

Impacts of psychological stress on high dose-rate radiation acute effects in a mouse experimental model

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ABSTRACT

Psychological stress affects health. Radiation workers in the medical field or astronauts living in space have possible risks of exposure to radiation, and psychological stress is considered to be easily induced in them due to activities performed in small areas or stress conditions. The impact of psychological stress on the effects of radiation was evaluated in senescence-accelerated mouse prone 10 (SAMP10) mice and ddY mice using a confrontational housing model, which makes dominant and subordinate mice in a cage live together without severe quarrel. Mice of ddY and SAMP10 have been previously demonstrated to be influenced in terms of acute and late effects, respectively, under psychological stress by this model. In SAMP10 mice, irradiation with 4 Gy induced the death of irradiated mice under psychological stress. In ddY mice, irradiation with 5 Gy X-rays alone had almost no effect on the mouse survival, but irradiation in conditions of psychological stress promoted acute death of irradiated mice. In addition, hypocellular bone marrow was also observed histopathologically in irradiated ddY mice under stress. Psychological stress may promote damage caused by radiation through modulation of radio-sensitivity in bone marrow in mice. This model would be useful for evaluation of modulation of radiation-induced various effects by psychological stress.

Keywords: radiation; psychological stress; acute effects; survival; bone marrow

INTRODUCTION

Chronic psychological stress has adverse effects on health [1–3]. In some cases, we may need to address both radiation effects and psychological effects. In occupational situations, radiation workers in the medical field have a risk of exposure to radiation while under occupational stress factors (stressors) inducing psychological stress [4, 5]. For radiologists or radiographers, it is recommended to reduce work-related stress and potential burnout [4–7]. Training for gaining knowledge about radiation is also important for preventing psychological stress in radiation-related workers [8, 9]. Moreover, in space, psychological stress by living in confined environment like the International Space Station as well as cosmic radiation including sparsely and densely ionizing radiation may influence human health [10, 11]. These findings suggest that evaluating the effects of radiation under psychological stress is necessary to assess the radiation risk.

To evaluate this, an appropriate experimental model is needed for modulation of radiation effects by psychological stress. Some experimental models have been developed and tested [12–16]. Some research on the effects of psychological stress on human health has been performed epidemiologically [1, 2]. In animal experiments, for example, using the restraint stress model, it was demonstrated that psychological stress modulates the effects of radiation [12]. These models have demonstrated many effects of psychological stress on animals, although some concerns about evaluation remain. For the evaluation of psychological stress, exclusion of physical stress induced by physical contact (touching, sensing a temperature, etc.) as much as possible is important. Psychological stress models that exclude physical stress are appropriate experimental models [13–15].

We have selected a psychological experimental model of confrontation that was developed and improved by Unno *et al.* [15, 16]. Mice are

kept in a cage with a partition for some period, and then the partition is removed, and mice are exposed to confrontation stress as a psychological stressor [15, 16]. Though they do not see the other mouse in the cage before removing a partition, they recognize each other's odors in the same cage. Male mice are known to fight aggressively against mice who have unfamiliar urine odors [17]. In this model, as mice have time to recognize odors of the other mouse in the cage before removing the partition, mice have no fights and can live together for a long time. It was also demonstrated that the stress by this model induces causes adrenal hypertrophy, decreases of thymus weight and changes in stress markers such as corticosterone and adrenocorticotropic hormone (ACTH) in two mice in the cage though it has no marked effects on body weight [16]. Using two different strains of mice, Unno *et al.* demonstrated the psychological stress effects in mice in this model [15, 16]. In the experiments, senescence-accelerated mouse prone 10 (SAMP10) and ddY as a normal strain were used. In ddY mice, adrenal hypertrophy and lowered thymus weights were observed under stress. SAMP10 is a stress-sensitive strain and the confrontation stress induced not only adrenal hypertrophy but also accelerated the aging and cognitive dysfunction, resulting from the persistent stress. Radiosensitivity of mice is dependent on strains [18, 19]. Modulation in radiation effects by psychological effects should be evaluated in several mouse strains and it is necessary that the stress model has already demonstrated to be effective on the mice. In addition, though SAMP10 is senescence-prone, radiosensitivity of SAMP10 mice has not been demonstrated. Evaluation of acute radiation effects using SAMP10 would give us precious information in the strain, leading to the following studies for radiation late effects. Moreover, SAM10 mice appear to have high sensitivity to psychological stress similar to BALB/c mice or C57BL [16], while ddY mice are different from SAMP10 in sensitivity to psychological stress [20]. Thus, as it is proved enough that psychological stress using confrontation stress is effective in the mice (a normal strain and specific senescence accelerated strain mouse), which have different sensitivity to the stress, in our current study, we evaluated the effects of this type of stress on acute radiation-induced damage using the two strains, SAMP10 and ddY.

