2013;12(12):1955-1963.10.
56. Simone BA, Dan T, Palagani A, et al. Caloric restriction coupled with radiation decreases metastatic burden in triple negative breast cancer. *Cell Cycle*. 2016;15(17):2265-2274.
57. Lipman JM, Turturro A, Hart RW. The influence of dietary restriction on DNA repair in rodents: a preliminary study. *Mech Ageing Dev*. 1989;48(2):135-143.
58. Wang C, Maddick M, Miwa S, et al. Adult-onset, short-term dietary restriction reduces cell senescence in mice. *Aging*. 2010;2(9):555-566.