MATERIALS AND METHODS

Mice

All animal studies were reviewed and approved by The Institutional Animal Care and Use Committee of the National Institute of Radiological Sciences (NIRS), and were performed in strict accordance with the NIRS Guidelines for the Care and Use of Laboratory Animals. Four-week-old male ddY and 4-week-old male SAMP10 mice, which are senescence-prone, were obtained from Japan SLC, Inc. (Hamamatsu, Japan) and housed four or five mice in a cage (floor area: 15 cm × 28 cm) for adaptation before performing the experiments. Concerning the strains, ddY is non-inbred strains and has been maintained as a closed colony [21]. SAMP10 is one of 9 SAMP strains developed through selective inbreeding of AKR/J strains [22].

Confrontation stress experiments

Mice (about 5 weeks old) were divided into groups of confrontation, single, or group housing (four mice in a cage). The mice in each group were divided into two groups (irradiated or not). For the experiment of

confrontation housing, two mice were randomly selected for housing separately in a partitioned cage for 1 month for SAMP10 or for 1 week for ddY mice. A standard aluminum cage (TOKIWA KAGAKU KIKAI Co. Ltd., Tokyo, Japan) was divided into two identical subunits with a stainless steel partition made as a custom-ordered product (IKEDA SCIENTIFIC Co. Ltd., Tokyo, Japan) according to the method of Unno *et al.* with minor changes [15, 16]. These mice were individually housed to allow for the establishment of an individual territory. The mice could not see or contact each other as a result of the partition. Then, the partition was removed to expose the mice to confrontation stress, and subsequently, the two mice coexisted in the cage (confrontation housing). In the cage, these mice were both residents and intruders to each other. The model which makes mice recognize each other's odors in the cage suppress aggressive fights in the cage. After removing the partition, dominant and subordinate mice are determined in two mice in the cage. Some offensive behaviors and defensive behaviors were occasionally observed in male mice immediately after the confrontational housing was started, however, neither subordinate nor dominant mice were injured in this model. The mice were observed carefully during the experiment daily in terms of health conditions such as fur state and injuries, to see whether or not the mice had aggressive fights. Mice for single housing or group housing in which four mice were housed per cage were used as controls.

X-ray irradiation and survival observation

The mice were held in acrylic containers and irradiated with a total dose of 3–8 Gy at a dose rate of 0.85 Gy/min using a Pantak 320S machine (Shimadzu Corporation, Kyoto, Japan) equipped with a 0.50-mm Al + 0.50-mm Cu filter and operated at 200 kVp and 20 mA. An exposure rate meter (AE-1321 M; Applied Engineering Inc. Bismarck, ND) was used for dosimetry measurements.

Histopathological observations

From control or moribund mice, the tissues were fixed with 10% neutral-buffered formalin. All samples were embedded in paraffin, sectioned transversely (4 μm thick), and stained with hematoxylin and eosin (HE) [23]. Histopathological diagnosis was performed by pathologist using J-SHARE (Japan Storehouse of Animal Radiobiology Experiments; NIRS, QST), in which data from autopsy observations and HE specimens prepared by the above methods were registered [24].

Statistical analysis

Statistically significant changes were evaluated using the log-rank test. Kaplan–Meier survival curves were plotted and the evaluation were performed using the software EZR (<https://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html>) [25].

RESULTS

The confrontation stress used in this study was investigated in SAMP10 and ddY mice previously [15, 16]. Here, using SAMP10 mice, radiosensitivity was evaluated after irradiation under confrontation stress. SAMP10 mice are senescence prone and exhibit acceleration of brain damage under chronic confrontation stress [15]. Here single-housed

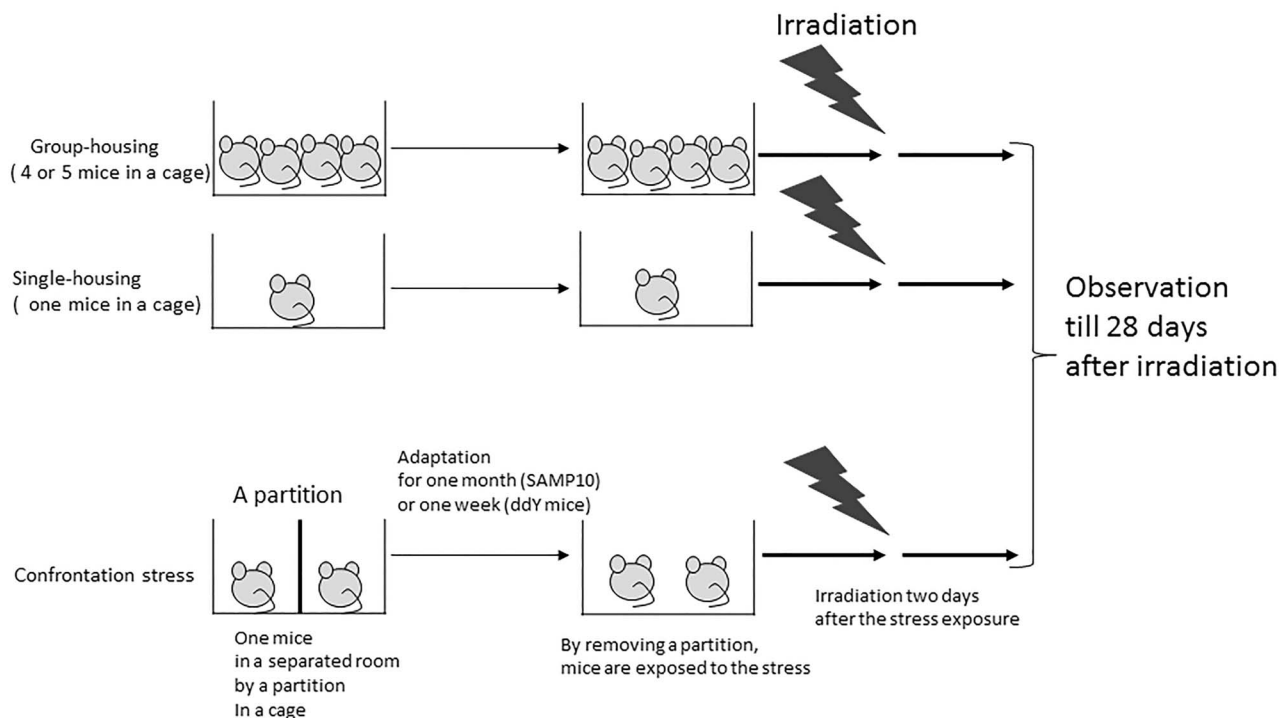


Fig. 1. A schematic view of housing conditions and experimental design.

mice were used as a control. For the confrontation model, one model of psychological stress, the mouse was housed in a cage with a partition for a month, and 48 hours after removing the partition, half of the confrontation stressed mice were irradiated. Half of the control mice were also irradiated, and all mice were observed for about a month. This experimental design is described in Fig. 1.

The result of survival after irradiation in SAMP10 mice is shown using Kaplan–Meier survival curves in Fig. 2. When the mice were irradiated with 4 Gy alone, the effects on survival were not severe, but irradiation under confrontation stress resulted in a decrease in survival to almost half of the control. Thus, psychological stress rendered the mice radio-sensitive.

SAMP10 is a specific strain. To demonstrate the phenomenon in a more general strain, we used ddY mice for evaluation of the effects of the stress condition. Single-housed mice and group-housed mice were used as controls, and the effects of confrontation stress on the radiation effects were assessed. In single-housed mice and group-housed mice, irradiation with 6 Gy or more had severe effects on survival in the mice (Fig. 3A and 3B). Whereas Fig. 3 shows that in mice irradiated with 6 Gy or more, no difference was found between irradiation alone (Fig. 3A and 3B) and irradiation plus stress (Fig. 3C). However, with 5 Gy irradiation, mice under confrontation stress were more radio-sensitive compared to 5 Gy alone. To clarify the difference, two independent experiments were performed and it was demonstrated that, in the case of 5 Gy, the confrontation stress renders the mice more radio-sensitive (Fig. 4). Although SAMP10 mice appeared to be more radio-sensitive under the stress, this experiment using ddY mice indicated that the stress made mice radio-sensitive in general.

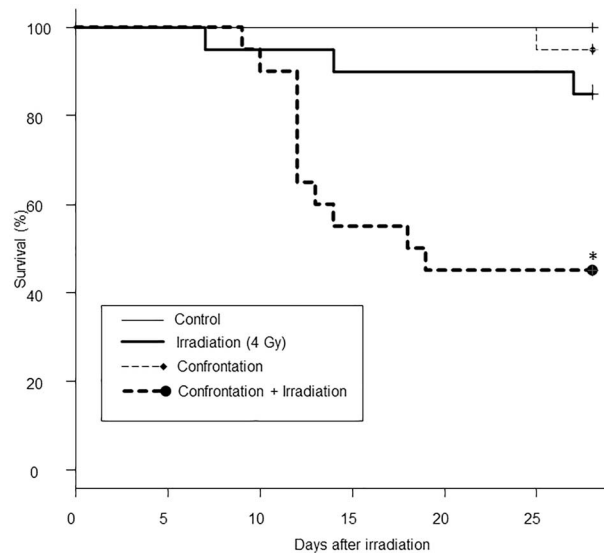


Fig. 2. Modulation of the effects of radiation in SAMP10 mice by psychological stress. The data were described using Kaplan–Meier survival curves. Single-housed mice were used as a control. $n = 20$, Logrank Irradiation vs Confrontation + Irradiation $*P < 0.01$ ($P = 0.00767$).

Next, we investigated how psychological stress modulates acute radiation effects. To evaluate the effect of psychological stress on an ability to recover of hematopoietic cells after radiation exposure, histopathological changes were observed. The histopathological

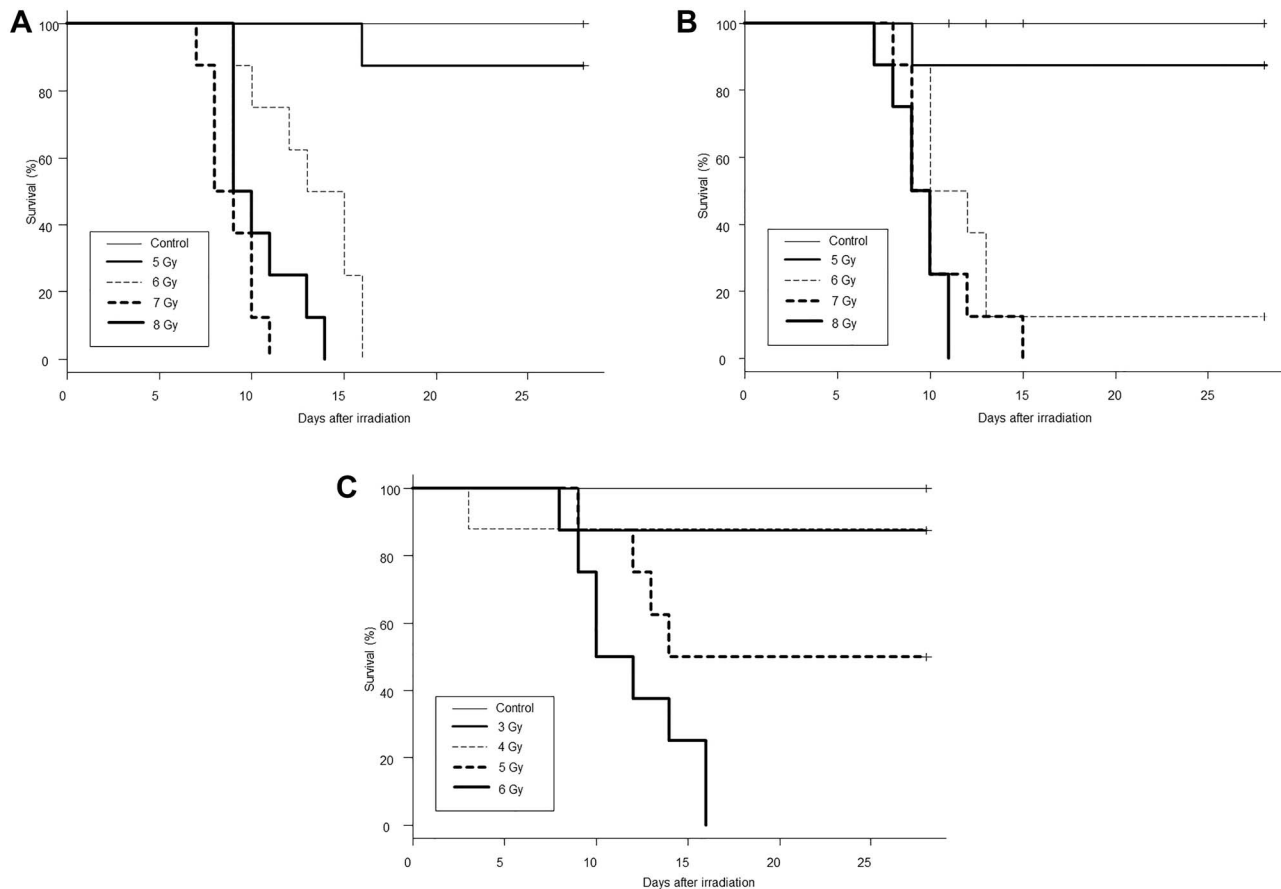


Fig. 3. Acute effects in ddY mice under psychological stress after irradiation with 3–8 Gy. The data were described using Kaplan–Meier survival curves. Single-housed (A) ($n = 7-8$), group-housed (B) ($n = 8$), and confrontational housing conditions (C) ($n = 8$) were used.

images of bone marrow from femur and sternum in non-irradiated ddY mice (control) and moribund irradiated ddY mice under stress were shown in Fig. 5. The compared to the histopathological findings in control mice (Fig. 5A, E, C and G), moribund irradiated mice under the stress at 13 days after irradiation (Fig. 5B, F, D and H) showed a marked decrease of hematopoietic cellularity in the bone marrow both of femur and sternum. These findings suggested that modulation of radiation effects by psychological stress seems to be due to a decrease in the restorative ability of hematopoietic cells after irradiation. Because the irradiation with 5Gy alone did not induce outstanding effects on the survival (Figs 3–4), the histopathological analysis was not performed. Though irradiation with the dose alone probably induces some damage, it does not appear to cause severe damage leading to acute death in the mice.

DISCUSSION

People with a potential risk of exposure to radiation such as radiation workers in the medical field are under psychological stress [4–9]. Psychological stress is considered to influence humans during exposing to radiation or after irradiation. In this experiment, mice are irradiated

under psychological stress and are also exposed to the stress after irradiation. In addition, as the psychological model we used includes almost no physical stress, it is appropriate for evaluation of modulation of the effects of radiation by psychological stress [15, 16].

Here, we demonstrated the effects of psychological stress on acute radiation damage using two mouse strains. The dose, which is not sufficient to induce acute radiation death in either strain, caused death in mice under stress. The observed period of acute death by irradiation with or without the stress is around when bone marrow death is induced after high-dose radiation exposure in mice [18]. In histopathological analyses, the moribund mice exposed to radiation under stress had hypocellular marrow. The results suggest that promotion of acute radiation death by psychological stress is due to bone marrow dysfunction including suppression of hematopoietic activity in bone marrow. Previously it was demonstrated that psychological stress induces a decrease in the thymus weight of the mice even in a week after start of confrontation stress [16]. This may suggest that psychological stress influences hematopoietic activity in bone marrow related to the thymus recovery [26] and lowers a threshold dose for hematopoietic acute radiation syndrome. As higher radiation doses such as more than 5 Gy are sufficient to induce severe damage for hematopoietic

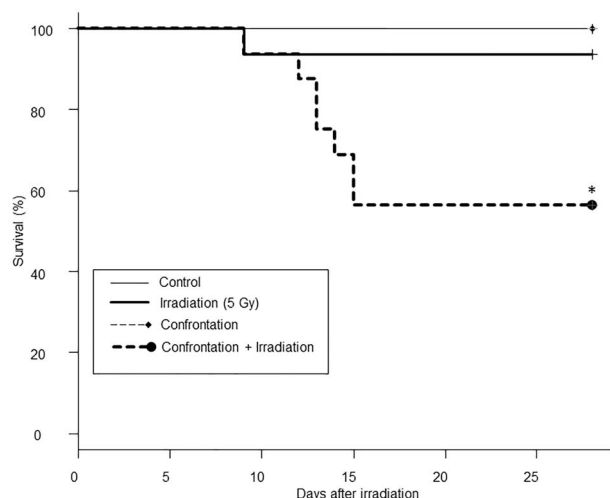


Fig. 4. Modulation of acute effects of radiation in ddY mice by psychological stress with 5 Gy X-ray irradiation. Group-housed mice were used as a control. The data were described using Kaplan–Meier survival curves. $n = 16$, Logrank Irradiation (group) vs Confrontation + Irradiation * $P < 0.05$ ($P = 0.0201$). The data include parts of Fig. 3 data. The line of the confrontation stress alone is the same line as that of the control.

acute radiation syndrome in ddY mice [19], modulation by psychological stress may not be apparent. Effects of confrontation stress alone or irradiation alone on bone marrow are interesting points. It seems that the restoration after irradiation is somehow suppressed by confrontation stress. In the further studies, how confrontation stress suppresses the restoration will lead to understanding the mechanism of this modulation.

We have analyzed modulation of radiation effects under the stress using irradiated mice in a cage under confrontation stress as a group. Indeed, as reported previously [15, 16], both mice in the cage have stress responses in this model. We have also observed the death of both mice in the cage due to irradiation. There are slight but not significant differences between dominant and subordinate mice [15, 16]. Though there may be differences in responses to radiation between them, here we evaluated both mice as a group and observed modulation of radiation effects under the stress. We will perform further research taking this point into consideration.

In the present study, modulation of effects of radiation by psychological stress was observed in the two strains, indicating that psychological stress generally influences acute effects of radiation. In confrontation stress alone group in SAMP10 mice (Fig. 2), a mouse died. SAMP10 mice start to die somehow from around 2–3 months after birth, even under conventional conditions [27]. The period when the mouse in the group died was close to the period (around 3 months). The death could result from the genetic background of SAMP10. SAMP10 mice were influenced by 4 Gy irradiation, which was less than the dose that was effective in ddY mice under stress. SAMP10 mice appear to be radio-sensitive. This result demonstrated that evaluation of radiation late effects by psychological stress using SAMP10 mice should be used by irradiation with doses less than 4 Gy. As SAMP10 mice have immune dysfunction, the mice may be also stress sensitive in the case of DNA-damaging stress like radiation [28]. Effects in terms of aging and brain functions were observed in SAM10 mice even after the lapse of a long period under psychological stress [15]. Further investigation will be needed for understanding radiation effects on SAMP10 mice.

In addition, modulation by psychological stress on late effects such as radiation-induced cancers is also a concern as some cancers may be influenced by psychological stress [1, 2]. Using the restraint model, a model of psychological stress, Feng *et al.* reported that

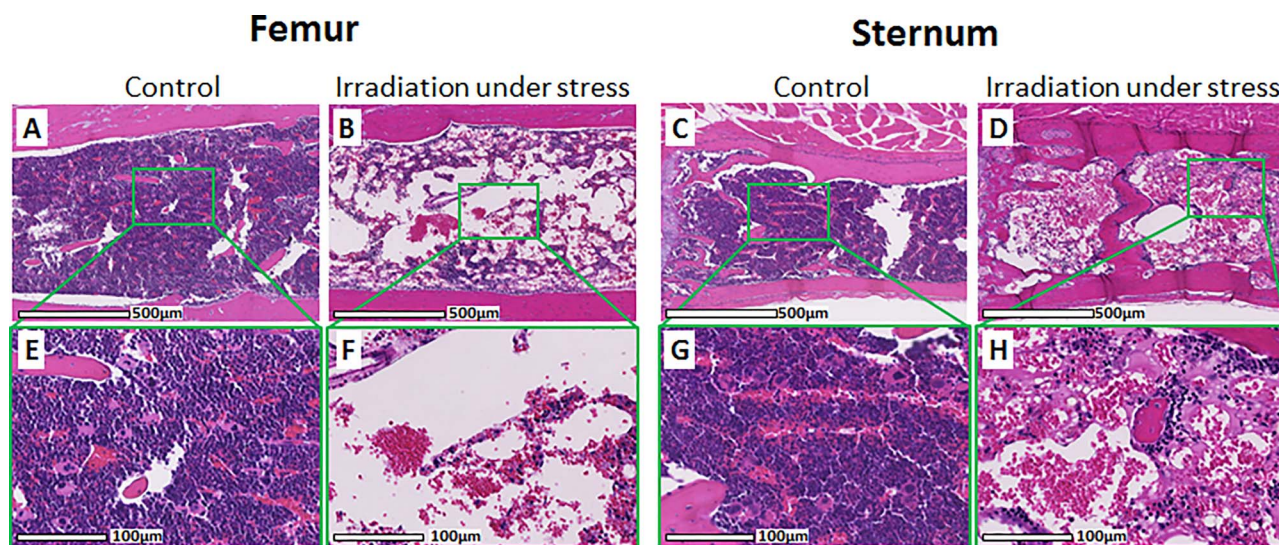


Fig. 5. Representative histological images of bone marrows from femur and sternum. In control ddY mice (A, E, C and G) and irradiated ddY mice at 13 days after irradiation under psychological stress (B, F, D and H). The photomicrographs of E–H were shown at higher magnification of red color squares in A–E, respectively. All sections were stained with HE.

psychological stress promotes radiation-induced thymic lymphoma in p53 heterozygous mice [12]. In terms of p53 functions, radiation-induced chromosome aberrations have also been investigated under restraint stress in normal p53 mice [29–31]. However, the restraint model is a very strong stress, appears to be accompanied by physical stress, and be a temporary experimental model of stress. Though we have to investigate effects of psychological stress using a variety of animal experimental models [12–16], confrontation stress has almost no physical stress, and the stress appears to persist for a long time [15]. Therefore, this model may be also appropriate for evaluating modulation of psychological stress on radiation-induced late effects such as cancers, leading to an understanding of radiation effects in the case of astronauts or people such as cleanup workers exposed to radiation in nuclear accidents [10, 32–34]. Evaluation of modulation of radiation-induced carcinogenesis in mice by confrontation stress is under investigation. On the other hand, it has been reported that psychological stress has a beneficial effect in some diseases [35]. Various approaches are necessary to assess the effects of psychological factors on health. Moreover, SAMP10 and ddY mice were used for evaluation in this study. As radio-sensitivities in mice are dependent on strains [18, 19], modulation of radiation effects including carcinogenesis by psychological stress demonstrated in C57BL wild mice or the p53 heterozygous mice may have differences depending on the mice used [12, 30, 31].

Radiation effects may be dependent on exposure conditions, such as lifestyles or mental conditions when people are exposed to radiation. Understanding modulation of the effects of radiation by psychological stress will contribute to the evaluation of an individual's risk of radiation exposure.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this study.

PRESENTATION AT A CONFERENCE

The parts of this study have been presented at the International Conference of Radiation Research 2019.

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